Profile of Tom A. Rapoport

Inside any cell, errant, misfolded proteins must be confiscated and destroyed. This process does not occur inside the endoplasmic reticulum, where secretory proteins are folded and packaged for export; instead, misfolded proteins must retrotranslocate—cross the endoplasmic reticulum membrane back into the cytosol. Biochemist Tom A. Rapoport, elected to the National Academy of Sciences in 2005, has spent much of his career studying the membrane channel Derlin-1, which exports proteins from the cytosol. In the last five years, he has studied the reverse protein movement process of retrotranslocation. In his Inaugural Article in this issue of PNAS, Rapoport identifies a class of proteins associated with the retrotranslocation complex (1).

Tom A. Rapoport

Personal Translocations

Now a Professor of Cell Biology at Harvard Medical School (Boston, MA), Rapoport was born in Cincinnati, OH, in 1947 to parents who had fled the Nazi regime in Austria and Germany. When he was 3 years of age, his parents, a pediatrician and a biochemist, left behind the McCarthy era in the United States to return to Austria. In 1951, the family settled in East Germany.

"We had a scientific household," Rapoport says. "My parents are responsible for my interest." According to Rapoport, he performed his first experiment at the age of 5, while making pudding. His mother likes to recall how the young Rapoport stood in the kitchen cooking pudding and coloring it blue. He carefully labeled it "blue pudding," and his mother made him write up the experiment in a notebook. He recalls this as his first laboratory report.

Mathematics held Rapoport's interest in his early teenage years, and he recalls, "There was a short period where I was interested in being a test pilot, but that didn't last long." Rapoport majored in chemistry in his first three years of college and switched to biochemistry in his fourth year, when his father was the head of the Institute of Physiological Chemistry at Humboldt University (Berlin). Although some may have expected favoritism, Rapoport thinks his father probably treated him more harshly than anyone. For example, one evening Rapoport accidentally left a faucet open. The resulting pressure burst pipes and flooded three floors of the institute, with water raining down into his father's office. When his father found out the flood was Rapoport's fault, he meted out punishment and made Rapoport renovate all of the affected spaces.

Science in East Germany

At Humboldt University, Rapoport entered a special research student program. Instead of the traditional five years of undergraduate work followed by three years for a Ph.D., he combined the two, shortening the period to seven years. He received his Ph.D. in 1972 at the age of 25. For his thesis, Rapoport studied the enzyme kinetics of inorganic pyrophosphatase. With Peter Heitmann as his adviser, Rapoport carried out mathematical modeling to understand the mechanism by which the enzyme cleaves diphosphate into phosphates. Over the course of his doctoral work, Rapoport published three papers in European journals, a feat he remembers as unusual because most East Germans published only locally (2–4).

After completing his doctorate, Rapoport left the field of enzymology, wanting to leave his father's institute and to learn more about molecular biology. A former student of his father's, Sinaida Rosenthal, offered Rapoport a position. Rosenthal had been charged with establishing the first molecular biology group for gene technology in East Germany at the Academy of Sciences in East Berlin. Despite the switch, Rapoport stayed in his same physical location because work on the new institute's building was not yet complete. Rapoport considered this fortunate because it allowed him to work on two projects simultaneously, gaining him a second postgraduate degree in 1977.

Rapoport and Reinhart Heinrich, whom he still considers a good friend, followed the advice of Rapoport's father to look at the modeling of enzymes' roles in systems. Together, Rapoport and Heinrich developed control theory, a quantitative method of assessing an enzyme's importance in a pathway and to this day a major way of describing metabolic networks (5).

At the same time, Rapoport had been assigned to clone insulin at the Academy of Sciences in East Berlin. "The situation was bad. Even though gene technology was an important area, we had very low resources," he says. He recalls a typical annual budget in the early 1970s as 5,000 marks, or about $2,500. Most supplies had to be bought with western currency, which was constantly in short supply.

To clone the insulin gene, Rapoport needed a source for the protein. "It didn't take me long to figure out that this was impossible," he says. The most promising source of insulin for study

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This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 14132.

was derived from about three cases of insulinomas in East Germany each year; however, this supply was not sufficient, and Rapoport looked elsewhere. Rapoport used islets from carp because, as a popular holiday food in East Germany, it was the only fish he could obtain in sufficient numbers. The islets of Langerhans in fish not only are larger than those in humans, but they can be seen with the naked eye and easily isolated. At the height of the research, he remembers killing 2,000 carp at a time. The fish were dumped in a small pool, and the entire department was present to isolate the islets. Rapoport remembers the custodian standing at the head of the table and killing the fish with a whack on the head. People lined up outside to buy these otherwise fine fish "rather cheaply." Word spread throughout Berlin, and crowds often formed on fish days.

Rapoport eventually cloned insulin mRNA from carp, despite the fact that the amino acid sequence for the protein was unknown (6, 7). "Everything we did, we had to develop ourselves," he says. According to Rapoport, this was East Germany's first instance of identifying the amino acid sequence of a protein, as well as the first nucleotide sequence of a gene. But it was not the first sequence of the insulin gene; Gilbert *et al.* had published on rat insulin two years earlier, in 1978 (8). "We were aware of where the field was," Rapoport says, "and, more or less, we knew we were behind." In the late 1970s, Rapoport made his first trip to West Germany to attend a course. When he was invited to meetings or to give seminars, he used the proceeds to buy chemical supplies to bring back to the institute. Colleagues including Peter Walter, Harvey Lodish, and Nobel Laureate Gunter Blobel helped him purchase such needed research supplies.

Moving to Translocation

Cloning insulin first brought Rapoport into the field of protein translocation, where he has spent the balance of his career. In the 1970s, as he tried to isolate insulin mRNA, he found himself needing to develop functional assays. Normally, when proinsulin is cleaved by trypsin it produces two linked chains. Rapoport performed this assay on the proinsulin he had generated *in vitro* and instead found three separate chains. He heard Blobel speak about the then-recently discovered signal sequences at a meeting in Reihartsbrunn, Germany, in 1975. Rapoport says, "I realized this is it. This is the explanation." His *in vitro* protein had not been translocated, so it had not lost its signal peptide and had not formed disulfide bridges, which causes the unique trypsin cleavage pattern. Rapoport went back to the laboratory and showed he had a pre-proinsulin product with an N-terminal signal (9).

He then asked himself, "How is this signal recognized?" and began to gear his projects toward answering this question. Collaborating with Sascha Girshovich, Rapoport searched for a channel in the membrane for translocation. To find such a membrane channel, Rapoport and Girshovich conjectured that if a protein chain had the ability to crosslink anything in the neighborhood upon excitation by light, they might be able to freeze it in the channel. However, adding a crosslinking reagent to a nascent polypeptide chain is difficult, and adding a modified amino acid is impractical because the tRNases are highly selective in recognizing and binding amino acids. "[tRNases] pay a lot of attention to the side chain," says Rapoport. Instead, Rapoport and his colleagues used a technique for first attaching an unmodified amino acid to a tRNase and then modifying it (10). "That trick worked very well," he says.

"The best year of my life [was] watching the x-ray structure of the channel slowly come to light."

By 1987, Rapoport's laboratory at the Central Institute for Molecular Biology (Berlin) had used the crosslinking technique to identify the signal sequence in signal recognition particle (SRP) and to identify channel proteins (10, 11). His laboratory was small, but the publication of these papers, coupled with his stature in the research community, allowed Rapoport to perform basic research at an institution that was often pressuring its scientists to do mainly applied work.

In 1990 the Berlin Wall fell, and Germany was reunified. The days of squirreling away money for supplies were over for Rapoport. Dirk Görlich, who joined the laboratory, which was now part of the Max Delbrück Center for Molecular Medicine (Berlin), worked on reconstituting the translocation channel outside the cell. Görlich purified the translocation components and put them back together *in vitro* (12). The machinery needed only three proteins to work, which surprised Rapoport, who says, "Everyone expected it to be very complicated, but it was very simple." Only SRP receptor, TRAM, and Sec61p are required to form a functioning channel *in vitro*. In addition, one of the three components, Sec61p, was similar in structure and topology to SecY from *Escherichia coli*, suggesting that the translocation machinery is conserved across evolution (13).

The two modes of translocation are cotranslocation and posttranslocation. In cotranslocation, the polypeptide is simultaneously translated and exported. In posttranslocation, the protein is made but is translocated only upon completion. Posttranslocation requires another complex, made of a tetramer and a luminal component, called BiP. Rapoport and his group reconstituted this post-translational process in 1994 (14).

Coming to America

In Germany, Rapoport's research group "had all the money in the world" but questionable status. Prior to reunification, he was a professor and had been elected to the East German Academy of Sciences. This academy dissolved in 1990, requiring Rapoport and his colleagues to reapply for their positions. Turned down twice for professorships for what he calls political reasons, Rapoport says, "I was annoyed and decided I would look elsewhere." After giving a seminar on the translocation channel's reconstitution, Rapoport joined the faculty of Harvard Medical School at the invitation of Marc Kirschner. Rapoport's entire laboratory, including eight students, moved to Boston in 1995.

Over the next 10 years, Rapoport pursued several avenues to understanding translocation. His laboratory performed mechanistic studies of the translocation apparatus to clarify how post-translational machinery works. "It's a ratcheting mechanism," he explains, where BiP binds to the incoming polypeptide and prevents it from moving (15). Rapoport also collaborated with Christopher Akey at Boston University (Boston) on structural examination of the translocation channel and ribosome channel complexes (16, 17). "There was a protein that looked like a channel, it had a big hole in the middle," says Rapoport, and a given polypeptide chain was believed to go through this ring. However, the polypeptide does not go through this hole as imagined, but instead goes through the center of a single copy of the Sec61p oligomer (18). "We're still agonizing about what the results mean," he says.

In 1997, the same year that Rapoport became a Howard Hughes Medical Institute Investigator, elucidating the x-ray structure of the translocation channel

seemed a lofty goal. "Everyone thought it was out of reach," he says. "Normally lots of people are working on a problem, but we were alone." He converted one-third of his laboratory to structural biology and collaborated with "x-ray guru" and fellow faculty member Stephen Harrison. Finally, in 2004, Rapoport had "the best year of my life, watching the x-ray structure of the channel slowly come to light." He describes the channel, viewed in its closed form, as an hourglass, with a helix that plugs the center and moves away to make room for the polypeptide (18). One of his next goals is to obtain another x-ray structure view of the channel. "We want [to see] the channel in action," he says. "It's a really long-term goal."

Against the Grain

Rapoport's Inaugural Article focuses on retrotranslocation, a problem he began studying about five years ago. "People knew for a long time that if proteins were not translated correctly, they would get destroyed," he says. "When it was found this happened in the cytosol, that presented an interesting problem because it meant movement of a protein in the opposite direction." This reverse-direction phenomenon is the basis for retrotranslocation. "We became interested in the molecular mechanism of how this works," says Rapoport.

In 2001, Rapoport's laboratory found a cytosol ATPase, p97, that pulls proteins out of the membrane into the cytosol (19). In 2004, his group had a "semi-breakthrough": while studying how p97 binds to the membrane, they found a protein complex. One protein in the complex possessed homology to a yeast protein known to be involved in degradation. The yeast protein was Der1, and the mammalian protein was thus named Derlin-1 (20).

Rapoport's next question was whether Derlin-1 was a component of the retrotranslocation channel. Referring to the Inaugural Article, he says, "This paper is a little step forward in support of that idea." The Derlin-1 protein is associated with p97 and ubiquitin ligases and helps direct the movement of proteins into the cytosol (1). "It seems like everything is in one big complex," says Rapoport. The paper also discusses the stepwise assembly of this retrotranslocation complex. "Derlin is on first, and then the others join," he explains.

This year, Rapoport was awarded the Otto Warburg Medal from the German Society for Biochemistry and Molecular Biology, and he shows no signs of slowing down. He wants to carry out the same characterization of retrotranslocation that his laboratory did with the forward process in the 1990s, but "we're still at the point where we're identifying the components," he says. His goal is to reconstitute the retrotranslocation machinery *in vitro*.

Even as he studies retrotranslocation, Rapoport continues to look forward. On the horizon, Rapoport is concentrating on a different question, asking, "How is the characteristic shape of organelles generated and maintained?" He would like to know, for instance, why the endoplasmic reticulum is not spherical in shape, since a network of tubules is biologically expensive because of the high energy costs of the convoluted curvature. Rapoport's laboratory has begun identifying the components involved in generating this organelle curvature, continuing his pursuit of answers to scientific questions concerning the cell's elegant machinery.

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