Advice to a young scientist (by someone who doesn't know how to give it)

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ABSTRACT **While trying to extract original and general advice from the details of my career, I realized this might not be possible. My path, like those of so many others, had too many idiosyncratic twists and turns that had to work out just the way they did to be mined for generally useful strategies. So I abandon the conceit of advice and simply give you my story. There are many like it, but this one is mine. Take what you wish from it.**

AND YET IT COLLAPSES

In Belgrade, where I grew up, I was a mediocre science student, unlikely to spontaneously improve. I have to believe this was because the subject was taught by rote memorization, but regardless, I was more interested in the indolent pursuits of disaffected youth in latter-day Yugoslavia, like stealing car radios (easier than you might think) and pilfering supermarket baguettes (harder). In an attempt to alter my steady course toward juvenile delinquency, my mom sent me to live with my dad, then in the throes of his second marriage, in the mythic land of affluent high school kids I had been watching on television: the United States. For the next year, despite being in rural Pennsylvania, I lived a new life that seemed as glamorous and as far from post-Tito Belgrade as the one Brandon and Brenda Walsh were living in Beverly Hills.

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One day, in Mr. Patterson's chemistry lab, I finally took notice of science. The task: explain why a soda can containing a dollop of boiling water collapsed when inverted in a beaker of ice water. What was remarkable to me was not that the can collapsed, but that Mr. Patterson refused to confirm or refute my explanation. Instead, he challenged me to devise an experiment that could falsify my working model. I returned the challenge: "Doesn't this way of thinking call into question all the other stuff in the textbook?" Smiling mischievously, he retorted, "What do you think?" I didn't have an answer, but what I should have said is "I think, therefore I am … a working model."

Learning that I, rather than the authorities (textbooks, Mr. Patterson himself) could be both originator and verifier of hypotheses was one of the most empowering revelations of my life, a quiet and melancholic form of resistance against my parents' divorce, against the authoritarian system back home, and, I realized as I got older, against the dying day itself. As summer began, Steven Spielberg fed my growing interest in science by genetically resurrecting the dinosaurs. Soon thereafter, I was sent back to Belgrade, just in time for the war in Bosnia. I dodged the draft by immigrating to New Zealand, where I attended college. Perhaps inspired by the velociraptors—clever girls—I majored in biochemistry.

"DEFINITELY. IT TAKES ANOTHER 3 HOURS BY PLANE FROM SYDNEY"

Near the end of college, I started reading recent papers in major journals. One study that caught my eye described how unfolded proteins in the endoplasmic reticulum (ER) send a signal to the nucleus to activate genes encoding ER chaperones (Cox and Walter, 1996). This feedback loop required the ER transmembrane protein kinase Ire1 and a new transcription factor, Hac1. Instead of activating Hac1 by yet another kinase cascade, Ire1 splices an intron from

DOI:10.1091/mbc.E15-06-0341

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Abbreviations used: ER, endoplasmic reticulum; VLCFAs, very-long-chain fatty acids.

the *HAC1* mRNA to relieve a block in Hac1 synthesis by ribosomes. The work was done at UCSF (closer to Beverly Hills) in the lab of Peter Walter. This was not the first molecular biology paper that I read, but it was the first that made me dream. I cold-called Peter from a phone booth in Auckland and asked him to let me work in his lab. He initially demurred, suggesting half-jokingly (or, knowing Peter, not jokingly at all) that a competing lab that had recently relocated to Australia was sending me as an infiltrator. Ultimately, he assented, but only after I convinced him that New Zealand and Australia are different countries.

When I left New Zealand after my third year of college, I planned to return at the end of the summer break, but I never did. Instead, I worked as an intern in Peter's lab for little over a year before joining the graduate program at UCSF. Living in San Francisco over the next 10 years, I came of age both personally and scientifically.

THE LONELINESS OF THE LONG-DISTANCE GRADUAL STUDENT

I pursued my PhD in Jonathan Weissman's lab, a scientific paradise that I managed to turn into a personal scientific hell—but I'm getting ahead of myself. I was attracted to the Weissman lab partly because it was new and relatively small, so Jonathan was often available to hang out in the lab and discuss science. However, discussing science with Jonathan meant always being a few steps behind. My brilliant solution was to insist that I work, essentially in isolation, on a problem that was at best tangential to Jonathan's main research interests. I had also somehow gotten the idea that a mentee should be petulant and jokingly dismissive of his mentor's scientific ideas. Despite my recalcitrance, Jonathan offered me several projects that were guaranteed to work, but I turned them down in favor of pursuing my own ideas.

Cut to four years, several "clever" genetic screens, and zero publications later. Jeffery Cox, one of the students who revealed Ire1 and Hac1's unique relationship in Peter's lab, once said (to someone else), "If you can't clone the gene you love, love the gene you clone." What he didn't say is what to do if you don't know what love is.

In my case, that meant not knowing how to explore the other worlds of cell biology that lay in the direction my cloned genes were trying to take me. In part, my resistance was based on fear that pursuing the obvious questions would require me to master biochemistry, which at the time I considered to be both less elegant and more laborious than genetics. By my sixth year of graduate school, the dream of crushing my own can of science was slipping away. But then something unremarkable happened: existing projects in the lab needed an extra pair of hands to get finished. My hands, idled by disillusionment, were available. I got some results. Results became figures. Figures became papers.

Year 7. Some of the aforementioned results had suggested that the uncharacterized gene *YJL097w* was involved in sphingolipid metabolism. In a previous clever (but fruitless) genetic screen, I had cloned two other genes involved in sphingolipid metabolism. As that project collapsed into irrelevance, I had occupied myself by accumulating an absurdly disproportionate familiarity with the sphingolipid literature. On the basis of that knowledge (heretofore useless to me), I intuited that YJL097w might be the missing biosynthetic enzyme for very-long-chain fatty acids (VLCFAs), the building blocks of sphingolipids.

Contemporaneously, a couple of publications from another lab had argued that the plant homologue of YJL097w was a protein phosphatase involved in the cell cycle. I was unconvinced by these data and felt that all of the phenotypes associated with mutations in the plant homologue could be explained by a defect in VLCFA synthesis. Thus, finally, I hit my stride: from my first tenuous baby steps in Mr. Patterson's chemistry lab, to a few Bambi-on-ice moments while finishing other people's projects, to making what was by far the coolest science prediction I had ever made, which—cherry on top!—was at odds with the accepted view. The exhilarating thought of testing (and possibly even confirming) this hypothesis motivated the next 6 months of labor—at the end of which a peak on a chromatogram showed me that purified Yjl097w had made a dehydrated VLCFA product. My working model had worked! We submitted our paper to a major journal, where it was rejected on the grounds that it lacked general interest.

Still intoxicated by my discovery that Yjl097w was the missing dehydratase, I decided that the general reader would be generally interested in total VLCFA synthesis in vitro using Yjl097w and three other enzymes. Unfortunately, all of these enzymes were integral membrane proteins sensitive to detergent. Groping for a path forward, I was inspired by a paper written by Görlich and Rapoport on an unrelated topic (Görlich and Rapoport, 1993). In their approach, one places several pure membrane proteins in detergent, mixes them with detergent-solubilized synthetic phospholipids, and then removes the detergent (with something called "biobeads") to yield proteoliposomes containing the desired proteins. Despite the strategy's straightforward logic, the remarkably detailed methods section suggested that there might still be some magic involved (for example, only lot number 810017 of Big CHAP worked), so Jonathan put me in touch with a former UCSF student, Manu Hegde, who was making proteoliposomes regularly in his own lab at the National Institutes of Health. Manu and I spent hours on the phone, like teenagers ("Did you know how much humidity in Bethesda affects my biobeads?" "Tell me about it. No, seriously, tell me ALL about it."), and a few weeks later, I was making proteoliposomes that were making VLCFAs. We submitted our work to another major journal, where it was rejected on the grounds that it didn't demonstrate anything new.

Meanwhile, I had figured out how two different versions of yeast VLCFA enzymes synthesize VLCFA products of different lengths. In a "natural experiment," I noticed that evolution had changed the distance between the active site on the cytosolic end of the synthase (where carbon building blocks feed the growing end of the fatty acid–chain substrate) and a lysine near the luminal end of a transmembrane alpha helix. Remarkably, I could make new VLCFA products of predictable lengths by "sliding" the position of the lysine, like molecular calipers, up or down the helix.

Several months later, Jonathan and I compiled the data for the molecular caliper story and sent it to the journal that issued our first rejection. (This felt a bit like trying to convince your ex-girlfriend to take you back because you spent a year in the gym.) A few weeks after the submission, as I waited in line in my favorite San Francisco bakery, Jonathan called to tell me that the paper had been accepted without revisions. The moment was ecstatic, but also sentimental, because it meant that our mentor–mentee relationship was finally coming to an end. It was the culmination of nine years of Jonathan's patience with me, during which he cheered me on, just as loudly every time I fell down as when I finally won the race.

GO EAST(?), YOUNG MAN

I spent my last six months in the Weissman lab helping another project in the lab get finished, lining up a postdoc in Japan, and hedging my career bets by applying for a job to a few departments that expressed interest in me after the caliper work was published. In the end, I bailed on Japan and started my lab at Harvard University. At

the time that I was contemplating taking the Harvard job, the word on the street was not good ("They eat their young"). Why did I still choose to go there? First, I really enjoyed my interview interactions with several senior members of the department (I know what you are thinking: senior, not junior; red flag), who convinced me that they could be decent Jonathan substitutes for this phase of my career. Second, I believed that bad reputations are often the disproportionately long shadows of atypical events (I know what you are thinking: shadows grow long when it is too late in the day for change to occur). And admittedly, my own hubris came into play: even if the place was bad for junior faculty, I thought I would be somehow different (I don't even wanna know what you're thinkin').

After a year at Harvard, however, progress was not swift. Only two students had rotated with me, and they both joined other labs (run by senior faculty). Self-doubt and fear spread through my veins like poison. As an antidote, I considered an offer from another department with a better reputation for cultivating junior faculty. Why did I stay, in the end? An old saying: "wherever you go, there you are." So, rather than entertaining Borgian fantasies about my senior lab "competitors," I tried to improve my own contributions to the process of attracting talented students. I got myself on the student radar by spearheading a journal club for first-year students and faculty, modeled on one I had enjoyed at UCSF, and started pitching projects with the unabashed verve of a used car salesman.

Over the next five years, our group figured out how tail-anchored proteins are inserted into the ER membrane by the GET pathway. Before I left the Weissman lab, I had developed a cell-free system for studying this pathway, which my group stripped down to its purified components. These were exhilarating times, because we were racing against several fantastic labs to answer the same mechanistic questions. Even though I was a newcomer to the membrane protein insertion field, I was encouraged by more senior figures—especially Manu Hegde, who taught me that scientific competition and criticism are not mutually exclusive with scientific openness.

As the lab established itself, we started parallel work on autophagy. My interest in this field arose during grad school, when I read a paper from Yoshinori Ohsumi's lab (where, incidentally, I had planned to do a postdoc). Autophagy is a half century–old puzzle in cell biology: How do cells wrap targets with a membrane to make a vesicle that then delivers targets to the lysosome? Many imaging methods have been used to track the formation of this membrane, but few biochemical approaches had been attempted. After a couple of years, we built a cell-free system that allowed us to initiate autophagosome membrane formation in situ using a purified autophagy target. Other protein-targeting fields have been transformed following the development and rapid adoption of cell-free systems. Our work adds selective autophagy to this list and will hopefully accelerate the elucidation of key mechanisms underlying this process

A TALE TOLD BY AN IDIOT, FULL OF SOUND AND FURY, SIGNIFYING NOTHING

Here is where I intended to summarize my tale, with the implicit purpose of inspiring younger scientists to do as I did. But what would that advice be? "Here's what you need to do, kids: Fail repeatedly for years, alone, but then get serendipitously lucky and pick a winning horse years in advance of a final payoff. Then sit back and wait by the phone for a job offer from Harvard, which despite everything you've heard will give you exactly the kind of support you need to succeed as junior faculty. You're welcome [mic drop]." But one person's rose-tinted view of their own idiosyncratic story does not constitute "advice," especially not in an endeavor where we value reproducibility; I'm not sure I could reproduce my own good fortune, much less expect someone else to reproduce it from the same set of initial conditions.

The only thing I know for sure is that the support I was repeatedly given at every stage of my career was critical to what success I did have throughout my career. That support enabled me to stay with it through failures and to do something productive at those times when I needed, more than anything else, to produce something. Not everyone who had the support with which I was privileged would have reached the same result, but I know that I wouldn't have succeeded without it. And so, for that, I am truly grateful.

ACKNOWLEDGMENTS

I thank Chris Patil for helping me write this essay and for helping me finish my college education in San Francisco. I dedicate this essay to my mother for giving me actually useful advice my whole life.

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