QnAs with Nenad Ban

Farooq Ahmed, *Science Writer*

The central dogma of molecular biology describes the flow of information in a cell from DNA to RNA and onto proteins. Key to this flow of information, ribosomes act as the site of translation, taking in genetic information and producing polypeptides made of amino acids. Although they are complex macromolecular machines that consist of RNA and proteins, ribosomes are highly conserved across kingdoms of life, attesting to their evolutionary importance. Nenad Ban, a professor of structural molecular biology at the Swiss Federal Institute of Technology in Zurich, Switzerland, has been studying ribosomes for more than two decades. His work on the structure and function of the prokaryotic large ribosomal subunit contributed to the 2009 Nobel Prize in Chemistry, which was shared by structural biologists Ada Yonath, Venki Ramakrishnan, and Thomas Steitz. More recently, Ban's team has leveraged innovative techniques, such as cryogenic electron microscopy (cryo-EM), to reveal the structures of eukaryotic, mitochondrial, and chloroplast ribosomes and their functional complexes to explain how the ribosomes assemble, function, and target proteins to specific cellular locations. Ban was elected as an international member of the National Academy of Sciences in 2021. PNAS spoke to him about his current research.

PNAS: Your Inaugural Article (1) details the assembly of the mitochondrial ribosome small subunit. Why is it important to understand mitochondrial ribosome structure and assembly?

Ban: The proteins responsible for producing the energy—ATP—that fuels all biochemical processes in our cells are made by specialized protein synthesis machines located inside mitochondria. Mitochondrial ribosomes have a distinct architecture and, like their cytosolic counterparts, are very complex cellular assemblies consisting of two subunits and many proteins and ribosomal RNAs. Their correct assembly requires a complex multistep sequence of events. Currently, relatively little is known about this essential process in mitochondria, and our study contributes to an understanding of how the small subunit of mitochondrial ribosomes is assembled to be able to participate in translation.

PNAS: What are some of the similarities and differences between the mitochondrial ribosome and those of prokaryotes and eukaryotes?

Ban: Because of their evolutionary origin, mitochondrial ribosomes are more closely related to bacterial ribosomes than to eukaryotic cytosolic ribosomes. However, they have undergone extensive structural and compositional remodeling during evolution, reflected in striking variations in RNA content and the acquisition of a large number of mitochondria-specific ribosomal proteins.

Furthermore, although the active site of the large ribosomal subunit and the decoding center of the small ribosomal subunits are conserved, mitochondrial

Nenad Ban. Image Credit: Regula Ruckstuhl (University of Zurich, Zurich, Switzerland).

This is a QnAs with a member of the National Academy of Sciences to accompany the member's Inaugural Article, e2114710118, in vol. 119, issue 3.

Published January 20, 2022.

ribosomes are structurally and functionally specialized to accomplish many mitochondrial-specific tasks. For example, in human cells mitochondrial ribosomes exclusively synthesize membrane proteins and do not have to switch between synthesizing soluble and membrane proteins, as is the case for cytosolic ribosomes.

PNAS: What did you discover about how the mitochondrial ribosome assembles?

Ban: Based on the insights reported in [the Inaugural] Article (1), where we studied mitochondrial ribosome assembly in trypanosomes, which are unicellular eukaryotic parasites that cause a range of diseases, and based on our previous work on the assembly of human mitochondrial ribosomes, we learned that this is a very intricate process. The precise incorporation of ribosomal proteins and the folding of ribosomal RNA during ribosome assembly is driven by specialized proteins, called assembly factors.

We also discovered that the process proceeds through the formation of many structurally distinct intermediates. The assembly factors act as chaperones to help the folding of ribosomal components; they serve to temporally control the process and to prevent immature subunits from prematurely participating in translation.

We also report on the surprising discovery that an initiation factor called mitochondrial initiation factor 2, [which] usually participates in the first stage of protein synthesis, is a component of one of the assembly intermediates. Our study uses a combination of high-resolution electron microscopy and cellular experiments to reveal the structural basis of trypanosomal mitochondrial small-subunit assembly and to demonstrate the role of an initiation factor in the process.

PNAS: How is mitochondrial ribosome formation tied to human health?

Ban: Mitochondrial dysfunction caused by mutations in the components of the mitochondrial translation machinery leads to many clinically and genetically heterogeneous multisystem disorders, such as Leigh syndrome, sensorineural hearing loss, encephalomyopathy, and hypertrophic cardiomyopathy. Defects in the assembly of mitochondrial ribosomes directly affect the ability of these ribosomes to synthesize the mitochondrial membrane proteins that are responsible for the production of ATP. Our insights about their assembly and a structural explanation of the effects of certain mutations will be helpful in understanding how these diseases develop and how they might be treated.

PNAS: How will these findings advance research on ribosomal structure and assembly?

Ban: Our work on the assembly of mitochondrial ribosomes in trypanosomes and in human mitochondria will help unravel the basic principles of the process across different organisms. In addition, our Inaugural Article (1) provides an interesting structural insight into the dual role of a translation initiation factor that plays a key role in translation and at the same time moonlights as an assembly factor. It will be interesting to investigate whether this is a more general principle in other organisms and for other translational factors.

PNAS: From X-ray crystallography to cryo-EM, the tools you have used to investigate protein synthesis have evolved over the past two decades. How have technological advances aided the understanding of nucleoprotein structure and function? What technologies on the horizon are you are most excited about?

Ban: My research in the field of protein synthesis is driven by the motivation to uncover the function of the participating cellular assemblies and the mechanism of the process. I have always considered the methodology as a means to get to these answers.

Initially, X-ray crystallography was the only method capable of providing us with high-resolution information on ribosomes. In recent years, cryoelectron microscopy combined with single-particle reconstruction has advanced to the stage where they can provide similar structural information but on much more heterogeneous samples, so now we predominantly use this method.

In my opinion, to understand the process of protein synthesis in the complex cellular environment will [increasingly] require a combination of methods at different resolution ranges, for example combining high-resolution electron microscopy with electron tomography, and methods capable of providing dynamic information on the process, for example single-molecule experiments, as well as approaches that allow us to monitor translation on a global cellular level, like ribosome profiling.

PNAS: Does this work reflect how your research interests have evolved?

Ban: For decades, the key focus of my [laboratory] has been to investigate the process of protein synthesis to understand it in terms of the underlying mechanisms and in the cellular context. Over the years, due to new developments in experimental methodologies and technologies, we gradually expanded our interest from studying bacterial protein synthesis machinery to investigating how the process works in eukaryotic cells.

1 T. Lenarčič *et al.*, Mitoribosomal small subunit maturation involves formation of initiation-like complexes. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2114710118 (2022).