Finding My Purpose in Life: Science as a Path for Self-discovery

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Abstract Life is all about making choices. While the degrees of freedom that one may have to choose from can vary greatly between individuals, a common dilemma for people is to choose a career path as one transitions from youth to adulthood. How we approach this is a very personal journey, and often, one does not really know the destination of the path that we chose when we start out. I mean, how "could" anyone know what the best career opportunity may be 15 or 20 years down the road? Or how our own perspectives and values may change as we mature and age? As technologies evolve ever more rapidly while our planet's environment and geopolitical outlook continue to deteriorate, how are we to attain financial security and maintain self-fulfillment at the same time? In other words, to get to a happy space with contentment when we become an adult. I am not wise enough to give specific answers to these questions, but I believe over the years, I have made some observations and encountered experiences that may speak to these common anxieties about making an important choice at certain junctures of our life. By sharing these anecdotes and opinions alongside to those of the other accomplished scientists in this volume, I hope my personal journey down the path of scientific discoveries would add more "food for thought" and assist our young readers to make their brave move with conviction and optimism. A caution: NOT choosing is in itself a choice and procrastination will likely makes the work needed to realize your goal much harder later on.

1 Motivations: How I Have Developed an Interest in Science

FINDING MY PATH IN A NEW LAND. If I look back at my chosen career path of science, and more specifically Plant Biology, I think my interest in understanding the natural world probably took its firm hold in the late 1960's around the time when

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D. Breviario and J. A. Tuszynski (eds.), *Life in Science*,
https://doi.org/10.1007/978-3-031-23717-1_8

I was 10 years old. Those were the heady days of the Moon Landing by the Apollo missions and the launch of the original Star Trek series on U.S. television. My family could not afford our own television set in Hong Kong at the time, and I remember hanging around after dinner outside shops and stores near my apartment building that may have a black-and-white television set turned on. There I watched with fascination the amazing events such as touching the surface of the moon for the first time by humans from Earth and imagined exploration of the galaxies with fantastic technologies in the not-too-distant future. Little did I realize then, my innate affinity for exploration and wonder about our place in the cosmos were being awaken. My curiosity was kindled and fortunately for me, this flame remains still to this day after more than half a century.

In 1970, my parents took the family from Hong Kong and immigrated to New York City. I was twelve and the youngest child along with a brother and a sister. I did not understand at that time what courage my parents must had to transplant the whole family to a new land, and the amount of sacrifice that they would have to make in the decades afterward. All that for the sake of better opportunities for their children. For us, and likely for many other families that moved to the United States without higher education, resources, or marketable skills, immigration was a traumatic experience financially and psychologically. We basically had to start from the bottom of the social ladder and work our way up, learning all the intricacies of the new society that we transplanted into, starting with learning to speak English—my parents never did learn English in part because of their more advanced age and daily struggles to make ends meet. During this tough time of my passage from adolescence to adulthood is when I first began to question my purpose and what is life's meaning. One can wake up quickly to the fact that the "American Dream" can be fraught with pain and suffering, when one began to encounter prejudice, bigotry and layers of bureaucracy. While my parents did not leave me with material riches, what they taught me is their work ethics, pride, kindness and optimism about the future. Their firm belief that education and knowledge is the most important key for our success and well-being shaped the course of my career choice. I was a good student in elementary and high school, despite my poor English in the first couple of years, while I have always enjoyed and did well in art. I especially liked my science classes and my high school biology teacher, Mr. Lucas, who was the first person that introduced me to photosynthesis and the world of plant biochemistry. I was fascinated by this fundamental process that provides the energy and oxygen which all animals depend on to stay alive. Growing up, I heard personal tales of survival from my mother about famine caused by the Japanese invasion and occupation of China during World War II and later on during the Cultural Revolution time in China. I thus have an early appreciation of agriculture as the bedrock for human society and the most fundamental of our essential needs. My interest to understand plants better was further sparked by the intricacies and beauty of the first steps of the process to capture energy from the sun and convert it into chemical energy as food stuff while making the oxygen that we need to stay alive.

After graduating from high school, I attended the State University of New York at Stony Brook for 3 years as an undergraduate with a double degree in Chemistry and

Biochemistry. While there, I conducted research for two years, first working with *Euglena* under Dr. Lyman and later focused on mitochondria bioenergetics under Dr. Tu. There, in lab classes and in my first taste of real laboratories, I found that I enjoy laboratory research immensely and have the aptitude for devising experiments independently. I was fascinated by how discoveries were made in different fields with very diverse sets of tools and model systems and thus read scientific journals voraciously. I especially enjoyed browsing the *News and Views* section of *Nature* for example in order to learn the cutting-edge development in many fields of science. Reading is also fundamental for me to learn how to write a scientific report as well, as I understood early on. However, the experience of trying to publish one's findings could be daunting. I still remember typing excitedly my first short paper for submission to a journal and drafting the graphs and charts for the data by hand in the wee hours of several nights. Then in a few months, I experienced the bitter taste of my first rejection with critiques from reviewers. After a week of gloom, overcoming my dejection and feelings of injustice, I went back to work and addressed the issues raised, followed with resubmission, etc. Thankfully, after another six months, the joy of receiving its acceptance by the journal and finally the ecstasy of seeing my name as an author in print! This experience along with subsequent successes in publishing additional papers during my undergraduate years solidified my decision to follow a career path in science. The competing choices at the time was becoming a painter or professional tennis player, both activities I enjoyed since my high school time. However, the odds for my ability to sufficiently excel in these two professions to enjoy some degree of financial security are much lower than that of getting a degree in the sciences. Another key deciding factor for me is that I found the process of knowledge discovery a truly exhilarating experience. For a moment in time, one could be the first person to hold on to a new scrap of knowledge about nature and which could add incrementally to our understanding of the world that we are in. To me, this is a special feeling that touched a personal chord in my conscience and relates to my later reflection about life's motivational force. I resolved then to pursue a career as a scientist and run my own laboratory to discover new knowledge in the area of Plant Biology.

2 Work Done: My Personal Scientific Approach

PATH TO MY "OWN" SELF-ENLIGHTENMENT (A 40-YEAR LONG JOURNEY). During the third year of my undergraduate work at Stony Brook, I applied to graduate schools with advice from Dr. Lyman, who strongly suggested that I study for my Ph.D. diploma at the University of California at Berkeley. This choice was made because of my interest to carry out photosynthesis research to make contributions that would improve agriculture and help alleviate famine in the world. During the 40-year period between 1950 and 1990, UC Berkeley was one of the hotspots of basic photosynthesis research in the world, starting with Melvin Calvin's Nobel Price-winning research that utilized carbon isotope to trace the biosynthesis

of carbohydrates and other organic compounds from fixation of atmospheric carbon dioxide. Enhanced by my successful research effort as an undergraduate, supported by having 3 publications already in press or in print at time of application, I was accepted to the Biophysics Ph.D. program at UC Berkeley with a full scholarship. In 1980, I left my family for the first time and traveled to Berkeley to begin my research career on the West Coast. I fell in love with the famous California sunshine and the laid-back atmosphere of the Berkeley campus. Since I did not know any of the professors in the program nor did I know what research topic interests me the most, I decided to attend introductory seminar classes related to photosynthesis in the first year to get to know more about the program and professors before committing myself to a laboratory. In the meantime, however, I secured a spot in the laboratory of Dr. Lester Packer in the Physiology department to study the mechanism of bacteriorhodopsin, also known as purple membrane. Dr. Packer was a well-known researcher in the field of oxidative stress, free radicals and human health. However, he also had a broad interest in bioenergetics that included the mechanism of light-dependent charge separation model systems, such as bacteriorhodopsin. This remarkable protein from a salt-loving bacteria called *Halobacterium halobium* is the simplest light-dependent proton pump known and uses retinal as the pigment for light absorption, in a fashion very similar to the opsins in our retina. I have known of Dr. Packer's laboratory in this field from my readings during my undergraduate days and wanted to learn about this interesting protein while getting settled into the new environment. Having my own support and armed with my short but convincing vitae, I was accepted into the Packer laboratory as a rotation student in my first semester and started working with a project to study the structure–function relationship of this simple transmembrane proton pump. With my prior experience working with rat liver mitochondria and membrane protein purification in the Tu laboratory, I quickly learned to purify the purple membrane patches from the bacterium and carried out chemical modification studies with various chemical reagents in an effort to determine which type of amino acid residues are important for the physical properties and function of this protein. During the 1970's, the age of molecular biology has just begun, and site-specific mutagenesis and protein structure determination were hardly routine. Thus, while the approach of chemical modification would be considered clumsy and inconclusive by today's standards, I was able to work hard and generated several small papers describing the effects observed upon treatment of purple membrane with reagents that modify different types of amino acids, with some showing interesting shifts in the chromophore's spectral properties. While my hard work and results from this project have been productive in terms of publications, I was dissatisfied with the ambiguity of my results, and I did not see a clear vision of how I could progress any further to reach more definitive conclusions without additional technologies to provide the tools needed.

By the end of my first year at Berkeley, I have attended several classes in plant biology and one special topics class on photosynthesis light reaction pathways given by Richard Malkin (hereafter referred to as Dick). During the early '70s, working together with Alan J. Bearden in Berkeley, Dick has applied the technique of electron spin resonance (ESR) to discover and study electron acceptors in the path of

energy transduction from absorbed photons in the reaction centers to their ultimate chemical carriers in the chloroplasts. I was fascinated by the new revelations that this technology has enabled and was also encouraged by new reports from several laboratories that have successfully isolated membrane protein complexes containing different components for the electron transport system from Photosystem II (PSII) to Photosystem I (PSI). It seemed to me that this complex pathway was then poised for new discoveries which can reveal the molecular mechanisms for energy capture and utilization by the chloroplast. I approached Dick in the Spring of 1981 to request a rotation in his laboratory starting at the end of my first year and he accepted me after an interview. I worked in Dick's lab that summer and began in earnest my Ph.D. thesis project to unravel the biochemical and structural complexity of proteins required for conversion of light to chemical energy while producing oxygen as a byproduct. It also began my long friendship with Dick and his family that continues to this day. While I appreciated all the great mentors that I have been fortunate enough to encounter throughout my career, the time that I have spent in Dick's lab and the warmth and sincere care that Dick and his family have bestowed on me have changed my life perspective. They showed me what mentors ought to do to help nurture the younger generation. In addition to the camaraderie that we had, such as sharing coffee in the morning before we start work in the lab, Dick and I often have informal chats about all-things photosynthesis. Most importantly, Dick encouraged me to strike out and explore my own ideas and often let me try things that he was not too convinced of or interested in. This is one of the most enjoyable time periods that I have had during the formative years in my career, with little responsibilities except to explore and learn while having little external pressure. In addition, as I mature in my knowledge in the field and begin to make significant contributions, Dick generously supported my attendance to national and international meetings, as well as to introduce me to many top researchers in the field at the time. These important activities helped to lay the foundation for the next step of my career.

My graduate work in the Malkin laboratory focused on the optimization of methods to purify active complexes involved in the light reaction of photosynthesis. These included PSII with or without the oxygen evolving complex, the cytochrome b_6/f complex, and PSI. Once these membrane-associated complexes were solubilized and purified from each other, they were then systematically characterized biochemically and functionally to define the minimal functioning units. For my thesis work, one of the achievements that Dick and I accomplished was the total reconstitution of photosynthetic electron flow from water oxidation by PSII to the reduction of NADP to NADPH by ferredoxin via the soluble ferredoxin-NADP reductase enzyme, together with all the resolved complexes and purified cofactors (Lam and Malkin 1982). This system allowed us to test competing hypotheses at the time on the specifics of donor/acceptor relationship between various components of the complex electron transfer pathway. Nearing the end of my thesis work in 1983, I attended one of the Photosynthesis Congresses and heard a lecture by Mary-Dell Chilton on the successful demonstration of stable plant transformation by a soil bacteria called Agrobacterium, using its newly discovered Ti-plasmid as a vector to direct DNA transfer and integration into the plant genome. This dramatic feat excited

me tremendously since it opened the door for much more precise experimentation than ever before to study genes and proteins in a living plant. I was convinced then that the molecular approach to precisely alter the sequence of a gene or its encoded protein would be the way to unravel the secrets of plant biology and advance agriculture. This conviction led me to seek out Dick's advice as to where I ought to go for my postdoctoral training. At the time in the early 1980's, it was the dawn of plant molecular biology when cloning of a plant gene was a high impact achievement worthy of a publication in *Nature* or *Science*. The race was on to isolate promoter elements of such genes that are active in plants and define the architecture of the controlling elements and relevant transcription factors, much like the bacterial promoter system worked out by pioneers like Jacob and Monod in the early 1960's. The sentiment in the field at the time was that like in the case of bacteria, we expected getting our hands on the promoter element and its corresponding transcription factors will quickly resolve their regulatory circuits. We were rather naïve in retrospect, as we learned in the subsequent decades.

Several prominent plant molecular biology pioneers in the U.S. that focused on nuclear encoded genes at the time were Robert Goldberg (UCLA), Nam-Hai Chua (Rockefeller University, referred to as Nam), and Elaine Tobin (also at UCLA), to name some of the top researchers. Dick turns out to know both Nam and Elaine quite well through their common interest in photosynthesis and plastid biogenesis. I first met Elaine because of my growing interest in duckweed, an aquatic macrophyte that was considered a model plant at the time, back in 1983. Our interest in duckweed was aroused due to the discovery of a duckweed mutant that was impaired in photosynthesis, and we suspected that it might have defects in electron transfer between the two photosystems based on its phenotype. Part of the effort we spent in the Malkin lab during the early 1980s was to generate antibodies to major components from each of the 3 electron transfer complexes. From a discussion between Dick and I, we thought it would be interesting to examine this mutant duckweed using both the ESR technique and immunoblot assay to query the components within the cytochrome b_6/f complex. After a phone call with Elaine, who was working with this plant at the time to identify and clone genes from duckweed, I soon went down to Southern California for a visit and obtained both the wild-type and mutant duckweed strains from her lab. Our work positively identified loss of the Riske iron-sulfur protein in the cytochrome b_6/f complex as a likely lesion in this mutant. In addition, we found that most of the other peptides within this membrane protein complex became unstable and thus are present only at very reduced amount, likely indicating the importance of this protein in mediating inter-subunit contacts. After I published this work before leaving Dick's laboratory, I stopped working on duckweed until thirty years later, as my career's focus came back full circle which I will mention later in this story.

At the end, I applied to work with Nam at the Rockefeller University, in part because I was impressed by his seminar at Berkeley during his visit in the early 1980s and because I wanted to move back to the East Coast to be closer to my family. I first know of Nam's name from his excellent work on organellar protein synthesis and transport in the days when coupled processing of signal peptide sequences of nuclear encoded proteins destined for organelles was being elucidated. However, his

seminar on his laboratory's more recent projects to combine tissue culture and in vitro molecular biology for deciphering the secrets of gene regulation using transgenic plants impressed me. I arrived back to New York City in the summer of 1985 to start the next phase of research training at the venerable Rockefeller University where much seminal work in biology had been carried out. While it is a small university in terms of student numbers and campus size, it boasts probably one of the highest numbers of Nobel Laureates per capita for a single institution that I have known. It featured many large laboratories that took up better part of a whole floor in some of its buildings. The Laboratory of Plant Molecular Biology is on one of the top floors of the Tower Building, located in midtown Manhattan, with views of the East River flowing by outside some of its windows. In this venue, I learned how big science is done in a large laboratory with more than 15 postdoctoral researchers that are smart, eager and industrious, all working on various research topics in plant molecular biology. We have access to state-of-the-arts equipment and reagents, including our own in-house oligonucleotide synthesizer when it first became available. Nam's laboratory that I worked in, and learned molecular biology techniques and approaches, opened my eyes to what it takes to compete at the highest level of science. Rubbing shoulders with top-notch postdoctoral researchers such as Robert Fluhr, Ferenc Nagy, Steve Kay, Pamela Green, and Cris Kuhlemeier, to name just a few of my contemporaries at Nam's lab, helped me to think deeply about research problems and properly frame the questions. The four and a half years that I spent at Nam's lab complemented in many ways my graduate training in the comparatively small lab of 6–7 people at Berkeley. I believe I have benefited from both settings to teach me about the pros and cons of both types of laboratories.

My projects at the Rockefeller University revolved around the many facets of cis-elements and trans-acting factors that combine to regulate gene expression. I spent several years devising ways to make transcriptionally competent nuclear extracts from various plants and tried to establish a robust in vitro transcription assay. While we have some successes, it was never quite good enough for large scale purification of the individual components required for promoter-specific transcription. What activities we had with crude extracts often were rapidly lost as we started purification procedures. However, using the nuclear extracts that we have learned to make, I was able to optimize the assays for monitoring and characterizing sequence-specific DNA binding proteins that interact with active promoter sequences. After 3 years of research, we have generated functional results for different cis-acting elements, showing that a single copy or multimers of a simple 21 base pair sequence can produce strikingly distinct patterns of promoter activity in transgenic plants. For example, addition of one particular 21-basepair sequence, called *as-1* for Activating Sequence-1, can turn a normally leaf-specific promoter to become highly active in the root of plants. In the last couple years of my work in Nam's lab, a big leap forward for me was the cloning of one of the first sequence-specific binding plant transcription factors, TGA1a and TGA1b, from tobacco. This was a collaboration with Fumiaki Katagiri, then a graduate student in Nam's lab, who adopted quickly the new method, devised in Philip Sharp's lab in 1988, of cDNA phage libraries screening with binding site multimers to isolate the genes for the binding factors

of interest. The result was my first publication in *Nature* as a co-author (Katagiri et al. 1989). These key advances also enabled me to rapidly generate many additional results with other cis-elements that I have already characterized and begin to formulate the next series of questions and projects that I could tackle in my own lab. I started my job search in academia at the Spring of 1989 and accepted an offer to start my own research group at a new center (the AgBiotech Center) that was being built in the nearby Rutgers University of New Jersey, just across the Hudson River from New York.

I started my own laboratory at Rutgers University in the summer of 1989. My first two years at New Jersey was a hectic time both in terms of working to set up my laboratory from scratch, writing grant proposals, recruiting people, and to start new experiments for different projects. By then, I also had three young children and we bought a modest house near the university with financial help from my brother. It was a busy, but also exciting time in my career. It was the heyday of biotechnology, and the U.S. economy was good, which translated into ample Federal and State funding for research and education in the plant molecular biology area. I was fortunate to be awarded with a coveted NIH grant in 1990, as well as additional grants from NSF and the USDA over the next couple of years. Together with my support from the AgBiotech Center, my research was well funded, and I was able to recruit students and postdoctoral researchers to my team without much trouble in those days since my publications were at the front of the field at the time. In the remaining paragraphs of this section, I would like to summarize what I think are my key independent contributions to plant biology and focus my description on how and why I ventured into these areas as I navigate my own path of research after leaving the wings of my graduate and postdoctoral mentors.

1) *Targeted disruption of a non-selectable gene in plants.* Back in the early 1990s when I started my lab, functional genomics tools were essentially limited to increase or decrease of a gene's transcript by overexpression of its sense or anti-sense coding region. We quickly found that this approach is confounded by a plethora of limitations such as position effects of the transgene, inadequate suppression, potential off-target effects, and unintended gene silencing, to name some of the most obvious issues. After working very hard with these approaches for four to five years, we found that few if any clear functional insight for our cloned transcription factors could be gleamed. I was convinced then we need to establish targeted gene knockout in the field to enable more definitive approach for determining gene functions. Working with my postdoc Zhonghe Miao for several years, we developed a gene targeting vector and reported its efficacy with Arabidopsis callus tissues for targeted disruption of the TGA3 locus in 1995. This vector was used in 1997 for *in planta* Arabidopsis transformation in Marty Yanofsky's lab to successfully generate a targeted disruption of the AGL5 locus after screening more than 750 transgenic lines. This was the first report of targeted disruption in plants of a non-selectable gene via homologous recombination (Kempin et al. 1997).

2) *Programmed cell death in plants.* In 1994, I was promoted to Associate Professor with tenure and was feeling more adventurous to explore risky ideas. One that came to me at the time is whether we could endow plants with a novel photoreceptor that can enable it to utilize photons in the green spectrum since chlorophyll absorbs light only in the blue and red spectral regions of visible light. Bacteriorhodopsin, a protein that I have worked on as a young graduate student, appears to be a perfect fit for this task since it absorbs light specifically around the yellowish-green region and reflects blue and red light. I convinced a new postdoc in my lab at that time, Ron Mittler, to take on this idea and determine if transgenic tobacco plants expressing bacteriorhodopsin can be made to absorb green light upon addition of the missing retinal chromophore. We found and reported in 1995 that our transgenic plants expressing this foreign gene resulted in ectopic cell death lesions and later demonstrated that these plants are more disease resistant to plant pathogens such as tobacco mosaic virus. This chance discovery demonstrated that expression of this single ion channel in plants can activate innate immunity that included spontaneous cell death activation. Since then, my lab has been working to understand the complex pathways that link programmed cell death to plant defense activation and stress responses (Lam 2004). Over the past twenty years, we have focused mostly on examining the structure and function for two types of cell death regulators that are highly conserved across multiple phyla: the metacaspase protease family and the Bax Inhibitors-1 cell death suppressor. This story is an example of serendipity that led us into the cell death and disease resistance field.
3) *Chromatin and genome organization.* By 1995, I have also begun to appreciate the difficult challenge for reverse genetics approach in organisms such as higher plants where gene families exist for many of the transcription factors, and crosstalk between pathways known and unknown makes mild quantitative effects very ambiguous. While we have shown that gene targeting for any locus is in principle feasible in Arabidopsis, the amount of work required is daunting as a routine method. By then, effort from multiple laboratories in Europe and the U.S. to create insertion mutant libraries using either transposons or T-DNA as mutagens were also beginning to be contemplated for Arabidopsis. In parallel, whole genome sequence for this model plant was also underway. The combination of these development made it likely that libraries of mapped insertion lines could become available for the community soon. I thus decided to turn the effort of my young lab to explore new grounds that I believe will be more fertile for novel discoveries. A key difference between eukaryotes and prokaryotes is the increase in DNA content per cell as well as their packaging into chromatin within the nucleus of eukaryotes via histones and other accessory factors and scaffold proteins. While the architecture of the nucleus and its enclosed genome has remained mysterious for decades, earlier observations in *Drosophila* and mammalian cells have shown that activation of certain genes can be correlated with changes in their subnuclear locations. This and other related reports on the effects of physical location of a gene with respect to the other parts of the genome on its activity suggested to me that the ability to track a gene's 3-D

location in a living plant could be a key tool to begin to address this aspect of gene regulation. This was a feat that would have been difficult to imagine in earlier days before 1995. However, it was just a year before that Marty Chalfie reported the remarkable discovery of a portable fluorescent protein, the green fluorescent protein (GFP) from jellyfish, with no requirement for addition of a chromophore (Chalfie et al. 1994), and the biology community was all excited about its possibilities. I began to try to come up with ways of utilizing GFP for tracking chromatin DNA in situ for live plants. The obvious challenge is to increase the number of GFP molecules to obtain sufficient fluorescence signals that can be localized to a specific sequence embedded in a known location of the genome. Eventually, my experience with multimerizing DNA binding elements to increase their strength of enhancer activity suggested that insertion of multimers for a heterologous cis-element can be used as a beacon within the genome that we could then "light up" with the corresponding DNA binding protein fusion with GFP, so long as it would not bind significantly elsewhere in the genome. But what sequence to use and how many copies would be necessary? As it happened, the exact same strategy was being contemplated by the labs of Andrew Belmont and Andrew Murray working with mammalian cell cultures and the budding yeast *Saccharomyces cerevisiae*. They demonstrated the efficacy of the method by using the well-characterized bacteria DNA binding protein *Lac* repressor and multimers of its target sequence *Lac* operator. This was further developed by 1997 in John Sedat's laboratory to monitor and quantify constrained movement of interphase chromosome for the first time. By then, GFP application in plants was finally getting a foothold from the realization that the original coding sequence of the jellyfish gene contains a cryptic splice site which prevented its ability to be expressed in the intact form when using plant cells. Discovering this critical issue, Jim Hasloff and colleagues in the U.K. finally solved the GFP expression problem in plants with mutated versions that removed this splice site. I remember visiting Jim's lab soon afterwards and obtained his latest mGFP vector as well as discussed with him my interest. With encouragement from Jim, I began to assemble the required pieces for this system from Aaron Straight and set out to test them in my lab. In 1999, I wrote an application to the new Plant Genome Research program of the NSF and obtained my first multimillion dollar grant to implement a chromatin charting project where we would map and quantify the transcription potential and diffusion coefficient for many insertions which we will create with a multimerized *Lac* operator tag. With this funding that enabled us to purchase one of the most advanced 3-D imaging system available at the time and recruiting a talented postdoctoral fellow, Naohiro Kato, we reported in 2001 the first transgenic plants in which we could visualize and track the movement of individual insertions. For the next seven years, I assembled a team in collaboration with Robert Martienssen and David Spector's groups at Cold Spring Harbor Laboratory to carry out the Chromatin Charting project. It culminated in a publication that described a collection of 277 transposant lines with our custom designed construct for insertion tracking and transcription potential monitoring using a luciferase reporter gene (Rosin et al. 2008). Examining a set

of transposant insertions clustered on one end of Chromosome 2, we carried out detailed studies that generated information about potential modes of epigenetic regulation of gene activities. Unfortunately, our project was not renewed because our transgenic approach and use of repeat elements were criticized as prone to artefacts. In addition, the advent of chromatin structure investigation using a new technique of proximity crosslinking combined with Next Generation DNA sequencing technology to query chromatin organization was cited as a more superior approach. While I do appreciate the advantages that the new technology (which is called HiC) has to offer, the functional data and mobility information that we could generate from our insertion lines remain a unique resource to study chromatin behavior in live plants. As an example, the quantitative description of DNA mobility along the length of a chromosome arm that we reported for different cell types in live plants is still an uncommon set of results to this date.

4) *Duckweed as a novel model crop plant for research and applications.* When our chromatin charting project ended around 2008, it coincided with the time that I was promoted to Distinguished Professor and was appointed as the Director of the AgBiotech Center. I was also awarded a sizable training grant from the NSF to support a large cohort of graduate students for over 6 years in the renewable energy area of research at Rutgers University. Through managing this large, cross-campus interdisciplinary training grant, I gained a deep appreciation of the looming crisis of Climate Change and our unsustainable dependence on fossil fuels. These are clearly existential challenges for our generation and all the scientific evidence that have been gathered since the 1970's indicate that our species would be running out of time very soon to find the right set of solutions for avoiding a global catastrophe that will confront humanity later in this century. In addition to formidable challenges in the geopolitical and policy fronts, finding solutions for feedstock production that is environmentally friendly while economically attractive are especially difficult. While billions have been invested in algal and cellulosic biofuels, they remain non-competitive because of the high costs for their production. In early 2009, I was reunited with duckweed as a research subject due to a combination of circumstances. A central figure in this turn of events is my colleague Todd Michael, who joined Rutgers as an Assistant Professor in 2007. Todd's main research interest is plant genomics and we quickly became good friends sharing common interest to develop advanced NGS at Rutgers, which culminated in getting support from the Waksman Institute at Rutgers to purchase the first SOLiD platform on campus at the time. Among the myriad plant species that Todd started genome sequencing projects on, he added duckweed (aka water lentil) to his list from a suggestion by Randy Kerstetter, who was also a professor at Rutgers at the time. However, in 2009, Todd decided to move to work at Monsanto in St. Louis but called me one day to ask if I would be interested to manage a large collection of duckweed germplasm. It turns out that the company Biolex, which had been working to commercialize duckweed-based human therapeutics, was going out-of-business and they were essentially cleaning out their stock collection of duckweed strains that were brought over to the U.S. from the Landolt collection at Switzerland decades ago by Anne Stomp.

Todd had been trying to get these strains from Biolex before but without success, and just before he was to leave Rutgers to move to Monsanto, this opportunity arose but has a short timetable for us to decide. We both agreed that this is too valuable a resource to refuse, in which case Biolex will likely discard them and these clones could be lost forever. At that time, I happen to have resources available from the Biotech Center as well as students from the new NSF training grant. So, I agreed to take care of this duckweed collection and that is how I restarted my research on these tiny plants in 2009. Since the ~650 clones of duckweed arrived to my lab from that time, it became a work of love for me to take care of them over these past 13 years. What sustained me over these challenging times of initiating a new area of research from the ground up is the firm belief that these plants could hold the key to a powerful new platform for feedstock production that will be cleaner, more productive and climate resilient than traditional crops. Their small size, simple aquatic habitat, and growth by vegetative budding make them amenable to scalable production without need of arable land (Fig. 1). On the other hand, unlike microalgae, their floating nature and size enable their easy harvesting by simple filtration and hydrology.

As I learn more about these tiny plants in the past dozen years, I became convinced that they offer an untapped natural resource that can play a key role to transform the way agriculture can be carried out. However, to make this plant model competitive for external funding has been more challenging than I expected in 2009. Chief among the needed foundation to enable the duckweed community to thrive in terms of attracting support for research or development are unified standards for nomenclature, broad access to well-curated germplasms, high quality genomic resources, and integration of societal engagement through commercialization efforts. The latter is especially a key to sustaining the support and expanding the impact from research with these plants far into the future. Working with a core group of duckweed research colleagues, including Todd Michael and Klaus J. Appenroth, I believe we have achieved many of these aims over the past dozen years, which are described in an invited review

Fig. 1 Duckweed in the wild and as a crop. *Left panel*: a pond in a garden is covered with the duckweed Wolffia (Hamilton, New Jersey) with the author shown on the left next to his brother Roger. *Middle panel*: freshly harvested duckweed (Landoltia spp.) grown on hydroponic system. *Right panel*: dried duckweed from hydroponic cropping system

published in the *Plant Cell* (Acosta et al. 2021). Currently, my own research on duckweed has two prongs: one is to elucidate the induction mechanism for turions, a form of dormant duckweed that sinks to the bottom of lakes and ponds and allow the plant to "hibernate" over the cold winter months. Another topic is the characterization of the duckweed associated microbiome, with the objective to uncover the basis for assembly of a stable microbial community that can benefit plant heath. Over the past seven years, I have also tried to learn how to farm duckweed effectively in order to understand the challenges for its commercialization. While I first started with ponds at a local nursery in New Jersey, I switched about five years ago to focus on establishing a modular, vertically integrated system with the objective to build a scalable platform that can be replicated quickly as well as fit for automation. The vision is to pave the way for eventual creation of a hands-free agricultural platform that can be driven by all the advanced technologies of the 21st Century such as robotics, artificial intelligence and wireless controls. Working with a graduate student Shawn Sorrels, we have founded a company Planet Duckweed (www.Planetduckweed.com) two years ago and are now moving toward the fund-raising phase to make duckweed products a reality in the marketplace. There is a lot of new tools and knowledge in the business arena that I must learn in the coming years, and I look forward to those challenges. Overcoming them should help make the duckweed field a vibrant one for investment by government agencies as well as commercial ventures.

3 Science Today and Tomorrow

HUMAN VERSUS MACHINE. In my own experience over the past 45 years working in science, especially in the biological disciplines, I have witnessed truly remarkable progress in knowledge acquisition—not only in the number of game-changing discoveries, but also the rapidity that these new advances have been realized. While I mentioned a few in describing my own journey in the last section, another simple tool that took over Biology practically overnight is the method of DNA amplification called polymerase chain reaction which was first reported in 1985. Another more recent technology is the famous CRISPR/Cas gene editing method that almost became household names in biology and medicine since its first publication in 2012. Of course, a more dramatic advance that truly permeated our society is the advent of digital technologies driven by computer hardware, software and satellite communication capabilities. Artificial intelligence will enable the use of big data that are being accumulated worldwide at a truly remarkable speed that would not have been thought possible merely a decade or so ago. A case in point is the recent advance of the AlphaFold platform published in 2021 that made significant improvement in our ability to predict protein folding structure based solely on the amino acid sequence (Jumper et al. 2021). These examples signify to me that scientists will need to have broader knowledge to utilize and assess what various data and machines can provide, as well as their limitations. In other words, a generalist with broad visions and skillsets may be more able to work effectively with interdisciplinary teams having two or more

complementary approaches or capabilities. The state of information availability and their proper "filtering" is another interesting shift of the paradigm between human memory needs and personal judgement. With a simple smartphone, an average person on this planet can now readily access more information on most topics than one can digest completely—think how many pages of a Google search output does an average person bothers to read. So, a new challenge for us in this information age is finding the right data and knowing the way to assess their validity as well as to integrate them. This challenge is true for us in this epoch for politics as much as with science. For scientist, the ability to design the right experiments for the proper questions, and to then assess the data with the right methods will be the "art" behind the laboratory bench that gives our work its personality. I do not think that this will be replaced by a computer algorithm in the near future, just yet.

TECHNOLOGIES: OPPORTUNITIES AND DANGERS. At the societal level, the awareness of how powerful technologies can offer both prosperity and catastrophes at a global scale has never been more acute in human history than in the past century. Advances in particle physics directly led to the creation of nuclear weapons in addition to nuclear power plants. The arms race during the Cold War era directly threatens the survival of the human species along with other life forms on a planetary scale. To this day, stockpiles of nuclear warheads continue to exist in an increasing number of nations and the threat of a nuclear holocaust remains. In contrast to the clear and present danger of the "bombs", the existential threat posed by the unintended consequence of rampant use of fossil fuel is much more insidious and challenging for human nature to deal with. While the rate of ocean temperature warming and rise of atmospheric carbon dioxide content has been steady and alarming during the post-industrial era, there is little political will to make serious adjustment to our fossil fuel-driven economy worldwide. Introduction of agriculture about 10,000 years ago was perhaps the first technology that changed much of the landscapes and altered the representation of plant and animal species on arable land of our planet over the millennia. In contrast, global warming catalyzed by greenhouse gas emission from massive fossil fuel utilization is impacting all parts of this planet's surface chemistry in merely a couple of hundred years. Humanity is currently undergoing an experiment on a planetary scale since this rate of rapid warming and rise in carbon dioxide in the atmosphere have not been seen before from ice core studies that go back 800,000 years. The outcome is uncertain, because like all good experiments it has no precedent. If unchecked, most models predict dramatic rises in sea levels, coupled with large extremes in climate patterns and rapid loss in biodiversity globally. These predicted effects is already evident over the past decades with dramatic disappearance of most glaciers worldwide as a vivid example. While policies and human nature appear too slow to change in response to this threat, I believe good technologies based on new science are urgently needed to mitigate the effects of Climate Change as well as to provide new tools that can disrupt our historic dependence on arable land and a predictable climate. Thus, new technologies and their associated science for the next millennium would need to be cognizant of their potential impact on the earth system as much as their immediate societal benefits. Some of the current efforts to minimize pressure on global deforestation to create pasture for meat production by

advancing plant-based or artificial meat products is a forward-looking approach that is gaining acceptance in the marketplace. With more optimization of the relevant food science and product development in the future, I could imagine perhaps in a decade or two, when we could revert pastures back into carbon sequestering forests all over the world. Similarly, improvement in vertical farming technologies could help minimize excess fertilizer runoffs while producing fresh produce close to the consumers, which could decrease transportation cost while lowering their carbon footprint for production. These are but a few examples of topics for investigations that could produce important solutions needed for climate resilience in the coming decades.

ECOSYSTEM OF SCIENCE AND BUSINESS. Another aspect of the evolution in scientific enterprise is a shrinking division between basic and applied research. One reason for this tendency is that while basic research driven primarily by curiosity of the natural world serves as a foundation for novel discoveries, those topics that are most relevant to societal needs typically garner significantly more funding from either government agencies or commercial sources. Human health related topics and key crops for agriculture are two examples of subject areas that are usually among the more well-supported. In the last two decades, there is also a growing trend of academics turn entrepreneurs who start their own commercial startups to develop their academic research results into products, often with early support by their institutions. This has created a growing ecosystem between the business and academic worlds where the potential for conflicts of interest can be substantial. That being said, I believe a good scientist can be an excellent Founder to translate good research results to benefit the neediest segment of the population, and not necessarily for the most financial gain. While there are examples of academic researchers who became successful entrepreneurs, it is very rare for a successful businessperson to shift successfully into academic research. This is in part due to the rather lengthy process required to gain the experience and track record needed to compete for a tenure-track position in an academic setting. My own introduction to the commercial aspects of basic research is through involvement in filing patent protection for discoveries that I have made in the Chua lab as a postdoc. Later, I have also learned to file patent protection for discoveries made in my own laboratory at Rutgers University. From this experience, and now with my aim to ensure that we could help develop duckweeds into a new sustainable crop, I have grown to appreciate that the business world works quite differently from academic research and a critical skill for a successful entrepreneur is to be able to build a core team with complementary skills in order to deliver a successful product. A big challenge is consumer education through advertisement, which one can leverage the available social media in today's world to reach the global market effectively. In the long term, my vision is to create a successful automated agriculture platform that can integrate basic research directions, such as duckweed microbiome research, together with optimizing a hands-free plant production system that can produce high quality food anywhere by everyone. Getting this technology to disadvantaged populations in the world to help democratize availability of nutritious food would be realizing the dream that I had 45 years ago to help mitigate famine by the fruits of my research.

4 Advice to the Next Generation of Scientists

HOW EVOLUTION HELPS ME TO RATIONALIZE MY OWN COMPASS ON THE PATH. I like to finish this chapter with a note of optimism. Stay curious, I'd say to the reader, and maintain your wonder about our world and our lives. In this story, I have shown two examples of serendipity that have consumed a large percentage of my career to work on: the topics of cell death in plants and duckweed as a new plant model. What they served is to illustrate that often we do not know the actual destination of the journey that we may take, but one has to trust one's own heart or intuition. Looking back, I chose these paths because I thought they were not crowded already with competing labs and I believed that I could do something interesting that has not yet been done by others. Most importantly, I felt they could have a big impact if my work is successful. This last point is a crucial one for me, and it circles back to the end of Sect. 1 when I described why I chose the path of science as a career.

A famous quote from the physicist Steven Weinberg is that "the more the universe seems comprehensible, the more it also seems pointless" meaning that there is no proven design for our existence and our destiny has not been prescribed (Weinberg 1977). However, that is not to say that our existence is meaningless—we come, we go and eventually the sun goes dark, and humanity will disappear in time. As the great science communicator Carl Sagan aptly puts it "our species is young and curious and brave and shows much promise" (Sagan 1980), what we need is for some of us to do the right thing at the right time, and keep humanity as a whole on the evolutionary path to a greater future for our species. That, I believe, is what our "purpose" could be. Knowledge, provided by good science, is like candles in the dark of the cosmic universe, and one after another, they can add to light the way for our next step in a path without fear and prejudices. At this juncture of our evolution, it seems that humanity is likely at the brink of a Climate Catastrophe which we are seeing the dramatic beginnings of over the past decades, with worsening climatic patterns recorded every year and species are disappearing at an alarming rate. While politicians are continuing their debate as to what policies to enact, we as scientists, artists, engineers, or entrepreneurs could all find our own niche to create and to build solutions, both small and big, to help make our planet more hospitable and peaceful. That perhaps would be the best mental driver to help you stay focused and persevere through difficult challenges on your career path: believing in what you want to do is important for now and that it will lead to a better future for all.

Once you have made your choice to pursue science as your calling, how could one start to learn all the necessary knowledge and skills required for an independent position? I hope Sect. 2 of my chapter has illustrated my own personal journey through this formative phase of a science career. Chief among the important ingredients for cultivating your skillsets is an interactive and vibrant laboratory as well as a research-active institution with a strong science culture. Through finding the most interesting publications in your field, I believe you would be able to make a list of the laboratories in the world that you admire the most in terms of the quality and depth of the work that they have published. Try to visit them if possible and if you can attend scientific

meetings, listen to their talks to get an impression of their intellectual and personal qualities to see if they are someone you like to emulate. In other words, are they likely to be a role model for you? While you are contemplating these people and places that you are likely to aim for, prepare yourself as best that you could by working hard at where you are at the present time. Assuming that you are at the undergraduate stage, volunteer to do research at a lab that is working on a subject of your interest and just take a deep dive into the work to see if you can learn it well enough to produce some significant data by the time you are nearing graduation. This would help you to compete for entry into a good laboratory. Once you are in a laboratory, bear in mind that there will be competition, either real or perceived, in any setting. You need to demonstrate your abilities and accomplish the goals of your project, but you also need to be considerate of others in your laboratory environment so that you can help create camaraderie in the lab for the benefit of all. Often, the network of colleagues and fellow students/postdocs that you work together with would be in the same field with you for many years to come. What I learn over the years is that productive collaborations with other laboratories can help me accomplish much more of my goals without having to support many people in my own laboratory. In other instances, it enabled me to compete effectively for grant proposals by bringing in technologies that I have no experience in. Your reputation as a good and fair colleague is just as important as your scientific abilities in your peers' eyes, so learning good people skills is an important asset for your career. In spite of all good intentions, however, there are always unfortunate situations when friction can occur. My advice is to treat others with kindness in these situations and never let the challenging times change your values and goals. Remember that we are all in this evolutionary path together and our lives' purpose may be more similar than most of us realize.

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