Proteins on the Move: Gateways into Mitochondria

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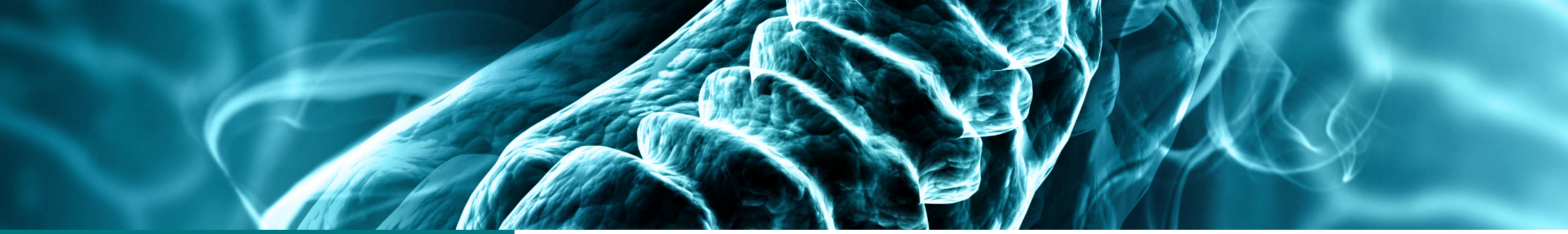


Proteins on the Move: Gateways into Mitochondria

Eukaryotic translation initiation factor 5A (eIF5A) is an essential translation factor found in nearly all living organisms. It helps ribosomes, the molecular machines that build proteins, in translating proteins containing specific amino acids. The lack of eIF5A is related to various diseases as well as ageing. Although eIF5A is known to be essential for mitochondrial function, the specific mechanism linking them has not yet been described. Professor Paula Alepuz and former PhD student Marina Barba-Aliaga (University of Valencia) work with Professor Brian M Zid (University of California San Diego) to investigate the fascinating world of eIF5A activity and its connection to mitochondria.

The Mighty Mitochondria

Mitochondria are complex organelles found inside eukaryotic cells, such as those of plants, animals, and fungi. These minute structures are critical for energy production and the synthesis of vital molecules, as key metabolic processes for cell function and survival happen inside them. Commonly known as the 'powerhouse' of the cell, the function of these organelles plays a role in health and disease development and is also a hallmark of age-related disorders. Understanding the complex interweaving processes within the mitochondria paves the way for novel approaches in medicine.

Professor Paula Alepuz carries out her ground-breaking research in the Department of Biochemistry and Molecular Biology at the University of Valencia, along with former PhD student Marina Barba-Aliaga (currently a postdoctoral fellow researcher at the Department of Molecular and Cellular Biology at Harvard University) and Professor Brian M Zid from the Department of Chemistry and Biochemistry at the University of California San Diego. Together, the team delves into the role of eukaryotic translation initiation factor 5A (eIF5A) activity in ensuring proper transport of proteins inside the mitochondria and, therefore, maintaining overall mitochondrial health and function.

Bringing in the Mitoproteins

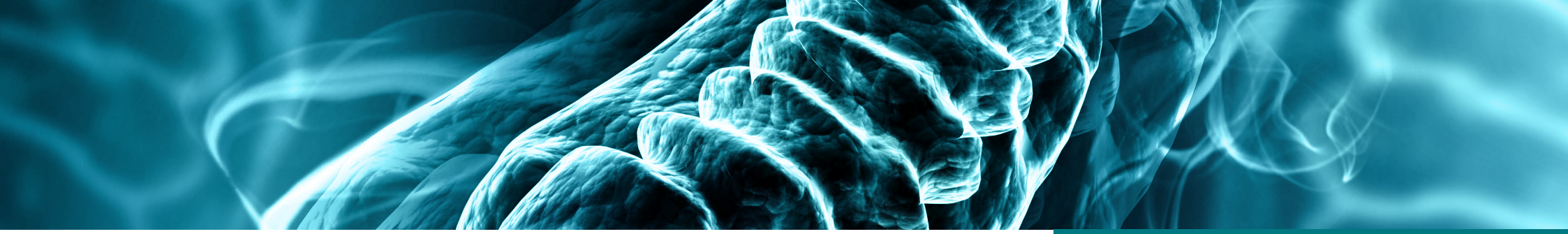
The team uses yeast cells, a type of fungus, to conduct their investigations. Around one thousand distinct proteins are found in the mitochondria of yeast, a collection called the mitochondrial proteome. Mitochondria hold their genetic material, which encodes for 1% of the proteins of the mitochondrial proteome. However, the other 99% are encoded in the genetic material found in the nucleus—a different structure within the cell.

These proteins, called mitoproteins, must be transported into the mitochondria to carry out their crucial biological functions. The importation of mitoproteins is a key process for optimal mitochondria function and involves both post- and co-translational mechanisms. Co-translational mechanisms involve coupling protein synthesis and import at the mitochondrial surface with a genetic molecule called mRNA, which is vital in protein synthesis.

Tom and Tim: The Gatekeepers

Protein translocases are found in the mitochondrial outer and inner membrane. These are molecules that help with the importing and sorting of the mitoproteins into the mitochondria. Initially, the mitoproteins enter through the universal entry gate, the general translocase of the outer membrane (TOM complex). They are then moved through the inner membrane space by interacting with the main translocase on the inner membrane (TIM23 complex). The mitoproteins hold specific markers called N-terminal positively charged presequences that allow them to be recognised by the translocases, with the most common being the mitochondrial targeting sequences. Importantly, the essential protein Tim50 works as the receptor protein in the TIM23 complex, which specifically recognises the mitochondrial targeting sequences.

Failure in the import of mitoproteins can lead to toxic effects inside and outside of the mitochondria, as unfolded mitoproteins may accumulate on the translocases, which is detrimental to the cell and linked to various diseases. Fortunately, yeast cells are specially equipped to deal with this problem with several stress responses, including reduced protein synthesis and increased removal of accumulated molecules from the translocases through proteasomal degradation.



The Essential Protein eIF5A

The protein eIF5A is an essential component of eukaryotic cells such as yeast. Its job is to help ribosomes translate messenger RNAs (mRNA, the nucleic acids obtained by transcription of DNA genes) to obtain proteins. Ribosomes are vital structures for constructing protein molecules, which work in tandem with mRNA. However, eIF5A only helps ribosomes when they have to translate mRNA sequences encoding for certain amino acids that are difficult to bond. Thus, eIF5A allows the formation of specific new protein molecules and, thus, prevents stalling of ribosomes during the protein-building process.

The production of eIF5A is regulated according to the cell's metabolic needs; for example, in yeast, production is altered in response to changes in oxygen levels. The researchers highlight that this alteration in the production of eIF5A shows the essential nature of this molecule in the function of the mitochondria; for example, a reduction in eIF5A will trigger a reduction in the rate of respiration in the mitochondria. Until now, there have been some potential explanations for the role of eIF5A in mitochondrial function, although the precise mechanism has remained unclear. Professor Alepuz and her colleagues now propose a molecular mechanism by which eIF5A impacts mitochondrial activity.

The researchers demonstrated that eIF5A plays a vital role in maintaining mitochondrial function. They found that eIF5A helped to prevent ribosome stalling during the translation or construction of the Tim50 protein, which has a crucial role in the import of mitoproteins. They reported that depletion of eIF5A triggers a cascade of events that involves the accumulation of precursor proteins, activation of mitochondrial stress responses, and, eventually, a reduction in the production of many mitochondrial proteins.

A Newly Discovered Role

Excitingly, eIF5A is much more than a simple translation factor since its role in mitochondria has a significant impact on cellular metabolism. eIF5A prevents ribosomes from stalling on Tim50 mRNA, which encodes a proline-rich sequence and is translated at the mitochondrial surface. This eIF5A's activity prevents the accumulation of proteins within the import systems and the initiation of a stress response, thereby ensuring protein import goes smoothly. As a result, eIF5A helps maintain the proper function and overall health of mitochondria. They add that this newly discovered role of eIF5A represents a distinct pathway through which cellular homeostasis, the carefully crafted balance of the processes within the cell, is achieved in terms of the activity of the mitochondria.

Professor Alepuz, Dr Barba-Aliaga, and Professor Zid's ground-breaking research has provided insights into the complex mechanisms of protein production and transport to mitochondria, and deepened our understanding of the eIF5A-mediated intricate systems at play within mitochondria and how their functions impact the overall health of the cells that they reside within.

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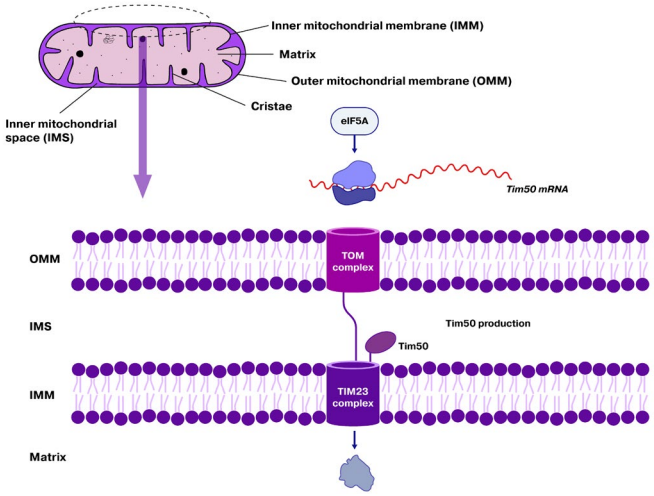

Article written by Luisa Postlethwaite, MPharm.

MEET THE RESEARCHERS

Professor Paula Alepuz
Instituto de Biotecnologia y Biomedicina (Biotecmed) & Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Biológicas, Universitat de València, València, Spain

Professor Paula Alepuz obtained her doctorate in Chemistry at the University of Valencia. During that time, she also spent 5 months studying at Johns Hopkins University in Baltimore, USA. From 1998 to 2001, she undertook a postdoctoral fellowship at the University of Vienna in Austria. In 2003, she began her career as an independent researcher after obtaining a contract from the competitive Spanish Ramón y Cajal programme, returning to the Department of Biochemistry and Molecular Biology at the University of Valencia. In 2009, she was appointed a tenured university professor, eventually becoming a full professor in 2023. She leads the Eukaryotic Gene Expression: from DNA to Protein (EGE: DtoP) group, studying gene expression with an integrated approach to the different stages, mainly gene transcription and mRNA degradation and translation. Over her well-published career, Professor Alepuz has presented at multiple conferences, lectured at various research centres, and participated in local, national, and international projects.

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Dr Marina Barba-Aliaga
Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts, USA

Dr Marina Barba-Aliaga obtained her PhD in Biomedicine and Biotechnology at the University of Valencia in 2023. Her research focused on gene expression regulation, mainly transcription and translation. During this time, she also gained international research experience at the University of California San Diego (USA) under the supervision of Professor Brian M Zid. Dr Barba-Aliaga has published numerous research publications and review articles in reputable journals. In addition to having several years of teaching experience, she has actively participated in national and international conferences, and she has taken on some leadership roles. She currently holds the position of Postdoctoral Fellow Researcher at the Department of Molecular and Cellular Biology at Harvard University (USA), where she is researching protein folding dynamics.

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Professor Brian M Zid
Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California, USA

Professor Brian Zid obtained his PhD at the California Institute of Technology in 2008, where he investigated the molecular mechanism of lifespan extension of Drosophila due to dietary restriction using genetics, genomics, bioinformatics, and biochemical methods. He undertook a postdoctoral fellowship at Harvard University from 2008 to 2015, during which he investigated the mechanisms of translational control upon nutrient starvation in S. cerevisiae using next-generation sequencing, microscopy, and biochemical methods. In 2015, he became Assistant Professor of Biochemistry at the University of California San Diego, where, in 2023, he took up his current role of Associate Professor of Biochemistry. His research has been published in several journals, and recent work focuses on gene expression and protein import.

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FURTHER READING

M Barba-Aliaga, V Bernal, C Rong, *et al.*, eIF5A controls mitoprotein import by relieving ribosome stalling at TIM50 translocase mRNA, *Journal of Cell Biology*, 2024, 223(12), e202404094. DOI: https://doi.org/10.1083/jcb.202404094