Fifty Years Excitement with Science: Recollections with and without tRNA

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I was born on April 2, 1942 in Strasbourg in the sad time when Alsace was occupied and ruled by Hitler’s Nazis. Like many in Alsace, my father, who was in the French Army in 1939, became a malgré-nous during Nazi occupation and was forcibly conscripted into the Wehrmacht in 1943. This was retaliation for refusing to wear the German uniform of railway employees while working at his stationmaster job near Strasbourg. He was quickly sent to the front in Russia and killed near Minsk in January 1944. I grew up fatherless in Strasbourg, but my childhood was happy and rich because I received love and affection from my mother and grandparents, and the memory of my father was always present.

From my family and also as a pupille de la nation (a designation given to minors in France whose parents have been killed while serving the country, including malgré-nous children), I received encouragement to be studious in school. I had excellent teachers in elementary school and in Lycée Kléber, where I was accepted at the age of 9. Reading books and listening to music were among my favorite hobbies. At Lycée Kléber, I discovered many facets of civilization I had never suspected, and I became interested in all the disciplines that were taught there, although with some preference for the sciences. I prepared for the baccalaureate in mathematics with optional courses in biology and participated in many cultural activities outside of high school.

The usual follow-up was a math-focused preparatory class, but I did not like its hyperspecialized and competitive training, which left no time for cultural and other activities. Thus, after two months I made the difficult decision to leave the lycée for the science faculty at Strasbourg University, where I followed an atypical curriculum combining biology with chemistry and physics. I rapidly understood that biology was moving from purely descriptive toward more molecular aspects. This belief was strengthened by the enlightening organic chemistry lectures given by Guy Ourisson on natural compounds.

Having graduated with a chemistry degree, I discussed my interests with Guy Ourisson, and he gave me the good advice to obtain the new certificate of biological chemistry before starting my doctoral studies in either Strasbourg or Gif. I never regretted his advice, and as the first student from a chemistry Licence I followed the avant-garde teaching of Jean-Pierre Ebel on metabolic pathways, enzymology, and protein synthesis, including the premises of tRNA (soluble RNA at the time) biochemistry. Only a dozen students attended his teaching, all of them already engaged in research. I discovered a moving wonderland where discoveries occurred each day.

After a successful oral examination (on the Michaelis-Menten equation!), Jean-Pierre Ebel offered me a position in his laboratory, where work on protein synthesis had just begun. (I had another opportunity in physics, having also obtained the certificate of experimental physics.) I accepted the challenge with pleasure, although with some apprehension, and began my career in science as an assistant (assistant professor in charge of the practicals) of biochemistry in September 1964.
After two years of practical teaching and my first steps in research, I got a permanent job at the Centre National de la Recherche Scientifique (CNRS) in January 1966. In retrospect, I realize that I was fortunate to join an excellent laboratory working on hot topics in the golden age of molecular biology.

My journey in the universe of tRNA began with observations on the amino acid-accepting capacities of some yeast tRNAs that were dependent on the growth state of the yeast. A lack of integrity of the terminal CCA sequence of these tRNAs accounted for the variations (1). The underlying idea, still pertinent at present, was to find the structural reasons that account for perturbed functional properties. My doctoral thesis, “Recherches sur la Spécificité de Reconnaissance des Acides Ribonucléiques de Transfert par les Aminoacyl-tRNA Synthétases” (2), followed this rationale. Thus, I used the approach of chemical modifications (by in vivo incorporation of nucleotide analogs into tRNA) to study the aminoacylation capacity of the modified tRNAs. I also studied the accuracy of tRNA aminoacylation under different assay conditions (with the assistance of Daniel Kern, a freshman in the laboratory) and found that aminoacylation errors (tRNA mischarging) are common, a conclusion that generated skepticism at the time. By comparing the sequence of tRNAs with similar aminoacylation properties, I found, among other things, that aminoacylation by phenylalanyl- or valyl-tRNA synthetase is sensitive to anticodon. Altogether, and as a conclusion of my thesis (defended in January 1972) (2), I proposed the existence of a second genetic code: “en d’autres termes, il pourrait exister un second code génétique responsable de la spécificité de reconnaissance”; in other words, a second genetic code could exist that is responsible for the specificity of recognition (between tRNAs and aminoacyl-tRNA synthetases (aaRSs)) and, more precisely, “que les groupements fonctionnels constituant ce code soient dans une séquence ou dans un assemblage discontinue formé par la structure tridimensionnelle,” that the functional groups constituting this code would lie in a sequence or in a discontinued assemblage within the three-dimensional structure (that of tRNA).

I realized later that Peter Lengyel had already proposed this idea in 1966 (3). It was rejuvenated by Christian de Duve in 1988 (4) after the explicit characterization by Paul Schimmel, Bill McClain, and their colleagues of the first identity determinants in a tRNA, namely the G3-U70 pair, which defines alanine identity. Later, in 1993, in a collaboration with Paul Schimmel, Dino Moras, and Shigeyuki Yokoyama, the concept was refined, and we proposed the alternative idea of an operational RNA code for amino acids (based on the aminoacylation capacity of tRNA minihelices and the modular structure of aaRSs) that connects tRNA aminoacylation with the canonical code (5).

Meanwhile, extending my doctoral work, I continued to explore different facets of tRNA mischarging in collaborations with Henri Grosjean in Brussels (6), Brian Clark in Cambridge (7), and Mathias Springer in Paris (8); and in an attempt to rationalize the apparently contradictory data on specificity and errors in tRNA aminoacylation, I proposed, with Jean-Pierre Ebel and colleagues from Strasbourg, the concept of kinetic specificity in tRNA aminoacylation in a 1973 *Biochimie* paper (9).

In 1973, Alex Rich, during a visit to Strasbourg and stimulating discussions on protein synthesis with Jean-Pierre Ebel and Jean-Marie Lehn, invited me to Massachusetts Institute of Technology (MIT), where the crystal structure of yeast tRNA^Phe_ had just been solved. He was seeking to investigate the three-dimensional structure of a tRNA-aaRS complex and thought I would be the ideal candidate to crystallize such a complex because of my expertise in tRNA and aaRS purification. I accepted the challenge, and the same year, I went with my wife and children to the Boston area to join Alex’s laboratory. However, I failed miserably because I refrained, for seemingly rational reasons, from using a high salt concentration in crystallization assays. Indeed, it was then standard practice to use salts to disrupt tRNA-aaRS complexes, and therefore, I never used them as crystallants. It was only at the end of 1979 that I realized ammonium sulfate should be the crystallant. This was during a meeting in Göttingen, where Friedrich von der Haar described the purification of aaRSs on Sepharose columns eluted by reverse ammonium sulfate gradients. The key observation, not highlighted in his talk, was a modification of elution when tRNA was present in the elution gradient. For me, the interpretation was obvious: complexes are stable at high ammonium sulfate concentrations. One week later, this expectation was verified at the Institut Laue Langevin (ILL) in Grenoble in a small-angle neutron scattering (SANS) experiment. One week after that, crystals of the yeast tRNA^Asp_-aspartyl-tRNA synthetase (AspRS) complex were obtained readily in my laboratory at the Institut de Biologie Moléculaire et Cellulaire (IBMC) (10). It took, however, a long and painful time to obtain adequate crystals to solve the structure of this complex, but that is another story.

Despite this failure, my stay with Alex Rich at MIT was fruitful and had a profound impact on my career in science (Fig. 1). Importantly, I became convinced that answering
fundamental questions, especially in biology, requires interdisciplinarity and adequate experimental tools. Furthermore, I learned from Alex that one should not be afraid to engage in high-risk projects but that they should be conducted in parallel with more feasible ones. I always followed his advice.

Thus, while trying to crystallize a tRNA-aaRS complex (working next to the bench of Alex McPherson, who was studying the crystallization behavior of a variety of proteins), I was also comparing the Raman spectra of soluble and crystalline tRNA\textsuperscript{Phe} with Michael Chen in the laboratory of Richard Lord (11). The aim was to demonstrate that crystal and solution structures are equivalent. Today, it seems unbelievable that in the 1970s many traditional biologists were still convinced that crystal structures were artifacts. I also collaborated with Paul Schimmel on the photochemical cross-linking of tRNA on aaRSs (12) and had contact with Tom RajBhandary and many other tRNA and aaRS experts working in the Boston area, at Yale University, and elsewhere in the United States. The scientific exchanges I had with them and with my laboratory fellows, visitors, and scholars from other disciplines were very stimulating for me.

Back at the IBMC in autumn of 1974 and encouraged by Jean-Pierre Ebel, I started the saga of structural biology in Strasbourg with Dino Moras and Jean-Claude Thierry (Fig. 2). This was not an easy task, but it was an exciting one. As training, we decided to crystallize a free tRNA, taking advantage of the countercurrent samples gathered by Guy Dirheimer and his associates. We were lucky and rapidly obtained crystals of several yeast tRNAs, those specific to tRNA\textsuperscript{Asp} being the most promising (13). Soon after that, we also obtained crystals of AspRS (14) and thought we had grown crystals of the tRNA\textsuperscript{Asp}-AspRS complex using PEG as the crystallant. Indeed, a charging assay on a dissolved crystal incubated with radioactive aspartate agreed with a 1:1 tRNA\textsuperscript{Asp}:AspRS stoichiometry.

Unfortunately, it was an artifact explained by covalent attachment of aspartate on AspRS due to the lability of the AspRS-adenylate complex. Our disappointment grew as we encountered other difficulties with optimizing the tRNA and aaRS crystals (the structures of free tRNA\textsuperscript{Asp} and free AspRS were solved in 1980 and 2000) (see Refs. 15 and 16, respectively). Therefore, I decided to start systematic studies on nucleic acid and protein crystallization. For similar reasons, a few other colleagues, notably Alex McPherson and Charly Carter, followed the same path.

In parallel with my high-risk project, I continued to investigate structure-function relationships in tRNAs and aaRSs using more conventional biochemistry and enzymology techniques. I also initiated three novel research lines for which the support of Bernard Jacrot, Jean-Pierre Ebel, and Léon Hirth was essential. They concerned (a) studies of tRNA-aaRS complexes by SANS at the ILL in Grenoble, (b) solution probing of free tRNAs or tRNAs in complex with aaRSs by chemical and enzymatic probes, and (c) investigations on the tRNA-like properties of viral RNAs. Many interesting results were obtained and put forward at several tRNA workshops, notably at Cold Spring Harbor in the United States in 1978, at Hakone in Japan in 1983, and at Bayreuth in Germany in 1985, the traditional encounters of the tRNA community (Fig. 3). Let me illustrate each of these topics with a few results and memories.

Bernard Jacrot has advocated convincingly for the SANS tool as the appropriate means to obtain unique structural information on nucleic acid-protein complexes in solution, either globally or focused on the individual partners after contrast variation with H\textsubscript{2}O\textsubscript{2}. Although great amounts of material were required, I was convinced that the method was worth trying with tRNA-aaRS com-
complexes. Experiments started in 1976 and continued until the mid-1980s with great scientific and human benefit.

The yeast tRNA\textsuperscript{Val}–valyl-tRNA synthetase complex was the first model system I investigated with Dino Moras and Jean-Claude Thierry and with the help of Joe Zaccaï and Bernard Jacrot, our experts in SANS (17). Joe and Bernard also assisted our colleagues from Palaiseau, Sylvain Blanquet and Philippe Dessen, who were competing with us but working on other models (tRNA\textsuperscript{Met}–methionyl-tRNA synthetase complexes from \textit{Escherichia coli}) (18). For practical reasons and because of a high demand for access to the small-angle camera D11, we got joint packages of beamline time, and we spent many full-time sessions of two to three days at the ILL together. This was stimulating and generated animated discussions, mutual assistance collecting and interpreting data, and memorable gastronomic and wine-tasting sessions in small Grenoble restaurants. Instead of being competitors, we became friends, and this friendship endured over the years, as did the links between the Strasbourg and Palaiseau laboratories.

As mentioned above, the existence of a tRNA-aaRS complex at high ammonium sulfate concentration was demonstrated at the ILL in December 1979 and soon later biochemically confirmed (19). I should add that the ten years of excitement about tRNA and aaRSs at the ILL certainly influenced Stephen Cusack, who abandoned neutron biophysics for a successful move to the crystallography of tRNA aminoacylation systems.

As a follow-up to tRNA solution mapping with ethyl-nitrosourea, which was launched in 1979 with Valentin Vlassov, I was able to demonstrate in 1985 with Pascale Romby that the structural differences seen in the crystal structures of tRNA\textsuperscript{Phe} and tRNA\textsuperscript{Asp} can be revealed by chemical solution probing because the differential alkylation of the phosphate residues of tRNA did not depend on the kinetics of the chemical reaction but solely on the accessibility of ethyl nitrosourea to its targets (a conclusion later extended for probing with dimethyl sulfate and diethyl pyrocarbonate). In my opinion, this was the study (in which Dino Moras and Eric Westhof, who had just arrived in Strasbourg, participated) that established the validity of the chemical probing approach for studying RNA structures in solution (20). Chemical probing of RNA remained active for years in the laboratory (21). This was for the design and assay of new cleaving reagents, mainly with Valentin and his associates from Novosibirsk (e.g., imidazole-containing constructs) (see Ref. 22) and with Sid Hecht for RNA conformation probes (e.g., iron–bleomycin) (see Ref. 23), and for diverse applications in the tRNA field. The method also revealed a magnesium-dependent alternate folding of \textit{E. coli} tRNA\textsuperscript{Glu} (in collaboration with Jacques Lapointe) (see Ref. 24).

Unraveling the bizarre aminoacylation properties of turnip yellow mosaic virus and other viral genomic RNAs was another venture. I thought that this question had a broader scope that went beyond virology and was crucial for understanding the biology of tRNA itself. Catherine Florentz, who joined my group in 1980 on the recommendation of Léon Hirth, developed the topic splendidly. Thus, tRNA-like domains were isolated, and their kinetic and structural features were studied with the tools employed for tRNA (25). This turned out to be very fruitful and generated several doctoral theses (first by Catherine, followed by Joëlle Rudinger, Brice Felden, and Pierre Fechter) and friendly collaborations with colleagues from France (Simon Litvak) and abroad (Kees Pleij, Theo Drehmer, and Alex McPherson). A significant result published in 1987 by Philippe Dumas (with Kees, who discovered pseudoknot folding) was the experiment-based computer model of the tRNA-like domain from turnip yellow mosaic virus (26). Many astonishing findings followed, culminating with the discovery in 2005 (by Magali Frugier) of the structure and role of an intricate tRNA-like domain in the mRNA of yeast AspRS (27).

Now, a few words about Zong Qi Li (Xavier Lee) and Sheng-Xiang Lin, two Chinese scientists I met in the late 1970s in Grenoble and Strasbourg when they arrived to prepare a thesis in France after their difficult years in Mao’s China. I was amazed by their charming personalities, iron will to succeed in structural biology, and real interest in tRNA and aaRSs. Xavier was supervised by Joe Zaccaï while learning the SANS technique, and during one
of my visits to the ILL, we planned a collaborative project on the solvation of tRNA\textsubscript{Ph}\textsubscript{e} for his thesis. Xavier rapidly produced original results. Notably, he demonstrated that the tRNA is surrounded by a shell of solvent whose structure depends on salt conditions and is significantly denser than bulk solvent (28). The result is important, considering that the structure of the solvent shell should affect the properties of the tRNA, but strangely, the role of solvent in functional tRNA has not been taken into account seriously to this day. This should be done in the future.

I took care of Sheng-Xiang Lin (best known as Lin at the IBMC) after his arrival in Strasbourg at the request of Jean-Pierre Ebel. Lin was especially interested in structural biology, but a new crystallographic project in the laboratory appeared too risky. Thus, he prepared a thesis on the enzymology of aaRSs with Pierre Remy, but he conversed continuously with me about crystallization and the structural biology of tRNA aminoaacylation systems. Our scientific exchanges lasted over the years, and while in Quebec, where his main topic concerned the structural and molecular biology of enzymes from the estrogen pathway involved in cancer, Lin continued to have an interest in what was going on in Strasbourg. He even continued research on the structural biology and enzymology of aaRSs, such as arginyl-tRNA synthetases, and was active in the field of crystallogenesis; among other things, he organized the eleventh International Conference on the Crystallization of Biological Macromolecules (ICCBM) in Quebec (see Ref. 29). Today, both Xavier and Lin have installed x-ray crystallography in their laboratories in Cleveland and Quebec, as was their wish when they came to France. I acknowledge their tenacity and have great esteem for their achievements.

Incidentally, a funny story should be recalled at this point and concerns my only negative project, which resulted in a publication in Biochimie in 1988 (30). At that time, it was thought that aaRSs contain essential lysine residues in their catalytic site because of affinity labeling of several of them by periodate-oxidized ligands concomitant with the presence of a conserved KMSKS sequence. The straightforward idea was therefore to search for such lysine residues in AspRS with the objective of cross-linking adenosine, ATP, or tRNA\textsubscript{Asp} on the protein for crystallization assays. It was the job of Anne Théobald to detect these residues, but despite her efforts, Anne did not find them. Soon after, the reason for the failure became crystal clear when it was discovered that there are two classes of aaRSs. AspRS is in class II, in which the lysine-rich KMSKS signature is absent. Later on, Anne collected many positive results. I am convinced that her sad experience with AspRS was beneficial for the future of her career in research.

In the 1980s, access to the microgravity environment was certainly overadvertised by the space agencies in both industry and academia and created many controversies in the structural biology community. It had, however, the virtue of stimulating strategic discussions between biologists, physicists studying crystal growth, and engineers. I participated in several such strategic debates. As an outcome, I was able to start dedicated research in the laboratory and to initiate interdisciplinary cooperation with the financial help of the Centre National d’Etudes Spatiales (CNES) and the European Space Agency (ESA), the French and European space agencies that showed interest in basic research on the physical chemistry of protein crystallization but also in tRNA and aaRSs. This also facilitated the birth of the ICCBM, which started in 1985 with the first meeting at Stanford and is presently in its fourteenth edition, with ICCBM14 held in September 2012 in Huntsville, Alabama. In 1987, I organized ICCBM2 at Bischberg near Strasbourg and was proud to have Dorothy Hodgkin (Fig. 4) and Ada Yonath among the participants. In addition, I was involved in activities of general interest during this period, such as the publication, with Arnaud Ducruix, of the book Crystallization of Nucleic Acids and Proteins: A Practical Approach (31) and the design and testing of a European crystallization facility in microgravity (32) with the participation of Madeleine Riès-Kautt from Arnaud’s laboratory and Naomi Chayen from David Blow’s laboratory. Altogether, significant experimental
results were obtained. In my view, a particularly significant example was Vincent Mikol’s development in the late 1980s of a diagnostic tool for protein crystallization by dynamic light scattering (33), a method well known by the local polymer physicists in Strasbourg. The same research trends occurred in a few other places, and today, dynamic light scattering is in current practice in all structural biology laboratories, although with much improved equipment adapted for measurements on very small samples.

Other results followed, combining physical chemistry and structural biology, and were produced by motivated doctoral students (such as Bernard Lorber and Claude Sauter, who both became recognized experts in the field), postdoctoral collaborators (including Joe Ng and Christophe Charron), and visitors on sabbatical (Alex McPherson and Abel Moreno). As highlights, I would like to mention the Ostwald ripening of AspRS crystals, the characterization by atomic force microscopy of temperature-dependent growth modes of tRNA\textsuperscript{Phe} crystals, and the estimation of crystal perfection by x-ray topography. Scrutinizing crystal-solution phase diagrams was another trend that gave good results, most notably in the hands of Claude Sauter, who solved the structure of free yeast AspRS with crystals optimized after exploring the phase diagram of the protein (16). Systematic studies on the effects of pressure on protein crystal growth (with Bernard Lorber in collaboration with Gérard Jenner, a local expert in piezochemistry) also proved productive.

In my opinion, however, the essential output was the demonstration by Joe and Claude that diffraction data collected on AspRS crystals grown in space led to improved electron density maps (34). This did not imply that microgravity is the panacea to obtain better protein structures but encouraged researchers to find ways to mimic microgravity conditions on earth by reducing convection in crystallization devices. Bernard and Claude thoroughly explored the idea and developed user-friendly crystallization strategies in gels and microfluidic devices based on counter-diffusion growth (the compelling method invented by Juan-Ma García-Ruiz) for \textit{in situ} x-ray data collection that show promising possibilities (35).

I also should say a few words about the paradigmatic changes that occurred in my research when gene technologies were massively implemented in the laboratory, first for tRNAs and later for aaRSs. The meeting on the structure and dynamics of RNA held 1985 in Renesse, The Netherlands, where Olke Uhlenbeck gave a visionary talk, “A Better Way to Make RNA for physical Studies” (by means of RNA polymerases) (36), gave me the boost to use this technology in Strasbourg for studies on tRNA and tRNA-like domains. I convinced Catherine Florentz to be at the forefront, and in 1986, she went to College Station, Texas, to learn with Tim Hall and Theo Dreher how to prepare RNA molecules by transcription of synthetic genes.

At home, results came rapidly, first in the context of the doctoral theses of Angela Garcia and Véronique Perret. This was the start of an intellectually rich decade that also benefited from the sabbaticals of Henri Grosjean (in 1988–1989) and Kees Pleij (in 1989); the rewarding postdoctoral fellow Jody Puglisi, who came in the fall of 1989 (after memorable discussions at a Gordon Conference on biopolymers in 1988); the participation of temporary visitors (Christian Baron, Johan Edquist, Hong Xue, Osamu Nureki, Ya-Ming Hou, and others); and the progressive arrival of a bunch of excellent doctoral students (including Joëlle Rudinger, Magali Frugier, Joern Pütz, Brice Felden, and Marie Sissler) (Fig. 5).

Having access to unmodified tRNA transcripts provided new ways to explore tRNA biogenesis (as splendidly initiated in Strasbourg by Henri, who became the world reference in this domain) and tRNA identity. It also opened many lines of questioning about the role of modified nucleosides in tRNA. Thus, the demonstration of a relaxed aminoaacylation specificity of tRNA\textsuperscript{Asp} transcripts was of prime importance and led me to propose the anti-determinant concept.

In this context, I would like to mention the visit of Tat-suo Miyazawa, the mentor of Yuki Yokoyama, during his stay at the Centre de Neurochimie (next to the IBMC) in the spring of 1988. I was excited to tell him our first results on the functional role of the methyl group at position 37 in yeast tRNA\textsuperscript{Asp} that prevents its mischarging by arginyl-
tRNA synthetase (37). He showed great interest and, to my surprise, reported a similar finding, yet unpublished at the time, made by Yuki with an *E. coli* isoacceptor tRNA^Sec^ protected against methionylation by lysidine 34 in the anticodon (38).

The visit of Professor Miyazawa was also important for another reason, as it was the origin of the tripartite Strasbourg-Tokyo-MIT project on the molecular recognition of tRNA that quickly received support from the Japanese New Energy Development Organization and later from the HFSP. This project, coordinated by Yuki and in which I had the honor to be principal investigator with Jean-Pierre Ebel, Dino Moras, and Paul Schimmel, was a success and benefited all of the members of the three groups. It generated joint publications (e.g. Refs. 5 and 39) and, in my opinion, had a positive impact on the tRNA field in general. In this context, Osamu Nureki completed his Japanese doctorate with a stay in Strasbourg in 1991, and Ya-Ming Hou from MIT was on sabbatical with me in 1991–1992.

Sadly, however, unexpected events clouded this flourishing period. The first was the sudden passing away of Jean-Pierre Ebel in June 1992 just after an HFSP meeting in Strasbourg. Less dramatic but still disturbing were the doubts raised by the CNRS about the future of tRNA and aaRSs at the IBMC, considering that the domain already belonged to textbook knowledge. I disagreed vigorously. Indeed, I was convinced to the contrary because of the ancestral presence of tRNAs and aaRSs in all forms of life, which implied unavoidable openings in fundamental biology, medicine, and biotechnology. It was not too difficult for me to convince my closest collaborators to follow my reasonable arguments. The future showed that I was on the right track.

As anticipated, the future in the laboratory was rich and varied. The first aim was to consolidate the idea of a second genetic code and to unify results on tRNA identity as well as to explore tRNA or aaRS functions outside of tRNA aminoacylation and to study atypical tRNAs or aaRSs. I was relieved when results accumulated again. In this short survey, I cannot quote all of these results and their contributors. Here, I will mention just a few examples, notably predictions about the properties of tRNA and other RNA molecules with modified identity sets (multiple, switched, shifted, and partial) or alternate architectures (resected or modified L-shapes) that were validated by experiments (for example, see Refs. 40–42). Also important, in my view, were the efforts to strengthen the understanding of tRNA identity either within a given organism or through evolution and the functional plasticity of identity rules (43, 44). This feature is essential and accounts for the overall conservation of identity determinants in life. The generalization of the antideterminant concept for the nonrecognition of tRNA^Sec^ (where Sec is selenocysteine) by prokaryotic elongation factor was another accomplishment (45). In parallel to all these, dedicated work on AspRS crystals, crystallizability, and crystalline features of aaRSs was going on and confirmed, among other things, that crystal plasticity (notably local disorders in otherwise well diffracting crystals) is a common theme in aaRS crystallography. Many postdoctoral fellows and temporary visitors, as well as interactions with external colleagues (such as Ruslan Aphasizhev, Lev Kisselev, Paul Schimmel, Peter Schuster, Dieter Söll, Mathias Sprinzl, and Glauco Tocchini-Valentini), contributed to the good atmosphere that was prevailing in the tRNA/aaRS community.

The field broadened in the 1990s when Catherine Florentz started her independent work on mammalian mitochondrial tRNAs in synergy with the other projects going on in the laboratory. This was pertinent for two main reasons, notably the unexplained presence of idiosyncratic structural features in mitochondrial tRNAs and the discovery near the end of the 1980s of human diseases associated with mutations in mitochondrial tRNA genes. Such diseases were predictable because the dysfunction of any step along the cellular life of a mitochondrial tRNA should likely have harmful phenotypic consequences. However, a lack of adapted tools and a lack of a theoretical understanding of tRNA biology explain why this field remained unexplored for years.

At present, many mutations in tRNA as well as in aaRSs and other factors of protein synthesis have been detected as the cause of human diseases and disorders in organisms all along the tree of life. Given these facts, I am particularly happy to see how well the topic of the structure and function of mitochondrial tRNAs has developed and is fundamentally important for medicine and biology in general. The characterization by Mark Helm (who joined the laboratory, coming from Würzburg on the recommendation of Hans Gross) of a structural chaperone needed for cloverleaf folding in a human mitochondrial tRNA was an important result (46). Likewise, the resolution by Luc Bonnefond of the crystal structure of human mitochondrial tyrosyl-tRNA synthetase (TyrRS) (47), important in its own right, helped a medical group in Australia (John Christodoulou and his associates) to obtain deeper insight into the effects of mutations in the *YARS2* gene that cause a severe neurological and metabolic syndrome (48).

In this context, it is worth noting the collaboration with Chantal Abergel and Jean-Michel Claverie from Marseille...
that allowed comparison of the serendipitous TyrRS encoded in the genome of the giant mimiviruses with other TyrRSs. Functional studies by Joëlle Rudinger-Thirion confirmed the TyrRS specificity for tyrosine and conformity with the identity rules for tRNA^{Tyr} for Archaea and Eukarya, although the viral TyrRS presents a unique dimeric conformation and significant differences in its anticodon-binding site. Also fruitful were functional studies on other atypical tRNAs (tRNA^Sec and tRNA^Pyl (where Pyl is pyrrolysine)) and on protein ligands of tRNAs other than aaRSs (e.g., tRNA maturation enzymes that were studied in continuing collaborative ventures with Henri Grosjean and Jacques Lapointe and in new ones with Christian Cambillau, Mike Holmes, and Anita Marchfelder). A more recent outgrowth of my dedication to the tRNA/aaRS field is the new topic launched by Magali Frugier (with Joëlle Rudinger-Thirion and Anne Théobald-Dietrich) on protein synthesis in *Plasmodium* and correlatively in its human host. I am proud of Magali and her associates, who rapidly made significant findings, such as deciphering atypical structural and functional properties of plasmoidal AspRSs (49). I am also happy to see how the contributors to this new topic are connected synergistically with those working on mitochondrial tRNAs and aaRSs in sharing laboratory space, expertise, and occasionally publications.

As a scientist, I became gradually convinced that my duty in research should not be restricted to producing knowledge but also to sharing it with others and, importantly, to teaching it to the younger generation. Therefore, in 1985, I enthusiastically participated in the launch of the *Magistère de Chimie-Biologie* (a selective undergraduate teaching program with focused practicals in both chemistry and biology research laboratories) at Louis Pasteur University. The aim was to provide a multidisciplinary education to motivated students essentially based on the hottest research topics going on in Strasbourg at the intersection of chemistry and biology. Managing the *Magistère* and establishing its study program with my colleagues (among them, my thoughts go especially to Maurice Goebl and to the late Claude Benezra) were gratifying because many of the students found their way into research. I also found it enjoyable to teach occasionally at various French Master of Advanced Studies programs and doctoral schools as well as at practical schools on macromolecular crystallization all around the world.

I also found it important to be involved in activities of general interest for the community. My part-time job of project manager at the Life Sciences Department of the CNRS in Paris (2000–2005) was a great experience. I acted together with Christiane Branlant and Jean-Pierre Henry and was especially interested in interdisciplinarity, either within the CNRS to establish links with chemistry and physics or externally with the Commissariat à l’Energie Atomique (recently renamed Atomic Energy and Alternative Energies Commission) for a multidisciplinary program on nuclear toxicology, merging scientists from all French science organizations. In my opinion, this program was a success (see the special issue of *Biochimie* in 2006) (50). I also acted within the Société Française de Biochimie et Biologie Moléculaire, the Federation of European Biochemical Societies, the European Molecular Biology Organization, and diverse other organizations, and I tried to be useful to the community. This gave me the opportunity to encounter many wonderful, open-minded colleagues who shared my determination to promote the interplay between basic questions in science and technology but also showed me how difficult it is to smooth the egos of some individualists and to convince larger groups to accept innovation. Despite such difficulties, I remained an optimist, and today, at the end of my career, I am very happy to see how many of my students and postdoctoral fellows are on the forefront in academia and active research. Likewise, I was pleased to see the active participation of French-German undergraduates at the Perspectives in tRNA Biology Symposium organized on the occasion of my seventieth birthday. Students represent the future, and it is my dream that many of them will enrich the science of tRNA.

Acknowledgments—I express my gratitude to my mentors in science and to all of my students, postdoctoral fellow, collaborators, and staff members at IBMC Strasbourg as well as to my worldwide friends and colleagues from the tRNA, aaRS, bioorganic chemistry, and crystallogenesis communities for exchanging ideas and results over the years. With all of them, I spent fifty rewarding years in the wonderland of science. This Reflections is based on a talk I gave at the Perspectives in tRNA Biology Symposium that took place on October 28–30, 2012, in Strasbourg and was organized by Catherine Florentz, Joern Pütz, Claude Sauter, and Eric Westhof to honor my scientific contributions to the field of tRNA biology. Particular thanks are addressed to the sponsors of the symposium, notably the CNRS, the Société Française de Biochimie et de Biologie Moléculaire (SFBBM), the Université de Strasbourg, the City of Strasbourg, Région Alsace, and the Université Franco-Allemande.

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