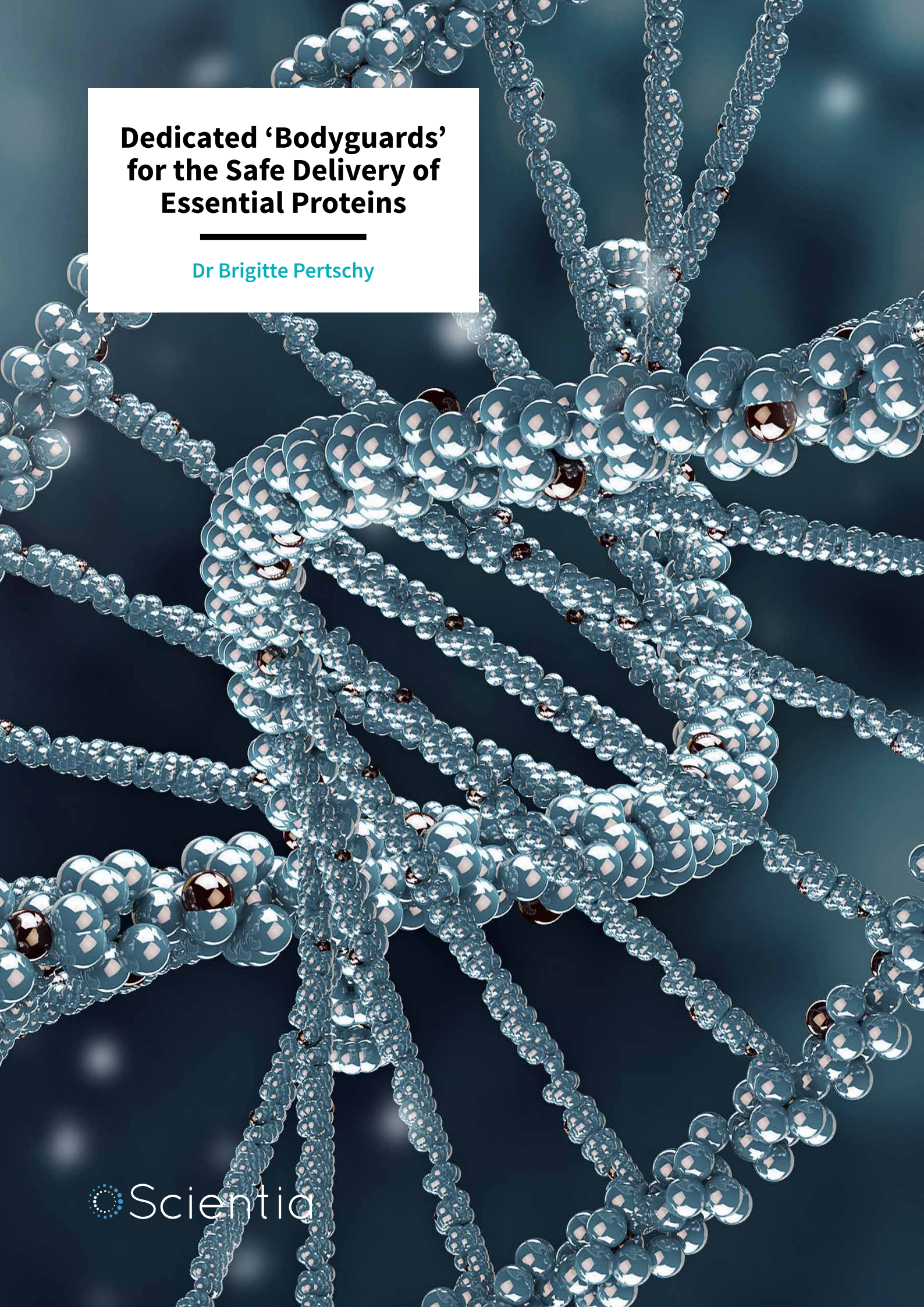


Dedicated ‘Bodyguards’ for the Safe Delivery of Essential Proteins

Dr Brigitte Pertschy

Scientia

DEDICATED 'BODYGUARDS' FOR THE SAFE DELIVERY OF ESSENTIAL PROTEINS

Ribosomes are undoubtedly one of the most essential cellular components in life. These macromolecules are responsible for the synthesis of proteins in all living cells. **Dr Brigitte Pertschy**, Dr Ingrid Rössler and Jutta Hafner at the Institute of Molecular Biosciences at the University of Graz, Austria, have discovered that the safe delivery of essential ribosomal proteins that make up the ribosomes is dependant on 'private bodyguards' or 'chaperones'.

Nascent Ribosomal Proteins Journey Across the Cell to the Nucleus

The ribosome is the intricate nano-machinery that translates messenger RNA strands (mRNA) into protein. Our DNA holds the instructions for building every protein needed for our bodies to function. Initially, DNA is transcribed into mRNA, which contains the amino acid sequence of a particular protein. This mRNA strand is processed or translated by the ribosome whereby the amino acids that correspond to the mRNA sequence are recruited and added to a growing peptide chain. The amino acids are linked via peptide bonds and once the full mRNA sequence is translated and the amino acid chain is complete – the protein is now formed and released for use.

Proteins are essential for countless critical functions throughout the body, from cell structure to the regulation of tissues and organs. Ribosomes can be thought of as 'protein-factories' and these extremely important components are made up of ribosomal RNA (rRNA) molecules and ribosomal proteins (r-proteins). The assembly of ribosomes, known as ribosome biosynthesis, is a highly complex, multi-step process and is the specific interest of Dr Brigitte Pertschy and colleagues Dr Ingrid Rössler and Jutta Hafner, from the Institute of Molecular Biosciences at the University of Graz in Austria.

Ribosome synthesis is an important and continuous process. Dr Pertschy describes that a growing cell requires up to 1,000 ribosomes to be synthesised per minute. The r-proteins are produced in the cell cytoplasm by the ribosome itself (that way, the ribosome participates in its own reproduction). From there the r-proteins must travel to the cell nucleus where in a complex maturation process they are joined with the rRNA to form a nascent ribosome. This precursor ribosome further matures and is transported back to the cytoplasm where the mature ribosome performs its function in protein synthesis. During their transit to the nucleus, r-proteins tend to aggregate and become non-functional. Dr Pertschy and her team had been studying the assembly path of r-protein Rps3 when they discovered that certain chaperone proteins could prevent the aggregation of r-proteins. These chaperones accompany the r-proteins on their journey from the cytoplasm to the nucleus and aid their successful incorporation into new ribosomes.

Dr Pertschy and her colleagues explain that there are a number of mechanisms to counteract the aggregation of newly synthesised r-proteins. These include general mechanisms used by many cellular proteins like a general chaperone network that is involved with protecting most new proteins from degradation at the very early stage of their synthesis and proper folding of the proteins. Importins have also been reported as aides in the import of proteins to the cell nucleus as well as in protecting proteins from aggregation.

The team speculated that since r-proteins are produced at extremely high amounts and their correct functioning is so critical for a cell, these general mechanisms acting on most proteins might be insufficient to fully protect r-proteins, and that there must also be more specific mechanisms by which r-proteins are protected until they arrive at their final destination. Further investigations by Dr Pertschy's team revealed that some r-proteins have their own personal chaperones to protect them from aggregation on their journey to the nucleus to join their rRNA counterparts.

Dedicated R-Protein Chaperones to Help Along the Journey

Other research groups, including Dr Pertschy's collaborator, Dieter Kressler and his team at the University of Fribourg's Department of Biology, have also observed the existence of dedicated r-protein chaperones. Dr Pertschy, Dr Rössler and Ms Hafner explain that these dedicated chaperones are able to protect their specific client r-proteins by exploiting different structures and binding mechanisms.

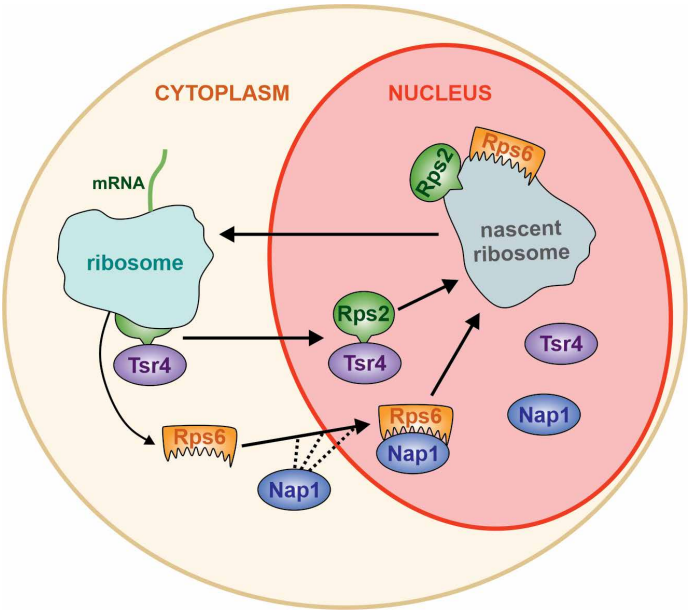
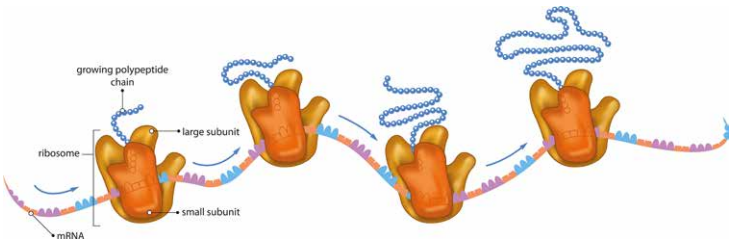


Figure 1: Tsr4 and Nap1, the dedicated chaperones of Rps2 and Rps6. While Tsr4 binds Rps2 already while it is being synthesized by the ribosome, Nap1 binds Rps6 at a later timepoint (after it has been synthesized and released from the ribosome). Whereas Tsr4 binds to a very small region on Rps2, Nap1 requires almost the entire Rps6 protein for binding. After delivery to the nucleus, both ribosomal proteins are incorporated into nascent ribosomes. These undergo a complex maturation process and are transported back to the cytoplasm, where the mature ribosomes can start their job in translation. Credit Brigitte Pertschy.



There are around 80 different r-proteins in eukaryotic cells of which dedicated chaperones of only nine had been identified. Dr Pertschy and colleagues had already studied and reported the importance of these dedicated chaperones and realised that there were many more yet to be discovered. They presumed that the reason why many dedicated r-protein chaperones had remained unidentified was that r-proteins are bound to their dedicated chaperones only during a very short window of time in their lifetime. This means that at a given time point, only a very small fraction of the r-protein is together with its dedicated chaperone, while the majority of the population of this r-protein is bound to its interaction partners within the mature ribosome. With this, the team led by Dr Pertschy set out to establish a method suitable to identify novel dedicated r-protein chaperones among all the other, much more abundant interaction partners of r-proteins. Their rationale was that while these abundant interaction partners are usually common to all r-proteins, dedicated r-protein chaperones should be found only with one or few different r-proteins. Having this in mind, they employed a strategy in which they purified more than 20 different r-proteins and identified all interaction partners by semi-quantitative mass spectrometry. In the subsequent analyses, they ignored all the abundant interaction partners present in all purifications and searched for those interaction partners which were specifically co-enriched with only one, or very few different r-proteins (which were present in the preparations only at low quantities).

The team first investigated if the known chaperones of r-proteins Rps3 and Rps14 were specifically co-enriched in this approach. Results showed that Yar1 had strongly co-enriched with Rps3 and this further confirmed Yar1 is a chaperone dedicated to Rps3. Furthermore, the dedicated chaperone Fap7 was also strongly enriched with its client r-protein Rps14.

The Search for Novel R-Protein Chaperones

Dr Pertschy tells us, 'we are on the one hand exploiting our data to identify additional, so far undiscovered dedicated ribosomal protein chaperones, and on the other hand investigating the cellular function of already identified dedicated ribosomal protein chaperones.' Having demonstrated the direct interaction between some r-proteins and their known dedicated chaperones, Dr Pertschy and her team next focused on analysing the data of two 40S r-proteins for which dedicated chaperones had not yet been identified, Rps6 and Rps2. A protein called Nap1 was, in comparison with all the other r-protein purifications, clearly enriched with Rps6. Nap1 is indicated in DNA repair and as a regulator of cell division. The latest results from Dr Pertschy's team now indicate that Nap1 is also likely to function as an r-protein chaperone for Rps6.

In the Rps2 purification, the team found a protein which was absent from all other r-protein purifications, Tsr4. Tsr4 is known as an acidic protein conserved in eukaryotic cells. Dr Pertschy notes that Tsr4 was previously reported by others to lead to defects in the maturation of the 40S subunit when depleted. However, the nature of its function in ribosome biosynthesis had not been known until now and these new data supported the notion that Tsr4 may be a novel Rps2 chaperone.

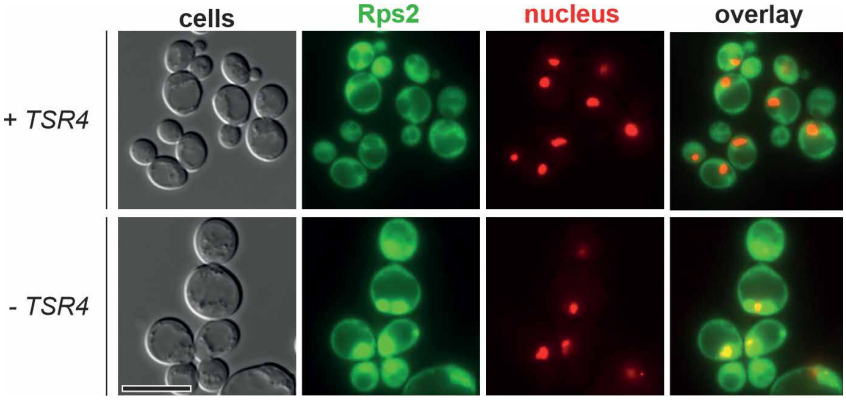


Figure 2: In the presence of Tsr4, Rps2 is mainly present in the cytoplasm, where mature ribosomes are found (no overlap with red nuclear signal). When Tsr4 is absent, Rps2 gets stuck in the nucleus (overlap of green and red signal), likely because it cannot be incorporated into nascent ribosomes and therefore does not travel back into the cytoplasm. Credit Brigitte Pertschy.

Subsequent experiments confirmed the direct interaction between Rps6 and Nap1 and also between Rps2 and Tsr4. It was found that when Rps6 was expressed alone most of the protein was insoluble, whereas when it was co-expressed with Nap1, the r-protein became almost completely soluble. Similarly to Nap1, Tsr4 also increased the solubility of its r-protein client, Rps2. These results suggest that Nap1 and Tsr4 are indeed novel dedicated r-protein chaperones. Through genetic mutation studies, the team also demonstrated the importance of Nap1 and Tsr4 in ribosome biosynthesis, leading to the model that Nap1 and Tsr4 are crucial for the efficient assembly of Rps6 and Rps2 into 40S particles.

Considering that dedicated r-protein chaperones are a very new protein class, there is not much knowledge about how they are functioning. With the thought in mind that a better understanding of the function of Nap1 and Tsr4 may also help to better understand the function of dedicated r-protein chaperones in general, the team sought to further analyse the direct interaction of the newly identified chaperone proteins with their r-protein clients.

When studying in more detail how Nap1 and Rps6 bind, the team found that almost the entire Rps6 protein is contributing to binding to Nap1. On the contrary, for Tsr4 to fully interact with its r-protein Rps2, a very small area of Rps2 is completely sufficient (Figure 1).

Differences were also observed concerning the timing of binding of the dedicated chaperone to its ribosomal protein. Nap1 does not bind Rps6 immediately when it is synthesised, but at a later time point, although it is unclear if that is still in the cytoplasm or after transport of Rps6 into the nucleus. In contrast, Tsr4 binds to Rps2 already during its production in the cytoplasm (Figure 1). The team moreover observed that when Tsr4 is deleted, Rps2 can nevertheless be transported into the nucleus, where it gets 'stuck' and is not assembled into a ribosome, indicating how critical Tsr4 is for Rps2's incorporation into ribosomes (Figure 2).

This study demonstrated that Nap1 and Tsr4, although sharing the function of protecting their r-proteins from aggregation, thereby aiding the efficient execution of ribosome biogenesis, greatly differ with respect to their binding spectrum, binding mechanism and timing of action. This highlights the diversity of the members of the group of dedicated r-protein chaperones, which employ different kinds of mechanisms to reach a similar goal.

Implications That Go Beyond the Cell

The research of Dr Pertschy, Dr Rössler, Jutta Hafner and their colleagues highlights the importance of dedicated chaperones for r-proteins during ribosome biosynthesis. This work paves the way for further studies to identify the yet undiscovered chaperones of the remaining r-proteins and understanding the common mechanisms of this unique class of protective proteins. Dr Pertschy tells us that 'ribosome biosynthesis defects can lead to diseases such as cancer and bone marrow failure,' so a deeper understanding of the intricacies of the process will undoubtedly contribute to a better understanding of certain diseases and the development of potential treatments.

Meet the researcher

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Dr Brigitte Pertschy received her PhD from the Institute for Microbiology at the University of Graz in Austria. Following several years of postdoctoral research at the University of Heidelberg in Germany and the University of Graz, she is currently a research group leader at the Institute of Molecular Biosciences at the University of Graz. Dr Pertschy has focused her research over the years on the ribosome biogenesis pathway including the ribosome assembly path of ribosomal proteins, their nuclear import, and the function of dedicated chaperones of these proteins. Dr Pertschy is a reviewer of several scientific journals including Nature Communications, editorial board member for the journal Microbial Cell and a member of multiple scientific societies.

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FURTHER READING

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