Preface

"Don't be so humble—you are not that great."
—Golda Mier

THE COLD ROOM is one of biochemistry's most formidable abodes, a stingy environment that can be as chilly metaphorically as it is thermally. Many a researcher has been drawn by the siren-like lure of classical protein studies, only to fall prey to a stark reality: what is true inside the cell cannot always be easily recapitulated outside the cell. Protein biochemistry abounds with cold room stories that span the gamut from glorious to hilarious, and I have a few of my own (read on).

Re-wind the reels back to the second year of graduate school at UCSF. The year is 1986 and the boss is Keith Yamamoto. There were no laptop computers, no cellular telephones, no commercial Internet or e-mail, and the human genome sequence was just a pipedream. You remember those days. Those were wonderfully nostalgic days both in and out of the laboratory, and arguably the golden days of transcription factor studies. Roger Miesfeld had just isolated the rat glucocorticoid receptor, a hormone-dependent gene regulator that quickly found its way to center stage in the race to understand how animal cells turn their genes on and off. With cloned receptor in hand, I set out to test whether glucocorticoid receptor function could be recapitulated in yeast cells, the idea being that fungi would allow us to test evolutionary conservation in eukaryotes, and forthwith unleash the "awesome power of yeast genetics" Guthrie-style on this unsuspecting animal gene if we were able to demonstrate functional conservation. Remarkably, the rat receptor sprang to life on the first attempt, producing a diagnostic blue color change in yeast cells expressing a β-galactosidase fusion and a broad smile on the face of a nervous young scientist. Conclusions? There were exactly four: (1) the cellular transcriptional apparatus had apparently retained function across a daunting 1 billion years of evolutionary time, (2) yours truly was incredibly brave to work for the hard-driving Yamamoto even in light of having rapid success in the laboratory, (3) yours truly was incredibly gullible to attempt such a ridiculous set of experiments with no obvious chance of a positive outcome, and (4) scientific success requires a perplexing combination of skill, intelligence, naïveté, passion, chutzpah, bravado, luck, childishness, and *je ne sais quoi.*

Receptor experimentation in yeast necessarily involved grinding up yeast cells, fractionating the proteins by denaturing polyacrylamide gel electrophoresis, transferring the proteins onto nitrocellulose, probing the immobilized proteins with a monoclonal antibody, and examining the filter to confirm the presence of the expressed rat protein. But "western blotting" can be tricky, particularly with heterologous proteins, which are notoriously unstable even if the yeast cells expressing the foreign protein have been engineered to remove the fungal protease genes. Such studies are also challenging because in spite of the grinding action of glass beads, yeast cells are surprisingly sturdy and orders of magnitude tougher than their mammalian counterparts. To maximize our chances of detecting the rat receptor protein, we decided to hedge our bets by moving as quickly as possible through the process. In the interest of speed, I quickly loaded up a bucket with ice, cold water, and about a dozen culture tubes containing yeast cells, and rushed off to the cold room.

Anyone who has ever worked in a cold room is familiar with the stainless steel floors common to such enclosures, as well as the damp environment and the frequent use of glycerol buffers designed to stabilize proteins during extract preparation (the sage protein biochemists among you probably know what's coming next). Instead of walking slowly and carefully into the cold room, I dashed in with great haste and promptly found a small puddle of buffer on the cold room floor. Cold steel, humidity, and glycerol conspired to produce something more slippery than ice, and before the former hockey player had time to react, he found himself flat on his back, sending a spectacular shower of ice, water, and yeast cells flying in every direction, including down the front of his lab coat. Dazed and disoriented, I spent a memorably miserable hour disrupting yeast cells while wearing a lab coat soaked with ice water. Only San Francisco summers and UCSF oral examinations were colder. Upon hearing about the mishap, a colleague in the lab spent the next few weeks referring to me a "cool guy" who really knew how to "chill out," phrases that many found funny, but I found less so!

An equally amusing story involved the same cold room, an unfortunate rodent, and a smattering of 20s-something tomfoolery. Not surprising, rat glucocorticoid receptor experiments necessarily involved the occasional use of rats, and apparently one such rodent attempted to transcend its model organism status by escaping into the cold room, only to meet its fate underneath an unsympathetic cold room refrigerator. Some time later during a routine cleaning, a colleague in the Yamamoto lab found the deceased rodent under the refrigerator, mummified but otherwise intact. He appropriately noted that such a find certainly added new meaning to the term "lab rat." After deliberating a few minutes, he decided that the best use of the dehydrated rodent was as a frisbee, a decision that sent him running from the cold room back into the laboratory for testing. He proceeded to fling the newly found rat mummy across the room, striking an unsuspecting lab mate who let out a shriek that sent the entire lab howling. Not to be outdone, the startled lab mate picked up the shriveled projectile and disappeared for about 5 mysterious minutes, only to return to the lab with the rodent suspended in a bucket of liquid nitrogen. He then fired up a cassette player blaring a favorite rock song, and flung the frozen rat back at the perpetrator, sending the mummy sailing across the lab once more. This time, the brittle object struck the lab floor with a crackle, sending small pieces of shattered rodent sliding in every direction. Embellished slightly, but all true, those were cold room stories for the ages!

Nearly two decades have passed since the early days of graduate school, and one is struck by the changes in high-level science. I sometimes pine for those days insomuch as the profession seems to have gotten much more serious over the years, perhaps because the tools are so much more powerful now and perhaps because so much more is at stake in terms of the fame and fortune that await scientists who are able to negotiate its sharp corners with dexterity. But in terms of the sheer thrill and excitement of doing science, science has never been richer and more exhilarating. And while we have lost some of the lighter moments and nostalgia that were more characteristic of decades past, and relegate such memories to lapses of introspection, there is probably no looking back, and rightly so. With the rise of microarray technology, we are unraveling the complexities of the human genome at an unprecedented pace, and unraveling such mysteries is affording safer medicines, better diagnostics, and a deeper understanding of the biochemical basis of development, disease, aging, behavior, and many other aspects of life. The latest manifestation of microarray technology extends our studies beyond genes and messenger RNA molecules to the ultimate purveyors of the genetic code, the proteins. It is this coveted class of molecules that provides the focus of a dynamic new book *Protein Microarrays*.

Protein microarrays are analytical devices that contain collections of proteins printed in rows and columns on glass substrates. The protein equivalent of DNA microarrays,

these tiny chips allow the massively parallel analysis of protein function in a highly miniaturized and automated manner. Tens of thousands of recombinant proteins, antibodies, synthetic peptides, and other protein derivatives can be used to examine labeled extracts from cells, patient sera, and other sources. Specific binding between target molecules on the chip and proteins in solution yields a quantitative measure of the proteins expressed in a biological sample. In one format, antibody microarrays provide specific binding reagents for protein expression monitoring, the logical extension being a chip that would allow the simultaneous measurement of every protein expressed in the proteome. In another type of assay, microarrays of recombinant proteins allow the identification of protein binding partners in a highly precise manner. Indeed, all of the fundamental activities of proteins carefully delineated using traditional approaches appear to be amenable to protein microarray analysis; protein-protein binding, enzyme-substrate catalysis, receptor-hormone recognition, target-drug binding, and so forth appear to work as well on chips as in solution. In essence, a protein microarray is a cold room on a chip (without the slippery floors and rodents!). Protein microarrays appear to be poised to replace many of the current in vitro diagnostic tests that rely on large reagent volumes and plate assays. This highly interdisciplinary technology also promises a new genre of tests based on genomic and proteomic information.

Jones and Bartlett Executive Editor Steve Weaver initiated this project by telephone, followed by a lively dinner at Spago in downtown Palo Alto where we finalized plans for the book. Steve and I have worked together successfully in the past, so the decision to take on this ambitious project was easy. Steve's intellect eclipses his physical stature (which is impressive because he barely fits in the passenger seat of a Porsche!), and I am indebted to Steve for all of his guidance and insight along the way. A dept of gratitude is also owed to the entire team at Jones and Bartlett including Rebecca Seastrong, Anne Spencer, Elizabeth Platt, Pam Thomson, Louis Bruno, Dean DeChambeau, and many others who have made this project enjoyable and rewarding.

I continue to build on the solid foundation provided by my incomparable scientific mentors Dr. Daniel Koshland, Jr., Dr. Keith Yamamoto, and Dr. Ronald Davis, and thank them for their nurturing and wonderful knowledge of protein biochemistry. I must also thank my publicist Paul Haje for insisting that I take on this project, and my colleagues at TeleChem International, Inc. for their remarkable expertise and innovative ArrayIt products. I also owe a special acknowledgement to my family and friends for their enduring kindness, and to René Schena whose love is an unwavering source of inspiration during these demanding projects.

Make no mistake that the contributors are the real heroes in *Protein Microarrays*. We were anticipating a total of twelve chapters for the book and received exactly twenty-four. The project expanded to twice its anticipated size, which is a testimony to the excitement in the field and to the energy and generosity of the contributors. By assembling two-dozen chapters from the top protein microarray laboratories in the world, we essentially guaranteed the success of the book before it was even published. It has been our experience that a compendium from the world's foremost authorities allows us to convey information and data that are virtually impossible to obtain from any other source. We are confident that *Protein Microarrays* will help usher in a new era in protein biochemistry, and I ask you to join me in embarking on this wonderful journey together!

Mark Schena
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