Getting Along With a Little Help From My Friends

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The successful launching of the Russian satellite, Sputnik, in October 1957 changed the course of American Science and my future. The shock waves reached my high school in Freeport, N. Y., a complacent suburb on the south shore of Long Island notable for its easy access to New York City and Jones Beach State Park. The school was uninspiring---its heroes were the athletes and cheerleaders, who were objects of admiration and respect especially by those outside their circle.

Fear gripped the nation. We had been told we were winning the missile race, but the glittering new star in the evening sky blinked undeniable evidence that we were behind. Something had to be done. Overnight, science became a national priority. Our principal, a former high school athletic star, grasping for familiarity, announced to the school that the Russians had launched something the size of a basketball. Local engineers from Grumman and Republic Aviation took the challenge more seriously and, energized by the national dread of Soviet technological superiority, taught advanced math courses pro bono at our school. One even switched jobs and became a teacher at the high school. Overnight, the cheerleaders took an interest in the smart kids.

A family friend, Jacob Yamins, perhaps the only Bohemian in town, noticed my growing interest in science and invited me to visit his laboratory at National Dairy. An organic chemist trained at MIT, Yamins was working on a test for phenylketonuria and the synthesis of L-DOPA on which he pinned his hopes for a cure for Parkinson's disease. His stories about similar studies in laboratories working on the same problems in Baltimore, St. Louis, and Berkeley sparked my interest in metabolism and supported my growing awareness that there was a lot going on outside of our town. Yamins took an interest in my education and channeled me towards his alma mater, Amherst College. A friend from high school, Harold Varmus, then a freshman at the college, encouraged me to come and avoid becoming "...just another grind in Harvard Yard."

College—a first taste of the regulation problem

In my junior year at Amherst two lectures weighed heavily as I thought about my future. One was by Linus Pauling from Caltech, who was a guest speaker in the physical chemistry class. Pauling peppered his explanation of the ideal gas laws with anecdotes about his youthful interest in measurement as he watched his father work in the pharmacy. He bobbed up and down excitedly, punctuating sentences with a broad smile, and weaving his personal observations together with the science in a way that made everything seem vital and clear. Making the difficult so simple and entertaining was alluring.

Another influence was a lecture by Professor Benjamin DeMott, who taught a course on the 19th century continental novel. DeMott often asked one of us to read the class a passage from the novel under study so that we could feel the power of the words. On prom weekend DeMott himself read the Grand Inquisitor scene from The Brothers Karamazov. The bell rang signaling the end of class, but stunned by the drama of his performance, no one rose. Slowly, one, then another of the couples released from his spell, ambled silently out of the room.

These experiences confounded my decision--should it be science or literature? I am not certain what pushed me towards science, but my impending marriage at the end of my junior year and the award of an NSF summer fellowship to start a research project...
on Drosophila in the laboratory of Dr. Philip Ives, probably played a big role.

The Amherst/NSF research program extended into my senior year and included a student seminar program. My assignment was to present the now classic JMB paper by Jacob and Monod (1) on the operon model for the regulation of metabolism. The omniscience of the paper mesmerized me; it seemed to explain everything from the regulation of bacterial physiology to animal development. I bought into a comment attributed to Monod, "What's true for E. coli is true for the elephant." My fascination with gene regulation was also fueled by the NSF weekly seminars presented by visiting scientists from other institutions. I was particularly intrigued by one presented by Yale's Norman Giles, who was trying to determine whether Jacob and Monod's ideas applied to the regulation of metabolism in fungi. He suggested that, given my interests, I should come to Yale and work with him.

**Pursuit of the Eukaryotic Operon**

When I began my graduate work in Giles' laboratory in 1962, the science community was still abuzz with the implications of the Jacob and Monod paper. My savvy lab mates, Mary Berlyn and Asad Ahmed, and I obsessed over every new paper on regulation and decided that the most interesting question was whether anything like the bacterial operons existed in eukaryotes. To a first year graduate student, the histidine pathway in yeast appeared to be the ideal system in which to test for the existence of a eukaryotic operon. Many histidine mutants were available in yeast and the organism had a tractable genetics. Moreover, Bruce Ames' laboratory had developed the perfect standard for comparison: in Salmonella all 10 steps in the histidine pathway were encoded in the quintessential bacterial operon transcribed into a single mRNA (2).

So, the question was: Were any of the yeast histidine genes organized into an operon? Although a number of histidine genes were located on different chromosomes, some of these loci might encode more than one protein and be suitable candidates for an operon. The first step in determining gene organization would require associating each of the known histidine mutants with its enzymatic defect. As a novice, I had failed to appreciate that the substrates for the requisite assays were not commercially available. My enthusiasm was replaced by the discouraging reality that I would be bogged down in synthesizing the substrates, unstable phosphoribosyl derivatives of ATP, before I could answer any question about eukaryotic operons. My thesis advisor, Norman Giles, turned down my $1500 order for PRPP, politely intimating that this was too steep an investment for an untested first year graduate student.

With some trepidation, I called Bruce Ames at NIH, already a luminary, and asked him for advice on the synthesis. His response was completely unanticipated. He invited me to come down to NIH and carry out the synthesis of these intermediates in his laboratory. He said it would take a week and that I could stay with him and his wife, Giovanna. He mentioned that efficient synthesis would actually require 2 grams of PRPP. When I told him that I could not pay for it, he seemed unperturbed and offered to foot the bill so long as I shared half of the histidine intermediates with his laboratory.

Bruce's hospitality and enthusiasm made it obvious where I would do my postdoctoral work. Moreover, my successful synthesis of these histidine intermediates at NIH allowed me to assign each of the genes in the yeast histidine pathway to an enzymatic reaction. Most of the genes controlling these conversions were unlinked; however, three of the steps were localized to a single locus (HIS4), leaving open the possibility that there was an occasional operon in eukaryotes (3, 4). Bruce's largesse let me finish my graduate work rapidly and in 1965 and I headed down to NIH, with my wife, Rosalie, and daughter, Julia. My goal was
to learn enough about bacterial operons and protein biochemistry so that I could determine whether the yeast HIS4 locus qualified as an operon.

Postdoctoral Research---The Bacterial Operon

The Ames laboratory in Building 2 at NIH was heaven, but a very small heaven. Bruce and I shared one of his two tiny adjoining labs, which also served as his office. Every day around coffee time, people from the floor would drift in to this cramped lab/office to schmooze. Inevitably, idle chatter morphed into a scientific discussion that could ignite into a heated debate. Bruce usually provoked the dialogue with a fanciful idea about regulation delivered with an impish grin: "Suppose regulation really works like this." And he would eagerly present his pet conception on a small chalkboard glued to the refrigerator door. First to contest the provocative new idea was Bob Martin, a brilliant MD trained in physical chemistry. The postdocs (John Roth, David Silbert, David Berkowitz, and I) might wait for a pause in the action to interject a comment, and ultimately others from the floor (Marty Gellert, David Davies, Gordon Tompkins or Gary Felsenfeld, attracted by the commotion, would stick their heads in and offer opinions. As the audience enlarged, people squeezed into the remaining space so they could see what the fuss was about.

These spontaneous forums were energizing, but one had to be bold and thick-skinned to offer an opinion---and new postdocs rarely got the floor for more than a comment. One night I came home very excited and regaled my wife with my success at proposing an alternative to one of Bruce's new models. My way of looking at the problem had captured the discussion. While recounting the day's events, uncertainty struck me; I was not sure that the great idea of the day was mine. I had been a participant in the fray, but on reflection I was not confident of my authorship. In retrospect this is the mark of a great laboratory---new ideas evolving from the interaction between articulate, respectful, but critical colleagues, who are not easily offended. Often no one owns the idea; rather, it is born from the synergy of the debate.

While in the Ames laboratory, I became familiar with the experimental methods used to study regulation in bacteria. I discovered that regulation of the histidine operon in Salmonella was intimately connected to the role of tRNA in Salmonella (collaborating often with John Roth and Bob Martin) and published several papers (5-9). As I contemplated my future directions, the excitement of the work on bacteria began to take a back seat to the lure of the unsolved operon question raised by my graduate work.

An Independent Career: Eukaryotic Operons --- Do They Exist?

Armed with the nuances and technology used to study bacterial operon regulation, I began my independent career at Cornell in 1967 determined to solve the operon puzzle in yeast. Although the yeast HIS4 region, a locus that encoded three steps in the histidine pathway, appeared to be the closest eukaryotic analog to a bacterial operon, the genetic and biochemical data I had obtained were compatible with two possibilities: the locus could be an operon encoding three separate polypeptides bound together in a multi-protein complex, or just a single gene encoding a multifunctional protein. My attempts to resolve the puzzle by purification were frustrated by proteolysis: on some days the three activities could be separated into different fractions, each capable of catalyzing one of the three reactions; on others a small percentage initial activity remained as a single polypeptide containing all three activities.

Of course, the DNA sequence of the HIS4 region could easily resolve the issue and show unambiguously whether the region encoded a single gene or an operon. But, in
1974 there was no method for isolating yeast genes. The "operon or not" question motivated my attempt to devise a transformation system for cloning specific DNAs in yeast. As I have written elsewhere (10), Albert Hinnen and Jim Hicks, two postdocs in my lab succeeded in developing the first yeast transformation system. This technological advance permitted us to clone the HIS4 gene and obtain its sequence (11, 12), which showed that the gene was an uninterrupted reading frame encoding a single multifunctional polypeptide. This discovery solved the problem: yeast did not have a bacterial-like operon for histidine biosynthesis and likely had no operons at all.

Feeling that the operon question had been solved, I turned my attention to another aspect of genome organization and regulation. While still a graduate student at Yale, I had tried to determine whether regulation of histidine biosynthesis in yeast was similar to that in bacteria. My experiments consisted of measuring whether the levels of the enzymes of histidine biosynthesis changed upon starvation for histidine. My control in the experiments was the levels of the arginine biosynthetic enzymes that were not expected to respond to histidine starvation.

The baffling result was that my controls didn't work: starvation for arginine derepressed both the histidine and arginine pathways, which were not only unlinked but metabolically unconnected. I had never published this part of my graduate work because at the time it made no physiological sense. Why would you turn on arginine biosynthesis when you needed histidine? Moreover, the yeast results conflicted with similar experiments in bacteria indicating that each biochemical pathway was controlled only by its cognate inducer or repressor. When I first presented this yeast work at a Regulation Gordon Conference, the talk was relegated to the last day of the meeting, when most of the bacterial scientists had left, in a session called "Unexplained Phenomena in Unusual Organisms".

Emboldened by our success at solving the operon problem we pursued this bizarre observation (13). Our subsequent isolation of the GCN4 transcription factor, which binds promoters at histidine, arginine and many other unrelated genes, provided direct evidence that this general regulation had a mechanistic basis (13-16). The confusion arose because we mistakenly assumed that yeast and bacterial physiology were the same. Alan Hinnebusch, my former postdoctoral fellow, went on to show that this general control response in yeast is really a global stress response that connects transcription and translation (17). Subsequently, the utility of general transcription factors for controlling vast networks of biochemically unrelated and genetically unlinked genes was shown to be characteristic of eukaryotic systems from fungi to humans.

Genomics Resurrects the Mystery of Chromosome Organization

In 1982, after I moved to the Whitehead Institute at MIT, my postdocs Jef Boeke and David Garfinkel showed that the Ty elements, repeated DNAs in the yeast genome, were retrotransposons that moved via an RNA intermediate (18-21). Our discovery of Ty element transposition deepened the mystery concerning genome organization. Most genomes contain repeated DNA elements that can rearrange the chromosomes. Yet, despite these forces that can scramble the genome in laboratory time, the order of genes (synteny) is preserved between very different species over millions of years of evolution. This conundrum was drummed home to me by Barbara McClintock, who had an uncanny sense of genome organization long before modern technology demonstrated the prescience of her intuition.

The current sequencing of complete genomes has definitively established the
remarkable synteny among fungal species as well as that among evolutionarily distant mammals such as mouse and man. Moreover, in eukaryotes the order of genes is preserved even though they bear no functional relationship to each other. Most striking to me is that Ashbya and Saccharomyces, predicted to have diverged from a common ancestor more than 100 million years ago, have enormous stretches of conserved gene order despite some genome rearrangement (22, Figure 1).

Our recent studies have shown that despite the conservative nature of DNA sequences in the heart of the chromosomes, genetic mayhem occurs at the telomeres. Many genes of related function are found at the telomeres, but they are found in no particular order and even within a species can be shuffled from one chromosome to another. This conservatism in the central portion of the chromosome and radicalism at the telomeres may permit the rapid evolution of new genes, especially those related to interactions with the environment.

Without operons, eukaryotic genomes seem disorganized by comparison with their bacterial counterparts. In eukaryotes genes of related function are in general arranged willy-nilly about the genome with their position bearing no obvious relationship to their biochemical function. But, the conservation of the order of this seemingly disorganized genome sequence in the face of the potential destabilization by mobile elements like Ty retrotransposons suggests that there must be other countervailing forces that maintain the order. As some have suggested, the chromosome should not be considered as an array of beads on a string, but rather as an organelle in which all of the components interact with each other. Perhaps there are interactions of the chromosome, currently invisible to our technology that constrains the wanderlust of genes. Thus, the problem that intrigued me when I first encountered Jacob and Monod almost 50 years ago has reemerged center stage today. And the answer could provide insight into a question that has tantalized humans from the earliest times: "Where have we come from?"

Epilogue

The personal recollections in Reflections resonate with a common theme---appreciation for mentors who were critical to a successful scientific career. Arthur Kornberg notes that Severo Ochoa's "enthusiastic and optimistic personality was engraved in his memory" and contributed to "his most formative year in science". Maxine Singer eschewed a personal essay to write a paean to her postdoctoral advisor, Leon Heppel, whose personal dedication to work at the lab bench and support of his postdoctoral fellows "set a remarkable tone for the group of researchers under his general oversight." Perhaps Bruce Ames' contribution sums it up best when he notes that despite his varied undergraduate record he was accepted at Caltech "... because Srb, or one of my other references, saw potential in me." Seeing potential and promoting the career of a young inexperienced student doesn't jive with the popular view of scientific competition. But, as these essays recount, mentoring is at the heart of scientific culture, as teachers pass down their idiosyncratic vision, style, and zest to their students.

In addition to the mentors I described in my own recollection, many people with whom I had chance encounters at scientific meetings or casual discussions about a topic of mutual interest provided opportunities for my scientific development. When I began as an Assistant Professor at Cornell, Jim Watson asked me to teach the Yeast Molecular Biology course at Cold Spring Harbor. This course catapulted yeast into the forefront of molecular biology research and provided the setting for my long friendship with Barbara McClintock, whose constant reminder of the lability of the genome spawned my interest in transposable elements (23). Watson and Paul Berg recommended me for a Guggenheim fellowship that supported my
sabbatical at Cold Spring Harbor where John Roth, David Botstein, and I spent a wonderful year in 1974 (24). A chance interaction with David Baltimore, was interested in solving the operon question in metazoans, led to a close friendship and his invitation to join him in starting the Whitehead Institute at MIT, where I was Director from 1990-2001.

Missing from the current collection is recognition of two groups critical to scientific success. The first are the co-workers, sometimes called technicians, who are responsible for the practical details of carrying out experiments and managing a laboratory. But "technicians" conjures up the wrong image: these co-workers not only perform many of the experiments that lead to scientific achievements, but they are the cultural leaders in the laboratory, setting the tone for standards of behavior, accuracy, and safety. I have been blessed by two collaborators, Cora Styles and Paula Grisafi, both of whom had not only a remarkable grasp of experimental science but also the managerial know-how to create the communal glue that held my laboratory together.

The other group is our families, who have supported us in our personal quest for understanding. Our spouses, often not scientists themselves, provide the connection between our scientific devotion and everyday life. They are companions in our success and balm in our failures.

References


Figure 1. Gene order is conserved between two fungi that diverged over 100 million years ago. Saccharomyces is a budding yeast and Ashbya is a filamentous fungus, as shown in the Figure. The depiction of the chromosomes shows the order of genes in each organism. The Petri plates on the left show the mode of growth of each on solid medium. The function of each of these genes has no obvious relationship to those residing on the same chromosome (see below). There are some gene differences interspersed between those that are syntenic (see reference 22 for complete data). GRX1-disulfide oxidoreductase, LSB5 binds a protein involved in actin patch assembly and polymerization, YCL033C-methionine-R-sulfoxide reductase, STE50-adaptor protein involved in mating type signal transduction, RRP7-protein involved in ribosomal RNA processing, HIS4-multifunctional protein encoding the 2nd, 3rd, and 10th steps in histidine biosynthesis, BIK1-a microtubule associated protein, RNQ1-a prion like protein,