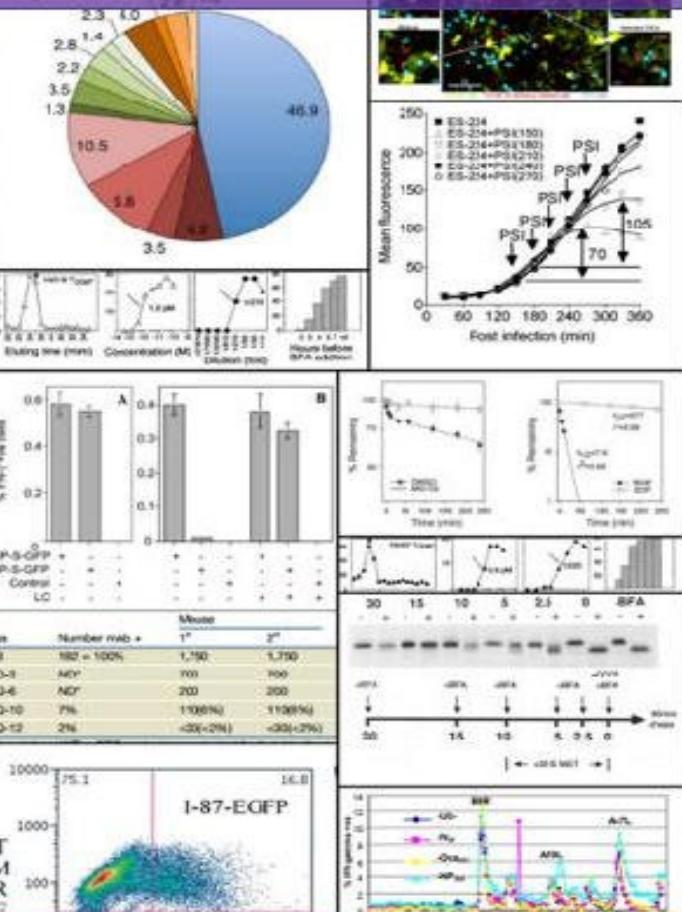
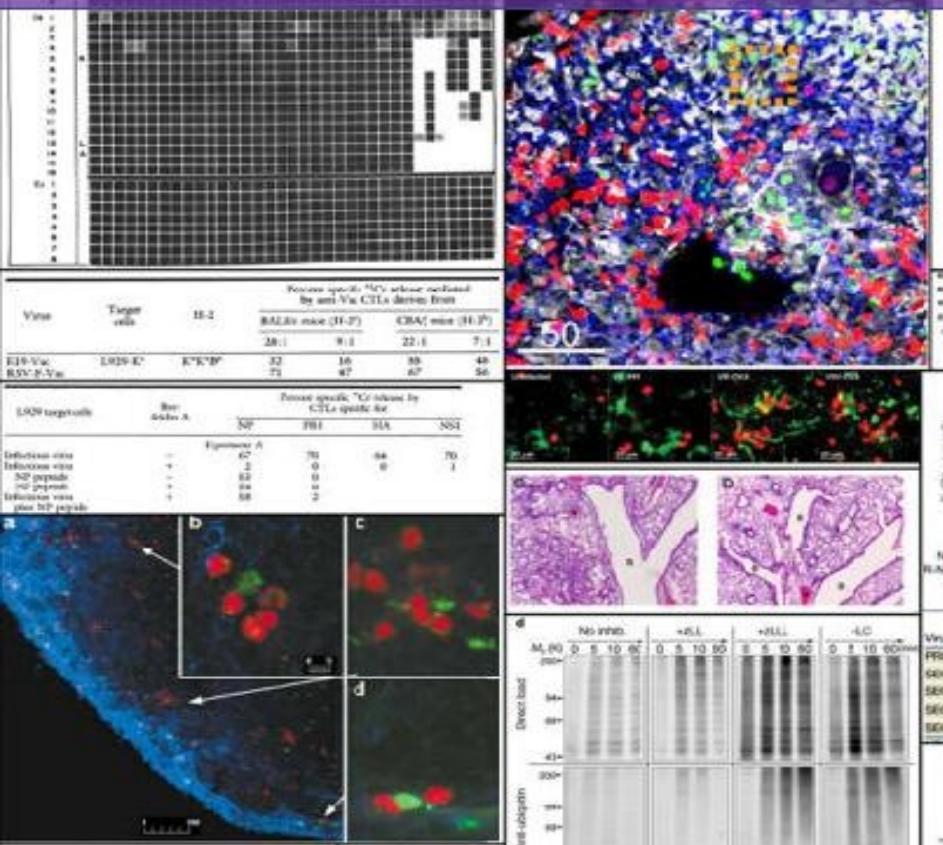


# TRUTH WINS

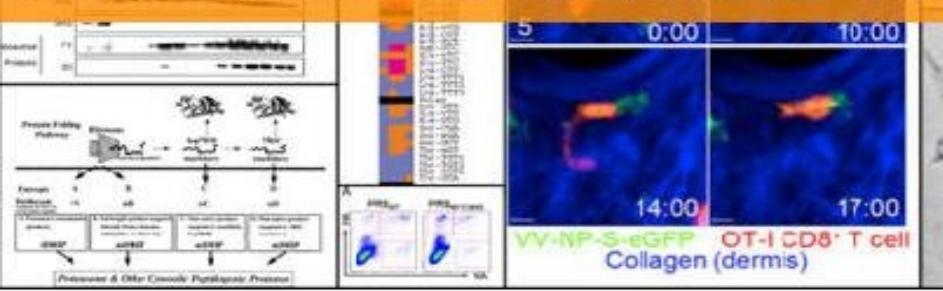
## A Practical Guide to Succeeding at Biomedical Research

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# Succeeding at



# JONATHAN W. YEWDELL MD PHD



# **Truth Wins**

A Practical Guide to Succeeding at Biomedical Research

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## Book Cover:

The cover image is a collage of data panels, each representing a discovery published in one of the author's scientific papers dating from 1979 to 2017. The joy of making discoveries is the beating heart of a successful scientific career. The power of the scientific method to reveal truths about nature through iterative rational experimentation and interpretation inspired the title of the book.

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## Introduction

A career in biomedical research can be amazingly fun, challenging, highly creative and deeply rewarding, while making a substantial contribution to humanity. It is not, however, for the faint of heart. For those seeking the most arduous and competitive career with the lowest salary, academic biomedical research is certainly in the running (with art, music, and dance, of course). Getting started is particularly difficult, with long hours, low pay, poor benefits, and zero job security.

When I was a graduate student in the late 1970's, things really were much better. Although biomedical research always demanded dedication, it was nearly guaranteed that if you were reasonably talented and somewhat sane, a rewarding career awaited. Most importantly, science was fun, so the long hours didn't seem like work. Just the opposite, you felt fortunate to be paid for something you loved doing.

But during the next 35 years, perversely, as the biotech industry began and boomed, making many leading scientists millionaires, and universities expanded biomedical research faculty and facilities at a breakneck pace, starting scientists became victims of this financial success. The odds today of a PhD student eventually running their own research group as a principal investigator (PI), are less than [1 in 10](#). And this is only after serving a long apprenticeship (typically 5 years) as a post-doctoral fellow (post-doc) after obtaining a PhD.

Post-docs are the cutting edge of biomedical research. They typically work 50+ hours a week, generating new ideas, performing experiments, and writing papers describing their discoveries. They are the most productive members of the research enterprise, making most of the breakthroughs that generate the new drugs and treatments that enrich their supervisors, universities, and biotech companies.

Despite the productivity of post-docs, their demanding training (5.5 years on average to obtain their PhD), and age (average starting age of 31), the post-doc starting salary in the USA is currently around \$42,000 per year. This is \$6,000 less than the average nationwide starting salary of college graduates. Compounding the financial penalties, since post-docs typically do not have official employment status (even in the Federal Government), they don't receive

either Social Security or retirement benefits, reaching their late thirties with a nest egg of zero dollars.



Saving the  
world with the  
power of his  
pipetman!

I've always had empathy for the powerless (particularly when I was powerless). Around 2000, I took it upon myself to become an advocate for post-docs, who at the time were paid less than \$30,000 a year. I began to vent to other PIs, who mostly agreed wholeheartedly. As fate would have it, as I started to seriously consider what I might tangibly accomplish in improving the situation for post-docs, Jeff Frelinger, of like mind, was visiting my lab on sabbatical. Wise from long time service as Microbiology Department chairman at the University of North Carolina, and with detailed insider knowledge of science politics, Jeff provided invaluable advice.

In his capacity of chairman on the public affairs committee at the American Association of Immunologists, he provided me an opportunity to advocate for substantially increasing post-doc salaries in the annual request that the Federation of American Societies for Experimental Biology (the major scientist run biomedical advocacy group in the USA) submits to Congress to prioritize NIH funding.

With the doubling of the NIH budget underway, there was a real opportunity at hand, and I played a role in securing the first sizable increase in NIH sponsored-post-doc salaries in many years. This taught me that reforming US science is possible. Encouraged, I began to write essays agitating for reforming the system, and then created a 1-hour "*How to Succeed in Science Without Really Trying*" talk. I've given this talk more than 100 times in North America, Europe, Asia, and Australia. The talk resonates with both young and old scientists.

In 2007, I spoke at a [Harden](#) biochemistry conference in England. Fortuitously, in the audience was an editor from *Nature Reviews in Cellular and Molecular Biology*, who encouraged me to convert the talk to essays, which they would publish. With important editorial assistance from the journal, and a few hilarious lab-life cartoons from [Alex Dent](#), the essays were an immediate hit, and have been read by tens of thousands of young scientists. Some PhD programs provide the essays as essential reading for incoming students.

I've always tried to focus on the most important things in science and in life. After giving the talk dozens of times and writing the essays, however, it dawned on me that I hadn't seriously considered my most important job as a scientist. By default, I had thought that making discoveries tops the list by a wide margin.

I'm proud to have personally made or contributed to a number of reasonably important discoveries in the past 35 years. With time, however, it becomes clear that at best, your older colleagues vaguely recall what you discovered years ago, and younger scientists haven't a clue. This is how it should be. The fierce

competition in science belies its essential nature as an intensely collaborative exercise. Unlike business, where secrets are closely guarded or patented to maximize profits, the ethos of science is exactly opposite. Discoveries are shared with the whole world as soon as possible, and in a form, that enable others to test their veracity and build on the work.

All things being equal, smarter scientists will uncover truths faster than others, but sooner or later the truth will emerge. If I hadn't made the discoveries, somebody eventually would have. What's more important is that in pursuing discoveries, I practice the scientific method at the highest standard.

It is easy to underestimate the [scientific method](#), i.e. formulating hypotheses and testing them by designing and performing experiments with the goal of uncovering basic truths of nature. It seems obvious, even trivial. But while we *Homo sapiens* have had similar mental capacity for at least 50,000 years, the scientific method was only conceived and developed over the past 500 years. And it took WWII, largely won based on advances in basic understanding of physics and chemistry, for the clear pragmatic benefits of the scientific method to be fully embraced by enlightened governments in the form of organizing and funding basic and applied research.

While the scientific method might seem to be an integral part of modern culture, every country has citizens who would deny its legitimacy on economic, political, or religious/philosophical grounds. The scientific method is analogous to a torch. Its flame enlightens humanity but is easily extinguished. While there are those who harbor quaint notions of returning humanity to its animal roots, there is no going back to nature at this point, not if we are to coax the earth to sustain 7+ billion of us (increasing at 2.3 people per second).

I've come to realize that my most important job is to help maintain the flame of the scientific method. By working to make science a decent enough career to attract the best and brightest young people. By doing science properly and imparting this knowledge to the young scientists in my laboratory. By giving the *How to Succeed...* talk, and now by writing this book.

The book is mostly intended for young people either contemplating a career in biomedical research or those who have already embarked on the journey. I hope that others might find it interesting and useful in understanding how scientists tick and what they do all day.

Please note that you don't have to read the book in the prescribed order, or for that matter in its entirety. If you are a student contemplating a career in science, you might want to skip the biographical introduction and jump to Part II. If you have little tolerance for science biography, also skip Part I. If you went to high school with me, you'll definitely want to start with Part I (and probably

end there, too).

Finally, a few words of thanks. First, to the National Institute of Allergy and Infectious Diseases for generously supporting my career since 1987, and creating a culture where creative, curiosity driven basic research can thrive. Second, to Donald and Mary King and [Clare Hall College](#) for providing a fellowship to spend a sabbatical year at this idyllic setting in Cambridge University, where I wrote the first draft of the book surrounded by beauty, truth, and wonderful new colleagues. Third, to Tom Daley, my Princeton roommate and FFL, who carefully read and expertly edited the first draft of the book. Lastly, to Alex Dent (Indiana University School of Medicine) for his generous permission to sprinkle his insightful and hilarious cartoons throughout the book.

## Part I

# Lessons from One Career in Biomedical Research

# CHAPTER 1

## *High School*

**L**ike most scientists, I was born curious. Unending questions. Not terribly discriminating, for nearly everything was (and is) interesting to me. Why, I don't know, and I can't explain it to the incurious.

Curiosity is useful for many things, even social interactions. Nearly everyone welcomes interest in their own life. Ask a few genuine questions and people generally open up. You almost always learn something interesting, and even useful. Curiosity-driven knowledge tends to be better much retained and integrated than force-fed knowledge. Ultimately, curiosity is the beating heart of science. Propelled by the joy of discovery, one decent question begets another, *ad infinitum*.

Despite this natural proclivity, I had no idea of becoming a scientist. My father owned and operated a small business with his two brothers. Although both my parents were college educated, they had no ties to academia or any sort of research. We lived in a tranquil neighborhood in Eastchester, NY, an inner suburb of New York City, where academic types were well dispersed among a large population. Science was not on anyone's radar in my family's social circle.

I did not stand out academically at first in school, but by 5<sup>th</sup> grade or so, overcoming mild dyslexia (which nearly resulted in repeating first grade, a blow that would have been difficult to overcome, given how teachers' expectations can influence outcomes). I had become a voracious reader, and devoured books, magazines, corporate annual reports<sup>1</sup>, anything really. This too, I think was driven by curiosity, not just about physical things, but also people and their stories. My parents were readers, and books and magazines were plentiful at home. Local libraries were excellent, and tapping the magic of a library card, I'd come home with piles of books. Free knowledge!

My reading was essentially unguided and I covered a lot of ground. Reading taught me many things, not the least of which was writing. I don't do well with rules of any sort, including grammar, but by dint of thousands and thousands of hours of reading (3 hours a day = 1000+ hours a year), I absorbed the basic rules

of English and also a sense of style. Reading history and classic British and American novels also taught a lot about human nature. Since science, like everything else in life (probably more so), requires team work and insight into people you are working under, over, and with, this was also unwitting training for my future career.

Reading Austen, Dickens, Dos Passos, Dreiser, Steinbeck, *et al.* reinforced my natural sympathy for the downtrodden and mistrust of the powerful. Sinclair Lewis's Arrowsmith introduced me to the idealism and thrill of science<sup>2</sup>. Most importantly, I learned the essential lesson that you can teach yourself almost anything by reading. At the very least, reading provides a foundation of knowledge that can be built on by experience.

At 12 or so I became interested in the human body<sup>3</sup>. I loved all sorts of machines (airplanes held a particular fascination), and living things are by far the most amazing machines. One of the few books I ever asked my parents for was a stunning [human anatomy book](#) (I still have it) aimed at the general public that caught my attention at a bookstore. At some point soon after, I read an inspiring novel ([The Last Angry Man](#), by Gerald Green<sup>4</sup>), about an idealistic general practitioner (GP) in Brooklyn who treats his indigent patients, often for free, with insight and compassion. I set my sights on becoming a GP.

Like many future scientists, my curiosity bred intense skepticism. Each of my grandparents had fled Europe to escape its pervasive anti-Semitism. My parents were not particularly religious, but I was expected to attend Hebrew school and become a [bar mitzvah](#) at the traditional age of 13. Despite my tender age, I already considered religion to be an obviously fabricated fairy tale, and made a deal with my parents that I would seriously study for bar mitzvah, but could abandon organized religion from that point on.

We both honored the deal. Becoming a bar mitzvah turned out to be a turning point in my life<sup>5</sup>. At 13 I was empowered to read and sing from the torah, and even give a sermon to the gathered friends, family, and regular synagogue attendees. I put my heart and soul into the sermon, and was greatly aided by the cantor<sup>6</sup>, my first real mentor, who took me under his wing. Together we crafted a speech chiding the attendees for focusing more on the celebratory than the religious aspects of the bar mitzvah<sup>7</sup>.

I have the speech on tape buried somewhere in my basement. It's pretty impressive, not so much for its content, but for its courage. It's not like I wasn't nervous; I was terrified. But still, I had the guts to stand up and speak truth to power, at least from the viewpoint of a callow 13-year old. This is an essential trait for anyone who hopes to accomplish positive change in any endeavor,

including science. And I was fortunate that my parents, the cantor and rabbi provided the freedom to speak my mind. Societies do well to allow their so-inclined young to be difficult<sup>8</sup>. This is a small price to pay to foster individuals who will help create a dynamic society aiming to always improve.

In New York State, local education is controlled and funded on a town-by-town basis. Our school district had been created to segregate lower-income kids from Tuckahoe from the very special children of Bronxville, an extremely wealthy community that was excluded to Jews, African Americans or Italians by real estate practices (Bronxville was and still is, I believe, essentially a *de facto* private school district, though now discrimination is strictly income based).

Tuckahoe had just one elementary school and one junior/[senior high school](#). Both were well below average for Westchester County, home to some of the best public schools in the country. The small size of the school, less than 100 students per grade, limited the academic possibilities and narrowed the talent pool of both students and teachers. On the other hand, knowing nearly everybody for years provided a tremendous sense of community<sup>9</sup>.

My science education was not terrible, but it failed to really engage me. Attending an undemanding school, however, provided the significant advantage of leaving plenty of free time for independent reading and thinking. One of the most difficult balances to achieve in schooling is to educate kids (particularly bright kids) without killing their curiosity. Over-education is a major problem with top-scoring educational systems around the world, where students memorize enormous amounts of material at the expense of developing creativity, empathy, humor, or irony.

I was ranked 5<sup>th</sup> out of 92 graduating seniors (due to low interest and ability in mandatory French, which killed my GPA). Normally only the valedictorian at Tuckahoe would have a chance at Ivy League schools. But with the top scores in the SATs and New York State Regents exams<sup>10</sup> I had big ambitions. I reflexively set my sights on Harvard, then as now, the most prestigious college in the USA, with the most diversely talented undergraduates. I made it onto the waiting list, not just at Harvard, but 3 other outstanding colleges as well. Harvard, alas, was not to be, but after a few anxious weeks, I was accepted by each of the others.

## CHAPTER 2

### *College*

**T**hough I had a lot of catching up to do at Princeton with kids from high-flying prep and public high schools, many were burned out from their rigorous education and had acquired a jaded attitude towards education. Coming from a less academic environment accentuated the tremendous opportunity that Princeton offered. Then as now, Princeton is highly demanding academically; each year the workload and intellectual challenge ratcheted up. Unlike most other top universities, Princeton doesn't have medical, law, business or most other professional schools, and the focus is squarely on undergraduate education. For faculty, teaching undergraduates is an important requirement for obtaining tenure; even academic superstars teach introductory courses. For academically inclined kids, the 4-year process converts their curious, undisciplined minds into effective tools for exploring the world and making contributions to knowledge.

From the get-go, the stiff competition made it clear that a major effort would be required. A strong ego can be useful if complemented by honest self-appraisal: pride drives effort. Eventually realizing that I could compete with Princeton's best provided life-long confidence that I could compete with anybody. In science, as in life, being confident (even to the point of overconfidence) is essential to reach your potential. Skiing provides an apt analogy: standing at the top of a steep chute, if you don't firmly believe you can ski it well, you won't.

I entered Princeton planning to take the courses required for medical school while majoring in history. Why history? I am not really certain. I liked reading history books, but then I liked reading almost everything<sup>11</sup>. Probably, I was influenced by Miss Solo, one of my few outstanding high school teachers, who taught American History. But a funny thing happened on the way to history. As I took the preliminary history and other liberal arts courses at Princeton, I was troubled by the flimsy factual basis that conclusions were based on. It seemed that the truth depended strictly on who could make the best argument, and that

bullshitting was the key skill in achieving top grades.

Science was different. There were real facts (though not so real as I believed at the time), and real problems that the facts could help solve. Princeton's science education was superb, with emphasis on problem solving and quantitative conceptual insight. The introductory courses featured weekly problem sets, with problems that often stretched my intellect past the breaking point. Looking back, the first sign that I might be suited for a scientific career is that I would become obsessed with a difficult problem and cogitate and fret for hours, using futilely, but with complete concentration. I worked at least as twice as hard as my liberal arts major friends. I believe that this is was generally true at Princeton, and still applies to most colleges in the USA.

A good deal of the extra work came from the practical labs that accompany science courses. This lengthened the actual class time considerably and also entailed a major effort in writing lab reports. I doubt that the labs are worth the time and money (they are very expensive to run). Labs greatly increase the workload for science majors, discouraging students, even industrious ones, from trying science. Labs typically also impart the exact wrong lesson, that there is a "correct" answer to an experiment. I was so intent on getting the right answer that I lost sight of everything else. No doubt this applies to most pre-med students, who treat the labs (and the coursework as well) as simply an obstacle to be cleared on the way to medicine.

I started to see the light sophomore year, taking my first advanced biology course, microbiology, taught by Frank Johnson. Prof. Johnson was a genial and gentlemanly southerner with a twinkle in his eye. Graduating from Princeton in 1930, he did his doctoral work there as well. A classical microbiologist with the demeanor of an Andy Griffith Show character, he seemed completely outclassed by the other high-powered biology faculty who were working at the molecular level.

But, be careful how ye judge! For despite his distinctly lower key, had he lived long enough, he would have likely received the Nobel Prize for discovering the basis of bioluminescence (the generation of light by living organisms), his life-long passion (his post-doc received it for work he performed with Prof. Johnson).

This perfectly illustrates the value and central importance of curiosity-driven research to society. It would have been quite easy for someone like Senator William Proxmire, famous at the time for the "[Golden Fleece Awards](#)" he awarded to scientists for wasting taxpayers' money, to lampoon Prof. Johnson's research. What possible significance could glowing jellyfish have for human health or other pragmatic applications? Well, enormous significance, since their

naturally fluorescent molecules have revolutionized nearly all fields of biology in the past 15 years.

Prof. Johnson's joy for science was contagious. Emphasizing the practical applications of microbiology alongside the academic, he guided trips to sewage plants, dairies, and even the Budweiser brewery in Newark. His course was lab centered, but rather than pursue a set exercise, on the first week of the course we each isolated a bacterial colony from soil and spent the rest of the semester characterizing it. Unlike other labs, there were no correct "answers": you had to figure out for yourself what bacterial species you had isolated<sup>12</sup>.

Prof. Johnson encouraged us to work on our own in the lab at any hour, whenever the mood struck (other labs had set hours). One night I found myself alone in the lab and had my first original practical scientific idea. Unfortunately, my scheme for improving the microscope nearly destroyed it, but I had a first taste of having an original idea and testing it. As trivial as it was, it was intoxicating (and terrifying until I restored the scope to a semblance of working order).

Still, I had no idea of my real talents or inclinations. I had every intention of majoring in chemistry, but then I learned of the mandatory two-semester course in analytic chemistry, whose fearsome laboratory was infamous for requiring dozens of hours of work per week. I saw no point in this, and majored in biochemistry instead.

Unbeknownst to me, Princeton had a superb biochemistry department. It was filled with rising young stars in the emerging field of molecular cell biology, nearly all of whom would make their names after leaving Princeton over the next decade. Princeton's leaders apparently had no idea of the coming importance of molecular biology or the top quality of their faculty. They were blindsided by the revolution in biomedical research, which would generate enormous sums of money for universities through research grants, licenses and patents.

There were many fewer facts to learn in cell and molecular biology in the 70's than there are today, and the curriculum was much more conceptually based. Some wise soul in the biochemistry department recognized the critical importance of physical chemistry for understanding biology, making this a required course. In my naïveté, I thought this was a complete waste of time. Wrong!

A famous physical chemist, Walter Kauzmann taught the course. Prof. Kauzmann authored a revered textbook on the subject used by many undergraduate physical chemistry courses. But not ours—Prof. Kauzmann believed that his own book was too bogged down by mathematical equations and he wanted to focus on the concepts. Lesson imparted: don't let your ego (or bank

account) interfere with your goals.

I loved this course. For budding biologists, getting an intuitive feel for the relatively simple physical rules of chemistry that govern life is more important than memorizing details about the cell's myriad molecules and pathways. In any event, the number of facts are increasing at such rate, that at some point in the near future, biology education will *have* to become more concept oriented. Not that the facts can be ignored. Rather, electronic devices will become integrated into every aspect of the way we think, to the point of becoming a functional extension of our minds, and perhaps even be directly wired into our brains in the coming centuries.

Though I didn't realize it at the time, the most important skill to acquire during college is how to communicate your thoughts and ideas to others both in speaking and writing. This is essential for success in virtually every career, including science. The liberal arts tradition in US higher education confers a significant advantage over the many other systems in the world where college students immediately focus on their intended career. Princeton, like many schools, had "distribution requirements", requiring all students to take courses away from their majors and academic strengths, including basic competency in a foreign language (three semesters of French, ugggh!!). These courses featured small tutorials (10-20 students) where we discussed and defended our ideas, and wrote many, many papers. Winning arguments may not have been based on real facts, but that was not the point. The point was to learn how to make an argument in speech and writing.

My father, a wise man, made only two recommendations in my academic life that I remember. One was to take typing in high school; in those days, the near exclusive domain of girls (which will seem strange to today's students who grow up typing and texting). Second, though brave, stoic, and physically tough, my father was terrified of speaking in public, and believed that a course in public speaking was essential for future success in life.

Outstanding advice! An enthusiastic young English professor taught Princeton's public speaking course. We 15 or so students prepared and gave speeches to each other and provided truly constructive feedback. In the pre-computer/PowerPoint era we were taught to organize our talks on note cards. And while practice was emphasized, we were taught not to memorize the talks to preserve a good measure of spontaneity, and to maintain eye contact with members of the audience to keep their interest. These basic principles are just as true today, where public speaking is more important than ever, not just in formal occasions like seminars or conferences, but also in work and community meetings. A semester's instruction should be mandatory for nearly every college

student. Further, high school debating teams and drama are an excellent place to build the foundation for effective public speaking.

I am naturally voluble, which had earned me a slot on [WPRB](#), the Princeton student run radio station as the color man for football and basketball games. Despite this, just before my first speech in the public speaking course, I was so nervous I vomited. Important lesson: everyone is nervous speaking in public. There is likely Darwinian evolution at work here: for groups to be cohesive, there has to be some limits on how many voices can be heard in making decisions. In any event, experience may never completely calm the nerves, but with sufficient effort and practice nearly everyone can become an effective public speaker.

Equally important in science, as in many careers, is the ability to write succinctly, logically and with some flair. In part, there is no substitute for reading, particularly fiction, for writing with style. Even the science courses at Princeton honed writing skills, with tests often being essay based. For introductory Physics, I wrote a 10-page paper on star formation! Junior year Biochemistry students were distributed randomly to 3 professors who assigned an original scientific paper to explain and critique in a few thousand words. This was a tremendous introduction to critically reading the scientific literature, and the first baby step in learning to write scientifically.

But this was just the opening salvo. From the 3 professors, you selected a mentor (with their agreement) for your senior [thesis](#), a very big deal at Princeton. To graduate, all students (except some of the engineering students) must produce a thesis, a 50 to 200+-page paper based on original research. The first three years at Princeton are designed to prepare students for the thesis. Rather than the standard 4 courses per semester, seniors take 2 courses and direct the rest of their efforts (and all their worries) to their thesis. No thesis, no degree.

Thesis work begins second semester junior year by becoming familiar with your mentor and the research project assigned to you. For budding scientists, this meant hanging around the mentor's research lab, and getting to know their PhD students and post-docs. Back then, virtually none of the undergraduates had any experience working in a research lab [13](#). I put together a notebook describing the methods I was likely to use, and began to read the relevant original research papers.

I had the good fortune of landing in Professor Arnold Levine's lab. Arnie was in his mid-thirties, recently tenured, and though he hadn't yet made a truly important discovery (which would come as co-discoverer of [p53](#), a protein that plays a key role in many human cancers), was a rising star.

Arnie was interested in "tumor viruses". At that time, limited to viruses that

cause cancer in birds and rodents (a number of viruses are now known to cause cancer in humans). It was clear that viral cancers were rejected by the immune system—but how this happened was a complete mystery. Although Arnie was not an immunologist, he obtained his virology PhD from the University of Pennsylvania (“Penn”), which had an excellent immunology faculty, and one of Arnie’s best friends at Penn, Norman Klinman, was a superb immunologist.

In collaboration with Norman, Arnie thought it would be interesting to study how mice and rats reject virus-induced cancers. So that was my project. I was initially assigned to work with a well-meaning graduate student. But I chafed at taking direct instructions, and within a few weeks was basically working independently. Arnie was a genial and inspirational leader, and though I did not see too much of him on a daily basis, he taught me many important general lessons.

First, he made me call him Arnie, not Dr. or Professor Levine. This was actually quite difficult at first. Not until decades later did I really understand why this is so important. If the PI is Dr. So-And-So and everyone else is Pam or Jim, this creates a barrier for intellectual discourse. In a good laboratory, the work has to be based simply on the best ideas, not the source of the ideas. Indeed, at a lab meeting presented by one of the PhD students, I had an insightful idea to test the proposed hypothesis. And without missing a beat, Arnie said, “that’s a great idea —let’s do that”. Wow!

Second, he showed that humor and humanity were an integral part of science. A poignant example: sitting on his desk was a set of teeth that he had (poorly) carved as a fledgling dental student. Arnie was not cut out to be a dentist; he didn’t really like using his hands (many years later, one of his graduate school lab-mates told me he was all thumbs in the lab). The jaw on his desk was a reminder of the misery he would have faced as a dentist (and a harbinger for my future as a MD).

Third, more darkly, every so often Arnie would arrive in the lab and scream at nearly everyone. As events unfolded, this was likely due to [alcoholism](#), but whatever the cause, I learned that acting out is never appropriate in the work place, and that the relationship between the PI and their lab members is asymmetric. The PI’s words have far more weight than the lab members, and a PI must take extreme care in not expressing negative thoughts unless they are absolutely necessary.

Finally, and most importantly, upon leaving the lab, Arnie wrote me a letter, still tucked into my bound thesis, that I cherish. In terms of making publishable or even just positive findings, my time in his lab was completely non-productive. Although the question of how the immune system detects cancers was

profoundly important, our approach was hopelessly naïve, which became clear during the next decade. Every experiment I performed in Arnie's lab generated meaningless data, but this was in many ways advantageous. A scientist's most important skill is designing and interpreting experiments. The experiments I was doing were relatively easy, which allowed me to design and interpret several experiments a week. Since nothing worked as expected, I had to constantly troubleshoot the method and question my assumptions. For every experiment, I had the thrill of breathlessly watching the data as it came off the machines we used to measure the outcome.

How different from the standard course related-laboratories. Here, there was no "answer": rather the goal was to pry a tiny bit of truth from nature. And I didn't do it alone: I had the joy of sharing the puzzle with Arnie and the other lab members. One, in particular had a huge influence on my career. [Art Levinson](#) was in the second year of his PhD, and he took me under his wing, becoming a mentor and good friend. Extremely bright, iconoclastic, acerbic, funny and as socially awkward as me, we hit if off from the start. Art not only discussed my (and his) experiments on a daily basis, he also greatly expanded my knowledge base, by sharing his enthusiasm for the classic experiments in molecular biology. By the end of the year, although I didn't know it, I had become infatuated with science.

Back to Arnie's letter. Like most Princetonians, I took the thesis extremely seriously (it also accounted for 25% of the ranking that determined who would graduate with honors). In addition to describing my failed experiments in detail, I made a major effort to summarize the current knowledge in the field, and thought hard about the future of our approach in particular, and the topic in general. I labored to express my thoughts logically, cogently, and succinctly. All this was not lost on Arnie, who lauded the job. He also quite honestly appraised the actual experimental findings, which were uninteresting but carefully performed and analyzed. And then the words that changed my life: "*it is likely you would make a first-rate scientist if you choose that route in medical school*".

## CHAPTER 3

### *Medical School*

**A**s today, medical school admission was highly competitive in the mid-70s. Coming from Princeton with excellent grades (I was near the top of the biochemistry majors) and [MCAT](#) scores, and aided by a highly enthusiastic recommendation letter from Arnie, who still had many ties to Penn<sup>14</sup>, I was accepted to Penn Medical school in Philadelphia. The oldest, and one of the best US medical schools, I was delighted to join Penn's illustrious ranks. Unlike most of the other top medical schools, Penn Med was located right on the main university campus, pulsing with undergraduate energy. The campus had just metamorphosed from gritty to a green urban oasis, with pedestrianized streets and open lawns with contemporary sculptures from top artists. It was only a short walk from the heart of Philadelphia, then as now, a cool city with lots of character.

With the science bug firmly planted from my senior thesis, I arrived looking to do independent research in a basic science lab as time allowed during the summers, but had not really seriously thought about a future in science. Medical school immediately proved to be a bit different from the dreams of a 12-year old. Completely clueless, I had made no effort to find out over the next 10 years what medicine and medical education actually entailed.

The first two years of medical school (shoehorned into one year at Penn) entails intensive classroom teaching of the basic science underlying medicine. This meant approximately 40 hours of lectures and labs a week (and another 20 to 30 hours of studying). There was a mind-numbing amount of memorization. I had an excellent memory (alas, time has taken its toll), so this was only a matter of discipline. But even though Penn is one of the most academic medical schools in the country, there wasn't much real thinking required.

Despite the emphasis on basic science, we had opportunities starting in the very first semester to begin to interact with patients and doctors in the clinic. This experience was fairly innocuous, but somehow, I was immediately repelled. There was an enormous degree of regimentation. Medical students were

basically at the bottom rung of a long career ladder. Unlike the lab, the activities of the group were not directed by who asked the best question, but rather by who wore the longest white coat. Attending physicians had knee-length coats with their names rendered in beautiful red sewn-in script, while students wore humiliating bikini coats with crappy plastic nameplates pinned on awkwardly. The hierarchy was understandable. Patient care is often complex in a teaching hospital, but it is typically by the book, and knowledge and experience easily trump creativity.

I really enjoyed meeting the patients and learning of their medical issues and lives. I had immediate issues with the doctors. The poor overworked house staff (the junior doctors, interns and residents, who did the heavy lifting in patient care) were in desperate need of help for routine, essentially menial “scut” work. This fell to the medical students, who were expected to do the work eagerly. “Thank you, sir, may I have another”: Kevin Bacon’s famous line from *Animal House*, well describes the servile attitude expected from medical students.

I had other ideas. I naïvely believed that medical school should focus on education, not errands. Typically, the attending professors were nowhere to be found except during rounds, an hour so each morning when the attending physician would grandly visit the patients trailed by the adoring residents and students they supervised. Though designed as a teaching platform, this often became an exercise to enforce the academic hierarchy by humiliating junior doctors and students who couldn’t answer a question on the patient or their disease or asked a dumb (or too clever) question.

At other times, few of the harried residents had any inclination to discuss pathophysiology, as they were overwhelmed with taking care of patients, who were generally extremely ill, if not at death’s door. Further, until 2003 with the adoption of the [Libby Zion Law](#), there were no national standards for how many hours house staff could work consecutively or per week. It was routinely expected that young doctors go without sleep for 36 hours or more during their 100+ hour workweek.

To me this was nuts. I function poorly without 8 hours of sleep, and had no intention of spending the night on the wards (I never did). The medical establishment insisted that sleep deprivation was required for junior doctors to demonstrate their dedication to their patients and profession, and essential for the continuity of patient care. I saw it as was a patently hypocritical ruse to staff the hospital at minimal cost. Residents were (and still are) paid relatively poorly, since the job was considered a temporary training position on the road to a lucrative 30+ year career.

Somehow, within a month or two of starting I intuited that clinical medicine

held little interest for me. Penn had one of the first medical scientist training programs (MSTP) in the USA. This program, initiated by the National Institutes of Health (NIH) in 1964, was designed to train physicians in science by having students simultaneously pursue MD and PhD degrees. By the early 60's, it had become clear that advancing medicine required massively increasing the knowledge base of how life works at level of molecules and cells. (It still does, more about this later). The goal was to generate a new type of physician who could bridge the very different cultures of science and medicine. Though most MSTP slots were given to incoming students, Penn, in its wisdom allowed students to apply after starting medical school. Thanks to my experience in Arnie's lab (and very likely, Arnie's extensive ties to Penn), I was admitted, and could start the program immediately after my first year had ended.

The MSTP was an incredibly good deal. The US government, bless its soul, would pay Penn's hefty tuition for medical and graduate school, and provide a stipend to boot. My parents, who were paying for medical school, were beyond ecstatic. While the \$3600 stipend seems meager today, it covered my living expenses, thanks to the low cost of living in West Philadelphia, which with the exception of a few OK streets, was basically a slum. James Ferguson, a distinguished physician scientist who led the program had the refreshing attitude that MSTP students were adults who could decide what they needed academically. If you wanted to take lots of PhD courses to solidify your scientific knowledge, fine. If not, Dr. Ferguson was cool with it. Further, Penn Med was quite happy if you wanted to learn a minimal amount of medicine in the MSTP. This was perfect for me. I was allowed to graduate with only 13 months of clinical experience, which meant I could complete the program 5½ years after starting med school.

I was near the top of the class academically after the first year, but my medical school performance deteriorated rapidly thereafter. Although I really enjoyed interacting with my patients and took joy and pride in taking and writing up their clinical histories, I had next to no interest for the nuts and bolts of medicine. Deciding that clinical medicine was not for me was liberating. At the same time, I largely cast off the insecurities that fueled my competitiveness. I decided I would no longer be a slave to tests, and would spend my time and energy learning only what truly interested me, which was basic research.

Naturally, this led to conflicts with the medical residents and attending physicians, who expected respect and deference based strictly on their position in the hierarchy. To wit: the first day of Medicine 200: *the* course for eventually lining up a prestigious residency in the many varied sub-disciplines of internal medicine. High anxiety among the medical students, selected from the most

competitive pre-meds in the nation. Larry Beck, the dashing young attending, rising star in the Medicine department who ran the course, opened by saying: “this is where Penn separates the men from the boys”. Though just a throwaway statement, this betrayed the ethos of much of the clinical faculty. Medicine wasn’t about helping people or advancing the science of medicine, it was a *cojones* contest. Particularly prized was the ability to quote the conclusions of published papers without any consideration of their validity. Skepticism was not valued.

Penn Med did not give traditional grades (Honors, Pass, and very, very rarely Fail<sup>15</sup>), but students were still ranked with great precision. The rankings had real currency since they were critical to obtaining prestigious residencies. Along with the nominal grade would be a summary of the student’s performance. Some of mine were remarkably, even humorously negative. The worst recommended psychiatric counseling for me, based on my negative attitude. This stung, but helped me develop a thick skin for essentially pointless criticism<sup>16</sup>. This is an important attitude for young scientists to develop. Challenging dogma, even in science, comes with a price. Those whose dogmas are challenged rarely respond with generosity and grace.

There is an old med school joke: what do they call the lowest ranking student in the class? Doctor. For the Penn class of 1979, that would be Doctor Yewdell. And this was an accurate appraisal of my knowledge of medicine. With intelligent guessing, I knew just enough to pass part II of the national medical board, which was required for graduation. I had no intention of further clinical training, not to mention practicing medicine, so my MD was not a license to kill<sup>17</sup>.

But the MD education was extremely valuable from every standpoint. My original interest in medicine was sparked by curiosity in how our bodies work. This I learned during the first two years of medical school; at least the basic outline. Further, medicine, in dealing with every aspect of highly complex organism (us), provides the big biological picture. Organisms evolve as organisms, not individual cells. Diseases affect an entire organism, not individual gene products, pathways, cells, or organs. Cells are constantly communicating, locally and distantly due to the endocrine system and the omnipresent nervous system, which innervates every organ. Having an appreciation of the entire system facilitates asking deeper and broader questions as a biologist.

It was also practically useful to learn some medicine. Being exposed to diseases and strategies of diagnoses has allowed me to provide medical advice to

myself, my family, lab members, and friends whose medical problems have been poorly handled or misdiagnosed<sup>18</sup>. A month of psychiatry (fantastically taught by Professor Irma Csanalosi, a commanding 5-foot Hungarian dynamo, a legend at Penn), provided invaluable insight into mental illness, which unfortunately, at some point of your life, is likely to affect members of your lab or department, friends or family.

Having an MD immunizes against the chip on the shoulder attitude of some PhDs, who (rightfully) resent the entitled, know-it-all attitudes of many MDs. Further, it provides life-long admission to the MD club, whose members have inside knowledge and access to the experts in any given disease, wherever they may reside in the world.

Medical school also provides a profound emotional education. Being on the wards exposes students to the realities of life. It enriches the soul to be at the bottom of a career ladder. In my case, being a simply awful medical student was a valuable lesson in learning to deal with failure, a critical life skill. Interacting with the human zoo that staff the hospital (janitors, nurses, junior and senior doctors), provided tremendous insight into human nature. Seeing how gifted doctors dealt with emergency situations graphically demonstrated the importance of grace under stress.

There were more lessons from the patients, who represented every social and economic class. Their plight as very sick individuals raised empathy and provided an invaluable perspective on how fortunate it is to be healthy. Sharing these intense experiences with fellow medical students, including my future wife, made for strong friendships, one of the most positive features of medical school.

## CHAPTER 4

### *Graduate School*

The plan for MSTP students was to be a traditional medical student for the first two years and then take 3 years to complete the PhD before returning to the clinics for a year. I decided to enter the immunology program strictly based on Arnie Levine's advice, who knew the department well and also had the foresight that the field of immunology would grow in importance in the coming years. At that point, and even today, I wanted to do rigorous, highly creative science and did not fret about the exact topic of research. So, when Arnie recommended that I work with Norman Klinman, I took his advice with no second thoughts.

Norman was a rapidly rising star who had already made major contributions to immunology <sup>19</sup>. His lab was a bustling, burgeoning enterprise, and I was initially assigned to work with a recently arrived post-doc. Norman probably assumed that if I didn't need help I would just begin to work independently, which is what I immediately did. I had decided to get a PhD because I was disinclined to take orders, and the future was now.

I spent the summer in Norman's lab after my first year in med school becoming familiarized with the technology and the people. I had every intention of working with Norman, but during the next year he decided to move to the Scripps Institute. Norman left Philly, where he had grown up and attended local universities (Haverford and Thomas Jefferson for his MD), for the sunny skies, gentle breezes, and other many attractions of La Jolla, surely on the short list of the best places to live in the USA.

Who should I turn to? One door closes, another one opens. Norman had recommended that I talk to Walter Gerhard, a well-trained Swiss MD. Walter had a distinguished pedigree. He first worked with Jean Lindenmann, co-discoverer of interferon, then did a post-doc with Stephan Fazekas de St Groth, a pioneer in understanding influenza A virus (flu) antigenic variation. A second post-doc brought him to the Klinman lab to generate monoclonal antibodies to influenza using the system that Norman had developed.

Due to this unique background in working with anti-viral monoclonal antibodies, Walter had just been recruited as an Assistant Professor to the Wistar Institute. Like most Wistar professors, Walter had a joint appointment at Penn and could supervise Penn students for their PhD training. By the time I had arrived as Walter's second PhD student (the first, a wonderful veterinary MD/PhD student named Dwight Lopes, would never actually get his PhD, for reasons I never fathomed), Walter had already produced the first hybridoma anti-viral monoclonal antibodies.

Hybridoma technology was invented in Cambridge, England, by Georges Kohler and Cesar Milstein, who would soon win a Nobel Prize for the method<sup>20</sup>. With hybridomas it was finally possible to harness the full power of antibodies, one of the most important elements of the immune system. Though Kohler and Milstein are typically portrayed as visionary geniuses for developing hybridomas, many other scientists had the same idea, including Hilary Koprowski, the Wistar director who had nabbed Walter to help make hybridomas. I had wandered into an ideal situation: a brilliant young PI with a new technology that would ultimately revolutionize biology and medicine<sup>21</sup>.

The lab at that point consisted of Walter, Dwight, Mark Frankel (Walter's first post-doc), two excellent technicians who worked closely with Walter, and me. Walter was highly organized, diligent, and enthusiastic. Working side by side at first, he taught me the craft of being a scientist. There was a bit of friction initially, as Walter learned to deal with me, an independent minded American graduate student. Though I took Walter's advice seriously, I trusted my instincts enough to select his best suggestions for projects and experiments. Within a few months I had learned enough to generate my own ideas and began to initiate projects on my own volition.

Looking back through the lens of supervising many young scientists over the years, I now appreciate that due to my independent mindset I was a royal pain in the ass to supervise, as any decent PhD student should be. My goal was to become a scientist who makes fundamental contributions to knowledge. I had to think for myself, that's the whole point (and fun) of basic research. This was not going to miraculously occur when the Science Fairy sprinkled dust on my head. Rather, it was good to think independently as soon as possible as a graduate student.

Ultimately, I spent more than a decade at Wistar, as a student, post-doc, and Assistant Professor. Wistar was a most peculiar place: a first-class biomedical research institute that fostered a personality cult surrounding Koprowski, a Polish émigré who resurrected the institute in the late 50's from what was

essentially an obscure anatomical museum. Reflecting the benign, jovial aura he charismatically projected, he was known to the staff simply as HK.

Wistar is located in heart of the Penn campus. Just across the street lurks College Hall, rumored to have inspired Penn student Charles Addams to create his famous cartoon mansion. Fittingly, Wistar had an unmistakable Addams ambience. Unlike Penn buildings, entering Wistar required negotiating guard stations, manned 24/7. Defying statistics, nearly all guards were named Bob. There was crossword Bob (capable of filling in the NY Times crossword like a simple form, even when inebriated, which was always), nice Bob, and sadly, dead Bob. Actually, all the Bobs were nice, except for an officious young Bob, who made you glad that the guards weren't armed. Negotiating past a Bob led to remnants of the [anatomic museum](#), where you were greeted by prized human fetal skeletons (Cyclops and two-headed were the stars) among many other anatomic curiosities. Even ascending grand stairway in the central foyer was unsettling, as various benefactors and founders lined the wall both figuratively, in paint, and literally, in ceremonial urns beneath their dark images.

Late at night in the cozy library you might find one of the janitors reading the NY Times financial section, of all things. Pungent, disheveled; these would be kind adjectives. All too easily underestimated, in a high, unmistakable voice he would freely provide insightful financial advice on various stocks and world commodities ("Titanium futures look promising...."). The librarian was perhaps the most eccentric among an eccentric group. He dressed like Gomez Addams, but lacking any discernible sense of fun or humor. Became quite cross when disturbed during his daily siestas on his prized reclining beach chair, strategically located among the stacks to peep up skirts through the glass floor above. His main task appeared to be to pen epic poetic essays on HK's virtues and accomplishments.

HK had a particularly close relationship with our lab, routinely attending our lab meetings. On paper, he was the official supervisor of Lou Staudt, an outstanding MSTP student who I helped recruit to the lab<sup>22</sup>. Walter's lab was a marvelous place to train. Having the first monoclonal antibodies meant that every new experiment I conceived was likely to provide new insights. Walter had already shown that monoclonal antibodies could be used to select for mutant influenza viruses that were resistant to antibody neutralization. Working together, we now measured the frequency of the mutants in the viral population, and also determined the number of different sites that the virus offered to immune system for antibody neutralization. Our findings had important implications for how influenza virus changes ("drifts") to avoid immune control. This is clinically important, since drift still prevents long lasting vaccination

against influenza, which costs tens of billions of dollars and tens of thousands of lives in the USA alone.

These findings were published in two papers in *Nature*, then as now, probably the most prestigious journal for publishing original biological discoveries. The review process in 1979 was greatly streamlined: the paper was either rejected, accepted, or nearly accepted with a short list of reviewer comments and suggestions for improvement. Today, a *Nature* paper can be in review for 2 years with endless debates with the reviewers about the appropriate experiments and conclusions. An industrious student in my day could easily publish 2 or 3 papers during their PhD in which they performed most of the experiments and wrote all (or most) of the papers. Due to the increased expectations and demands of reviewer, students today often publish a single paper during their PhD with multiple authors from multiple labs, and may only have a chance to write an early draft of the collaboratively written paper.

Although the science culture 35 years ago was in most ways much better than today, Wistar was a strange and troubled institution; a window into the dark side of science. The area just outside of HK's office was an integral part of the personality cult. Professional quality black and white photographs captured HK in dozens of scenes; working at the bench in a very long and very white lab coat, frolicking with beautiful people in the snow around a giant snowman (clear Freudian implications), immunizing children in deepest Africa, well-tanned while sailing in exotic locales, beaming at important conferences surrounded by equally famous scientists. HK really was one of the world's most interesting men, and he made sure that you knew it.

HK treated Wistar as a personal asset. Employees staffing the lunchroom doubled as HK's housekeepers at home. The Wistar driver functioned as a private chauffeur who made solo runs to NYC to collect delicacies unavailable in Philly, or drove hundreds of miles to provide local transportation to a jetting HK. The Wistar computer "expert" was pretty useless at computers (then a novelty), but took care of HK's home sound system and other personal electronic needs.

HK personally determined every employee's salary. Christmas would bring an engraved card from HK informing you of your new salary signed simply (and still bizarrely incomprehensibly) "I am". Toward the end of HK's reign, Pete Wettstein, a free-spirited member of the faculty, created an uproar by posting a printout of all Wistar salaries, obtained from a mole in the accounting office. Some post-docs favored by HK were paid more than Associate Professors.

In the meeting/dining room used for special occasions (lavish state dinners with expensive wines and real Cuban cigars), hung a giant tapestry by a well-

known artist commissioned by HK at considerable expense to the institute. Wistar as a solar system. A dominating HK as the sun lounging in a natural scene artfully surrounded by the talented young scientists he had recruited, supported, and was genuinely proud of. But as time passed in the real world, some of the more junior planets, no longer favored by HK's gravitational field, careened out of the Wistar to parts unknown, a clear sign of the dangers of being captured by HK's gravitational field.

In the wider world, HK led a culture shift in biomedical research. It became common for scientists to profit personally from their discoveries, nearly all of which were funded by the US and other governments. This was not illegal; indeed, the opposite was true. The 1980 [Bayh Dole](#) act of Congress encouraged universities and private institutes to profit from NIH and other US government funded research. Bayh-Dole played a central role in the development of the US biotech industry, which has enhanced the US economy and lessened the burden of diseases and improved the quality of life for hundreds of millions around the world.

Every law has unintended consequences, however. In creating the biotech industry, Bayh-Dole transmuted universities into business parks, and academics into entrepreneurs. The jackpot nature of business success has created tremendous inequities among scientists (the few reap the benefits of the work of many). Bayh-Dole has played a major role in the degradation of biomedical research as a career and in the exploitation of young scientists, who often toil at poverty wages for millionaire lab heads. The conflict of interest of principal investigators pursuing personal wealth at public expense is a serious ethical issue that has essentially been ignored.

And HK led the way. Personally banking tens of millions from establishing Centocor<sup>23</sup>, Wistar wound up with shockingly little of the payout. The fury of the Wistar board upon learning the details of this outrageous deal led to HK's [forced](#) retirement as Director. Had HK been more generous to the institute in commercializing the hybridoma patent, he could have had his cake and eaten it too, as he would be remembered as the great champion of Wistar in all aspects.

But all this was in the future. I had heard vague notions of the deal from Walter during our West Philly neighborhood watch walks (two skinny geeks armed with flashlights, what were we thinking?). Walter, as a co-inventor, received a much smaller, but still significant piece of pie. And I would swear that Walter was motivated only by extremely exciting science that the hybridoma revolution generated<sup>24</sup>.

While HK had the mountaintop view of the medical importance of

monoclonal antibodies, I never saw that he had any notion of how to actually use the antibodies to make discoveries. Attending our lab meetings, he was a genial and supportive presence, but I can't remember him asking a single relevant question or making any suggestions. The rabies virus group that he headed quickly adopted approaches that we pioneered with influenza virus. It was strictly a one-way street for the information flow.

The rabies work was solid, but was not going to win HK a Nobel Prize. A Nobel, however, would surely await the discoverer of a virus that caused multiple sclerosis. Over the years, HK and colleagues reported no fewer than 5 distinct viruses as causative agents, all as a result of sloppy science. This work formed the core of a substantial, long running program project grant that initially paid my Assistant Professor salary upon returning to Wistar from a post-doc in London. My only duty, fortunately, was to attend the monthly meetings in the tapestry room, which like all of HK's meal meetings featured excellent food. Though the "Slow Virus" group had many outstanding scientists, the science it produced was pretty awful, in part because its existence depended on confirming a hypothesis rather than asking open-ended questions. HK was also an eager participant in the "Emperor Has No Clothes" field of [idiotypic antibody networks](#), which greatly tarnished the entire field of immunology.

HK cultivated scientific acquaintances who could possibly nominate and support him for prestigious prizes. On visiting, they were placed on a pedestal for us to admire, whether they were outstanding scientists (some were), or charlatans with little regard for the truth. Though he could clearly appreciate it in others, seeking scientific truth did not appear to be very prominent on HK's scientific agenda. It was all about the power and prestige of being a great scientist, without the burden of worrying about truth or ethics.

HK taught me that unchecked power and ego result in disaster. That in science as in politics, absolute power corrupts absolutely. I learned that you can't fool nature, and that the truth is indifferent to the incorrect opinions of the self-important. I saw a number of junior scientists, drawn by HK's power and prestige, whose careers were damaged or terminated by uncritical judgment of the projects they were required to participate in. I learned to keep a safe distance from HK and others like him.

HK had real charisma and was a natural leader, but leadership is a double-edged sword. Perhaps if he had received proper credit for being the first to develop an effective polio vaccine, his later leadership would have been more positive. HK created a world-class institution that published outstanding papers and developed treatments and vaccines that have saved millions of lives. Resurrecting Wistar, however, did not entitle HK to loot it. Further, it is difficult

to conclude that the talent he recruited would not have been equally or even more productive in a more inspiring environment. HK did not destroy all the science around him, but he exerted a corrosive, corrupting influence.

By negative example, HK taught me that:

The single most important aspect of science is that it must be practiced correctly.

Our most important mission as scientists is to pass the torch of the scientific method to the next generation.

If we teach our trainees to do science properly, truths will continue to emerge.

If we cut corners, science will be damaged and possibly even destroyed.

The mission of science is accomplished by a largely anonymous group of individuals sincerely dedicated to its value to society.

By their very nature, great men tend to damage science.

Seeking glory at the expense of truth puts the entire scientific enterprise at risk.

Icarus had a choice!

## CHAPTER 5

### *Post-doc*

I may have given the impression that my relationship with HK was strained. Au contraire! HK was a genuinely fun and likable individual. I possessed sufficient hypocrisy and self-survival instincts to maintain a good relationship with HK, but at a waxed-wing-preserving distance. As an excellent judge of talent, like many others he was smitten by David Lane, who having recently discovered [p53](#) (co-incidentally, Arnie's lab discovered it around the same time, along with at least 3 other groups), was starting his lab at [Imperial College](#) in London, and had an open post-doc position. My wife, a Penn and Children's Hospital of Philadelphia trained pediatrician<sup>25</sup>, managed to win a coveted fellowship at the Great Ormond Street pediatric hospital in London, so there we landed for a year, in an apartment<sup>26</sup> attached to the hospital.

David, genial, whip smart, and with real charisma, would eventually be knighted, a rare honor for a British scientist. Naturally (but charmingly) disorganized, he was just learning how to run a lab (a key step would be finding a lab manager) and from his growing pains, I learned first-hand many of the possible mistakes in starting a group. Imperial eventually became a biomedical powerhouse by merging with various London medical institutions, but at that time there was virtually no modern molecular or cellular biology. Getting into the game, they had just hired four young faculty members (David, Peter Rigby, David Glover and Jean Beggs), all of whom would go on to fantastically productive careers, but only after leaving Imperial for greener pastures.

Despite its limited size, Imperial was an exciting place, and like most Americans in England, I enjoyed the traditional teatime, where the entire department gathered to talk science and life. There was in general, a lot more discussing, and a lot less doing than in the USA. This was not due to a lack of money; the labs were better funded than our lab at Wistar (for a while at Wistar, we washed our “disposable” Finnpipette tips for re-use). Rather, the work ethos was just down a few notches. There weren't many foreign post-docs, just one very nice German, as I remember. He and I worked about twice as hard as the

British students and post-docs. Although I appreciated the British attachment to discussing experiments in detail, this only goes so far. Since the most important discoveries typically cannot be anticipated, it is generally a good strategy to do lots of experiments. Indeed, it is all too easy to overthink and miss doing good experiments while trying to conceive perfect ones.

Starting out in a new lab was another useful exercise in humility and boot strapping. I went from being a world's expert in one field (viral immunology) to knowing nothing in another (cell biology). Although p53 would eventually become one of the most medically important proteins to study (it is involved in 50%+ of all human cancers), and has dozens of known functions, the field was fetal at that point, and David and I struggled to generate useful hypotheses to test.

Oncogenes were the rage then, and it was clear that there would be many proteins that are involved in cancer. The standard approach was to identify oncogenes by genetic methods. Many high-powered labs were pursuing this approach, and I was unlikely to be competitive. Further, I've never had a knack for molecular genetics. For whatever reasons, many scientists become comfortable with some approaches and not others<sup>27</sup>. David and I, however, were both adept at making hybridomas and working with antibodies. Could I find new cancer related proteins using antibodies? This would have a number of advantages over gene-based methods in enabling us to identify changes in protein localization and amount. So, I immunized mice with different types of cancer cells in numerous permutations and modified an assay (to increase throughput) that David had developed to screen antibodies specific for cancer cells.

Although I could screen thousands of antibodies per week, this required my examining every antibody for its binding pattern by eye. To ease comparison between normal and cancer cells, I would put both in the same well, but at different ratios so I could easily distinguish them. For hours on end I would peer down the microscope looking for differences in staining patterns. This taught me that there is more to seeing than looking. It takes dozens of hours of looking at cells to begin to be able to discern all but the grossest differences in patterns. When I would close my eyes at night I would see the staining patterns that were engraved on my visual cortex that day.

Looking at cells for hundreds of hours that year, I learned the power of microscopy to make discoveries in cell biology. Because it samples so many proteins in so many contexts in a cell, microscopy greatly favors serendipity, perhaps my favorite word in the English language. Coined by the English author Horace Walpole, it conveys the spirit of the Princes of Serendip, who roamed

their island (modern day Sri Lanka) looking for some arbitrary thing but inevitably finding something better. Not simply by luck, mind you, but by wisdom and insight. In the words of Louis Pasteur, chance *only* favors the prepared mind.

So, what did I find? I recovered many hybridomas that secreted antibodies that stained cancer cells but not normal cells; exactly what I was looking for. In starting to characterize these antibodies, I learned a lot more biochemistry from David, which combined with microscopy, would form the technical foundation of my independent career. I also produced three new antibodies specific for p53. Dimly, we were not terribly excited about them, since David and others had already produced several p53-specific monoclonal antibodies. From my PhD, I should have known that the more monoclonal antibodies the merrier, since we had made hundreds of antibodies specific for flu, and found that antibodies specific for the same flu protein typically had widely divergent properties that provided great insight into the function of the target flu protein. After I left London, David's lab went on to show that one of the monoclonal antibodies I had made was able to distinguish two very important forms of p53: a form that functioned normally in cells, and an alternative form that was intimately involved in cancer.

After 6 months in London, with child #1 *in utero*, my wife and I decided to return to Philly, in part because I thought I was ready for complete independence (in those days, prolonged post-docs were the exception, and it was not unusual to even go directly from a PhD to a tenure track position). I left London without publishing a paper, but was quite happy with what I had learned technically and socially. As an added bonus, several years after leaving, David made me first author on a paper describing the p53 monoclonal antibodies that I had generated. Though I had genuinely earned the position with hard work, it is my only first author paper that I did not write the bulk of (I didn't write a single word, in fact).

The paper was published in a solid but not terribly prestigious journal. The cachet of the journal has nothing, however, to do with the value of the papers published in the journal. This this paper been cited more than 333 times (the average molecular biology paper published is cited ~50 times over the same period), in part because the antibody (which was quickly made commercially available) proved to be extremely useful to many labs studying p53. Another lesson learned: what I had thought to be a non-productive publishing year proved to be the opposite; you never know!

## CHAPTER 6

### *Tenure Track PI*

Wistar was happy to take me back, but, not on the terms that I had thought I had negotiated by phone. I intended to work on the cancer cell specific monoclonal antibodies that I had generated in London. But Walter (and thus HK) wanted me to return to flu research, at least for most of my effort. Though things worked out for me in the end, it is typically a bad idea to start your independent career where you trained. It is exceedingly difficult for your former mentors to treat you as a fully independent scientist. And indeed, I was given a raw deal compared to every other tenure track recruit at Wistar. I had no lab space of my own, no new equipment, or any equipment of my own, for that matter. I had to fight to get a tiny private office. My salary was less than many post-docs at Wistar.

And worse of all, Walter somehow expected to be included as an author on my papers. While this was typical for European labs, it was not the tradition in the USA, where young investigators were given their wings. And I was independent-minded, even for an American. It was not as if I was not contributing to the overall group effort. I was bursting with good ideas, and shared them freely with Walter's much larger lab without expecting (or receiving) credit. So goes it. I focused on the science, which was going very well.

My group at this point was a technician, Amy Yellen, who had just graduated from Penn, and me. Hiring is tricky, and I still haven't figured out a foolproof method for choosing good people<sup>28</sup>. It is always good to be lucky though. Amy had little practical experience, but she was energetically positive, funny, smart, careful, and an extremely quick learner. Most importantly, she wanted to squeeze the truth out of nature, and not just an easy answer. She would go on to be an outstanding graduate student at Penn and a terrific post-doc<sup>29</sup>.

For four years Amy and I constituted my "group". Although I didn't give it much thought, this was fortunate, as it meant that I spent virtually all of my time working at the bench. This was much more fun delegating the job to others.

There is nothing like the thrill of making a discovery with your own two hands. Further, you think much better about the potential artifacts and implications of the data when you perform the experiment yourself.

Many newly independent scientists make the mistake of sitting in their office and recruiting students and post-docs to do the work. It is unlikely that a starting scientist will attract students or post-docs who are anywhere nearly as capable with their hands as the scientist themselves, who were selected, after all, based on their bench skills. This is more prevalent today, in large part due to the onerous demands we place on “young”<sup>30</sup> faculty members, who are expected to teach undergraduates, serve on committees, and most damaging, obtain grants to completely fund their labs (typically including a large proportion of their own salaries).

The [Basel Institute](#) provided an excellent model for how young scientists should be fledged. Funded from 1971 until 2000 through the largesse of the family that controlled Hoffman La Roche, a Swiss pharmaceutical giant, the Institute’s mission was to make basic discoveries about how the immune system works. Its director, Niels Jerne, a Nobel laureate<sup>31</sup> had a discerning eye for talent. The idea was that young scientists would run nearly all the labs, typically fresh from their PhDs. They would be provided a trained technician, free access to central services necessary for their work, and essentially an unlimited budget. They could work on whatever interested them. The small size of their groups meant that they had to work at the bench, and also catalyzed collaboration between groups. Collaboration was fostered by the thoughtful architecture of the institute. To enter the institute meant having to walk through the cafeteria, which featured subsidized food and coffee. Hanging out there often lead to chance encounters that sparked many fruitful collaborations. The institute was built on two floors, but the top floor was connected to the bottom by spiral staircases located in many of the labs. This too created a large neighborhood for interactions. The labs were highly productive, and many of the alumni would become leaders in their fields after leaving Basel for traditional academic institutes.

Alas, when the bean counters inevitably took over the reins at Hoffman La Roche, they could not justify the existence of the Institute, since it had not been designed to generate knowledge of immediate practical use. Still, the Basel institute provides a shining example of how a small organization dedicated to basic science and fostering young careers can function at a bargain price. Keys to Basel’s success were the rapid turnover of its staff, which limited the sequestering of resources by senior scientists, and the lack of a crippling

administrative bureaucracy. If a scientist had a major need or problem, they could just see Jerne or a trusted deputy, and they immediately rendered a decision<sup>32</sup>. Usually, however, the problem could be resolved quickly by just walking down the hall to see the relevant administrator or staff member.

This may sound trivial, but a major problem in large scientific organizations is the anonymity of the administrative staff. Personal relationships between administrative staff and scientists they ostensibly serve<sup>33</sup> are key to a well running organization. Knowing scientists personally is crucial to connecting the administrative staff to the mission of the organization, which in the case of a research institute is to make discoveries<sup>34</sup>. When this contact is severed, there is a natural tendency for administrators and scientists to view each other as enemies bent on thwarting each other's best intentions.

One of the young scientists at Basel was Jack Bennink. Jack had done his PhD at Wistar with Peter Doherty, who had relocated from Australia shortly after making the discoveries with Rolf Zinkernagel that would win them a Nobel Prize in 1996<sup>35</sup>. Walter collaborated closely with Peter, and my medical school roommate, Neil Greenspan, was a PhD student in the lab with Jack, but I didn't know Jack well.

Profiting enormously for the Basel setup, Jack had learned how to make T cell clones from neighboring colleagues who were pioneering the technique. These clones made it possible to begin to answer the question that I had pursued in my Princeton thesis: how does the immune system recognize virus-infected cells? I had continued to work on this question during my PhD. Though my [findings](#) pointed to interesting features of cell biology that to this day remain mysterious, it turned out that they were the classic [red herring](#), and completely irrelevant to T cell recognition. But from this work, I had all of the flu virus tools to determine which of the flu proteins the T cells recognized.

Jack, toting his T cell clones, returned to Philly from Basel, and together we worked for an intense month. I'll never forget the day we did the critical experiment: one of the least likely viral proteins was recognized! We were stunned. But correct. And the [Nature paper](#) we published set the stage for what was to become our joint obsession for understanding anti-viral T cell specificity, which rekindled upon my return from the UK.

Jack, alas, had made the same mistake of returning to his training ground, but at least in his case, Peter had departed for Australia, so Jack inherited a nice new lab and a better deal. While I was in London, Jack had initiated a collaboration with Bernie Moss from NIH who pioneered the insertion of foreign genes into viruses. Such "recombinant viruses" represented the greatest advance in the

principle of viral vaccination since Pasteur's time. They were also perfect tools for deepening understanding of T cell recognition of virus-infected cells.

Just to illustrate the randomness of scientific careers (like the rest of life), the collaboration resulted from seminar serendipity, *viz* Jack's misunderstanding of the title of Bernie's seminar. Jack, who had used vaccinia virus as a model virus to study T cell recognition, thought that Bernie had generated vaccinia viruses akin to the influenza viruses we had used to map flu specific T cells. Rather, Bernie described how he had inserted foreign genes into vaccinia virus, including a flu gene.

Recognizing this is exactly what was needed to map flu specific T cell specificity, Jack initiated a collaboration with Bernie. Though many accounts of science focus on intense competition between investigators, this is highly misleading. Science is the most communal among all human activities. The whole point of science is to publish papers, which by the very ethos of publication, divulge to the entire world all of the hard-earned expertise and truths extracted from nature. On top of this, a high fraction of scientific discoveries are made by teams of collaborating scientists, often scattered around the globe. As knowledge and techniques inexorably grow in sophistication and complexity, collaboration becomes a necessity. Individual labs typically need others to best address their research goals. So it was with Bernie: he provided the viruses that expressed flu genes, and Jack and I provided the expertise in cellular immunology and eventually cell biology to determine which flu genes are recognized and how this works. The result was a classic series of papers that were the first to describe how recombinant vaccines induce T cell responses and use the recombinant viruses to understand the specificity of the T cell response at the molecular level.

# CHAPTER 7

## *Tenured PI*

In the early 80's gay men began to die from otherwise extremely unusual infections, normally only seen in immunosuppressed patients. This disease was acquired from other affected individuals, and hence AIDS came into the lexicon, for acquired immune deficiency syndrome. Stupidly and dangerously ignored by the Reagan administration on religion-based, homophobic nonsense that gays deserved their fate, eventually the government greatly expanded funding to understand and treat the disease<sup>36</sup>. As part of the NIH effort, the “[intramural](#)” program of National Institute of Allergy and Infectious Diseases (National Institute of Allergy and Infectious Diseases) was allowed to recruit a number of new investigators to study the newly discovered viruses that cause HIV and similar diseases in animals. Wisely, Tony Fauci, the NIAID director, was happy to recruit scientists interested in other aspects of virus host interactions, with the idea that expanding the general knowledge base would play an important role in preventing and treating HIV and other infectious diseases.

The call went out to intramural NIAID lab chiefs to recruit scientists. You have probably heard of the NIH, but may have little or no idea what *intramural* refers to. The NIH was established in 1930, though Its roots date back to a Staten Island infectious disease laboratory founded in [1887](#). It was first expanded in 1937 with the creation of the National Cancer Institute (NCI). In 1938 the nascent NIH moved to its present campus in Bethesda, just a few miles from DC, thanks to the generosity of a couple who donated much of their estate. President Roosevelt officially dedicated the new campus in 1940, with [stirring words](#):

The National Institute of Health speaks the universal language of humanitarianism. It has been devoted throughout its long and distinguished history to furthering the health of all mankind, in which service it has recognized no limitations imposed by international boundaries; has recognized no distinctions of race, of creed, or of color.

When NIH was founded, science was dominated by Europe, and the NIH was

set up on the European great man model: a prominent scientist was given enormous resources, including many PhD-trained scientists, to support their research. At NIH, these units were termed Laboratories with the great scientist denoted as Chief. With a small army behind them, many chiefs would publish more than a 1000 papers during their careers, putting them in the top 0.01% of all scientists in the world.

WWII was waged and won largely on the basis of scientific advances that led to the development of blood transfusion, antibiotics, radar, sonar, jet and rocket engines, and the atomic bomb. This convinced the US government of the value of basic research, a cause championed by Vannevar Bush, who led the scientific effort during the war and became a passionate supporter of the [value of basic research](#) after the war<sup>37</sup>.

Thanks to Bush, NIH funding increased enormously after WWII, and over the years, the NIH expanded by leaps and bounds to include 27 institutes and centers that cover the entire spectrum of human diseases. Seven thousand or so scientists perform intramural research on the now 310-acre Bethesda campus. The 90 buildings are scattered on a hilly, beautifully landscaped oasis with many trees dating to the original estates (the donors' gracious house remain in use as offices). The US government directly funds intramural research. The approximately 1100 PIs are federal employees. Several thousand post-docs, recruited from all over the USA and the world, conduct most of the research. At its founding in the '30s, NCI began to award research grants to academic investigators. This "extramural" program expanded to other institutes and eventually came to consume more than 80% of the total NIH budget, which now stands at 30+ billion dollars per year. These funds are doled out to academic investigators at universities and institutes in the form of grants (I'll discuss the merits of the grants system later). This has fueled the enormous expansion of US medical schools and spawned biotechnology industry, and provided the basic discoveries providing the drug pipeline to big pharma.

Due to the vagaries of Congressional funding<sup>38</sup>, the NIH sometimes does not know when (or even whether) new programs will be funded. Jack and I had heard from Bernie that it might be possible to join his laboratory as independent investigators in an off-campus building that NIAID was considering to rent for expansion. The idea was that I would spend 25% of my effort making and studying monoclonal antibodies specific for HIV; basically extending the work I had been doing on flu, to this new virus. The institute was happy to let my curiosity guide the rest of my work.

Jack and I were both looking to leave Wistar. It was clear that I could do

better elsewhere. Even though I had a precious R01 grant from NIH (the gold standard in extramural funding), I still had no lab space of my own. Less accomplished scientists recruited to Wistar were getting their own labs, lots of new equipment, and at least 50% more in salary. What was I, [chopped liver](#)?

When the phone call came from Bernie offering us positions, we had just a few weeks to decide. On the plus side: I'd get a permanent position in the government at nearly twice the salary, a huge amount of space, an essentially unlimited budget for equipment, supplies, and salaries to hire a technician and 3 post-docs. Although my wife and I were living in a charming (but tiny) house located in downtown Philly between Fitler and Rittenhouse Squares, we were about to add child #3, and were already contemplating the traditional move to the suburbs.

On the minus side: the new lab would have to rise Phoenix-like in what was essentially a dilapidated warehouse in Twinbrook, Rockville, 5 miles from the main NIH campus. It was uncertain what other PIs would be recruited to Twinbrook. We were particularly concerned that the institute would use the new facility as a live burial ground for non-productive scientists they wanted to relocate from the main campus to free up space for new recruits. We were assured that this would not be the case<sup>39</sup>.

I was ready to leap, but first I went to HK to see if I could improve my situation at Wistar. I basically asked for what Wistar was offering new recruits at the same career level (I would be up for promotion to Associate Professor that year). HK was friendly and understanding and seemed willing. From what I could gather afterwards, HK deferred to Walter's judgment and declined to make a reasonable counter offer. So, NIH it was!

Jack had reached a similar decision. Since we were moving our labs to a relatively remote location and shared an interest in the intersection of virology and immunology, we decided to form a single lab that we would jointly lead. This is uncommon, but not unheard of. Although the renovation was delayed and there were many battles with administrators over the set up and running of the new building<sup>40</sup>, we greatly profited by the enormous increase in material resources, and had nearly instant success, making some of the most important discoveries in our careers.

A year or so after we had started the lab, Tony Fauci toured the Rockville facility and had a brief introduction to each of the labs. When we explained that we ran the two groups as one, he said "that's cool" or something to that effect, and that was basically the last word on the topic. Over the years we've found that there are tremendous advantages in dual stewardship. First and most

obviously, there is always a first-rate mind with different knowledge and perspective to discuss the science that the lab is doing or contemplating. Second, the most difficult decisions in a laboratory typically involve how to handle younger scientists through the ups and downs of the normal graduate or post-doc experience. Much like in a family with two parents, having two opinions about the problem and proper course of action alleviates much of stress of making difficult decisions, and also is likely to result in a fairer outcome for the individual. Third, with time, each person can specialize in scientific and administrative areas according to their strengths and tastes, increasing the enjoyment and efficiency of running the lab.

So that's how I arrived at NIH in 1987. Little did I know how lucky I was. The level of support and freedom to pursue my instincts and best ideas has been fantastic. I believe that my work would have suffered had I stayed in the extramural world. I am most proud of thinking out of the box and challenging prevailing wisdom. In the grant system, this would have been more difficult, since scientists whose wisdom is being challenged typically comprise the NIH study section that decides whether your work should be funded. It certainly would have been riskier to speak out against the scientific *status quo*, since I could have easily faced retribution by offended individuals reviewing my grants. I will return to the important topic of the relative strengths of intra- vs. extramural approaches to research funding in Part II.

At NIH, I have worked almost exclusively with post-docs, roughly half from overseas, representing (to date) Argentina, Australia, China, Egypt, England, France, Germany, Israel, India, Iran, Italy, Mexico, Oklahoma<sup>41</sup>, Scotland, Slovakia, Spain and Sweden. NIH is a post-doc magnet, and from our first recruit (Ike Eisenlohr) to the current crew, there has been a steady stream of fantastic young scientists in the lab. About half of the former post-docs now have jobs doing academic research, with most rising steadily through the ranks of academia. The others have a variety positions, ranging from teaching secondary school to patent lawyer, with many going on to work for NIAID extramural, the FDA or other government science agencies.

Working with this talented group of young scientists and seeing them prosper in their careers has been a great joy. Being a PI is being Peter Pan: due to the constant flux of post-docs, Never-Never Lab is filled with people who never get old, with all of the energy, enthusiasm, and fresh outlook on life and science that youth brings.

So that's how I began my career. After arriving at NIH, it's basically been the living happily ever after part. I could have departed for different challenges or more money, but why abandon a decent salary, outstanding colleagues both in

my department, institute and elsewhere on campus, extremely generous research budgets, zero grant writing (!)? In short: scientific Nirvana.

I will leave describing the 30+ years of science I've been a part of at NIH for another day. What follows from here are the general lessons that I have learned about how to think about and do science.



## **Part II**

# **A Practical Guide to Succeeding at Biomedical Research**

# CHAPTER 1

## *Honoring the Scientific Method*

**H***omo sapiens* has existed in present form for perhaps 50,000 years. From the fossil record, our ancestor's brains are indistinguishable from our own. We can surmise that if we could time transport a newborn from that era to today, it would learn to function in the modern world just like today's babies. Why did it take 50,000 years to invent the computer that I am using to write this sentence?

The answer is that the brain's hardware had a 50,000-year head start on the software, *viz.* language and culture. Clearly, the invention of written language, starting about 7,000 years ago with the use of symbols, was a critical step towards rational technology development. This made it possible to record knowledge for transmission to other people and succeeding generations. But still, progress was painfully slow by today's standards.

A key step occurred with the introduction of the scientific method, whose roots go back at least 1000 years to Arab scholars. Bacon and Descartes first formally formulated the scientific method in the 1600s. Underlying the scientific method is the notion that the natural world is subject to invariant laws that our brains can understand. The key to deciphering the laws is observation and experimentation. A scientist starts with an observation.

Here's one: apples fall to the ground when released from apple trees. It is essential that the observation is actually correct. In the case of falling apples, this is trivial, but there are many, many examples of scientists wasting their time (and your money, if they are funded by your government) studying observations that are incorrect. Sometimes, preposterously, comically, incorrect (*e.g.* cold fusion and homeopathy). Having made a correct observation, the next steps are to make an intelligent guess<sup>42</sup> as to what accounts for the observation, and then devise an experiment to test the guess. Next come more observations, where again, accuracy is at a premium. Finally comes the conclusion, when the scientist decides whether or not the hypothesis is "proven".

"Proven" is a loaded word. Although scientists frequently utter the "P -word"

in interpreting their observations, they really shouldn't. In the formal mathematical sense of the word "prove", it is impossible to prove anything about nature, a system that created us, not *vice versa*. It is even impossible to prove that observations reflect reality, though here, statistics can be used to demonstrate that it is exceedingly unlikely that the observations are incorrect. What experiments accomplish is not proof, but rather evidence that either strengthens or weakens prevailing concepts about whatever it is the scientist happens to be interested in.

For scientists, thinking you have proven something closes the door to deeper understanding. It is essential to always be ready reconsider your conclusions based on new evidence or even new thinking. At the heart of good science is deep skepticism. Nobody should know the flaws of your work better than you do, and it is your mission as a scientist to constantly seek the truth. If you discover that your observations are incorrect, it is your duty to correct to the published record in a future publication or by issuing a correction in the original journal of publication.

If you have a long enough career as an experimentalist, there's a good chance that sooner or later, another lab will publish a paper that questions your data or conclusions. You will likely be upset at first; very upset. You are certain that the other lab is wrong. But, a day, week, or perhaps years later you may realize that the fault, dear Brutus, is with you. The appropriate response at this point is gratitude to your colleagues who questioned your results. They have gotten you closer to the truth, which is your Holy Grail, after all.

Non-scientists can use the "prove test" to identify charlatans. A first-rate scientist never believes that they have proven anything. So, their words are carefully chosen, and their statements hedged. This does not mean that they are wrong, rather, that you should listen closely to what they have to say. By the same token, do not trust scientists (or anyone, for that matter) who claim absolute knowledge.

Now, I have to be careful here not to overemphasize the lack of certainty in science. There is a school of thought (typically promulgated by academics with little or no scientific training), that there is no objective reality in science. That in essence, interpretation is so tainted by the investigator's biases (cultural and otherwise) as to be meaningless. I won't mince words about this idea: it is completely stupid. While proof may be unattainable, the scientific process nonetheless uncovers approximations of the truth that are sufficiently accurate to enable practical applications. Like microwave ovens. Or jet planes. Or vaccines that save billions of lives<sup>43</sup>.

Although it seems like a cop-out, the greatest strength of science is that is

inherently self-correcting. The truth exists inviolate and is completely impassive. It cares not a whit about what we humans believe. If mistakes are made in experimentation or interpretation they will eventually be corrected by those dedicated to uncovering the truth. It follows that the most important mission of science and scientists is to honor the scientific method. It is far better to do science right and get the wrong answer than *vice versa*, for the latter imperils the scientific enterprise.

It is no coincidence that the formulation of the scientific method occurred in lockstep with the principles of rational democratic governance. This period, known as the Enlightenment, was enormously influenced by the ability to science to explain the natural world (Newton linked falling apples to the movement of planets and stars). It led to the birth of the USA and other representative democracies, which while imperfect<sup>44</sup>, have greatly improved living conditions for both rich and poor alike. Governments (corporations or any non-religious organization, for that matter) that abandon evidence for faith will inevitably lead their constituents to disaster.

Enemies of science exploit every opportunity to denigrate its value to society. Motivations vary, ranging from corporate greed (*e.g.* climate change denying oil and coal companies), religious belief (*e.g.* evolution deniers), to well-meaning ignorance (*e.g.* vaccine deniers). If science is done unethically, even by the odd rotten apple, it imperils the entire enterprise. Human nature being what it is, there will always be some scientists who cheat, lie, and steal. Protecting science requires improving systems for identifying, punishing and removing such individuals<sup>45</sup>.

Public trust is essential since science must be funded by governments. The public needs to be better informed that with few exceptions<sup>46</sup>, private enterprise is unwilling to invest in basic research. This is wise from a corporate viewpoint, since the practical application of discoveries is highly unpredictable, and in any event, requires decades to reach the marketplace. Since basic research is the key to improving standards of living and provides enormous widely shared economic benefits for society, this is the one area that all rational political parties should agree on as a positive benefit for government funding.

Even with the enthusiastic support from the biopharma lobbying goliath<sup>47</sup>, science is losing the funding battle in the USA to other interests. Despite enormously increased opportunities for breakthroughs in applied and basic biomedical research, NIH funding has decreased in real dollars by 25% in the past 10 years!<sup>48</sup>.

How can this be remedied? Given the competitive instincts of Americans,

perhaps we will come to our senses when China and India, with the energy and aggressiveness of emerging economies, begin to eat our biomedical lunch. These countries already supply a significant fraction of biomedical researchers in the USA. At some point, likely the near future with xenophobia making its periodic reappearance, the best individuals will begin to return to their native lands as the opportunity curves begin to cross. When the money follows discoveries patented overseas, this may convince the USA to put biomedical research back at the top of government funding priorities.

## CHAPTER 2

### *Becoming a Life Scientist: College*

**L**ife can be complicated, and there are myriad paths to biomedical research. The simplest path however, starts by majoring in a scientific or engineering discipline in college that enables you to take the required courses for entering a biomedical doctoral program. Having a doctorate is almost an absolute requirement for running your own lab in academia, or for having a reasonable degree of autonomy in industry. Typically, this will be a PhD, but it is also possible to pursue a scientific career with a medical, veterinary or dental doctorate. In all cases, you will need further experience as a post-doc.

College isn't just about learning the basic science that you'll need in graduate school. In fact, the most important general skills you'll need in science, and nearly all the other interesting careers you might pursue, involve thinking rationally and being able to effectively communicate your thoughts in speaking and writing. For this, a liberal arts education is perfect. Even if you choose a more focused undergraduate program (*e.g.* engineering), I strongly advise taking courses from diverse disciplines. If you are going to be an active participant in a democratic society you will need to have a decent knowledge of history, politics, and economics. Further, exposure to literature, music, and art will help you to appreciate and enjoy life's myriad activities. Literature will teach you the most about human nature, which you will use on a daily basis in and out of the lab.

Unless you become a professor at a small liberal arts college yourself<sup>49</sup>, college is likely to be the only time in your life to live in a truly academic environment. To attend talks on crazy subjects that turn out to be fascinating. To have great libraries a short walk away. To have easy and inexpensive access to music, art, and theatre. To have professors and friends who are passionately engaged in subjects that are alien to you. To argue with your friends about what makes a valuable life. Once you enter a profession, your intellectual horizons will narrow considerably. Once you have a family, your worries will be center on them. Enjoy your intellectual freedom!

Assuming the finances can be arranged, I would strongly recommend one of the many small colleges in the USA where the faculty is devoted to teaching. Most students would profit by attending one of the more selective schools, since on average the faculty and students will provide a more challenging intellectual atmosphere and raise your expectations. Indeed, the small elite liberal arts schools in the USA provide a disproportionate share of PhD students. Since the outstanding faculty run productive research labs staffed by undergraduates, they provide a perfect mix of research experience with dedicated teaching.

Due to finances and other factors, most students will attend larger public universities. These can provide a first-rate science education, but students will need more discipline in both their social life (it is all too easy to get overly involved in the party culture, which tends to dominate the social scene) and in academics. Lectures will typically be large and perhaps even taught by teaching assistants of variable quality. Still, for an intellectually aggressive student, these schools can offer outstanding research opportunities. Students will likely have to seek out professors by asking questions during their office hours and volunteering to work in their laboratories. Large universities will have many laboratories to potentially join, but try to find one where there will be decent training, which will typically be provided graduate students or post-docs working in the laboratory.

All things being equal, it is best to attend an elite college or university and work in a world-class lab. You will graduate brimming with confidence along with a thorough and practical experimental knowledge of science imparted by your courses and independent research experience. Your research advisor will write a strong recommendation letter that will get the attention of the graduate programs you apply to.

But all things are never equal. You might not be able to afford to attend an elite university<sup>50</sup>. You might not be able to get in. Critically, what's important in your college education is what you learn, not the name of the institution that issues your degree. Even if the course work is sub-par, nothing prevents you from reading on your own. With proper motivation, you can teach yourself almost anything. Professors are generally delighted to help students so keen for knowledge that they read above and beyond that standard material. An essential ingredient for success is ambition, drive, and enthusiasm.

In fact, I am a bit wary of graduates of elite universities, since they are often freighted with attitude issues. These include, “a what can you do for me” mentality, and a focus on obtaining high profile findings rather than a deep curiosity of how things work. Many of the best future scientists have an independent streak that results in a checkered academic record. They simply

won't do the work if they don't like the subject or respect the professor. This will basically exclude them from MD-PhD programs (and also from elite colleges, where the competition is equally intense), which focus on students with the very best grades. In perfectly matching the expectations of their elders, such students often lack creativity and the independence to challenge authority. At the end of the day, good scientists can come from any educational background.

What science do you need to learn as an undergraduate? First and foremost, you need a solid foundation in chemistry and biochemistry. You will need two years of chemistry (inorganic and organic) to understand pH, buffers (which you will use every day), and the basic principles of the chemical reactions that govern life. Biochemistry (which may be folded into molecular cell biology courses) provides the essential basic knowledge of biological molecules and their participation in the enzymatic pathways that create life. In addition, I strongly recommend that you take at least the first semester of physical chemistry to deepen your understanding of thermodynamics, essential for getting a feel for how biological molecules interact, and chemical kinetics: crucial to understand the concept of rates and steady states<sup>51</sup>. If at all possible, take courses in cell biology, classical genetics, and molecular genetics, topics you'll need to have a thorough knowledge of before beginning your lab work in earnest.

For students who won't be attending medical or veterinary schools, courses in physiology, anatomy, histology or pathology would be extremely useful for getting the big picture of how humans and other animals function. In the current funding environment, where every project must demonstrate immediate clinical relevance, some basic understanding of human biology is both practical as well as a useful introduction to true systems biology.

Ideally you will get hands-on independent research experience as an undergraduate. As I mentioned in part I, I am skeptical about the value of course associated laboratories, where there is usually a pre-ordained answer arrived at by following cookbook protocols. Although some basic skills may be acquired (pipetting, dilutions, running gels, thinking about buffers *etc.*), didactic practical labs give the wrong idea about how science is performed and rewarded. I encourage you to pursue an independent project in an established lab and do an experimental based honors thesis if this is possible at your school.

First and foremost, this experience will tell you whether you actually like doing research and whether you have a knack for it. Given some guidance to get going, can you understand what the project is about? Can you develop ideas for experiments? Can you design experiments to test the ideas? Can you perform experiments in a reproducible manner? Can you analyze the data? Do you love the whole process of designing, performing, and interpreting experiments? If so,

getting a PhD is the next step.

## CHAPTER 3

### *Becoming a Life Scientist: Graduate School*

**O**K, you are pretty sure that you love science to the point where you can't live without doing it. If your parents happened to be a Walmart heir, you'd become a scientist for fun. So, a PhD it is.

The good news about getting a biomedical PhD is that it should be tuition free, and even pay you to be a student. For students in the USA, this is very good news, since a professional degree in law, business, or various medical fields will likely set you back several hundred thousand dollars. The flip side is that you will have to earn the stipend by teaching and/or performing research at world-class level. This will typically entail 6 years of 60+ hour weeks with minimal vacations. The worse news is that even if you are the best PhD student the department has ever produced, you will still have to be a post-doc for 3 to 5 years before you qualify for a true entry level position (Assistant Professor). Post-docs are paid a fraction of true value to society—in the USA an average about \$43,000 per year<sup>52</sup>, and they are rarely accorded employee status. Post-docs almost always receive health insurance, but hardly ever social security or 401K contributions. So, on average, you will be 37 years old before you put any money away for your future.

But you are still gung-ho, right? OK, two intimately related decisions, which school, and what discipline. We'll deal with the second question first. If you are passionately interested in a topic, say, immunity to HIV, then this will dictate which programs you apply to and figure out what to study after you arrive and settle in. In general, it is best to go to the best program that will accept you. Better programs will on average have better professors who can raise more money for their research so your discoveries will be limited by your smarts and efforts rather than your resources, *i.e.* the ideal situation.

If you are like me, you might not have a passionate interest at this stage in your career. Anything might do. It's all science, after all. And you may be more interested in an approach than a topic. For example, you like cell biology, which

basically means you can choose any field. Or proteins. Or nucleic acids, whatever. What's important then is to find a good mentor. In fact, even if you subject driven, you should still focus more on finding a good mentor. Despite what you might think, science is a craft, and you want to learn from a master craftsman. Good scientists and mentors tend to congregate at the best graduate schools. They also tend to treat their students as colleagues, and not employees/slaves.<sup>53</sup>

## The 12 types of graduate students

<b>EAGER BEAVER</b>	<b>PARTY ANIMAL</b>	<b>STONER</b>
 Science is cool! I want a Nature paper!	 Dude! Let's get beers while this gel runs	 I'm synthesizing a highly active THC isomer
<b>PLAYBOY</b>  Knows all the female grad students well	<b>NERD</b>  The project they are working on is the most important research project in the university — or so they think	<b>UNDECIDED</b>  I don't really know what I want to do. Grad school sounds better than working...
<b>MED STUDENT WANNABE</b>  I didn't get into med school but I'll show them by curing cancer!	<b>THE QUIET ONE</b>  Don't see them much Don't know what they do...	<b>MARRIED</b>  Doesn't socialize much. Works 9-5.
<b>FOREIGN STUDENT</b>  America is a strange and wonderful place	<b>CAREER-MINDED</b>  The future is mine!	<b>MARRIED-SWINGER</b>  Very social... perhaps too social

The National Institutes of Research on Like Bad Stuff that Happens to People

But be careful. Hopefully, you'll have a choice of graduate schools. When you visit<sup>54</sup>, be sure to talk to as many of the PhD students that you can. Things to find out from them:

What is the cost of living? Can you afford to live there on the stipend offered?

How much course work will there be before you start on your research project?<sup>55</sup>

What are the teaching responsibilities?

What is the average time to PhD?

Who are the best professors to work with—not necessarily the professors who publish the best papers (this you should find out yourself anyway by reading them), but who are the best mentors.

What happens to the graduates of the department? What percent do post-docs? Go to industry? Become Assistant Professors vs. taxi drivers?

Most of all—is everyone miserable<sup>56</sup>?

Although every PhD program is different, in terms of required course work, teaching, and lab rotations, my advice is to start your thesis work as soon as possible. The goal is to finish a good thesis as quickly as possible without sacrificing quality or depth. Why quickly? Because the process of educating young scientists has become obscenely prolonged. The average PhD begins their tenure track position at age 37 and receives their first major NIH research grant (the treasured R01) at age 43! This is not mandatory, however, and it is possible to traverse the entire pipeline in eight or nine years, and start earning a real paycheck at 30 or so.

This will require not taking time off between college graduation and graduate school. Post-college gap years are increasingly popular. From my own children, I know that they can be extremely rewarding. But, several clocks are ticking. Starting a family will be easier with a real paycheck and also will benefit from youthful energy. You will be best at the bench in your 30s, and it would better to be working for yourself than working as a post-doc for someone like me.

Obviously, the sooner you can start your thesis work the sooner you can finish. Don't waste time on courses or rotations if you don't need them and aren't forced to take them<sup>57</sup>. You can't be in a hurry when choosing a lab, however.

Choosing which PI to work in is like choosing which graduate school to attend, only more important. Your experience in grad school will depend which lab you join more than anything else. Obviously, the lab should work on

something you are or can get interested in. And the lab members should be generally happy with the mentor and with each other. There should be an open spirit and regular lab meetings.

Lab meetings are important for many reasons. They provide the major forum for communicating the approaches and findings of the laboratory. They provide a major opportunity for the PI to impart lessons on the field of study, the methods used, and the philosophy of science. Not the least, they will provide you with most of your opportunities for learning how to communicate your science with the outside world. They also provide the first public feedback on your work.

Although it will be difficult at this stage in your career<sup>58</sup>, you should try to find a PI whose personality and approach to science mesh with yours. This might be a younger scientist. Newly minted Assistant Professors can provide the very best experience for a number of reasons. First, they should be highly enthusiastic, energetic, and idealistic<sup>59</sup>. Second, ideally, they will be working at the bench alongside you, so you will be learning at arm's length from a world's expert<sup>60</sup>. Third, since they have a hive with only a few worker bees, their success depends on your success <sup>61</sup>.

Of course, there are risks and drawbacks with working with a younger scientist. They have trained few, if any graduate students, so it will be difficult to judge their mentoring qualities. They might be terrible at training students. Every PI has to learn by making mistakes, and many of the mistakes will involve you. A smaller lab means there is less to learn from the other members and less opportunity for collaboration.

Once you have a lab and a project, go at it like a tiger. To succeed in science there is no substitute for hard work. Making progress in your project will take a sustained serious effort. The relationship between effort and productivity (in millipapers per week; *i.e.* 1000 mP = a first author publication) is not linear in science, it's more like as shown on the graph below. Note that the hours plotted is the time you are actually working and not just the time that your body happens to be in the laboratory while doing other things. Also, note the units for the Y axis: millipapers per week.



At 10 hours per week, you are wasting the time of everyone who is helping you directly or indirectly, so your net contribution to the lab is actually negative. At 20 hours per week, at least you are not hurting the overall effort. With each additional hour spent there is an exponential increase in productivity. This is due to a number of factors. The harder you work, the more you learn and all of your general and specific skills improve. The harder you work, the experiments can be bigger, enabling more replicates, more controls and more adjustments to the protocols. Probably the most important factor, however, is that if you are obsessed with your work, you will naturally work harder. Obsession is an essential factor in having great ideas bubble up from your subconscious, and also in driving you to perform the experiments with the requisite eye for detail. You will note that somewhere after 80 hours per week, output declines. Like everything else in life, there can be too much of a good thing. At 120 hours per week (17 hours per day), we are back to a net negative contribution, as the lab deals with your insanity.

Another reason for hard work: biomedical research is competitive, and if you are working on something interesting or important, it is very likely that others are too. Getting scooped is not a disaster as a graduate student, where the focus should be on acquiring the skills and knowledge that you will need for the rest of

your career. As a post-doc however, getting scooped by another lab can greatly impact your job opportunities.

If you are put off by the effort required, you need to rethink bench research as a career. The hours should not be an issue if you love science. Where else would you rather be? You really should feel fortunate that you are actually paid to do something you love.

Enthusiasm is essential for success and happiness. As quickly as possible you need to own the project. Within a year or two you should know as much, if not more about the project than your mentor. Why? First, on average you should be just as smart as they are. Where do you think mentors come from anyway? You and your classmates! While you don't have as much knowledge as your mentor, knowledge can be an encumbrance that limits vision and smothers new ideas. You can learn quicker than your PI too, since your memory is better and your mind is nimbler.

Second, while your mentors typically have many other projects and collaborations and other responsibilities (teaching, administration, family) you have this one project and nothing else to expend your brains' ATP supply.

So, you need to:

Learn the history and background of the question you are investigating.

Know all the recent literature on your topic.

Know all of the methods you are using, not just the how, but also the why (more about this below).

Analyze your data in depth and recognize the flaws in your logic and limitations in your conclusions.

Come up with ideas to mitigate these problems and drive the project forward.

For a few lucky students, this will come easily and quickly. For some, it will never come, and these students will need to seriously consider non-bench related careers. But you will need to give yourself some time to see your potential. You might also need your mentor's opinion on the matter, since like the rest of humanity you are likely to be overconfident or under confident.

It is much, much better to be overconfident in science. It may even be required to be successful. It is difficult to challenge dogma or break new trails, and it helps enormously if you have unrealistic expectations for success. Accomplishing great things typically requires great ambition, which is typically accompanied by overconfidence.

Under confidence is a career killer. At the very least, you have to have sufficient confidence to believe your own findings. If not, you are just wasting your time doing experiments. Under confidence limits your aspirations and your capacity to impress others. In a perfect world, it wouldn't be necessary to

impress others. But by now you should know that we don't live in a perfect world<sup>[62](#)</sup>. To get your ideas across, your papers accepted, and your grants funded you will have to make others believe in you. This is only possible if you believe in you!

Whatever your confidence levels, during the middle to latter stages of your PhD, you need to have serious discussions with your PI about your strengths and weaknesses in the lab and your potential as a scientist. If things are not working out, you need to start thinking about non-bench based careers. While these may still be termed "alternative" careers, this is a complete misnomer, since only a small minority of PhD students stay on the academic track all the way to starting their own labs.

Indeed, the really good news about doing a biomedical PhD is that you will be excellently trained for many high paying, interesting non-bench, or even non-science jobs. In the information based economy we live in, the skills you acquire during your PhD: critical thinking, data analysis, written and oral communication, working in a team, can be applied across multiple careers. Further, most of these jobs will offer better pay and job security than academic jobs.

So even during the bleakest periods of your PhD, when nothing is working and things seem hopeless, relax. Better times are ahead, and if they don't involve bench research, someone will be quite happy to have your well-trained mind join their organization before you are driving for Uber.

Finally, some specific advice for MD-PhD students. Thirty years ago, it was possible to finish both degrees in less than 6 years. But everything was easier back then, not the least being publishing papers, which required maybe 10% of the effort of today's papers <sup>[63](#)</sup>. Today, 8 years is about average for MD-PhDs. If you do a full residency and fellowship, we are talking about 5 or 6 more years, and even more if you throw in a basic science post-doc. Here's a frightening number: 45. This is the average age of MD-PhDs when they obtain their first NIH R01 grant! If you had gone straight to Wall Street after college, you could be settling comfortably into your second retirement career at this point.

So, time is of the essence in your graduate school training, and every point that I made above about getting going ASAP counts double for you. Most importantly, try to decide as quickly as possible where your passion truly lies. It is difficult enough to succeed at one career, and while not impossible, succeeding at both medicine and science, will exact a serious toll on your personal life. There are only 24 hours in a day, and to have some semblance of a normal life, you need to decide where your passion is and focus on that. As a

MD-PhD student, you have typically succeeded at every academic task at hand and have not dealt with serious failure. But since succeeding at science or medicine requires a different skill set from acing tests, you may well be deficient in some essential skill for one or the other path.

If you choose basic research, you don't necessarily need any further medical training. Your medical education is not wasted in any sense. You now have excellent knowledge of how an organism actually works, which in other words, is real systems biology. You know what diseases are, and which ones affect the most people. You have a good idea of how to think through a clinical puzzle, which will put you in good stead when your family, friends and colleagues have a medical issue that is not being effectively dealt with. You have the emotional intelligence that comes from working with a wide variety of individual (doctors, nurses, orderlies, patients and their close ones) under highly stressful conditions. You have a set of excellent friends whose bonds were permanently forged in the crucible that is medical training. These friends will provide a lifelong network of expert advice as they fan out into myriad medical specialties after life. You don't have the PhD chip on your shoulder for not being a real doctor. Speaking of which, your mother can refer to you as my daughter/son the Doctor, while experiencing maximal kvell.

If you want to be accepted your lab as an equal as a student, you will need to avoid having the MD attitude, *viz.* that you are the special one, whose work is the most important in the lab, and that you are above performing the more menial tasks that enable a lab to run smoothly. Indeed, if you make a special effort to do more than your fair share in common lab tasks your lab mates will cherish you as an atypical MD. You also can't expect that your PhD will require anything less than the standard for single degree students.

By the same token, if you decide on a basic science career as a medical student, you no longer have to be a good little boy or girl on the wards. If you are not worried about getting that desired residency, the system has lost all its power over to control you. You can then focus on learning on what you need or want to learn, and not waste a molecule of ATP on pleasing those who are evaluating you. The liberating effect of this freedom is amazing, trust me.

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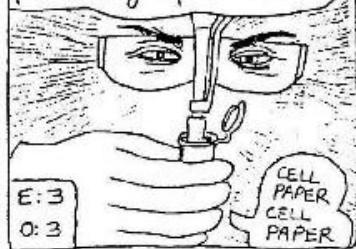
THE EXPERIMENTAL LIFE CYCLE

Advisor tells Post-Doc about  
project that could lead to a  
CELL paper



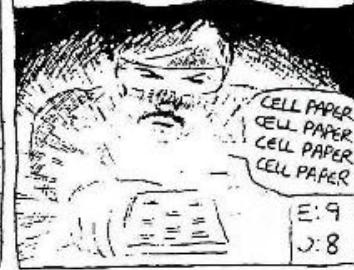
Excitement level (E): 1  
Optimism level (O): 0

Post Doc happily prepares  
reagents and starts  
preliminary experiments



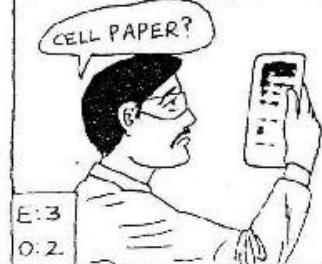
E: 3  
O: 3

Dent  
Early results suggest  
possible favorable outcome



E: 9  
O: 8

However, further experiments  
don't support the initial results



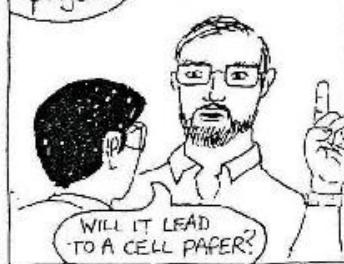
E: 3  
O: 2

Post Doc realizes critical  
control is missing...



E: 0  
O: 0

Project Crashes. Post Doc  
goes to advisor for new  
project



## CHAPTER 4

### *Doing Experiments: Getting Started*

**O**K, you are now settled in a good lab and ready to make discoveries pursuing your PhD. Before getting into the nitty-gritty, let's celebrate a milestone. One of the most important in your life, in fact, if rarely recognized. At this very moment, you are transitioning from *learning from* what is known to *contributing to* what is known. In other words, having spent your whole life being sheltered from the terrors of nature by the wall of the scientific knowledge, you are now ready to repair the wall<sup>64</sup> and even add to it.

You will need something to work on. As a PhD student, it is likely that your PhD advisor project will suggest/assign your project<sup>65</sup>. Hopefully, you have chosen a lab that pursues basic research. This will provide the best environment for you to learn how to do science. The key to earn ordination as a Priest of Scientific Methodism, is learning to design, perform and interpret experiments. This must be done at the altar of the bench, not in an armchair.

Unfortunately, many students wind up in labs doing translational research<sup>66</sup>. Often this choice is deliberately made by the student, who with the very best of intentions, wants to contributing to curing or preventing some terrible disease (cancer, AIDS, TB etc.), typically one that has felled a family member or friend.

Problem #1: making a contribution that directly contributes to improving medicine is unlikely in an entire career, much less a PhD. It is extremely difficult to find something a drug/treatment/therapy that works without causing unacceptable side effects. Example, in 2015, 19 truly novel drugs [were approved by the FDA](#)<sup>67</sup>. And, this was the most in 66 years.

Problem #2: you will have little or no latitude in changing the methods, approach, or problem studied. Typically, you have been recruited to work on this one translational project, and that's it. If you make a discovery that shows that the approach is wrong/seriously flawed you will be more likely to be a pariah than a hero.

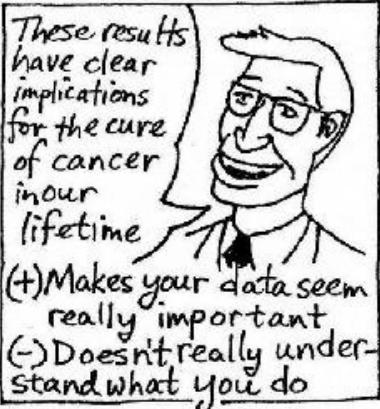
Problem #3: You will essentially be trained to be a technician, not a scientist. You need to have a project where you can think about the designing a new

experiment once you have the data from the last experiment, not just trouble shoot the technical difficulties with the last experiment.

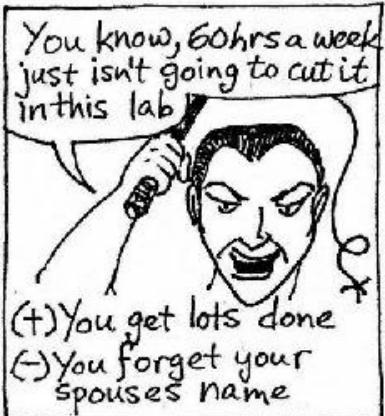
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## THE NINE TYPES OF PRINCIPAL INVESTIGATORS

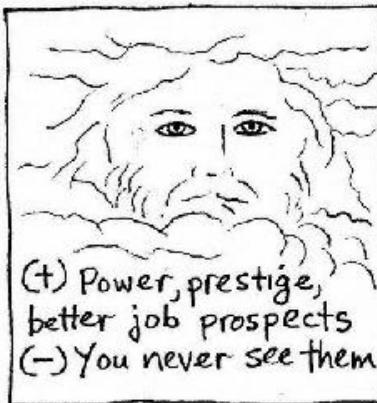
### Big Talker



### Slave Driver



### Demi God



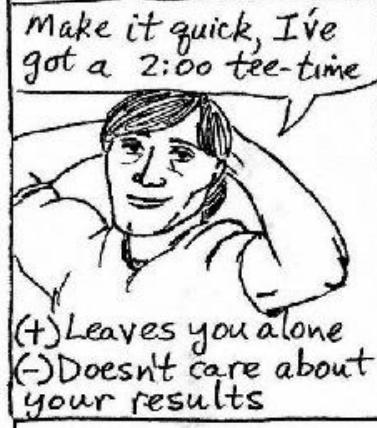
### Control Freak



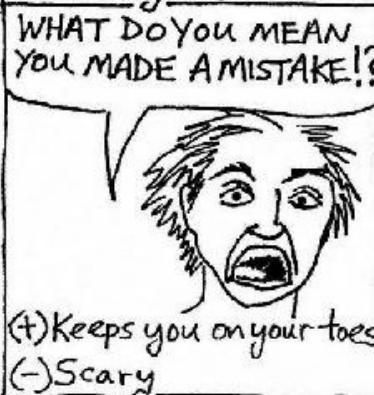
### Science Wonk



### Laid-Back



### Psycho



### Small Town Grocer



### Rising Star



Designing the next experiment is one of the best parts of science, and it involves many skills. One of the most important is devising controls. To the laymen (and many clinicians), controls seems trivial, a book-keeping exercise. Wrong! Controls are what allow you to believe in your interpretation. Nearly every experiment<sup>68</sup> should have two types of controls. Positive controls are needed in case the results are all negative. How do you know *anything* would have worked? Negative controls are needed in case the results are all positive. Maybe *anything* would have worked. To make it clearer I'll have a tangible example.

Let's say we are doing an experiment to see if T cells from a patient infected with influenza recognize a given influenza protein, let's call it NP (for nucleoprotein). The experiment is to determine if T cells will kill cells infected with a recombinant non-influenza virus that is genetically engineered to synthesize NP. The positive control (*i.e.* there should be killing) is cells infected with influenza. This demonstrates that the killers can kill and the target cells can die. The negative control is to take a recombinant virus that expresses something other than an influenza protein (we can't take another influenza protein since this could be also be a target for some of the killer cells). This demonstrates the specificity of the response. The killers might just kill uninfected cells (we'll include this negative control too!). The killers might recognize the recombinant virus we used to express NP (this is interesting, and might lead to a new project, but is not what we are querying with this experiment). Oh, we need some more controls. We need to know that the recombinant NP is actually expressed, so we will stain cells with antibodies to be sure. We need to know that cells infected with the different recombinants are infected to the same extent, so that they really control for each other. This we will do in two ways. We will check with antibodies and we will also make another population of T cells specific for the vector virus.

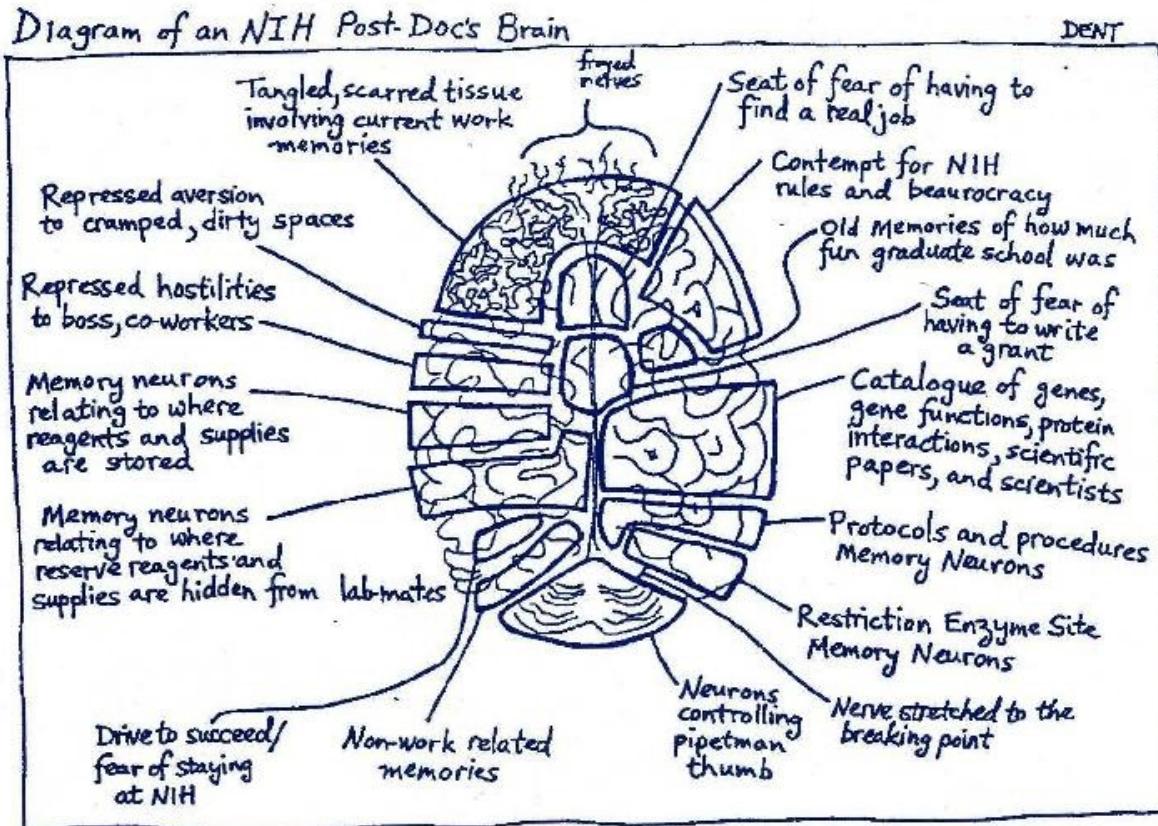
Whew!! The experiment has way more controls than experimental groups. This is called careful science. This is what you are supposed to learn as a student. You have to think about everything that could possible confound your results and design a control for it. If you think this is boring, you should choose another career (maybe medicine?).

Being obsessed with controls is essential to being a good scientist. Don't feel bad if someone else suggests an important control<sup>69</sup>. This happens all the time, even to the very best scientists. If it happens at a meeting when you are giving a talk, it might be a bit embarrassing, particularly if it turns out that your

conclusions are wrong, or even worse, meaningless. If a reviewer of your paper suggests a good control, well, this is what reviews are for. In either case, whoever suggests a good control under whatever circumstances, the appropriate response is gratitude. Your goal in science is to find the truth, anyone who helps you has done you a great favor.

A crucial ingredient to thinking of controls is a thorough<sup>70</sup> understanding of what you are doing. Science is not cooking. But even in cooking, a master chef knows exactly why they do what they do. Attention to and understanding the details are crucial. To further illustrate, we're going to plan a biochemical experiment together.

This is going to take some effort, and likely a higher blood concentration of caffeine, so take a break and meet me in the next chapter. In the meantime, here's a preview of your brain if you make to the post-doc stage.



## CHAPTER 5

### *Doing Experiments: Details, Details, Details*

**W**arning! Warning! I know that this chapter will be rough sledding for many readers who lack extensive lab experience, but don't worry about getting it all. My goal here is to show you how much knowledge you will need to become an experimentalist adept at rationally modifying existing methods and devising new ones. If at some point, if it is too painful to slog through, just skip it, and maybe try again in 6 months.

OK, let's begin. To illustrate the importance of understanding the exact methods used in an experiment, we are going to study the biogenesis of influenza hemagglutinin (HA) by biochemical analysis exploiting the remarkable ability of monoclonal antibodies to distinguish fine details in protein structures<sup>[71](#)</sup>. Our overall strategy is to use the antibodies to recover forms of the HA whose properties we will analyze in gel electrophoresis.

HA is inserted into the endoplasmic reticulum as it is translated. Once translation is completed, HA is exported to the Golgi Complex, where it traverses the stacks of the cis, medial and trans Golgi (each with unique enzymes that modify HA as it moves outward), and then ultimately to the cell surface, where if other flu proteins are present, it is incorporated into progeny viruses that are released from the cell.

The first thing we need to do is to express the HA in our cell type of choice. For this we will just<sup>[72](#)</sup> infect the cells with flu virus itself. Now we have to choose a time after infection to study. This is our first complication. Ideally, we could study every time after infection, since the rules of making HA may change as the infectious cycle proceeds. But we can't do this, for the obvious reason that we only have two hands, and must tailor the experiment to what a person can actually do in a given period of time. One of the first principles of experimentation is to keep experiments to a manageable size. Experiments that are too big will be done poorly and are likely to produce gibberish. Experiments that are too small won't provide enough data, and worse, won't have the proper

controls. What's manageable is personal, will increase with your general level of experience with a method, and also within the confines of a specific set of experiments.

OK, to get an idea of where to start, we will do a pre-experiment to determine the kinetics of HA synthesis. This should be something relatively quick and easy<sup>73</sup>. Let's say you already know the basics of flow cytometry<sup>74</sup>. We can start then by infecting the cells for various times and measuring expression of HA on the surface of viable cells. This will only take a day to perform and analyze. Plus, if we include the antibodies we are going to use for the biochemical experiment, we will already have an important positive control that the antibodies work<sup>75</sup>. Depending on your skill level, we might also be able to investigate the effect of virus dose on HA expression kinetics, so we can choose an appropriate dose for the biochemistry experiment. On the other hand, we can do this in a second preliminary experiment, once we know the approximate time window to test.

But wait a minute. What cells are we using? Ideally, we'd use the cells that flu evolved to infect<sup>76</sup>. This is generally not possible with human or animal viruses, since biochemical analysis is virtually impossible to do on the real tissues that support viral infections<sup>77</sup>. We have to use cultured cells as a model system. Typically, there isn't a large choice of cell lines. The lab (and field of study), will have selected a standard cell type (or maybe a few). The development of cultured cells was a key advance in modern biology, but there are limitations you should always be aware of. Since you are likely to spend much of your career working on cultured cells, it is worth a few paragraphs of discussion.

Cells did not originally evolve to grow on plastic flasks in a monolayer in synthetic media<sup>78</sup>. Most cell lines exhibit enormous changes in their genomes, with a large variation in chromosome number per cell, with consequent imbalances in gene dosage and expression that vary between the cells. Many cell lines used were derived from cancers; in this case many/most of the genetic changes occurred in the unfortunate donor. HeLa cells, probably the most extensively used human cell line, have chromosomes so damaged they are referred to as shattered. Indeed, most cultured cells are aneuploid, *i.e.* they exhibit a statistical distribution of chromosomes, with resulting imbalances in gene expression that vary among the cell population.

Biologists propagate cells in air supplemented with CO<sub>2</sub> as a convenient way to buffer the media. But air has 21% oxygen, a toxic concentration for mammalian cells, which never see more than 10% or so (arterial blood) and

more typically 5% or less, in tissues (even less in pathological tissues like tumors or reactive immune tissues). Cultured cells have to adapt to deal with increased levels of reactive oxygen species, which are highly damaging. They also have to adapt to synthetic media, which is very different from their natural media<sup>79</sup>. Synthetic medium is typically insufficient to maintain growth, so must be supplemented by serum, typically from fetal cows, adding batch-to-batch variation to the list of variables in cell culturing.

Cultured cells are constantly changing <sup>80</sup> and are typically the most variable part of an experiment. You should be obsessive in your culture technique, adhering to a split schedule that prevents overgrowth. Your sterile technique should be good enough that supplementing media with antibiotics is not needed<sup>81</sup>. You should have a good feel for how cells should look when they are “happy”. Particularly at the start of your career, you should look at the cells every day, to start your visual memory bank for cell phenotypes. If you don’t already know, immediately learn how to optimize the phase contrast settings on the tissue culture inverted microscope so you can maximize the information the scope is providing. Cells are incredibly beautiful machines, and looking at them with a good quality microscope still thrills me<sup>82</sup>.

Where were we? Oh yes...HA biogenesis. Our preliminary experiments revealed that we should infect cells with a certain dose for a certain time. OK, the idea is that we label cells for a brief period with a radioactive version of methionine (Met), one of the standard 20 amino acids cells use to make proteins. Why Met? For two reasons. First, cells can’t make it, so they load it more efficiently, which means we can label cells for a shorter time. This is important if we want to study early events in HA biogenesis. The ribosome will finish each copy that it is making in less than 2 min (~6 amino acids per second, 528 residues to synthesize the entire protein), so if we want to study early events, we need to label at their time scale of occurrence<sup>83</sup>.

Second, we need to detect HA in a SDS polyacrylamide gel (more about these later) either by an X-ray film or a machine that visualizes radioactivity in the gel, so we need to label HA with a radioactive isotope that emits high energy electrons. For the standard amino acids, this is only sulfur 35, which limits our choices to Met or cysteine (Cys). Why not Cys? Cys is not incorporated into proteins as efficiently as Met, since it has less immediate access to the appropriate tRNA<sup>84</sup>.

How to actually label the cells? Most scientists will just follow published methods without giving them much thought. The standard protocol entails incubating cells for 10 min to several hours in media lacking Met, to increase the

amount Met taken up by cells. This is completely unnecessary, since the intracellular Met pool is very rapidly depleted. Worse, Met-starvation changes the cells, which correctly and rapidly sense that they are missing Met employ two remedies. First, they greatly reduce protein synthesis and focus on making only the proteins they need until they get more Met. Second, they start degrading their own expendable proteins to recycle into free amino acids (all for the Met) into proteins they immediately need<sup>85</sup>. So, if the point is to study HA biogenesis under “normal” circumstances<sup>86</sup>, the one thoughtless step of starvation has compromised the whole experiment.

Next, we are going to “chase” the cells: wash off the label<sup>87</sup>, and add excess unlabeled Met so that we can biochemically follow the pulse labeled material. At various times, we put the cells on ice to stop their metabolic activity and after one final wash, we can proceed. Here we have a choice. If its lateish in the day (most young scientists work well into the evening and often the night), or you don’t have time to continue, we can flash freeze the cells and then continue tomorrow, or later, since the cells will be fine at -80°C, the standard storage temperature for sensitive biological material<sup>88</sup>.

Now, for the tricky part. How do we release HA from the cells? We have to break the cells open. We can do this physically, by basically smashing the cells into glass beads squeezing them very hard, but this won’t release the HA, which is inserted into the various membranes of the cells. So, we have to use a detergent (just like the ones used for home cleaning), to dissolve the membrane and release the proteins. But which detergent, as there are dozens we might use? And it would be a lot of work to test them all. Our basic decision is to weigh the efficiency of extracting the HA from the cell vs. maintaining HA structure. We’ll have to err on the side structure, since this is the whole point of the experiment. So, we’ll chose Triton X100, (TX100) a mild detergent typically used for this type of experiment. We might comfort ourselves that losing HA by insufficient TX100 extraction will be constant throughout the maturation process. This is a reasonable assumption, and people made it for decades.

Like many reasonable assumptions, it turns out to be wrong. The detergent solubility of HA changes as it moves towards the cell surface, becoming more difficult to extract with TX100. This is one of many reasons why the experiment will not be perfect, but then again, experiments are never perfect, and experimental science is the art of the possible. To make discoveries you have to do experiments, and waiting for the perfect experiment will simply mean you don’t perform any experiments. It is, of course, critical to strive to understand as many of the imperfections, as possible but you cannot let this paralyze you. DO

## EXPERIMENTS!!

Sorry for that. It's just that we all know very smart people who excel at criticism<sup>89</sup>, and who will tell you excellent reasons why this or that won't/cant' work. Ignore them, they will never make a discovery. Yes, at the end of the day, you won't have proven anything. This could be wrong, that could be wrong, etc. This is what science is. Not only do you have to live with ambiguity, you ought to find it to be one of the most compelling aspects of science. Since we never know the real truth, we can never stop thinking. And thinking is what makes science fun.

For now, we will stick with TX100<sup>90</sup> to release HA. OK, what else is in the extraction buffer? Buffer is a clue, since biological molecules evolved to be stable in a narrow pH range, so we need a chemical that buffers around pH 7. There are many choices. Phosphate is typically used to buffer cells, but [freezing lowers its pH<sup>91</sup>](#), so is generally avoided in favor of Tris. Biological molecules evolved in salty solutions, so it's generally a good idea to include sodium (or potassium<sup>92</sup>) chloride at 150 mM, the osmolality of most cells and body fluids. What else?

Cells are filled with proteases, which can destroy proteins, particularly when the proteins are not fully folded, oligomerized, or otherwise assembled. Proteases include lysosomal proteases, released by the detergent, and also proteasomes, the major cellular protease<sup>93</sup>. So, we will include a cocktail of protease inhibitors, including a proteasome inhibitor, serine protease inhibitor, and a chemical (EDTA) that tightly binds divalent cations, which metalloproteases require for activity<sup>94</sup>. If we include a chemical (N-ethyl maleamide) that modifies free sulfurs we can kill three birds with one stone<sup>95</sup>, since this will prevent spurious disulfide from forming post-extraction and disulfide bond rearrangement (both of which happen to HA, so we there is a method to the madness), and will also inactivate ubiquitin hydrolases, which remove ubiquitin molecules from proteins<sup>96</sup>.

Now we have half a dozen tubes containing HA at various stages in its journey to the cell surface (0 min ('pulse'), 5, 10, 20, 40, 80 min chases). To compare between samples, it's important to be sure to have the same amount of cellular extract in each tube. HA may be degraded at some point, and if recover less we'd need to be sure it isn't because we lost cells or extract along the way somewhere. There are several ways to control for this. The best way is to normalize the samples before we start working with them. In principle, this is easy: we can take a small amount of the sample and put it in a machine that counts the radioactivity. If we are really obsessive<sup>97</sup>, we can make sure that the

radioactivity is in protein and not free methionine (or some other molecule that has managed to acquire the radioactive sulfur through cellular metabolism) by acid precipitating the proteins and counting them. But there is a problem with this. Cells degrade 20-30% of their new proteins within 10 to 30 min of their synthesis, and this can vary to the point where we can't confidently account for it. Instead, then, we can perform a standard protein assay, which will give us an accurate reading of the amount of cellular material, if we have washed the cells well enough before the extraction. The chase media is loaded with protein (~ 4 mg/ml) from the 10% fetal bovine serum present, and will skew the result if not completely removed.

With normalized extracts, we are ready to do the actual experiment: using different monoclonal antibodies to collect HA from the extracts. This is typically, and incorrectly, referred to as immunoprecipitation (IP), which correctly describes the forerunner of this technique. A century ago, immunologists discovered that Abs would literally precipitate (*i.e.* fall out of solution) when added at the “equivalent” point to antigen. The chemical basis for this phenomenon still defies description, but it proved to be a useful method for purifying Ab-Ag complexes, and formed the basis for a simple, beautiful, and relatively powerful method termed immuno-diffusion when performed in simple agarose gels<sup>98</sup>. In primordial IP, the goal was to find the equivalence point and then collect the precipitate for further analysis. This was painful to perform<sup>99</sup>, and not very quantitative.

A major advance came with the discovery of protein A, a *Staphylococcus aureus* protein that binds to the constant region of antibodies. At this time, it cleverly recognized that, quite conveniently, staph expresses protein A in large amounts on its cell surface<sup>100</sup>. If you grew up 20-liter batch of staph and killed the bugs (which can give you a very nasty infection, so you had to be careful, at least the relatively lax standards of the day), you could use them to display antibodies on their surface (or to purify the antibodies, since the antibodies will elute at pH that does not damage them)<sup>101</sup>. With eventual the development of recombinant technology, protein A (and other bacterial natural or engineered proteins with similar but broader activities, making them even useful<sup>102</sup>) could be produced relatively inexpensively and coupled to synthetic beads, which led to much lower “background binding”, *i.e.* sticking of proteins to the beads and not the antibody.

Rather than precipitating the HA, then, we will immunocollect it<sup>103</sup>, with monoclonal antibodies that we have already bound to the beads. Binding the monoclonal antibodies has one disadvantage typically outweighed by an

advantage. The disadvantage is that the Abs are not freely diffusing, so they may have limited access to the HA when they are bead-bound. Immobilizing them might also change their binding properties in some way, since antibodies normally function as free proteins. The (big) advantage is that we don't have to worry about recovering all of the HA in the extract, due either to having too much antibody present for the beads to completely capture it<sup>104</sup>, or the presence of antibody that can bind HA but for whatever reason, not the protein A beads. Speaking of which, how much antibody per bead do we have to add?

Enough to recover all of the HA. Which poses a multidimensional max-min problem. The basic variables: amount of HA vs. the amount of antibody. We are aiming to use as little as possible without compromising the experiment. The longer we infect the cells, the more unlabeled HA there will be to compete with the labelled HA, meaning we will have to use more of the antibody bead mixture. Ideally, we can choose a time after infection when the rate of HA synthesis is high, but early enough so that the amount of unlabeled HA is relatively low. This will also minimize the amount of [<sup>35</sup>S]-Met and antibody we have to use. [<sup>35</sup>S]-Met is relatively expensive and monoclonal antibodies can either be almost free (if you have a cell line that makes the monoclonal antibody<sup>105</sup>), or very expensive (if you have to buy it from a commercial vendor<sup>106</sup>). More factors, the number of cells needed per time point, which will depend on how much HA is synthesized per cell, how many Met residues HA has<sup>107</sup>, and how quickly you would like to see the results<sup>108</sup>

Did someone mention money? As a PI, I am glad to have the opportunity to raise the issue of cost. Every lab has finite resources. As biological techniques have grown in power, so too their cost has increased enormously. It behooves you as a member of a lab to minimize expense without compromising the science too severely. This means designing experiments to use the minimal amount of expensive materials without sacrificing the controls. This typically entails performing a series of typically modest pre-experiments to optimize the amounts of precious materials that will be used in the main experiments. On the other hand, saving money on reagents can incur opportunity costs in the form of missed discoveries. The goal is to optimally balance minimizing cost and maximizing productivity.

We are making progress. Which monoclonal antibodies should we use? Hopefully, this will be obvious based on the prior work you have done. For example, you have a number of HA specific antibodies that give different patterns of staining of the various compartments of the infected cell, when you perform immunofluorescence. In this case, we might choose an antibody that

only binds HA in the ER, one that binds in the Golgi Complex and cell surface, and one that only binds HA in lysosomes. Again, the experiment should be designed to extract maximal information (more Abs = more chance for discovery and also will control for each other if they give different patterns) but without making the experiment so large that you can't do it well.

We also have to think about how we will analyze the sample. If we have 6 time points per monoclonal antibody, and three monoclonal antibodies, that's 18 lanes of a gel. Most gels these days have 15 lanes or less, and particularly when we are starting out, it would be nice to run all of the samples in the same gels, since there can be considerable variation between gels, and we are very interested in small differences in mobility between HA species. So maybe we should only use two antibodies, or maybe three monoclonal antibodies but less time points. I've left out the controls we'll need, but they can be run on a second gel, since these are controls are of the grenade-horseshoe-atomic bomb type (close is good enough).

One last question before we add the monoclonal antibody-loaded beads to the infected cell extracts: what temperature and how long should we incubate. Generally, cold is good. Ab-antigen interactions are typically not terribly sensitive to temperatures between 0 and 37 °C, and to minimize protease activity and protein unfolding, it is generally a good idea to perform the incubations between 0 and 4 °C. But, it's still worth thinking about. One of the monoclonal antibodies we are going to use exhibits the unusual property of binding to fully folded HA only at elevated temperatures, because it recognizes a region of the HA that is only exposed by HA "breathing", which is proportional to the temperature (and time) of incubation. Again, we should keep things simple, and leave the effect of temperature on monoclonal antibody binding for its own experiment.

Ding. The timer has just gone off after the 4-hour incubation (this step is not terribly time sensitive, we could have left it overnight if that's more convenient). I hope you spent the 4 hours doing something useful. Surfing sports- or news-websites doesn't count. Gossiping about lab mates doesn't count either. Going to the gym counts, since it is good for your mental health, but 4 hours working out is too long to spend on a routine basis. Reading papers counts. Talking to your lab mates or PI about results or future experiments counts. Working on a different experiment counts double, but don't try this until you have some experience under your belt. Doing more experiments will be counter-productive if it compromises quality. Experiments must be done carefully enough to believe negative results, *i.e.* the true outcome is negative, and the negative result is not due to careless work. Why negative results? Because typically these won't be

repeated, whereas positive results will be repeated as a necessary step to confirmation and extension.

We have to now wash the samples. Another difficult choice. We want to minimize the non-specific interactions of the labeled proteins with the antibodies, so we might ramp up the strength of the detergent we use or increase the salt concentration. On the other hand, we don't want to miss the chance of discovering that HA binds to a surprising cell protein, so to start, with let's wash with the same solution we used for the IC<sup>109</sup>. This type of wash is based simply on dilution. We add a solution, centrifuge the beads and aspirate the supernatant with a small hypodermic needle attached to a cut syringe at the end of a vacuum tube. We place the needle all the way into the tube to make sure that we remove as much liquid as possible<sup>110</sup>. Sounds easy, but you have to be careful not to suck up any of the beads (though we will control for this, but still, better not to have to do too much fudging), which means that the bevel of the needle has to point AWAY from the beads and squarely face the wall of the tube (we have to bend the needle into an "N" shape to make this easy).

Samples are washed, and now we deliberately do something to the HA we have studiously avoided. We are going to analyze the collected HA based on its molecular weight, which we will do by having HA migrate through a gel based on its electrostatic charge. Since the charge of HA changes as it is modified in the secretory pathway, we have to do something to neutralize this effect. Killing multiple birds, we will use SDS, a very strong detergent that will: 1) denature the protein, converting if from a compact folded structure, into an extended string of amino acids (more or less), so that its shape doesn't affect its migration in the gel 2) surround the protein with negative charges, to minimize the inherent charge differences between different maturation forms of HA.

Taking no prisoners, we will boil the proteins for 5 min. As you should be well aware from its effects on eggs, boiling is Armageddon for most proteins<sup>111</sup>, the thermal energy overwhelms their relatively weak bonds, and they become a string like structure with a high propensity for the exposed hydrophobic residues to aggregate due and fall out of solution. This would be a disaster, since HA and all the other proteins will just sit as a blob at the top of the gel and not even enter. But since we cleverly used SDS, this highly charged molecule will surround the proteins all will keep it in aqueous solution.

I've mentioned gels a few times now, and you've probably used them or at least seen them, but like your phone or TV or any of today's technological marvels, I bet that you have little idea of how they work. You simply order them from any number of vendors and open the foil pack they come in before using

them. Oh, for the bad old days. When men were men<sup>112</sup>, and rolled (errrr...poured) their own. Though simple in principle, PAGE (polyacrylamide gel electrophoresis) took decades to develop. Originally, natural products (like agarose or starch) were used for protein electrophoresis. These substances are charged however, and sensitive to minor fluctuations in temperature. Polyacrylamide is a pure chemical, so not subject to natural fluctuations in composition, and is uncharged. Further, the degree of cross-linking (hence sieving capacity) can be controlled by balancing the two components used to create gel. And it can be poured as a liquid at room temperature, since it only forms gel after a catalyst is added (the time to gelling is controlled by how much catalyst is added). This delayed gelling allows the simply production of “gradient gels”, gels with increasing concentrations from top to bottom which greatly increases the ability to resolve small differences in molecular mass.

In the early days of SDS-PAGE, the gels were hand poured into little tubes that were inserted into a custom tank with a divider that separated the buffers at the top and bottom of the gel. Given their hand made nature, there was considerable variation between tubes to make fine distinctions between samples difficult. Saving lots of time, and greatly improving sample to sample consistency, someone had the bright idea of making a slab gel between two plates that held apart by plastic spacers at the sides and sealed using a silastic tube or by pouring agarose all around the perimeter. The lanes were cleverly but simply created by making a rectangular indentation at the top of the gel by inserting a comb into the gel when it was poured. It sounds trivial, but this represented a major advance that facilitated an enormous number of discoveries. A similar physical gel setup was crucial to nucleic acid sequencing (with other clever chemical tricks), leading to even more discoveries.

One more critical invention to further increase resolution and improve sample to sample reproducibility. The stacking gel. This allows you to fill the sample well with a relatively large volume without losing resolution in the gel. It's fun to watch a stacking gel in progress. To monitor how far the proteins have migrated, you include a blue dye which migrates at the gel front. When the gel starts, the dye is uniformly dispersed in the sample. As it moves through the stacking gel the dye, along with the rest of the sample, becomes concentrated into a very thin line. So, when the samples hit the resolving gel all of the proteins start at the same time<sup>113</sup>.

Don't worry, I won't make you pour your own gels, you can still use the McInstagel. You carefully remove the “sample buffer” from the beads and meticulously load it into the well (it sinks because of the large amount of

glycerol in the buffer). Be careful not to introduce any bubbles or beads into the well! They will make a very ugly streak in your gel, betraying your sloppy technique to your lab mates, PI, and reviewers. Turn the gel on, set the timer, and take another break while the gel runs for an hour or two.

Ding. Ok, time to be very, very careful, you don't want to blow it now, not after all of the work you have done up to this point. And now is the time when disaster can strike in an instant. Carefully separate the plates and gently remove the gel without tearing it. We could dry it right way for analysis, but we are more careful than this, right? We want to be as sure as possible that we have loaded equivalent amounts of sample in each of the lanes. So first, we will stain the gel with a protein staining dye in a solution that fixes the proteins the proteins in the gel (they will diffuse, albeit slowly, if we don't fix them). The protein staining dye allows us to visualize and quantitate the amount of antibody eluted from the beads<sup>114</sup>. Another triple whammy. We not only have a positive control that the antibody actually was bound to the beads, but we can control for sample to sample variation in recovering the sample buffer (and other errors you might have made along the way causing you to lose beads), and also have a way to be sure that you used the right antibodies (antibody light chains often migrate with different apparent molecular weights).

The last lap. To visualize and quantitate the [<sup>35</sup>S]-Met in the gel, we need to vacuum dry it onto a piece of paper. We very carefully lift place it on the paper, and smooth it lovingly, before placing it on the gel drying apparatus, which we have checked to make sure is pulling a strong vacuum<sup>115</sup>.

A few hours later we remove the perfectly dried gel, and visualize the radioactivity using one of two methods (or often both, one after the other). For the best resolution of the bands, we take the gel into the dark room and place gel under an x-ray film in a light-tight metal cassette. As the [<sup>35</sup>S]-Met decays, it emits  $\beta$ -particles that convert the silver ions in the film to metallic silver, which is amplified into a far greater number of silver atoms when the film is developed. These silver atoms are visible as a black precipitate on a clear background. We are in the dark room since light (or X-rays) also causes the silver transformation. A day or two (or more, if we haven't recovered much radioactivity), we return to the dark room to remove the film. Now for the moment of truth. We put the gel in a standard x-ray film developer, the type used in hospitals<sup>116</sup>. Less than a minute later, the film pops out at the other end. If this is a new experiment, my heart is beating quickly in nervous but happy anticipation of an interesting result.

The x-ray film makes a high resolution of the pattern of bands, but it is difficult to determine how much radioactivity is in each band. To precisely

quantitate radioactivity, we use a sophisticated machine, invented less than 20 years ago, that measures ionizing radiation collected on a special plastic screen incubated with the gel<sup>117</sup>. Having struggled for years to obtain accurate measures of radioactivity by x-rays, the introduction of this machine was a milestone: what was difficult and crappy became easy and excellent. Every time we use the machine I smile: you gotta love technology!

Now for the fun part. Sometimes the answer to the question posed by the experiment is obvious. There is one band (*i.e.* a protein species that migrates uniformly through the gel giving a single band). But usually there are many bands, some, likely different forms of the same protein, but other bands that are other proteins that stick to target protein. This is where the thinking occurs. By looking at the pattern of the bands and how they change during the chase, you form a hypothesis about what is happening.

In this case, one monoclonal antibody, the temperature sensitive one, recovers a single band that is most intense at right after the pulse and then decays with the chase, without changing its mobility. The other monoclonal antibody, doesn't recover any species right after the pulse, but recovers a band 5 minutes later, which becomes stronger with time and also changes its mobility to a slower migrating form.

How to explain this? It turns out that the first monoclonal antibody binds the monomeric HA, as it is released from the ribosomes and continues to bind until the HA "oligomerizes", that is binds to two other HA molecules to form a trimer, which is the mature form of the HA. The 2<sup>nd</sup> monoclonal antibody will only bind to HA after it trimerizes, hence the lag in its binding. Further, as the HA moves from the inside of the cell to the outside, it is modified by a number of enzymes that add sugars and lipids that make the HA run slower in the gel, and we can see this nice orderly progression from lighter to heavier forms. A third monoclonal antibody, which binds all forms of the HA, gives a pattern that is the sum of the other two patterns.

To correlate these biochemical findings with intracellular location we can do more biochemistry by isolating various cell organelles (endoplasmic reticulum, Golgi complex, plasma membrane), but there is another technique that is way easier, more definitive, and esthetically spectacular. Here, we infect cells while they are attached to glass, stabilize their proteins and organelles by chemical fixation, and gain access to the inside of the cell by briefly treating with the same detergent we used to extract the HA in the pulse chase experiment. We then add the monoclonal antibodies to the cell and localize their binding by virtue of having labeled the antibody with a fluorescent dye. We then look at the cells in

fluorescent microscope, which allows us to visualize the dye in the cell. This reveals that the monomer specific Ab binds the HA in endoplasmic reticulum but no further in the secretory pathway. The trimer specific Ab binds to HA in the Golgi complex and plasma membrane.

These complementary experiments establish that HA oligomerizes in the Golgi complex. This sounds simple and obvious, just like every discovery does once you work out of the details. It took me about 5 years to figure this part out<sup>[118](#)</sup>. You might be thinking, who cares, why is this important? Good question!

For many reasons. First, if you want to make a vaccine, these findings indicate that full immunogenicity requires HA to trimerize into its native form. So, you can't use short peptides or even full length monomeric protein that you produce in bacteria. Second, prior to this discovery it was generally thought that oligomeric proteins can't leave the ER without assembling there. Since about a third of all proteins made by the cell follow this pathway, our findings suggest that a substantial fraction of cell proteins oligomerize in the Golgi complex. Third, defining this system laid the foundation for other findings from our lab, including the first demonstration that proteins can travel backwards from the Golgi complex to the ER, which changed the way the world thought about the process of intracellular transport.

I think that the conclusions are cool and even important, but I didn't describe the experiment in such excruciating detail (sorry!) to discuss their implications (don't worry if the preceding paragraph is completely opaque, it's a topic for specialists). Rather my goal was to illustrate the amount of knowledge, skill, and attention to detail needed to perform biological experiments. You need to eventually this level (or greater) understanding of each method you use. I have described just one method out of dozens that we routinely use in my lab. And for each method, enormous amounts of incompletely overlapping background knowledge are required to perform the experiment properly and most importantly, to analyze the results. All that schooling may seem like a long grind: but you will eventually use the knowledge that you begin to accrue in high school<sup>[119](#)</sup>!

At some point in your career your friends and family will wonder out loud what exactly it is that you do all day. Science seems so easy from the armchair, reading about discoveries in the popular press. When you tell them that your experiments “aren't working” it sounds trivial. It isn't. It's crucial to convey to laymen (including many MD's doing clinical research) what it actually takes to make a significant discovery. Aside from making you feel better, it is a critical step in improving science as a career. It is all too easy to underpay you when

there is precious little appreciation for what is involved in what you do.

## CHAPTER 6

### *Doing Experiments: Thinking*

**Y**ou've been working on your dissertation research for 6 months now, and I hope that you are beginning to come up with your own ideas.

There is a saying “ideas are cheap”. Bad ideas, yes. Indeed, this phrase is typically uttered by people who never have good ideas, and consequently can't value them<sup>120</sup>. Ideas are everything in science. Though the hard-wired basis for creativity, like every other form of intelligence, is distributed unequally among individuals, it can be enhanced in all of us through software input.

First, is the laboratory environment. Creativity can be squelched in “plantation” labs where members are treated as machines, who merely exist as extensions of the PI's nervous system. Conversely, creativity is contagious in labs with an appropriate combination of freedom and encouragement from the PI. I've never seen a creative lab that wasn't also a happy lab, which benefits from a classic positive feedback loop.

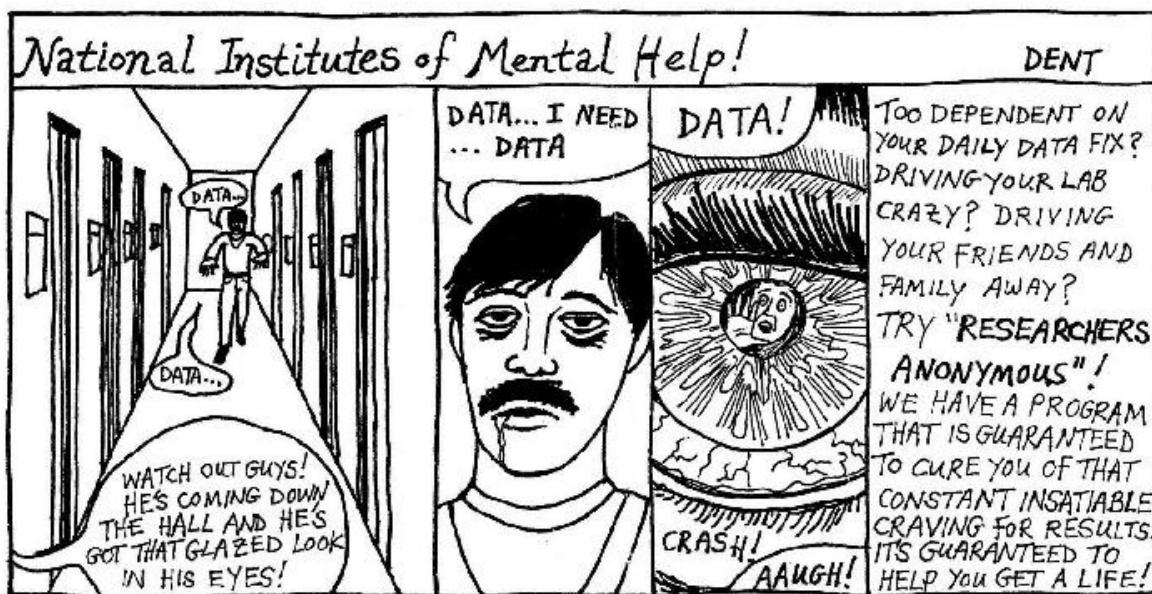
Second, is your knowledge base. Ideas are based on applying new knowledge to a problem. The knowledge often comes from experimental results (DO EXPERIMENTS!!). Knowledge can also come from reading, of course. Textbooks might seem an unlikely place to stir imagination, but even if a finding is 50 years old, if you didn't know it, it can prompt an idea. Even if you think you know a field, it is useful to occasionally take a look at the textbook descriptions<sup>121</sup>.

Your main source of ideas will be research papers, of course. But not just new papers. Reading old papers can be extremely rewarding. Authors had much more freedom to express their styles and ideas, and papers published before 1980 are generally much more readable. Since much less was known in earlier days, discussions tend to consider the big picture rather than minutiae. Papers published before 1970 focus on phenomena, and it can be really fun to read the author's speculations as to mechanism. Some are comically removed from reality, but many are inspiringly perceptive. Further, many of these beautifully described phenomena have never been explored, so if you are in a field with a

long history, there are phenomena just waiting to be worked out with today's amazing tools<sup>122</sup>.

It is essential to read new papers, of course, but I would counsel you not to read them as you have likely been trained in journal clubs. Journal clubs are part of the normal life of graduate students. A group of students (and often post-docs) gather in an informal setting once every few weeks or so to discuss a paper, often with the participation of a faculty member. The point of the journal club is to learn how to critically read (and write) scientific papers. This is a worthy goal, but journal clubs typically turn into a trashing competition as each participant showcases their knowledge and sophisticated insights in over criticizing each experiment.

Rather than focusing on the implications and importance of the findings, participants can easily take home the message that all science is BS. I've met university training program directors who banned journal clubs since it was teaching their students to hate science.



My advice is to read papers selfishly. Don't over worry about validity of the paper's conclusions. It's the authors' job to get things right. Nearly all published experiments from good labs<sup>123</sup> report valid observations. Serious scientists make a tremendous effort to get things right, and almost always do. The experiment trashed in journal club in a few minutes discussion was typically just one of many experiments performed, and represents the net result of hundreds of hours

of labor and thought by the scientist doing the experiment, the PI, and typically many other lab members. The findings were discussed in excruciating detail in multiple lab meeting over several years. Don't carelessly dismiss them!

In any event, even if the conclusions in a paper are incorrect, the idea it sparks might be useful. So, here's how to read papers. Start with title. Interesting? Read the Abstract? Still intrigued? Read the Introduction. Now skip the results. This will save an enormous amount of time, since reading the results with due diligence takes hours. Go right to the Discussion. Hopefully, by now you have an idea, or start of an idea. If so, now you can read the Results to see what the supporting evidence is. You should also take a look at the Methods, since the paper might describe something new that might be useful or an improved (faster or cheaper is also better!) version of what you are doing.

Which papers to read? The luxury journals for sure [124](#). Also, the bread and butter journals in your given field, which publish many outstanding papers in addition to the typical solid papers that report essentially incremental advances. I also recommend that you subscribe to the services that search PubMed and other sources and send you the titles of papers with whatever key words you specify, including individual authors who work in your field. Google Scholar provides a free search for papers that cite a given paper or author. I use this to find papers that cite publications from my lab. This is a wonderful way to find links between your work and new fields and to catalyze unexpected collaboration with the citing authors [125](#).

Though much inferior to reading, seminars are also good for sparking ideas. If you are at a large research center, there might be dozens of seminars each day that you could attend. In addition to the seminars that are obviously related to your work, make an effort to attend seminars that seem to be related (some creativity may be required). You might look at the publications of the author to see if your hunch is right. You might just show up, since you never know what new findings the speaker might present, or who you might meet in audience (many collaborations begin from such random interactions). In case the seminar is completely off target or otherwise terminally boring, you can sit in the back and quietly make your exit, or read some papers you have brought just in case.

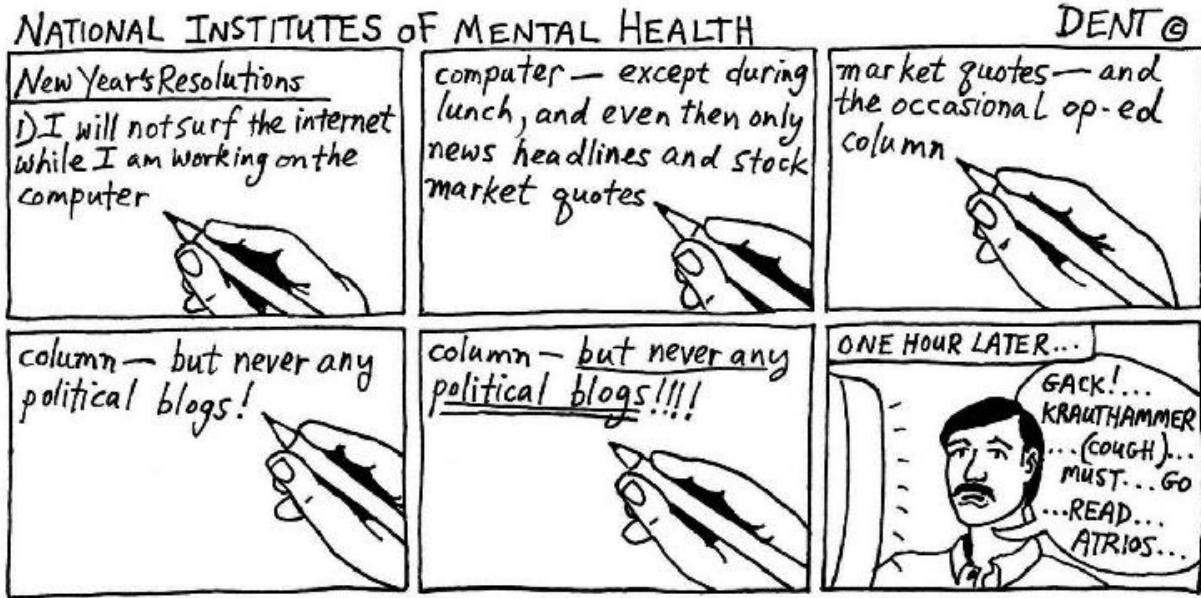
It goes without saying that you should never pass up an opportunity to give a seminar on your work. Just preparing the talk gives you a good opportunity to view the project from 30,000 feet, and might spark questions and ideas. The questions you get during the seminar provide invaluable feedback about the work and often raise critical questions about controls or provide novel ideas about the impact and implications of your findings.

In addition, you should be talking about your research to whoever will listen. Nearly every time you explain what you are doing to another human some novel aspect of the work will occur to you. Even better, since everyone carries around different knowledge and a uniquely wired brain, they are likely to provide insight into flaws in your conclusions, new methods, and connections that hadn't occurred to you. This is also an excellent way to initiate collaborations, which are more important today than ever, due to the rapid increase in knowledge and technology.

Hopefully, you have lots of ideas. I encourage you to share them with others in your lab<sup>[126](#)</sup>, to get their feedback, but not to the point where depend entirely on the judgment of others as to whether an experiment is worth your effort. A typical scenario. You wake up at night with a fantastic idea<sup>[127](#)</sup> and can't wait to discuss with your PI. As you are describing your idea, you can see that the PI's initial enthusiasm has given way to a more neutral affect. For the next 30 min, the PI patiently explains why the experiment won't work, or will be uninterpretable even if it does.

You will leave the office a bit depressed, wondering about the possibilities of driving a cab for a living, or possibly managing a Radio Shack. But, if you are a good student or post-doc, on many of these occasions you will eventually regain your good opinion of the experiment and instead question the wisdom of your mentor, who, undeniably, is getting older and may have lost a neuron or two. So, without further discussion, you do the experiment.

Here's a PI secret. Like your family members, we too have no idea what you do all day. In my scientific travels, I have noted a world-wide phenomenon: although PIs fight tooth and nail for more space for their labs, you hardly ever see anyone in the lab, and those that you do see are typically engaged in non-scientific activities (watching YouTube, ESPN on their computers *etc.*). So, when I step into my lab and someone is pipetting or using the flow cyclometer I am really happy: they might discover something! I don't know that they are doing the experiment that I encouraged them to forget, and frankly, I don't really care.



PIs, including myself, are not complete idiots, and most of our advice is sound. So, when the experiment doesn't work, just don't tell us, we don't need to know<sup>128</sup>. You almost certainly learned something valuable from the experiment, so I am not overly concerned. And if you have a competitive streak (I hope you do), your failure will spur your efforts to do better. Sooner or later, if you don't give up pursuing your ideas, you will have the great pleasure of entering the PI's office with your notebook open to the results from the experiment that they counseled against. With a shit-eating grin, you will ask whether they would like to see your data. Rather than being upset, they will be delighted; both because of the discovery, but more so, because you are taking your first steps towards being an independent scientist. For many young scientists, such "forbidden" experiments lead to the most important discoveries in their PhD or post-doc, sometimes opening the door for their future careers.

Indeed, don't be overly concerned about being "right". A good working attitude is that hypotheses are mostly excuses for doing experiments. A good idea is an idea that leads to a discovery: period. Indeed, it isn't likely to be an important discovery if you could predict it! This is why serendipity plays such an important role in research. Fortune is just one part of serendipity; the far more important part is being smart enough to see that you are lucky. Many great discoveries could have been made by multiple labs, who made the same observations. Often, it is one clever individual, who the creativity and insight to imagine what the observation *could* mean.

While naïveté is useful before embarking on a project, once promising data appears you will need to master what is known about the topic. This can save

you from rediscovering wheels<sup>[129](#)</sup>, and also is a rich source of new ideas (read the Discussions of old papers carefully!). Once the story starts to fill in, I encourage you to start writing the paper. Even if completion of the project is years away, confronting what is known and what you have done that's new in writing will force you to think about your work at a deep level that is difficult to achieve otherwise.

## CHAPTER 7

### *Doing Experiments: Being Organized*

No matter how many hours you work per week, your accomplishments will be proportional to the discipline and organization you put into the work. There are many elements to being organized in the lab. At heart of everything is your laboratory notebook.

There are legal requirements for keeping notebooks that apply if your work for the government or some other organizations, or if it supports a patent application (you never know!). Your notebooks will also be central in protecting you if you are suspected of scientific misconduct. But beyond this, your notebook is essential to planning, analyzing and interpreting experiments.

Scientists typically take enormous pride in their notebooks, and they are typically on prominent display in the PIs office. This communicates to the students and post-docs the that PI at one time was on the other side of the royal “we”, and could actually perform experiments and make discoveries. Also, the sheer number of notebooks is used to demonstrate the type of sustained effort it takes to become a PI. But more than just display items, the notebooks are typically living documents that reveal methodological details and discoveries that didn’t make it to publication.

Actually, I should change the previous sentence to haven’t yet made it into publications. You never know, in science. A number of recent papers from my lab start with data from experiments that I performed more than 30 years ago. So, clearly I did a reasonable job of keeping decent notes. But I wish I had done better. I rarely recorded what I was thinking either before or after performing the experiment. I have a very good memory, or used to anyway, but even so, looking back over decades I can’t decipher what exactly I had in mind.

Many of the post-docs I’ve worked with have done much better. One of our post-docs set the standard that everyone should strive for. I keep her notebooks on even more prominent display than my own so I can use them for instructional purposes. Each experiment described begins with a brief statement of the hypothesis being tested along with relevant background information. The

methods are described in intricate detail, and are typically printed from a word document to avoid handwriting ambiguities (overkill, since her handwriting is calligraphic in quality). The data are recorded perfectly, and important values are highlighted. Considerable effort is made to display the data in a manner that facilitates interpretation. This may entail presenting the data in several formats to bring out important differences between experimental groups.

Let me expand on this point. I am amazed sometimes at the lack of effort expended on data analysis, relative to the protean efforts put into physically performing the experiment. Data analysis is a critical part of the experiment, and wake up, it's the fun part! Your pride in your experimental work should be reflected in the attention you lavish on the data. Think about the mathematical relationships of your values, and where appropriate, plot the data meticulously [130](#). Tears of joy should stain your printed graphs, whose design (including colors and symbols) facilitate making discoveries. Play around with various curve fitting parameters to gain insight into the processes underlying the phenomenon studied.

Once you have completed the initial phase of analysis (going back again and again to reconsider the data is all part of the process of discovery), force yourself to write down the conclusions. What are you thinking? What could the data mean? What may have gone wrong? How can you improve the experiment next time? How have you modified the hypothesis? It can be easy to fall into the trap of working very hard while thinking very little.

These days much of your data will reside in a hard drive somewhere. Make certain you have multiple copies of all of your files! Your working assumption should be that your hard drive will become irretrievably damaged before you leave the lab for the day. Ideally, your institution provides an off-site storage facility for storing copies of data. Lab fires happen, and if they do, years of work can literally go up in smoke. With the cost of cloud-bytes plunging, the lab should invest in off-site storage if it is not provided. Data are the fruits of research, and must be protected at all costs. Be disciplined about moving the data into safe storage ASAP. Other lab members (not to mention institutional network minders, equipment service technicians) can inadvertently (or even advertently) delete your files. It is also unfair to keep your massive files on a machine dedicated computer, since this can compromise the function of the machine as the hard drive reaches its capacity.

When you are about to share you precious data with your PI, be sure that it is in a form that facilitates understanding, INCLUDING THE CONTROLS<sup>[131](#)</sup>. Clearly label the various data groups in graphs and figures. If the protocol is

complicated, make a diagram that explains the work flow and sample handling. In explaining the experiment, assume the PI has forgotten all the discussions you had regarding the experiment, even if the experiment was mostly designed by the PI<sup>132</sup>.

You will save lots of time if you have a system for organizing your data. Name your files in a way that meaningfully identifies them and organize them in a rational folder system (e.g. Immunoblots, ELISA, Cloning, etc.). Since computers seem to derive joy from arbitrarily modifying file creation and modification dates, it's a good idea to put the date of the experiment in the file name (also date your notebook entries). At some point, technology will consign written notebooks to science museums, but like virtual reality glasses, this always seems to be a few years away. If you are early e-notebook adapter, be sure to have an up to date copy (or ten) of your e-notebook at all times ([Dropbox](#) and similar programs that automatically update to the cloud and download to your other computers should be perfect for this).

Samples too must be organized. Think hard about how you label and store your samples and reagents. Ideally, you will use a computer to print labels that you adhere to your stock reagent and experimental tubes and vials. Try to fit as much information as you can read on the label to minimize errors while you are working with the tubes. For temporary tubes (working dilutions, dilution blanks) put enough information on the tube with a fine tipped marker so that if you are distracted you don't have to guess which tube is which<sup>133</sup>. Organize your samples into boxes rationally and keeping in mind that space in lab refrigerators and freezers is a typically precious commodity. And on that bittersweet day when you leave the lab for greener pastures of starting your own lab or a new job, your task will be much, much easier<sup>134</sup> if you have kept accurate records of your samples and have been sure to have duplicate samples of critical reagents (one to take to start your new lab).

Your stock buffers should be neatly organized on the shelves above your bench and clearly and accurately labeled. Labeling the top caps is a good way to avoid mixing the caps between bottles and the resulting cross-contamination. Be aware of the shelf life of buffers, and play close visual attention to clarity color and presence of microbial contamination. Know which buffers need to be refrigerated or frozen. As with your biological samples, your reagents should be well organized in the refrigerator or freezer, preferably in racks that allow efficient packing and easy visual localization.

While on the topic of liquids, virtually every experiment that you do will depend on the accuracy of your pipetting. It is a simple task to insure the

physical accuracy of the pipet itself by weighing a given volume of pipetted water every 6 months or so. An accurate pipet is just the first step in accurate pipetting. Be sure to put the tips on firmly (and remember that tip brands vary in their compatibility with pipet brands), and develop the habit of visually checking that the liquid is properly expelled. This is particularly important for multi-channel pipettes, where it is easy to have a few tips that are not properly sealed and do not completely discharge (or take up) the full volume. When pipetting dilution series, you can save time, money (less tips), and increase accuracy (less loose tips) by starting with the most dilute volume and adding more concentrated samples without changing the tips. Even with different samples, it is possible to avoid changing tips by rinsing the tips in between samples (whether this is kosher depends exactly on the experiment plan).

You should have a schedule for each day in the lab. See what meeting/seminars are happening and plan your experiments in advance for an incubation or stopping point to coincide with the seminar. Once your experimental skills have advanced, you can often dovetail two experiments to maximize your daily efforts. When you have a break in an experimental protocol, you should be either doing another experiment, analyzing data, talking or reading science.

As information increase at an ever-increasing pace, it is crucial to be able to efficiently search the internet for work that can amplify your own. This is harder than it may seem. Searches need to be crafted to narrow results to a scannable number. PubMed is a good place to start, but often you will do better by using either Google, or better yet, Google Scholar, which expands PubMed searches to include text in the many papers that are available. Creativity is essential in effective searching. Also, allow for serendipity in searching. Your institution probably does not have access to all of the journals that contain potentially interesting articles. There are now websites that will give you access to virtually all fire walled publications.

Pre-internet, serendipity happened in the library<sup>135</sup>. Oh, I'm sorry, young whippersnapper. A library is a place where journals and books used to be stored exclusively. You would have to actually get out of your chair and walk to the library! Once inside, you would have to use card catalogs, or that most ancient of books, the [Index Medicus](#), to search the literature. When you found an article of interest, you physically copied it, took notes with a pen, or sent the author a reprint request by mail (no, that's not a typo). These were not the good old days, but in looking for whatever it was that motivated your visit, you would inevitably find something more interesting. These serendipitous discoveries positively affected many careers, and still do, only now it's, much, much easier

for serendipity to occur with the internet. Let your curiosity guide you!

## CHAPTER 8

### *Doing Experiments: Reproducibility*

**W**hen I serve as external examiner in PhD defenses (see chapter 10), perhaps the most important question I ask the student is whether it is more important to be correct in their observations or their conclusions. Only once out of more than dozen times did the student answer “my conclusions”. Ouch. I winced, as did all of the faculty members on the examining committee. Wrong!!

Conclusions are expected to be at least partially wrong, since you can't possibly know what you don't know. But science is built on a foundation of accurate observations, without this, progress is impossible. Obviously, this applies to positive findings. But it applies nearly equally to negative findings. It is extremely important to know that the failure of an experiment is likely to have to do with the hypothesis tested and not the lack of skill of the experimentalist. Supervising unskilled scientists, you don't know what to believe, which is extremely frustrating.

Findings must be reproducible. It may seem obvious what reproducibility means, but there are essential subtleties. Reproducing an experiment does not necessarily mean doing the experiment the exact same way. Experiments are typically complicated, and each repetition should provide insights on how to do it better next time. Changes can be minor (*e.g.* which dilutions are used and how exactly they are made, time intervals used, order of how samples are treated, number of replicates, *etc.*) or relatively major (constitution of buffers used), but up to a certain point, all count as a replicate.

These non-exact repeats drive statisticians crazy, since in their world-view, an experiment should follow the one protocol as precisely as possible. And I would definitely agree that this does provide the tightest error bars. But the calculated statistical error in experiments bears an uncertain relationship with the truth. Let's say we do an experiment the exact same way 4 times and find that the statistical difference between two experimental groups has a very low p value.

What is the p value? This is the statistical likelihood that the null hypothesis

(no difference between the groups) is true, assuming a normal distribution (in the statistical sense) of outcomes for each experiment. Normally this set at 0.05 for biological work. Young scientists (and MD's) often misunderstand how arbitrary this is. In the real world, there is no reason to believe that an experiment with a p value of .04 is more believable than one with .06. Why?

First, how do we know that outcomes actually follow a normal distribution? To establish this, we would have to perform the experiment dozens of times (if not more) and the effort that goes into each experiment is more like flipping a house, not a coin. There is not enough time or money to do this even for simple experiments.

Second, there are hidden variables in experiments that can completely confound the results. With a large experiment, there can be a considerable time interval (minutes to tens of minutes) between the various samples. Ideally, we would have a number of replicates for each that we could stagger to account for this, but typically, this is not feasible. Many assays use multi-well plates, and the mechanics of the experiments often dictate the replicates will be adjacent. There is a well-known, if poorly understood phenomenon where the wells on the edge of the plate give different values than the interior wells. So, if you repeat your experiment 10 times but always put experimental group A in edge wells and experimental group B in interior wells you might see a highly significant statistical difference even though you would have seen the same difference if you had just put group A in each location<sup>[136](#)</sup>.

In experimental biology, it is much safer to reach a conclusion based on different approaches that reach the same general conclusion rather than multiple repeats of the same experiments (even with extremely low p values). This is often lost on clinical researchers, whose conclusions by necessity depend entirely on statistics due to the inherent inflexibility of clinical research. [John Ioannidis](#) has done a great service in clearly showing that most clinical studies of treatment outcomes are seriously flawed<sup>[137](#)</sup>. There is a common misconception in the general public that this analysis equally applies to basic science studies.

Clinical studies almost always suffer from the inability to perform proper controls. As emphasized in Chapter 4, controls are the most important part of experiments. I am even willing to go out on a limb and state that one well designed experiment with clear positive and negative controls can be much more believable than multiple repeats of a less well-designed experiment.

I am getting into dangerous territory here, but I believe it is important to be completely honest, so I will dauntlessly forge ahead. A [paper](#) by two former employees of Amgen, a highly profitable and prestigious biotech company,

claimed to have attempted to confirm the major findings of 53 “landmark” studies, succeeding in only 6 cases. Though they criticized the rigor of the 47 non-reproducible studies, ironically/hypocritically they provided no details on their attempts or on what they deemed confirmation to be, making it impossible to judge the validity of their conclusion. Although I agree with many of the problems that they pointed to regarding the perverted incentives in contemporary science that favor sloppy or even dishonest science, I do not believe that the problem is anywhere nearly as prevalent as they conclude.

I want to particularly focus on what a failure to reproduce an experiment means. Biology is distinct from physics and chemistry, disciplines the identity of the reagents can be verified from experiment to experiment. Living organisms are complicated and are constantly changing. Biological reagents are also complex and not easily characterized in terms of their composition and activities. Consequently, it is common when performing a series of experiments that not all experiments give the same result. Often, it is possible to weigh the believability of individual experiments based on the outcome of the control groups. Let me clear: this is not “cherry picking” the data to obtain the desired outcome.

Many experiments performed in my lab are sufficiently difficult that it takes months to years for the scientist performing the experiment to develop the skill to demonstrate a given phenomenon. I have experienced several instances when after the departure of the post-doc who perfected an experiment, new post-docs cannot reproduce the findings. Although I can see that the new post-doc is not as skilled as the departed incumbent, the new post-doc, after making a solid but unsuccessful effort, typically comes to believe to their core that only fraud can account for their failure. My explanation that I reviewed the raw data as it was generated, and moreover, that the previous post-doc did not even necessarily see the conclusion or its importance, is considered a rationalization by the new post-doc, who believes that I am biased by the credit that the positive result generated.

This is stressful, to say the least. But if the PI genuinely believes in the validity of the data, it is their duty to defend it. Indeed, in one of these instances, our published findings were validated by another lab, ironically, *before* we had published the findings<sup>138</sup> (it was also reproduced by several later post-docs who came to our lab). So, the inability of Amgen to reproduce 46 or 53 experiments is very likely due in part (or even in large part) to the inexperience or experimental clumsiness of those attempting to reproduce the experiments.

There is important factor in reproducibility inherent to biology. Cultured cell lines, which are used in 95%+ of cell based studies are moving targets for which it is difficult to control. Here’s another example from my own lab. Two post-

docs observed a clear phenomenon in a certain cell line completely independently, and separated by 6 years. The second post-doc built on this phenomenon in a series of well-controlled experiments to generate a published paper with important conclusions. I carefully reviewed the raw data as it was generated<sup>139</sup>, worked with the post-doc on its software analysis, and would swear to its validity.

In attempting to extend these findings, a few years later, post-doc number three was unable to reproduce the original phenomenon. In this case, I do not doubt the technical competence of the post-doc three, who was superb. And all of the many controls we devised together pointed to the phenomenon just not being there, despite using the “same” cells. At the same time, I can’t just discard the very careful data from post-docs one and two. My best explanation is that something changed in the cells, perhaps intrinsic to the cells, or more likely, related to changes in the media in which we grow the cells. It could also be due to a tiny detail in how the experiment was performed; *e.g.* precisely how the cells were pipetted or resuspended could perhaps induce different stress pathways.

Whatever the reason, I don’t know why we can’t reproduce the findings, but I stand by the paper we published, since I strongly believe that the observations were accurate. Many scientists would disagree with me, believing that the phenomenon is meaningless unless it can always be reproduced. But this is anything but black and white. Different cell lines typically generate different results. Some of this is artifact, but much is real. Vertebrate organisms have thousands of different cell types, each specialized for certain function. It is expected that many phenomena will differ widely between cell types.

It should be a given that *in vitro* studies can only inform what *might* be going on *in vivo*. In Chapter Five, I went to lengths to describe the artifacts associated with cultured cells. It is not shocking that findings obtained with a given cell line are not universal, it is expected. Moreover, the fact that a given phenomenon can only be observed under highly specific conditions *in vitro* does not mean that phenomenon is irrelevant *in vivo*. My working philosophy is that it is much easier to destroy phenomena *in vitro* than to create them, and that sooner or later, the phenomena will turn up *in vivo*, at which point you will be quite glad to have made the *in vitro* findings. Indeed, as biology returns to studying phenomenon *in vivo*, reproducibility should increase. Animals are far better at tissue culture than we biologists, and if animals are maintained under conditions that minimize infections and behavioral stress there should be less differences between work from different laboratories.

I’ve seen several other phenomena in my lab that stopped working. And in

my travels, I have heard of many examples from other labs. I've met several starting tenure track scientists, whose lives were hell at the time, since new post-docs in old labs were unable to repeat the phenomena that basically landed them their jobs. In both cases, technical competence in the new post-doc was the issue.

This isn't to say that fraud doesn't happen. It happened in the lab of a good friend of mine. A brilliant student in his lab, who had made a series of *bona fide* observations leading to an important publication, fabricated an entire second story that was published in a highly prestigious journal. And when a new post-doc in the lab couldn't repeat the findings, the culprit returned to the lab and reproduced the findings by breaking into an office to decode the samples. The sleuthing of a collaborator eventually caught the student red-handed, at which point he confessed everything. And guess what? He was thrown out of graduate school and his scientific career was over<sup>140</sup>. This is what nearly always happens to pathologically dishonest people, who are typically responsible for the most egregious fraud. They will eventually be entangled in their web of lies.

Fortunately, such individuals are rare. But basically honest people also perpetrate fraud, particularly given the wrong lab environment. There are steps that PIs can take to minimize fraud in their lab

Make it clear when recruiting post-docs and students that the lab is only interested in the truth, no matter the consequences, and emphasize this in lab meetings and in one on one interactions.

Don't' ostentatiously favor lab members whose projects are likely to lead to publication in luxury journals while neglecting (or even ignoring) other projects in their lab.

When a lab member makes an exciting finding, give them an easy way to retreat from the summit if the finding turns out to be an artifact. Even honest people can be trapped by expectations from their PI. This accounts for many cases of fraud.

Scrutinize the primary data generated by lab members and not just the processed graphs, micrographs, figures *etc.*

Have a yearly session that uses real cases of misconduct to discuss research ethics <sup>141</sup>.

The Amgen studies and Ioannidis's work has prompted a campaign at NIH to increase reproducibility in basic biomedical research. In principle, this is a fine goal, but there is a great danger of mandating a huge increase in effort for reviewers and submitters of papers and grants in providing overly detailed methods and raw data for what amounts to window dressing. The scientific community is already wasting too much time on reviewing as opposed to

discovering. At some point, we just have to trust scientists<sup>142</sup>. If we can't, then we have a systematic failure that must be addressed at a far more basic level. Though the idea has somehow been devalued, science is truly self-correcting. Discoveries must stand the test of time of confirmation and extension, or they will be discarded from the body of knowledge.

If you have a long career in science, there is a good chance that you will personally deal with fraud. This is always a painful process. Even in what turn out to be black and white cases, there is often a considerable amount of doubt about the truth. As high priests of Scientific Methodism, it is our responsibility to report fraud if we suspect it happening, and not just sweep it under the rug. But, at the same time, be very careful in what you say and who you say it to.

Start with your PI. If you are not satisfied with their response, try to find a sympathetic faculty member in the department that you respect and who can provide advice. The next step is the Department chair, and after that, the Dean, or someone in the Dean's office who deals with scientific misconduct. Remember this: we do not live in perfect world. Whistle blowers typically suffer from fraud cases in proportion to the power and prestige of the accused. Be extremely careful of launching a crusade. It is all too easy to be blinded to the truth by overly simplistic idealism; life is painted in shades of grey.

## CHAPTER 9

### *Playing Well with Others: Lab Life, Collaborations, and Conferences*

**A**ny illusions that science is different from other human endeavors was dispelled long ago by James Watson's [The Double Helix](#), which recounts how he and Francis Crick solved the structure of DNA, marking the beginning of modern molecular biology. Highly readable, The Double Helix revealed that scientists are driven, by ego, jealousy, pettiness, sex; i.e. are normal people. Watson combined deep insight into human nature (except his own) with a remarkable scientific story<sup>[143](#)</sup> in the first, and still the best description of what biomedical research is really like<sup>[144](#)</sup>.

Biomedical research is as dependent on interpersonal relationships as any human endeavor, and probably more than most, since progress is so dependent today on collaboration. A crucial part of your success in the lab (and outside the lab in non-academic careers) is your ability to get along with others. Your general attitude and demeanor is important. Although we are all born with a given baseline affect, you should try to be as positive as possible<sup>[145](#)</sup>.

You will accomplish much more with a positive attitude and will be happier, to boot. You will deal with failure better, which is important, since most experiments only work by patiently refining the methods following repeated failures. You will be more popular among your colleagues, and more sought after as a collaborator. You will have a much more open and friendly relationship with your mentors, who will look forward to meeting you rather than dreading it. You will be a better friend, spouse, and parent. So, grit your teeth and smile, goddammit<sup>[146](#)</sup>.

An excellent way to improve your mood is to make it a habit to get some exercise every single day. Ideally, you can cycle, run, or walk to work. In high traffic areas, this can even save you time. If not, carve out some time to get outside during the day or to visit the gym. You don't have to spend hours on this. My first law of exercise is that 10 min is better than nothing. Aside from the health benefits of being fit<sup>[147](#)</sup>, you will have more energy and stamina, and a

feeling of accomplishment for showing your self-discipline. The personal time during exercise is often when ideas bubble up from the unconscious incubator.

I also urge you to take your vacations, and don't bring any work with you. How much time to take off? I think 4 to 6 weeks is fine as long as you are working really hard when you are in the lab. If you are single or don't have kids, this is the perfect time to explore the world, or at least go as far as your limited resources can take you. Take advantage of the freedom!

Accepting criticism of your work is an essential part of science. Your *raison d'être* (and the lab's too) is to make discoveries. Feedback that facilitates this is good. Being overly polite in not offering constructive criticism is bad. Being a good colleague means raising questions and making suggestions for improving the work of your lab mates.

You will not get along equally with everyone in the lab. In a large enough group, someone is bound to rub you the wrong way. The best working attitude is to let as much go as you possibly can. Nothing good comes from fighting with people, and it is best avoided unless absolutely necessary. Most arguments arise from misunderstandings. Which brings me to one of my favorite stories.

In a state of extreme hunger, I was rushing to catch a train in Germany, where I was attending a meeting. I was only able to purchase a small bag of potato chips. While arranging my luggage and getting my computer out to work, I put the bag on the little table that separates the row of facing seats. To my horror, as I sat down, the guy facing me opened the bag and started to eat them. I gave him an incredulous look, which he replied with a quizzical expression. Quickly approximating the number of chips and his rate of consumption, I realized that I had better start eat the chips, too. Now the look of surprise was on face, followed by resignation, as we quickly finished the bags of chips. I got to work, but thought, what a jerk!

Hamm. Who was the jerk? Upon arriving in my hotel at the end of the day's journey I opened my pack, and you know how this ends. I was eating his identical bag of potato chips<sup>148</sup>. The point of my story, of course, is that you might actually be the cause of the problem with X and not the other way around. This is a good default position, particularly if you are a man. One of the traits that segregates with the Y chromosome is the assumption that we are always right. So, let things go, if possible.

Sometimes, it really isn't you, and you have to deal with the person. But plan the confrontation carefully, and if possible, have it in person and in private. Body language can be very useful in such circumstances. If this is not possible, then deal with it on the phone. At least, the details of the fight will have to be remembered by your antagonist. NEVER, EVER, EVER start (or continue) a

confrontation by email, which should never be used to send emotional messages.

Be extremely careful with emails, which for all practical purposes are messages sent to all of humanity until the end of time. If you fight with someone verbally, they eventually will have a fuzzy memory of what happened. Most people are well meaning, and this good spirit, given sufficient time will eventually fill in the cracks in your relationship. If, on the other hand, you are foolish enough to send an emotional email, they will never forget the details of the fight, since there it is, right in front of them, whenever they happen upon the message. Be careful as well with what you download onto your computer. Big brother is watching, I have seen people lose their jobs due downloading pirated software and inappropriate videos.

You need to focus on your work. Don't waste time on lab gossip or fretting about how unfairly your PI is treating you. It is perfectly normal to believe that your PI is treating everyone better than you. Even if your PI was doing a perfect job, they would be giving each lab member what they need, not treating everyone equally. But certainly don't expect your PI to be a perfect manager, or even a decent one, for that manner. They were not selected based on their diplomacy, psychological insight, or skills in handling people. They were selected based on their science.

Who are PIs? They are you, plus however many years separates your careers. Someday this may well be you. Are you perfect? And just like your parents, your PI has the asymmetric generational advantage of experience. We have been in your position as a student or post-doc, but you haven't been in ours<sup>[149](#)</sup>.

If you are genuinely unhappy about something in the lab, think about it carefully for at least a few days, and then arrange to discuss it with the PI in private. State the problem with as little emotion as possible, and give the PI time to ask questions and ponder solutions. Offer a solution, if possible.

A major source of lab gossip is inter-lab romances. I know this is asking a lot, but if at all possible, avoid lab romances. True, there are plenty of wonderful children in the world who owe their very existence to lab catalyzed love. Unfortunately, for each marriage, there are dozens of couples who eventually fall out, creating an awkward situation for themselves and everyone else in the lab<sup>[150](#)</sup>. I am a practical person, and I know that my other advice on how hard you need to work will limit your romantic possibilities to the geographic vicinity of your bench. Just try to meet someone working as far from your bench as possible. A different Department is good. At least a different lab. OK, a distant bay in your lab.

Another source of lab rancor is spoiling the commons. This will sound

obvious, but clean up after yourself! Your bench should be neat and well organized. If you can't keep your immediate space clean and organized, how can you perform a complicated experiment? This is usually not a problem, but the cleanliness of common spaces and the general state of common equipment is a universal problem (common use =common abuse). This is why communism failed as an ideology. I know I am swimming upstream here, but please leave common areas at least as clean as you found them, if not cleaner. Every so often, shock everyone by spontaneously cleaning up the area around the pH meter and scales, or a water bath or two. You might embarrass others into following suit, and I guarantee that you enjoy the sense of accomplishment of tangibly seeing the fruits of your labors.

You should take extreme care in using common reagents, including chemicals and biological stock solutions (antibodies, enzymes, inhibitors etc.). Cross-contamination between reagents can undermine the work of everyone in the laboratory. Always use a new pipette tip to remove such reagents and a new (or well cleaned spatula) to remove powders from their bottles (it is often quickest and cleanest to carefully pour the powder onto the weighing boat, but you have to be careful and be sure to clean up wayward material).

Being a good lab citizen will increase your attractiveness as a lab collaborator. Collaboration is nearly always win-win situation for each party, and in a good collaboration, 1 + 1 can really equal 11. In addition to gains in efficiency and productivity by sharing the work, there are enormous intellectual advantages to having two brains obsessed with the same problem. For most people, collaboration increases the excitement and fun.

Your lab mates will work with you only if they trust your work. When a new member of my lab is sought as a collaborator by the incumbent post-docs, this is a very good sign for their future success in the lab. Trench level peer review! The only potential fly in the ointment is possible conflict over principal authorship when the story is published. This is generally easily solved with co-authorship.

Authorship issues can be a major drag on lab cohesiveness and are also a major source of lab gossip. In some labs, co-authorship requires at least contributing a figure that appears in the published paper. I take a different approach. Since each lab member's time is precious in building a c.v. that will enable their career progression, I err on the side on inclusion<sup>151</sup>. This is excellent for lab cohesion, and does not detract from the credit accruing to the principal author<sup>152</sup>. Co-first authorship can be a tricky situation, but typically, it is obvious that this is warranted by the efforts expended by the lab members involved.

Within lab collaborations typically develop spontaneously. While inter-lab collaborations can be even more valuable, before they have gone too far, ground rules should be established in a face-to-face meeting (best in person, but OK by internet). Although it is not possible to predict the precise outcome of a collaboration, including how the effort will be eventually divided, the general ground rules should be established. Discussing authorship issues up front may seem awkward, but is usually much less awkward than discussing them later on, and can save a great deal of trouble. Since this is science and not business, there will be no contracts involved, and trust is part of the process<sup>[153](#)</sup>. It might sound dicey, but collaborations typically provide some of the most rewarding moments, both scientifically and personally.

Many collaborations are initiated at meetings. Indeed, this is one of the major reasons for attending meetings. Although meetings are organized around talks, these are the least important part of the meetings<sup>[154](#)</sup>. Meetings are called meetings since they are designed with the specific purpose for scientists to meet each other. Take every opportunity to meet others. Don't be afraid to sit down at meals with complete strangers. Introduce yourself, try to bring them out if they are shy. It's all good practice for your future career, which whatever path you will take will almost certainly involve interpersonal interactions. The poster sessions are the perfect place for increasing your acquaintances. Their sole purpose is for discussion. Obviously, you should talk to the presenters. But standing around and discussing the poster is an excellent opportunity to engage others viewing the same poster. Big shots in the field often attend the poster sessions to recruit post-docs to their lab, they are anxious to meet you. Oblige them. Introduce yourself. Telling them you are great admirer of their work will effectively lubricate the conversation.

But be careful what you relate to strangers. It is natural to want to impress others with your brilliant intellect and amazing ideas and results, but try not to share ideas/findings that can be easily co-opted by other labs. Not everyone is scrupulous. Older scientists might be perfectly scrupulous, but their memories are imperfect, and a day, or a week, or a month later they may remember your idea but forget the "your" part<sup>[155](#)</sup>. One hard and fast rule: never provide unpublished information or ideas from someone else's project in the lab. If you want to risk being scooped on your own project, fine, but it is not fair to jeopardize your colleagues' work, even if you are collaborating on the project.

Some meetings are organized to encourage attendees to perform non-scientific activities together. Don't pass up this opportunity, which is perfect for finding collaborators and making new friends. Spend an afternoon skiing under

Colorado bluebird skies at a Keystone Symposia meeting with a stranger, and you may well have a friend for life<sup>[156](#)</sup>.

# CHAPTER 10

## *Writing & Presenting*

**T**here is a general misperception under the rubric of “Publish or Perish”. For professors who principal job is teaching undergraduates there may be some reason to view the ties between publication and career advancement with skepticism. But for scientists, publications are what we actually produce. There are many reasons for this, not the least is that you only fully consider what you have actually discovered when you are forced to write it down in a form that other human beings might understand<sup>157</sup>. Questioning the importance of producing scientific publications is akin to farmers questioning growing and selling their crops <sup>158</sup>.

So, while it may be fair to criticize pressure to publish inconsequential papers<sup>159</sup>, your focus as a scientist should be on publishing good papers. The published product should be in your mind when you still in the process of designing and performing experiments. What points can you make with the data in hand? What points would you like to make with further experiments?

What makes a good paper? First, the observations must be reproducible<sup>160</sup>. Second, the findings should add information to what is known or sometimes just as importantly, subtract information from what is known in the form of correcting erroneous published findings.<sup>161</sup>. Third, the paper should be written succinctly<sup>162</sup> and lucently in logically describing the experiments and their outcome, and creatively considering their implications.

Before getting into the nitty-gritty for each section of the paper, a few general points. Probably my greatest deficiency as a mentor is that I do not expend any effort in formally teaching post-docs in my lab how to write<sup>163</sup>. In addition to laziness, there are other reasons for this. Topping the list is that I believe it is *your* responsibility to learn how to write by the time you finished college, or at least graduate school. To write well, you need to be well-read, and I can't correct a lifetime of not reading or reading the wrong things<sup>164</sup>. You also must have to have something to communicate, and I can't correct your lack of knowledge of the field, and most of all, your failure to organize your thoughts logically. Good

writing takes lots of practice and must be driven by an obsession to write better. “That’s good enough” just won’t do. Perhaps in days gone by you could have a career without being an effective writer<sup>165</sup>, but no longer. Writing is essential to getting your papers accepted by good journals and most importantly, in getting your grants funded.

While you absolutely need to be a competent writer, I also urge you to be an elegant one. Read the old literature and you will see that scientists had a much more personal style of expression that greatly added to the flavor and value of a paper. Although reviewers today typically criticize writing that veers even slightly from completely sterile, this trend can and should be reversed. Sterile writing takes the humanity out of the science, and also the fun. Science is still performed by humans and not robots, and we detract from the art and beauty of science by insisting on boring and humorless papers.

## Manuscript Writing: Section-by-Section

**Title:** This is most important part of a paper per word by several orders of magnitude. Well before you are finished with the experiments described in the paper, you should be thinking about the title, which is the focal point for the work. It is not at all selling out to having a clever, provocative title. You want the maximum number of people to read the paper, and in a world where everyone is way too busy, it’s worth the effort to craft a title that attracts readers. Given that editors often make life or death decisions based on superficial criteria, having a nondescript, dull title will not help your chances of having the paper reviewed.

**Abstract:** The second most important section, per word. Here, you need to boil down the problem you are addressing, your findings, and their implications to the bare essentials. This, along with the title may be the only thing the editor reads before giving your paper the death sentence. This is not the place to point out the flaws or limitations in the study. Rather, without overselling your story, you want to put in the best light. The first sentence of two sets up the question. The bulk describes your most important result. The last sentence either makes an important point or implication, or better yet, both.

**Introduction:** Provides a succinct summary of what is known about the topic in a manner to leads naturally to the posing the questions that the paper addresses. It is now traditional that the Introduction ends with a summary of the major

findings of the study. I suppose that this arose to ensure that the reviewers comprehend the major findings of the paper. But to be blunt, this is just stupid. The points of the paper are presented in the Title, Abstract, Results and Discussion! So, don't waste words and the reader's time by fattening the Introduction.

**Materials and Methods:** Accurately describes what you did. This is important if you want to avoid the cross-hairs of the Amgen crowd, who might eventually claim that your findings are not reproducible. It's fine to refer to previous papers where you described the same method with two caveats. First, the method should actually be described *in the paper you refer to* and not a reference to another paper<sup>166</sup>. Second, if you have made changes to the original protocol you must describe them. You should write this, along with the rest of the paper, in the active voice.

**Results:** This is the heart and soul of the paper. Inasmuch as a typical paper these days describes many man-years of work and hundreds of thousands of dollars of labor and materials, you should make a major effort to effectively describe what you found. A good paper, like a good story has a narrative. You want to present the story not necessarily chronologically, but in a way that will make it easiest for the reader to follow and builds towards the important conclusions, ideally ending the paper with the one experiment that ties it all together.

You can never mislead the reader in the Results, but it's kosher not to mention all the experiments you have done that do not clearly support the story. Getting to the truth is not a simple process, and there can be many misleading findings along the way. You must make an honest judgment as a scientist what you believe to be true and relevant<sup>167</sup> and not torture the reader with all of the arguments for why the experiment you presented should not actually be believed<sup>168</sup>.

In describing the experiments, the Goldilocks rule applies: you need to describe the important parts of the experiment, neither too little nor too much. This takes practice, and it requires a deep knowledge of what you are doing to be able to decide what the most important aspects of the experiment actually are. You need to give deep thought to how exactly you should visually present the data, with an eye to making it easy for readers to understand what you discovered. When presenting gels or micrographs<sup>169</sup>, you need to arrange the data to make it easy to understand the findings. This is much easier if you have

carefully considered the order of the samples when you are planning the experiments. In labeling the images in the figures, use text and not just numbers and letters to identify lanes or images. If the protocol is complicated, diagram it on the figure.

**Discussion:** Here the goal is weave the conclusions (referring to specific experiments and figure panels for emphasis) into the web of existing knowledge with a good dose of insightful speculation and cross-field fertilization. This is the fun part! Or more accurately, it used to be, since many reviewers today believe that they have the right to dictate what is discussed. I agree that the reviewers have a right to censor truly outrageous speculation and unjustified conclusions, but in general the authors should be given wide berth. Indeed, the major goal of the Discussion is to challenge dogma and present truly novel concepts. Let's say the Discussion is truly crazy/stupid/wrong: if the authors want to show the world how dumb they are, they should have the right. Try whenever possible to end the Discussion on the most important general or practical point. You want to leave the reviewers in a state of excitement, not boredom.

**References:** Just in case: you are insane if you don't use computer software to organize the references<sup>[170](#)</sup>. In a rational world, the journal would all use the same formats. In the world we live in, changing the format as the paper bounces between journals will cost you many hours if you do it manually (as will adding and reordering references as the need arises).

The number of references is often limited by the journal, which will influence your latitude and choice. While it is easy to refer to a review to support a statement, avoid this whenever possible. It isn't fair to the original discover<sup>[171](#)</sup>, and moreover, reviews often get the facts wrong<sup>[172](#)</sup>. You should at least read the Abstract of each paper you reference to be sure that you have it right. It is an art to know which findings need to be referenced. In general, references should focus on the newer or more central papers.

Be absolutely sure to include the relevant papers of potential reviewers! Even if they are fair-minded, they will be more favorably inclined towards your work if you properly include their work<sup>[173](#)</sup>.

## Getting Papers Published: A Twisted Path

In the early days of biomedical research there were few scientists and few journals, and the publishing process was highly collegial<sup>[174](#)</sup>. *Nature* instituted formal peer review only in 1967<sup>[175](#)</sup>. Even until the mid-eighties, publishing papers was a relatively stress free-task. My first paper as PhD student consisted of 3 tables that simply reported frequencies of virus escape mutants using different monoclonal antibodies that represented about 6 months of work by only me. Students won't believe it, but the paper was published in *Nature*. This was normal. Nobel prize winning papers in *Nature* represented a similar amount of work.

The last *Nature* paper from my lab had over 100 panels of data representing the combined efforts of 18 co-authors exerting more than 10 man-years of effort. The paper was at *Science* for about 18 months, eventually satisfying the reviewers but leaving the professional editor "uncomfortable". Sadly, this too is typical. The publication process, especially for high profile journals, has become a siege, often lasting years as the paper bounces back and forth between the authors and the reviewers, with the professional editors who run these journals unwilling to make a final decision.

Despite this extra effort, today's papers, while larded with more data, are not really more consequential than the svelte papers of yesteryear. Good papers report a discovery. Typically, the added data in papers reinforces conclusions that are solid to begin with. Indeed, in many ways today's papers represent a step backwards in an effective publication process. Reading papers from the first 60 years or so of modern experimental biology (1900-1960), when the foundations of biochemistry, cell biology, microbiology and immunology were established, reveals a much more lucid writing style. This includes liberal use of the first person (many studies were individual efforts), and a flowing description of the thought processes that connected experiments. Discussions were free flowing and genuinely speculative, and were often brutally honest about the limitations of the study, and its merits relative to studies from other investigators.

Despite the introduction of electronic media, ironically, modern papers are corseted by strict page limits that force an artificial narrative in the main body of the paper to tell a "story" in the most efficient manner, often leaving out details that provide clues to alternative interpretations. Any mention of the potential flaws of the study is strictly avoided, since reviewers will likely use it to club the paper to death<sup>[176](#)</sup>. Indeed, reviewers tightly control the Discussion, using "speculative" as a pejorative, and fighting to keep the Discussion as close to (their) accepted truths as possible. Typically, reviewers are angling to prevent the spread of ideas that threaten their own models and hypotheses, a problem

exacerbated in by tight funding, when grants are rejected because the hypothesis might not be “correct”<sup>177</sup>.

Why we allow this censorship baffles me, since it undermines one of the principal purposes of publishing, *viz.* to spark the creative processes of the reader. The most egregious examples occur in the most prestigious journals. *Nature* and *Science* have the tightest page limits, which essentially limit the Discussion to a paragraph or so. For a few highlighted papers in these journals, outside experts in the field are invited to describe the paper to a more general audience and allowed to provide a much more detailed discussion than the authors themselves<sup>178</sup>!

There is much to dislike about the review process (see Chapter 13), but until the entire system of publication, hiring, and promotion is overhauled<sup>179</sup>, this is what you will have to deal with. As a PhD student, the name of the journal is not of tantamount importance to your careers. Publishing even one solid paper where you have done all or nearly all of the work, in a well-respected journal, should, with a highly enthusiastic letter from your PhD advisor extolling your virtues, get you interviews at potential post-doc destination labs.

While it doesn’t hurt to have a boutique journal paper adorning your c.v. As a PhD student, it is a luxury<sup>180</sup>. Here, there is a natural conflict of interest with your PI, who garners many career points from publishing in such journals that not only buff their ego, but increase their salary, and most crucially, their fundability. They may push to send the paper to the highest possible journal. While flattering, this may not be optimal for your training. If you are not near the finish line of your PhD you might be entangled in a war with the reviewers, who often request difficult and time-consuming experiments that only incrementally advance the story. Working on the real extension of the findings in the paper or another project will likely teach you more, and perhaps yield another manuscript to hone your writing skills on.

As a post-doc, it’s a different story. At this stage, you definitely need to have your papers in the highest ranked journals that will take them. Although organizations may pay lip service to considering the quality of work and not the impact factor of the journals that publish it, this is a thinly veiled lie. Look at the c.v.’s of young faculty members at places you’d like to start your career. Likely it is littered with luxury journal publications.

Here’s how you play the game. You aim high and take your licks. Something important to know: a paper is not truly rejected by a journal until it is accepted by another journal. Nearly all of the papers from my lab that have appeared in the luxury journals were initially rejected by that very same journal, sometimes,

robustly. There is an art to reading a rejection letter, but policies also vary between journals. If your lab is not familiar with these unwritten rules find someone in your department who is (typically they publish papers in these journals).

You have to decide during the siege however, whether you are ever really likely to crack the defensive walls. At some point, it is best to try another journal. Sometimes you will actually have a better chance in a journal with a higher impact factor, depending on what the referee's comments are. In general, the best refereed journals are those run by and for scientists, with no pretensions to being "news makers" and with limited income from advertisers, who subtly (or more energetically) lobby for papers that will sell more of their reagents<sup>[181](#)</sup>

In responding to the referees' comments be careful not to offend the referees. Remember that they are not being paid, and are basically volunteering their time to help you publish a better paper<sup>[182](#)</sup>. There a number of strategies to gain their blessing:

1. Never, ever, just blow off a referee's comments. You don't have to perform the experiments they request, but if you don't, carefully explain why (cost and time are legitimate answers). Try as hard as you can<sup>[183](#)</sup> to preface your remarks with positive words, "we thank the referee for this insightful suggestion", even if you wonder whether they actually understood anything about study.
2. It is nearly always useful to exclude some non-critical data in the original submission, particularly if you think the referees will request them. While you may find this odious<sup>[184](#)</sup>, a balance between idealism and realism goes a long way in actually having a career.
3. Editors almost always honor your requests for excluded reviewers and typically select at least one of your recommended reviewers. It is commonplace that authors will rail against the opinions of reviewers they actually requested! With rare exceptions, it is difficult to guess the identities of reviewers, and authors usually get them wrong. Be very careful in choosing your selected reviewers. Reviewing is a crap shoot, as is evident from the near impossibility of having three reviewers positively review a paper<sup>[185](#)</sup>.
4. If you have real beef with a referee, make your case by communicating with the editor only.
5. Despite the vicissitudes of the reviewing process, never forget that the value of the paper is intrinsic to the paper, and does not depend on the journal it

appears in. With the power of internet-searches, interested scientists will eventually find and digest your paper, which is, after all, the goal of the entire process.

## Dissertation

With the enormous increase in the amount of data that comprise a “publishable unit” in biomedical research, you may not have much chance to write papers as a PhD student<sup>[186](#)</sup>. You will, however, have a chance to produce a major document in the form of your PhD dissertation. A dissertation is essentially a long form version of a manuscript in which you describe your PhD studies, even the experiments that did not work.

You may be a student in a program where the Dissertation is not a big deal; you may even be allowed to essentially staple your papers together, adding a short Introduction and Discussion (this is common in many European countries). Having spent 5 to 6 years at the bench dealing with the inevitable frustrations in experiments and seeing your precious papers rejected, you are likely to suffer from serious burn out and will be sorely tempted to put the minimum amount of work into your Dissertation.

Avoid the temptation!!! The Dissertation provides a unique opportunity to hone your writing and cognitive skills and to put the work into its proper historic context. Make the effort, if you haven’t already (you ought to have, but better late than never), to come to grips with what others have reported. If you are lucky, your field will stretch back 100 years (or more), and you will have great fun of knowing the answers to many of the puzzles that prior generations were wrestling with. The Introduction should be a comprehensive literature review that can easily exceed 25,000 words<sup>[187](#)</sup>. The Material and Methods is particularly important. This is not the place to refer to previous publications, since the lab will use this as a biblical reference, particularly if you are a good student. Put every detail in. Imagine that an idiot will be using it to repeat your experiments. The Results can and often should be written chronologically, since in this case the order of the steps and missteps is important to record. Provide more explanation than in a paper regarding the rationale of the experiments and what you were thinking along the way. Point out the intriguing findings that are worth further investigation. The Discussion is your opportunity to show the world just how brilliant you are.

Oh, one more thing, the Acknowledgements (usually up front) is a good

opportunity for you to express your appreciation for those who helped along the way. Don't be afraid to show emotion. Without being maudlin (humor helps), you can relate just how important your family, friends, colleagues, and even mentor, are to you.

Though it varies between universities and countries, students must "defend" their PhD dissertations in front of an evaluating committee. To insure academic standards, an outside expert is typically invited to serve on the committee and given the lead role in questioning the student. In the USA, students typically give a 1 hour public presentation of their work followed by questions from the audience, and then meet in private for up to several hours with the committee. Here the committee is free to determine the depth of the student's general and specific knowledge, and also to make specific comments on the dissertation itself (including the quality of writing). Typically passing is a foregone conclusion, but a really good performance can be a sling-shot for the next phase of your career, so make an effort to shine.

In Sweden, the defense is a fully public mano-a'-mano affair, with the student facing an opponent for 2 hours or more. It's a big deal, with the audience including family members, friends, students, post-docs and faculty in the department. After two rounds of sparring in the form of a brief introduction to the field from the opponent and then a summary of the thesis from the student, the gloves come off and the questions and answers fly. There is a race car element to the event, with some in the audience eagerly anticipating the crashes.

I have had the great pleasure being an opponent three times, and it was a blast. My goal was to showcase the student's depth of knowledge to their families but also stretch their capacities to beyond breaking, with questions on science philosophy, general biological knowledge and deep knowledge of their field<sup>[188](#)</sup>. I wanted them to walk away proud but a bit humbled, not by me personally, but by our needs as scientists to recognize the limits to our knowledge and always strive to know more.

## Presenting

Learning to give effective presentations is a critical aspect of your training as a PhD student, and is one of the major generally useful life skills imparted during the training process. Most interesting and well-paying jobs in today's economy require an ability to effectively communicate your ideas, both in formal presentations and in informal conversations. While some people are born with

the “gift of gab”, everyone with the intelligence to obtain a PhD can become an effective communicator. It takes practice with effective feedback on your performance.

A good place to start is to take public speaking courses in high school and college<sup>189</sup>. This will help you get over stage fright, which is perfectly natural, and likely to persist well into your career (most scientists never get over it). A little bit of nerves is good for adding energy and enthusiasm to your talk. If you perform independent research in college you will probably have an opportunity to give your first real presentation with graphics and data. Every time you give a presentation you will feel a bit more comfortable and will get a bit better, so never pass up an opportunity to present in public. This includes presenting to a non-scientific audience, which will force you to boil down your research to its essential features and prepare you as well for preaching the gospel of the scientific method to the benefit of society.

There are many excellent sources on the web to learn how to optimize scientific presentations, but I will give you ten pointers about how to give a good talk.

At the top of the list, you have to know what you are talking about! When deciding on hiring post-docs or faculty a key element is their ability to answer detailed questions about their work. It’s impressive when a question opens a floodgate of supporting information that the speaker could not present due to time limitations. At the same time, if you don’t know the answer to a question, just say so, after prefacing your remarks with “that is a really good question”. Don’t get into a prolonged discussion with questioners; if the issue isn’t resolved, politely end the exchange with “lets discuss this over coffee etc.” Humor is the most effective tool for disarming aggressive questioners. NEVER LOSE YOUR COOL!!

The most impressive and effective speakers demonstrate exquisitely chosen words that do not overstate or undermine the findings. Until you are well into your career you should rehearse your talk to the point of nearly memorizing the exact words you will use. Later on, you can sacrifice precision for the energy and excitement that more spontaneous word choices provide<sup>190</sup>.

Your genuine enthusiasm for your work should be obvious from your demeanor and energy. Approach each talk as an actor approaches each performance: the audience should feel that this is the very first time they are seeing the performance. If you seem bored, they will be too.

You should raise intriguing questions as you go that demonstrate your insight and also why you would be an excellent addition to the lab or department if it’s a

job talk.

You should stress the big picture: Why are you studying this topic? What do you hope to learn? Why is it important? Why is it important<sup>[191](#)</sup>? Sadly, these days you need to tell the audience how your work will help cure which horrible human diseases. Make sure to provide background information at the start of the talk commensurate with the knowledge of your audience.

Humor as part of the formal presentation is good, but use it sparingly at the start of your career. If I'm in the audience, the funnier the better, but science has many stodgy individuals, particularly those who make decisions on hiring and promotion. You can adjust the level of humor to match your audience.

If some of the work you present is not your own, make sure you acknowledge the person in your lab who actually did it, and why you need to show their work in the first place. Ideally, everything you present will be product of your two hands, but sometimes this is not possible.

Find out beforehand if someone in the audience (or at that institution) works on the same field and bend over backwards<sup>[192](#)</sup> to give them proper credit, both verbally and in slides.

Keep your slides as simple as possible. Just show the relevant data. Every data slide should have the conclusion as the title. The audience should get the gist of your talk and most important conclusions just by reading the slides on autoshow!

Try to enjoy it. It's a kick to perform in public, and you should feel drained but on cloud nine afterwards.

# CHAPTER 11

## *Post-doctoral Fellowship*

### **Choosing a Lab**

Your post-doc should be the most enjoyable time in career. You should have little or no responsibility in running the lab, raising funds, or teaching<sup>193</sup>. You should be working nearly as an equal with a world leader in their discipline who burns with curiosity and wants to see you succeed. You should be surrounded by other smart, funny, happy people who will become the core of your scientific network for the rest of your career. Choose carefully! Preferably a year, but at least 6 months before you plan to defend your PhD, you should write to PIs you would potentially like to work with as a post-doc. This presupposes that you plan to actually do a post-doc, which you need to carefully think about. If you are contemplating an academic career, you had better love science, because the career at this point has many drawbacks.

First is the low salary you will earn as a post-doc, generally around 40-45K per year, with only health insurance as a benefit<sup>194</sup>. Second, is the uncertainty of getting a tenure track job. Third, is the difficulty in funding your lab and keeping your position if you happen to get a job. Fourth, is the huge effort you will have to make your entire career to remain competitive. Fifth are the battles you will constantly lose with the administrators and regulators who run the world<sup>195</sup>.

OK, you can't live without the sheer thrill of making discoveries? I understand, but please don't blame me if things don't work out. What's a good lab for a post-doc? First, you need to choose a topic for research. Now is the time to make a major switch in fields, which can be very good for both the post-doc and the lab, due to the two-way influx of new knowledge. Unfortunately, you need to have an eye on what you envisage as a topic that you can continue as a newly minted PI. Study sections are risk averse, and are skeptical about young scientists changing fields, so your first grant will almost certainly be a continuation of your post-doctoral work. Consequently, you need to choose a topic that the funding agencies are interested in.

This is not that difficult. Viruses, for example are cool things to study: just choose a virus that kills people in the country you envisage starting your career. Cancer is always a good choice, since it will never be cured, and covers just about every topic in modern biology. Neurobiology is another can't miss. In addition to the infinite complexity of the CNS, nothing is known about the causes of the myriad psychological and degenerative diseases that plague humanity.

Take time in writing to your potential new PI. The letter should be personal and clearly demonstrate that you know their work and suggest ways that you would be good fit for their lab. If they don't reply, send the email again. PI's are busy/disorganized and miss many emails. If they don't answer again, call them. The worst they can do is to tell you they don't have any positions. Nothing lost on your end.

If they express an interest, you need to visit the lab. In addition to extensively talking to the PI, you need to talk with the people in the lab. Things you need to know:

What are the salaries and benefits? Is it negotiable? How many years of research will be supported?

What will your standard of living be given the salary?

How are post-docs generally treated by the institution? Is there a career office that will help you find a suitable job? Is there a strict time limit on how long you can be a post-doc<sup>196</sup>

If you are changing fields and will need help in learning a new system find out if this is likely to happen.

Are people in the lab happy? Do they get along with each other? Do they collaborate extensively? Is there laughter?

Does the PI allow or even encourage competition within the lab?<sup>197</sup>

What is the policy on co-authorship? Or collaboration?<sup>198</sup>

Is the PI only interested in publishing papers in the boutique journals<sup>199</sup>?

Is the PI only interested in interacting with lab members whose projects are going well? What happens if nothing is working for people in the lab? Are they ignored, shunned, or mysteriously abducted?

At the other end of the spectrum, does the PI micromanage everything, and leave you no freedom to explore your findings and plan experiments?

Which brings up an important point: how much freedom do you want? Try to choose a PI that matches your expectation.

Who is responsible for really running the lab? It is well organized? Is it everyone strictly for themselves?

Does the lab have enough money and resources to support your project?

What sort of opportunities will you have to present your work at lab meetings, department meetings, outside meetings?

Will you be able to take reagents and projects with you when you depart?

Does the PI support their post-docs after they leave<sup>200</sup>? Will they be disappointed if you don't follow in their footsteps?

In addition to talking to the people in the lab, ask the PI for the email addresses of all of their former trainees. If they won't provide it, find another lab. Contact former lab members to arrange for a time to talk by phone<sup>201</sup>.

## Landing the post-doc

Aside from your c.v. and letter to the PI expressing your interest in the lab, your recommendation letters play a large role in the level of interest your application will generate. If your PI is not firmly behind you, you are in trouble. Often, one or more supporting letters are required. A good choice are members of your PhD committee, but only if they think you are really good. Don't leave this to chance: ask them directly, if they can enthusiastically support you.

Ok, you have one foot in the door if the lab wants to interview you. If they won't pay for your domestic travel expenses, either they are cheap, which is bad, or have limited financial resources which is worse. You should have a reasonably detailed knowledge of what the lab you are visiting has published in the last 5 years<sup>202</sup>. In many labs, the lab members will have a large influence in the post-doc selection process. In addition to gleaning the information you need about the lab atmosphere, be sure to ask about their projects, and treat their replies as the most interesting things you have ever heard<sup>203</sup>. Try to have some questions prepared beforehand, if possible, but be sure to ask questions that demonstrate how much fun you will be to have as a colleague. Treat this entire exercise as excellent practice for the end of the post-doc tunnel when if all goes well, you will repeat the process (with higher stakes) for a PI position.

## The National Institutes Guide to the Nine Types of Post-Doctoral Fellows

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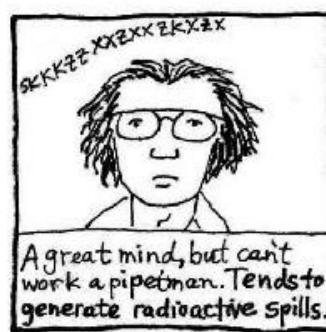
The M.D.



The 9 to 5-er



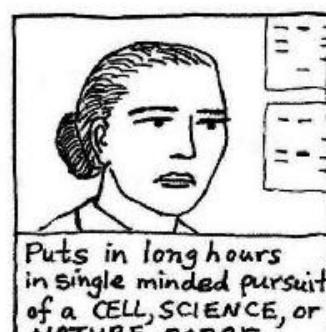
The Parent



The Biohazard



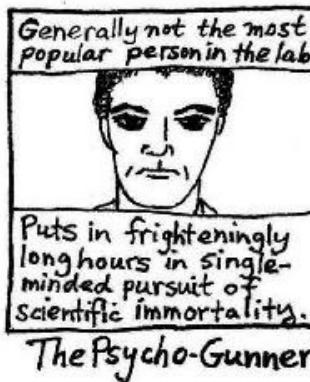
The Robot



The Gunner



The Pseudo-Gunner



The Psycho-Gunner



The True Psycho

The most important part of your visit from the lab's standpoint is your seminar. Typically, 45-60 min, plus questions. If you are visiting my lab, your seminar will likely take 2 to 3 hours since members of the lab, with me leading the charge, will ask you many, many detailed questions. These are designed to gauge the depth of your knowledge about all aspects of your work. Your talk should be extremely well organized and rehearsed with your PI and other lab members, who should be urged to question you intently.

## Getting Started

Whenever I've changed jobs, or even moved between buildings for that matter, for the first few weeks to months, I've questioned the wisdom of my decision. Different is almost always good, but it takes some getting used to. Likely, things will feel weird when you being your post-doc. You have gone from being one of the sages in your old lab who the newbies look up to and seek advice (even the new post-docs), to the baby in the new lab. Where you were the trusted side-kick of your old mentor, you are unsure of the new relationship. Just remembering where your bench is in a big lab (not to mention the reagents and various instruments) can be challenging for the first few weeks.

Just relax, and be yourself. If the project has not been lined up, before launching into a project, do extensive reading, thinking, and discussing with the PI and lab members about the plan. Maybe you can already start a collaboration with lab members that will lead to a quick co-authorship. It's always good to get quick publication to get that monkey off your back. I now encourage post-docs to start two projects: one relatively easy that is likely to give a quick publication (12-18 months is quick for my lab), the other riskier and challenging, which if all goes well will launch an independent career.

It is particularly important at the start of your post-doc to focus on lab work. Do not volunteer for various committees and activities that are available for post-docs. If things go well, there will be time for this. If you have a family, you will need to spend time making sure that they are properly settling in. High stress is normal for the first few months.

Between 6 months to a year after starting, you should have a private meeting with your PI to discuss your progress. It's a good idea to have these meetings annually. You need to hear their honest opinion of how you are doing in the lab, and what they think your potential is. Older PIs will be much more comfortable having this conversation than younger PIs, but it's worth pushing their comfort level. As with all advice, you don't have to take it<sup>204</sup>.



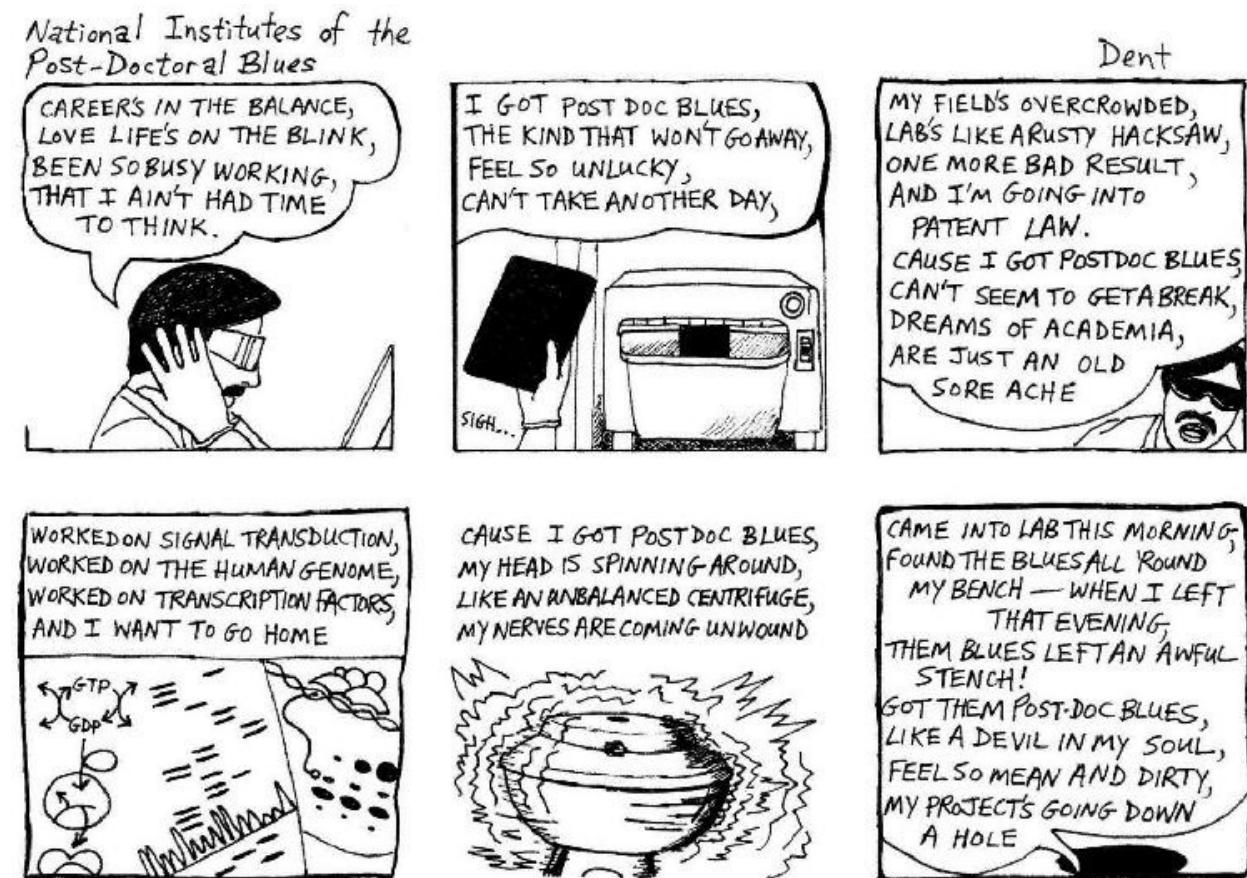
A year or two (but no longer) into your post-doc, if things are not going well you need to make a decision. Do you still want to pursue an academic career, or do greener pastures beckon? Don't be afraid to disappoint your PI (or spouse or parents), you have one life, and you should be happy in your work. In any event, as I have emphasized throughout, if you don't have a burning passion for science it is very unlikely your will have the obsession it takes to succeed.

## Landing a real job<sup>205</sup>

If everything goes perfectly, after 3 years you will have enough experience and publications to begin your job search. This generally takes about a year, so you need to plan ahead. In addition to the replying to advertised jobs, don't hesitate to write to departments to inquire about your possible recruitment<sup>206</sup>. Landing a tenure track position was always challenging, and is now more difficult than ever. You will greatly enhance your chances for a research professorship<sup>207</sup> if you come with funding, typically in the form of a K22 grant. These are very competitive, and it often takes several tries (if ever) to obtain one. If you are one of the lucky few, you will have a year to find a position. Science policy jobs are equally or more competitive, and you need to find out the application deadlines. Industry tends to the opposite; the process is typically bang-bang. You apply, are interviewed, and have to decide and begin relatively quickly<sup>208</sup>.

Whether your target job is in policy, industry, policy, academia, networking is key. We have had post-docs with relatively modest achievements who landed excellent jobs (where they are happy and successful) by dint of effort and networking genius. This is why I have emphasized the importance of

interpersonal skills throughout and making every effort to meet people. Obviously, this is much easier and more enjoyable if you actually like people and are curious to learn about what they do and who they are. Under these circumstances, networking is not just a cynical ploy for self-advancement, but a genuine reflection of your personality [209](#).



You should apply for every job that looks interesting, or at the beginning even close. The interviewing process for academic and non-academic jobs is intense and exhausting. As with running a marathon, training is crucial. It is good to have a few interviews under your belt before the really juicy job comes up. Typically, you will meet over a day or even two with many members of the department, who will be trying to decide how smart you are, how likely you are to be funded, and how you will be as a colleague and collaborator.

You need to prepare. Get a list of your interviewees ASAP and read their recent papers [210](#). You will make a very favorable impression if you ask a series of insightful questions, and will earn valuable bonus points if you subtly show them how wonderful it would be to have you just down the hall to bounce ideas

off of and to collaborate. If you make it past the first round, there will be time later in the process get the lowdown on the department chair and local politics. During the first encounter, you want to present yourself in the best possible light. Even if this is not your dream job, you need to act as if it is<sup>211</sup>. Your goal is to get the job, at that point you can make a decision.

In addition to 4 to 8 (or more) interviews with the faculty, you will probably have lunch with the PhD students, and of then of course give your all-important seminar. Just re-read what I advised for your post-doc seminar and multiply by 20. The seminar is EXTREMELY important. The audience may include various Deans and Deanlets who can greatly influence the hiring process. If you believe that university administrators are only interested in money, you will never be disappointed. So you need to convince them in your seminar (and the Chair, of course) that your work is fundable. It would be hard to over stress the need to clearly explicate the importance and general interest of your work. And your enthusiasm should be obvious and even contagious.

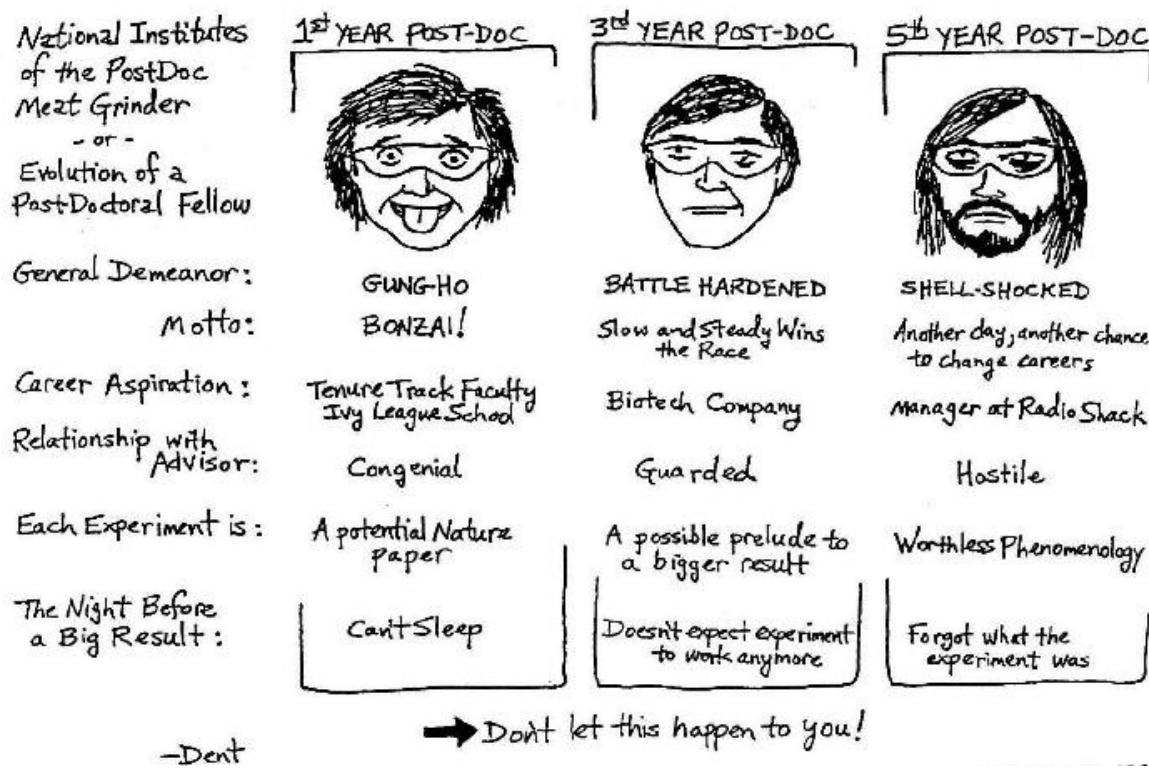
You will probably also have a 1-multi hour “chalk talk”, i.e. no slides, in which you astound the committee with your brilliant experimental strategic plan for the first few years of your tenure. They will pepper you with questions to test your mettle and their ears will be perked for fundability, which you must be cognizant of.

If anointed by the selection committee as “The One”, on to round two, where you return to talk turkey with the Dean or Chair regarding compensation and research support<sup>212</sup>. Given the difficulty in getting external funding, the institution will have to provide you with 3 to 5 years of support, and also the essential equipment you need. Typically, the total package exceeds a million dollars, which the institution hopes to recoup with the overhead costs that will fill their coffers by your success in obtaining NIH grants<sup>213</sup>. This is good news, since your institution will be desperate for you to succeed and ascend the academic ladder. If you fail, they will have to begin the whole process again, shelling out another mil, and spending time on recruiting the next bright eyed, bushy tailed candidate.

You need to strike a balance in the negotiation. You will typically be armed with a complete list of the equipment you need along with the number of animals and a budget for supplies and services. Your current mentor is a good source of advice, as are other willing faculty members in your department<sup>214</sup>. While standing firm about what you really need to get started in terms of equipment (or access to equipment), supplies (including animals and animal space), and salaries, you can't be unreasonable or too aggressive. There won't be a lot of

room on salary, unless you have multiple offers, in which case you benefit from the competition for your services<sup>215</sup>. At research dominant-institutions, you can negotiate how much teaching you will be expected to perform in the startup period, when you will be extremely busy get your research going and above all, writing grants to fund the lab.

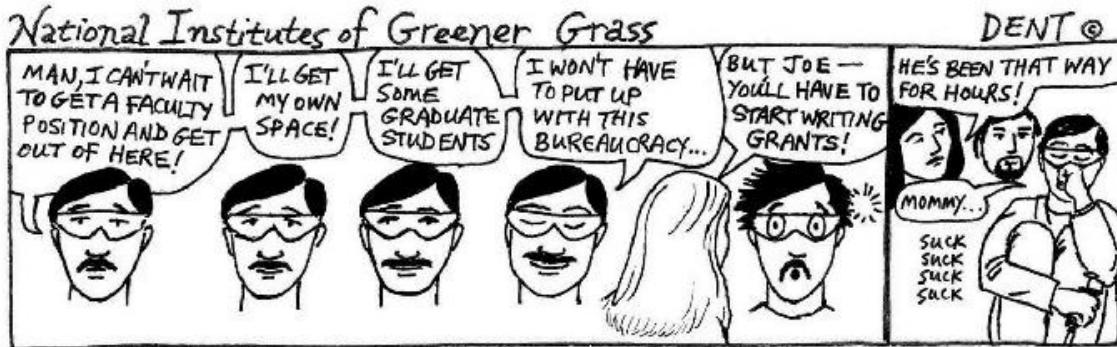
Given the realities of life, you might face the two-body problem: i.e. trying to find two academic jobs within commuting distance. This will make the job search much more difficult<sup>216</sup>. Ideally, each partner can find a good job. If not, compromises will be necessary.



## CHAPTER 12

### *Starting your own lab*

For the lucky few that obtain a prized junior group leader position (typically tenure track in North American universities), this is just the beginning of a long journey. In all but the actual lab work, you are basically starting from scratch: nothing has prepared you for the challenges ahead, teaching classes, dealing with mentees, administrators, budgets, writing grants, writing grants, writing grants, writing grants, writing grants.



While there is an enormous amount of useful advice to be gleaned, I will not provide much of it. And not purely out of laziness. When starting to write book, I happened to visit Emory University to give a seminar and meet collaborators and colleagues. At a highly collegial and convivial dinner, Jerry Boss threw a book across the table that he had co-authored with Susan Ecker, and said something to the effect of “I heard you were writing a career book, but don’t bother, bitch”<sup>217</sup>. The book had an unpromising title: [Academic Scientists at Work](#) but I dived in just the same that night when I returned to my hotel.

Wow! This is a fantastic soup-to-nuts book on how to succeed as a tenure track scientist. So good, that universities and institutes should provide it every starting PI. It is packed with good practical advice and also good humor and overall gestalt. Realizing that this book is better than could ever write myself <sup>218</sup>,

I scaled back my ambitions to focus on a few important topics.

## Focus on your research

Many newly minted PIs hire a technician and recruit students and post-docs and leave the bench work to them. Although I understand that you will have to make a major effort writing grants to raise funds<sup>[219](#)</sup>, you should spend as much time as possible doing experiments yourself. There are many reasons for this:

You were chosen for your skills as a bench scientist. It will be many years (if ever) that you have someone in the lab as gifted at performing experiments as you. Indeed, it is difficult/impossible to tell which students will have the knack of doing research: general experience is that about half are pretty much useless at bench work, and will be difficult, at best, to train. Even the good ones will take time to train and otherwise get up to speed. Post-docs are more of sure thing, but you are competing with well-established PIs, and you will tend to get the bottom of the barrel. Your own two hands are your best asset as a young PI. Use them!

Once you don't do the experiment yourself, you are never sure of its validity. Even the good people in the lab will miss observations that you are likely to make that can greatly influence the outcome of the experiment, including avoiding artifacts.

You will think better and more creatively about the experiments and the problem itself if you are actually doing the experiments.

If you sit in your office all day, you will not be aware of all of the things going wrong in the lab, including who is working and who is not, who is a slob vs. a real team player etc.

Working at the bench provides an excellent role model for your lab members. You can show them the standard of quality required in performing experiments and analyzing the data. If they are reluctant to doing a certain type of experiment, you can shame them into it be doing it yourself<sup>[220](#)</sup>.

To the extent possible, you should avoid committing to university/institute community tasks that will take you away from the lab. This includes reviewing papers and grants. It may be difficult at first, but you need to take Nancy Reagan's advice: Just Say No. At this time in your career, you need to be selfish. If you don't make tenure (or equivalent) you won't have an academic career. You need to publish papers. There will be plenty of time later to contribute to the community.

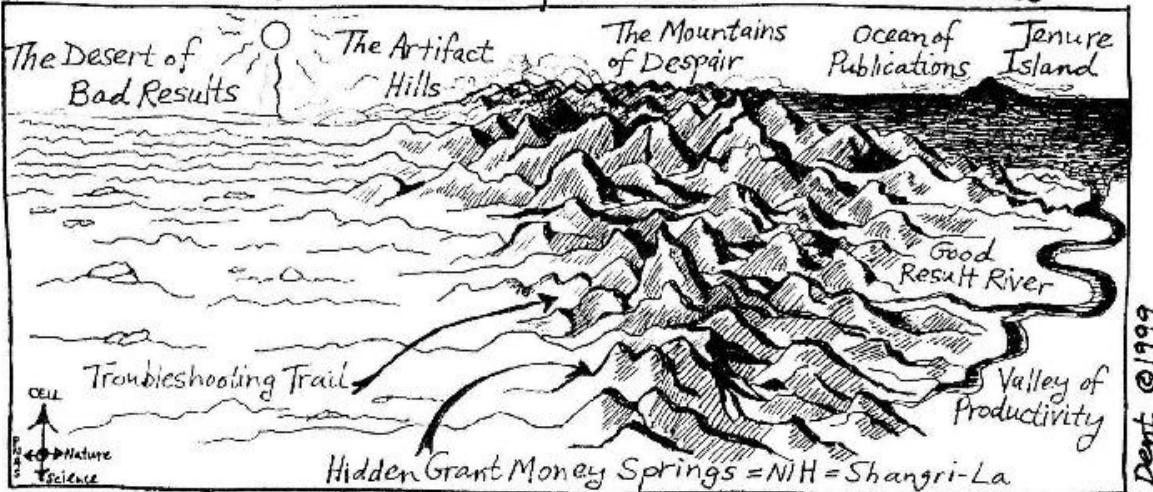
The one thing you need to do is to gain sufficient fame<sup>221</sup> to increase your odds of recruiting good people to your lab (eventually), and have your papers and grants accepted. Never turn down opportunities to:

Give a seminar at a university, even if you are invited to what you would consider inferior institutions. Every NIH-funded department in the country has smart, industrious, and creative people you have never heard of. If you have an open mind, you will learn something useful from everyone on your schedule.

Present at a legitimate meeting. There are many illegitimate meetings run for profit. I get at least one invitation per day. There is a very simple way to distinguish a legitimate meeting: they will cover all of your expenses. Don't worry that the meeting is centered on a different field: you will learn new things, meet new people, and likely come away with new ideas and collaborators.

Write a News and Views type micro-review. This takes a relatively small amount of effort and establishes you as an expert in the field<sup>222</sup>. If you write a pithy, witty, and insightful review, you will probably be asked again, and also by other journals once you have a reputation for producing good pieces<sup>223</sup>.

### *The Junior P.I. Landscape - Lost in the Wilderness*



## Mentoring

As young scientists join your lab, you owe it to them to be a good mentor. Never forget that they are not your employees, they are your junior colleagues who have chosen you to inspire and teach them. As with parenting, leading by example is the most effective way of imparting your values. If you demonstrate

integrity, curiosity, and enthusiasm on a daily basis they will absorb these qualities by osmosis. You owe it to lab members to create an environment where people can genuinely enjoy science. Happy and fun labs are typically the most creative labs.

A good lab pursues meritocracy of ideas. The source of the idea, be it a high school student or the PI, is immaterial to whether the lab will pursue it. One of the best features of US science has been the flat hierarchy in basic science labs<sup>[224](#)</sup>. This is why you must insist that the lab members call you by your first name and not Professor or Doctor Last-name. Using an honorific establishes a barrier between you and your lab, setting a bad tone that will harm your scientific output.

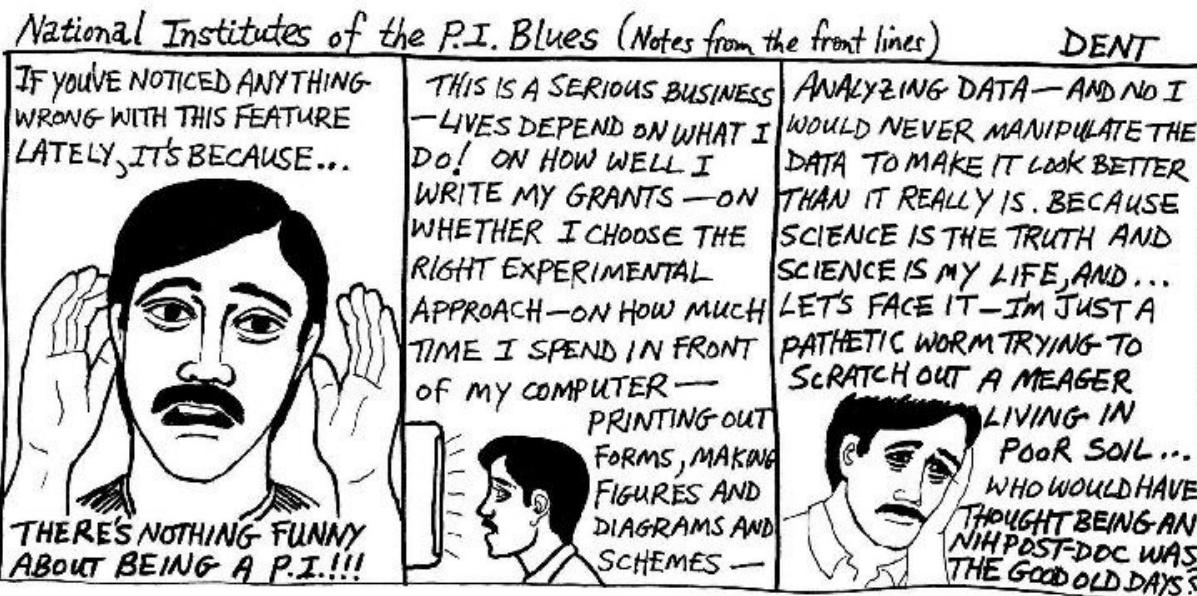
In recruiting lab members, you want to be sure that they have a good idea of what your values are and what you expect. The last thing you want is to have unhappy people in your lab. Their downer mood and non-productivity will infect the others and make your job much more difficult. So you need to be honest about yourself while recruiting, and should encourage the candidate to contact your previous mentees to get their take on your style. It is important to impart what sort of effort you expect in terms of hours per week and vacation days per year<sup>[225](#)</sup>. If there are any specific safety issues (radioisotopes, infectious diseases) you need to let them know. Also, if animal studies are likely to be involved in their work, or even the work of others in the lab, you need to be sure that they are comfortable with the use of animals in research<sup>[226](#)</sup>.

Obviously, new graduate students will require a lot of input at the start. You need to establish from the start the importance of reproducibility and the inclusion of appropriate controls in every experiment. Post-docs are a different story, but those that are changing fields will likely require a lot of feedback until they get on their feet. The amount of your daily involvement in lab member's experiments should vary according to what they need and want<sup>[227](#)</sup>. Some lab members will want to discuss every experiment with you. This is fine (it can be really fun) as long as you feel they are developing the capacity to work independently. Others will go for weeks or months without setting foot in your office. This too is fine, as long as they are on the right track and are productive.

The overall productivity in your lab is best tracked in lab meetings, which have enormous value. Lab meetings should occur weekly, if nothing else to discuss general lab issues (equipment issues, supplies, cleaning etc.). In some labs, each person describes what they have accomplished in the previous week. In others, (typically bigger) each lab member gives a semi-formal presentation, in which they briefly introduce the topic and their previous results and then bring

the lab up to speed on their new data. This provides excellent practice in giving presentations, which will be valuable to lab members regardless of their future occupation. Further, having to describe their progress (or lack of it) will help non-productive lab members to recognize a need for improvement. Encouraging everyone to join in productive, well-meaning criticism, questioning assumptions and out of the box thinking will improve everyone's research and enhance inter lab collaboration. If questions or comments become aggressive, you need to step in quickly to stop it and then deal with the problem behind closed doors.

Keeping up enthusiasm is one of your major tasks as PI. Negative adjectives should not be used in commenting on the efforts of your mentees. Personal criticisms should be reserved for private meetings, with very, very few exceptions. Take every opportunity to give positive feedback. Even if someone has a dumb idea or question<sup>228</sup>, start your response with "that's a good idea/question" before carefully explaining why it isn't. At the same time, it's fine to ask tough questions and point out findings that have to be shored up or performed at a higher technical standard, as long as you use the right words and tone of voice<sup>229</sup>



To deal with private meetings and phone calls you need a sound-proof office. Though there are legitimate reasons to close your office door for prolonged times if you have to concentrate on a task, it is a good idea to have an open-door policy. This will encourage your mentees to share their ideas, findings, and concerns over their progress or lab problems. If you don't enjoy these encounters, you are missing a large part of the joy and pleasure of being a PI. It's

not all roses, of course. Sooner or later (probably sooner), you will have to deal with tears. You'll get used to it.

After a reasonable period of time<sup>230</sup>, you owe your mentees your honest appraisal of their work and career potential. In NIH intramural we are now mandated to meet yearly with each mentee to discuss these issues. This is a good policy. Left to their own, most PIs will avoid such meetings, fretting over giving bad news. If you are sure, however, of your concerns<sup>231</sup>, you are wasting both your time and the mentees time by not trying to improve their performance or allowing them to succeed in another lab or job. Even talented and productive mentees can get in a rut and need a jolt of reality to reach their potential.

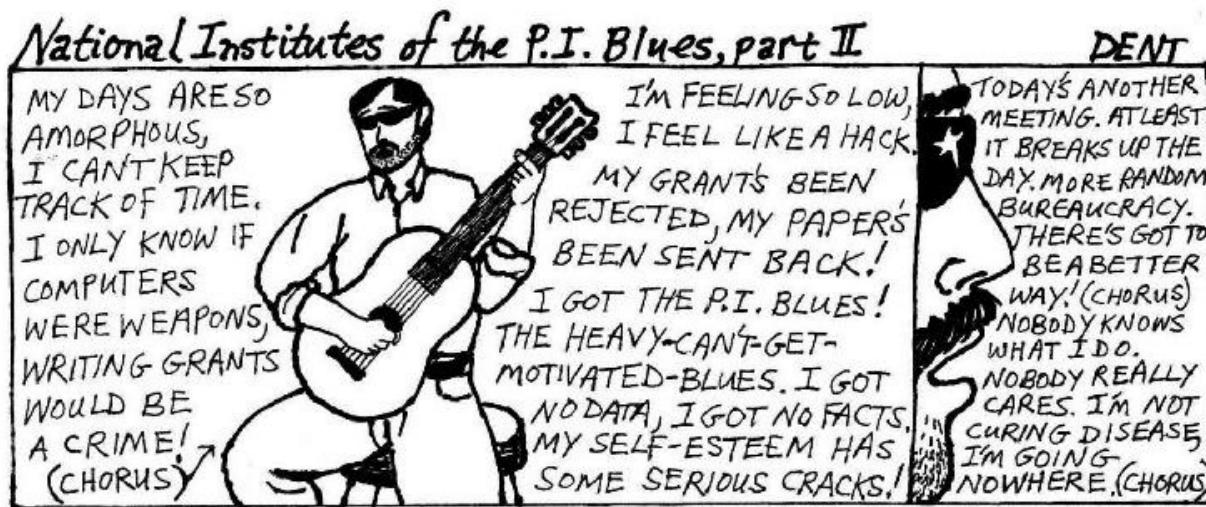
Some people are just not suited for academic research, and it is your job to inform your mentees when you are certain about this. This should never be delivered as a fact, but as your personal opinion, with the proviso that the person is free to try their luck in a new lab. The most common emotion I have encountered in such circumstances is relief. Often, the mentee has already reached this conclusion but has kept mum for fear of disappointing you. At this point, you can discuss other career options based on their strengths exhibited in the lab.

Unfortunately, mental illness is a common disease, particularly among young intelligent people. If you suspect that a lab member is mentally ill you need to get professional help ASAP. Most institutions mechanisms to help deal with these and other health issues. If mental illness (or other behavioral issues) jeopardizes the safety (or even productivity) of other lab member, it is your responsibility to effectively deal with the situation with alacrity.

One of the most difficult things to learn as a PI is how to fire post-docs or technicians<sup>232</sup> who are non-productive or disruptive<sup>233</sup>. If you have given the person a fair chance at improvement, you have nothing to feel guilty about. Your career, and the careers of others in the lab, depends on lab productivity. It is more selfish not to take appropriate action, so steel yourself, and cut the person loose. Be firm, and tell the employee that decision has been made and there is no room for appeal. It is never pleasant, but it will be easier with practice. Life goes on after a few sleepless nights.

Unless the employee is dishonest (or evil), you can in good faith, help them get another job. In your recommendation letter (or conversation) you can stress their qualities, putting less emphasis on their deficiencies. You will need to have a good answer to the question of why the person is leaving. You can couch your answer in an honest way that does not eliminate the person's chances at the new job. Usually, the person was not right for your job, not any job. You must

suspend your loyalty for dishonest people, who can cause great damage to society. Be careful, however, in what charges you level in writing, since the person can sue you personally. Even if you are correct (and you might not be, as in science, the truth with people can be slippery), you will likely suffer enormously emotionally and financially<sup>234</sup>.



You will have to decide how much you want to merge your personal and lab lives. Some PIs treat their labs as extended parts of their family, with frequent group outings and meals with all lab members and spouses and kids included. Some even have lab members babysit their kids. This is OK, but still it should be remembered that the relationship with lab members is asymmetrical. To some extent they will feel pressured into joining your extended family.

If you want to avoid this conflict of interest, it is fine to draw a line between your home and lab life. This is easier in the anonymity of a large urban area, where you will be unlikely to run into your lab members or colleagues or share schools, teams, or social activities.

## Department Life

Most lower level administrators and administrative assistants are well meaning individuals just trying to do their job without breaking organizational rules and regulations. At each end of the spectrum, some are passionately devoted to supporting your research, while others are lazy and interested only in a paycheck. In either case, you need to be nice to them, for they can make your

life hell. Never losing your temper applies even more to administrators than lab members, who will generally be more forgiving, since they see more of your good side.

If possible, you should develop personal relationships with support personnel. This social interaction serves as a general-purpose lubricant that will smooth potential conflicts. As you build time in an organization, you should be accruing a favor bank based on helping others when they are in need<sup>[235](#)</sup>.

Gravely concerned with your ability to raise grant supports, most departments will assign you a faculty mentor to provide advice on funding and getting your lab off the ground. You are free to seek other mentors as needed. Senior scientists, tempered by years of hands on experience, are particularly good at providing advice on personnel issues. It will help your decision-making process just to explain to a peer exactly what the problem is.

You need to have a positive relationship with the department Chair. If you are lucky, they will have time to spend with you freely discussing science and life, but given their other responsibilities, this might be a luxury they can't afford. You will need their support should funding difficulties arise, and when time comes for tenure review, their enthusiasm can tilt the balance if the decision is close. Indeed, if your Chair leaves prior to your tenure, you may be in serious trouble. Incoming Chairs, like male Lions<sup>[236](#)</sup>, have been known to devour the offspring of the departed Chair to make room for their own cubs. You can certainly inquire over the Chair's future plans when being recruited, but you might not have a choice between jobs, and in any event life happens, and Chair's plans can change.

If possible, acquire older scientist friends outside of your institution. I was tremendously fortunate to make two such friends during my graduate school days, Gustav Russ and Thomas Baechi. Gustav came in 1978 to our Wistar lab from Bratislava to learn how to generate monoclonal antibodies. Though 13 years older, we hit it off immediately, and began doing experiments together. At that time Bratislava was part of Czechoslovakia, and although Austria was just a few miles across the Danube, Bratislava was very much behind the Iron Curtain. As you'd expect, he had a refreshingly different take on science. Thomas, a boyhood friend of Walter Gerhard, my PhD advisor, came a few years later from his lab in Zurich to do a summer sabbatical. Once again, despite a 15-year age gap, we hit it off and began to work together.

In both cases, I was rewarded with life-long friends who could provide wise career and life advice from the invaluable perspective of experience. Gustav wound up spending 5 years in our lab at NIH as a visiting scientist. He

contributed to many of our most important papers and ideas and was a constant source of wisdom for our post-docs. Thomas was a collaborator for decades who I visited often, spending perhaps 4 months in Zurich over the years (he inherited a charming house in the center of Zurich). Both had<sup>237</sup> sophisticated senses of humor, certainly among the most valued traits in a friend. Both welcomed me into their families with open arms (and vice versa). Sharing the vicissitudes of life and science with these two very wise men is one of the very best parts of my career.

## CHAPTER 13

### *What's Wrong with Biomedical Research and How to Fix It*

#### **Eating our young**

Every career needs to constantly renew itself with an influx of young people. This is particularly important in science, where energy, creativity, and intensely focused effort are needed to challenge dogma and discover entirely new vistas. The current system in the USA and most other countries may well have been deliberately designed to minimize the contributions of young scientists and even discourage them from pursuing a research career.

Aside from being grossly unfair and hypocritical<sup>238</sup>, this is a suicidal social and economic policy. Knowledge, the product of research, is essential for improved standards of living and for economic competition. From every practical perspective, we should do everything possible to encourage the very best and brightest young people to enter science.

What discourages them? In the USA, the average time to a biomedical PhD is about 7 years. At least a PhD is not a terrible deal financially: students do not pay, and in fact are supported reasonably well (~25K per year). On the other hand, they do not receive retirement benefits or contributions to social security.

Second, is the length and pay of the post-doc fellowship. Once upon a time a year or two interlude, the post-doc has swelled to a 4-to 6+year ordeal. Here, pay is a huge part of the problem. The reward for 50 hour+ weeks and low income for your PhD is another 50+ hour job that typically pays less than what cabbies make (\$40K per year is the mandated NIH starting wage) with no retirement or social security benefits. At this point (average starting age of over 30) many post-docs start their families (or would like to). Unlike their college peers who are buying houses, they often qualify for low income housing.

How can we justify this? I mean, really? Post-docs are the most productive people in the lab (good students become better post-docs), meaning they make most of the discoveries. And we agree that the future of humanity depends on

the knowledge they generate. So, how can we possibly justify paying them so dismally?

# National Institutes of Hysteria



**ANOTHER NIH POST-DOC IS SWALLOWED BY THE SCIENTIFIC FOOD CHAIN**

It gets worse. It's not as if a lucrative academic career surely beckons. The

official NIH statistic for the fraction of PhD students that become a PI is 14%. Most scientists believe that this is at least twice the real rate. But even if it accurate, the odds of becoming an independent scientist are slim.

OK, so maybe you are thinking, “yeah, it’s hard, but then they have it easy”. Really? The starting salary for a tenure track biomedical researcher is OK (~90K per year), but this is only attained at age 38 on average, and then a career is not guaranteed. Rather, unless the new PI can attract sufficient funding for their research they will be unceremoniously dumped. And even if the “young” PI manages to clear the tenure hurdle, this does not typically guarantee employment. Rather, the fight to obtain funding persists for the entire career. And though the salary is decent (topping at ~ 150K per year), riches are not in the offing unless you happen to hit the jackpot and obtain a lucrative patent. Further, upon retirement at 65, there will only be less than 30 years of accumulated investment, as opposed to 45 years for those who immediately entered the work force.

Although “young”<sup>239</sup> scientists are older and older, the field is still tilted against them in the funding wars. While NIH makes a special effort to increase the success rate of first time R01 applicants, the success rate for renewing the first grant is abysmal. Further, many young PIs, unable to raise sufficient funds, are forced into (or even recruited into) large collaborative grants, where their freedom can be severely compromised. NIH has increased the fraction of the budget going to various program grants (typically P or U awards), that are typically headed by politically savvy senior scientists.

Some of these program grant leaders are truly excellent scientists who are devoted finding the truth and are genuinely interested in supporting “young” scientists and willing to give them the freedom to challenge dogma, even the dogma that the grant is based on. Others, however, are less saintly. Many powerful scientists are just that, powerful scientists, who have little or no interest in the truth or in other people, for that matter.

I know of NIH funded program grants where each participating investigator must submit their publications to a publication committee for approval. What are the project leaders afraid of: that someone in the group will dare to challenge the premise by which the grant was obtained? In what sort of world is this OK<sup>240</sup>? If we were trying to destroy the independence, creativity, and enthusiasm of “young” scientists we could hardly do better.

Faced with these difficulties, many of the best and brightest will choose another career. What we owe undergraduates contemplating entering a PhD program is a near guarantee<sup>241</sup> that if they are talented, work hard, and produce

good papers that they will have a long and joyous<sup>242</sup> career in science. Anything less is not acceptable: not if we wish to have a prosperous country with a continuously rising standard of living.

## Publications

Publishing papers is at the very heart of science, and if the process is corrupted, science will be corrupted. There are major problems in the publication process that compromise the entire field of biomedical research.

First is the power of the luxury journals. Publishing has turned into a highly lucrative business for several large houses, with profit margins nearing 40%<sup>243</sup>. This enables them to hire former researchers to run the journals as professional editors, journalists to provide interesting content, and artists to produce arresting images and diagrams. Although with online publishing, these journals could publish every good paper they receive with little additional costs, they maintain high selectivity to maintain their cachet, exploiting humanity's weakness for the elite.

Publications are the currency for scientific careers, and having papers in luxury journals greatly increases the chances of getting a PI job, being promoted, and obtaining grant support. The gatekeepers of these journals, therefore, wield great power in shaping career prospects, but also in scientific priorities. If luxury journal editors consider a field boring, they will not even send papers out for review.

Let's examine this in more detail. Who are the editors? They are generally highly dedicated, well-meaning individuals who love science and who have the very difficult job of dealing with distraught irate scientists whose work they must routinely reject<sup>244</sup>. But why are they editors and not PIs? Often, they failed to demonstrate sufficient talent to stay in the lab. In many cases, this was a failure to appreciate what is truly novel and important.

Why would we entrust these people to have a large say in the course of scientific research? Further, why would we allow for-profit journals to have this power, in any event? Because they are never swayed by the interests of their advertisers in selling reagents and machines that are particularly useful for given areas of research?

The journals only wield this power because we scientists give it to them<sup>245</sup>. Search-, promotion-, and funding committees don't have to base their decisions on the impact factor of the candidate's publications.

Second, is the prolonged tortured process that publication has become, particularly in the luxury journals. Papers can easily be in review for more than a year. The journal editors are the final gatekeepers for which papers are published based on the advice of scientist reviewers. Typically, the luxury journals send papers to three reviewers, and recently I've heard from colleagues that *Nature* now typically sends their papers to four reviewers. Since the journals are not paying the reviewers a dime (for many hundreds of dollars of advice, even at the hourly rate of scientists), there is no financial disincentive for using more reviewers. Getting three scientists to agree on anything is unusual, and adding one more makes it nearly impossible. Reviewer disagreement is one of the major impediments for more rapid publication.

On the other hand, most of the problems in reviewing papers come from the reviewers, not the editors. Scientists have become increasingly demanding and negative in the past 20 years. A likely major cause is a negative feedback loop: your paper/grant is trashed, reducing your enthusiasm for praise. Another factor is the increasing trend to using less experienced reviewers (as busy PIs recruit their lab members to review papers they are ostensibly reviewing themselves [246](#)). Young scientists are typically taught in journal clubs that every experiment is terribly designed, poorly performed, and incorrectly interpreted. Being a bit insecure and wanting to show their erudition, they typically are overly critical of the work and overly ambitious and unrealistic in suggesting additional experiments.

In a strange psychological phenomenon, scientists often forget that they too submit papers and suffer from the demands and harsh words of reviewers. Young scientists are particularly sensitive to irresponsible comments of reviewers. Most papers represent years of intense effort in generating the data and months of intense effort in writing the paper. Words like *careless, sloppy, poorly written, ill-conceived*, can damage young scientists and destroy their ambitions. Reviews should be as constructive as possible. The job of the reviewer is not to serve as gatekeeper, but to improve the paper by making suggestions that can be done with a reasonably modest effort. Further, the authors should have the right to interpret their data the way they see fit: they have earned the right to show the world how dumb their ideas are.

Reviewers, particularly young reviewers, tend to view papers in their field as threats to their careers. This is nearly always a short-sighted and incorrect attitude. Competition these days is largely between fields, not within them. A rising tide in a field raises all of the boats. Further, your own work will go faster and be better if you are in a competitive field. Competition is needed to generate and test hypotheses in an efficient manner. Other labs will generate data and

reagents that your lab will profit from. If you field does not have this attitude, it is your job to instill it<sup>[247](#)</sup>. This is what leadership is about.

The ultimate solution to the problem with publishing is that biomedical scientists must change their ways. There is no reason we must be in thrall to luxury journals. We can adapt the mores of physics and math where papers are first posted to an internet archive for all to read<sup>[248](#)</sup>, and only then submitted for publication. We can publish all, or nearly all papers in journals run by scientists for scientists. We can refuse to review papers if there are more than two reviewers. We can base hiring, tenure and promotions on actually reading a candidate's papers and listening to how they present their ideas and plans. We can base on self-worth on our treatment of our trainees and colleagues and not the number of papers we have published in luxury journals.

## Grants<sup>[249](#)</sup>

Biomedical research in the USA is in a crisis. Established scientists with decades of accumulated knowledge and wisdom are permanently losing their research programs or positions due to their inability to obtain research grants. After graduating from college, junior scientists spend ten to fifteen years in "training" at wages and circumstances that repel many of the most talented and ambitious individuals. The trainees that manage to obtain coveted tenure track positions (perhaps 10% of PhD recipients) face severe pressure in obtaining their initial grants (rewarded at the astonishing average age of 43), and particularly their first renewals, forcing them to abandon promising research careers (with an uncertain future at nearly 50 years old). Those who manage to survive the funding crunch perpetually face the imminent threat of defunding, and spend a significant, and ever-increasing portion of their time writing grant proposals to maintain their labs.

These problems are obvious to every working scientist who relies on the NIH extramural grant system to support their research, but who is to blame? Is it the executive and legislative branches of the US governments, who simply don't provide enough money to support the research establishment? Funding can certainly be increased enormously with easy justification in terms of scientific opportunities and economic benefits, this avoids the major flaw in the system.

The fundamental problem is that with every increase in the NIH budget, the US biomedical establishment expands well beyond what the increase can support. This bubble-based cycle exerts an enormous toll in both human misery

and inefficient use of human resources.

This pattern, which has persisted for at least 20 years, principally results from the unregulated expansion of US biomedical research, where every dean is a potential entrepreneur angling to increase the size and prestige of their institution. Merging of the biomedical training and labor pools (*i.e.*, nearly all of the work is done by graduate students and post-docs) compounds the problem. Expanding universities recruit far more graduate students and post-docs than the number of new faculty positions created, *decreasing* the fraction of trainees who attain assistant professorships, and prolonging the time until independence, as competition for the limited number of tenure track positions increase and c.v.s of applicants lengthen.

Uncertainty in successfully funding research grants leads investigators to maintain a super-optimal number of grants to buffer against dipping below the optimal funding level: *i.e.* the amount necessary to maintain a lab of sufficient size and expertise to progress on a research topic. Such overfunding of the most successful labs (defined in terms of funding, not necessarily science) fuels a negative feedback loop of decreasing grant success and increasing pressure to obtain them.

Here's the big picture of the current situation. We biomedical scientists have created a system in which highly intelligent, extremely hard-working individuals who spend years in training at low wages must struggle to maintain a career that serves the public good. Bluntly put, this is crazy. Why should talented and ambitious youngsters sign up for such misery? Is there any doubt why so many wind up on Wall Street? We scientists can do better than this for ourselves and for the future of science, which is inextricably linked with the economic, health, and social well-being of our society and nation.

Ending the boom-bust cycle of NIH funding requires rationally planning the number of NIH supported investigators and trainees. But, how to create such order from the chaotic system in place? I suggest extending the model of the NIH intramural funding system to the entire NIH funding system.

US government of funding of biomedical research is so pervasive through direct and indirect mechanisms, that with exceptions (*e.g.* HHMI investigators, public employees of state universities), NIH funded investigators are already essentially *de facto* employees of the US government. The idea is for the government to employ grantees *in situ* (like HHMI investigators), where they would maintain their positions in their institutions and their participation in teaching and other institutional obligations.

Rather than submitting grant proposal to fund their research, investigators would be allocated funds based on four-to-five year reviews of their productivity

and general direction of their research.

This would have enormous benefits. It would end the fiction that important discoveries can be accurately predicted in advance by grant proposals. It would end the corrupting prevarication that the “proposed” research hasn’t already been started (if not finished), and the common practice of using grants to fund more promising ideas not included in the proposal (which is increasingly running afoul of government auditors, clueless of how science actually advances). It would save enormous amounts of time in writing and reviewing grants. It would prevent grant reviewers from filching ideas from proposals<sup>250</sup>. It would free the imagination of investigators, who would be able to pursue their *best* ideas, and not their most *fundable* ideas.

But here’s the deal. No more super-sized labs, unless mandated by special circumstances. Again, the model is the intramural program, with an average group size of approximately 8 investigators (one PI, one technician, one PhD level staff scientist, 5 post-docs/students). Although it is common knowledge that large labs are typically inefficient, their PIs typically have mastered the art of funding. Trimming such labs would free resources to support more independent investigators. It would also greatly encourage collaboration between groups with different expertise, increasing collegiality and scientific excitement in institutions. Efficiency should increase too, since expertise will be shared between groups and not have to be created by each group.

The productivity of the proposed “extra-intramural” system is a critical issue. Given the advantages of a direct funding mechanism described above, true productivity, as measured in important discoveries should increase. A key to maintaining productivity will be the effectiveness of the review process. The NIH intramural system provides an example of fair yet rigorous peer review process that occurs on a quadrennial basis. Non-productive laboratories are typically closed after two consecutive substandard reviews (*i.e.* over an 8-year period), and the resources re-assigned to a newly recruited, typically tenure track, investigator.

Such junior investigators are hired by the standard process employed by universities: a nationwide search is conducted to identify the best candidates who are selected by a committee composed of senior scientists in the field of interest, with the ultimate hiring decision made by the department chair (whose leadership is also subject to quadrennial review). The potential of junior scientists is judged after a 5 to 6-year period principally by the quality of their publications, with advice sought from 8 to 10 world experts in their field. Candidates must have demonstrated that their productivity is largely due to their efforts, and is not based on collaboration. The offer of “tenure”, *i.e.* becoming a

full government employee, is made only on the advice of two Promotion and Tenure committees: one at the Institute level, the other at the NIH level. Further promotions through the academic ranks (with commensurate salary increases) are made via the Institute committee, but with oversight from the Institute and NIH intramural directors.

The greatly relaxed competition for funding will have huge payoffs in the psychological state of individual scientists and the entire scientific enterprise. Today's intense pressure brings out the worst in human nature, eroding the integrity of the research culture: fudging and outright fraud is increased in such circumstances, particularly in grant proposals, where data are preliminary and not subject to the crucible of reproducibility by other labs. The cycle of pain that accompanies repeated grant rejections contributes to a poisonously critical atmosphere that saps creativity and kills the spirit and joy of science.

Don't get me wrong. Constructive criticism and vigorous competition are essential to the scientific process. The truth can only be approached by constantly proposing, testing, and remolding hypotheses. Human nature is such that this process is greatly accelerated by competition between investigators with common interests. The competition must be collegial, however, and based on respect, not fear.

Obviously, it is not possible to immediately remedy the present state of affairs. We can, however, begin to offer top level investigators as well as tenure track investigators the opportunity to become extra-intramural investigators as a carefully monitored pilot program to compare the productivity and happiness of investigators in the new vs. traditional grant system. If warranted, over a 20-year period, we can gradually move to the new system.

## Endnotes

<sup>1</sup>Once I learned that companies would mail them gratis upon request, I received dozens and dozens, to the dismay of our postman, whose back bore the brunt of my curiosity.

<sup>2</sup>[Arrowsmith](#) should be required reading for scientists and MD's alike. Arrowsmith's description of science and medicine rings true 90 years after publication. Paul De Kruif, a working microbiologist, gave considerable assistance to Lewis. His [Microbe Hunters](#) is another must read, particularly for future immunologists and microbiologists.

<sup>3</sup>Could puberty have had anything to do with it....hmmmm?

<sup>4</sup>Thanks to Tessa Campbell, for finding the title and author of this book. It eluded me for years.

<sup>5</sup>An important lesson in life is that it really hard to predict how things will turn out. Often, some seemingly terrible thing turns out be a real blessing, and *vice versa*.

<sup>6</sup>The assistant Rabbi who leads the singing and traditionally is responsible for educating the bar mitzvah students.

<sup>7</sup>A lavish party typically accompanies an American bar mitzvah. In typical youthful myopia, I missed the irony of an atheist making this accusation.

<sup>8</sup>The Japanese have a saying translated as "*the nail that sticks out gets hammered down*" meaning difficult people will be marginalized and ostracized. This attitude may be good for social cohesion, but is awful for encouraging the kind of creativity that spiritually and materially enriches society.

<sup>9</sup>Mitigated somewhat by not being able to escape your past. Even events from

kindergarten lingered in collective memories.

[10](#) Except in French, my bête noire, whose Regent's exam my teacher bribed me not to take by inflating my grade, an early lesson on life's grim realities.

[11](#) I still don't get poetry. No one's perfect.

[12](#) Today you could do it in a few hours by nucleic acid sequencing....but that wasn't the point, anyway.

[13](#) This is marked contrast to today, when nearly all high school seniors aspiring to major in science at top universities, typically on their way to medical school, have spent summers, at least, in research labs. There can certainly be too much of a good thing. There is plenty of time in life for organized research. High school should be a time for self-guided exploration, not building an impressive c.v. by 16.

[14](#) Never underestimate the old boy network!

[15](#) Only clinically psychotic students fail to graduate medical school in the USA if they make any sort of effort.

[16](#) The pediatric resident who wrote it was a small-minded control freak. Stanley Plotkin, the attending physician who signed it, should have paid attention to whether I was really in need of psychiatry. I had barely interacted with him.

[17](#) To practice medicine in the USA, a year of internship is required in conjunction with passing part 3 of the national boards

[18](#) All too common in the USA, where physicians focus on the typical and often miss the unusual and interesting

[19](#) Norman was the first scientist to truly work with monoclonal antibodies, *i.e.* antibodies derived from single B cell clone. This was years before hybridoma technology was invented.

[20](#) A classic example of awarding the prize to one team for a discovery based on the groundwork laid by a number of laboratories, including Norman's.

[21](#)More than half of novel drugs brought to market today are monoclonal antibodies, for treating infectious diseases, autoimmunity, and cancer.

[22](#) Walter was only officially allowed to supervise two Penn students, so a little bending of the rules, a Wistar specialty, was required to recruit Lou. Ten years later, I also helped bring Lou to NIH, where he's had a spectacular career pioneering cancer genetics.

[23](#)Among other products, Centocor developed Remicade, the first effective monoclonal antibody for autoimmunity, which has enormously benefited humanity. This story is not black and white.

[24](#)Yes, I would have gladly taken the money, too!

[25](#)Romantically, we met under a giant toxic megacolon, at a med school welcoming party at the [Mutter museum](#).

[26](#)The Brits would call it a flat, of course. In medical school, I had a friend who returned from a few months in Cambridge affectedly using every possible UK version of words (lift for elevator, aluminium for aluminum, *ad nauseum*). From this I decided to stick religiously to American usage, even while in Rome!

[27](#)Indeed, looking back on a career centered on virology and cell biology, the courses that I did best in at Princeton were virology and cell biology, where the concepts came naturally to me. It didn't hurt that each was taught fantastically well, respectively, by Arnie Levine and Fred Meinz.

[28](#)OK, there is one method has always worked on the few occasions when the stars have aligned a close colleague enthusiastically recommends their PhD students for a post-doc. If nothing else, the colleague doesn't want to have deal with your disappointment/ire when you run into each other at meetings.

[29](#)As in the movie Chinatown (Oscar nominations for Jack Nicholson and Faye Dunaway), she is both my scientific daughter and granddaughter, since she became the first post-doc of our first post-doc, Ike Eisenlohr.

[30](#)Today a young scientist is anyone under 40. The average starting age in a

tenure track job is 37. The first RO1 is received at age 43 (45 for MD PhDs). I was 29 when I went back to Wistar, and 30 when I received my first R01. This was normal in 1983. We are robbing 10 years from young scientists, making them work for some else for their most productive and creative years as a bench scientist. For more on this, listen to [this](#).

[31](#)Though with [questionable scientific skills and a deeply flawed personality](#).

[32](#)This was also a positive feature of the Wistar Institute, where Koprowski was the decider in chief.

[33](#)The relationship is typically inverted in large organizations.

[34](#)Not to follow rules and regulations, which increase each year (along with administrators), causing a general sclerosis and malaise among the scientific staff.

[35](#)As I said, Koprowski had an excellent eye for talented young scientists

[36](#)In large part due to the heroic efforts of the Surgeon General C. Everett Koop, ironically selected by Reagan due to his religious orthodoxy. Dr. Koop was one of my professors at Penn. From a student's perspective, not a very likable man, with no discernible sense of humor and clearly in love with himself. But, a hero of mine just the same for his courage in speaking truth to power and in exerting real leadership, which always come with a steep personal cost. This is a test of personal integrity, and Koop passed with flying colors.

[37](#)“Discoveries pertinent to medical progress have often come from remote and unexpected sources, and it is certain that this will be true in the future. It is wholly probable that progress in the treatment of cardiovascular disease, renal disease, cancer, and similar refractory diseases will be made as the result of fundamental discoveries in subjects unrelated to those diseases, and perhaps entirely unexpected by the investigator. Further progress requires that the entire front of medicine and the underlying sciences of chemistry, physics, anatomy, biochemistry, physiology, pharmacology, bacteriology, pathology, parasitology, etc., be broadly developed. Progress in the war against disease results from discoveries in remote and unexpected fields of medicine and the underlying sciences.” How could our leaders in science have forgotten this message?

[38](#)In the past 35 years, Congress have passed a regular appropriations bill only 4 times. During “continuing resolutions” the government must essentially guess at the level of funding based on recent funding history.

[39](#)You can guess how this turned out. Hint: “trust but verify” is generally good advice.

[40](#) Which lacked amenities like an intercom system (the lab was separated from the offices by 100 feet or so), loading dock or ancillary employees to perform standard tasks like pushing newly delivered large equipment up the stairs or taking care of the antiquated air handling system, which routinely needed to be reset. The animal colony would only be finished in two years, which meant fighting tooth and nail with administrators to have a government car to reach the temporary facility located 5 miles away.

[41](#)Just joking.....

[42](#)In formal terms, the “hypothesis”, which scientists usually avoid saying out loud using for fear of sounding like a 6<sup>th</sup> grader.

[43](#)This may seem like an exaggeration, but it is not. Smallpox killed more than 300 million people in the 20<sup>th</sup> century alone, before its vaccine-based eradication in 1979. With a mortality rate of 30% and an infection chance of near 100% over a lifetime, it would kill more than 2 billion of the 7 billion people alive today. And that’s just smallpox.

[44](#)“Many forms of Government have been tried and will be tried in this world of sin and woe. No one pretends that democracy is perfect or all-wise. Indeed, it has been said that democracy is the worst form of government except all those other forms that have been tried from time to time” Winston Churchill, to the House of Commons.

[45](#)Unfortunately, as in other careers, the same personality type is favored by their clever manipulation of the reward system.

[46](#)Basel Institute, Bell Labs, Roche Institute...there may be a few more, but not many.

[47](#) NIH funding provides trained workers and discoveries that lead to drugs (and often the actual drugs themselves) at no cost. What's not to like?

[48](#) This contrasts with a historic average of annual increases of 3.3%. Cutting NIH funding (taking inflation into account) is beyond stupid, though in 2016, Congress passed the first permanent inflation adjusted increase in NIH funding in 12 years, with [Newt Gingrich](#) (yes Newt) pushing for rapidly doubling the budget. Newt, aside from FDR, is probably the politician who has done the most for NIH. He played the key role in the doubling that occurred during the Clinton administration. [John Porter](#), a republican congressman from Illinois, was at the tip of the doubling spear, and dedicated his post congressional life to supporting biomedical research. He is in my pantheon of scientific heroes: there is no science without \$\$\$!

[49](#) Which is a fantastic job for those who passionately enjoy imparting their knowledge to young people and shaping their aspirations and lives.

[50](#) The very best universities typically provide financial assistance based strictly on need. If you come from a lower income family, these are actually the last expensive schools to attend, since they provide scholarships that cover room and board and work study opportunities to cover other living expenses.

[51](#) The weird, weird world that is quantum mechanics is fun, but optional. Eventually biology will have to be reconciled with quantum physics, but the day seems far off, unless you are interested in biophysics.

[52](#) To put this in perspective, the average starting salary for a science/technology job for newly minted college graduates is approximately \$60,000.

[53](#) I've noted a distinct direct relationship between the rank of the graduate school and the respect afforded to graduate students, that I attribute to the confidence that faculty have about themselves. Confident people tend to treat their junior colleagues much better. They have no need to establish a dominance hierarchy.

[54](#) They are more worried about you than you are about them! They are about to invest a lot of time and effort into you, and they don't want knuckleheads or emotional cripples. So, they will pay for your visit so they can interview you in

person. They will also treat you well, very well indeed. If they like you, they want you to like them and accept their offer. But don't be fooled. You will not be treated this well until you are wooed for an assistant professorship in 10 years, if all goes according to plan.

[55](#)There might be too much, if you were a serious science student as an undergraduate, or too little, if you have a lot of catching to do.

[56](#)I visited a famous university where a number of people told me that half the PhD students were on anti-depressants. My advice: don't go there! In any lab or department, there will always be some unhappy individuals. But get a feel of the overall vibe.

[57](#)This may be negotiable with the department. You are going to need to be a bit aggressive in your career to make headway, and this would be a good place to start. Don't assume that administrative rules are set in stone without testing the boundaries.

[58](#)For several reasons. You probably don't know your scientific style since you are just getting started. At this age, you are not as good a judge of character as you will be later on. Indeed, one of the few mental areas that improves with age (nearly all others decay) is insight into human nature.

[59](#)Which will soon be beaten out of them by overly competitive system of grant funding and publishing.

[60](#)Particularly these days, when there is so much competition for tenure track jobs.

[61](#)In large labs, a few highly productive individuals can produce enough data to fund a large enterprise. In such labs, it's often every man for themselves.

[62](#)And that there is no such thing. Every system devised by man (like biology itself) involves making compromises between opposing ideas and properties. People who believe in utopia are dangerous, and more so in leadership positions where their single mindedness inevitably leads to unmitigated disasters.

[63](#)I finished in 5.5 years with three first author papers where I did all of the

experimental work. I was a good student, but this is probably impossible today, even with a herculean effort.

[64](#) By correcting errors.

[65](#) Particularly these days, when graduate students serve as a major part of the biomedical labor force. Money for research requires grants, and grants require research plans that increasingly, must be followed to renew the grant. More about this in Chapter 14.

[66](#) Many translational researchers sincerely believe they are doing basic research. Admittedly there is a fair amount of gray between basic and translational research. The major difference is that basic research is driven by curiosity about nature and has no immediate practical goal in mind.

[67](#) Big pharma plays all sorts of [games](#) to maximize their profits. Most of the “new” drugs approved by the FDA are variations in formulation, dose or combination, of old drugs.

[68](#) Like everything else, there are always exceptions.

[69](#) This is one of the major reasons for lab meetings. It’s also why you should tell your findings to whoever will listen. More about this later....

[70](#) Complete would be nice, but there are always things you don’t know.

[71](#) You should be thinking, why is this interesting or important? This is important, because flu costs a lot of money and kills a lot of people because of the ability of the HA to avoid antibody recognition. It’s interesting, because it addresses the basic question of how proteins fold (or misfold), oligomerize and are modified as they transport the secretory apparatus (or are degraded by the ubiquitin-proteasome pathway, the cell’s principle garbage disposal system)

[72](#) Just, if you happen to be a lab with freezers full of well characterized flu virus stocks, and work in laboratory approved for biosafety level (BSL-2) work.

[73](#) Of course, when you are just starting, nothing is easy, since you don’t know how to do anything, yet. But, remember that every expedition to a high, remote

summit begins with simple steps in the valley.

[74](#)This is something you need to learn anyway. Flow cytometry is not just for immunologists.

[75](#)Why wouldn't they, you might ask. There might not be any functional antibody in the tube. Tubes get mislabeled or mistreated (someone left it overnight at 56 °C and then put it back in the fridge). The antibody might not bind your virus, because your virus isn't what you think it is, or what you think you know about the antibody specificity is wrong.

[76](#)This gets tricky. The flu strain we are using may have originally come from humans, but it has been passaged extensively in mice and eggs, so its evolution is murky. Still, it's good to consider this.

[77](#)Unless you happen to study a virus that infects common cell types in human blood. Humans are very large animals (more than a 1000-fold larger than a mouse), and its relatively easy to get large numbers of blood cells.

[78](#)They do, however, evolve to grow optimally under these conditions once they are transplanted from an organism to a flask.

[79](#)Whose composition, remarkably (except for blood), is not known! So, if you'd like to know the "normal" concentration of say, amino acids, you are out of luck.

[80](#)Even if you clone them from single cells (not possible for many lines) they will soon diverge into populations, and because you might choose a strange clone to begin with, this is dangerous. This is a problem with making permanent transfectant lines, which often display clonal variation unrelated to the gene of interest, confounding analysis. Conversely, when experiments are going well, it is a good idea to freeze a large batch of cells that you can get back if/when things go south.

[81](#)The last thing you want is for antibiotics to mask poor technique. Not every infection is obvious, and visible bacteria are a good sentinel for breaks in technique. Here's something else: ribosome targeting antibiotics induce [mistranslation in mammalian cells](#).

[82](#)If a lab nearby has a cell microinjection apparatus, spend a day or two learning cell injection. This is a very useful technique, and more importantly, will give you a very good feel for how cells should look. Playing around with the location and volume of injection is super fun too (make the nucleus explode!) Better than video games. Try it.

[83](#)To study early folding of the protein, we have to catch the HA on the ribosome, since folding occurs co-translationally

[84](#)Surprisingly little is known about how amino acids are incorporated into proteins. We just [published a study about Cys](#). You would have thought that this was worked out decades ago. Scratch the surface of biology and you often strike the ignorance layer.

[85](#)[Soylent Green](#) is methionine!.... its methionine!!!! (or would be if Charleston Heston's character in the [eponymous movie](#) had been a cell biologist).

[86](#)Which really means as normal as experimentally feasible.

[87](#)Taking great care to dispose of the radioactivity properly, which is closely monitored by radiation safety personnel, who will revoke your privilege to use radioactive chemicals if you are careless and contaminate the lab. Interestingly, while in the USA liquid waste must be collected into storage vessels and radioactivity in the sink will be *severely* punished, in the UK, it must be flushed down the sink on its way to being diluted into the Atlantic Ocean.  
Hmmmmmmmmmm....interesting.

[88](#)But definitely not fine if you freeze them after the next step, detergent extraction. If cells are intact when frozen their highly crowded nature, several hundred mg of protein per ml, protects proteins from denaturation.

[89](#)I am not being snarky, they really do.

[90](#)Comparing different detergents can be another experiment in the series that will build, step by step, on knowledge from previous experiments. Patience is essential in science. If you are in a hurry, you will miss important details and often even discoveries. One of the major goals of the PhD is to teach you how to methodically examine a topic in detail.

[91](#)Bet you didn't know this!

[92](#)Sodium is the major cation (positive ion) outside cells, potassium inside. Often, potassium is used to mimic intracellular conditions. Antibodies evolved to work outside cells, so we will use sodium. For many applications, the two are interchangeable.

[93](#)Proteasome degradation generally requires a highly choreographed series of events that is disrupted by extraction, but residual activity is still a concern.

[94](#)Unless divalent cations are needed for stability of the protein of interest. For example, flu neuraminidase (NA) requires calcium for stability, and if you are studying NA, you will have to choose between the lesser of two evils.

[95](#)But don't tell PETA (people for the ethical treatment of analogies); they might get upset.

[96](#)Ubiquitin is a small protein that is used for many purposes (including targeting proteins for proteasome degradation). We would very much like to know if and when HA is covalently attached to ubiquitin.

[97](#)Bonus points!!

[98](#)Tiny wells are cut into the agarose to contain antibody or antigen. At the equivalence point, a beautifully delicate precipitate forms that can be seen clearly by eye when viewed with a light box on a black background. The similarities between antibodies or antigens can be judged by whether lines from different wells fuse with other, completely ignore each other, or form spurs. This elegant method is named after its inventor, Ouchterlony.

[99](#)I still bear the mental scars to this day.

[100](#)This very clever bug uses this mechanism to avoid the immune response, but pointing Abs molecules the “wrong way” to protect itself from anti-staph antibodies binding the “right way”. Nature is so cool!

[101](#)I've used the past tense advisedly. There was a time, when children walked to

school barefoot through 3 feet of snow and graduate students made their own reagents! There were few biotech companies, and basically you could purchase chemicals, but not proteins of any type, including antibodies. The downside: progress was much slower. The upside: you knew exactly what you were working with, and learned the underlying details of how the reagent was made. Today, many reagents and assays are purchase as “kits”, where the manufacturers’ greed precludes them revealing what is actually in the kit. I know of one example where a several hundred dollar “extraction kit” consisted of a few cents of a common detergent. I swear. Of course, the same companies base nearly all of their products on information provided to them for free through the publication process.

[102](#) Antibodies use one of a half dozen or so different constant regions, and protein A does not bind well to some common ones. This varies considerably with the species the antibodies derive from.

[103](#) IC should replace IP!

[104](#) We can control for this by pre-experiments where we determine how much antibody to add to how many beads, but we’d have to do this for each monoclonal antibody we want to test and for each batch of the same monoclonal antibody. More bonus points, if you are up for it.

[105](#) Many investigators, imbued with the proper spirit of science, provide cell lines or genes gratis to other investigators with essentially no strings attached.

[106](#) On the other hand, Abs are typically ridiculously expensive relative to their production costs. This being capitalism, companies price antibodies just below the cost of producing them yourself, regardless of their production costs. Unpurified polyclonal rabbit antibodies, useful for many things, are typically priced at \$3,000 per ml. Rabbits can produce 100 ml of serum, meaning each rabbit is worth a house or so, minus the cost of producing the antigen and serum, typically in the few thousand-dollar range, or less.

[107](#) On average, Met represents ~ 2% of the amino acids used in a protein. But it can vary widely (some proteins don’t have any).

[108](#) The more Met, the sooner the signal will be distinguishable from the noise

when we finally see the results! The sooner you get the results, the sooner you can plan and perform the next experiment.

[109](#) Immunocollection, remember?

[110](#) this reduces the number of washes we have to do, which improves reproducibility and reduces the work load, ultimately meaning we can do more experiments, because more experiments = more discoveries, right??

[111](#) Unless they happen evolved in thermophilic organisms, where proteins are quite happy at temperatures higher than boiling! The chemistry behind this is surprisingly easy, taking a few extra weak bonds here and there.

[112](#) Oops, I can't use this phrase anymore. But maybe irony is OK?

[113](#) I wonder what fraction of scientists who routinely use SDS-PAGE understand how the stacking gel works? [Here's](#) an excellent description.

[114](#) In this type of experiment, there is usually much more Ab than antigen, so we only detect the Ab

[115](#) A true story. I learned how to work with slab gels from one of my graduate student friends lucky enough to be working with an early adapter of the technology. Everything was new and awkward, but I made it all the way to the end with no major mishap. I was testing the very first monoclonal antibodies to be made to viruses, and was super excited, since this could be the first definitive evidence that we had monoclonal antibodies specific for any internal viral protein. So excited, that I wanted to watch the gel dry (think of paint drying for equivalent excitement). In front of my very eyes, like an airplane exploding, the vacuum seal broke and the gel exploded into hundreds of tiny pieces. My friend, a true trooper, seeing my despair, said, don't worry, we can put it back together. So, for the next few hours we played radioactive jigsaw puzzle and then redried it. It still sits in my notebook, along with the messy but clear x-ray film showing that we indeed had made monoclonal antibodies to the internal flu proteins, a key part of my PhD thesis. Booyaaaahhh!

[116](#) Indeed, in the 70's when this was all worked out, x-ray film developing machines were only available in hospitals (the films can be developed by hand,

but this is messy, slow, and poorly reproducible.) I would take my cassettes to the x-ray department at the main Penn hospital at night when things were slow. The technicians would kindly run the films through for me. Amidst the various broken bones and upper GI series, there were the images of radioactive flu proteins.

[117](#) Since [ $^{35}\text{S}$ ] has a half-life of nearly 3 months (i.e. in 3 months, half the radioactive particles have decayed), we can keep recording the radioactivity for months. For some experiments, it may take months to obtain a reliable signal. These are painful experiments, since waiting for an answer is unsettling and you can't plan the next experiment without knowing the answer from the present one.

[118](#) I had started working on these monoclonal antibodies as a graduate student. They had a number of interesting and inexplicable features that gradually led to the biochemical experiments. 40 years later we are still working on the remaining inexplicable features! Science takes time and patience.

[119](#) So, if you happen to be reading this as a college student and are contemplating a scientific career, pay attention! To wit: I had an outstanding post-doc in my lab who (like Dick Cheney), had "other" priorities during their first two years in college, and consequently graduated with limited knowledge of basic chemistry and biochemistry. They could overcome this by their work ethic, excellent hands, and creativity, but at considerable cost in fundamental understanding and power of analysis.

[120](#) It's also self-serving and convenient way for managers to take the credit for the output of the creative workers they supervise. This attitude is not uncommon among MD's who run large research groups and hire PhDs to perform the basic research components.

[121](#) If nothing else, to see what's wrong and see how much progress has been made, or the opposite: finding a topic that is long due for an update with new techniques and information.

[122](#) We recently published a highly cited paper which basically just extends on a classic series of studies in the 1950s with new technology. We went so far to obtain the PhD thesis describing these studies on microfilm and used the major protocol essentially verbatim.

[123](#) What's a good lab? A lab with a track record in a given field of getting things right. This may seem cryptic, but it's like at high school where everyone knows who the good teachers are. A tip off to identifying unserious scientists: they frequently change fields as mistakes pile up and reviewers in each successive field wise up to poor quality of their work.

[124](#) These are the most highly cited journals, which for basic science include: Cell, Nature and Science. Each discipline has its own members as well: for immunology, for example, this would include Nature Immunology, Immunity, and The Journal of Experimental Medicine. Though criticized for their superficiality and frequency of errors, they still publish a disproportionate share of the most interesting and important papers in many fields, so they can't just be ignored.

[125](#) These inter-disciplinary collaborations are typically the best, since there are minimal competitive issues between the labs and provide the best chance for breakthroughs due to their novel approach to the problem.

[126](#) But not necessarily with those outside your lab. As I said, ideas are everything, and in the real world, you have to be careful about being scooped. More about this in Chapter 9.

[127](#) It's a good idea to write these down, particularly as you get older and your memory decays. The fact that you are waking up with ideas is a good sign that you are appropriately obsessed with your work. Many ideas bubble up from the subconscious netherworld of obsessed minds.

[128](#) But I do want to know if you found something weird, which can lead to something new, if you have done the experiment carefully.

[129](#) Though it's hard not to finding something new to add to an old story, particularly if you are taking a new approach.

[130](#) Easier than ever, with fantastic software like [Prism](#).

[131](#) The most important part of the experiment!

[132](#)For PIs >50 this is a pretty safe assumption.

[133](#)Yes, guilty of this infraction too many times to admit comfortably.

[134](#)And your PI's stress level much lower...

[135](#)

[136](#)Ahem.....you should have thought of this control!

[137](#)He's not pulling any punches either: his most cited paper is entitled: [Why Most Published Research Findings Are False](#)

[138](#)The paper, though outstanding, was published in an exceedingly obscure journal and had escaped my very best intentions to know the published literature on the topic. For a more detailed account of the story, which has many other interesting aspects, see [this](#).

[139](#)Warts and all: experiments are never perfect. If they are, you worry.

[140](#)He was fortunate to have avoided jail, since scientific fraud performed with public money is legally a criminal offense in the USA and many other countries.

[141](#)Of the many mandated training sessions at NIH, this one is actually extremely valuable, and even fun.

[142](#)The way it works in the real world, is that when you are well established in a given field, you have a reputation that you cannot escape.

[143](#)That by today's standards is a pretty clear example of scientific misconduct. Watson and Crick didn't generate any of the data in the paper, and basically worked on it without the permission of Rosalind Franklin, who did the hard work to generate the beautiful X-ray diffraction data, or her supervisor, Maurice Wilkens (who did share the Nobel prize).

[144](#)For a deeply insightful analysis of Watson and Cricks relationship, and a fantastic description of the history of molecular biology, see [The Eight Day of Creation](#) by Horace Judson, still the best general interest science book I've ever

read.

[145](#)More wisdom from Dr. Csanalosi, my psychiatry professor at Penn Med. In discussing mania, she pointed out that the luckiest people are born with hypomania. Their view of the world may be rose colored, but they are never discouraged, and yet not so manic as to interfere with their daily functioning.

[146](#)There is [evidence](#) (OK, not great evidence), that the act of smiling makes you happier (conversely, frowning makes you sadder). After reading this, as a true skeptic, I tried it. It did make me happier, not the least because I began laughing at myself for trying it. Whatever works!

[147](#)I have exercised pretty much every day since graduating from college. I started running in grad school, and then as various inflammatory conditions set in, switched largely to cycling in my late 40's. I also lift weights a few times a week to maintain upper body strength. My family history of cardiovascular disease caught up to me in my 60's, and I may have avoided a serious heart attack by having a high level of fitness. I was cycling 100+ miles a week up right up until I needed quadruple bypass surgery. I was back to jogging (very slowly) 3 weeks after surgery, and cycling 100 miles a week 5 weeks later.

[148](#)Yes, I am well aware that there are suspiciously similar stories on the internet, but this really happened to me, too, more or less as I have recounted.

[149](#)My father used to tell me that as he got older his father got a lot smarter. Similarly, once you become a PI you might rue how clueless and unappreciative you were as a post-doc.

[150](#)The NIH police once needed to visit our floor to break up a fight between two otherwise gentlemanly scientists who represented two sides of a love triangle. Not a great week for lab productivity. The third side wound up with another dashing scientist on the floor, and I have to admit, they have actually lived happily ever after.

[151](#)Negative experiments when performed well can be essential in advancing a story.

[152](#)Particularly if the PI relates in their recommendation letter exactly how

important a role you played as first author in the relevant publications.

[153](#)People talk, and if you develop a bad reputation it will harm both your attractiveness as a future collaborator and more generally, your career.

[154](#)Unless you are giving a talk at the meeting. In this case this is by far the most important part of the meeting, since it is your chance to shine on the stage. Giving an outstanding talk can open the door for future opportunities.

[155](#)I've done this, with zero memory of the origin of the idea.

[156](#)Who often live in really cool places to visit.

[157](#)There are also legal requirements that require a publication (or a patent) to ensure that the work has complied with relevant legal obligations.

[158](#)Oh, sorry, bad example, since in many countries farmers are paid not to grow their crops. While this not the practice in science, it would save a lot of trouble if careless labs could be paid not to publish their findings!

[159](#)Which you shouldn't was your time with, since they don't count for anything if you are at a decent university or institute.

[160](#)Remember, we covered this in Chapter 8.

[161](#)There is also a general misconception that “you can’t publish negative findings”. Completely untrue. But remember, that your negative findings should be correcting an important error and further, that the burden of proof is on you. You don’t necessarily need to show exactly where the original description went wrong (the authors may just have been incompetent), but the correction must be based on data that are highly convincing. And remember, since we can’t prove anything in science, the original conclusion in the end may be correct, so be very careful in the Discussion. Always remember, it is far better in science (unlike business) to be wrong for the right reasons than right for the wrong reasons.

[162](#)The best writing uses the minimal number of words necessary to communicate your thoughts. The unattainable goal is not a word too many nor a word too few.

[163](#)The post-docs get to write the first draft. I keep what I see fit, but my editing often entails re-writing the entire paper. I am up front with post-docs during their interview that that my prerogative as PI, with ultimate responsibility for the paper (personally and legally), is to have the final say on what is published. I am open to discussion, but at some point, I exert my droite de Roi.

[164](#)Or reading the wrong things. The right things include classic novels and well written non-fiction. The best constantly refreshing source of non-fiction in the English language world is the New Yorker, which I have read religiously since graduate school.

[165](#)Actually, you probably always had to be a decent writer. In fact, on average older papers are much better written than today's papers.

[166](#)Which sometimes refers to another paper in a chain that often dead ends.

[167](#)But you must be brutally honest with yourself, and never ignore inconvenient truths!

[168](#)The idea that all of the data in its entirety from a project should be made public so that other scientists can come to own conclusions is just nuts. At some point, we have to trust each other. I don't to wade through another lab's garbage: if it's a good lab, the PI takes enormous pride in getting things right. I just want them to tell me what they think. Because I too am a scientist, I won't blindly believe that their conclusions are true. I don't blindly believe my own conclusions are true!

[169](#)A few points on micrographs. 1. Reviewers may insist, but showing background staining of immunofluorescence after black level zeroing is pointless. 2. The human eye is much more sensitive and accurate in viewing grey scale images than color. So, show the images in grey scale and only use color for the merge. In general, avoid blue whenever possible. This is the worst color for the eye and also for printers and screens.

[170](#)There are now many low cost and even free options available  
[http://en.wikipedia.org/wiki/Comparison\\_of\\_reference\\_management\\_software](http://en.wikipedia.org/wiki/Comparison_of_reference_management_software).

[171](#) Whose careers these days often teeter on the number of citations their papers garner.

[172](#) There are many examples of chains of wrong citations due to the author's laziness in actually knowing the salient conclusions of a study.

[173](#) A few suck-up adjectives in describing the work, *e.g.* elegant, seminal *etc.* doesn't hurt either.

[174](#) Though Jenner's treatise on smallpox vaccination in 1798 was rejected by the Royal Society, and was published privately. Fittingly, this is probably the most important scientific publication in history, in terms of providing the first example of non-surgical medical intervention helping and not harming people, and in describing something very close to the vaccine that eradicated the most dangerous virus in recorded history.

[175](#) [http://www.nature.com/nature/history/timeline\\_1960s.html](http://www.nature.com/nature/history/timeline_1960s.html)

[176](#) “Although the Discussion is refreshingly frank, it points to severe limitations with the data that preclude their publication at this point”.

[177](#) Yes, the exact opposite of science.

[178](#) Hypocrisy alert. Yes, I have written many such pieces myself, which are easy and actually fun to produce. These are also excellent c.v. fodder, establish you as an expert in the field, and helps you to recruit students and post-docs to your lab. Like Gore Vidal's advice (never pass up opportunities for sex or to be on TV), you should never turn down the opportunity to write a “News and Views” type piece, even when you have to hold your nose and praise a paper you really don't think much of.

[179](#) Definitely don't hold your breath!

[180](#) Indeed, if a post-doc applicant has several publications in these journals I am immediately suspicious. Are they fabricating data? Are they only interested in appearances and riding the wave of the latest hottest topic? Are they truly committed to finding the truth, wherever their experiments take them (which is typically into important but not very sexy details)?

[181](#)Captain Renault in Casablanca: “I’m shocked.....shocked.....”..

[182](#)On occasion, referees make suggestions that greatly improve studies. I’ve been on both ends of these: when you do it as a reviewer you wonder about contacting the PI to inquire about being included as an author!

[183](#)Sometimes the suggestions are so ridiculous you can’t do this and maintain a shard of self-respect.

[184](#)I am sympathetic here.

[185](#)See <https://www.youtube.com/watch?v=-VRBWLPYCPY>, but don’t blame me if you break a rib laughing.

[186](#)Thirty years ago, most PhD students were first authors on several papers where they performed all or most of the experiments. Today, you might be first author on a study with 10 other authors who collectively performed the bulk of the work in the paper. One of the lamentable trends of modern research is that students get very little hands -on practice in writing real papers and getting them accepted.

[187](#)I have been an external examiner for students whose Dissertation Introductions were publication quality reviews, and indeed, they can and should be submitted for publication if they are really good.

[188](#)Only if there are egregious errors in the Dissertation do I probe in detail the actual work described. The student should have covered this ground many times in lab meetings, meetings with the advisors, and in the manuscript submission and publication process.

[189](#)Drama is good for this too.

[190](#)If you give a lot of talks, as senior scientists do, this helps to keep the talk fresh and challenging.

[191](#)No, this redundancy isn’t a typo, you really need to stress this at the beginning and at the end of the talk. Indeed, the old advice for giving a talk still holds

today: tell them what you're gonna tell them, tell them, and tell them what you've told them.

[192](#) But avoid being unctuous!

[193](#) Unless you volunteer for these tasks, which you might, since they can be very good learning experiences.

[194](#) Not that you have any spare money, but even if you do the IRS will not allow you to put it in a 401K plan since you are a post-doc “trainee”. No, you won’t even get social security if you are paid by NIH in some manner.

[195](#) If you are willing to risk a stroke, take a look at [this](#).

[196](#) This is good, not bad. Without such a rule, your post-doc could extend for too many years. Your career has to get going at some point. If you live in the USA, you need to put money away for your retirement.

[197](#) Never go to a lab like this!! Support national legislation to incarcerate such PIs.

[198](#) There are some PIs who prevent their lab members from talking to other labs. Choose another lab.

[199](#) A very bad trait that emphasizes superficial research and encourages fraud.

[200](#) I actually know of some PIs who won’t write letters of recommendation for their post-docs. Don’t go to their lab. This is the least they can do for you.

[201](#) They, like you, would never put such sensitive information in an email!

[202](#) This seems obvious, but many candidates I have interviewed (yes, we pay!), have only a vague idea of what we do and have published. How do they think this comes across? I mean, it isn’t a surprise that they are visiting my lab. Come on, people!

[203](#) This is standard advice for [winning friends and influencing people](#). It really works.

[204](#)You should have had this conversation with your PhD advisor in deciding between a bench career and other options!

[205](#)i.e. a job that pays a salary commensurate with your education, skills, and dedication, and provides benefits that your contemporaries take for granted (social security, 401K).

[206](#)This is more likely to work if you (or your PI) is friends with a faculty member, but nothing is lost in inquiring. Rather, the department will be flattered.

[207](#)There are also good job where teaching is the sole or principal activity. In this case, obviously, your communication skills and ability to connect with young people is paramount. There are a large number of prestigious small colleges in the USA where teaching includes running a good lab staffed with undergraduate doing research projects. Here, you will be expected to attract funding from NSF or foundations to fund the lab. These provide much less money than NIH grants, so your goals will have to be more modest, and you will be much better off working on less expensive systems, generally non-mammalian systems.

[208](#)The start date can be flexible in some situations. If they really want you, they will be more flexible.

[209](#)I hope you can see where I am going here.

[210](#)If the department is small, you can just look up the members on the department web site.

[211](#)Funny things can happen, and this may actually turn out to be your dream job when you learn more about it.

[212](#)They will probable arrange time with a real estate agent so you can gauge the local market for affordability based on your salary.

[213](#)Few other funders provide overhead costs, at least anything close what NIH provides, which averages ~ 50% of the funds directly awarded to the grantee.

[214](#)Particularly if there are tenure track investigators who have just gone through

the experience. You can also tap former lab members starting out on their own.

[215](#)But factor in the cost of living: 90K per year in Iowa equals 190K per year in Manhattan.

[216](#)And possibly could have been avoided had you taken my advice about avoiding lab romances!

[217](#)Actually, that's not at all what he said, which was not negative in the least. But that's how I inappropriately interpreted it. Emotions color everything in life.

[218](#)If nothing else, I have been ensconced in the NIH cocoon for nearly 3 decades, and have only anecdotal knowledge of what its really like in academia these days. Further, Jerry and Susan bring a wealth of knowledge from the perspective a Department Chair and Dean with a research interest in faculty development,

[219](#)At least until the revolution! See Chapter 14 for details.

[220](#)If it doesn't work, don't tell them!

[221](#)But no more, which will only be a distraction and will ultimately make you less happy, not more.

[222](#)Generally (but not always), you have to review the paper to write one of these. While you shouldn't spend a ton of time reviewing papers, it's a good idea to review papers for the luxury journals if they should deign to ask you.

[223](#)One rule you should not violate: you can raise questions about the paper, but treat the paper with kid gloves and keep the review positive. This is not anonymous, and the authors may seek revenge, which will not do your career any good.

[224](#)Clinical labs are typically much more hierarchical.

[225](#)This can be an issue with Europeans who can expect 6+ weeks off per year.

[226](#)AN animal rights supporter in a lab that performs animal work is a ticking

time bomb.

[227](#)When you first start, you will have to fight the temptation to see your mentees data before they have a chance to analyze it (I still have to fight the temptation). However difficult for you to wait, they deserve the private thrill of digesting the findings

[228](#)Contrary to the maxim, “there are no stupid questions”, there are, of course, very stupid questions.

[229](#)Yelling, essentially unbridled verbal aggression, is never acceptable behavior by you or anyone in the lab. Although it can be challenging, you should never lose your temper at work, particularly in front of your lab. If you are going to blow, seal your lips and seek solitude.

[230](#)Months to years, depending on the circumstances.

[231](#)Erring on the side of waiting to give negative feedback.

[232](#)You can't just fire PhD students! This is a much more involved process. Unfortunately, in many universities, it is virtually impossible to remove a PhD student, no matter how unmotivated or incompetent, even when it is their best interest.

[233](#)Many never learn how.

[234](#)Many organization, the federal government included, will not take sides, and you will be on your own for legal fees. If you are a federal employee you can purchase [insurance](#).

[235](#)Ideally, you will take joy in helping others.

[236](#)<http://www.youtube.com/watch?v=xZRw0IYdf3g>

[237](#)Thomas died tragically at the age of the 65. He had the most wonderful way about him of making instant friends in whatever country he happened to be in. I miss him dearly.

[238](#) Today's leaders entered the field under starkly different conditions that were much more favorable to youngsters.

[239](#) Sorry, but I just can't write "young" when referring to 38 year olds. To keep your attention on how ridiculous this is, I will put quotations around "young" in this context.

[240](#) If you answered "The Soviet Union and other authoritarian countries" you get a free week at re-education camp in the countryside.

[241](#) Life is too complicated for guarantees, even under the best of circumstances and goodwill.

[242](#) Being happy is never part of the equation of policy makers. It's always about efficiency. Happiness is arguably the most important value for a career to strive for, and even for bean counters, happy people are more creative and productive.

[243](#) The average [profit margins](#) for all industries is 6%, for publishers in general, its 3%

[244](#) Though again, they don't have to reject any good paper with the advent of on line journals.

[245](#) Along with many millions of hours of free labor in the form of content and reviewing articles.

[246](#) There is a proper way to do this. It is good to introduce young scientists to reviewing papers, but you have to at least read their reviews and temper their tendency to be too critical. I usually ask a post doc to help review papers, and I at least take their opinions into account in writing my review. When they write a substantial part of the review, I inform the editor of the fact, along with my assurance that I agree with their opinion.

[247](#) A true story. My second paper as a student was ruthlessly and unfairly rejected by a senior scientist in the field whose identity I could guess. While at a conference, this scientist, who was actually a very nice guy, lost his wallet and desperately needed some cash, which I provided. Seeing the confused look on his face as I offered the money was priceless. I not only learned a valuable

lesson (the best revenge is kindness), but I had a friend and champion for life.

[248](#)This establishes priority for the authors, protecting them from being scooped in the publication process. There is now an [internet archive](#) for biologists

[249](#)I [previously published](#) parts of this section.

[250](#)As older scientists can attest, even honest people can be guilty of this. You get an “original” idea, forgetting that you heard it or read it previously.