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The Fires of Life

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Abstract

This retrospective recounts the hunt for the mechanism of mitochondrial ATP synthesis, the early days of research on mitochondrial formation, and some of the colorful personalities dominating these often dramatic and emotional efforts. The narrative is set against the backdrop of postwar Austria and Germany and the stream of young scientists who had to leave their countries to receive postdoctoral training abroad. Many of them—including the author—chose the laboratory of a scientist their country had expelled a few decades before. The article concludes with some thoughts on the uniqueness of U.S. research universities and a brief account of the struggles to revive science in Europe.

Contents

CHILDREN OF MARS

I am a child of Mars, the god of war. His Second World War overshadowed my early youth and still sways my thoughts and actions. I only discovered this when I no longer lived in Europe and saw my former self in the befogged mirror of early memories: the childhood among barbarians, bombast, and bombs; the teenage years in an impenitent Austria; the tortuous path to science; and the restless life that marks my scientific generation. No wonder I am the eternal misfit. I live in Switzerland, carry an Austrian passport, and was born at the Hungarian border where the sounds of Hungarian, Croatian, and Romani blended into the singing German dialect of the local peasants. Yet my German lacks local color because my mother, a schoolteacher, grew up speaking Hungarian and taught me the textbook German expected of her profession. My wife is Danish but delivered each of our three children in a different country. They converse with her in Danish, with me in English, and with their friends in Swiss German, German, English, or French. As they have chosen Swiss, Russian, and Romanian partners, our panoply of passports would almost do for a domestic poker game. And two universities anointed me Professor of Biochemistry, even though I never attended a course—or passed an exam—on this subject.

That is fine with me. In lopping my roots, father Mars gave me critical distance and freedom. I abhor processions, parades, sermons, uniforms, incense, national celebrations, drum rolls, gun salutes, and official headdresses of any sort. This may not be a shortcut to popularity, but at least it gave me a headstart in spotting the rigid traditions that split the Austria of my youth into a clerical republic and a Marxist counterstate. Rigid traditions stifle innovation, pave the way to prejudice, and are the archenemies of science. Science is an expedition into the unknown where flawed maps and excess baggage can mean danger.

For us children, the war was not all that bad. We often went hungry and spent long nights in cold bomb shelters, but this was a small price to pay for the abandoned steel helmets, live ammunition, and brand-new bayonets we could pick up almost anywhere; they made our war games much more realistic than home-made wooden swords, bows, or arrows. But the ultimate kick was a nocturnal dogfight with its screeching plane engines, barking antiaircraft guns, probing fingers of search lights and—as the eagerly awaited climax—the fiery crash of an interceptor or bomber. But shortly before my ninth birthday, everything was suddenly over. We did not see it as a liberation, but as a defeat. In our war games, being "the Germans" was out and being "the "Americans" was in. Food and coal became even scarcer, and we spent much of our free time begging nearby farmers for bread and milk or ransacking bombed-out houses for firewood. During these years, Mars branded me forever with his coordinates. On leaving a room, I unconsciously turn off all lights, a gourmet shop makes me dizzy, and a restaurant guest griping about his salad dressing makes me angry. To a Mars child, the emotional coordinates of children of peace are out of kilter.

The myths portray Mars as a poor lover, yet he fathered many Austrian and German children. Most of them lead perfectly normal lives; I can only spot them by their age and their

home country: We children of Mars lack telltale birthmarks. We neither caused nor fought our father's war and survived it without grasping its horrors. Most new generations dream of a new world; we just tried to patch up the old one. We would have loved to turn back time and watch the bombs and bullets hurtle back into the planes and guns they had come from, and see the rubble reassemble into the houses, schools, and shops it had once been. Our goal was not to invent but to repair, to reconstruct a past that none of us had ever known. During the turbulent first half of the twentieth century, each decade spawned its own generation of Austrians and Germans. The three generations before me created a brilliant culture, proclaimed revolutions, waged civil wars, committed abominable crimes, suffered unspeakable horrors, or laid the foundations for a united Europe once the guns fell silent. The generation after me espoused the visions of my British and French contemporaries who tried to transmute their countries into social utopias. My own generation can offer nothing of this kind. We are the generation without qualities. History seemed to have condemned us to rebuild our ravaged countries, help the next generation back into the saddle, and then drift into oblivion without leaving our trace in the sands of time.

Reconstruction of our cities and roads was astonishingly fast and made everyone forget that the war had also devastated our cultural heritage. Universities were prime examples of this invisible havoc. Having expelled their Jewish or otherwise "politically unacceptable" faculty during the Nazi insanity, they were now mere shadows of their former selves. Their scientific fires were dead. To rekindle them, the children of Mars had to leave their Austrian or German homes to find sparks elsewhere—often with the help of scientists their country had expelled three decades earlier. Many of these fire seekers never returned, and for some of them, their voyage turned into an odyssey whose plights no Muse has ever sung. Those who returned often faced the envy and hostility of those who had sat pretty at home and slipped comfortably into a professorship. Yet, some of those who returned did eventually rise to key positions and revived the scientific fires that now safeguard the future of Austria and Germany. Perhaps, after all, my generation without qualities did leave its mark in the sands of time.

Beginnings

I was born on August 18, 1936, in the tiny village of Strem near the Austrian-Hungarian border. A kind fate had placed the village to the west of this border, which made all the difference when the border turned into a forbidding barrier guarded by dogs, mines, and barbed wire fences. My father was the son of a local peasant whose wife had borne him thirteen children. Six of them died early, five emigrated to the United States, and the oldest surviving son inherited the farm. My father Andreas, "the smart one," studied agricultural engineering because one of his older brothers kept sending him money from New York. My mother descended from a long line of teachers, who, for generations, tried to bring the light of education to border villages such as Strem. Before her marriage in 1935, she was Panika Lantos, talked to her parents in Hungarian, and kissed their hands when she greeted them.

When I was two years old and Austria became part of Hitler's "Thousand Year Reich," the government transferred my father to the Styrian city of Graz, which by then had a well-deserved reputation as a Nazi haven. My grade school teacher, a kind and intelligent woman, genuinely liked us, but also kept telling us with glowing eyes of the glorious advances of "our" armies into Poland and the Soviet Union. After the war, my high school gave me an excellent training in the classics, but essentially none in the sciences. History courses were eclectic: We heard a lot about Greece, Rome, and the crusades, much less about contemporary history up to 1914, and not a peep about what happened later. Books on the Nazis and their war were difficult to come by, but second-hand popular science books were plentiful and cheap—so were chemicals. Today this may

be hard to believe, but back then, some drug stores stocked white phosphorus, mercury, and sometimes even potassium metal and sold them to a teenager like me. Soon I spent most of my allowance on chemicals and glassware for the "laboratory" I had set up in our kitchen. I can only guess what today's safety inspectors would have to say about it, but I still recall vividly what my mortified parents said after I had successfully detonated my first device of red phosphorus and potassium chlorate. These quibbles aside, my parents were wonderful. Having left their world at the Hungarian border, they struggled to become city people, but the rigid class structure of the time placed this goal beyond their reach. They stood unflinchingly behind me, showed me their love, and gave me confidence. They also helped me assemble a respectable collection of college textbooks on inorganic chemistry, which I knew forward and backward by the time I finished high school. In the end, they even tolerated my kitchen experiments, which acquainted me with the brilliant yellow of zinc sulfide, the infernal smell of hydrogen selenide, the chameleon-like color changes of freshly precipitated manganese (II) hydroxide, and the lurid glow of a sulfur flame. Chemistry was my enchanted garden.

So was music. When I was about five, my maternal grandfather—a teacher, like all of his relatives—briefly placed his shiny new violin under my chin and got me hooked for life. My first violin teacher was a cobbler, but I soon attended the Graz conservatory as an external student. It was there that it dawned on me that Austria must once have been a much more exciting place. Where had all the great violinists gone? Most of them had funny names such as Menuhin, Heifetz, Szeryng, Goldberg, Stern, Milstein, and Rostal, and they now lived in England or the United States. Why didn't they perform in Austria? There were just too many things I did not understand, and the grown-ups around me were reluctant to explain them. By the time I was sixteen, I had become deeply frustrated and wanted to get away. Thanks to the American Field Service, a private foundation fostering student exchange, I spent the school year of 1952–1953 as an exchange student in Rochester, New York.

That year changed my life. In the early 1950s, the Soviet Union was not yet a credible foil to the United States, which savored its role as the undisputed ruler of the globe. Every day was a new adventure. I valued the freedom I had at school, even though the low academic standards astonished me. Music was a different matter, though. Rochester was home to the first-rate Eastman School of Music, which generously offered me a scholarship for its violin master of arts program. I also tried to conquer the basics of jitterbug and, at the insistence of my American foster family, spent evenings with church groups discussing the deep questions of life such as "Should one kiss a 'date' on the first night out?" The answer was a firm "no," of course, but as I was much too shy for dating, the kissing issue was moot. I had never heard of most of the other things that we were told not to do, but they sounded enticing, and I tried to remember them for later. I was still the diffident Mars child with dislodged coordinates, but now I had another vantage point from which to triangulate the world. I worked as a newspaper boy for the local Rochester newspaper, the *Democrat and Chronicle*, as an usher at a local movie theater, and as a helper at Sears, Roebuck and Co. during the Christmas shopping season. After Christmas, all helpers were laid off; I had no idea what that meant and went back to work the next day. My boss explained to me that I was fired and that I should quit coming back. I did not understand that either, but eventually got the message. This year was not always easy, but it made the United States my second home and was to help me immeasurably during my scientific career.

Returning to Graz felt like being plunked back into a dark hole. My new stereoscopic vision showed me how backward and prejudiced the city was, and I finished my last year in high school as a difficult and rebellious student. But the Eastman School of Music had made me aware of what first-rate violin playing was like, and I began to channel much of my pent-up energy into becoming a better player. Today,

Austrian string players again rank among the best in the world, but at that time, their general level was quite low. I learned only later how effectively the Nazi purges had crippled Austrian and German violin playing for which Jewish artists had set the standard for the past century. As gramophone records were essentially nonexistent, Graz with its imposing opera house, concert halls, and university buildings was not even aware of its intellectual and artistic poverty. The ravages of war and the stifling grip of the Iron Curtain had made it sink into provinciality.

This provinciality could not, however, diminish the music of the great masters. The hours I spent playing as a substitute with the Graz Philharmonic or in the orchestra pit of an opera house were among the happiest of my life. There is no way to describe how a professional orchestra sounds from within or how a successful performance can send a tingling down the spine. Playing string quartets or sonatas with friends transported me into yet another enchanted garden. Yet even this garden was not without blemishes: Most musicians at the time abhorred modern music, which to them was music written after Johannes Brahms. Quite a few of them were also overt anti-Semites, even though the objects of their aversion were gone. Luckily, my violin teacher, a former concertmaster of the Vienna Symphony Orchestra, was a true cosmopolitan untainted by prejudice. He became my first personal role model, and his photograph still comforts me in my office at home.

Desert

"Have fun with them super-duper specials of yours, *Herr Doktor*" growled Aloysius Zacherl across the loan desk of our Graz university library and reluctantly pushed two battered "*Physiologische Chemie*" textbooks a few millimeters in my general direction. According to my well-informed aunt, Herr Zacherl was the illegitimate son of a blue-blooded "*Hofrat*" of local prominence and a decidedly red-blooded peasant's daughter from the nearby hamlet of Sinabelkirchen. This mésalliance of two discordant worlds had spawned an Austrian centaur whose nobly sculpted paternal mouth was condemned to issue the atrocious sounds of the maternal dialect. After his ill-fated attempt at studying law, Zacherl had become a librarian who liked books, but not students, and who did his malicious best to keep the two apart. By facetiously addressing me as "*Herr Doktor*," he was trying to remind me that a lowly undergraduate such as I had no business requesting books that were not required reading.

As a seasoned Austrian, I took his spiteful officialdom in my stride, but the biochemistry textbooks he had dug up for me were pre-World War II vintage. Now, in 1958, they were just useless clunkers. The chemical processes in living cells had fascinated me already as a teenager, but my high school teachers knew nothing about them. I had hoped to study biochemistry at the university, but at that time, the University of Graz had not a single biochemist on its faculty and offered no biochemistry courses of any kind. As my parents could not afford to send me abroad and as international fellowships were essentially nonexistent, I had decided to enroll as a chemistry student and to master biochemistry on my own. Thanks to Herr Zacherl I had now learned that textbooks from our university library were not an option. The bookstores in Graz carried only a single modern, but also outrageously expensive, textbook by the Swiss biochemist Franz Leuthardt and were unable—or unwilling—to find for me what British or American publishers might have to offer.

Such was the intellectual splendor of postwar Graz, which had once been home to such scientific giants as Ludwig Boltzmann, Karl Cori, Karl von Frisch, Viktor Hess, Otto Loewi, Ernst Mach, Erwin Schrödinger, Nikola Tesla, Alfred Wegener, and Richard Zsigmondy. The university faculty still boasted a few first-rate scientists, such as the physicist Adolf Smekal and the physical chemist Otto Kratky, but they were exceptions and could not change the facts that the general level of science in Graz was at best mediocre and that my training as a chemist was rudimentary. The situation with music was

not much better. Austria has always prided itself on its musical legacy, yet it held its breath for almost two decades before daring to allow Gustav Mahler's bandmaster music and the degenerate sounds of the New Viennese School back into its concert halls.

I have often wondered why the collapse of the Third Reich left Austria stymied for decades, whereas that of the Austro-Hungarian Empire triggered revolution, civil war, and a dazzling, if tragically brief, cultural flowering. The general hardship after 1945 cannot explain this difference: After 1918, the hardship was even more severe, and the loss of national identity and social fabric much more dramatic than after 1945. But the Third Reich and its aftermath had depleted Austria's intellectual resources twice over: first through the Nazi purges, and then through the efforts of postwar Austria to cleanse its cultural institutions of former Nazis, not all of whom were incompetent. The unhappy outcome was a Golden Age of Mediocrity: Those ready to sail with the prevailing winds landed key positions despite their lack of talent, paralyzing Austria's intellectual life for at least a generation. This period of intellectual, political, and moral anomie robbed my generation of its ideals and laid the foundation for Austria's present political immaturity. Democracy, fairness, minority rights, and tolerance meant little or nothing to us. We wanted to help our relatives and friends, travel to faraway lands, and build ourselves a future.

My future was biochemistry, and I had no choice but to master it on my own. After many false starts, I concocted a six-step course: First, I worked my way through the biochemistry section of *Chemical Abstracts*, a now defunct abstracting periodical that our library did carry. Second, I jotted down the names and addresses of the authors whose articles interested me. Third, I bought several dozen picture postcards of Graz and sent them to these authors with the lapidary handwritten request: "Please send me all your reprints." For the fourth, fifth, and sixth steps, I waited, waited, and waited because I could not afford the luxury of airmail and sent all postcards by surface mail. Looking back, I am amazed that anybody answered me at all. Yet some did, sending me a few of their most recent reprints. Not so David E. Green, a leading researcher in the field of mitochondrial biochemistry, who ran a huge and successful laboratory with several dozen collaborators at the Enzyme Institute of the University of Wisconsin-Madison. Green liked to do things the big way and sent me a hefty package with more than 200 reprints on the function and structure of mammalian mitochondria. Some of these papers are now classics, and all of them bore the mark of Green's polished scientific prose. I devoured these articles, mostly on a solitary bench in our local park, and soon lost myself in an enchanted world of electron-conducting membranes and colorful cytochromes. What could be more exciting and important than the pathways giving energy to life? My private biochemistry course had swung into high gear. Its balance of subjects may have been open to question, but it kindled my lifelong fascination with cellular respiration and mitochondrial biogenesis.

I met Green for the first time on a visit to his Madison laboratory in the early spring of 1965. His generosity as a host, his charm, and his scientific flair immediately impressed me, and it was obvious that his collaborators liked and respected him. After obtaining a biology degree from New York University, he spent eight years at the University of Cambridge, where he acquired not only a doctorate in biochemistry, but also an elegant British accent, which, however, still betrayed his Brooklyn roots. His intellect and experimental skill had quickly won him attention, and when, at the tender age of 27, he published his outstanding monograph *Reconstruction of Chemical Processes in Living Cells*, he established himself as a biochemical *wunderkind*. After his return to the United States in 1940, he briefly joined Harvard University and published his second book entitled *Mechanism of Biological Oxidations*, which brilliantly summarized the knowledge of the time and influenced research on this topic for years to come. He soon left Harvard for Columbia University where he made seminal discoveries on a variety of processes, including

the oxidation and transamination of amino acids. His seven years at Columbia, the most productive of his life, propelled him into the top league of biochemists in the United States and the world. In 1948, the University of Wisconsin chose him as one of the three directors of the new Enzyme Institute, where he remained until his premature death in 1983. The Madison campus offered Green fabulous resources, which he exploited to the full. Soon he directed a large team of outstanding collaborators, including Helmut Beinert, Fred L. Crane, Youssef Hatefi, Frank Huennekens, David H. MacLennan, Henry Mahler, Jesse Rabinowitz, Alan Senior, Salih Wakil, Alexander Tzagoloff, and many others. But when I met him in 1965, his had already passed his apogee. Managing his big laboratory occupied so much of his time that he had lost touch with research, acquiring the reputation of someone whose ideas were often no longer rooted in reality. Yet he was one of the great biochemists of his time (1). Future generations may underestimate his scientific contributions because many were not published under his name: Unlike most of his colleagues, he only put his name on a paper if he had participated in the research directly. This exemplary habit was very much appreciated by his collaborators and may partly explain why so many of them rose to international prominence. I remember Green as a generous and engaging man whose magnanimous response paved my way to biochemistry.

Oasis Years

Well before finishing my PhD studies, I decided to leave Graz as soon as possible. But where was I to go? Luckily someone recommended me to Hans Tuppy, who had just been appointed professor of biochemistry at the University of Vienna. Tuppy was a fabulously gifted, imaginative, and dynamic young biochemist who had been a key player in Fred Sanger's Nobel Prize–winning work on the amino acid sequence of insulin. In setting up his department, Tuppy had attracted some of the best young Austrian biochemists. We admired and tried to emulate him, and I am still amazed at how much his small and underfunded laboratory accomplished in the early 1960s.

When I joined Tuppy in the spring of 1961, Vienna was still scarred by the war and the Soviet occupation, and the once famous Vienna Medical School had become a scientific backwater. Tuppy showed us that one man can make a difference. His contacts with Cambridge University kept us in touch with many exciting discoveries in molecular biology, and his impeccable credentials as an anti-Nazi opened the doors for us into the international biochemical community that included many Viennese refugees. We worked day and night for seven days a week, asked cocky questions at seminars, and were both envied and heartily loathed by outsiders as "Tuppy's arrogant Mafia." However, this successful period did not last long. Tuppy had his eyes on a political career and became in rapid succession Rector of the University, President of the Austrian Academy of Sciences, and, finally, Federal Minister for Science and Research. Even for a workaholic like him, a day had only 24 hours, leaving him less and less time to discuss our research with us. Moreover, most of us left the laboratory, one after the other, for postdoctoral training abroad. Rarely has a small laboratory sent out such a large and motivated group of pilgrims, and rarely has such a group been as successful. All of us became professors at universities or research directors at international pharmaceutical companies. Only three of us did not come back: Peter Palese is now a renowned virologist at Mount Sinai Medical School in New York, Manfred Karobath directed drug development at Sandoz in Switzerland and at Rhône Poulenc in France, and I ended up as a member of the Biozentrum in Basel.

MITOCHONDRIAL DNA

By the late 1950s, biochemists already knew that cellular respiration is mediated by a chain of colored cytochromes, flavoproteins, and nonheme iron proteins that pass on electrons from nutrients to dioxygen gas, reducing this gas

to water. The best known of these chromoproteins was the intensely red cytochrome *c*. Tuppy and his assistant Günter Kreil, collaborating with Emanuel Margoliash and his group at Northwestern University, had just elucidated the complete amino acid sequence of this cytochrome, awakening Tuppy's interest in mitochondria. He readily agreed with my proposal to work on these organelles. But when he asked me for specifics, he drew a blank: I knew far too little biochemistry to come up with a credible research plan, but I saved my skin by promising him a detailed proposal within a week.

I spent the following days racking my brains trying to come up with a specific and credible research project. Luckily, I did not yet know how crucial the choice of the first project can be for a research career. A clearly defined, safe project promises quick results, but it usually lacks originality and the chance of a scientific coup. An innovative project, by contrast, may be the "launchpad" for a successful career, but it is usually long and risky. The choice is a question of courage. No wonder that the famed biochemist Harold C. Urey once declared courage as the key ingredient of scientific talent. And yet courage alone is not enough; scientific success also demands passion and patience. It takes passion to tackle a problem everybody else considers intractable, and it takes patience to allow courage and passion to wield their force. Innovative research is an expedition into unknown waters. Those who shun them will rarely discover anything new. There is no better advice for a young researcher than the words of John A. Shedd: "A ship in harbor is safe. But that is not what ships are made for."

After several days of soul-searching, I decided to work on how yeast cells form their mitochondria. By felicitous serendipity, David Green had included in his reprint package to Graz a brief paper on this topic by his former postdoctoral fellow Anthony Linnane, and I found the paper fascinating. Few, if any other, biochemists seemed to work on this problem, and my struggles to produce something resembling a doctoral thesis in Graz had at least taught me how to grow and handle yeast cells. In the end, I let my heart decide. Why do we so often ignore its advice? In science as well as in art, the insouciance of youth is usually keener than the wisdom of old age.

In 1961, the heroic age of mitochondrial research had just drawn to a close (2). During the previous two decades, Albert Claude, George H. Hogeboom, Walter C. Schneider, and George E. Palade had worked out methods for isolating liver mitochondria by differential centrifugation in isotonic sucrose solutions; Eugene P. Kennedy and Albert L. Lehninger had discovered that mitochondria contained the complete enzymic machinery for oxidative phosphorylation, the tricarboxylic acid cycle, and fatty acid oxidation; George E. Palade, Fritjof S. Sjøstrand, and Keith R. Porter had shown the world the first high-resolution electron micrographs of mitochondria; Britton Chance, applying his rapid and ultrasensitive spectroscopic methods, had dissected energy coupling in the mitochondrial respiratory chain into three discrete steps; David E. Green and his colleagues had established the role of mitochondria in fatty acid synthesis and had isolated and characterized four distinct subcomplexes of the respiratory chain; and Harvey S. Penefsky, Maynard E. Pullman, and Efraim Racker had discovered and purified F_1-ATPase, the first defined part of mitochondrial ATP synthase. Mitochondrial researchers ("mitochondriacs") saw themselves, and were also seen by others, as the biochemical elite. As the respiratory chain was no longer a mystery, everybody was convinced that, a few years down the road, the same would be true for oxidative phosphorylation. But the question "How are mitochondria made?" aroused little general interest and, at any rate, seemed beyond the reach of biochemistry. In 1958, Simpson and his colleagues (3) had observed that isolated rat liver mitochondria incorporated labeled amino acids into protein, but for reasons mentioned later, it seemed a hopeless task to identify these proteins.

Enter yeast genetics. In France, the Russian Boris Ephrussi and the Polish man Pjiotr P. Slonimski had spent the preceding

15 years studying strange mutations that abolished the respiration of yeast cells. The mutations never reverted and were not inherited according to Mendel's laws. Because these respiration-deficient yeast mutants utilized glucose less efficiently than respiring cells, they formed smaller colonies on plates containing low glucose levels. Ephrussi baptized them *petite* mutants (the French word for yeast, *levure*, is a feminine noun). Ephrussi and Slonimski were convinced that these mutations reflected the inactivation or the loss of some extrachromosomal factor that controlled the formation of the respiratory system. They took it for granted that the respiratory system of yeast was housed in typical mitochondria and suspected that this might also be true of the mysterious genetic factor. For those of us who studied mitochondral formation in yeast at that time, the slim monographs by Ephrussi (4) and Slonimski (5) on this topic were "holy scripture." But few biochemists knew about them, and their general impact was low. Interest in the genetic control of mitochondrial formation was still an exotic hobby. The reasons for this parochial attitude show how much biochemistry has changed during my lifetime.

In the early 1960s, biology was still fragmented into scientific fiefdoms, and yeast genetics was defended as an arcane calling for the chosen few. Yeast geneticists loved to intimidate outsiders with their obfuscating and often unnecessary patois. On the other side of the border, mitochondriacs were so busy chasing after nonexistent chemical intermediates of oxidative phosphorylation that they would never have deigned to read the *Journal of Molecular Biology* or, God forbid, the journal *Genetics*.

Also, many biochemists did not accept yeast as a model for mammalian cells and refused to believe that the "respiring yeast granules," studied by Slonimski and a few others, had anything to do with mitochondria. In their eyes, yeast was just another microbe, not much different from *Escherichia coli*. There was no general awareness of the profound divide between prokaryotes and eukaryotes. This awareness spread slowly in the late 1960s as more cells were examined with the electron microscope or with the powerful new tools of molecular biology. Today, we know that respiring membrane vesicles isolated from bacteria are vesicular fragments of the bacterial plasma membrane, but at that time, they were often considered to be preexisting intracellular organelles. Finally, although mitochondria had been discovered and first studied in Europe, research on them in the early 1960s was very much an American enterprise. Europeans trying to learn the trade had to embark on a *hadj* to the mitochondrial meccas in New York, Madison, Philadelphia, or Baltimore. The mitochondrial research centers in Amsterdam, Stockholm, and Munich had already begun to flex their muscles, but they were not yet a match for their powerful U.S. rivals.

In sum, when I decided to work on the formation of yeast mitochondria, I had no idea that I would be separated from the mainstream of mitochondrial research by the Atlantic Ocean, the continental divide between yeast and mammals, and the lack of travel funds. Toward the mid-1960s, however, the general ignorance about yeast mitochondria slowly lifted, and the skeptical remarks about "yeast respiratory granules" gradually subsided.

One of the turning points was the discovery of mitochondrial DNA. In about 1962, it became clear that chloroplasts, the green sisters of mitochondria, contained their own DNA. Hans Tuppy and I reasoned that, if this also held for mitochondria, this DNA might well be the mysterious extrachromosomal factor controlling mitochondrial formation in yeast. Together with Ellen Haslbrunner, my first graduate student, we decided to look for DNA in yeast mitochondria. Just at that time, Sidney Brenner and his colleagues at the Medical Research Council in Cambridge devised a simple gadget for generating linear sucrose gradients. By the time-honored Viennese method of offering a bottle of wine, I persuaded our local mechanic to copy this device from a hand-drawn sketch. I then purified yeast mitochondria by isopycnic centrifugation in a sucrose gradient, collected 15 fractions by puncturing

first my finger and then the centrifuge tube with a syringe needle, and analyzed each fraction for DNA by the color reaction devised by Karl Diesche. Did I find DNA! Every fraction had lots of it, without any visible peak where the brownish mitochondria had equilibrated as a turbid band. Clearly, DNA had leaked out of the nucleus, and the sucrose gradient had failed to separate it from the mitochondria. But when we replaced sucrose by the X-ray contrasting agent Urografin, the bulk of the DNA sedimented to the bottom of the tube, and a tiny part of it cofractionated precisely with the mitochondria. The DNA in the bottom fraction was easily digested by DNAase and probably represented nuclear DNA. The DNA in the mitochondrial fraction was not readily digested by DNAase unless the fraction was first mistreated with trichloroacetic acid; presumably, it represented DNA enclosed by mitochondrial membranes. Its amount per milligram mitochondrial protein was remarkably constant between different experiments. We submitted our findings under the cautious title "DNA Associated with Yeast Mitochondria" to *Biochemical and Biophysical Research Communications* (6), the newest and "hottest" biochemical journal of the day, and soon, an avalanche of reprint requests and telephone calls descended on our laboratory.

For the first time, I felt the thrill of discovery. But was this discovery important? When a journalist asked me how it would benefit the general public, I answered him that I did not know. What a short-sighted answer! Today, I would not know where to start. I could tell the journalist that the DNA in our own mitochondria carries the blueprints for 13 essential proteins of our body's power plants; that mutations in it can cause terrifying diseases; that it offers unique advantages for tracking the origin of modern humans and their spread across the globe; and that analysis of this DNA from human bones has posthumously exposed the woman by the name of Anna Anderson as a fraud: She was not the "daughter Anastasia of the last Romanov," as she had claimed, but a false Anastasia of Polish peasant stock.

Shortly before our report on mitochondrial DNA appeared, we learned that Margit M.K. Nass and Sylvan Nass, two electron microscopists at the University of Pennsylvania in Philadelphia, had described thread-like inclusions within the matrix of chick embryo mitochondria. As these threads were sensitive to DNAase, but not to RNAase or protease, the authors had correctly identified them as DNA (7). In those days, U.S. journals took up to half a year to reach our Vienna university library, and the electronic grapevine across the Atlantic was still in its infancy. Some reviews had already cited us as the sole discoverers of mitochondrial DNA, and so the two excellent papers by the Nass team left us crestfallen. In retrospect, however, these concordant reports accelerated the general acceptance of mitochondrial DNA and even got some mitochondriacs interested in mitochondrial biogenesis.

What was this mitochondrial DNA doing? My colleague Erhard Wintersberger found that yeast mitochondria also harbored a DNA-dependent RNA polymerase as well as ribosomal RNA hybridizing to mitochondrial DNA. He suspected that this RNA was part of mitochondrial ribosomes, which were discovered by others a few years later. But what about protein-coding genes? The amount of DNA we had measured in yeast mitochondria was insufficient to encode all of the hundreds of mitochondrial proteins. Most of these proteins had to be encoded by nuclear genes, synthesized on cytosolic ribosomes, and then imported into mitochondria—a bizarre scenario!

Yet we knew from the work of Simpson that mitochondria made some proteins themselves, and we suspected that they were encoded by mitochondrial DNA. Simpson's team had gone to great lengths to exclude contaminating microsomes as the source of the observed activity, and a few years later, others found that protein synthesis by isolated mitochondria was insensitive to inhibitors of microsomal protein synthesis, such as cycloheximide, but sensitive to inhibitors of bacterial protein synthesis, such as chloramphenicol and erythromycin. However, efforts to identify these mitochondrially

synthesized proteins ran into an insurmountable roadblock. Amino acid incorporation by mitochondria was very low, the labeled proteins were too insoluble to be analyzed by the available methods, and a fraudulent claim that one of them was cytochrome *c* severely blemished the field's reputation. Perhaps the most frustrating obstacle was the obstinate refusal of the incorporated radioactivity to cofractionate with any of the known mitochondrial enzymes. Because the non-Mendelian *petite* mutants lacked cytochrome oxidase and succinate-cytochrome *c* reductase, we suspected that these enzymes were synthesized by mitochondria. But when a few heroic researchers labeled isolated mitochondria with radioactive amino acids and then purified cytochrome oxidase or cytochrome *b* from them, neither enzyme was labeled. In 1964, it was even claimed that mitochondria could not synthesize any proteins at all and that the observed activity merely reflected contaminating bacteria. I should have stayed in the game to exploit our discovery of mitochondrial DNA, but the time had come to leave for my long overdue postdoctoral training in New York City.

Postdoc in New York

My decision to go to New York was born in the summer of 1961 during a visit by the famed biochemist Efraim Racker. It was Racker's first postwar visit to the city he had grown up in and fled after obtaining his MD degree 23 years earlier. After brief sojourns in Denmark and Great Britain, he had finally made it to the United States, where, after brief interludes at the University of Minnesota, the Harlem Hospital in New York City, and Yale University, he was now working at the Public Health Research Institute of the City of New York on biological energy conversion. His discovery of an "energy-rich" thioester intermediate in glycolytic ATP formation by glyceraldehyde-3-phosphate dehydrogenase had propelled him to fame and prompted his conviction that ATP formation by oxidative phosphorylation in mitochondria occurs by a similar mechanism. I had heard that he conducted his research not as a highly organized campaign but as an intuitive and almost artistic endeavor. Indeed, after graduating from high school, he had been accepted into the prestigious Vienna Art Academy, but he had soon left it to study medicine. Later, he used to quip that this academy, by twice rejecting Adolf Hitler, had caused World War II. He remained a passionate painter throughout his life and gave each of his students and postdoctoral fellows at least one of his paintings as a farewell gift.

When I first met Racker, he was only 48 years old. His thinning white hair and wrinkled face made him look older, but his lively demeanor and quick wit soon revealed his true age. As he then still refused to speak his native German, we conversed in English. "How come you speak English so well?" he wanted to know. When I told him that I had spent a year in a U.S. high school, he quickly retorted, alluding to my German accent, "How come you speak English so badly?" When both of us erupted in laughter, we spontaneously shook hands and laid the foundation for our lifelong friendship. We spent the next evening strolling through the streets of Vienna, which evoked in him many long-buried memories. His reminiscences showed me a city I had only known from books: its rich musical life, the legendary public readings by Karl Kraus, and the rising Nazi tide at the university that led to violent attacks on his Jewish student friends. When I told him that I had learned about these events only recently through books by the German publishing house S. Fischer and that most of my colleagues abhorred these crimes as much as I did, he at first would not believe me, but many years later, he confessed to me that our talks that evening had persuaded him to make peace with Austria's young generation. He even started to speak to me in halting German. Science had begun to bridge the gulf between our two generations, but it could not completely heal the wounds from his youth. Shortly before his death, we both attended a scientific meeting in Vienna and, rushing to the conference center, crossed the busy Ringstrasse on a red light. The angry honking immediately attracted a police

officer who reprimanded us sternly, but politely. I was relieved to be let off the hook that easily, but Racker became unreasonably aggressive and might have caused both of us considerable grief: Insulting an Austrian official is almost as grave an offense as not paying a Swiss hotel bill.

I had initially planned to train with David Green in Madison, but after that evening with Racker, I spontaneously asked him whether I could work with him. He immediately agreed, but warned me that his laboratory was already full and that I could only join him in 1964. I was more than willing to wait, and so my wife, our one-year-old daughter, and I boarded the little Dutch steamer *Westerdam* on June 27, 1964, for a leisurely 10-day voyage to New York. It was to be the beginning of a restless life that made us change residence 10 times—a tribute Mars exacted from his children who tried to escape his shadow. We arrived in New York with great expectations, but our first impressions were disappointing. My fellowship had seemed lavish if expressed in Austrian Schillings, but now barely met our basic needs. We were as poor in New York as we had been in Vienna. I shall never forget the humiliation of having to postpone the purchase of a larger baby carriage after a New York bank had turned down my application for a $50 loan. Racker's laboratories were another shock. They were housed in the "Public Health Research Institute of the City of New York, Inc.," which, despite its name, was a serious public health threat. It was a ramshackle, dirty building at the foot of East Sixteenth Street, a dead-end slum street abutting the East River. At first I could not even find the place, and when I asked a taxi driver to get me there, he asked suspiciously, "Now why would you want to go *there*?" A coal-fired power plant of "the Confederated Edison Co." supplied our Institute with barely enough electricity; it was the decade of the brownouts but with a surplus of black soot, which settled as a grimy black film on window sills and lab benches. Cockroaches of ghastly dimensions lurked everywhere—even in the circuits of our centrifuges and blenders—where their electrocution often led to mysterious short circuits. But there was nothing ramshackle about Racker's research group in which I spent two and a half exciting years. Racker was then in his early fifties. He looked even older than during our first encounter in Vienna, but he was in superb physical shape: He excelled in competitive sports, such as tennis, ping-pong, and volleyball, and loved to argue about just everything. His presence radiated a competitive and creative restlessness that I found invigorating, even though I soon learned that it was not to everyone's liking. He came as close to reading other people's mind as anyone I have known, and he delighted in getting his coworkers and colleagues off balance. A lack of self-confidence was a dangerous platform from which to approach him. Yet, I have met few human beings who could be as sensitive, helpful, and empathic as he, and none who were as broad-minded, or who had a keener sense of humor. When he felt at ease, he regaled his audience with his quick wit, child-like playfulness, and genuine charm that were irresistible. His humor reflected his youth in Mazzenstadt, the Viennese ghetto district, playing on human folly and life's dark and surrealistic sides. It would do him an injustice to say that he harbored two souls because he had so many. He could be incredibly rude and disarmingly gentle, overbearing and unassuming, stunningly petty and royally generous. His artistic temperament made him approach a scientific problem intuitively rather than methodically, making it often frustrating to discuss science with him. He was the epitome of a scientist-artist and a genuine humanist. In his private universe, the human spirit was both a center of gravity and a point of reference. He became my mentor, then a father figure, and finally one of my closest friends. When he died of a stroke in 1991, so did part of me. I still talk with him, as I do with the ever-growing family of others who are no longer with me. I have learned that the dead can be closer friends than the living. They are always there to offer advice or consolation; they belong to a world that is uniquely mine.

In Racker's lab, I learned how to prepare submitochondrial particles, extract specific

proteins from them, and measure oxidative phosphorylation as well as how to keep my calm when challenged in a scientific discussion. This could be quite a trial because the field of oxidative phosphorylation had a well-deserved reputation as a no-holds-barred war zone dominated by weak data and strong personalities. The verbal duels between David E. Green and Efraim Racker were legendary; both lost little time in going straight at each other's jugular, entertaining and shocking everyone by their personal attacks and biting repartees.

When I joined Racker in 1964, it was still a mystery how the energy released during respiration drives ATP synthesis from ADP and inorganic phosphate. Racker's colleagues Harvey S. Penefsky and Maynard E. Pullman had just isolated a key component of this process: By treating submitochondrial particles with ultrasound, they had solubilized a protein that cleaved ATP to ADP and inorganic phosphate and, when added back to the mistreated particles, restored their capacity for oxidative phosphorylation (8, 9). Penefsky and Pullman suspected correctly that this ATPase was just one of many components of the mitochondrial oxidative phosphorylation system and baptized it "factor 1," or F_1. "Ef" Racker was apparently quite satisfied with this designation, as its sound reminded everyone of his nickname and his self-perceived rank in the scientific hierarchy. But this seminal discovery had also led to tensions because Penefsky and Pullman had discovered F_1 on their own and resented Racker's insistence that he be the senior author on their papers. These turbulences aside, all of us expected that the remaining parts of the ATP-forming machine would soon be known and that we would then accompany our master on his well-deserved winter trip to Stockholm.

At first everything progressed well. Racker's postdoctoral fellow Yasuo Kagawa identified a complex of insoluble membrane proteins that tightly binds soluble F_1 and restores its native properties. In mitochondria, F_1 is sensitive to the antibiotic oligomycin and, like most proteins, stable on ice. Once solubilized, however, it is oligomycin insensitive and cold labile. As Kagawa could not separate the insoluble F_1-binding proteins from each other, he called the complex "oligomycin-sensitivity factor" or, in short, F_o. Had we only known that the complex between F_o and F_1 represented the entire ATP-forming machine! But we did not, and so we lost precious years hunting for additional factors that would stimulate oxidative phosphorylation by mistreated submitochondrial particles. This approach led nowhere. Like most of our competitors, we were looking for a protein that, by reversibly adopting an energy-rich state, could transfer energy from the respiratory chain to F_1, allowing it to make ATP. We were all mesmerized by Slater's "chemical hypothesis," which predicted such an intermediate. Racker was particularly partial to this hypothesis, as it corresponded to his earlier discovery of an energy-rich thioester intermediate in the first ATP-forming step of glycolysis. This *fata morgana* led hundreds of researchers—us included—down the garden path, seduced one of Green's collaborators to falsify data, and tarnished the reputation of our field. The British microbiologist Peter Mitchell had warned as early as 1961 that the link between respiration and ATP synthesis was not an energy-rich substance, but a proton electrochemical potential gradient across the mitochondrial inner membrane (10). Most biochemists, however, ignored this heretical view. Maynard E. Pullman and I published the first comprehensive review of Mitchell's "chemiosmotic hypothesis" in 1967 (11), yet it took 10 more years for the leading researchers in the field to publish their famous "peace treaty" in which they declared their agreement with Mitchell's concept (12).

In New York, I lost almost an entire year trying to confirm reports from David Green's laboratory on protein factors stimulating oxidative phosphorylation at specific regions of the respiratory chain. Using an existing assay for ATP synthesis in the cytochrome oxidase region and developing a novel one for the NADH dehydrogenase region, I proved these claims to be incorrect. In the early spring of 1965, my postdoctoral colleague June M.

Fessenden and I took a plane to snowed-in Madison in a last attempt to confirm the published results in the lion's den—again without success. Some of them were the result of wishful thinking, but one turned out to be a deliberate fraud. Sadly, fraud is no longer a rarity in today's hypercompetitive atmosphere, but in those more tranquil days, it was a shocking exception that seriously shook our field. Luckily, my next project was successful: Together with Harvey Penefsky and Ef Racker, I purified F_1 from yeast and used it to show that F_1 was not only a catalyst for ATP synthesis, but also a structural "plug" for the proton pore of F_o, thereby preventing a short-circuit of the respiration-driven proton flow (13).

With his 17-year-long lonely battle against virtually the entire mitochondrial community, Peter Mitchell wrote one of the most bizarre chapters in the history of science. He confirmed that fundamentally new ideas emanate from creative individuals rather than from groups or institutions. But Mitchell also acknowledged his intellectual debt to Robert Robertson, Robert Davies, Alexander George Ogston, and others. Scientific discoveries, like works of art, are children of solitude, yet are not born in isolation.

Promitochondria

When I returned to Vienna in the fall of 1966, I could not wait to do what my move to New York had put on hold: to identify the proteins encoded by mitochondrial DNA. On the hunch that some of them might be part of Kagawa's $F_o.F_1$ complex—the "ATP synthase"—I checked whether this complex was altered in the extrachromosomal *petite* mutants of yeast. The result was striking: The mutants still had F_1, but it was oligomycin insensitive and cold labile even in the intact mitochondria. F_1 itself seemed to be normal because it became oligomycin sensitive and cold stable when I mixed it with F_o from beef heart mitochondria (14). As it was already known that the *petite* mutation caused massive alterations, or even the complete loss of mitochondrial DNA, I reasoned that mitochondrial DNA encoded at least one of the F_o proteins. I was getting close! My next steps should have been to isolate F_o from wild-type yeast, to characterize its protein subunits, and to check whether they were made by mitochondria. But two things got in the way: my desire to settle the controversial fate of mitochondria during anaerobic growth of yeast and my decision to leave Europe for good and settle with my family in the United States.

The fate of mitochondria during anaerobic growth of yeast had been a long-standing and emotional issue. The Australian biochemist Anthony Linnane had reported that yeast cells growing by fermentation in the absence of oxygen lose mitochondria, but regain them upon aeration (15, 16). I believed that anaerobically grown cells could not respire and that this defect was reversed by aeration, but I thought it very unlikely that this physiological change reflected the disappearance and reappearance of mitochondrial organelles. Shortly before leaving for my postdoctoral U.S. stay in 1964, I had shown that the anaerobically grown cells still had mitochondria-like structures, which contained succinate dehydrogenase and an oligomycin-sensitive ATPase. I had termed these structures "promitochondria" but had failed to convince others in the field. Now, however, I had better insight and better tools. I showed that the ATPase activity of promitochondria was inhibited by an antiserum against purified F_1 and that it was oligomycin sensitive in promitochondria from wild-type cells, but not in those from *petite* mutants. Together with my old school pal and colleague Fritz Paltauf from the University of Graz, I then went on to show with Richard S. Criddle (17) that promitochondria contain the mitochondria-specific lipid cardiolipin, and Helmut Plattner from the University of Innsbruck helped me (18) to identify promitochondria as double-walled structures with typical cristae in electron micrographs of freeze-etched anaerobically grown cells. Independently and at about the same time, Hewson Swift and colleagues (19) at the University of Chicago published similar

electron micrographs of anaerobically grown yeast cells. Later on, my coworkers and I (20) used pulse-chase experiments and quantitative electron microscope autoradiography to prove that promitochondria from wild-type cells were structural precursors of the respiring mitochondria that arose upon respiratory adaptation. This put an end to the claim that mitochondria can arise de novo, even though many details of promitochondrial maturation remained to be worked out. It seems that a key event is the oxygen-requiring formation of heme that stimulates not only the transcription of many nuclear genes for mitochondrial proteins, but also the assembly of oligomeric cytochrome complexes of the respiratory chain. Mitochondria contain so many vital enzymes that a cell cannot lose these organelles—even when respiration is not necessary. Many years later, Michael P. Yaffe and I (21) exploited this fact in a screen for yeast mutants defective in mitochondrial protein import.

Cornell Bliss

In 1966, Racker had accepted a prestigious Albert Einstein professorship at Cornell University in Ithaca, New York, and had persuaded many prominent membrane biochemists to join him there. This cast included internationally known senior figures such as Quentin H. Gibson, Leon A. Heppel, and André T. Jagendorf as well as younger scientists such as Richard E. McCarty, Peter C. Hinkle, June Fessenden-Raden, and David C. Wharton. He had also invited me, but I had already planned to return to my old Public Health Research Institute in New York City. I soon changed my mind, however, and, in the fall of 1968, moved with my family to Ithaca. Like most of the other young staff members there, I was eager to make my mark and cared little about Cornell traditions. Not only did I not lunch at the Faculty Club, I did not even know that such a Club existed. The address of my small basement laboratory, Wing Wing G-1, would today suggest a *Drosophila* mutant or an oncogene. After I received my own grant, I hired my first technician, Jo Saltzgaber, and, in a spell of hubris, even bought an automatic pencil sharpener and an electric typewriter.

The excellent facilities at Cornell renewed my determination to track down the proteins made by mitochondria. My previous attempts at incorporating ^{14}C-labeled leucine into isolated yeast mitochondria had taught me two things. First, I would never get the protein products hot enough without five additional National Institutes of Health grants. Second, the methods for preparing yeast mitochondria were so lengthy, and our common centrifuges at Wing Wing so dirty, that I would always have to worry about protein synthesis by contaminating bacteria. Why not label the mitochondrially synthesized proteins within their proper environment—the living yeast cell? The protocol was simple enough: Inhibit the cytosolic ribosomes of yeast cells with cycloheximide, incubate the cells with glucose as an energy source and labeled leucine, and then isolate and analyze the mitochondria. The results made us dance; virtually the entire incorporated label was in the mitochondrial fraction, and all labeling was abolished when we inhibited the cells with both chloramphenicol and cycloheximide (22). This in vivo labeling protocol was fast, minimized bacterial contamination, and labeled the mitochondria at least one hundred times more efficiently than protocols using isolated mitochondria.

With this new labeling tool, we showed that mitochondria of extrachromosomal *petite* mutants of yeast could no longer synthesize proteins. Stefan Kužela et al. (23) at the University of Bratislava, then in Czechoslovakia, had reached the same conclusion by examining the incorporation of labeled amino acids into the isolated mitochondria. This could only mean that the *petite* mutation prevented expression of all the genes that were still left on the defective mitochondrial DNA molecules. Now, we finally understood why all *petite* mutants lacked the same set of enzymes even though they had widely different deletions in their mitochondrial DNA, or no mitochondrial DNA at all.

SPELLBOUND BY CYTOCHROME OXIDASE

When we analyzed the labeled mitochondria by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, which the virologist Jacob V. Maizel pioneered in 1966, we found six broad radioactivity peaks, but none of them coincided with a major stained protein band. The mitochondrial protein products were clearly not major mitochondrial proteins. But what were their properties and their functions? It was at this critical stage that Thomas L. Mason joined my laboratory as a postdoctoral fellow. Tom knew about my earlier work on the biosynthesis of the $F_1.F_o$ complex and was disappointed to hear from me that this project was already well advanced in Alexander Tzagoloff's laboratory in New York City. He therefore wanted to check whether cytochrome oxidase—another enzyme missing from *petite* mutants—was made by mitochondria. Cytochrome oxidase (also termed cytochrome aa_3) is the last enzyme of the mitochondrial respiratory chain. It had already been purified by detergent extraction of mammalian mitochondria, but its subunit composition was unknown. Tom and I decided to ignore earlier reports that cytochrome oxidase was not labeled by isolated mitochondria. We expected to see things others had missed because we now had better eyes. Our Cornell colleague David C. Wharton, an expert on cytochrome oxidase, helped us purify the enzyme from yeast mitochondria and resolve it into three large and four small subunits. The three large ones were labeled in cycloheximide-poisoned yeast cells and, therefore, made by mitochondria; the four small ones were labeled in chloramphenicol-poisoned yeast cells and, therefore, made in the cytosol. Presumably, these small subunits were imported into the mitochondria and only then assembled with the three large subunits (24, 25). Once again, we were not alone. Hanns Weiss and his colleagues (26) at the University of Munich concluded that only a single large subunit of cytochrome oxidase from the mold *Neurospora crassa* was made by mitochondria. This result turned out to be an artifact of electrophoresis. After Meryl S. Rubin & Alexander Tzagoloff (27) had confirmed our results, many subsequent studies by different groups (28) established that mitochondria of most eukaryotes synthesize three large subunits of cytochrome oxidase. As discussed below, these subunits are the catalytic "heart" of the enzyme.

Tzagoloff (29) also unraveled the biogenesis of the yeast $F_1.F_o$ complex, now commonly referred to as ATP synthase. He established its subunit composition and showed that the smallest subunit was made by mitochondria. He published this finding in 1971 (29), the same year we first reported our results on cytochrome oxidase at a Gordon Conference. His landmark contribution did not immediately receive the attention it deserved, perhaps because ATP synthase was not yet considered as well-defined an entity as cytochrome oxidase.

For a while, many colleagues believed that these mitochondrially made proteins were not functional parts of either cytochrome oxidase or ATP synthase, but hydrophobic contaminants. Hydrophobicity was the angel with the flaming sword that protected the mitochondrial magic garden from unworthy intruders. We tried to purify these hydrophobic subunits; they precipitated like balls of steel. We tried to sequence them; they merely scoffed. Strange legends started to grow around them. Some claimed that the large cytochrome oxidase subunits were in reality tight aggregates of smaller proteins. Others considered them as structural scaffolds without specific function.

Gradually the fog lifted (29). When my postdoctoral fellows Eberhard Ebner and Tom Mason isolated nuclear yeast mutants that specifically lacked cytochrome oxidase activity, they noted with delight that these mutants also lacked one or two mitochondrially made subunits of the enzyme (30). Then Robert O. Poyton showed that an antiserum specific for the second largest cytochrome oxidase subunit inhibited the activity of the purified enzyme [see Poyton & Schatz (31)]. Both findings implied that these large subunits were essential for the function of the enzyme. A few years later,

Thomas D. Fox used the nuclear yeast mutants to uncover a translational control mechanism by which nuclear genes govern the expression of mitochondrial genes (32). And once several groups worked out the primary sequence of the large cytochrome oxidase subunits, these turned out to be normal, albeit extremely hydrophobic proteins. Perhaps the most decisive breakthrough was the discovery of specific mitochondrial DNA mutations that altered only a single mitochondrially made protein (33). Some of these mutations made ATP synthase or cytochrome *b* resistant to antibiotics; others selectively inactivated one of the enzyme complexes of oxidative phosphorylation; and still others had no physiological effect, but altered the electrophoretic mobility of a mitochondrially made protein. Analysis of these specific mutations led to the first genetic map of yeast mitochondrial DNA (33). This map, however, did not tell whether a gene affecting a mitochondrially made protein was regulatory or structural, but Slonimski's laboratory and ours showed that one of the genes affecting cytochrome oxidase function was, in fact, the structural gene of the second largest cytochrome oxidase subunit (34). All of this became history when Fred Sanger's group (35) at Cambridge deciphered the complete nucleotide sequence of human mitochondrial DNA, Giuseppe Attardi's group (36) provided the matching transcript map, and several groups sequenced most of the remaining mitochondrially made proteins. When the three-dimensional atomic structure of bovine cytochrome oxidase arrived on the scene (37), it showed that subunit I carries the two heme *a* groups as well as the two copper atoms, whereas subunit II binds cytochrome *c*. The function of the other subunits, particularly that of subunit III, is still unclear. Today, we know that human mitochondria make 13 proteins, all of them encoded by mitochondrial DNA: 3 subunits of cytochrome oxidase, 1 subunit of the cytochrome bc_1 complex, 7 subunits of the NADH dehydrogenase complex, and 2 subunits of the F_o part of ATP synthase. Mitochondria of the yeast *Saccharomyces cerevisiae* make only 8 stable proteins, those of plants at least twice as many and mitochondria of the protozoon *Reclinomonas americana* make no fewer than 62. Most or all of these mitochondrial genes bear a striking resemblance to their counterparts in bacteria—dramatic evidence for the long-held view that mitochondria evolved from bacterial endosymbionts.

My Cornell years showed me what a first-rate university can offer. By comparison, most European universities were grossly underfunded, granted their young researchers little independence, and clung to antiquated hierarchies and rigid faculty divisions. My colleagues in Europe had much more power than I at Cornell, but wielding this power left them little time for research. Running a modern university with public funds has become so complex that Wilhelm von Humboldt's ideal of a "scholars' republic" should be abandoned in favor of an enlightened presidential system, which frees researchers from the burden of administrative and political duties. The prime goals of a university president should be to select the best faculty and then to guard its precious time, but neither goal seemed to be a priority at the European university I visited at that time. I am afraid that this situation has not changed much.

My years at Cornell also dropped me smack in the midst of the hippie revolution. This proved to be a challenge—particularly for someone coming straight from conservative Austria. Although most of my students badly needed a haircut, a shave, or sartorial advice, they were outgoing and idealistic. With my friend Stuart J. Edelstein, I taught the introductory biochemistry course to a class of several hundred unruly students, which called for the equanimity of the Dalai Lama and the guts of a lion tamer. We stopped at nothing to keep our students attentive. To explain the role of ATP, I held up a huge wooden model of the molecule, which could fire a spring-loaded red Styrofoam™ ball representing the γ-phosphoryl group into the audience. To make the Krebs cycle less boring, I went through its steps by rotating the hand of a monstrous clock-like device. Whenever the hand reached a decarboxylation reaction,

teaching assistants hidden behind this "Krebs cycle clock" released a green helium-filled balloon and fired a starter's pistol. I had heard that, in the United States, shooting always helps. The students seemed to appreciate our efforts and even took my German accent in good humor. Their comment "Hey Prof, you sound just like Henry Kissinger" was a compliment because the German-born U.S. secretary of state was notorious for his political craftiness and alleged allure for the opposite sex. Everything was questioned; everything tried. It seemed that a new society was just around the corner—until the flowers of this exuberant revolution wilted in the chill of the rising political violence, the oil shock, and the bitterness of the Vietnam defeat.

BASEL FOR BIG BIOLOGY

My wife and I had intended to move up the evolutionary tree from resident aliens to full-fledged U.S. citizens, but evolution has its own ways. In 1972, it made me accept a visiting professorship in Zurich, which, to most Austrians, conjures up hard-working burghers, discreet bankers wearing gold watches, and a nightlife reminiscent of Vienna's municipal cemetery. But our very first evening stroll along the elegant Bahnhofstrasse and a brief glance at the calendar of events in the venerable *Neue Zürcher Zeitung* quickly showed us the city's wealth, its cosmopolitan flair, and its rich cultural life. Later, I was equally impressed by the level of science at the university and at the Swiss Federal Institute of Technology. This visit made me realize that my image of Europe still reflected my years in postwar Austria and that the tranquil life in a small U.S. college town was worlds apart from the splendor and the intellectual excitement of Zurich. When the University of Zurich offered me a professorship, I was about to accept but evolution intervened again in the guise of the molecular biologist Thomas Hohn, who invited me to visit the newly created Biozentrum at the University of Basel. I had heard of this new institute through the grapevine and a British science magazine, which had run a highly laudatory article entitled "Basel for Big Biology." Indeed, this visit convinced me that the Biozentrum was the place for me. Even though it had just started operation, it radiated the informal and international atmosphere I so loved at Cornell. When my future Swiss colleague Max M. Burger phoned me at my Cornell office shortly upon my return and asked me to join the Biozentrum as a professor, I forewent the time-honored mating dance of academic courtship and simply said "yes"—provided my wife would say the same. She said nothing of this sort, however, and could not understand why we should now move our family across the Atlantic for the fifth time. Her loaded question "Is this move really necessary?" dominated our evening discussions for several weeks until I finally convinced her that this move would benefit us all.

In setting up the Biozentrum, the penny-wise citizens of Basel and their venerable university had outdone themselves—but not without insistent prodding by Basel's pharmaceutical giants F. Hoffmann-La Roche, Sandoz, Ciba, and Geigy. When these companies got wind of the university's plan to appoint a new professor of biochemistry, they pressed for an entirely new multidisciplinary institution in which biochemists, cell biologists, physical chemists, and pharmacologists would attack biological problems in a concerted manner. At first Basel's city fathers thought this idea to be plain wacky, but once they had decided to go ahead, they did so with their customary thoroughness. In no time, they erected an attractive building in which wide-open staircases invited casual interactions between scientists from different floors. By offering very generous financial support, they also had little difficulty hiring first-rate scientists. Most of them were either foreigners or Swiss nationals who had spent many years abroad. The cell biologists, Max M. Burger and Walter J. Gehring, came from Princeton and Yale, respectively; the crystallographer, Johan N. Jansonius, left the University of Groningen; the biophysicists, Jürgen Engel, Kasper Kirschner, Joachim Seelig, and Gerhard Schwarz, had all been trained at the Max-Planck-Institute in Göttingen; and the

microbiologists, Eduard Kellenberger and Werner Arber, came from the University of Geneva and, together with the Roche Research Director, Albert Pletscher, gave the Biozentrum the legal statutes and the career structures of a modern and open research institution.

It is a rare privilege to belong to the founding generation of a scientific institute. All of us were eager to put our international experience into practice and make the Biozentrum one of the best research centers in the world. Although we did have our occasional squabbles about teaching and budgets, we never knew the internecine feuds common at European universities. Yet our presence did not please everyone: Colleagues from the university's other departments reproached us for wasting money, flouting university traditions, skipping faculty meetings, and—the ultimate sin—embracing American manners. Even our habit of working late into the night raised some eyebrows: An anonymous resident from across the street complained to the government that the nocturnal lights from our laboratory windows "interfered with his marital life." This being Switzerland, the government made us install outrageously expensive time-controlled Venetian blinds to protect the marital bliss of its population. But when Werner Arber received the Nobel Prize in 1978, Basel staged a city-wide celebration replete with a fearsome battalion of *Fasnacht* drums. After such an event, our critics could no longer denounce us as a bunch of money-squandering show-offs. As the Biozentrum's international reputation grew and we collected more and more scientific honors, Basel's citizens started to take pride in their Biozentrum. Today, it has become a firm part of the city's scientific and cultural scene. Still, if Basel University were to conduct a popularity poll among its faculty, most of us at the Biozentrum would rank near the bottom. Old resentments die hard.

TACKLING PROTEIN IMPORT INTO MITOCHONDRIA

During our first years in Basel, I continued to study the mitochondrial genetic system and its protein products. The Biozentrum proved to be a magnet for young scientists from around the world, and once again, I was lucky to have many outstanding students and postdoctoral fellows join my laboratory. The first one was Thomas D. Fox with whom I never coauthored a paper because he preferred to follow his own nose. This nose led him to the startling discovery that the genetic code of yeast mitochondrial DNA diverges from the general code (38) and that the nuclear genes affected in Ebner's cytochrome oxidase-less yeast mutants (30) encoded translational activators of specific mitochondrial mRNAs (39).

As the size of my research group grew, I felt the itch to tackle a new problem, but which one? In the fall of 1977, my good fairy sent me Maria-Luisa Maccecchini who became my first Swiss PhD student. At that time, my laboratory was already full, but she would not take "no" for an answer. "Maria-Luisa," I said finally, "if you will teach me how to spell your last name, I will teach you how to study protein import into mitochondria." It was a deal, and the start of a long trek into new territory.

Several laboratories had already described systems for studying uptake of proteins by isolated mitochondria. In particular, Walter Neupert and his colleagues (40, 41) at the University of Munich had translated total mRNA from *Neurospora crassa* in a cell-free extract, then added mitochondria, and found that some mitochondrial proteins (notably cytochrome *c* and the ADP/ATP carrier) were taken up by the organelles. However, uptake was difficult to prove convincingly as the measured radioactivities were low, and neither cytochrome *c* nor the ADP/ATP carrier was proteolytically altered upon import. Aided by a visit to Günter Blobel's laboratory at Rockefeller University, Maccecchini approached the problem with a highly efficient reticulocyte lysate. She translated total mRNA from yeast in the presence of radiolabeled methionine, then added yeast mitochondria, and checked whether these would import the α- and β-subunits of yeast F_1. The choice of these two proteins was a stroke of luck: Both were made as larger precursors and

then cleaved in a single step to their mature size upon import. This modification was readily seen as a mobility shift in sodium dodecyl sulfate-polyacrylamide gels and offered a fast and reliable way to monitor import (42). Susan Gasser, my next graduate student, then established many key features of this import process (43). When she graduated with honors in 1982, Bruce Alberts and his colleagues handed her the best graduation gift a student could desire: almost two pages of their magisterial textbook *Molecular Biology of the Cell* describing the results of her thesis. Susan is now the successful director of the Friedrich Miescher Institute for Biomedical Research in Basel and a widely admired role model for young women scientists.

The discovery of mitochondrial precursor proteins marked the beginning of our 20-year-long effort to unravel the complex mitochondrial protein import system. In 1980, my Swiss student, Peter C. Böhni, identified a soluble metalloprotease in the matrix that removed the transient N-terminal sequences of precursors destined to cross the mitochondrial inner membrane. He partially purified the enzyme, but we obtained the pure enzyme only eight years later after a circuitous detour involving yeast genetics. In 1981, Michael Yaffe and I decided to isolate yeast mutants defective in mitochondrial protein import, but how were we to find such mutants? We reasoned that mitochondrial protein import should be necessary for life because it is a prerequisite for mitochondrial function, and our earlier work had shown this function to be vital. We therefore screened for conditional mutants. During the Christmas recess of 1982, I produced a collection of about 2,000 temperature-sensitive yeast mutants, which Yaffe then tested for the accumulation of an uncleaved precursor to the F_1 β-subunit at the nonpermissive temperature. This brute force dragnet netted us two mutants (21), which later proved to be defective in the genes for the two different subunits of the matrix protease. In 1988, Gerd Hawlitschek in Walter Neupert's group at Munich finally obtained the homogeneous enzyme from *Neurospora crassa* [see Hawlitschek et al. (44)], and my student Meija Yang isolated it from yeast [see Yang et al. (45)].

This protease was not the only one participating in the import process. Akira Ohashi et al. (46) and Susan Gasser and coworkers (47) found that the precursors to the imported mitochondrial proteins cytochrome c_1 and cytochrome b_2 are cleaved twice: first by the metalloprotease just discussed, and then by another enzyme that was tightly bound to the outer surface of the inner membrane. Why would mitochondria resort to such proteolytic extravagance? As cytochrome c_1 and cytochrome b_2 are both located in the space between the two mitochondrial membranes, we suspected that their import requires two different signals: (*a*) a matrix-targeting signal at the extreme N terminus that, by itself, would direct the protein into the matrix, where it is cleaved off by the matrix protease; and (*b*) a downstream "stop-transfer" signal that makes the once-cleaved precursor get stuck across the inner membrane. Cleavage of the stuck intermediate by a protease on the outer face of the inner membrane then generates the mature protein. The doubly cleaved cytochrome b_2 is released into the intermembrane space, whereas cytochrome c_1 remains tethered to the inner membrane through its hydrophobic C terminus. This stop-transfer model sparked a drawn-out and emotional controversy, but it is now generally accepted (48, 49). A similar combination of signals also directs many proteins to the mitochondrial outer membrane. By attaching the corresponding targeting sequences, Eduard C. Hurt, Dolf van Loon, and others in my group (50, 51) could direct nonmitochondrial "passenger proteins," such as dihydrofolate reductase, to any of the four major mitochondrial compartments or redirect mitochondrial proteins between compartments. Following up on this work, Martin Eilers made the puzzling observations that import of fusion proteins containing dihydrofolate reductase as the passenger protein was blocked by the dihydrofolate reductase ligand methotrexate and that it was dramatically accelerated by denaturating the protein. As methotrexate stabilizes the native conformation of dihydrofolate

reductase, denaturation destroys it. Thus, Eilers concluded that proteins must enter mitochondria in the unfolded state [see Eilers & Schatz (52)]. But how do they find the mitochondria? Howard Riezman discovered that they bind to specific protein receptors on the mitochondrial surface [see Riezman et al. (53)], but it took several years before Walter Neupert's group in Munich and we could identify these receptors.

I will always remember the moment when my German postdoctoral fellow Eduard C. Hurt burst into my office and told me that even a targeting sequence as short as a dozen amino acids could direct a protein into the mitochondrial matrix [see Hurt et al. (54)]. How was this information encrypted? David A. Roise found the equally startling answer: All it takes is a positively charged amphiphilic helix [see Roise et al. (55)]. The exact amino acid sequence did not matter—even randomly generated sequences would do the job, as long as they could fold into such a helix. What then prevented cytosolic proteins from entering mitochondria? Our computers told us that evolution had apparently selected against such helices at the N termini of cytosolic proteins (56).

By the mid-1980s, our laboratory basked in the glow of a golden period, and it would take too long to recount all of what we found. But I cannot resist telling how Dietmar Vestweber tracked down the first subunit of a mitochondrial protein import channel [see Vestweber et al. (57)]. Vestweber's capacity for work has become a Biozentrum legend, and rumors that he once left the laboratory to get some sleep were never verified. To identify proteins of the import channel, he constructed an artificial precursor protein that gets stuck in this channel; he then photocross-linked the stuck precursor to any mitochondrial protein nearby; and he then identified the cross-linked protein. This may sound simple, but it required the construction of an artificial precursor stitched together by chemistry and genetic engineering from three different proteins as well as the synthesis of a trifunctional and photoactivatable cross-linker. The fish Dietmar finally caught was an integral outer membrane protein, the deletion of which proved to be lethal (58). We called it ISP42, but it was later renamed Tom40 and identified as the key subunit of the protein import channel across the mitochondrial outer membrane.

Vestweber's departure from our laboratory in early 1989 marked a new phase in our research. Having spent a decade sketching the broad outlines of the mitochondrial protein import system, the time had now come to flesh out the sketch. This phase saw again gifted young people and new hunts, as well as success and disappointment. But finishing a painting rarely matches the excitement of drawing the sketch. The sketch is an exclamation mark calling for attention, whereas the finishing phase is a more private battle, often too subtle for a riveting story. Yet it was this final phase that helped me become a mature scientist. Most of the insecurities of my early years had faded; I finally felt at ease leading a research group; and the honors that started to come my way in rapid succession sweetened the departure *d'un certain âge*. Just before I closed my laboratory, Carla Koehler and Sabeeha Merchant opened a new door by showing that a devastating human disease inflicting deafness, muscle weakness, dementia, or blindness is caused by a defective mitochondrial protein import system [see Koehler et al. (59)]. Our sketch of the protein import system showed us many nuts and bolts, but did not tell us how they functioned. To me as a chemist, this question was at the heart of the problem, but not everybody saw it this way. In the hothouse of today's science, discovering a new protein is great; cloning its gene a must; documenting its intracellular distribution by "red-green-merge" fluorescence imaging is politically correct; and coaxing the editor of a "high-impact" journal to pick the image for the journal cover is the ultimate victory. But deciphering the mechanism by which the protein works is a long uphill struggle with an uncertain outcome. Yet, when everything is said and done, it is the mechanism that counts. Unless we know it, we know nothing. And so we tried to identify the forces that move proteins into mitochondria as well as the mechanisms by which these proteins acquire

their mature structure within the proper compartment (60). These questions are still not fully resolved, but the fleshed-out picture revealed a fiendishly complex network of interacting receptors and channels, aided by ATP-powered chaperones and enzymes. On their long voyage from endosymbionts to well-behaved cellular citizens, mitochondria have left no stone unturned. They are not quite there yet because the oxidizing sparks from their furnaces damage their host, and when this damage exceeds a certain threshold, mitochondria order the host to kill itself. Mitochondria are not only obedient servants, but also angels of death.

SCIENCE: A CONTRACT BETWEEN GENERATIONS

In 1998, Swiss activists mounted a massive attack on genetic engineering. This "Gene Protection Initiative" was mainly orchestrated by educated, professionally successful women from the German-speaking part of Switzerland (61), and even though it was roundly defeated, it made me aware of the fragility of science in a democratic society and the rising tide of political and bureaucratic controls that was threatening the country's scientific innovation. Everyone bemoaned this danger—but why not fight it? In the fall of 1999, I decided to stop research, close down my laboratory and office, and follow the call of the federal government in Bern to head the Swiss Science and Technology Council. As the country's top science advisor, I hoped to persuade its political leaders to adopt a more enlightened science policy. I was then only 63 years old, and my decision surprised and even shocked many of my friends and colleagues. But my four years in science politics brought me face to face with the plight of European science. Switzerland is one of the world's top scientific nations, yet many of its universities are poorly governed and unaware of the damage their antiquated hierarchies inflict on young researchers. Universities should be ticking intellectual time bombs, yet many are among our most conservative institutions. Elsewhere in Europe, matters are even worse. Descending into the netherworld of science politics was a daunting experience, but at least our Science Council could stem the planned increase of politically inspired project grants, reaffirm the importance of long-term basic research, and persuade some universities to adopt a "tenure track" system for their young faculty. And these years of reflecting, lecturing, and writing on these issues gradually transformed me into the author of essays and books that I am today (62, 65).

Looking at science from the outside made me aware of how much it has given me. Mars had done its best to stunt me, but science had rescued me through the scientists who helped me on my way—foremost among them Hans Tuppy, Efraim Racker, and David E. Green. By introducing me to the fires of life, they lit the fires of science in me and let me witness at close range one of the greatest biological discoveries of the twentieth century. Science is a contract between generations. Rarely was a generation as dependent on this contract as mine. And rarely was a generation as entitled to renege on it as the Jewish refugees. In honoring this contract so generously, they changed my life.

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for Mitochondrially Synthesized Proteins" (28), "Coming in from the Cold: How Answering a Postcard Can Launch a Scientific Career" (68), as well as on my book *Feuersucher* (65). All required permissions were given. I wish to thank Michael P. Murphy and Heimo Brunetti for helpful comments and Jesslyn Holombo for her exceptional care in copyediting the manuscript.

LITERATURE CITED

1. Beinert H, Stumpf PK, Wakil SJ. 2003. David Ezra Green. In *Biographical Memoirs*, 84:1–34. Washington, DC: Natl. Acad. Sci.
2. Ernster L, Schatz G. 1981. Mitochondria: a historical review. *J. Cell Biol.* 91:s227–55
3. McLean JR, Cohn GL, Brandt IK, Simpson MV. 1958. Incorporation of amino acids into the protein of isolated mitochondria. *J. Biol. Chem.* 233:657–63
4. Ephrussi B. 1953. *Nucleo-Cytoplasmic Relationships in Microorganisms*. Oxford: Clarendon
5. Slonimski PP. 1953. *La Formation des Enzymes Respiratoires chez la Levure*. Paris: Masson
6. Schatz G, Haslbrunner E, Tuppy H. 1964. Deoxyribonucleic acid associated with yeast mitochondria. *Biochem. Biophys. Res. Commun.* 15:127–32
7. Nass S, Nass MMK. 1963. Intramitochondrial fibers with DNA characteristics II. Enzymatic and other hydrolytic treatments. *J. Cell Biol.* 19:613–29
8. Pullman ME, Penefsky HS, Datta A, Racker E. 1960. Partial resolution of the enzymes catalyzing oxidative phosphorylation. I. Purification and properties of soluble dinitrophenol-stimulated adenosine triphosphatase. *J. Biol. Chem.* 235:3322–29
9. Penefsky HS, Pullman ME, Datta A, Racker E. 1960. Partial resolution of the enzymes catalyzing oxidative phosphorylation. II. Participation of a soluble adenosine triphosphatase in oxidative phosphorylation. *J. Biol. Chem.* 235:3330–36
10. Mitchell P. 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* 191:144–48
11. Pullman ME, Schatz G. 1967. Mitochondrial oxidation and energy coupling. *Annu. Rev. Biochem.* 36:530–610
12. Boyer PD, Chance B, Ernster L, Mitchell P, Racker E, Slater EC. 1977. Oxidative phosphorylation and photophosphorylation. *Annu. Rev. Biochem.* 46:955–1026
13. Schatz G, Penefsky HS, Racker E. 1967. Partial resolution of the enzymes catalyzing oxidative phosphorylation. XIV. Interaction of purified mitochondrial adenosine triphosphatase from baker's yeast with submitochondrial particles from beef heart. *J. Biol. Chem.* 242:2552–60
14. Schatz G. 1968. Impaired binding of mitochondrial adenosine triphosphatase in the cytoplasmic "petite" mutant of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 243:2192–99
15. Linnane AW, Vitols E, Nowland PG. 1962. Studies on the origin of yeast mitochondria. *J. Cell Biol.* 13:345–50
16. Wallace PG, Linnane AW. 1964. Oxygen-induced synthesis of yeast mitochondria. *Nature* 201:1191–94
17. Criddle RS, Schatz G. 1969. Promitochondria of anaerobically grown yeast. I. Isolation and biochemical properties. *Biochemistry* 8:322–34
18. Plattner H, Schatz G. 1969. Promitochondria of anaerobically grown yeast. III. Morphology. *Biochemistry* 8:339–43
19. Swift H, Rabinowitz M, Getz G. 1968. Cytochemical studies on mitochondrial nucleic acids. In *Biochemical Aspects of the Biogenesis of Mitochondria*, ed. EC Slater, JM Tager, S Papa, E Quagliarello, pp. 3–19. Bari, Italy: Adriatica Editrice
20. Plattner H, Salpeter MM, Saltzgaber J, Schatz G. 1970. Promitochondria of anaerobically grown yeast. IV. Conversion into respiring mitochondria. *Proc. Natl. Acad. Sci. USA* 66:1252–59
21. Yaffe M, Schatz G. 1984. Two nuclear mutations which block mitochondrial protein import in yeast. *Proc. Natl. Acad. Sci. USA* 81:4819–23
22. Schatz G, Saltzgaber J. 1969. Protein synthesis by yeast promitochondria in vivo. *Biochem. Biophys. Res. Commun.* 37:996–1001

23. Kužela S, Smigan P, Kováč L. 1969. Biochemical characteristics of respiration-deficient yeast mutants differing in buoyant densities of mitochondrial DNA. *Experientia* 25:1042–43
24. Mason TL, Ebner E, Poyton RO, Wharton DC, Mennucci L, Schatz G. 1972. Participation of mitochondrial and cytoplasmic protein synthesis in mitochondrial formation. In *Mitochondria: Biogenesis and Bioenergetics*, ed. P Borst, EC Slater, GS van den Bergh, pp. 53–69. Amsterdam: North Holland
25. Mason TL, Schatz G. 1973. Cytochrome *c* oxidase of baker's yeast. II. Site of translation of the protein components. *J. Biol. Chem.* 248:1355–60
26. Weiss H, Sebald W, Bücher T. 1971. Cycloheximide-resistant incorporation of amino acids into a polypeptide of the cytochrome oxidase of *Neurospora crassa*. *Eur. J. Biochem.* 22:19–26
27. Rubin MS, Tzagoloff A. 1973. Assembly of the mitochondrial membrane system. X. Mitochondrial synthesis of three of the subunit proteins of yeast cytochrome oxidase. *J. Biol. Chem.* 248:4275–79
28. Schatz G. 1997. The hunt for mitochondrially synthesized proteins. *Protein Sci.* 6:728–34
29. Tzagoloff A. 1971. Assembly of the mitochondrial membrane system. IV. Role of mitochondrial and cytoplasmic protein synthesis in the biosynthesis of the rutamycin-sensitive adenosine triphosphatase. *J. Biol. Chem.* 246:3050–56
30. Ebner E, Mason TL, Schatz G. 1973. Mitochondrial assembly in respiration-deficient mutants of *Saccharomyces cerevisiae*. II. Effect of nuclear and extrachromosomal mutations on the formation of cytochrome oxidase. *J. Biol. Chem.* 248:5369–78
31. Poyton RO, Schatz G. 1975. Cytochrome *c* oxidase from baker's yeast: immunological evidence for the participation of a mitochondrially synthesized subunit in enzymatic activity. *J. Biol. Chem.* 250:762–66
32. Costanzo MC, Poutre CG, Strick CA, Fox TD. 1985. Yeast nuclear gene products required for translation of specific mitochondrial messenger RNAs. In *Achievements and Perspectives in Mitochondrial Research*, ed. F Palmieri, C Saccone, AM Kroon, pp. 355–60. Amsterdam: Elsevier
33. Tzagoloff A, Akai A, Needleman RB, Zulch G. 1975. Assembly of the mitochondrial membrane system. Cytoplasmic mutants of *Saccharomyces cerevisiae* with lesions in enzymes of the respiratory chain and in the mitochondrial ATPase. *J. Biol. Chem.* 250:8236–42
34. Cabral F, Solioz M, Rudin Y, Schatz G, Clavilier L, Slonimski PP. 1978. Identification of the structural gene for yeast cytochrome *c* oxidase subunit II on mitochondrial DNA. *J. Biol. Chem.* 253:297–304
35. Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, et al. 1981. Sequence and organization of the human mitochondrial genome. *Nature* 290:457–65
36. Attardi G, Chomyn A, Doolittle RF, Mariottini P, Ragan CI. 1986. Seven unidentified reading frames of human mitochondrial DNA encode subunits of the respiratory chain NADH dehydrogenase. *Cold Spring Harb. Symp. Quant. Biol.* 51:103–14
37. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, et al. 1996. The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 Å. *Science* 272:1136–44
38. Fox TD. 1979. Five TGA "stop" codons occur within the translated sequence of the yeast mitochondrial gene for cytochrome *c* oxidase subunit II. *Proc. Natl. Acad. Sci. USA* 76:6534–38
39. Fox TD. 1986. Nuclear gene products required for translation of specific mitochondrially coded mRNAs in yeast. *Trends Genet.* 2:97–99
40. Harmey MA, Hallermayer G, Korb H, Neupert W. 1977. Transport of cytoplasmically synthesized proteins into the mitochondria in a cell free system from *Neurospora crassa*. *Eur. J. Biochem.* 81:533–44
41. Hallermayer G, Zimmermann R, Neupert W. 1977. Kinetic studies on the transport of cytoplasmically synthesized proteins into the mitochondria in intact cells of *Neurospora crassa*. *Eur. J. Biochem.* 81:323–33
42. Macchecchini M-L, Rudin Y, Blobel G, Schatz G. 1979. Import of proteins into mitochondria: precursor forms of the extra mitochondrially made F_1-ATPase subunits in yeast. *Proc. Natl. Acad. Sci. USA* 76:343–47
43. Gasser SM, Daum G, Schatz G. 1982. Import of proteins into mitochondria. Energy-dependent uptake of precursors by isolated mitochondria. *J. Biol. Chem.* 257:13034–41
44. Hawlitschek G, Schneider H, Schmidt B, Tropschug M, Hartl FU, Neupert W. 1988. Mitochondrial protein import: identification of processing peptidase and of PEP, a processing enhancing protein. *Cell* 53:795–806
45. Yang M, Jensen RE, Yaffe MP, Schatz G. 1988. Import of proteins into yeast mitochondria: The purified matrix processing protease contains two subunits which are encoded by the nuclear *MAS1* and *MAS2* genes. *EMBO J.* 7:3857–62

46. Ohashi A, Gibson J, Gregor I, Schatz G. 1982. Import of proteins into mitochondria. The precursor of cytochrome c_1 is processed in two steps, one of them heme-dependent. *J. Biol. Chem.* 257:13042–47
47. Gasser SM, Ohashi A, Daum G, Böhni P, Gibson J, et al. 1982. The imported mitochondrial proteins cytochrome b_2 and cytochrome c_1 are processed in two steps. *Proc. Natl. Acad. Sci. USA* 79:267–71
48. Glick BS, Brand A, Cunningham K, Müller S, Hallberg RL, Schatz G. 1992. Cytochromes c_1 and b_2 are sorted to the intermembrane space of yeast mitochondria by a stop-transfer mechanism. *Cell* 69:809–22
49. Bömer U, Meijer M, Guiard B, Dietmeier K, Pfanner N, Rassow J. 1997. The sorting route of cytochrome b_2 branches from the general mitochondrial import pathway at the preprotein translocase of the inner membrane. *J. Biol. Chem.* 272:30439–46
50. Hurt EC, Pesold-Hurt B, Schatz G. 1984. The cleavable prepiece of an imported mitochondrial protein is sufficient to direct cytosolic dihydrofolate reductase into the mitochondrial matrix. *FEBS Lett.* 178:306–10
51. van Loon APGM, Brändli A, Schatz G. 1986. The cleavable presequences of two imported mitochondrial precursor proteins contain information for intracellular targeting and for intramitochondrial sorting. *Cell* 44:801–12
52. Eilers M, Schatz G. 1986. Binding of a specific ligand inhibits import of a purified precursor protein into mitochondria. *Nature* 322:228–32
53. Riezman H, Hay R, Witte C, Nelson N, Schatz G. 1983. Yeast mitochondrial outer membrane specifically binds cytoplasmically-synthesized precursors of mitochondrial proteins. *EMBO J.* 2:1113–18
54. Hurt EC, Pesold-Hurt B, Suda K, Oppliger W, Schatz G. 1985. The first twelve amino acids (less than half of the presequence) of an imported mitochondrial protein can direct mouse cytosolic dihydrofolate reductase into the yeast mitochondrial matrix. *EMBO J.* 4:2061–68
55. Roise D, Horvath SJ, Tomich JM, Richards JH, Schatz G. 1986. A chemically synthesized mitochondrial signal peptide can form an amphipathic helix and perturb natural and artificial phospholipid bilayers. *EMBO J.* 5:1327–34
56. Lemire BD, Fankhauser C, Baker A, Schatz G. 1989. The mitochondrial targeting function of randomly generated peptide sequences correlates with predicted helical amphiphilicity. *J. Biol. Chem.* 264:20206–15
57. Vestweber D, Brunner J, Baker A, Schatz G. 1989. A 42K outer-membrane protein is a component of the yeast mitochondrial protein import site. *Nature* 341:205–9
58. Baker KP, Schaniel A, Vestweber D, Schatz G. 1990. ISP42, a protein of the yeast mitochondrial outer membrane, is essential for protein import and cell viability. *Nature* 348:605–9
59. Koehler CM, Leuenberger D, Merchant S, Renold A, Junne T, Schatz G. 1999. Human deafness dystonia syndrome is a mitochondrial disease. *Proc. Nat. Acad. Sci. USA* 96:2141–46
60. Schatz G. 1998. The Swiss vote on gene technology. *Science* 281:1810–11
61. Schatz G. 2005. *Jeff's View on Science and Scientists*. Amsterdam: Elsevier
62. Schatz G. 2008. *Jenseits der Gene*. Zürich: NZZ Libro
63. Schatz G. 2008. *Jenseits der Gene*. Zürich: Kein & Aber. Audio version.
64. Schatz G. 2011. *A Matter of Wonder*. Basel: Karger
65. Schatz G. 2011. *Feuersucher. Die Jagd nach dem Rätsel der Lebensenergie*. Zürich: Wiley VCH/Weinheim/ NZZ Libro
66. Schatz G. 2000. Interplanetary travels. In *Comprehensive Biochemistry*, ed. G Semenza, R Jaenicke, 41:449–530. Amsterdam: Elsevier Sci.
67. Schatz G. 1993. From 'granules' to organelles: how yeast mitochondria became respectable. In *The Early Days of Yeast Genetics*, ed. MN Hall, pp. 241–46. Cold Spring Harbor, NY: Cold Spring Harb. Lab.
68. Schatz G. 2009. Coming in from the cold: how answering a postcard can launch a scientific career. *Nat. Cell Biol.* 11:364