

FIRST PERSON

First person – Ioanna-Maria Gkotinakou

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping early-career researchers promote themselves alongside their papers. Ioanna-Maria Gkotinakou is first author on ‘ERK1/2 phosphorylates HIF-2 α and regulates its activity by controlling its CRM1-dependent nuclear shuttling’, published in JCS. Ioanna-Maria is a PhD student in the lab of Panagiotis Liakos at Department of Medicine, University of Thessaly, Larissa, Greece, focusing on the molecular mechanisms of the cellular response to hypoxia.

How would you explain the main findings of your paper in lay terms?

Human tissues are exposed to reduced oxygen concentration (hypoxia) during both physiological processes and pathological situations. Deprivation of oxygen elicits a number of adaptive changes that involve the upregulation of genes so that their products promote metabolic reprogramming and oxygen availability, allowing cells to survive in the hypoxic environment. The response to hypoxia is orchestrated by a small family of proteins responsible for the control of gene expression: the transcriptional activators, hypoxia inducible factors (HIFs). A tissue-specific and less-well-studied member of this family of transcription factors is hypoxia-inducible factor 2 (HIF-2 or EPAS). Like its other isoforms, the HIF-2 α subunit is controlled by oxygen levels as well as oxygen-independent mechanisms. These mechanisms involve HIF-2 α modification by a group of proteins called kinases, whose main role is to collect signals and forward them to the correct target through a process called phosphorylation. We discovered that HIF-2 α is phosphorylated at serine residue 672 by the extracellular signal-regulated kinases 1/2 (ERK1/2). Moreover, we established that HIF-2 α phosphorylation by ERKs impairs its association with exportin CRM1, which acts as a delivery truck and mediates nuclear export of proteins. Taken together, our findings unravel a novel oxygen-independent mechanism that controls HIF-2 α nucleocytoplasmic shuttling as a means to finely tune HIF-2 activation in order to support cellular adaptation to an oxygen-poor environment.

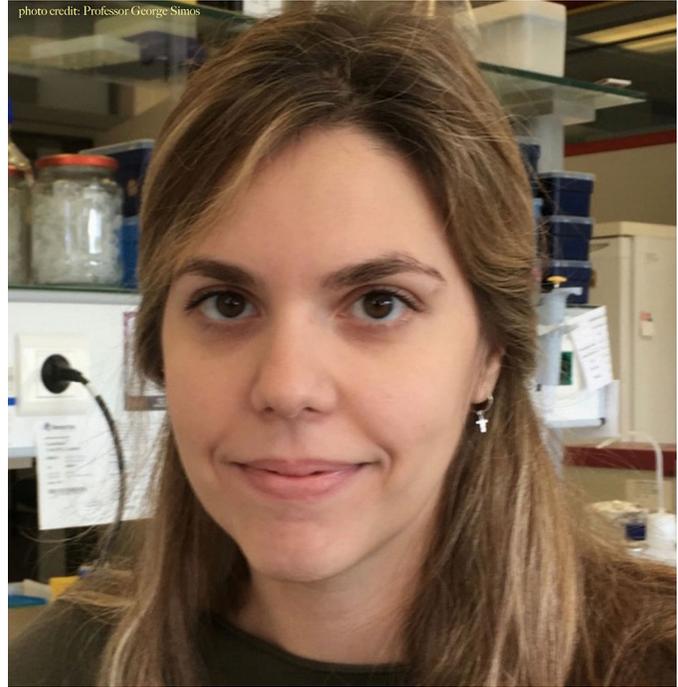
Were there any specific challenges associated with this project? If so, how did you overcome them?

The biggest challenge that I faced was that I couldn’t detect the endogenous HIF-2 α localization by immunofluorescence microscopy using the available antibodies. In order to overcome this obstacle, I performed cellular fractionation experiments.

When doing the research, did you have a particular result or ‘eureka’ moment that has stuck with you?

My ‘eureka’ moment was the discovery that HIF-2 α is phosphorylated by ERK1/2 at serine residue 672. This was both a novel finding and one that came after a long time of getting nowhere. I remember being super excited to see the *in vitro* phosphorylation results that clearly showed that phosphorylation

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levels of recombinant HIF-2 α S672A mutant form (HIF-2 α with alanine at position 672) were very reduced relative to wild-type HIF-2 α , indicating that ERK2 targets serine 672.

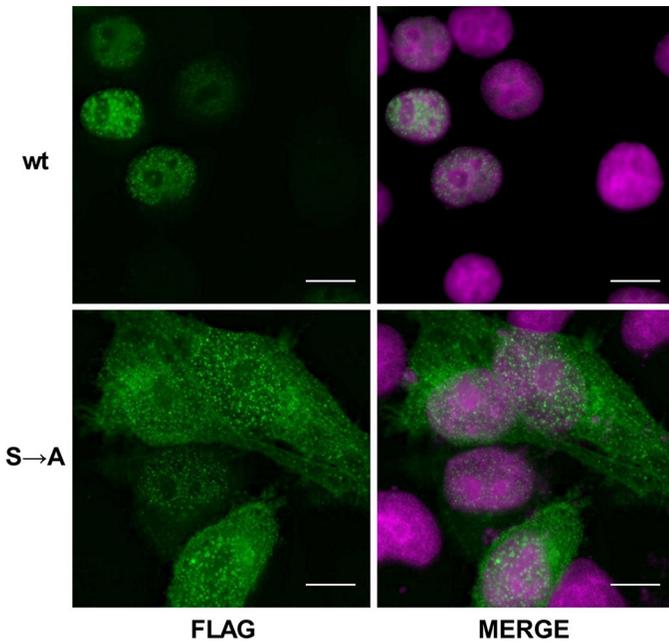
Why did you choose Journal of Cell Science for your paper?

Journal of Cell Science is a high-quality journal that covers a wide range of cell biology issues and addresses a broad scientific community. Personally, I have always found articles of interest in Journal of Cell Science. Moreover, our team has published in Journal of Cell Science in the past and we were very pleased with the clarity of the revision process and the reviewers’ constructive criticism and comments.

“I remember being fascinated by the people who conducted experiments and their dedication to science.”

Have you had any significant mentors who have helped you beyond supervision in the lab? How was their guidance special?

Dr Panagiotis Liakos, Associate Professor of Medical Biochemistry, has been my mentor throughout this early stage of my scientific career. He’s been continuously supporting and guiding me during my studies. Above all, I appreciate him for the confidence he has shown me over the years that helped me to rely on my own strengths and abilities. Another very influential person for me is the head of our laboratory, Dr George Simos, Professor of Biochemistry, whose advice and insightful comments contributed to my scientific development over the years. I feel that I should thank Dr Ilias



Immunofluorescence microscopy of HeLa cells showing the localization of overexpressed Flag tagged wild-type HIF-2 α (upper panel) and phospho-deficient S672A HIF-2 α mutant form (lower panel).

Mylonis, Assistant Professor of Biochemistry, for always being eager to answer my questions on both technical and theoretical issues. Finally, I would like to acknowledge the valuable help of post-doctoral researcher and friend Dr Christina Befani for our impeccable collaboration over the years.

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

I grew up in a science-surrounded environment. As a child, my mother often took me to her workplace, which was a genetics and plant-breeding laboratory. I remember being fascinated by the people who conducted experiments and their dedication to science. Later, I was drawn to biology and chemistry in high school and dreamed of following scientific research. Finally, it was during my diploma thesis that I realized that research was a fulfilling and enjoyable career that I was keen to follow.

What's next for you?

My immediate goal is to successfully defend my PhD thesis. In the near future, I'd love to be actively involved in cell biology research as part of an interesting project, preferably studying protein interactions and using a variety of microscopy techniques.

Tell us something interesting about yourself that wouldn't be on your CV

I really enjoy writing short stories with my friends, which we then adapt into short movies. I also love spending time with my cat, Lucy. Finally, I'm a collector of vintage dolls and I enjoy cooking.

Reference

Gkotinakou, I.-M., Befani, C., Simos, G. and Liakos, P. (2019). ERK1/2 phosphorylates HIF-2 α and regulates its activity by controlling its CRM1-dependent nuclear shuttling. *J. Cell Sci.* **132**, jcs225698. doi:10.1242/jcs.225698