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How WWII and the old Turkish mass standard led a Greek to a scientific career

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I was born in a small village near Olympia, on the western coast of Peloponnese, Greece, destined to become a farmer like my father and his father before him. However, WWII changed the course of my life in a profound way. The occupying Italian and German forces confiscated many of the farmers' crops to feed their troops. Later, angered by the continuous Greek armed resistance, they began to burn the farmers' fields in retaliation. As a consequence, my frustrated father decided to relocate our small family to Athens, taking advantage of the Red Cross distribution of Canadian wheat to the starving Greeks. Thus, my siblings and I grew up in the heart of the Athens slums full of ex-country folk like us. The Greek misery continued even after WWII, due to the equally devastating Greek civil war, pitting the communists against the conservatives who were supported by the British and Americans, and lasting until 1949.

When I was 8 y old, my father became a green grocer, selling his vegetables in a small stall in the central Athens market. I spent all of my after-school hours and all day Saturday helping my father in his business. At that time, and until 1959, Greece used the old Turkish "oka" as a mass standard (a remnant of the 400-year occupation of Greece by the Ottoman Empire). An oka was divided into 400 drams, a dram being equivalent to 3.2 g today. To further complicate things, an oka was divided into ½, ¼, 1/8, 1/10, and 1/25 fractions. My task was not only to weigh the buyer's groceries using the appropriate weight standards, but also to determine the price based on their weight. Because speed meant more grocery sales, I quickly became proficient in addition, subtraction, multiplication and division. This mastery of "baby math" at a relatively young age gave me confidence in my abilities, and later enabled me to win a competition, thereby earning me a full scholarship at Athens College High School, the best private high school in Greece.

At Athens College, one of my favorite professors was Tom Richardson, who was a Fulbright scholar and a graduate of Amherst College in Massachusetts. Richardson was my chemistry professor, a knowledgeable and effective teacher, who instilled in me a passion for doing exact, experimental science. Based mostly on his recommendation, I was awarded a full scholarship as an undergraduate at Amherst.

Although I majored in physics at Amherst College, I worked throughout my undergraduate years, including summers, as a laboratory assistant to retired biology professor Harold Henry Plough, in order to earn pocket money and to support myself during school recesses.

Plough was a multifaceted scientist. He obtained his PhD degree in 1917 at Columbia University working in the Drosophila lab of T. H. Morgan. Plough's scientific life was closely interwoven with that of Hermann "Joe" Muller, a fellow graduate student in the Morgan laboratory. According to Plough, who was one of the few who befriended him, Muller was as brilliant in science as he was

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difficult in his relationships with most others in the lab. As a consequence, Plough played a catalytic role in 1940 in the hiring of Muller as a biology instructor for 5 y at Amherst College. At that time, Muller was thought of as an "undesirable communist sympathizer" since returning from his years-long Russian adventure, and was thus unable to land a better academic job in the States. Muller's appointment was not renewed by the Amherst College Trustees, leading to his accepting a position at Indiana University in the summer of 1945.

A year later, and to the dismay of the Amherst College community, Muller won the 1946 Nobel Prize for his pioneering work demonstrating the induction of mutations in Drosophila by X-rays.[1] My future PhD adviser, Salvador Luria (see below), an avowed socialist/leftist like Muller, played an instrumental role in Muller's hiring by Indiana University. The award of the Nobel Prize catalyzed the immediate "resuscitation" and acceptance of Muller by the US government and biology community.

Because of Muller's influence and friendship, Plough became a member of the biological section of the Atomic Energy Commission and obtained a grant from the AEC to study the effects of X-ray irradiation on *Salmonella typhimurium* physiology. Thus Plough[2] showed that X-rays can cause mutations in *S. typhimurium*. He also discovered bacteriophage-mediated transduction,[3] at the same time as Zinder and Lederberg.[4] My 4-year undergraduate project in Plough's lab was to follow up on that discovery. I worked completely alone on the project, partly because by then Plough had redirected his own interests to sea squirts (aka tunicates) instead. Because he was a trustee of the Woods Hole Marine Biological Laboratory, Plough spent a few weeks every summer there, working with sea squirts instead of bacteria. He and his wife, Frances, often invited me on these and other scientific and social trips, exposing me to many aspects of science and culture. Thus, Plough, despite only a mild interest in my project, turned out to be the most influential scientist in my life, cementing my desire to follow a scientific career like his.

Following my graduation from Amherst College and accepting Plough's advice to "go to MIT and work with a young bacteriophage geneticist named Salvador Luria," I enrolled as a graduate student at MIT. Luria was a very colorful person, an intellectually stimulating and supportive mentor, encouraging all of his associates to work independently on their project, and occasionally offering key, insightful advice on the physiology of bacteriophage-infected bacteria. Because of my previous research background, I quickly finished my PhD degree, working on the biological role of bacteriophage T4 glucosylation. T4 replaces all of its cytosine residues with hydroxymethyl cytosine (HMC), and further modifies HMC using both of its α-glucosyltransferase and β-glucosyltransferase gene products. I isolated both missense and nonsense mutations in the T4 glucosylating genes and constructed many double-mutants.[5,6] It turned out that the T4-nonglucosylated mutants behaved like wild type T4 in all biological assays tested, except for their DNA sensitivity to prophage P1 and a novel *E. coli* restriction system.[7,8]

While in Luria's lab I worked not only on my PhD thesis work, but, intrigued by the existence of "super-suppressor" mutations in Yeast and the (then) newly discovered amber nonsense suppressors of *Escherichia coli*, I devised a selection to isolate and characterize my own suppressor mutants in *Bacillus subtilis*.[9] This was partly motivated by the need to isolate non-leaky, suppressible bacteriophage ϕe mutants, used by me and others in Luria's lab, e.g., ϕe was used by Losick and Sonenshein[10] to demonstrate changes in the specificity of the *B. subtilis* RNA polymerase during its sporulation.

During my 4-yr stay in Luria's lab, I saw him working only once at a lab bench. On that occasion, he wanted to show an undergraduate, the son of a friend, how to manipulate and assay bacteria and bacteriophage. As a consequence, a fellow graduate student, Linc Sonenshein, and I spent a whole Saturday afternoon tending to Luria's continuous string of commands: "Costa, you got some fresh Shigella culture? Linc, you got some fresh LB plates? Where do we keep the pipettes? Can you get me some?" That experience sealed my intention to never give up bench work. Shortly after this event, Luria shared the Nobel prize in Medicine with Max Delbrück and Alfred Hershey.

At the time I finished my PhD thesis, the smartest geneticists at MIT and elsewhere were working on the temperate bacteriophage λ. Thus, I decided to do my postdoctoral work in Dale Kaiser's lab at Stanford (following Luria's advice to "study under the most brilliant young λ geneticist"). I have previously described in detail how serendipity played a key role in my collaboration with the late Ira Herskowitz while we were both graduate students at MIT.[11] With Herskowitz, I

designed a direct selection and toothpicking technique to isolate *E. coli* mutants blocked in the propagation of bacteriophage λ at a step subsequent to adsorption and injection. I continued and expanded these original studies with the so-called *E. coli gro* mutants during my 2 y stay in Kaiser's lab (1969-71), and subsequently at the University of Geneva (1971-75), the University of Utah (1976-91), back to the University of Geneva (1991-2007) again, and finally at the University of Utah (2007-present). Despite all these shuttlings, my colleagues and I were able to characterize and study in detail 3 classes of bacterial mutants that were unable to support bacteriophage λ infection. One group was blocked in the λ N transcription antitermination mechanism (*groN*, Georgopoulos[12] and *groNB,* Keppel et al.[13]), a second group in λ DNA replication (*groP*, Georgopoulos and Herskowitz[14]) and a third group in λ morphogenesis (*groE*, Georgopoulos et al.[15,16]). In the above mentioned article,[11] I have reviewed in detail how the subsequent studies of *groP* mutants by my group and others led to the identification of the DnaK/DnaJ/GrpE molecular chaperone machine, and the studies of *groE* to the GroES/GroEL chaperone machine.

In 1986, during my only sabbatical year, I took advantage of the fact that both the DnaK/DnaJ/GrpE and GroES/GroEL chaperone machines were also shown to be heat shock proteins, i.e., their rate of synthesis increases with temperature to compensate for the concomitant increase in protein unfolding and aggregation (reviewed in Georgopoulos and Welch[17]). The idea was to devise a genetic screen for nonessential *E. coli* genes whose products are only essential at high temperatures. To do this in a facile manner, I created various *E. coli* transposon libraries at 30 C. These transposon-insertion libraries were subsequently screened by replica-plating and toothpicking to identify those candidates unable to form colonies at 42 C. The putative genes, inactivated by the transposon, were named *htr* for "high temperature requirement." These studies led my colleagues and me to identify and characterize the *htrA* protease gene (Lipinska et al.[18]; independently identified as *degP* by Jon Beckwith's group; Strauch et al.[19]). Our studies with the *htrB* gene[18] and its *msb* multicopy suppressors led us to identify the *msbA* gene as encoding an essential, putative Lipid A ATP-translocator.[20,21]

Since my obligatory retirement from the University of Geneva more than 8 y ago, my longtime collaborator and partner, Debbie Ang, and I have been attempting to decipher the biological roles of the plethora of uncharacterized, nonessential, mostly small open reading frames sprinkled throughout the chromosomes of T4-like, virulent bacteriophages. To do this, we again set up an appropriate genetic selection and toothpicking technique.[23] Thus far, we have succeeded in identifying the small T4 *39.2* gene (encoding a 58-amino acid peptide), whose function was completely unknown, as a subtle modulator of the GroES/GroEL chaperone machine. Both our genetic and biochemical studies suggest that the Gp39.2 protein shifts the equilibrium of the GroEL chaperone toward its so-called "open" conformation, capable of binding its host- and bacteriophage-encoded GroES cochaperone(s). Consistent with this interpretation, the growth of a bacteriophage T4 *39.2* deletion mutant, unlike its isogenic wild-type parent, is completely blocked in specific mutant *groEL* hosts.[23]

I would like to end this essay with a short story intended to encourage (relatively) young readers. Early one Monday morning, Luria came to my bench and asked me about my progress in finding the T4 mutant I had planned to isolate the previous week. When I told him that I found the mutant by toothpicking 3,000 plaques over the weekend, Luria exclaimed, "Costa, smart guys are a dime a dozen. I personally prefer doers like you." The moral of the story is that brute force experimentation may not be as intellectually pleasant and psychologically rewarding, but is often faster than trying to devise and implement a clever selection/screening technique. If I could do it, most of you out there can also do it and will probably do it better. Never give up. My limited understanding of the latest physics theories ensures that you will always succeed, in at least one of the seemingly infinite parallel universes continuously popping up…

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