Biographical Sketch

An Eclectic Career Steered by Discoveries in our Laboratory

"By the time most scientists have reached age thirty they are trapped in their own expertise."
—Francis Crick, What Mad Pursuit, 1988

Certainly, this claim is hardly applicable to me because my scientific career has followed an atypical multidisciplinary trajectory transitioning from organic chemistry, microbiology, biochemistry, immunology, immunogenetics, oncology, and biotechnology.

Most bizarre, in 2013, my career diverged even more dramatically: I became a professional investigative reporter heading a team of three research associates in different countries. Most startling was my finding that uncovering a novel historical fact produces eureka instants similar to those of the laboratory.

Why did I changed discipline

The changing was *not* due to any whimsical decision, instead the change resulted from using breakthroughs in my laboratory as innovative tools for solving pressing problems in other disciplines. For example, I was "forced" into Immunology when we developed a novel technology for measuring single molecules of β-galactosidase, which led to discovering "activating antibodies," an unprecedented class of immunoglobulins capable of activating (repairing) inactive mutant β-galactosidase molecules.

Advantages and drawbacks

A multidisciplinary career, however, has advantages and drawbacks. It is exhilarating to observe effects of applying a newly discovered tool to an "unsolvable" problem in another field.

As neophyte, one must enjoy both steep learning curves and humbling experiences. On the other hand, as newcomer, one can ask questions without fear of appearing ignorant (the fragility of scientists' egos is well known). On the negative side, changing disciplines presents challenges such as building a new reputation among different groups of peers and getting approval from grant reviewers who are unaware of one's past achievements. Another potential difficulty is the inevitable loss of contact with old colleagues.

Description

I describe here how specific discoveries directed me to new research disciplines. The publications pertinent to each of the discoveries are listed below.

After graduating as a chemical engineer from the Universidad Santa Maria (Valparaiso, Chile) in 1948, I moved to the United States to work as organic chemist in Sol Spiegelman's laboratory (http://profiles.nlm.nih.gov/PX/) at the University of Illinois. My particular task was to synthesize o-nitrophenyl- β-D-galactoside (ONPG), a chromogenic substrate for β-galactosidase (LacZ) introduced by Joshua Lederberg in 1950 (http://www.nobelprize.org/nobel_prizes/medicine/laureates/1958/lederberg-bio.html). My early work in carbohydrate synthesis provided experience that was invaluable to the development of my career (*vide infra*). I went to become Spiegelman's graduate student and post-doctoral fellow. My thesis centered on using cell-free extracts of galactose-positive yeast to transform galactose-negative yeast. The transforming factor produced a new phenotype stable for 3-5 generations, but the cells were not genetically altered. I often thought of revisiting this phenomenon in the near future and thus complete a full circle in my career. But old age has interfered with my plan.

The list of discoveries and the corresponding new discipline follow.

On Mechanisms of Protein Synthesis

Discovery	MECHANISMS OF PROTEIN SYNTHESIS in *E. coli* (1952-53)
Publication	Impact
On the origin of the carbon in the induced synthesis β-galactosidase in *Escherichia coli.* Rotman, B., and Spiegelman, S. 1954. *J. Bacteriol.* **68**: 419-429.	This paper empirically answered a fundamental question concerning protein synthesis: Are induced enzymes made from inactive precursors? *

*For unknown reasons, practically all the credit for this central discovery has been given to Hogness, D.S., Cohn, M., and Monod J. (Biochim. Biophys. Acta, 16 (1955) 99-116), who use our methodology to achieve the same goal. Awkwardly, they did not cite our paper, but duly acknowledged the use of our methods in the text (!).

Personal note.

Jacques Monod and Sol Spiegelman had an ongoing scientific feud fueled by their strong personalities. In this particular case, Monod claimed Spiegelman received the idea of this important experiment from him. Retrospectively, I believed Monod for two reasons; first, because Spiegelman told me that Monod had agreed to publish side-by-side. Second, I finished the project almost a year before Monod's group, but Spiegelman delayed publication. Finally, we published the paper in a secondary journal rather than Science or Nature where the article really belonged.

It may be of scientific interest to mention the two different approaches used to answer the fundamental question posed at the time: Are induced enzymes made from inactive precursors?

To answer it, Monod's group introduced *E. coli* cells into growth medium containing S^{35}-labeled amino acids and an inducer of β-galactosidase. After incubation to allow for enzyme synthesis, they look for labeling in isolated and purified β-galactosidase. This approach didn't work well due to S^{35} labeling non-specifically irrelevant proteins. We learned about the experimental failure and therefore I thought of a different methodology. Parenthetically, in contrast to modern times, in those days there was considerable informal scientific exchange between scientists.

I used an original approach. *E. coli* was uniformly labeled by growing cells in a mixture of C^{14} amino acids as sole carbon source. This experiment was extremely expensive, and to everyone's dismay, the first time, it literary blew off because the culture got contaminated with unknown bacteria that convert the expensive C^{14} amino acids into $^{14}CO_2$. Spiegelman wasn't happy about the loss, although our lab had plenty of financial support.

The second time, the labeling worked as expected. After washing the C^{14} cells free of external isotopes, I introduced them into inorganic growth medium containing enzyme inducer and cold amino acids as sole carbon source. After enzyme formation, I extracted the cells and purified their β-galactosidase.

This unorthodox approach had an important advantage, I was able to follow up enzyme purification by measuring diminishing enzyme radioactivity in the enzyme. At the end, the enzyme had practically no label. Other features of our experiments were using new purification techniques for proteins; namely, starch electrophoresis and antibody-mediated precipitation. We communicated these techniques to Monod, and his group used them to obtain better enzyme purity than before. In their paper, they supported their weaker experiments by mentioning our results.

Final recognition

Years after, I was invited to celebrate Monod's Nobel prize at Harvard U., and I had an opportunity to talk to him alone at a party. I was a very outspoken young man and angrily told Monod that he had no right to exclude my name from the references on account of alleged Spiegelman's misconduct.

Monod apologized, and after that discussion, I was integrated in Monod's inner circle. During my subsequent visit to Monod's lab, after lunch at a large communal table in the Institute Pasteur, Monod stood up for coffee and discussion at a separate room and called on several scientists to join him: "Mel, Suzanne, Gerard, Borís (notice French accent)."

During my postdoctoral years

Back to the subject. The paper on protein synthesis was crucial for my career because it resulted in a post-doctoral position in Joshua Lederberg's laboratory at Madison, WI, in 1953. Josh was already famous and a sure candidate for the Nobel prize.

While in Josh's group, he asked me to investigate a puzzling phenomenon he had discovered: Why do *E. coli* cells exhibit only about 1% of their intracellular β-galactosidase (LacZ) activity? The answer came two years after, but not from my lab but from Monod's group. They discovered the first bacterial transport system, which was termed "lactose permease" (LacY).

Second permease found

In 1955, I accepted a position at the Medical School of the Universidad de Chile in Santiago, where I continued to work on transport systems of *E. coli*. The work led to discovery of a second permease, the methyl-β-D-galactoside (MeGal) permease that turned out to be first known member of a broad superfamily of ABC transporters very different mechanistically from the LacY and.

Our laboratory continued studying the genetics and mechanism of the MeGal for another 25 yearsl Five years at the Syntex Institute for Molecular Biology, Stanford, CA, and 20 years at Brown University Medical School, Providence, RI.

ABC Transporters

Discovery	TRANSPORT SYSTEMS (PERMEASES) IN E. COLI (1955-1980)
Publication	Impact
Separate permeases for the accumulation of methyl-β-D-galactoside and methyl-β-D-thiogalactoside in *Escherichia coli*. Rotman, B. 1959. *Biochim. Biophy. Acta* ***32***:599-601.	The MeGal permease was the first transport system of a broad superfamily (across phyla) presently known as "ABC transporters."

Fluorogenic substrates

While still a post-doctoral fellow at Lederberg's lab, I reasoned that the sensitivity of the chromogenic LacZ assay could be improved by using a non-fluorescent compound that upon enzymatic action produced a fluorescent product. Since I had all the chemicals on hand, it took me only a few hours to synthesize fluorescein di-β-galactoside (FDG), a fluorogenic substrate for LacZ. I coined the term "fluorogenic" for this new type of substrate. As hypothesized, FDG turned out to be a better substrate, and was the tool for measuring single molecules of enzyme. Over the years, other laboratories synthesized hundreds of fluorogenic substrates for diverse biological applications.

History

FDG remained a curiosity on a shelf of my lab for about five years. I enjoyed showing my visitors how FDG colorless solutions become instantaneously fluorescent after I added one microliter of extract containing β-galactosidase.

A breakthrough occurred in 1959 while working as visiting Research Associate of Bernard Davis (Wikipedia) at Harvard's Microbiology Department. In a Harvard series of seminars presented by Bernie to a selected group of Boston biochemists, I discussed how the seemingly impossible feat of measuring activity of individual molecules of β-galactosidase can be accomplish (in theory) by using FDG in a microfluidic device. Surprisingly, the seminar was well remembered, and (to my embarrassment). Apparently the seminar resonated well because I was often asked "how is the single enzyme molecule work coming along?"

This outside interest spurred me to initiate in earnest experiments using FDG to develop a single molecule of enzyme assay (Wikipedia). Progress was slow until a breakthrough occurred during a short visit to the Medical Research Council (MRC) at Mill Hill (North London) in 1961. I was fortunate to meet John F. Collins who had developed a simple, novel microfluidic system using droplets dispersed in silicon oil for measuring penicillinase in single cells of *Bacillus licheniformis*. When we applied Collins's system for measuring β-galactosidase in the presence of FDG, we were astonished to discover that the system was not only capable of measuring single *E. coli* cells but also measuring individual molecules of the enzyme!

Single molecule studies

Discovery

Publications

Measurement of activity of single molecules of β-D-galactosidase. Rotman, B. 1961. *Proc. Natl. Acad. Sci.* USA **47**, 1981-1991.

Fluorogenic substrates for β-D-galactosidase and phosphates derived from fluorescein (3, 6-dihydroxyfluoran) and its monomethyl ether. Rotman, B., Zderic, J.A., and Edelstein, M. 1963. *Proc. Natl. Acad. Sci.* USA **50**, 1-6.

FLUOROGENIC SUBSTRATES FOR ENZYMES (1960-PRESENT)

Impact

The 1961 article demonstrated for the first time the feasibility of measuring single molecules of β-galactosidase using microfluidics and fluorogenic substrate. The second publication extended fluorogenic substrates to other enzymes.

The unique information offered by studying single molecules remained little known for about30 years. But a relatively recent surge of interest culminated in a 2014 Nobel Prize (Wikipedia). See Knight, Alex E. (2011) Single Enzyme Studies: A Historical Perspective. Methods Mol Biol. 778:1-9..

Fluorochromasia

In 1965, Ben W. Papermaster, a post-doctoral fellow at Stanford Medical School, made a one-afternoon visit to our laboratory to explore the possibility of measuring β-galactosidase activity in single cells of mouse lymphoma.

I vividly recall the occasion. It was a Saturday afternoon and Ben was busy doing his experiments. Out of curiosity—since I had never seen mammalian cells under a microscope—I mixed lymphoma cells with a series of newly synthesized fluorogenic substrates. To my enormous surprise, the cells in contact with fluorescein diacetate (FDA) became highly fluorescent in less than 5 minutes!

Ben and I thought at first that my observation could not possible be original because FDA had been synthesized almost a century before. However, after thoroughly searching literature and experimenting for one year, we concluded that we were dealing with an unprecedented cell membrane phenomenon. We termed it "fluorochromasia" (Wikipedia) for "becoming fluorescent."

Fluorochromasia (https://en.wikipedia.org/wiki/Fluorochromasia) has been widely used in many different laboratories to measure cellular viability of diverse organisms including animals, bacteria, molds, plants, spores and embryos. The original paper has been cited over 1,370 times, but the phenomenon suffered the fate of "Obliteration by incorporation" (Wikipedia) meaning that the reference is no longer cited (https://en.wikipedia.org/wiki/Obliteration_by_incorporation). From Google Scholar, it is estimated that there are 29,000 reports of measuring cell viability using FDA without mentioning either discoverers or fluorochromasia by name.

Cellular viability

Discovery

Publication

Rotman, B., and Papermaster, B.W. (1966). Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. *Proc Natl Acad Sci* USA 55, 134-141.

General Cellular Physiology

Impact

The outstanding feature of fluorochromasia is that it only occurs in cells that have a healthy membrane, which is essential for living cells.

Consequently, fluorochromasia has been widely used (more than 29,000 publications) to easily distinguish between living and dead cells. Over the years, fluorochromasia has been shown to occur in all types of cells including mammalian, vegetal, and microbial https://en.wikipedia.org/wiki/Fluorochromasia .

The Karolinska Institute, another turning point

The discovery of fluorochromasia in tumor cells was pivotal in sparking my interest in tumor cells. To learn more about tumors, I went on sabbatical leave to the Tumor Biology Institute at the Karolinska Institute in Stockholm in 1966. There, I learned about tumor cells from sharing a lab with Georg Klein, the Head of the Institute https://en.wikipedia.org/wiki/George_Klein_(biologist) .

George was one of the most impressive personalities I've met. It was fascinating to watch him perform in his laboratory counting living cells under the microscope while talking to Africa with the telephone on his shoulder using anyone of four different languages.

At the Karolinska, I met Franco Celada, an Italian immunologist working on tissue transplantation in mice. Franco was most enthusiastic about fluorochromasia, and together, we develop a new simple immune cytotoxicity assay (Celada, F., and Rotman, B. (1967) A fluorochromatic test for immunocytotoxicity against tumor cells and leukocytes in agarose plates. *Proc Natl Acad Sci* USA *57*, 630-636). This test did not compete with one developed by Walter Bodmer (https://en.wikipedia.org/wiki/Walter_Bodmer), who was one of the first scientists to realize the potential power of fluorochromasia. He made fluorochromasia popular for testing human histocompatibility (Bodmer, W., Tripp, M., and Bodmer, J., in *Histocompatibility Testing* (eds. Curtoni, E. S., Mattiuz, P. L., and Tosi, R. M., 1967).

While at the Karolinska, I had a lunch with Franco during which we discussed single molecule studies and he asked me, how one could measure single molecules of antibody? The question intrigue d me, and a few days later, I answered Franco. The solution is to find an enzymatically inactive mutant of β-galactosidase that becomes active in the presence of its specific antibody. I continued, "however, there is no reason that such a mutant exists." Afterword, I completely lost interest in the problem.

To his credit, Franco persisted with the idea and continually nagged me for months about doing the experiments. Finally, in 1968, Franco arrived in our laboratory at Brown University ready. My thought was, "I'm going to let Franco waste his time for a month and then we can think of decent experiments."

Eureka day

It was a Monday morning, John Ellis, our assistant, had prepared 12 test tubes with four ml of cell-free extracts of different *E. coli* mutants defective in β-galactosidase (from our extensive bacteria collection of more than a thousand specimens). Then he added to each tube one ml of rabbit serum anti β-galactosidase and one ml of o-nitrophenyl-β D-galactoside (chromogenic substrate of the enzyme), and incubated the tubes at 37°C for an hour.

I was in my office when Franco and John entered bearing huge smiles and holding a tube rack in which 12 tubes were colorless and one was bright yellow! A control tube without antiserum was also colorless.

A new type of antibodies is discovered!

Discovery

Publication

Rotman, B.M., and Celada, F. (1968). Antibody-mediated activation of a defective β-D-galactosidase extracted from an *Escherichia coli* mutant. Proc Natl Acad Sci USA *60*, 660-667.

IMMUNOLOGY, IMMUNOGENETICS

Impact

The discovery opened an extensive field of protein conformation mediated by immunoglobulin. For example, see paper collection "Protein Conformation as an Immunological Signal" by Celada, Franco, Schumaker, Verne N., and Sercarz, Eli E. 1983.

To be continued…

Publications of M. Boris Rotman

For publication's impact look https://scholar.google.com/citations?user=BMr4daMAAAAJ&hl=en

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