Protein Science (1999), *8*:1562–1563. Cambridge University Press. Printed in the USA.

OBITUARY

Oleg Ptitsyn 1929–1999

Oleg Ptitsyn was a pioneer in protein folding studies: his discovery of molten globule folding intermediates revolutionized the field. He died in England on March 22, 1999, after a heart attack. Oleg Ptitsyn was born in St. Petersburg (Leningrad) on July 18, 1929, and took his M.Sc. in Molecular Physics at the University of Leningrad at the age of 22. He remained in Leningrad, at the Institute of High Molecular Compounds, first taking his Ph.D. in 1954 and then his D.Sc. in 1963 in physical and mathematical sciences. In 1967, he became Deputy Director of the Institute of Protein Research and simultaneously Head of the Laboratory of Protein Physics at Pushchino, at the Academy of Sciences Institute for Protein Research. He retained his position at Pushchino after becoming in 1992 a visiting member of the NIH Laboratory of Mathematical Biology, in the section of Robert Jernigan.

In the early 1970s Oleg Ptitsyn was one of a small band of scientists dispersed around the world, each intent on solving the protein folding problem in his or her own way. Colleagues in other fields, who generally viewed the folding problem as insoluble, thought these people were nutty and frequently told them so. Chris Anfinsen, Charles Tanford, and John Schellman, who had played a major role in setting up the field, were moving on to new interests by the mid-1970s. Harold Scheraga wanted to predict protein structures from amino acid sequences by energy minimization. Kurt Wüthrich wanted to track the folding process by NMR, but he became diverted to determining protein structures by NMR. Fred Richards and also Jane and Dave Richardson were deciphering the secrets of packing arrangements in protein structures. Martin Karplus was developing a kinetic mechanism of folding that could solve the Levinthal paradox. Tom Creighton was isolating covalently trapped intermediates by coupling folding to disulfide bond formation, while I was intent on characterizing kinetic folding intermediates in reactions not involving disulfide bonds. Cy Levinthal and Michael Levitt, like Oleg Ptitsyn, wanted to predict protein structures by first predicting their folding pathways.

Photo of Oleg Ptitsyn courtesy of Mrs. Irina Ptitsyna.

In this group Oleg Ptitsyn was unique in having a strong background in polymer physics. At the age of 35 he had co-authored with T. M. Birstein a book entitled *Conformations of Macromolecules* (later published in English, Birstein & Ptitsyn, 1966), which summarized their theoretical studies on the relation between polymer flexibility and local order in the polymer backbone.

In 1967 Oleg Ptitsyn moved to the Laboratory of Protein Physics at Pushchino, near Moscow. He and his colleague Alexey Finkelstein very early proposed a method for predicting protein secondary structure from sequence (Ptitsyn & Finkelstein, 1970), a method similar in character to the popular Chou–Fasman method of 1974. Next he proposed a model for how proteins fold (Ptitsyn, 1973). His model might be called hierarchic folding today, but recently Ptitsyn preferred to call it the framework model, a term coined by Peter Kim and myself. In his model, folding starts in the backbone by first forming secondary structures, α-helices and β-strands, which then interact by nonspecific interactions, the hydrophobic interaction and hydrogen bonds, to form more advanced folding intermediates. Ptitsyn emphasized that in his model each stage of the folding process stabilizes the major conformation already present so that only native-like backbone structure is present, in addition to unfolded segments, at all stages of folding.

To show the potential of his model, Ptitsyn and Rashin used it to predict the dominant folding pathway of apomyoglobin (myoglobin without the heme), which then yields the structure of the native protein as the end product of this pathway (Ptitsyn & Rashin, 1975). They took the locations of the helices as being known from the X-ray structure and they assumed that each pair of neighboring major helices could act as a "crystallization center" initiating the growth of the overall structure. They predicted a major folding pathway, which yielded a product resembling native myoglobin (without the heme). Although later work did not confirm this prediction of the dominant folding pathway, nevertheless their paper was highly influential in forecasting the course of future work.

Next Oleg Ptitsyn and Alexey Finkelstein began work on the prediction of backbone topologies for β-strand proteins (see review, Ptitsyn & Finkelstein, 1980). They were particularly interested in the common occurrence of the Greek key fold in proteins such as immunoglobulins. Their approach was to generate all possible topologies for a protein, given the locations of the β-strands, and then to compare the actual with the possible topologies and use the results to generate heuristic rules for predicting the correct topology. This work was probably intended to be the start of a general attack on predicting protein structures, but unexpectedly Oleg Ptitsyn changed course.

The idea suddenly came to him that the partly folded forms that had been observed for a few proteins in non-native conditions (acidic pH or moderate denaturant concentrations) might be the folding intermediates he had predicted in 1973 (see his account, Ptitsyn, 1995a). He resolved to study these partly folded forms to find out if his hunch was correct. The simplest and best studied partly folded form was that of bovine α-lactalbumin, which had been investigated extensively by Kuwajima and Sugai. From measurements of intrinsic viscosity, Ptitsyn and his coworkers found the surprising fact that the partly folded form is compact, almost as compact as the native protein, even though it lacks rigid tertiary structure (Dolgikh et al., 1981). Oleg Ptitsyn argued that the key features held in common by the few partly folded proteins known at that time were compactness, high content of secondary structure (measured by CD), and lack of rigid tertiary structure. The name "molten globule" was provided by M. Ohgushi and A. Wada in 1983, following discussion with Ptitsyn of their work on the acid form of cytochrome *c*. The name was chosen because the side chains are molten and yet the protein molecule is a compact globule.

After satisfying himself that his hunch was correct, Oleg Ptitsyn had to convince the world. Proteins with the properties of molten globules were strange, unfamiliar beasts in the early eighties. One turning point came in 1990 when the molten globules of apomyoglobin and cytochrome *c* were found by NMR-hydrogen exchange to contain helices at precisely the locations they occur in the native structures. Consequently these molten globules contain some authentic native structure. Another turning point came in 1993 when a kinetic intermediate in the refolding reaction of apomyoglobin was found to have exactly the same exchange-protected peptide NH protons as the stable molten globule present at pH 4. Consequently the stable partly folded form corresponds to an authentic intermediate in the kinetic folding process. By 1993 half a dozen or more kinetic folding intermediates had been characterized by NMR-hydrogen exchange in conjunction with stopped-flow mixing, and the results fitted the same pattern found for the molten globules of α-lactalbumin, cytochrome *c*, and apomyoglobin. Most of this work was done in other laboratories, but Oleg Ptitsyn had won his battle to convince the world that molten globules are folding intermediates. He became a scholar of the subject and produced admirably clear reviews summarizing the entire literature, which was growing at an astonishing rate (see Ptitsyn, 1995b). Some simulations of the folding process have been used to predict that observable folding intermediates are dead-end, off-pathway intermediates. This question remains open, but current opinion in the field is swinging in favor of on-pathway intermediates.

Oleg Ptitsyn's interests were always broad, and in recent years he paid particular attention to the possible involvement of molten globules in molecular diseases and in the transport of proteins across membranes. He also became fascinated with the growing evidence that an important factor had been left out of his 1973 model (Ptitsyn, 1996): the formation of clusters of tight packing interactions among side chains (folding nuclei?), suggesting that specific packing interactions are important in stabilizing molten globules in addition to the nonspecific hydrophobic interactions and peptide hydrogen bonds.

Oleg Ptitsyn loved to debate issues connected with protein folding. Usually these were one-on-one debates. It was not easy to grasp what he said, not so much because of his Russian accent as his manner of speaking. Fortunately, it was always worthwhile pausing to think about what he said. Unlike some people who have good ideas and don't like to part with them, Oleg was always open in voicing his ideas and opinions. He twice visited my lab for a 2–3 day stay and he discussed with each lab member his or her research until Oleg felt he had got to the bottom of it. He thoroughly enjoyed doing this; he not only said so, you could see it by his face and by hearing him humming. He was like a bee going from flower to flower. On visits like these, Oleg's suggestions sometimes led to new research projects. I particularly remember hearing appreciative comments about his visits from Michel Goldberg, Neville Kallenbach, Kunihiro Kuwajima, and Roger Pain.

Oleg was very fond of foreign travel, for the contact with the culture as well as the science of the outside world. In the 1970s and early 1980s, it was not easy for him to get out of Russia. When he was scheduled to speak at a meeting, we never knew if he would appear until we saw him. Although he was keenly aware of the restrictions on his personal freedom, he loved his scientific life in Pushchino and Leningrad in that period, and he watched with dismay the modern breakup of the scientific system as he knew it in Russia.

References

Birstein TM, Ptitsyn OB. 1966. *Conformations of macromolecules*. Interscience.

Dolgikh DA, Gilmanshin RI, Brazhnikov EV, Bychkova VE, Semisotnov GV, Venyaminov SY, Ptitsyn OB. 1981. α-Lactalbumin: Compact state with fluctuating tertiary structure? *FEBS Lett 136*:311–315.

Ptitsyn OB, Finkelstein AV. 1970. Connection between the secondary and primary structures of globular proteins. *Biofizika 15*:757–767.

Ptitsyn OB. 1973. The stepwise mechanism of protein molecule self-organization. *Dokl Akad Nauk SSSR 210*:1213–1215.

Ptitsyn OB, Rashin AA. 1975. A model of myoglobin self-organization. *Biophys Chem 3*:1–20.

Ptitsyn OB, Finkelstein AV. 1980. Similarities of protein topologies: Evolutionary divergence, functional convergence or principles of folding? *Quart Rev Biophys 13*:339–386.

Ptitsyn OB. 1995a. How the molten globule became. *Trends Biochem Sci 20*:376–379.

Ptitsyn OB. 1995b. Molten globule and protein folding. *Adv Protein Chem 47*:83–229.

Ptitsyn OB. 1996. How molten is the molten globule? *Nat Struct Biol 3*:488–490.

ROBERT L. BALDWIN
Department of Biochemistry
Stanford University School of Medicine
Beckman Center, B400
Stanford, California 94305-5307