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A Serendipitous Scientist

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Abstract

Growing up in a middle-class Jewish home in the Bronx, I had only one professional goal: to become a physician. However, as with most of my Vietnam-era MD colleagues, I found my residency training interrupted by the Doctor Draft in 1968. Some of us who were academically inclined fulfilled this obligation by serving in the US Public Health Service as commissioned officers stationed at the National Institutes of Health. This experience would eventually change the entire trajectory of my career. Here I describe how, over a period of years, I transitioned from the life of a physician to that of a physician scientist; my 50 years of work on cellular receptors; and some miscellaneous thoughts on subjects as varied as Nobel prizes, scientific lineages, mentoring, publishing, and funding.

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As a youngster and later as an undergraduate and medical student at Columbia University, it never occurred to me that I would someday become a scientist. Rather, I was fixated from perhaps the age of 8 or 9 on the goal of becoming a practicing physician. Only through a serendipitous set of circumstances was I subsequently tempted and ultimately seduced by the Muses of science. Before I succumbed, however, I did mount some pretty serious resistance. But I'm getting ahead of the story.

CHILDHOOD

I was born in 1943 and grew up in the East Bronx, the only child of Rose, an elementary school teacher, and Max, an accountant, both first-generation Americans. All four of my grandparents had immigrated to the United States from Poland during the so-called great migration of Jews from Eastern Europe during the late nineteenth and early twentieth centuries. Growing up in the 1940s and 1950s among a very large and close network of multigenerational extended family, I was nonetheless devoid of any familial role models in either medicine or science. My interest in science and medicine was inspired, at an early age, by my family physician, Dr. Joseph Feibush. Accordingly, some of my favorite playthings were a functioning toy microscope with which I examined various insect parts, hairs, skin cells, and so forth and a chemistry set with which I prepared solutions and precipitates of varying color.

EDUCATION

I attended the Bronx High School of Science, a public high school that admitted students interested in science and mathematics who scored high enough on a New York City–wide competitive examination. From there, I went on to Columbia College, where I majored in chemistry and was premed, and then medical school at the Columbia University College of Physicians and Surgeons. All of this was on a clear trajectory leading me toward a career as a physician. Although I very much enjoyed my science courses, I never did any original research either in college or medical school, even though such electives were available. In fact, while in medical school, I filled every available elective period with clinical rather than research experiences. That said, in retrospect, two professors did kindle within me a deep interest in biological chemistry. The first was Dr. Ronald Breslow, then a junior faculty member in the chemistry department who single-handedly taught a seminar on Topics in Biological Chemistry. In medical school, a young hematologist, Dr. Paul Marks taught the introductory course in clinical diagnosis. His lectures included readings from the original scientific literature, for example, about hemoglobinopathies, which were just being elucidated at the time. Both of these young professors kindled in me a nascent and deep interest in the scientific basis of medicine. Both went on to highly distinguished, award-winning careers.

THE US NATIONAL INSTITUTES OF HEALTH

After graduation from medical school in 1966, I did two years of house staff training (intern, junior resident) in internal medicine at Columbia Presbyterian Medical Center before heading to the US National Institutes of Health (NIH) on July 1, 1968, for the experience that would change the course of my career. In the late 1960s, the Vietnam War had led to a draft of all young physicians. In general, after medical school graduation, the Berry plan [created in the 1950s by Assistant Secretary of Defense Frank B. Berry, MD (1)] allowed physicians to obtain a deferment for house staff and/or residency training before entering one of the armed service branches for a two-year period of mandatory service. In 1968, almost all medical draftees could count on a

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one-year deployment to Vietnam. The war was very unpopular, and many among my colleagues did not support it. However, there were very few options for avoiding service in Vietnam. One, however, was to gain a commission in the US Public Health Service and be assigned to one of their stateside research installations such as the NIH, the Communicable Disease Center (CDC, now named the Centers for Disease Control and Prevention), or others. The positions at the NIH were highly sought after and thus very competitive to obtain. Largely because of my strong academic record, and despite the absence of any research credentials, I was successful in gaining such a position and, on July 1, 1968, began work in the National Institute of Arthritis and Metabolic Diseases (NIAMD), as it was then called, under the joint mentorship of Dr. Jesse Roth and Dr. Ira Pastan in the Clinical Endocrinology Branch.

Things did not go well. Devoid of research experience of any kind, it was all new to me. My project was challenging—to develop a radioligand binding technique to study the adrenal adrenocorticotropic hormone (ACTH) receptor. The context at the time, it should be emphasized, was that there was no consensus that receptors for drugs and hormones even existed. The choice of the ACTH receptor by my mentors was dictated by the availability of an adrenal cortical carcinoma that was serially passaged in the subcutaneous tissue of nude mice. The assumption was that the ACTH receptors would be present and perhaps even enriched in the membrane fractions derived from such tumors. My first task was to develop a procedure for radioiodinating ACTH with $Na^{125}I$ that did not destroy the biological activity of the hormone. This in turn necessitated being able to separate the very small amount of mono-radioiodinated product from the much larger amount of unreacted ACTH so that its bioactivity could be assayed. It took me well over a year to achieve this goal, which was more than enough time to convince me that a career in basic research was not for me. I had never before in my life met with such sustained and unremitting failure. My father's unexpected sudden death in December 1968 at age 63 further darkened my mood.

In an effort to return to more solid ground, I made arrangements to resume my clinical training at the Massachusetts General Hospital (MGH) in Boston upon the completion of my service at the NIH. There I would follow a three-year program, one year as a senior medical resident and then two more as a cardiology fellow. Meanwhile, roughly 18 months into my time at the NIH, my project began to progress. In the course of a few months, I succeeded in purifying the mono-radioiodinated ACTH, demonstrated its bioactivity, and developed the radioligand receptor binding assay, one of the very first of its kind (2). This led to several publications (2–4) and a tremendous sense of relief and accomplishment, but not enough to change my plans or respond to the entreaties of my mentor Jesse Roth to extend my stay at the NIH.

I learned much about how to do research while at the NIH, which I credit to Jesse Roth and Ira Pastan. In addition to the basics, I learned a great deal about the science of chromatographic separation and, perhaps more importantly, about how to deal with failure, at which I was constitutionally not very good. Lessons learned during this time have helped me to counsel my own trainees as they face the inevitable frustrations of a scientific career. One particularly helpful piece of advice at the time was given to me by an NIH Senior Investigator whose laboratory was down the hall from mine on the eighth floor of Building 10, the Clinical Center. Sensing my deep frustration about my lack of success, he asked if I knew the difference between an average scientist and a world-class one. When I said that I did not, he said something to the following effect: "For an average scientist, perhaps 1% of experiments actually work. But for the superstar, it could be as high as 2%." I have often passed this insight along to trainees in a talk that I give each year to research fellows at Duke entitled "How to deal with failure and rejection in research." I do, however, add the proviso that I have come to believe that 2% is a bit high.

As I look back on my formative years at the NIH, it is worth commenting on the special and remarkable role that this institution played in training a whole generation of biomedical,

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in particular physician, scientists and academic leaders. The best analysis of this I have seen was published several years ago by my good friends Mike Brown and Joe Goldstein. In their short essay, "A golden era of Nobel laureates," they try to understand the magical—and thus far irreproducible—set of circumstances that led to nine Nobel laureates training in research at the NIH during the decade of the 1960s (5). All nine of us were MDs, and two also possessed a PhD. This generation of physician scientists who served as commissioned officers in the US Public Health Service during the era of the Vietnam war was often referred to as yellow berets, for reasons that are not too difficult to discern. Among my specific cohort who served between 1968 and 1970, four of us would eventually receive the Nobel Prize (Harold Varmus, Joe Goldstein, Mike Brown, and myself). I was by far the laggard, with Brown and Goldstein receiving the prize as early as 1985, Varmus in 1989, all in Physiology or Medicine, and myself not until 2012 together with Brian Kobilka in Chemistry.

Among the factors Goldstein & Brown (5) cite in their article as contributing to the success of the yellow berets were (*a*) the high competition for places in the NIH program, ensuring the selection of "the best and brightest" applicants (5, p. 1034); (*b*) the quality of our mentors; (*c*) the intensity and focus of our research experiences with very few distractions; (*d*) the emphasis on basic research in reductionist systems as opposed to today's emphasis on translational research; and (*e*) the focus of medical schools in the 1960s on basic science as the core of the medical curriculum. This is in contrast to the relatively short shrift given to the basic sciences in medical schools today and the rarity of teaching anything about how current scientific understanding was actually achieved. To quote Goldstein & Brown, "The joy of finding new facts or overturning old ones is no longer transmitted to students" (5, p. 1034). What is the prognosis that any institution in the future will succeed at the level of the NIH in the 1960s in training physician scientists? They conclude, and I agree, that if there is any possibility, it will require that lessons derived from that experience be applied going forward.

THE MASSACHUSETTS GENERAL HOSPITAL

I began my work at the MGH on July 1, 1970, as a senior resident in medicine. For the next six months, I was immersed in standard house staff rotations, for example, in the emergency room or on the consultation services of various subspecialties of internal medicine. There was a great deal of night and weekend duty, and the pace was frenetic. Although I enjoyed the clinical work and its challenges very much, unlike my earlier years as a more junior house officer at Columbia, something now seemed to be missing. I soon came to appreciate that this missing ingredient was experimental data. I began to realize that the taste of success I had at the end of my time at the NIH had hooked me. Like a junkie needing a fix, I was clearly having serious withdrawal symptoms. For someone whose sole professional aspiration since childhood had been to become a practicing physician, this was a very surprising development indeed. Accordingly, I secured a position to work in the laboratory of Dr. Edgar Haber during the second six months of my residency year. This was ironic indeed. Because residents were paid with hospital revenues, they were required to spend all elective periods (such as my second six months of residency) exclusively doing clinical work, not research. Despite this prohibition, I forged ahead. Thus, whereas in medical school I did only clinical electives and no research, later in my training when research electives were actually prohibited, I sought them out. Alas, my illegal work in Haber's lab was eventually discovered, and I was called into the office of the chairman of medicine, Dr. Alexander Leaf. Feigning anger, he eventually let me off with a very mild verbal rebuke.

Haber, a physician-scientist, was then the young chief of cardiology at the MGH. He had trained with Nobel laureate Christian Anfinsen at the NIH (thus, he was another yellow beret). In addition

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to continuing basic work on immunochemistry in his large laboratory, he had also developed an interest in the renin–angiotensin–aldosterone axis. Given my interest in, and hands-on experience, working with receptors, his wish was for me to try to develop a binding assay for the aldosterone receptor, a project in which I had no interest. I wanted to continue to work on a receptor coupled to adenylyl cyclase (i.e., a plasma membrane–bound receptor), not a soluble cytoplasmic steroid receptor. Being fairly strong-willed, I resisted his entreaties and finally convinced him to let me work toward a binding assay for the β-adrenergic receptor (β-AR). This seemed particularly timely in view of the then-recent advent of beta blockers in clinical medicine. This work would continue throughout my two-year cardiovascular fellowship and ultimately for my entire career.

I am often asked why I chose the β-AR as a model for my studies. There was no single reason. Rather, a confluence of considerations convinced me that it would be an excellent model system. First, it was coupled to adenylyl cyclase, as was the ACTH receptor. Earl Sutherland won the Nobel Prize in 1971 for the discovery of adenylyl cyclase and cyclic AMP, and much of the signaling community was now focused on elucidating all the various hormones and neurotransmitters that stimulated the enzyme's activity. Second, Sutherland's own studies focused on β-adrenergic–stimulated adenylyl cyclase activity. In fact, he felt that the adenylyl cyclase itself was the β-AR for adrenaline. Third, as noted above, there was a clinical resonance because of the recent introduction of beta blockers into clinical practice. Finally, unlike many of the peptide ligands that had been shown to stimulate adenylyl cyclase—such as ACTH, glucagon, secretin, parathyroid hormone, and vasopressin, among others—ligands for the β-AR, such as epinephrine and norepinephrine, as well as β-adrenergic antagonists, such as propranolol, were small molecules. Whereas for my earlier work on the ACTH receptor, I had to create my own analogues by chemical and enzymatic treatment of ACTH to perform structure-activity studies, there were many dozens of small-molecule ligands available for the β-AR. I anticipated putting these to good use as the basis for developing radioligands, photo affinity probes, affinity chromatography resins, and so forth.

While in Boston, I did develop a ligand binding assay, but it eventually became clear that it was not labeling the true β-AR binding sites. This would await work during my first year at Duke University (1973–1974). During the time in Boston, I also was one of several investigators who extended Martin Rodbell's new paradigm of a GTP requirement for glucagon stimulation of the cyclase to the β-AR–stimulated system. During the two years of my cardiovascular fellowship, I spent as much time as possible in the lab but also did the required clinical rotations. More than one experiment in progress was trashed during nights on call when I had to respond to one emergency or another.

DUKE UNIVERSITY

My decision to move to Duke at the end of my fellowship surprised my colleagues in Boston, all the more so because Duke in the early 1970s had not yet attained the national stature that it enjoys today. However, the offer was too good to refuse: a suite of labs totaling about 1,500 square feet in a newly constructed building, a startup package of $75,000, a salary of $32,000 per annum, and support for a technician and a fellow for up to three years or until I received grant funding to support them (which I did immediately, thereby cancelling that part of the deal). In contrast, at Harvard where I had already been offered a position, my lab would be constructed by ripping the shelving out of the supply closet in my mentor's laboratory and installing a single lab bench. This would be sufficient in size for two people to work side by side if they stood close enough so that they actually touched. Thus, in the end, this was not a difficult choice.

My career at Duke began on July 1, 1973, and I have remained at this institution ever since. During the first few years, imagining myself the consummate physician scientist, I devoted about

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25% of my time to attending clinics and making teaching rounds on the medical (internal medicine) service while also doing experiments at the bench. However, my lab grew rapidly so that by the five-year mark, I was no longer at the bench but was spending all my time working with my trainees, planning experiments, reviewing data, and writing papers. I had also cut my clinical work back to about 10%. I did, however, continue to make teaching rounds for 6–8 weeks a year for 30 years, only standing down from this responsibility in 2003. Nonetheless, for reasons that are not entirely clear, I continue to renew my medical and control substance licenses each year.

In the early 1970s, it was not yet generally accepted that drug and hormone receptors existed, at least in a physicochemical sense. This is illustrated by a 1973 quote from Dr. Raymond Ahlquist written shortly after he and I had met for the first time: "This would be true if I was 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" (6, p. 121). The remarkable irony of this statement resides in the fact that it was Ahlquist who, in 1948, first proposed the existence of distinct α- and β-ARs, based on classical pharmacological dose-response studies. Clearly, his concept of receptors was very different than mine.

Dr. James Black's description of the status of the receptor concept at the time he was developing the first clinically used beta blockers (for which he received the Nobel Prize in Physiology or Medicine in 1988) is also noteworthy: "In 1958, John Stevenson and I were given the chance to try to synthesize the first β-adrenoreceptor antagonist. At that time, almost no one in biomedical research recognized receptor as a useful concept. Even those who did, pharmacologists with a bent for mathematics, were apologetic. To them receptor was an invention they had to make to apply the Law of Mass Action to the actions of drugs. So, when Inderal was first marketed in 1965 as a β-adrenoceptor antagonist, receptor was still a mere idea, though a useful one" (J. Black, personal communication).

Finally, Earl Sutherland's concept of cell signaling at about the time I was beginning my work is summarized in **Figure 1*a***. Noticeably absent from his diagram is any independent receptor entity. Rather, he imagined that the adenylyl cyclase enzyme was the central target of extracellular ligands. This figure, taken from his Nobel lecture in 1971 (7), was in essence the jumping-off point for my own research. **Figure 1*b*** shows a simplified representation of current concepts and highlights how much has been learned over the past almost 50 years. Many laboratories have contributed to this progress. Alas, however, some of the major contributors from the early days of the field with whom I often interacted, such as Al Gilman, Marty Rodbell, Mickey Schramm, Zvi Selinger, Ora Rosen, Ed Krebs, Eva Neer, and others, are sadly now gone.

RESEARCH CAREER

I have had numerous opportunities to review in some detail the arc of my laboratory's research over the past years, from the early 1970s to the present, so I do not plan to reprise that here. Rather, let me briefly delineate the distinct phases of the work that have engaged me.

Development of Methods (1970s–1980s)

In the early 1970s, there were no methods for direct study of receptors nor, as described above, any wide consensus about their existence. It was clear that the way forward would require the development of a whole suite of new technologies that did not then exist. Using the β- and α-ARs as models, we developed radioligand binding methods, photo affinity probes, and affinity chromatography matrices for each of the then four known subtypes of adrenergic receptors (8).

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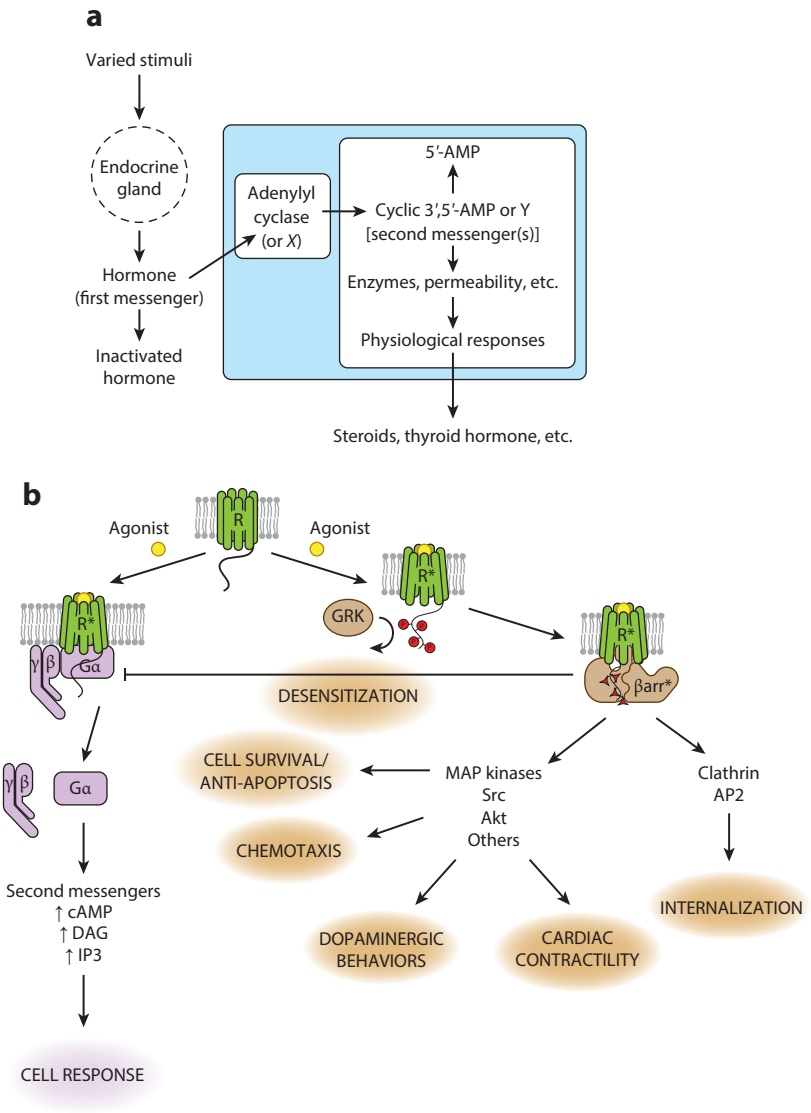

Figure 1

Models of cell signaling. (*a*) Model reflecting understanding in the late 1960s and early 1970s. Panel reprinted with permission from Reference 7. (*b*) A contemporary model featuring the activities of G proteins, β-arrestins, and G protein–coupled receptor kinases. Abbreviations: βarr, β-arrestin; cAMP, cyclic AMP; DAG, diacylglycerol; IP3, inositol trisphosphate; R, receptor.

Solubilization, Purification, and Reconstitution of the Receptors in Lipid Vesicles with Effector G Proteins (Late 1970s and Early 1980s)

This work served to establish conclusively that both functions of the putative receptor (specifically the β_2-AR) (i.e., binding of ligands with appropriate pharmacological specificity and activation of effector G proteins) did in fact reside in a single protein. Purification of the four adrenergic

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receptors (α_1, α_2, β_1, and β_2) to homogeneity was a daunting task requiring more than 100,000-fold purification and occupied my lab for more than a decade (9). Concurrent with this work, Gilman's lab identified and purified G_s (10). These two streams of research came together in reconstitution studies accomplished contemporaneously in both my lab and his (11, 12). Both laboratories demonstrated that the isolated β-AR was able to confer catecholamine responsiveness on the resolved catalytic unit of the adenylyl cyclase in the presence of the G_s protein.

Development of Techniques for Analyzing Complex Ligand Binding Curves for G Protein–Coupled Receptors (Late 1970s to Early 1980s and Early 1990s)

This work led to the development of the ternary complex (13) and extended ternary complex (14) models in which the cooperative interactions of agonists and G proteins with the receptor were used to explain biphasic agonist binding curves and the effect of guanine nucleotides to convert receptors from a high- to low-affinity state. At a fundamental level, the ternary complex model provided a simple, direct way of calculating alpha (i.e., efficacy) for any ligand as the ratio of its affinity for the low- and high-affinity forms of the receptor (K_L/K_H). These analytic approaches also provided a means for quantitating receptor subtypes by dissection of complex biphasic competition binding curves (15).

Cloning of the β_2-Adrenergic Receptor and other Adrenergic Receptor Genes and cDNAs (Mid 1980s to Early 1990s)

The cloning of the β_2-AR and other adrenergic receptor genes and cDNAs was made possible by our having obtained stretches of protein sequence from our purified receptor preparations, first for the β_2-AR (16) and then for the α_{2a} (17) and α_{1B} (18) ARs. Our first success was the cloning of the β_2-AR gene and cDNA in collaboration with a group at Merck Pharmaceuticals. When we recognized its sequence homology with rhodopsin and its analogous seven membrane–spanning domain architecture, we realized that this might be the defining structural feature of all G protein–coupled receptors (GPCRs) (16). This conjecture was quickly supported by our cloning of a total of eight adrenergic receptors and a serotonin receptor (19). The rapid expansion of the family of GPCRs over the next several years occurred largely through various homology cloning approaches. I believe that it was the purification of the adrenergic receptors, accomplished with great difficulty over a decade, that made this rapid progress possible.

Desensitization (1970s–1990s)

Throughout the 1970s and 1980s, I had been fascinated with the fairly universal phenomenon that agonist stimulation of GPCRs becomes dampened after receptor activation. The diverse mechanisms involved operate over time frames ranging from seconds to hours or even days and involve processes as diverse as covalent modifications of proteins, receptor internalization, and transcriptional and translational regulation. Although we studied several of these mechanisms, by far the majority of our effort was focused on the process of rapid homologous (i.e., receptor-specific) desensitization. Once we discovered that phosphorylation of the β_2-AR was intimately associated with its rapid desensitization (20), this became the focus of our effort. These studies led us to the discovery of the novel activation-dependent receptor kinase responsible, which we initially termed the β-AR kinase or βARK (now G protein kinase 2 or GRK2), and to a small family of related kinases, seven in number, that mediate analogous phosphorylation of most GPCRs (21). This in turn led to our discovery of β-arrestin 1 and 2, proteins that act in concert with the GRKs to rapidly desensitize the receptors and facilitate their endocytosis (22, 23). Contemporaneous

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work in the visual system on rhodopsin kinase and visual arrestin (24, 25) immediately suggested the potential generality of these mechanisms, amply confirmed by 25 years of subsequent work.

β-Arrestins: Multifunctional Adapter Proteins (Late 1990s–2000s)

Although we discovered the β-arrestins in the context of trying to understand the basis for rapid receptor desensitization, within a few years it began to be appreciated that they played much more diverse roles. In the late 1990s, the Caron and Benovic labs produced the first evidence that β-arrestins were clathrin adapters that facilitated the endocytosis of the receptors (26, 27). Shortly thereafter, we found evidence that, seemingly paradoxically, even as β-arrestins desensitize G protein signaling, they also initiate signaling in their own right by serving as adapters and scaffolds to assemble diverse signaling networks. Src (28) and ERK (29) were the earliest such pathways discovered, but the list has grown rapidly, and proteomics studies by our group and others suggest that a very large and diverse array of pathways can be activated by β-arrestin–dependent mechanisms (30–32). Many of these are the same ones activated downstream of G proteins, but often with different physiological results. β-Arrestin–dependent signaling is often entirely independent of G protein involvement, although in the case of G_i-coupled receptors, it appears to require concurrent G_i activation. The mechanisms of this cooperative signaling interaction are poorly understood. Other β-arrestin functions have also been discovered; for example, it serves as an E3 ubiquitin ligase adapter to facilitate ubiquitination of GPCRs and other proteins (33).

Biased Signaling (2004–)

To our surprise, we found ligands for the angiotensin receptor that completely lost their ability to activate G protein signaling but retained the ability to activate β-arrestin pathways (34). We termed these biased agonists and realized that their existence offered strong support for the notion of multiple activated receptor conformations (35). Subsequently, this concept has been very widely extended to dozens of GPCRs. There are therapeutic implications as well, as in some cases, the unwanted side effects and desired therapeutic effects of GPCR-targeted drugs are differentially mediated by G protein or β-arrestin signaling. Several such biased ligands are being clinically tested. Although most studies of biased signaling by G proteins or β-arrestin have used synthetic ligands, it is already clear that such bias operates in physiological signaling systems as well, for example, among various chemokine receptors (36).

Biophysical Studies (2010–)

In the most recent phase of my work, I have been focused on trying to apply a variety of biophysical approaches to an understanding of the conformational basis of β-arrestin signaling and biased signaling at both the receptor and β-arrestin levels. These studies, which involve techniques such as X-ray crystallography, negative stain, cryo-electron microscopy, hydrogen-deuterium exchange, and double electron-electron resonance, have involved collaboration with the laboratories of Brian Kobilka, Yiorgo Skiniotis, and Wayne Hubbell, among others. The first fruits of these efforts have begun to elucidate mechanisms of β-arrestin activation (37, 38).

SOME PERSONAL REFLECTIONS

The Nobel Prize

On October 10, 2012, at about 5 AM, I received a call from the Royal Swedish Academy informing me that I would share that year's Nobel Prize in Chemistry with Brian Kobilka (of Stanford

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University), a good friend, collaborator, and postdoctoral fellow in my lab during the 1980s. There is no doubt that such a call alters one's life in many significant ways. First, it changes one's name: Thereafter, you are known and always introduced as a Nobel laureate, regardless of the situation. You are expected and invited to pontificate on a wide variety of subjects, many of which you know nothing about, and to nonetheless be taken quite seriously. You receive innumerable invitations to give lectures and attend symposia, including many that are in fields completely outside of your areas of expertise. And you are asked many of the same questions over and over. In this regard, I strongly relate to a quip I recently heard attributed to the late Al Gilman (Nobel Prize in Physiology or Medicine 1994). When asked what was the best thing about winning the Nobel Prize, he is said to have responded: "Never again having to answer the question, when do you think you will win the Nobel Prize?"

Mentoring

One of the greatest satisfactions of my career has been mentoring my trainees and observing their subsequent successes in a variety of careers, including as independent scientists, scientific administrators, and leaders in the worlds of pharma and biotech. More than 200 trainees have passed through my lab at Duke University over the past 44 years. I am by nature a very social creature, and interacting day-to-day with my fellows and students about our projects, reviewing data, planning directions and experiments, exulting together when something actually works, and commiserating more frequently when it doesn't, are to me the essence of what I do. It is during these interactions that I share my own approach to the scientific process. The mentoring experience for a trainee is essentially an apprenticeship, an opportunity to live with their mentor in the lab and observe him or her in all the multitude of situations that arise in the day-to-day experience of doing science. During this apprenticeship, and from close proximity to the mentor, the trainee presumably absorbs values, approaches, attitudes, and even some relevant knowledge. I would argue, however, that specific facts or technical aspects are much less important than approaches to issues, such as how to choose a good scientific problem, when to pursue an unexpected finding or rather to ignore it as a distraction, when to push on in the face of repeated failure, and when to abandon a particular project. There are obviously no simple right or wrong answers to these types of questions. As I often say, "If it's really important, you can't look it up in a book."

That there are transferable elements in the process of doing high-quality science is supported by the existence of scientific lineages. These have been documented repeatedly, and there are even websites that publish such scientific family trees. **Figure 2** presents a condensed scientific family tree for each of the nine MD scientists who trained at the NIH during the 1960s and who went on to win the Nobel Prize (see the section above titled The US National Institutes of Health). The purpose of constructing this was to see whether there were other Nobelists either in their scientific forbears or progeny. The stunning result is that in every case, each scientist had a Nobel Laureate as either their mentor or their mentor's mentor. One tree, from Emil Fischer to Thomas Südhof, has a Nobel laureate in six of seven successive generations. Another has four consecutive generations of laureates.

One of the biggest challenges in mentoring, I have found, is finding the sweet spot between micromanaging a trainee's work on the one hand and being too aloof from it on the other. It can be a fine line, and one which varies not only with each trainee but during his or her tenure in the lab, depending on native ability and level of scientific maturity. I realize, of course, that there is no one ideal way to mentor. Rather, I think it is important that each person use their own unique personality to craft their best approach. Among the pieces of advice I find myself giving most frequently to my trainees are the following, for which I claim no great originality.

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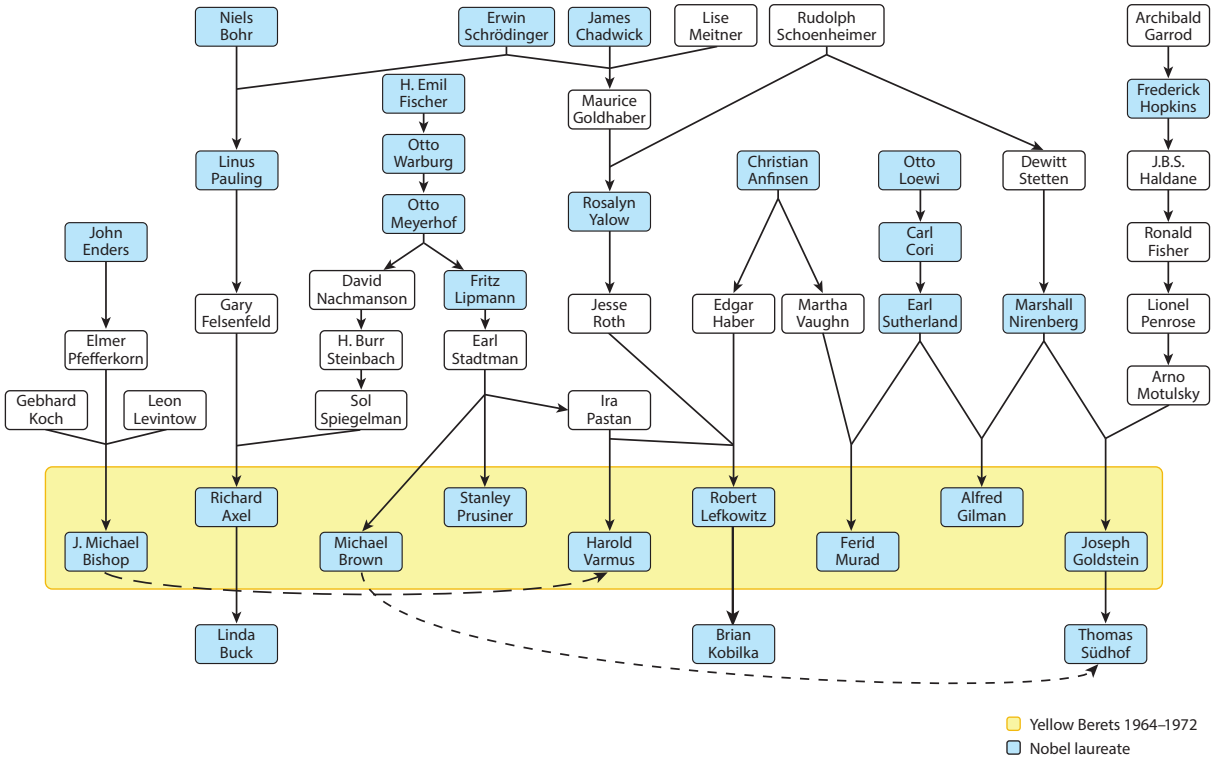

Figure 2

Scientific lineages of the author and eight other Vietnam-era yellow berets who trained in research at the NIH between 1964 and 1972 and later won the Nobel Prize. All were commissioned officers in the US Public Health Service in fulfillment of their obligatory military service. Light blue boxes indicate recipients of the Nobel Prize. Arrows indicate mentoring relationships. Many of the individuals shown have multiple mentors. For example, my mentors were Jesse Roth, Ira Pastan, and Edgar Haber. Not all mentors are shown. The data have been adapted and condensed for ease of presentation from Neurotree.org.

1. There are four keys to success in research: The first is focus, the second is focus, the third is focus, and the fourth you have to figure out for yourself.
2. Build your career around scientific problems that interest you, not around techniques or groups of techniques.
3. Do lots of experiments. Among the trainees I have had who were the most successful, a fairly general characteristic has been that they did more experiments than the average trainee.
4. Don't talk yourself out of experiments too easily. Even if you have numerous reasons why an experiment won't work, consider doing it anyway. Often you will be surprised at the result.
5. Be bold, take risks, and don't be afraid to fail.
6. Learn to tell a good story (see below).
7. Be ambitious.
8. Try to view your work as play.
9. Try to see the humor in everyday situations. If you have the right attitude, it is all around you. I find that humor is a great prod to creativity, and I try to leaven my lab and other meetings with a generous amount.
10. Be enthusiastic about what you are doing. If you are not, find something else to do. When new people come to the lab, I always tell them: "There are two conditions that are necessary

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but not sufficient for you to succeed here. First, you are enthusiastic about your project. Second, I am enthusiastic about your project."

11. Be optimistic.
12. Be doggedly persistent.

Role Modeling

I try to model as many of these traits as I can on a day-to-day basis. Moreover, I try to keep my activities in the office as transparent as possible. Thus, I include my trainees in such things as conversations with journal editors about manuscript reviews; teleconferences with collaborating scientists; or even early on, when I'm just seeking a collaborator's help. There are so many aspects of being a scientist that the more of these activities I can expose them to, the better. Almost everything is a teachable moment.

SOME THOUGHTS ABOUT SCIENTIFIC WRITING AND PUBLICATION

Telling a Story

The single most important thing I teach my trainees about writing a scientific paper is so simple that it may seem blatantly obvious: the primacy of the story. Data are not a story. Data must be assembled into a story. Once the experimental phase of the project is nearing completion, it is easy to assume that there is only one obvious storyline, but in my experience, this is often not true. A given set of data can often be assembled into stories in several ways, some of which are more compelling and interesting than others. So the place to start is by moving the pieces (of data) around to see what works best.

I learned this lesson on clinical teaching rounds as a third-year medical student from a very wise attending physician. As is standard practice, he would ask a medical student to present the case of a newly admitted patient. Then, after some brief discussion, he would ask the student to present the case again, this time telling a somewhat different story, but without changing any of the basic facts. When he first did this, my classmates and I were dumbfounded and unable to present another coherent story until he demonstrated the skill a few times. Our inability to respond initially was due to our naive assumption that the data were the story. The lesson is as true in the basic science laboratory as it is in the clinical realm, if not more so.

This focus on the story dictates the order in which I teach my trainees to assemble their scientific papers. First, rough drafts of the figures are assembled into an order that tells the best story. Then the title and abstract follow. Once these pieces are in place, writing the paper becomes much more straightforward. Although I would not argue that this is the best or only way to write a scientific paper, it is my own way of doing things. Most of my fellows are surprised at this approach and generally don't seem to want to worry about the title and abstract until the paper is almost done. My own view is that if you don't know the title, how do you know what the story is?

The Tyranny of Impact Factors

My trainees, like many biomedical scientists these days, seem to use a journal's impact factor as the most important criterion for selecting where they would like to publish their research paper. Thus, journals such as *Science*, *Nature*, and *Cell* become the preferred initial target for almost every paper. I have been privy to discussions in which a journal with an impact factor of 10 is chosen over

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one with an impact factor of 8.5 for essentially no other reason. Why has this happened? I would cite several factors. One is the mistaken notion that the impact of a research paper, as assessed by its frequency of citation, is determined largely by where it is published, rather than by the importance of what it reports. To the contrary, in my own case, for example, my two most highly cited papers (not reviews) were both published in the *Journal of Biological Chemistry* (*JBC*), which has a relatively unimpressive impact factor of 4.6. As an aside, in my career, the single journal in which I have published by far the greatest number of papers is *JBC*. So you can imagine my dismay when during a recent discussion with one of my fellows about where to send his paper (I had suggested *JBC*), he replied with evident chagrin, "I thought you liked this work."

A second related factor is the prestige of publishing in these top-tier journals. Acceptance of a paper in these journals is met with great elation, a reaction which relates to an important third factor. Tenure/promotion and search committees increasingly evaluate academic achievement by relying on where papers have been published rather than on the content of the articles. No wonder young scientists believe (with some validity) that their careers very much depend on their ability to publish in journals with the highest impact factors.

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Roads Not Taken

I have spent my entire academic career at Duke University. During that time I have devoted all my energies to my research program, mentoring my trainees, making clinical teaching rounds, and teaching the occasional class. I ended the clinical work 14 years ago. I have assiduously declined all the many opportunities that have come my way to become chair of a department (at Duke and elsewhere), head research institutes, and the like. I have few if any regrets about not taking major leadership positions. I think that I might have been successful in such a role, but my heart would not have been in it. With respect to abandoning my childhood dream of becoming a practicing physician, the feelings are more complex. But it is hard for me to imagine a more rewarding career than I have had. I am often asked if I regret the years I spent training in internal medicine and cardiology, given how my career eventually played out in the basic science laboratory. The answer is a resounding no. The opportunity to have brought comfort and sometimes healing to the sick, albeit for a limited number of years, was a high privilege for which I will always be grateful. And my clinical and medical outlook continues to influence my research to this day.

The Howard Hughes Medical Institute

Throughout most of my career, my research has been supported by the Howard Hughes Medical Institute (HHMI). I became an HHMI investigator in 1976. This more than 40-year run is, to the best of my knowledge, the longest continuous tenure of any HHMI investigator (together with my colleague at the University of Washington, Dr. Richard Palmiter). I am pleased to report that in 2016, my appointment was renewed for an additional five years. The renewal of these appointments is a rather stressful ritual involving a formal presentation to a daunting panel of experts and scientific luminaries. A Nobel Prize is no guarantee of success, and to date these panels have discharged at least five investigators who would later win the Nobel Prize and several who had already done so. Suffice it to say that these decades of continuous and generous funding by the Institute have played a very significant role in whatever successes I have enjoyed. I have served under every president of HHMI dating back to the first, Dr. George Thorn. The Institute was a very different organization when I joined. It was much smaller, with 56 investigators, about 50 of whom had an MD degree. When I reviewed data for my presidential address to the Association of American Physicians in 2001, it had grown to about 350 investigators, but still with only about

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50 MDs, many of whom also had a PhD. The number of HHMI investigators has not grown appreciably over the past 15 years. Thus, Institute support of the physician scientist has been less emphasized during this period. However, HHMI does have several programs aimed specifically at supporting the training of physician scientists.

The remarkable catalytic effect of HHMI support on my and many other careers, beyond the actual dollars committed, is due to the philosophy that underlies the funding. Unlike granting agencies such as the NIH (which has supported my ongoing R01 grant for 43 years), which fund specific projects, HHMI funds investigators. HHMI investigators are under no obligation to pursue a specific line of investigation. This provides a means that encourages creativity and risk-taking without having to seek specific support for one or another new or outside-the-box idea.

Final Thought

There have been times in my career when I was not sure whether I was directing my research, or whether it was directing me. At such times, the research seemed to be racing along at breakneck speed, and I have felt like I was riding a wild horse, holding on so that I wouldn't get thrown, so that I could see where it was taking me. As I near the fiftieth anniversary of my start in research at the NIH in 1968, I'm hoping that I can stay in the saddle a while longer.

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