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FIRST PERSON

First person – Luca Sardo

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. Luca Sardo is co-first author on 'Real-time visualization of chromatin modification in isolated nuclei', published in Journal of Cell Science. Dr Sardo is a Postdoctoral Fellow at the Department of Biological Sciences, University of the Sciences in Philadelphia, investigating the mechanisms of HIV neuropathogenesis and latency.

Luca Sardo

How would you explain the main findings of your paper to non-scientific family and friends?

The nucleus contains our genetic information in the form of chromosomes. Chromosomes are composed of chromatin, a complex structure formed by DNA, proteins and RNA. We know that the expression of the genetic code is also determined by the organization of chromatin in the nucleus. For instance, the spatial localization of chromatin can specifically regulate the expression of genes that are involved in the development of the cell and the insurgence of diseases. For these reasons, scientists are interested in understanding the nuclear architecture and chromatin organization in fine detail. The outcome of this research will allow us to better understand the biology of the cell and to find new strategies to cure diseases. In this paper, we developed a microscopy tool to visualize the content of the nucleus in real time. We did this by isolating the nucleus from the cell and observing chromatin by microscopy at the single-nucleus level. This method does not require chemical manipulation such as fixation, and maintains the nucleus intact and able to function. By using confocal and structured illumination microscopy, we visualized the architecture of the nucleus and chromatin at high resolution and were able to monitor changes of chromatin organization after treatment with drugs that inhibit specific nuclear functions.

"[…] students should just have fun being scientists."

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

The visualization of endogenous chromatin in real time was the main challenge we had to address in this work. Normally, chromatin modification is investigated by chromatin immunoprecipitation (ChIP). Despite being informative, ChIP cannot resolve changes in real time and at the single-cell level. Microscopy can visualize a single cell. However, current microscopic techniques require either fixation of the specimen or expression of exogenous fluorescently tagged proteins of interest. To overcome the limitations posed by these approaches, we developed a method to visualize live nuclear events by optical microscopy (NUCLEO-M). This method is relatively simple and can be applied to different cell types. Functional nuclei are isolated from cells and stained with immunofluorescent antibodies and dyes without fixation. This allowed us to visualize endogenous proteins and nucleic acids, and monitor chromatin changes at single-nucleus level in time-lapse experiments.

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

We were particularly excited the first time we observed that isolated nuclei are sensitive to histone deacetylase inhibitors. In those experiments nuclei isolation, staining and imaging procedures were performed in presence of a histone deacetylase inhibitor. We noticed that nuclei processed in the presence of the histone deacetylase inhibitor had higher levels of acetylated histones compared with the untreated controls. This observation suggested that isolated nuclei were functional, sensitive to drug treatment and could be used to study chromatin changes at the single-nucleus level.

Have you had any significant mentors, and how have they helped you?

I have had outstanding mentors during this early stage of my career. Among them, Dr Zachary A. Klase of the Department of Biological Sciences at the University of the Sciences in Philadelphia has given significant support to my professional development. When I was interviewed to join his newly formed laboratory, one of the first

Luca Sardo's contact details: Department of Biological Sciences, McNeil Science and Technology Center, University of the Sciences, Philadelphia, PA 19104, USA.

E-mail: l.sardo@usciences.edu

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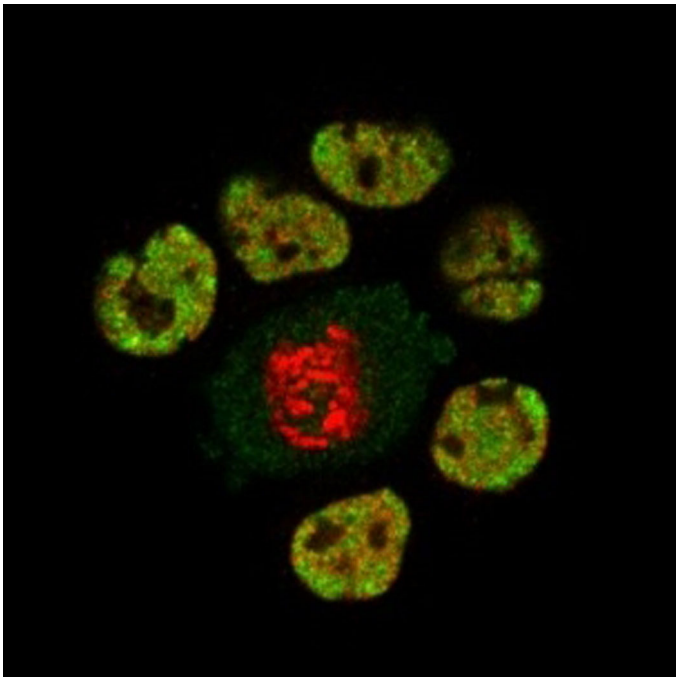

Dividing TZM-bl cell fixed and stained for acetylated histone 3 (red) and PolII (green).

questions he asked me was to define my career goal. Since then, he has tirelessly worked to help me reach that goal and to become an independent investigator. Not only he has provided creativity to the scientific process, but he has also offered opportunities for me to teach, to apply for funding and to experience academic life completely.

What's the most important piece of advice you would give first-year PhD students?

If I had to summarize my advice for a first-year PhD student, it would be to think about specific, measurable, attainable, realistic and timely (SMART) goals. Science is fascinating and many times when we undertake research projects to answer a scientific question, we are distracted by several other experiments and ideas that can be pursued. Sometimes these ideas can lead us to great discoveries, but sometimes they do not; this is part of the scientific process. However, with a clear objective in mind, science and careers can develop faster and with fewer risks. I think that a young doctoral student should read and perform experiments, but still dedicate time to visualize a career goal for the future. Besides that, students should just have fun being scientists.

What changes do you think could improve the professional lives of early-career scientists?

I believe that to select a supportive mentor and to define career goals early are paramount. Moreover, a solid professional network attracts opportunities and collaborations that lead to better careers. In this regard, I think that attending international conferences, grant-writing workshops and career-development seminars can really improve professional lives at all stages.

"[…] a solid professional network attracts opportunities and collaborations that lead to better careers."

What's next for you?

My goal is to become an independent investigator and educator in academia. I am reaching the end of my postdoctoral training and I am seeking an opportunity to start independent research in my own laboratory. My research interest is in understanding virus–host interactions with a focus on cellular regulatory functions and chromatin organization. As the reader may find evident in this article, I have a preference for live imaging techniques to address scientific questions.

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

I like to travel and sometimes I am lucky to visit places where I have never been before. I am interested in learning about new cultures and languages. In addition, I like all sort of technologies to capture images of the microscopic and macroscopic worlds. For this reason, photography became one of my favorite hobbies and reciprocally influenced my predilection for imaging techniques when I am in the laboratory.

Reference

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