TURNING POINTS

Heeding a mentor's advice: A lesson in persistence

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Four years into my PhD in the laboratory of Luigi Gorini, a world-renowned bacterial geneticist in the Department of Bacteriology at Harvard Medical School, I concluded that my data did not support the hypothesis of my thesis. I had accepted the project for two reasons: solving a difficult problem that had previously stumped two postdoctoral fellows was a welcome challenge, and with a young daughter, I did not want the stress of being in the tight race to discover why bacteria became resistant to streptomycin. Thus, while the rest of the lab worked on 'ribosomal ambiguity mutation' in *Escherichia coli*, I was battling Coccus P, a bacterium that secretes a potent protease. This is what we knew: the protease was stable, but was secreted only after addition of calcium. The level of secretion would eventually reach an apparent steady state, suggesting feedback inhibition. Luigi had hypothesized that calcium induced secretion of the protease, and I was to figure out how Ca^{2+} selectively allowed secretion of this enzyme and how the protease level inhibited its own secretion.

In 1967 we knew little about protein secretion and how bacteria distinguished between extra- and intracellular proteins. After four years and much frustration, I had a brainstorm late one night. Looking over the data we had accumulated over the years, I realized that all could be explained if we assumed that this protease was treading its way out of the cell 'unfolded', possibly from membrane-bound ribosomes. It was not detected in the absence of Ca^{2+} because bacterial growth required constant shaking and the protein, which had no disulfide bridges, was destroyed as it was secreted owing to surface tension and, possibly, transient proteolytic activity. Ca^{2+} would stabilize the protein by substituting for disulfide bridges. Although I had new data, Luigi dismissed the idea almost immediately, saying, "What do you think this protein is? Spaghetti?! Perhaps you should go back to ballet dancing, because you will not succeed as a scientist." I was devastated. I could not understand why he had not even bothered to refute my interpretation scientifically or ask for further proof. After all, he had a reputation of being open-minded and had handpicked me as a graduate student for winning an award in chemistry. The more I thought about it, the more annoyed I became.

The turning point occurred when I made an appointment with Elmer Pfefferkorn, a professor in the same department, who is now at Dartmouth College. He was a brilliant scientist, an inspiring teacher, and modest and generous to a fault: the antithesis of some other professors, then and now. I explained the history of the project, our massive amount of data and Luigi's reaction, and asked Elmer how to handle such complete rejection. I felt that Luigi had perhaps lost faith in my abilities after I had a child. Elmer listened patiently and said, "You may or may not be right about his lack of faith in you, but I think Luigi's reaction is because your hypothesis is too 'radical'. He is not convinced by your data and your explanation. You are challenging conventional wisdom and going out on a limb, so the burden of proof is on you. Instead of feeling annoyed, you should troubleshoot and do further experiments to prove your point beyond a reasonable doubt."

I let the advice sink in, and planned experiments to test the new model. By the fifth year of my PhD, I had shown that the enzyme was indeed secreted at all times, but because it was unfolded, it degraded in the absence of Ca^{2+}. I also showed that there was no 'feedback inhibition' of secretion, but that even in the presence of Ca^{2+}, the rates of secretion and destruction cancelled each other out owing to surface tension. Luigi and I went over all the experiments and agreed that only the new hypothesis could explain the data. A couple of years later, when I had moved to California, Luigi called me to report that others had published a model of 'co-translational secretion', and he was wondering why we had not published our important studies in a higher-impact journal. It felt good to remind him of 'spaghetti', but I also admired him for being able to change his mind in light of the evidence.

This episode, painful as it was at the time, has held me in good stead all these years, and has become a teaching point for my own students and postdoctoral fellows. Most of the 'radical' ideas we put out there during subsequent years were initially greeted with harsh judgment by some well-known colleagues. Each time, I reminded myself that the burden of proof is still on me, and I tried to inject more experimental rigour in our work to convince the sceptics. Some ideas have taken thirty or more years to be accepted, but with the help of thoughtful colleagues like Elmer who cared enough to listen and inspire rather than simply dismiss, I learned to persist. I have had many such memorable mentors and colleagues, such as David Shirley, former Director of Lawrence Berkeley National Laboratory, who literally saved my career. Without them I would probably be running a Persian restaurant now — it may have even been a good one!

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