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Profile

An Interview with Professor Robert J. Lefkowitz, M.D.

Robert J. Lefkowitz, M.D., is James B. Duke Professor of Medicine and Professor of Biochemistry at the Duke University Medical Center. He has been an Investigator of the Howard Hughes Medical Institute since 1976. Dr. Lefkowitz received a Bachelor's degree from Columbia College and an M.D. degree from Columbia University College of Physicians and Surgeons. After serving an internship and one year of general medical residency at the College of Physicians and Surgeons, he served as a Clinical and Research Associate with Drs. Jesse Roth and Ira Pastan at the National Institutes of Health. He then completed his medical residency and research and clinical training in cardiovascular disease at the Massachusetts General Hospital, Boston. During this time, he continued his research in the laboratories of Dr. Edgar Haber and was a teaching fellow at Harvard Medical School. On completing his training, he was appointed Associate Professor of Medicine and Assistant Professor of Biochemistry at the Duke University Medical Center.

Dr. Lefkowitz, what led to your interest in studying the G protein-coupled receptor (GPCR) family?

It's an interesting story. I began my career as a physician. I went to medical school, not as many aspiring researchers do today, as part of an M.D./Ph.D. program or as a way of getting some general biological background for a career in research. I went to medical school to be a practicing physician; that was all I had in mind. When I finished medical school and I began my residency, a lot of my colleagues were looking for ways to avoid going to Vietnam. It was the late 1960s, the Vietnam War was at its peak, and there was a doctor draft going on at that time. Depending on the arrangements you made, you either did one year of internship before you went to Vietnam, or two years if you made special arrangements. One of the attractive things for someone interested in academia, which I was—I was thinking about being an academic physician—was to get some research experience. The typical way to do that was to be accepted to the NIH, and then to enter the military via the U.S. Public Health Service, which is one of the branches of the Armed Forces. In that way, you could fulfill your military obligation. Since I was a very good student, I was able to win one of those appointments.

I graduated medical school in 1966, completed an internship, and went to the NIH on July 1, 1968. One day, in early 1968, I was sitting in the House Staff library reading a journal article. This was about four months before I was to start at the NIH. I happened to read a paper in the *Annals of the New York Academy of Sciences* by a very famous scientist named Earl Sutherland, who was a Nobel Laureate, having won the Nobel Prize for discovering cyclic AMP. In the article, he talked about his work on adenylate cyclase; he had shown that cyclic AMP levels could be raised by things like epinephrine, through what was called the β-adrenergic receptor. He speculated that the β-adrenergic receptor was part of the adenylate cyclase pathway. This was all very interesting to me.

When I got to the NIH and I talked with my mentors, Jesse Roth and Ira Pastan, they suggested that I consider a couple of different projects. One was to see if I could work out a radioligand binding assay for the adrenocorticotropic hormone (ACTH) receptor. At that time, there was no direct way to study receptors. Taking into consideration what I had just read about Sutherland's work, I had an intuitive feeling that this would be a good area to study. So I began to study receptors.

Your laboratory is sometimes viewed from the outside as an "army" of postdocs. What is the average ongoing size of your lab?

The average census of my lab, and this hasn't changed in 25 years, is about 25 people, which includes about three administrative personnel and six or seven technicians. Everybody else is a postdoc or a student.

Never a lack of applications?

Oh, no, more than I can use. Lately, it has gotten worse, as many of us have been flooded with applications from China, via E-mail. I probably get an average of two inquiries a day, ten a week, and 90% of those are from China.

Where do your various trainees come from (college, graduate school, medical school)?

I have several types of trainees. The major denizen of my laboratory is someone who has just completed his or her Ph.D. in a field such as biochemistry, pharmacology, or molecular biology. I also have physician/scientist trainees, who generally come out of the clinical program at Duke Medical Center. Over the years, I have had physician/scientists from virtually every branch of medicine, every subspecialty. Typically, they are trying to determine if they want to travel the career path that I did and see if research is for them. I also have graduate students and M.D./Ph.D. students and, occasionally, undergraduate students who spend a few hours a week in the lab.

When they leave your lab, what types of positions do they generally take? Do most of them go into academia?

Not any more, and that has changed over the years. When I started the lab in 1973, during the first decade, nearly 100% of my trainees went into academia. Beginning in the early 1980s, a significant fraction began to go into the pharmaceutical industry. Now, although they still go in those same two directions, about 50% go into academia and 50% go into the private sector, which now divides into two groups, big pharma and biotechnology. These days, probably the majority of that 50% are going into biotech. Some of those positions are really quite nice, as many of the biotechs are almost a hybrid between academic life and drug company life.

Can you remember all of your trainees? So many of them have gone on to great success in research. To what do you attribute that?

That is a very interesting question. I have been remarkably fortunate. In the area of GPCRs, which is currently one of the hottest topics and the focus of many conferences each year, it is not unusual for anywhere from 25% to 35% of all plenary speakers to be people who have trained with me over the years. And sometimes that percentage is even greater. If you look overall at the attendees at these conferences, the percentage that trained with me is amazing.

The key issue is why have my trainees had so much success. I have thought about that a great deal. I really do think there are key elements about doing good science that are transferable, but none of those elements can be written down in a book or even lectured about. They have to be taught by example. Studying with a research mentor like myself is an apprenticeship; you basically live with the person for three, four, or five years. You watch what he or she does. Therefore, it is important for the mentor to share himself with his students. Anybody who knows me knows that I am extraordinarily interactive. I thrive on the daily interactions and the relationships. My trainees get to see me in operation day to day. I do not function behind closed doors.

For example, for many years I have held small group meetings in my office three or four afternoons a week. Typically they last anywhere from two hours to three or four hours. These are groups of from four to a dozen people, sometimes more, who are working on related themes in the laboratory. We go over data, talk about the results, and plot strategy. I share myself with them, and they see me work, making decisions such as what projects to work on. They learn my thinking style; for example, when do I abandon a project for which I think there is no hope, and when do I stick with it even in the face of adversity? They learn by example. When they leave my lab, I like to think that there is a piece of me in each one of them.

If you look at the history of science, choose virtually any scientist who has really made a name for herself or himself, and look at the laboratory in which they began their career, you will find that it is almost always with someone equally or more prominent. These extraordinary lineages exist in science, and they are no accident. They simply reflect the fact that there are transferable elements about how to do research, and how to do it well.

With regard to the first part of your question, I think I do remember everyone who has worked in my lab. I am so interactive, and for me it is all about the relationships. Even though I have always been very focused on specific goals, in an almost mystical sense, the older I get, the more I realize that the science is almost secondary. It's all about interacting with the people who come to work with me, sharing a project, and struggling with goals. For the most part, I form very close personal relationships with almost everyone who comes to work with me.

Your work has probed fundamental questions of GPCR structure and function. Being the most important single class of pharmaceutical targets,

how do you believe your research has influenced this fact?

When I began my work in the early 1970s, there was no direct way to study any receptor. In those days, you studied an adrenergic receptor by giving drugs to an animal and seeing how it responded; you had to infer the characteristics of the receptors based on very downstream physiological effects. The first contribution I made, 30 years ago, was the development of direct radioligand binding techniques for studying receptors, which allowed you to study the receptors directly. That revolutionized the business of how drugs are developed. Now drugs could be screened simply based on their ability to compete in radioligand binding assays. In fact, while we never patented any of the receptors after we cloned them, we did make available, through a nonexclusive licensing mechanism at Duke, cultured cell lines that stably expressed all of the adrenergic and other receptors we had cloned over the years. These licenses made a lot of money for the institution.

In terms of drug development, that relatively simple notion had a major impact. Once we had cloned the β_2-adrenergic receptor, in 1986, and it was the first seven-membrane spanning drug target receptor to be cloned, we quickly thereafter cloned eight or ten other receptors. Based on that information, within a year or two, it became clear that virtually all the seven-membrane spanning receptors were members of a superfamily. They shared similar structures and amino acid sequences, and we later showed that they shared a universal mechanism of regulation in terms of desensitization.

That realization immediately accelerated the whole field. Now people realized that they could clone whatever receptor or drug target they were interested in studying simply by using homology techniques. The discovery of one receptor led to the discovery of the next. In our very first paper on the cloning of the β_2-adrenergic receptor—a classic paper in *Nature* in 1986—we speculated in one of the last paragraphs that all GPCRs would look like the β_2-adrenergic receptor. Our prediction turned out to be right.

What types of new drugs can you envision based on current knowledge of GPCR function and distribution and the size of the gene family?

People quote different figures, but one is that 60% of all prescription drugs sold in the world target GPCRs. All those drugs have the same mechanism of action: they target the ligand binding site of the receptors, as either agonists or antagonists. Agonists are things that stimulate, like adrenaline, and antagonists are things like β-blockers or α-blockers. When I think about potential novel ways of utilizing this information, I envision drugs that target other aspects of receptors: not the ligand binding site on the outside of the receptor, but maybe the inner surfaces of the receptors where they interact with various effector proteins, such as G proteins. If you could block the interaction of the receptor with a G protein, you would profoundly affect signaling. Currently, we silence a receptor by blocking its active site so it cannot bind its ligand. One of the main directions of my laboratory for the past several years, and undoubtedly the main direction now, has to do with delineating novel signaling pathways in which the G proteins are either less important or not important at all.

Wouldn't compounds that target an effector binding site tend to have less drug specificity?

I would think they would have less specificity, but there are clinical conditions in which one might want to target multiple receptors in a class. For example, consider hypertension, in which many of the receptors involved in hypertension (which control vascular reactivity) couple to G_q, which is one of the G proteins. Angiotensin receptors couple to G_q, for example, and, from a commercial perspective, some of the most useful drugs for treating hypertension are classical receptor blockers that block angiotensin receptors. α-Adrenergic receptors also couple to G_q and can cause vasoconstriction. Endothelins, too, couple to G_q and can lead to vasoconstriction, and people are developing endothelin receptor blockers. Suppose you had a generic way of inhibiting the coupling of receptors to G_q. You could give one drug and maybe take out ten different receptors that lead to increased vasoconstriction and hypertension, rather than giving an angiotensin blocker, an endothelin blocker, etc. It may be useful to be able to target panels of receptors.

Your laboratory has been a pioneer in many areas of research related to GPCR signal transduction, one example being your work on constitutively active receptors. Please tell us a little about why this is important and how knowledge of this mechanism might lead to new drugs.

Constitutively active mutant receptors were something we discovered about a dozen years ago. It was a completely serendipitous discovery. We were trying to make receptors that were impaired in their activity and, quite by accident, we made some that were superactive. Receptors are switches, and they are generally off in the absence of a stimulus. An agonist, such as adrenaline, throws the switch and turns it on, changing the conformation of the receptor. In general parlance, pharmacologists often talk about two different conformations of receptors, or basically classical allosteric theory applied to receptors. They call the conformations R and R^*, the inactive and active conformations, respectively; they are in equilibrium with each other. Agonists are drugs that bind

with higher affinity to R^* and push the equilibrium in that direction.

We discovered that making certain changes in the receptors changed that equilibrium. For a typical receptor, in the absence of agonist, the equilibrium is way in the direction of R, with very little R^*. However, certain mutations could completely reverse that, putting the majority of receptors in the active state even without an agonist. Therefore, those mutations led to signaling, even in the absence of a stimulus. From the perspective of drug development, this was interesting, because we were able to prove in a way that had not been possible before that, rather than there being only two types of drugs, agonists and antagonists, there had to be three. This had been worked out by others years before, but had never been demonstrated. The idea is that if you have two conformations, R and R^*, there are three possibilities for a drug: one with preferentially higher affinity for R^* (an agonist); one with equal affinity for R and R^* (an antagonist, which does not change the equilibrium but prevents an agonist from binding to the receptor); and one which has higher affinity for R than for R^*, which should turn the switch off if it is already on. In most systems, the basal activity of a receptor is so low that almost all receptors are in the R conformation, and it would be very difficult to demonstrate this third drug possibility. With a constitutively active mutant receptor, which is already on in the absence of any drug, you have a beautiful assay system for what are now called negative antagonists or inverse agonists. If you put such a drug on a constitutively active mutant receptor, it shuts off its signal, whereas if you put a classical antagonist on the receptor, you saw no such turnoff. This has direct implications for drug discovery in terms of situations in which a constitutively active mutation spontaneously forms, causing disease. You would want to design a drug to turn it off.

Another area that your laboratory initiated is that of "orphan" receptors. How did that come about?

This is another classical example of serendipity. The story goes back to about 1985, when we had just cloned the β_2-adrenergic receptor. That was a huge watershed in the field. We were beginning to think there would be other receptors like it, but this was not yet known. One of the first things we did was to try to clone the β_1-adrenergic receptor. We took some human genomic DNA and did a Southern blot, using as the probe the full-length cDNA for the β_2-adrenergic receptor. When we did the Southern blot at high stringency, as you would expect, we got only a single band, which was the gene for the β_2-adrenergic receptor. We then reduced the stringency, expecting to see the progressive appearance of other bands, which might represent other receptors. As we reduced the stringency of the Southern blots, we did pick up one other band, but we never picked up any others, for reasons I do not understand to this day. We assumed that the new band was the β_1 receptor, so we made a size-selected genomic library, cloned out that band, sequenced it, and found that it had seven-membrane spanning domains. It looked very much like the β_2 and we were quite convinced it was the β_1.

However, when we expressed it in cells and looked at its ligand binding ability, it did not bind the β receptor ligands, so we knew it wasn't the β_1. We published the sequence anyway, because we knew that it was only the second seven-membrane spanning receptor that had been cloned. We called it G21 and it was, in fact, the very first orphan receptor, because we did not know its ligand.

Nobody in the lab wanted to work on it, because they didn't know where to start. A year later, though, a new postdoc (Anick Fargin) came to work in the lab. That's an important time, because it is only during those first few months that people actually listen to me! I thought it was a great project, and I sent her off to the library to do some reading. I told her that what we know about the receptor is that it looks like the β_2 receptor and it weakly binds β-receptor ligands, but not with the appropriate specificity. She was to look in the literature and see if there were any other receptors known to weakly bind β-receptor ligands. A day or two later, she came back and reported that serotonin receptors could weakly bind β-receptor ligands. We set up a competitive binding assay and showed that serotonin could compete for receptor binding, and our orphan receptor turned out to be the first serotonin receptor, the 5-HT_{1A}. We published a paper stating that this was the first orphan receptor and the first orphan receptor "deorphanized." The orphan receptor field is now huge. Technically, of the 1,000 or more seven-membrane spanning receptors that we know of from the sequencing of the human genome, the overwhelming majority are orphans.

The serendipitous nature of all this continued. The band we had pulled out of the Southern blot and expected to be the β_1 had a sequence that was extraordinarily similar to the β_2 receptor. By the way, another fortunate break for us was that most of the adrenergic receptors have intronless genes. So we were able, right at the beginning, to get complete protein sequences from genomic clones. While we were sequencing this gene, I wanted to make sure we had a cDNA for it, so we probed a very good human placental phage cDNA library that I had obtained from a friend of mine. We screened that cDNA library with what we thought was the β_1 clone. To our horror, we pulled out a clone that, while clearly having seven-membrane spanning domains, was different in sequence than our presumptive β_1 receptor, and differed from the β_2 receptor. So now we had a third clone, another orphan. Some people in the lab wanted to just put it away, but I said, no, let's finish the job. So we ex-

pressed it, and it turned out to be the β_1 receptor! Isn't that a crazy story? When the smoke cleared, we had cloned the entire family of adrenergic receptors. We had stumbled and bumbled, but in the end, it all worked out.

The visual signal transduction field has many times foreshadowed discoveries of nonvisual signal transduction. How has your laboratory leveraged this literature and, in some cases, added to it?

That's a very good question. The reason that the visual system often gave clues as to what was going on in mammalian biology is a simple one: the abundance of these molecules in the visual system is thousands of times greater than in other systems. We now appreciate the fact that the visual signal transduction system works through a series of components that are functionally and structurally quite analogous to hormone receptor interactions. For example, the light receptor rhodopsin (like the β receptor) signals to a G protein called tranducin, and there is an effector enzyme, a cyclic GMP phosphodiesterase, analogous to the mammalian adenylate cyclase. These functional analogies were already appreciated in the early 1980s. In 1983, the sequence of rhodopsin was determined by Edman degradation (standard protein sequencing), which implies that there were grams of it available. From bovine retina you can get an unbelievable amount of rhodopsin. By comparison, to purify something like the β_2 receptor required 200,000-fold purification. We spent years figuring out how to purify such a receptor. When we published the sequence of the β_2-adrenergic receptor in 1986, we showed that the receptor looked like rhodopsin. Even though that should not have been a surprise, because of the known functional analogies, it shocked us and everybody else. Nobody really expected that rhodopsin and the β receptor would look alike.

I was, maybe more than other people, tuned in to the fact that the visual system might be providing clues. Let me give you a specific example. We had found in the early 1980s that the β_2 receptor was regulated by phosphorylation, and that this occurred via a unique kinase, which we named the β-adrenergic receptor kinase, or βARK. One of my graduate students (Jeff Benovic) eventually purified βARK.

At the same time, several laboratories were studying a mechanism for inactivation of rhodopsin and had identified a kinase that was involved in phosphorylating rhodopsin, which resulted in its inactivation. They called it rhodopsin kinase. In about 1986, the eye people found that rhodopsin kinase could not work alone. It seemed to require another molecule, a molecule that had been known for years as 48K protein. It was a very abundant molecule in the retina and it was extraordinarily immunogenic. It could lead to autoantibodies that could cause retinal degeneration. They found that when you stimulated the retina with light and rhodopsin became phosphorylated, the 48K protein translocated to the retinal membrane and helped to turn off the rhodopsin.

When they reported that in 1986, we were fretting over the fact that the more we purified βARK, the more it lost desensitizing activity. (We assayed βARK in a reconstituted system of β_2-adrenergic receptor and G_s). Some in the lab thought we were "barking" up the wrong tree, and that the kinase had nothing to do with inactivating the receptor. I was convinced we were losing some accessory factor. That is when the retina guys defined a role for the 48K protein, and they renamed it arrestin. I called the researcher in Germany who had purified arrestin (Herman Kuhn), and he sent me some of it. Sure enough, when we added that to the assay, it completely restored the ability of our highly purified βARK preparations to inactivate the β receptor. That convinced me that there must be related effector molecules in nonvisual tissues. We went searching for that and eventually discovered the β-arrestins, which function in an analogous way to their retinal counterparts.

By the way, Jim Inglese's connection to all this is that his first project in my laboratory, after we had just cloned the β-adrenergic receptor kinase, was to see if he could clone rhodopsin kinase, and he did. We were the first ones to clone rhodopsin kinase and we found that it looked just like βARK, once again suggesting the existence of a gene family.

When molecular cloning began to build momentum in the early 1980s, your laboratory forged a strategic collaboration with Merck Research Laboratories to clone the first mammalian GPCR, the β-adrenergic receptor. What were the key elements that each laboratory contributed to allow this landmark event in biology to occur?

That collaboration began when a recent graduate of my laboratory, Cathy Strader, went to work for Merck. She was very impressed with some of their high tech capabilities, such as a state-of-the-art protein microsequencing facility. Merck also had some good young molecular biologists that were very involved with cloning. In conversations with Cathy, she may have suggested that we collaborate, combining the expertise in our laboratory—the only lab in the world to have purified a G protein-coupled receptor—and the molecular biology capabilities of Merck. We agreed that we would provide the purified protein and Merck would do the cloning. Cathy cleaved the purified protein with cyanogen bromide, separated the peptides using HPLC, and then sequenced the peptides. That yielded five individual peptide sequences totaling about 80 amino acid residues.

Then the cloning work started; initially all of the cloning was done at Merck. As the months went by,

though, and many libraries were screened, no headway was being made. We never found any clones that contained the peptides we knew were in the receptor. I became increasingly impatient and frustrated with the whole endeavor. I had a young cardiology physician scientist in my laboratory named Brian Kobilka, and he was very anxious to get involved in the cloning effort. He made four one-week trips to Merck and learned the essential aspects of cloning. Then we started working in parallel with the people at Merck, but still we made no progress.

One day, Brian made a crucial decision. He suggested that we try to get a genomic clone instead of relying on the cDNA libraries. We broached the subject with the people at Merck, but they were not very enthused about it. So Brian went ahead and made a genomic library. He sent the genomic library to Merck, and both Merck and we screened it. The first screen done in both places immediately pulled out really good-looking clones. Within a week, we could see that our clones and Merck's clones were the same. Then the sequencing began. That went very quickly, and to our amazement no introns were present in the genomic clone. We had the entire receptor in one open reading frame, and one by one all of the peptides we had deduced from protein sequencing popped up.

Targeting of GPCRs with small molecules to mediate hypertension has revolutionized health care for cardiovascular disease. What is next in terms of drug development based on GPCRs or their signaling pathways? In particular, what is on the frontier, for example, for psychiatric disorders and GPCRs?

My feeling is that a huge unexplored area has to do with the systems that regulate GPCRs, and I have spent much of my career studying this. Virtually all the drugs we have today that target GPCRs target the ligand binding sites of the receptors. But, as I explained, there is a universal mechanism that regulates the receptors, and I think that system is a wonderful system for drug development. We have made knockout animals of most of the GPCR kinases and β-arrestins, and they have interesting phenotypes. For example, I think that inhibitors of GRKs (G protein-coupled receptor kinases) might be very useful drugs. I see possible indications in heart failure, asthma, Parkinson's disease, etc. A number of drug companies are currently working in this area. Knockout animals lacking β-arrestin 2 show a markedly prolonged and intensified action of morphine; the morphine is not able to desensitize the receptors, so you do not get tolerance. So one frontier for the future is the development of drugs targeting the systems that regulate these receptors.

When did you decide it was time to "leave" the lab bench, and when was the last time you actually demonstrated a laboratory technique to a trainee?

I set up the lab in 1973, and at the very beginning I was very active at the bench. By 1975, I was not doing much in the way of experiments. As the lab grew, I would spend the early part of the day getting people started, going over their experiments with them, etc. So my own experiments would start later and later in the day, until I was starting my experiments at four o'clock in the afternoon and I had to go around the lab trying to bum reagents from people.

I would guess, though, that not very much goes on in the lab that you do not know about.

That is correct. I basically live right here. I don't chair a department or lead a program, and I have refused such things because what I love is being in the lab.

Is it true that you nearly pursued a career as a pianist instead of going into medicine?

Absolutely not! I took piano lessons like any self-respecting middle class Jewish boy in the Bronx growing up in the 1940s and 1950s, but as best I could tell, I was totally devoid of talent, not to mention interest in the subject.

Your diet of whole grains and water is somewhat legendary in scientific circles. What advice can you give someone wishing to improve his or her dietary lifestyle?

Avoid saturated fat and eat healthy foods—fruits and vegetables. Avoid pastry and cakes. For a while, after I had bypass surgery about 10 years ago, I was on a fairly strict Ornish diet, and I am to this day a strict vegetarian. In the last year or so, I have been liberalizing my monounsaturated fats, adding some olive oil to my diet, and my latest passion is peanut butter. I do not mean the peanut butter you buy at the store, which has lots of hydrogenated fats in it. I go to a whole foods market that has a machine with which you grind your own peanuts.

Thank you, Dr. Lefkowitz.

—Interview by Vicki Glaser