

# Traversing the RNA world

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An invitation to write a “Reflections” type of article creates a certain ambivalence: it is a great honor, but it also infers the end of your professional career. Before you vanish for good, your colleagues look forward to an interesting but entertaining account of the ups-and-downs of your past research and your views on science in general, peppered with indiscrete anecdotes about your former competitors and collaborators. What follows will disappoint those who await complaint and criticism, for example, about the difficulties of doing research in the 1960s and 1970s in Eastern Europe, or those seeking very personal revelations. My scientific life has in fact seen many happy coincidences, much good fortune, and several lucky escapes from situations that at the time were quite scary. I have also been fortunate with regard to competitors and collaborators, particularly because, whenever possible, I tried to “neutralize” my rivals by collaborating with them — to the benefit of all. I recommend this strategy to young researchers to dispel the nightmares that can occur when competing against powerful contenders. I have been blessed with the selection of my research topic: RNA biology. Over the last five decades, new and unexpected RNA-related phenomena emerged almost yearly. I experienced them very personally while studying transcription, translation, RNA splicing, ribosome biogenesis, and more recently, different classes of regulatory non-coding RNAs, including microRNAs. Some selected research and para-research stories, also covering many wonderful people I had a privilege to work with, are summarized below.

My mother told me that I was born with a caul (in Polish, *wczepku urodzony*, which literally means “born in a bonnet”). This is traditionally the sign of a person who will always enjoy good luck. I am not sure that it turned out that way for others with this rare birth phenomenon, *e.g.* Lord Byron, Charlemagne, Sigmund Freud, and Napoleon, but my survival as a one-year-old of the 1944 Warsaw Uprising and the ensuing destruction of the city by German troops does nothing to destroy the hypothesis. My parents were actually very courageous to conceive me in that hell, where my father served in the underground army (Armia Krajowa) controlled by the London-based Polish government-in-exile. In 1946, with Warsaw destroyed, my family moved to the industrial city of Lodz, and my father became involved in setting up a Department of General and Physiological Chemistry at the newly formed Medical University of Lodz. He became its chairman in the early 1950s.

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Given my father’s position, I was exposed to biology and chemistry since my early childhood, and this greatly influenced my professional future. Our rather spacious apartment served as an extension of his laboratory and was usually full of mice. The poor animals were often not so attractive given that part of his research concerned vitamin deficiencies. Laying the white mice out on our black grand piano as a backdrop for photographic documentation didn’t necessarily improve their appearance. Perhaps because of this, I was more interested in chemistry than biology in high school, yet one should also give credit to my excellent chemistry teacher, who went far beyond the normal curriculum to prepare us for a prestigious school competition known as the “Chemistry Olympics.” As luck again would have it, I won this nationwide contest and was eligible to enter the university without sitting the entrance examinations.

## Undergraduate and graduate studies in Poland

Despite my interest in chemistry, I ultimately ended up studying medicine at the Lodz Medical University. The decision was certainly influenced by my mother, who was a dentist, and my older sister, who was just graduating as an M.D. and seemed to like her choice of profession. Although the medical curriculum was quite demanding, I started working in a laboratory as a second-year student in 1963/1964. The early 1960s were of course a fascinating time for anybody interested in biochemistry or molecular biology. For example, the genetic code was being deciphered live before my eyes, and mitochondria were being shown to have their own genetic apparatus. The latter was very significant to me because, among other things, I was purifying rat liver mitochondria to study intra-mitochondrial protein synthesis. We were “competing” with many well-known scientists, including Gottfried (“Jeff”) Schatz, who I had the privilege to meet and learn from 20 years later, after moving to Basel. Despite sacrificing dozens of rats and getting myself exposed to lots of radioactivity, I did not get far with my experiments. I will explain *why* later.

At one point, I became so frustrated with the progress of my experiments that I dropped out of the laboratory to become an amateur actor. The cabaret-like theater called Cytryna (a Lemon) was one of the most successful student theater groups in Poland. We were directed by a professional from the Higher School of Film and Theater in Lodz, which is famous for alumni such as Andrzej Wajda and Roman Polanski. We performed on our own stage in Lodz but also in other towns and even outside Poland. Poland then was probably the most amusing and laid-back wagon in the communist corral, with even travel to the West being granted occasionally (albeit *only* occasionally; see below). Luckily, with the cooperation of friends among the censors, we were able to smuggle dialogue into our plays that would

have been impossible in a professional setting. This continued cat-and-mouse game with the censors and the political system made my three-year-long association with Cytryna one of the most memorable periods of my life. More important, my acting experience proved invaluable at one of the turning points in my later career.

Surprisingly, despite this and other distractions, including very active participation in the Polish Students Union and the translation of popular life-science books from English into Polish, I managed to graduate from Lodz Medical University. Even before graduation, to test my medical skills, I worked during the summer as a trainee on a surgical ward in a hospital in Ayrshire, Scotland. During this visit, arranged through the Students Union, I found that medicine in the flesh was definitely not my cup of tea (or rather, glass of scotch). Rumors reached me later of a statistically significant drop in the population of Ayrshire dating back to my hospital assignment, and so the decision to drop medical practice was clearly the right one! In 1968, I moved to Warsaw to work toward a Ph.D. at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences. I joined its Department of Protein Synthesis, headed by Przemyslaw Szafranski.

In the late 1960s, many ambitious biochemists were working with bacteriophages to try to define the basic mechanisms behind the regulation of gene expression. I was one of them, and I began to study the *Escherichia coli* RNA phage  $\phi 2$ . My objective was to assess the usefulness of methoxyamine to modify the secondary structure of  $\phi 2$  RNA and so to study the possible involvement of RNA higher structure in the initiation of protein synthesis. The choice of methoxyamine as an RNA-modifying agent was based on its specificity for C residues; hence, AUG initiation codons were not affected. Research on RNA phages was then a very hot area, rather like microRNA studies these days. I vividly remember Joan Steitz's 1969 article in *Nature* describing the nucleotide sequences of the three ribosomal binding sites in phage R17 RNA (1). This was a milestone paper, laying the foundation for global analyses of translational regulation, developed decades later. Our competition with people at Harvard, Rockefeller, and the Medical Research Council (MRC) Laboratory of Molecular Biology in Cambridge was a rather exasperating experience. Of course, the people in those labs had no idea about our work in Warsaw, and we believed this to be to our advantage. We planned to take them by surprise and publish first! But, of course, it did not turn out that way.

As well as the shortage of funds, a critical problem with research in Poland at that time (and the same applied to my student experiments in Lodz: the roadblock I referred to earlier) was that we lacked information about progress in other labs. Even scientific journals had to pass the censors and were often delayed by a year before arriving in our library (it must have been torture for the censors to go through every issue of the *Journal of Biological Chemistry*!). We had plenty of great ideas, but very often, having done the experiments, we found out that others had already published similar findings. This was true for some of my methoxyamine results (2–4), which simply confirmed the already published data of Harvey Lodish with formaldehyde as the denaturing agent (5). This was one of my

“downs,” but such scientific frustrations during my postgraduate studies were more than compensated by a very important “up.” In 1970, I met and later married a wonderful girl named Aleksandra (Ola) Wodnar, who was a biochemist working in the same laboratory at that time. We worked together for several years (much too long from her perspective) and also published together (2–4). Ola eventually got her own lab and later became Professor of Experimental Hematology at the University of Basel, after our move to Switzerland.

### Postdoctoral time in the USA with Severo Ochoa and Aaron Shatkin

I defended my Ph.D. thesis in 1973 and moved with my family in early 1974 to Severo Ochoa's laboratory at the New York University (NYU) School of Medicine as a postdoctoral fellow. Severo Ochoa, a Spanish biochemist living in the USA, contributed significantly to the characterization of many metabolic enzymes and to the mechanism of translation. He also discovered — together with Marianne Grunberg-Manago — a polynucleotide phosphorylase (6). This discovery by Ochoa of an enzyme that later helped him and others decipher the genetic code had been rewarded with the Nobel Prize in Physiology or Medicine in 1959. I spent most of the first months in Ochoa's laboratory in the cold room, which was not at all bad during the hot and humid New York City summer. I was purifying and characterizing translation initiation factors from the brine shrimp *Artemia salina* (7), which was a model organism used by Ochoa at that time. Almost all the people in the lab were Spanish, and I was known there as the Científico Polaco (Fig. 1). In autumn 1974, Ochoa's lab moved to the Roche Institute of Molecular Biology in Nutley, NJ, where I was fortunate to meet Aaron Shatkin.

Aaron and a group of very talented collaborators including Hiro Furuichi had, with others, just discovered mRNA capping, the post-transcriptional addition of 7-methylguanosine ( $m^7G$ ) to the 5' end of mRNA (8). While continuing to work with Ochoa on translation factors and other problems related to the development of the brine shrimp, I then started to investigate the role of capping with Aaron's group. Together, we identified the first protein activity specifically recognizing the  $m^7G$  cap and characterized the methylation status of mRNAs and the methylating activities of undeveloped and developed embryos of *A. salina* (9, 10). The latter experiments were addressing the question of whether the modification status of mRNA changed during development, with capping perhaps being responsible for the activation of mRNAs; sadly, the duly recorded answer was “no.”

A couple of years ago, when giving a talk at the Skirball Symposium at my first postdoc home, I was reminded of one of those *other problems* I studied 40 years ago in Ochoa's laboratory. To introduce me at the Symposium, Joel Belasco, one of the professors at NYU School of Medicine, had dug out an old paper with my name on it published after my departure from the USA in 1977 (11). Joel showed its title on the screen and asked: “Why are you guys so shy? You had discovered small RNA regulators, probably microRNAs.” Honestly, I had completely forgotten about this work, which was probably a big mistake. The endogenous oligoribonucleotides described in the





**Figure 1. Severo Ochoa and his group in 1974 at the Roche Institute of Molecular Biology in Nutley, NJ.** From right to left: Jose-Miguel Hermoso, César Nombela, Nohelly Nombela, Severo Ochoa, Christa Milcarek, José Manuel Sierra, Witold Filipowicz, and Dallas George.

paper acted as inhibitors of translation and corresponded to RNAs of molecular weight  $\sim 6$  kDa, and hence they were  $\sim 20$  nt<sup>2</sup> in length! Stuart Heywood at the University of Connecticut in Storrs had reported similar RNAs in muscle cells even earlier (12). But all this work had remained inconclusive because cloning and sequencing were not that simple in those days.

With two such famous mentors as Ochoa and Shatkin, my time at the Roche Institute of Molecular Biology was very rewarding. Roche, as we called it, was then one of the leading institutes investigating gene expression and was teeming with interesting people. Through Severo Ochoa, I became acquainted with many prominent biochemists, especially at the very digestible “Enzyme Club” dinners at Rockefeller to which Ochoa regularly invited us. I also made many friends among my Spanish labmates (Fig. 1). Aaron Shatkin was just a couple of years older than I was, and his relationship to postdocs was much less formal than that of Ochoa. We very quickly became close friends and continued our collaboration over many years (Fig. 2).

### Back to Warsaw: RNA ligation part I

I arrived back in Poland in early 1976 and started my own laboratory at the Institute of Biochemistry and Biophysics in Warsaw. I had decided to continue the study of eukaryotic translation and the role of the cap in initiation. The start was stymied by the refusal of most of the *in vitro* extracts I used routinely in the USA to display any activity in Poland. The answer to this puzzle was the impurity of the ordinary salts purchased from local suppliers, so we recrystallized the salts and solved the problem. We relied largely on self-prepared



**Figure 2. Aaron Shatkin and his wife Joan, together with my daughter Magdalena, at the Jim Darnell 70th Anniversary Symposium in 2000 at the Rockefeller University in New York (courtesy of Hiro Furuichi).**

research tools. We purified RNase inhibitor from placentas obtained from the local obstetrics clinic and prepared reticulocyte lysates from hydrazine-injected rabbits. We carried out enzymatic decapping of mRNAs using nucleotide pyrophosphatase purified locally from potatoes (13); this enzyme was similar to the better-known tobacco acid pyrophosphatase (TAP).

Our early work focused primarily on the requirement for the m<sup>7</sup>G cap in the translation of different viral RNAs. Here we used plant RNA viruses because at that time working with animal viruses or cells was too expensive (13, 14). One issue that particularly interested us was the mechanism of eukaryotic ribosome recruitment to mRNA. It will become obvious later

<sup>2</sup> The abbreviations used are: nt, nucleotide(s); TMV, tobacco mosaic virus; pre-rRNA, precursor ribosomal RNA; pol II, RNA polymerase II; pol III, RNA polymerase III; RNP, ribonucleoprotein; snoRNA, small nucleolar RNA; snoRNP, small nucleolar RNP; m<sup>7</sup>G, 7-methylguanosine; miRNA, microRNA; miR-122, miRNA 122; IFN, interferon.



Figure 3. Arguing with Hans Gross (left). Basel 2012.

why I now describe the situation in detail. In 1978, Marilyn Kozak and Aaron Shatkin (15, 16) obtained the first evidence of a scanning model of eukaryotic initiation. According to this model, the 40S ribosomal subunit binds mRNA at the cap, travels along the 5'-UTR, and stops after encountering the first AUG; a 60S subunit joins, forming the 80S ribosome, and polypeptide synthesis gets started. The long ~70-nt 5'-UTR of the tobacco mosaic virus (TMV) RNA is devoid of G residues between the cap and the AUG. We and others demonstrated that a full-length TMV RNA or a capped AUG-terminated 70-nt-long fragment (prepared by digestion with RNase T1 that cuts RNA after G residues) associates under conditions of hindered elongation not with one but with two 80S ribosomes (Ref. 17 and references therein). Importantly, we found that the binding of both ribosomes was cap-dependent, which indicated that the second ribosome followed the first via 5'-UTR scanning (17). When the scanning 40S could not proceed to the already occupied AUG, an 80S complex assembled at an upstream AUG mimic, shown later to be AUU (18). I carried out some of these experiments during a three-month stay in the lab of Anne-Lise Haenni at the Jacques Monod Institute in Paris. The results not only offered support to the scanning model but also primed our interest in using the TMV RNase T1 fragment (referred to as  $\Omega$ ) as a convenient model to test the requirement for the free mRNA 5' end in translation initiation by eukaryotic ribosomes. Marilyn Kozak had already shown that synthetic polynucleotides do not initiate translation when converted to circular forms (19). We wanted to know whether a similar requirement for the free mRNA 5' end applied to natural mRNA fragments.

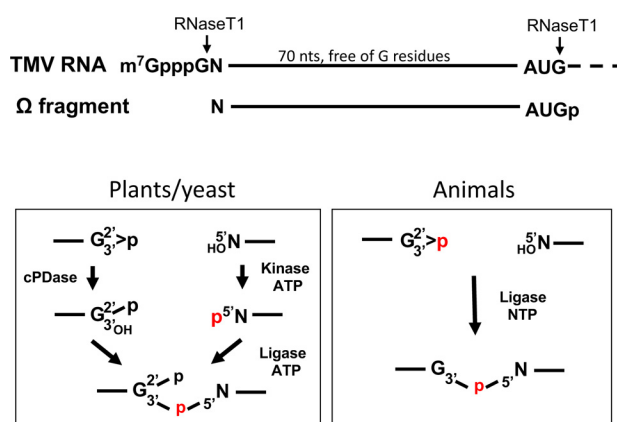
To address this and related questions, we initiated collaboration with Hans Gross at the Max-Planck-Institute (MPI) for Biochemistry in Martinsried (Fig. 3). Gross *et al.* (20) had just reported the sequence of the potato spindle tuber viroid (PSTV) RNA, the first example of a molecularly characterized circular RNA. In early 1979, Maria (Magda) Konarska, a recent graduate of the University of Warsaw, joined my laboratory as a Ph.D. student. After a few months, Magda moved to Martinsried to take advantage of Hans's experience with RNA circles and to test linear and circular  $\Omega$  fragments for ribosome-binding activities. As predicted, both types of fragments efficiently bound prokaryotic *E. coli* ribosomes, but  $\Omega$  circularization prevented association with wheat germ ribosomes, which was rein-

stated by linearization with RNase T1. These data confirmed that the free 5' end was essential for mRNA interaction with ribosomes, either capped or uncapped (we and others had shown previously that uncapped RNA can initiate translation *in vitro* quite efficiently at low  $K^+$  ion concentrations (14)).

We were happy with these results, but it was actually a side observation arising from the  $\Omega$  research that intrigued us most. While fiddling around with linear and circular RNAs, Magda and Horst Domdey (Hans's collaborator) noticed that not only T4 phage RNA ligase but also wheat germ extracts could (rather inefficiently) catalyze circularization of the linear  $\Omega$ . Unlike T4 RNA ligase, which joins the 5'-P and 3'-OH termini, the plant extract ligated RNAs bearing a 3'-terminal phosphate (prepared by RNase T1-mediated digestion of either the T4 ligase-generated circles or the linear molecules that were 3' end-labeled with radioactive [5'- $^{32}$ P]pCp). To our great surprise, digestion with RNase T1 did not linearize the covalently joined circles generated in plant extracts, despite the presence of a G residue. What on earth was going on?

It took a year to figure it out, with work going on in both Warsaw and Würzburg (to where Hans Gross had moved his lab). It wasn't easy to carry out experiments in the Warsaw of 1980/1981. The Solidarność (Solidarity) movement led by Lech Walesa had been at the peak of its activity, opening the first cracks in the communist system. We divided our time among attending pro-Solidarity demonstrations, searching for food (often the only thing available in the shops in unlimited quantity was vinegar), and working in the laboratory. Hans's generosity kept our science alive. He arranged for the shipment of radioactive compounds to Warsaw and invited us repeatedly to spend time in his lab in Würzburg. However, by coincidence, our shortage of reagents turned out to be a blessing in disguise. The yield of ligation in the wheat extracts differed considerably from experiment to experiment, and we eventually realized that the enforced limitation in the amount of RNase T1 used for substrate preparation actually tended to increase circle yield. In fact, it turned out that ligation depended upon the 2',3'-cyclic phosphate ends that accumulated as intermediates of the digestion by RNase T1, a cyclizing endonuclease. This was an important hint that helped us to understand the reaction mechanism. Detailed analysis of the ligation linkage finally showed that it corresponded to the 2'-phosphomonoester, 3',5'-phosphodiester bond. The 2'-phosphate, itself an explanation of the unusual resistance of the circular product to RNase T1, originated from the opening of the 2',3'-cyclic phosphate by the 2',3'-cyclic 3'-phosphodiesterase, while the bridging 3',5'-diester phosphate resulted from ATP-dependent phosphorylation of the substrate 5' end by the kinase activity (Fig. 4). We were all very proud of this work. The two 1981/1982 papers by Konarska *et al.* (21, 22) provided the first mechanistic insights into eukaryotic RNA ligation, while leaving a lot of room for interesting speculation. At that time, nothing was known about the enzymology of mRNA splicing or excision of the self-splicing intron from the precursor ribosomal RNA (pre-rRNA) in *Tetrahymena*. It was very clear to us in Warsaw that these projects could not have been realized without the collaboration and generosity of Hans Gross.





**Figure 4. Two distinct eukaryotic RNA ligation pathways identified initially in plant and animal (HeLa cells, *Xenopus* oocytes) extracts, using the  $\Omega$  fragment circularization assays (21, 22, 25).** Both pathways are used, in a kingdom-specific way, for exon ligation during tRNA splicing (Refs. 24, 26, and 30; reviewed in Ref. 57). The phosphate forming the 3',5'-phosphodiester linkage is marked in red. A scheme visualizing the TMV RNA 5'-terminal region and excision of  $\Omega$  is at the top. The *in vitro* 2'-O-methylation of the penultimate G residue prevents cleavage by RNase T1 and allows isolation of the capped  $\Omega$  used in the translational studies described in the text.

## Sabbatical year at the Roche Institute of Molecular Biology: RNA ligation part II

In October 1981, I left Warsaw for a sabbatical year in Aaron Shatkin's laboratory in New Jersey. Ola and our children, two-year-old Magdalena and eight-year-old Peter, joined me two weeks later. Unfortunately, political events in Poland caught up with us almost immediately. Martial law aimed at crushing the Solidarity movement was declared in Poland at midnight on December 13. By a strange coincidence, we learned of this during a dinner at which Lech Walesa's cousin, Walter Brolewicz, was also a guest. We were of course completely shocked and totally perplexed by this development. Back at work, I discussed the situation with the founding director of the Roche Institute, Sidney Udenfriend. He put me in touch with Frank Press, the President of the U. S. National Academy of Sciences, to whom I expressed my anger and concern, asking American scientists to condemn the act.

Despite the political situation, returning to Aaron's lab was like a welcome homecoming, with the lab again full of interesting people. The choice of research topic was left to me, and I decided to investigate the mechanism of tRNA splicing in HeLa cells, particularly the ligation of the two tRNA halves that resulted from intron excision, and in particular whether exon ligation followed the 2'-phosphomonoester, 3',5'-phosphodiester-forming pathway as in plants. There was already a hint in the literature from the lab of Eddy de Robertis working with *Xenopus* oocytes that this might not be the case (23). Indeed, we quickly found that HeLa cell extracts contain activity distinct from that operating in plants (24, 25). In marked contrast to the situation in plants, where the bridging 3',5'-phosphate came from the  $\gamma$ -phosphate of ATP, the "animal" pathway joined RNA ends via an orthodox 3',5'-phosphodiester, with a linkage phosphate originating from the 2',3'-cyclic end. The requirement for 2',3'-cyclic phosphate ends was hence common to both pathways (Fig. 4). Intriguingly, we discovered that cleavage by cyclizing endonucleases is not the only way to produce

RNAs with 2',3'-cyclic ends. They can also form in an ATP-dependent reaction by the RNA 3'-terminal phosphate cyclase, a novel enzyme that we identified in HeLa cells and *Xenopus* oocytes (25). In view of the observed need for cyclic ends in eukaryotic RNA ligation, which represented the first example of the "anabolic" function of the 2',3'-cyclic phosphate, identification of the RNA 3'-terminal phosphate cyclase was an important finding. We continued to investigate this enzyme for nearly two decades.

Our data on tRNA splicing in human cells were published in early 1983 (24). In the same issue of *Cell*, John Abelson's group reported in two very elegant papers that tRNA splicing in the yeast *Saccharomyces cerevisiae* (26, 27) was via the 2'-phosphomonoester, 3',5'-phosphodiester "plant" pathway. In continued collaboration with us, Hans Gross's lab obtained evidence that this pathway may also be involved in tRNA splicing in *Chlamydomonas reinhardtii* and in potato spindle tuber viroid circularization (28, 29). On the other hand, Phil Sharp's laboratory independently demonstrated ligation of tRNA halves via a usual 3',5'-phosphodiester in mammalian cells, corroborating our HeLa observations (30). Clearly, at least two different systems of enzymatic ligation of RNA ends operate in eukaryotes, now generally referred to as the "animal" and the "plant/yeast" (or, regrettably, just "yeast") pathways (Fig. 4).

A 1983 review written by Tom Cech (31) nicely summarized the current status of enzymes involved and the linkages formed in different splicing reactions. Evidently, there was a lot of interest in this area and, combined with my "exotic" East European origin, this promoted me to a temporary celebrity in the field. I was invited to give talks at many universities and symposia. Perhaps the most flattering was an invitation to the Industrial Affiliates Symposium at the Department of Biochemistry, Stanford University, where I was one of the three main speakers together with Phil Sharp and Roger Kornberg. The honor of the invitation, but also the stress, was amplified when they seated me at dinner between Arthur Kornberg and Paul Berg!

In late 1982, I gave a talk at the Rockefeller University in New York, where I was asked by Ed Reich, one of the professors, whether I would be interested in setting up a lab at the Friedrich Miescher Institute (FMI) in Basel, Switzerland. Reich was about to take over the directorship there. This was a very attractive offer, given the enduring martial law and the difficulties of doing research in Poland. In addition, the FMI had an excellent scientific reputation as a basic research foundation with generous support from Ciba-Geigy AG, the Swiss pharmaceutical company now known as Novartis. As I did not plan to leave Poland and my Warsaw lab permanently, I investigated the possibility of a part-time or temporary appointment in Basel, arguing that a commute between the two countries would have advantages for Polish science. Indeed, the Polish Academy of Sciences agreed and granted me, in writing, a leave of absence.

## Getting in and out of trouble: the Moscow connection

With this official permission in my hand and ignoring the warnings of many friends that martial law was still in force, I flew from New York back to Warsaw in March 1983, to take part in the Ph.D. thesis defense of Magda Konarska. By that time, I had already shipped reagents to Basel and hired two

people for the new laboratory. My family was due to move directly from New York into a rented apartment in Basel. The appointment was like a fairy tale, but reality and its complications soon caught up with us. Once in Warsaw, I was repeatedly denied a passport to travel to Basel, in fact to any symposium or seminar abroad. I suppose I should have expected the problem as I was twice prevented from traveling abroad for different periods during my student and Ph.D. time. The situation looked hopeless, and hence Ola and the children arrived back in Warsaw in June. Over the next year, many people in Poland and abroad tried to help, but to no avail, and it became more worrying when I was even refused permission to travel to Moscow.

Although this is hard to believe, the Moscow incident turned out to be the key to resolving my year-and-a-half detention in Poland. Together with Josif Atabekov, I was organizing a virology symposium in Moscow as part of the June 1984 Federation of European Biochemical Societies (FEBS) Congress. The main organizer of the FEBS Meeting was Yuri Ovchinnikov, a Vice President of the Soviet Academy of Sciences and member of the Central Committee of the Soviet Union Communist Party. As I still hadn't received my passport by the day of the virology symposium, I cabled him that the Polish authorities were denying me permission to participate in his congress. The reaction was instantaneous! The next morning, I received a call from the President of the Polish Academy of Sciences that my passport to travel to Moscow was ready. However, as the symposium had taken place the day before, I asked whether I could use the passport to travel to a meeting in The Netherlands starting a few days later, at which I was an invited speaker. Surprisingly, they agreed, and I collected my passport from the Academy office. I noticed, however, that the passport had been restamped from multiple to single-exit status, and it was now obvious that the trip to The Netherlands would not be the end of my battle against travel restrictions. By now, we were quite paranoid: Ola and I met in the middle of a nearby sports field to discuss clandestinely how to proceed.

At the invitation of our Spanish friends, Ola and the children had recently been granted a permit to travel to Spain for a vacation. Needless to say, my application for this trip had been rejected. Because the passport offices handling private and official trips were separate and possibly not in communication with each other, we decided to gamble and try to depart Poland on the same day, with me flying to Amsterdam and Ola taking the kids to Madrid. The only obstacle to this plan was the urgent need for Spanish visas for my family. Alas, the Spanish Embassy in Warsaw was besieged by people who had been waiting for a visa for weeks. This is when my former association with the Nobel Prize recipient Severo Ochoa, a prominent figure in Spain, and my acting experience from my student days came in very handy. Playing perhaps the most useful role in my acting career, I dressed up in black and, pretending to be a diplomat with an urgent appointment with the embassy scientific attaché, I stormed through the waiting crowd and past the policeman on guard. After I explained my case and mentioned my former connection with Severo Ochoa, the attaché arranged for the visas on a spot. Thus, without telling anyone, not even our parents, we checked in separately a few days later and boarded our planes. At the meeting in Renesse, a Dutch resort on the

North Sea, I offered a glass of beer to everybody in the audience of my after-dinner talk to celebrate the success of our conspiracy. I then flew to Madrid, and a month later, we received our Swiss papers. The restrictions, this time for travel to Poland, remained in force for the next seven years. I felt very bad about abandoning my students in Warsaw, but my worries dissipated somewhat when Magda Konarska departed for a postdoc at MIT with Phil Sharp, the start of her illustrious career, and when Kazio (Tyc) Tycowski, another brilliant Warsaw student, left shortly afterward to join Joan Steitz's lab at Yale.

### Happy landing in the Friedrich Miescher Institute in Basel, Switzerland

Forgive me this rather lengthy account of personal ups and downs in my early career. It makes perhaps more interesting reading than an everyday description of *normal* science (which will follow). More important, however, the enforced improvisation in the lab, the solidarity of my friends and collaborators, and the decision to move to Basel all had profound effects on my science for the next 30 years. Luckily, the position as a research group leader and the attendant lab space were still waiting for me at the FMI in Basel. Family-wise, events of the passing year were most difficult for our children. Moving between four countries, as well as changing languages and schools or kindergartens regularly, made them a bit confused. Early after our arrival to Basel, we remember our son waking up one morning and asking: "Where are we now? In Finland?" Wonderfully, Switzerland turned out to be very accommodating. After a few months of tutoring, our children, unlike their parents, were fluent in both German and the Swiss dialect. A bit later, Peter was a proud winner of the Ciba-Geigy Tennis Cup. As for my daughter Magdalena, I will talk about some of her activities further below.

As the tRNA splicing field had moved on during my "incomunicado" time, and I discovered how very pleasant the community of plant cell and molecular biologists working in the FMI was, I decided to investigate problems related to mRNA splicing and RNA metabolic enzymes in plants. Despite my previous experience with plant viruses and enzymology, the world of plant physiology was totally alien to me, but I was fascinated by the richness of plant RNA-related phenomena, as exemplified by viroid RNAs and the RNA viruses with bizarre gene expression properties. In addition, the genomes of plant mitochondria are much larger than those of vertebrates, and the processing of their transcripts is very complex. We were puzzled by the inability of plant cells to process mammalian pre-mRNAs despite the fact that plant and animal splice site consensus sequences are quite similar (32). Greg Goodall, an excellent postdoc from Australia, approached the problem by assembling a large collection of synthetic mosaic genes and testing the requirements for their splicing in plant protoplasts. It turned out that plant introns must be AU-rich throughout their length to be effectively excised. Particularly in dicotyledonous plants, marked differences in AU/GC distribution along gene transcripts allowed the reasonable prediction of intron positions (33, 34). We found that introns in some other organisms are also much more AU-rich than flanking exons (reviewed in Ref. 35). We characterized several families of

nuclear RNA-binding proteins that were probably responsible for the recognition of AU- or U-rich nucleotide stretches in plant introns and continued to study their activities until the late 1990s (Ref. 36 and references therein).

In parallel, we investigated U-snRNA genes and their products to better understand the intricacies of plant splicing, focusing mainly on the model plant *Arabidopsis thaliana*. Actually, the highlights of this work related not so much to the role of U-snRNAs in splicing but to the transcription of their genes. Much work had been done in the late 1980s on the promoters of snRNA genes transcribed by either RNA polymerase II (pol II) or pol III (37). Interestingly, the transcription of pol III-specific snRNA genes in both plants and animals was not dependent on intragenic elements, in marked contrast to tRNA and 5S rRNA genes. Equally surprising were the findings that “extragenic” pol II and pol III promoters were similar, which argued for a common ancestry of the pol II and pol III transcription systems (37, 38). Franz Waibel and Tamas Kiss in the lab discovered that the similarity of pol II and pol III promoters is most extreme in plants. Both promoter classes contained two identical elements, the upstream sequence element (USE) and a –30 TATA box, with the spacing between them being either four (pol II) or three (pol III) DNA helical turns. Franz demonstrated that it is possible to change the polymerase specificity of the promoter by manipulating the spacing between the two elements (39). Even more significant, Tamas found that such a change had occurred during evolution. In contrast to all known organisms in which U3 small nucleolar (sno) RNA contains the 2,2,7-trimethylguanosine (m<sub>3</sub>G) cap and is synthesized by pol II, in plants, it is synthesized by pol III and bears an  $\gamma$ -monomethyl phosphate cap (40, 41). Tamas was able to convert the plant U3 gene into a functional pol II-transcribed gene by extending the promoter element spacing by one helical turn (40). This was the first example of a gene that is transcribed by different polymerases in different organisms.

### Chasing functions of the RNA 3'-phosphate cyclase and other RNA metabolic enzymes

Although it was not a major theme in the lab, we also continued research on RNA metabolic enzymes related to our previous work on RNA ligases and tRNA splicing. We were particularly interested in the RNA 3'-terminal phosphate cyclase and enzymes opening 2',3'-cyclic phosphates to 2'-phosphates, similar to those participating in the ligation step of the plant/yeast pathway. With regard to the latter activities, we purified and characterized biochemically and structurally 2',3'-cyclic nucleotide 3'-phosphodiesterases from plants and yeast (42–46). These enzymes hydrolyze nucleoside 2',3'-cyclic phosphates and adenosine diphosphate-ribose 1'',2''-cyclic phosphate (ADPr>p), a side product of tRNA splicing. We identified two conserved His-containing catalytic motifs in the enzymes (45, 46), and they became the founding members of a large family of 2H phosphodiesterases (47). We concentrated more on the cyclase, with the ultimate aim of understanding its biological function. The cyclase work took many interesting turns, and we were side-tracked on many occasions. We established the mechanism of the cyclization, which involves covalent adenylation of the enzyme, followed by transfer of the

adenylate to the substrate 3'-phosphate, yielding the RNA-N<sup>3'</sup>pp<sup>5'</sup>A intermediate, and final conversion to RNA-N>p (Ref. 48; reviewed in Ref. 49). Purification of the human cyclase (50) enabled us to clone its cDNA (51), and this, in turn, revealed that the genes encoding cyclases are highly conserved in all three kingdoms. Pascal Genschik, a very gifted French postdoc, showed cyclases to be a novel protein family, although later crystallization of the enzyme revealed that subdomains of cyclase share an ancient fold with other proteins (52). We were fortunate to collaborate at that time with a very accomplished crystallographer (and also a good friend), Alex Wlodawer of National Institutes of Health (NIH), whose lab determined the structure of both the 2',3'-cyclic nucleotide 3'-phosphodiesterase and the RNA 3'-terminal phosphate cyclase (45, 52).

Identification of the cyclase gene in *E. coli* presented an opportunity to study its function by genetic means. Regrettably, the gene (which we named *rtcA*) was not essential for growth (53). *rtcA* formed part of a new operon containing two additional ORFs. The ORF immediately upstream of *rtcA* (named *rtcB*) encoded a protein that is also highly conserved evolutionarily. The third ORF (*rtcR*), which is transcribed in the opposite direction, encoded a transcriptional activator controlling the expression of *rtcA* and *rtcB* in a  $\sigma^{54}$ -dependent manner (53). The dispensability of the *rtcAB* operon was not that surprising given that almost all known  $\sigma^{54}$ -controlled operons are not essential for growth under standard conditions and their induction is part of different adaptive responses.

I enjoyed this transitory return to bacterial genetics very much, bringing as it did fond memories of my Ph.D. studies. However, the research on the *rtcAB* operon was also associated with probably the largest blunder of my career! When defining RtcA as a cyclase, it occurred to us that its co-regulated neighbor RtcB, despite lacking sequence signatures of any known enzymes, might be a cyclic phosphate-dependent RNA ligase. We overexpressed and purified RtcB and tested its activity but found no RNA ligation (53). Obviously, we did not try hard enough because about 15 years later the groups of Javier Martinez, Dieter Söll, and Stewart Shuman demonstrated independently that RtcB has RNA ligase activity (Refs. 54–56; reviewed in Ref. 57) and that its human counterpart is a catalytic subunit of the tRNA-splicing ligase originally identified by us in the early 1980s (54). This “epilogue” was extended very recently by the publication of data on a possible role of the *rtcAB* operon in *E. coli* in RNA repair and the maintenance of ribosome homeostasis (58, 59) and, in one specific case, in the re-ligation by RtcB of 16S rRNA cleaved by a stress-induced endoribonuclease (59). Equally inspiring are recent findings implicating cyclase/RtcA in neuronal functions in metazoa, in association with either RNA transport in mouse neurites (60) or the regulation of axon regeneration in *Drosophila* and rodent neurons (61). Clearly, we abandoned research on cyclase 20 years too soon!

I will return for a moment to the time we were chasing the cyclase function and describe how this chase diverted us to the field of ribosome biogenesis. When inspecting different genomes in the mid-1990s for sequences with similarity to the human and *E. coli* RtcA, we noticed that we could divide encoded proteins into two highly related subfamilies (51). Pro-



teins of one class, encompassing human and *E. coli* cyclases, were conserved in all three kingdoms, but proteins of the second class were conserved only in eukaryotes. We referred to the latter class as the RNA-3'-terminal phosphate-cyclase-like (Rcl) proteins. We were very excited to find that the Rcl1p protein was essential for the growth of *S. cerevisiae* (62) but soon learned, unfortunately, that the yeast Rcl1p had no cyclase activity. An answer to the riddle came from inspection of the sequences of the Rcl1 family proteins: these lacked a conserved histidine equivalent to the *E. coli* cyclase His-309, which is a residue that undergoes covalent adenylation in the course of the cyclization reaction (53, 63).

Although disappointed, we were still curious as to why the *RCL1* gene is required for the growth of *S. cerevisiae*. Eric Billy, Tomek Wegierski, and Fahd Nasr in the lab found that depletion of Rcl1p impairs pre-rRNA processing at sites A0, A1, and A2. This leads to a strong decrease in mature 18S rRNA and 40S ribosomal subunit levels (62). A two-hybrid screen identified a further novel protein (Bms1p) involved in pre-rRNA processing as an Rcl1p partner (64). Depletion of Bms1p, the first GTPase found to function in pre-rRNA processing, resulted in a phenotype identical to that seen upon depletion of Rcl1p (64–66). Underlining the important role of both proteins, we found association of the Rcl1p/Bms1p complex with U3 small nucleolar ribonucleoprotein (snoRNP), a particle orchestrating early pre-rRNA processing events (64).

The demonstration that the Rcl1p/Bms1p complex is active in ribosome maturation nicely complemented other ongoing projects in the lab focused on the biochemistry of U-snoRNPs involved in ribosome biogenesis. Curiously, Stuart Maxwell's lab in the early 1990s demonstrated that some U-snoRNAs (specifically U14) were encoded in pre-mRNA introns (67). I was fortunate to have Tamas Kiss as a postdoc in the laboratory. Tamas was a real maverick of RNA analysis. He identified several new intron-encoded U-snoRNAs with rather unusual properties (e.g. they were not associated with fibrillarin, a component of most U-snoRNPs known at the time) (68, 69) and investigated the mechanism of their processing from pre-mRNA introns. This led to a model according to which 5'→3' and 3'→5' exonucleases bring about the maturation of examined U-snoRNAs from excised and debranched introns (70). It was shown later that some intronic U-snoRNAs can use alternative, splicing-independent processing pathways (Ref. 71 and references therein). Tamas Kiss also studied RNase MRP (mitochondrial RNA processing), demonstrating that it acts in both plants and mammals in ribosome biogenesis in the nucleolus rather than in the processing of primers for DNA replication in mitochondria (72, 73), as originally proposed by others. After he had started his own lab, Tamas discovered that most U-snoRNAs, grouped into either C/D or H/ACA classes, act as guides of the rRNA modifications ribose 2'-O-methylation and pseudo-uridylation, respectively (74). Later, we continued to investigate in my lab the assembly of mammalian H/ACA snoRNPs, including the formation of the telomerase RNP (75, 76), whose RNA component was demonstrated earlier to contain the H/ACA domain at the 3' end (77).

It is quite evident above that the topics covered in the lab were quite eclectic. Although always working in the domain of

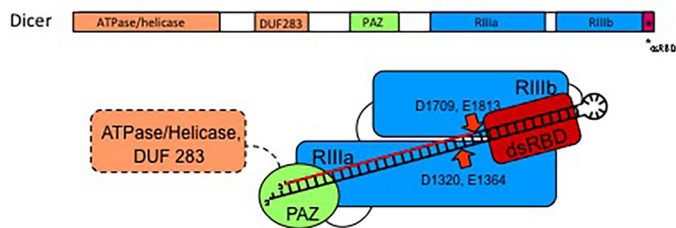
RNA, we experimented with many model organisms and studied transcription, translation, RNA splicing, ribosome biogenesis, sn(o)RNPs, and various RNA metabolic enzymes. But there was always some logic behind the moves from one subject to another. For example, our interest in RNA ligases was a consequence, quite accidentally, of the work on translation involving the use of  $\Omega$  fragments. Similarly, our interest in the formation and hydrolysis of 2',3'-cyclic phosphates was a result of earlier findings on the role of cyclic ends in RNA ligation. Excursions into the  $\sigma^{54}$ -regulated operon in *E. coli* or ribosome biogenesis in yeast were side products of our hunt for cyclase function. I always thought it good and healthy to follow potentially interesting leads arising from current experiments and to change the topic from time to time, to satisfy our evolving curiosity and to learn new things. Sticking to a problem for decades to understand its every detail was certainly not my favored philosophy. I believe that group members also enjoyed the rather broad coverage of RNA biology, particularly when working in an institute like the FMI that provided an excellent infrastructure and nurtured a great intellectual environment with lots of opportunities for collaboration.

### Looking for new challenges: RNAi and microRNAs

In the late 1990s, I decided we needed a more radical change in our research, for three reasons. First, the growing complexity of ribosome biogenesis, with hundreds of protein- and snoRNP-transacting factors, made me skeptical that we would soon, if ever, understand the molecular details of the process. Second, the absence of suitable *in vitro* systems made studies of plant transcription and mRNA splicing increasingly difficult. Third, we were rather tired of chasing the function of cyclases and cyclic phosphodiesterases. When, in 1998, Fire and Mello reported the discovery of RNA interference (RNAi) in worms (78), I was not immediately convinced about the generality of this phenomenon. However, I changed my mind entirely in 1999, when David Baulcombe's lab presented the first evidence of the formation of small interfering (si) RNAs in virus-infected plants (79). The decision in the group to throw most of our resources into investigating RNAi and, somewhat later, miRNAs in mammalian cells heralded probably the most rewarding episode in my career. As we have summarized this work in a number of recent reviews (80–83), I will discuss only very few aspects here.

After the discovery that siRNAs mediate RNAi response, many labs started to look for the enzymes slicing double-stranded (ds) RNA into siRNAs. With our experience in RNA-processing factors, we thought we were well-placed to enter the race. In fact, we lost that contest and published our first Dicer paper (84) several months after the ground-breaking work by Hannon's group (85), but we were in a good position to study the Dicer mechanism. Haidi Zhang, a Ph.D. student who I recruited during a visit to China, quickly demonstrated that human Dicer, an RNase III family multi-domain enzyme, requires a free dsRNA end to initiate cleavage and that it generates siRNAs in a stepwise manner, starting at the dsRNA end (86). However, it was not clear how Dicer cuts dsRNA and "measures" the ~20-bp intervals. There were several models on offer, but none seemed satisfactory. Haidi again took the lead





**Figure 5. Domain organization of the human Dicer and a model of dsRNA processing by the enzyme.** Dicer functions as an intra-molecular pseudodimer of two catalytic RNase III domains (RIIla and RIIlb). Arrows point to two independent catalytic sites in the enzyme processing center. Reprinted from Zhang, H., Kolb, F. A., Jaskiewicz, L., Westhof, E., and Filipowicz, W. (2004) Single processing center models for human Dicer and bacterial RNase III. *Cell* **118**, 57–68 (87), with permission from Elsevier.

and tested, together with Fabrice Kolb and Lukasz Jaskiewicz, two dozen purified mutants of both Dicer and its bacterial prototype, the *E. coli* RNase III. The results indicated that both enzymes have a single processing center containing two RNA cleavage sites and generating products with 2-nt 3' overhangs. These data and subsequent molecular modeling in collaboration with Eric Westhof indicated that Dicer functions through intramolecular dimerization of its two catalytic RNase III domains, with the PAZ domain likely involved in the recognition of the terminal 3' overhang in dsRNA and pre-miRNAs (87) (Fig. 5). It was gratifying that crystallographic studies of Dicer later fully confirmed the correctness of our model (88). In addition to the mechanistic aspects, we also carried out several studies on the function of Dicer in mammalian cells (reviewed in Ref. 82).

The most intensive effort of the past 15 years or so in our lab has been on the mechanism of miRNA repression. This took off with the arrival in 2003 of Ramesh Pillai, a very imaginative and courageous postdoc who had just graduated from the University of Bern. Using both Argonaute protein tethering and miRNA reporter approaches, Ramesh obtained evidence that miRNAs not only induce mRNA deadenylation and decay (as shown later) but also inhibit protein synthesis at the level of translation (89, 90). The work of several other excellent postdocs and students, including Suvendra Bhattacharyya, Petr Svoboda, Marina Chekulaeva, Julien Béthune, and Hansruedi Mathys, provided additional support for the translational effects of miRNAs, also with the demonstration that translational repression kinetically precedes mRNA degradation (91, 92). In addition, Suvendra Bhattacharyya demonstrated that miRNA repression can be reversed under stress conditions and that RNA-binding proteins interacting with the 3'-UTR can regulate miRNA activity (93). We were fortunate to have excellent collaborators in our mechanistic studies, in particular Nahum Sonenberg and Elena Conti. Our contacts and friendship with Nahum, who was also an alumnus of Shatkin's lab, dated back to the late 1970s (Fig. 6). We were proud to have attracted this renowned expert in protein synthesis to the miRNA repression field, when the connection with translation had become obvious. Nahum's lab, with modest input from us, quickly developed *in vitro* systems recapitulating miRNA repression. These were invaluable for gaining biochemical insights into the miRNA mechanism, pointing to the effect on the initiation step of translation (92, 94). Together with Nahum,



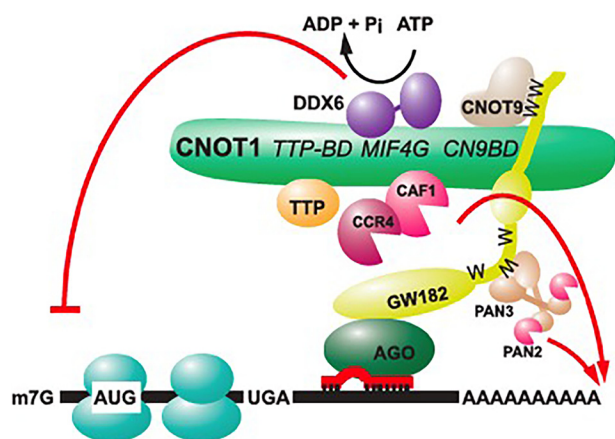
**Figure 6. Praying with Nahum Sonenberg at the 2007 meeting in Kyoto** (courtesy of Marv Wickens).

we published several reviews that discuss the miRNA mechanism (80, 83), including the most recent one on “the long unfinished march toward understanding miRNA-mediated repression,” which summarizes current models of miRNA repression (83).

Together with Elena Conti, a wizard crystallographer, we embarked on a project aimed at a structural understanding of the repression. From the work of several labs including those of Nahum and Elisa Izaurralde, as well as our own (reviewed in Refs. 83 and 95), we knew a lot about factors that work downstream of Argonautes, such as GW182 proteins or CCR4-NOT and PAN2/PAN3 deadenylase complexes. Moreover, Marina Chekulaeva and Hansruedi Mathys discovered that tryptophan-containing motifs (W-motifs) present in non-structured regions of GW182 are important for the recruitment of CCR4-NOT and PAN2/PAN3 complexes (96, 97). The structural studies with Elena not only revealed how the W-motifs recruit CCR4-NOT but also demonstrated that CCR4-NOT in turn recruits and activates the ATPase DDX6, and that DDX6 activation is important for miRNA-mediated translational effects (98) (Fig. 7). Further structural analyses implicated the DDX6 interactor, eIF4E-T, as a downstream effector in the repression. Among a number of DDX6-associating repressors, eIF4E-T was found to be the only one that interacted with DDX6 when the latter was also complexed with the CNOT1 subunit of CCR4-NOT (99). Despite extensive progress, there is still much controversy about the exact mechanism of miRNA repression and the relative contributions of translational and mRNA decay effects (83, 95).

### Back to the roots: some medical connections

Other collaborative studies led us to projects of some medical relevance. One such project particularly dear to my heart involved my daughter Magdalena, at the time an M.D./Ph.D. student in Markus Heim's lab at the University of Basel. Originally, her work aimed at explaining why only about half of hepatitis C virus-infected hepatitis C patients respond to interferon- $\alpha$  (IFN $\alpha$ ) therapy. Using liver biopsies of patients collected before and after IFN $\alpha$  administration, Magdalena found that non-responders expressed IFN-stimulated genes at elevated levels already *before* therapy. This not only explained the lack of



**Figure 7. Central CNOT1 region as a hub of protein-protein interactions important for miRNA repression and other types of post-transcriptional regulation.** The miRNA-Argonaute (AGO) complex interacts with GW182, which in turn recruits, via its W motifs (W), the PAN2/PAN3 and CCR4-NOT deadenylase complexes. The MIF4G region of CNOT1 recruits the ATPase DDX6, which contributes to translational repression. Reprinted from Mathys, H., Basquin, J., Ozgur, S., Czarnocki-Cieciura, M., Bonneau, F., Aartse, A., Dziembowski, A., Nowotny, M., Conti, E., and Filipowicz, W. (2014) Structural and biochemical insights into the role of the CCR4-NOT complex and DDX6 ATPase in microRNA-mediated repression. *Mol. Cell* **54**, 751–765 (98), with permission from Elsevier. For more details, see Refs. 83 and 95.

response to exogenous IFN $\alpha$  but also provided an opportunity to predict the success of the therapy by profiling the expression of selected IFN-stimulated genes (100). These were important findings then, but the IFN $\alpha$ -based medication with its side effects has since been replaced by highly specific antiviral therapies. We also used the biopsy material to analyze the status and response to IFN $\alpha$  of miRNAs in diseased liver. Rather surprisingly, the levels of miR-122, which had been implicated in hepatitis C virus replication, did not correlate positively to the viral load, and the patients without a response to IFN $\alpha$  therapy showed markedly lower miR-122. We also found that administration of IFN $\alpha$  had no significant effect on the levels of miR-122 or other miRNAs (101), arguing against a proposal based on *in vitro* experiments that IFN-induced miRNAs mediate therapeutic response to IFN $\alpha$  (102). I strongly recommend collaborating with your daughters: it is great fun (Fig. 8)!

In another collaboration, I have very much enjoyed working at the FMI with Botond Roska, an excellent retinal physiologist and molecular biologist. We first aimed at identifying light-regulated miRNAs in mouse retina and indeed found some, particularly among those expressed in photoreceptors (103). But once again it was a side observation that turned out to be more exciting. Jacek Krol, a very gifted postdoc in the lab, found in collaboration with others that miRNAs in mouse retinal neurons turn over much faster than in other cell types and that the same applies to primary hippocampal and cortical neurons of the mouse brain. At that time, miRNAs were considered to be very stable molecules, but our observations indicated that this is not always the case. Moreover, miRNA decay seemed to be regulated by neuronal activity (103). This work led to an interest in other aspects of miRNA metabolism, and Jacek found that the biogenesis of the sensory neuron-specific miR-182/96/183 cluster is the subject of very sophisticated developmental regulation; interfering with it results in a phenotype resembling a known retinal disease (104). In continued collaboration with

Botond, we investigated the role of miRNAs in the function of adult retina. The results were quite stunning! The cone-specific depletion of miRNAs, specifically those of the miR-182/96/183 cluster mentioned above, prevented formation of photoreceptor outer segments, *i.e.* the stacks of opsin-containing membranes essential for vision (105). Having had many problems myself with my own retinas, I was excited to learn the intricacies of retinal physiology and pathology.

### Enjoying the beauty of sunset

Despite being compelled, for age-related reasons, to gradually dismantle my laboratory, the last few years of my scientific activity have been full of very memorable moments. During the 2011 RNA Society Meeting in Kyoto, I was presented with the Life Achievement Award of the society, and this is no doubt the distinction I most value. In May 2012, my *official* retirement from the FMI was marked with a small symposium that gathered together a dozen of my professional colleagues and friends as speakers, as well as most of my former and current students, postdocs, and research assistants. This was a very moving experience, although unfortunately saddened by the last-minute cancellation by Aaron Shatkin. In the fall of 2011, with Aaron's health already deteriorating, most of the alumni of his lab met at the Rockefeller University in New York at a symposium organized by Hiro Furuichi and Jim Darnell. Aaron passed away in June 2012, and Nahum Sonenberg and I wrote a retrospective on his life for *Science* (106).

My farewell symposium at the FMI was also an appropriate moment to think about the past 30 years spent at the Institute. It was one of my strokes of luck that brought me to the place, with its remarkable staff and a multitude of superb students and postdocs selected from all over the world. I also admired and enjoyed the dynamism and the scientific diversity of the institute. I started as part of the plant community but then switched to the epigenetics and RNA biology program, and also collaborated with neurobiologists and stem cell researchers. With great satisfaction, I experienced how the FMI is continuously evolving — through the hiring of new staff and the building of supportive infrastructure — and how, with a cutting-edge attitude, it meets new challenges in biomedical sciences. Given the opportunity, I would no doubt immediately apply to work at the FMI again, as a Ph.D. student, postdoc, or even a group leader.

Another event that brought back memories of the “old days” in science was the celebration around the 80th birthday of Hans Gross in 2016 in Würzburg, at which I had the pleasure to speak. With the increase in my “free” time, I was also able to enjoy three recent sabbaticals in stimulating locations for RNA research: at the Max Perutz Laboratories in Vienna (with Renée Schroeder), at the Institute of Molecular and Cellular Biology of CNRS in Strasbourg (with Eric Westhof), and at the MRC Laboratory of Molecular Biology (LMB) in Cambridge (with Venki Ramakrishnan and Lori Passmore). Combined with a three-month fellowship at Trinity College, the Cambridge sabbatical was a particularly unique experience, also bringing back memories of the ground-breaking discoveries made at the LMB. Most important, such activities and various advisory functions continue to keep me almost as busy as before.





**Figure 8.** My most important life and work companions: my son Peter, my wife Ola Wodnar-Filipowicz, and my daughter Magdalena Filipowicz Sinnreich.

My 50 years of research on RNA have allowed me to witness many exciting developments in the field. Following the discoveries of mosaic genes and splicing, RNA editing, and catalytic RNA, it seemed unlikely that RNA could surprise us with more secrets of comparable caliber. We were wrong! What ensued was the unearthing of RNAi and the diverse classes of short and long regulatory non-coding RNAs, the RNA-based bacterial immunity CRISPR-Cas systems, and a multitude of new mRNA nucleotide modifications. I was also totally wrong in suspecting that we will never understand the molecular details of spliceosome dynamics or ribosome biogenesis. High resolution cryo-electron microscopy has changed it all! Doing RNA research in the last few decades has been very rewarding and full of fun. From my perspective, it was like being paid to pursue a hobby. The icing on the cake was the opportunity to interact with so many extraordinary people, whether professional colleagues and friends (Fig. 9) or members of my group. I have mentioned a few names from the lab when describing specific projects, but the list is of course much longer: my students, postdocs, and research assistants were responsible for most of the ideas and the experiments to test them. I am also proud of having run a rather open laboratory, collaborating (and publishing!) with more than 40 other groups from all over the world. With all my lab members and so many outside contributors, claiming a role for myself in this whole enterprise becomes somewhat questionable. Was I really needed? Probably not as much as I imagine.

Reflections articles often conclude with advice to young scientists who are approaching an independent career. I have already discussed my favored philosophy when choosing (and changing!) research topics. Certainly, the conditions of doing research in terms of funding, getting published, the amount of data, and the technologies, including bioinformatics, have changed a lot during the past decades. For example, I was exposed to the different “omics” only very late in my career and must admit that I was not very comfortable with them. Hence,



**Figure 9.** With Joan Steitz and Gideon Dreyfuss, my two role models, at the meeting in Crete in 2015.

many decisions about getting into research in the life sciences and navigating your way within the field must take into account the continuous transformations in the professional environment and must be adjusted to acknowledge talent and objectives. A very inspiring discourse on how to do good science is the article in the Reflections series by Avram Hershko, a discoverer of the ubiquitin system (107). I can add only very few suggestions: be generous in offering your help, advice, and tools to others. When entering a collaboration, avoid asking before the work is done about authorship or other benefits the collaboration may bring. Sooner or later, your generosity and openness will be reciprocated. Irrespective of whether you are a novice or advanced in your career, publish in good but not necessarily flashy journals; pay more attention to the quality of the work than where to publish. In particular, your Ph.D. students will appreciate producing one or two good first-author papers



rather than having their name hidden somewhere in the middle of a multi-author, monumental high-impact article. When running a group, be aware that success in the realization of the research project is more important for the future of your junior collaborators than for your own. Nothing has been more gratifying to me than seeing dozens of my students and postdocs succeeding in science and eventually running their own laboratories.

What would I do when starting my research right now? Doubtless I would choose to work on RNA. The range of new and fully unexpected RNA-related phenomena discovered in the last one to two decades is mind-blowing, and I am sure this trend will continue. More specifically, I would focus on studying RNA biology in the brain, perhaps starting with some of my own unfinished stories in retinal and non-retinal neurons, complemented by intercellular communication and the dynamics of RNA modification. To those who will take on these and other challenges, I wish you good luck!

*Acknowledgments—I thank Susan Gasser, the current Director of the FMI, for her continuous support and for tolerating me in the institute these days. I also express my deep gratitude to all my collaborators and members of the group over the years. They have made my time researching the RNA field truly memorable. I apologize to those whose names or work I have not specifically mentioned in the text or referred to in the list of references. In reality, the research topics covered by my group were even more numerous than the text implies. I thank Pat King for critical reading of the manuscript. I am most grateful of all to my family, who over many years have accepted my often lopsided sharing of time between home and laboratory.*

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