Profile of Joachim Frank

If you were to look at Joachim Frank's recent papers, you might think he had spent his entire career studying how the ribosome converts mRNA into protein. On that subject alone he has enough publications in *Nature* and *Science* to span several careers. But in fact, Frank was introduced to the ribosome only after he had become one of the world's foremost experts in digital image analysis and electron microscopy. For his contributions to these fields, and to our understanding of the ribosome—one of the molecular machines that makes life as we know it possible—Frank was inducted into the National Academy of Sciences in 2007.

The ribosome poses a triple challenge to those who would visualize it. It is hard to see: its diameter of 20 nanometers is well below the wavelength of visible light. The most ideal technique for imaging would seem to be electron microscopy. It is fragile, and electron bombardment at high magnification burns it up. At the same time, it is large for an organic molecule, with a mass of several megadaltons, and its size makes x-ray crystallography a formidable task.

Several crystal structures have nonetheless been obtained. However, emerging research shows that the process of translation is dynamic and involves the interaction of the ribosome with a host of catalytic factors. X-ray crystallography may be able to pinpoint atomic location, but it cannot show how the molecules ratchet, bend, and snap in the assembly line of translation.

To obtain a picture of the ribosome in a more natural environment, Frank uses a technique called cryo-electron microscopy (cryo-EM). In cryo-EM, a biological sample—say, of ribosomes translating mRNA—is frozen instantly into a thin layer of ice. "The ice is amorphous," Frank says. "This is very important because if the water has time to form crystals, the biological material is destroyed." Frank operates the microscope at ×50,000 magnification, effectively holding the ribosome at arm's length to avoid damaging it with the electron dose. Each image he takes is a picture of disorder, with thousands of ribosomes in all orientations. "An individual image of a molecule is very noisy," he says. "It's meaningless. Only averages over large numbers have any meaning." Mathematical methods that he has developed enable him to integrate such large-scale micrographs into three-dimensional (3-D) images of a single ribosome.

Joachim Frank

Soap Box Radios

Frank was born in Germany during World War II, in Siegen, a town in North Rhine-Westphalia. After the war, Siegen was incorporated into the American occupied zone. As an 8-year-old boy, Frank was fascinated by science and conducted chemistry experiments under the veranda of his family's house. Frank, like many scientists of a certain age, entered physics through the portal of amateur AM radio. "When I was 12 or 13," he recalls, "I bought the first stuff for building radios—very small devices. Later I took old radios apart and reassembled them."

Frank took a physics curriculum in high school and advanced to a program in physics at the University of Freiburg (Freiburg, Germany). He then enrolled for a "Diplom," a type of master's degree, at the University of Munich (Munich, Germany). This degree required a thesis, and Frank conducted a study of electron diffraction at the melting point of gold. "It was a pretty hairy experiment because it's hot and gold vapor is everywhere, coating everything and messing up the electrical signals," he says. "But I was already thinking in terms of electrons and electron bombardment and vacuum technology and so forth. So from there to electron microscopy was not a very big step." Frank became intrigued by the idea that it might be possible to use electrons to look at molecules, and he began to cast about for a supervisor who could offer him an interesting project.

He found one in Walter Hoppe, an x-ray crystallographer in Munich at the Max Planck Institute für Eiweiss- und Lederforschung. (Direct translation: "eggwhite and leather research." The institute was later incorporated into the Max Planck Institute for Biochemistry in Martinsried, Germany). Hoppe was also a professor at the Technical University of Munich, which would eventually grant Frank his Ph.D.

Frank wrote his dissertation on the use of cross-correlation to align carbon films. The fidgets of a Polish colleague, Anton Feltynowski, led to a key insight in Frank's research. "Feltynowski was incredibly excitable," Frank says. "In experiments, he would kick the column of the electron microscope, so he always got these very unsteady images." Frank examined the images by optical diffraction, a way of looking at the Fourier transform. "And then I saw these striped patterns. The image, during the exposure, had jumped from one place to the next."

Two things were immediately apparent to Frank. First, if the stripes appeared in the Fourier transform because the microscope had been jolted, then he could use the existence of stripes to diagnose image instability. And second, one could superimpose two images taken at different times in the optical diffractometer, look at the Fourier transform, and align the images to great precision by adjusting their position until the stripes vanished. Frank derived and published the equations underlying the formation of the stripes, which are related to Young's fringes in optics, for different types of image movements: smooth, jerky, oscillating. "That was my first publication," he says (1). "And my mentor was very generous. He didn't want to have his name on the paper. He said, 'That's completely your own.'" In later papers, Frank developed the concept into a method of image alignment.

Cannonball Run

Frank defended his dissertation in 1970. One of the professors on the examining board, impressed, nominated him for the prestigious Harkness fellowship. Under the terms of the Harkness, Frank was funded for two years' work in the United States at any laboratory that

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would have him, plus a generous stipend for traveling. On arrival in the United States, Frank and his wife bought a used car and headed for NASA's Jet Propulsion Laboratory (JPL) in Pasadena, CA. The JPL might seem to be an odd destination for a specialist in microscopy, but "at the time," Frank says, "they were the leading people in the world in image processing." He was able to study the JPL software, to which he made his own improvements.

From JPL, Frank and his wife moved to the University of California (Berkeley, CA), where Frank teamed up with Bob Glaeser, a pioneer in cryo-EM. "His work at Berkeley," Glaeser says, "helped to set his resolve to develop ways to average images of thousands of identical molecules." At Berkeley, Frank published a comprehensive review of image processing in electron microscopy (2). "It proved to be fateful, because on the basis of that, I got my later position in the United States," Frank says.

After six months in Benjamin Siegel's microscopy laboratory at Cornell University (Albany, NY), Frank returned to Germany to search for full-time positions. During this time, he developed a way to describe how the "partial coherence" of electron waves—their imperfect synchronization—affects how images are formed. "I found a very elegant solution to a very complex problem," Frank says. The paper he published on the "envelope function" describing partial coherence provided other researchers in the field with a tool that they soon found indispensable (3).

Because no faculty jobs opened up in Germany, he took a position as a research assistant at the University of Cambridge's Cavendish Laboratory under Vernon Cosslett, an electron microscopist. In Cosslett's group, working with Owen Saxton and Peter Hawkes, Frank focused on electron optics. On his mind were the experiments he had done with biological samples at Berkeley. He was convinced that images of large proteins could only be obtained by averaging methods. With Saxton, he began to calculate the lowest electron dose necessary to achieve images that they could align with enough precision to garner useful information. Out of their work emerged an equation relating contrast, resolution, and dose (4). "We convinced ourselves that for a certain size of a molecule, this is going to work," he says. "That was really the fundamental equation which was at the root of everything."

The term of Frank's employment at Cambridge was two years. He had not put much thought into what he was going to do next when he received a letter from the Wadsworth Center (Albany, NY) asking whether he would be interested in a position as a research scientist. The review article he had written at Berkeley had made him famous in his field. "People somehow associated my name with electron microscopy and image processing at this early time," he says.

3-D Vision

The Wadsworth Center is a large government laboratory that sits under a 42-story skyscraper facing the Hudson River in downtown Albany. When the decision was made to create the center, Donald Parsons, who had hired Frank, had lobbied the state governor and Wadsworth's director to purchase a 1.2-megavolt electron microscope, one of only three in the United States at the time.

After his original task failed, Frank turned to the challenge that had been obsessing him: how to combine low-dose electron images into averages and, ultimately, 3-D reconstructions.

> **"An individual image of a molecule is very noisy," he says. "It's meaningless. Only averages over large numbers have any meaning."**

Together with his students, Frank developed a modular tool for processing images, which could perform separate tasks such as selecting a window of an image or performing cross-correlations or Fourier transforms (5). "The acronym was 'SPIDER,' constructed in a very painful way," he says. (It stands for "System for Processing of Image Data in Electron microscopy and Related fields.") "The system grew over the years and became more and more powerful. . . it's being used by very large number of people around the world and there is now a whole user community. We just had a SPIDER workshop this spring with 50 participants."

Glutamine synthetase was the molecule on which all of this expertise and software was brought to bear. David Eisenberg, a colleague at University of California (Los Angeles, CA) who is now well known for his contributions in bioinformatics and protein folding, provided samples. Frank and Martin Kessel, a visiting scientist, began to collect data, but the averages were quite blurred. It was clear from this experience that averaging and 3-D reconstruction of molecules would require powerful methods of image classification. To address this problem, a Dutch student visiting from Groningen, Marin van Heel, began to write programs in Frank's laboratory. He had brought with him electron micrographs of hemocyanin molecules, which appeared in an unusual variety of shapes. Frank recounts the story of a key breakthrough:

> This French scientist recently hired by the Wadsworth Center, Jean-Pierre Bretaudiere, printed out large maps with symbols clustered on them. He analyzed blood samples for the amounts of different components. Since the list of components for each sample formed a vector, he analyzed the vectors statistically in a high-dimensional space. I went to Marin and said, 'Scrap the programs you are writing. Blood samples are images, and the components in the blood are pixels!' And in a single afternoon, we wrote simple programs that made our images appear like blood samples, so they could be read by Jean-Pierre's program. We got the most beautiful clustering of molecule images. From that moment on, averaging of molecule images became very powerful (6).

Enter the Ribosome

Frank's career had already gathered serious momentum, but it was about to receive a huge boost. At the time, cryo-EM was not widely used. Electron microscopists applied a negative stain to their samples to achieve good image contrast. At a conference at Rockefeller University (New York, NY), a scientist named Miloslav Boublik approached Frank. "He showed me pictures of the ribosome. They were negatively stained, they looked very crisp," Frank says. "These were eukaryotic ribosomes, from HeLa cells. And he suggested, 'is this something that would be useful for your method?'"

Frank ran the ribosomes through his system to produce side-view images that were greatly enhanced by averaging. The resolution was 20 Å. "That immediately got us a paper in *Science* [ref. 7] and generated a lot of excitement, and was a beginning for a very successful grantsmanship," he says. "I got grants from then on. That allowed me to build up a group." In fact, he has held one of his National Institutes of Health grants continuously since 1982.

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The assured funding allowed him to grow his group and hire specialists such as Michael Radermacher, who had also studied under Frank's Ph.D. mentor Walter Hoppe. Radermacher, Frank says, is "mathematically gifted" and writes elegant, powerful computer programs. Frank asked him to implement some ideas he had about 3-D reconstruction into SPIDER. The group began to apply Radermacher's 3-D reconstruction modules to several types of macromolecules, although they increasingly focused on ribosomes. In 1987, Frank published the first 3-D reconstruction of the large 50S ribosomal subunit, from a negatively stained specimen (8).

However, it was clear that this image contained artifacts. "The negative stain makes the molecule collapse," Frank says. To avoid this problem, they switched to cryo-EM, in which the ribosomes maintained their normal shape. The tradeoff was that, without the negative stain, the contrast was not as high, and high resolution was difficult to achieve. In 1990, they published the first cryo-EM reconstruction of the ribosome, at 45-Å resolution (9). Further improvements in image processing were required to advance beyond this point. Pawel Penczek, who had a background in medical image processing, was recruited from Warsaw by Frank. Many of the subsequent improvements to the software were a result of Penczek's efforts.

As Frank's group gained experience with cryo-EM, they found themselves graduating from simple snapshots of inactive ribosomes to investigating the process of mRNA translation. During translation, amino acids bound to tRNA molecules are shunted along the A, P, and E sites in the space between the ribosomal subunits. The anticodons on the tRNAs bind to the mRNA, and the amino acids are incorporated in the growing polypeptide chain.

Lost in Translation

Frank took a sabbatical in 1994, at the Max Planck Institute for Medical Research (Heidelberg, Germany). In the laboratory of Ken Holmes, known for his work on muscle, Frank traded his expertise with SPIDER for time on the laboratory's energy-filtering microscope. "We collected exceptional images," Frank says. He and his student Jun Zhu put together a reconstruction of the ribosome at 25 Å (10), from which it was possible to infer how elements such as the mRNA and tRNA interacted with the ribosome, as well as where the polypeptide chain emerged. "That was our first foray into serious functional work," Frank says. "And this particular paper was a sensation. . . there were many collaborations proposed. Everything snowballed."

Until then, Frank's group had been oriented toward computation. But Rajendra Agrawal joined the group during the Heidelberg sabbatical. Agrawal was a "real biologist," Frank says. "Raj had a real biochemical understanding of translation." His expertise was vital as the group sought to interpret their cryo-EM images. Agrawal stayed on for 10 years and contributed to several important papers as the group honed their ability to visualize tRNAs at the A, P, and E sites (11). In a key paper, Frank and Agrawal reported their discovery that the large and small subunits of the ribosome act as a ratchet to crank the tRNAs through the internal sites (12).

"Freezing" translation at different points enabled Frank and his colleagues to study the complex process as if they were looking at individual frames in a film reel. Måns Ehrenberg of Uppsala University (Uppsala, Sweden) is a collaborator who continues to provide Frank with "outstanding" biochemical specimens. In Ehrenberg's *in vitro* system, the experimenter can add antibiotics or GTP analogues to freeze translation at virtually any step. "So we know exactly where we are [in the process]," Frank says. Ehrenberg's system has proven particularly useful as the scientists explored the roles played by the various elongation and termination factors (13).

Continuing to refine his techniques and build on his previous work, Frank has been able to explore increasingly complex aspects of ribosomal action. One of his landmark findings is the structure of a ribosome bound to a protein channel that allows protein secretion through a membrane (14). To appreciate the magnitude of this achievement, one needs to understand that he was able to obtain a composite image of tens of thousands of identical assemblies, each consisting of a ribosome, two protein channels, the mRNA, three tRNAs, and the nascent polypeptide, all frozen at the same instant of translation.

Bright Lights, Big City

In his Inaugural Article (15), Frank reviews the contributions that cryo-EM research—much of it conducted by his group—has made to our understanding of exactly what happens during mRNA–tRNA translocation. Translocation is the process by which the tRNAs are bumped one codon forward as translation progresses. Frank and colleagues couple cryo-EM images with computer modeling to determine the best fit for the atomic structure. They have found that the Brownian jiggling of surrounding molecules powers the ribosome as it alternates between the "normal" and "ratcheted" conformations, advancing the tRNAs and mRNA one codon at a time. An elongation factor, EF-G, helps reduce the energy barrier between the two conformations. Frank speculates that the ribosome existed as a much less efficient machine before life forms evolved EF-G to speed things up.

Frank currently is working on approximately 20 different projects on the themes of translocation and decoding (how the correct amino acid-bearing tRNAs are selected from the churning soup of molecules surrounding the ribosome).

He is also beginning to collaborate with Nobel laureate Guenter Blobel in exploring the mechanics of the nuclear pore complex. In a new position in the Department of Biochemistry and Molecular Biophysics at Columbia University (New York, NY), Frank's research should produce even more remarkable, high-precision insight into the mysteries of giant molecules.

Kaspar Mossman, *Science Writer*

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