

First person – Vibhu Prasad

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. Vibhu Prasad is co-first author on 'Cell-to-cell and genome-to-genome variability of adenovirus transcription tuned by the cell cycle', published in JCS. Vibhu conducted the research described in this article while a Research Associate/bridging postdoc in Professor Urs Greber's lab at Department of Molecular Life Sciences, University of Zurich, Switzerland. He is now a postdoc in the lab of Professor Ralf Bartenschlager at the Department of Infectious Diseases, Molecular Virology, University of Heidelberg, Germany, working on identification of cellular factors and processes required for flavivirus and SARS-CoV-2 infection.

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

Viruses need host cells for their multiplication. Their entry into host cells, multiplication within the host and exit from the host cells occurs by exploitation of normal cellular processes. In a human body, there are several different types of cells and, even if a cell population consists of a single cell type, there are subtle differences in the cell functions. Hence, when a virus infects a cell population, the variability among the host cells results in subtle differences in infection efficiency when focus is set to a single-cell level. Using single-molecule RNA fluorescence *in situ* hybridization (FISH) to visualize viral transcripts and 5-ethynyl-2'-deoxycytidine (EdC)-labeled DNA virus adenovirus to see single virus genomes, we checked whether the number of incoming virus genomes determines the efficiency with which the virus gene expression starts in a given cell. To our surprise, we found only a rather poor correlation between the virus genome and transcript counts early in infection. Hence, we asked a simple question, 'What other factors might be involved?' To begin to answer this, we searched for cellular features that would improve the correlation, and discovered that the G1 cell cycle stage has a significant impact on viral gene expression. Although the increased viral gene expression in G1 cells improved the correlation of virus gene expression to viral genomes, the correlation was still far from 100%, justifying future work to find more cellular factors that adenovirus utilizes to initiate its gene expression program.

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

We were using an imaging approach to measure the number of viral RNA transcripts in infected cells. When we correlated these transcripts to the cell cycle stage of the host cell using fluorescent cell cycle indicator (FUCCI)-containing cells, we realized that we could not use these cells, since a limited set of fluorophores were available for the RNA-FISH probes of the viral transcripts and these interfered with the fluorescent indicators in FUCCI cells. To overcome this challenge, I utilized DNA staining with the DAPI fluorophore as a marker for DNA content in cells to determine the



Vibhu Prasad

cell cycle stages. This led to the discovery that G1 stage of cell cycle boosts viral transcription early in infection, without any more additional and complicated experiments.

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

My 'eureka' moment in research was when I looked in to the ultrastructure of a cell in an electron micrograph and related them to the biological processes and general things around in the world. A particularly long-lasting moment was when I performed a correlative light electron microscopy experiment and visualized how a fluorescent signal in a light microscopy image correlated to the ultrastructure of organelles and vesicles as seen in an electron micrograph. 'Seeing is believing' really came true for me that day. I remember walking through the corridors of the Sagrada Família in Barcelona, and looking at the giant structures on the ceilings made me wonder if I am walking inside a giant cell with all these active cellular processes frozen in space and time.

".. when I visualized how a signal in a light microscopy image correlated to the ultrastructure of organelles [...] in an electron micrograph. 'Seeing is believing' really came true for me that day."

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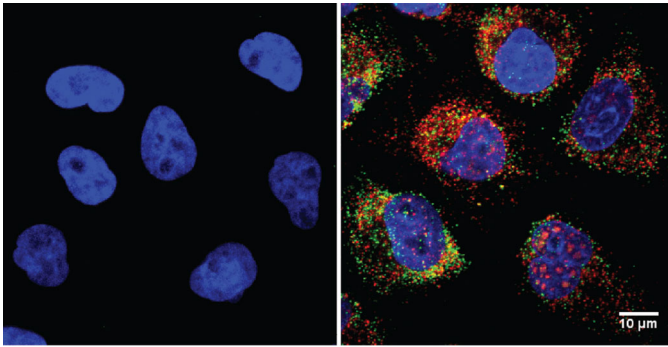


Image of adenovirus infected A549 cells. Shown is RNA-FISH staining for viral early mRNA, and for E1A (red) and E1B (green) staining. Non-infected cells are shown on the left; the cells infected with an average of 100–200 adenovirus particles per cell show an enormous number of viral transcripts per cell (right). Scale bar: 10 µm. Photo: Maarit Suomalainen, Vibhu Prasad.

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

We chose *Journal of Cell Science* due to its balanced and engaged readership of cell biologists, microbiologists and biochemists.

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

I am grateful to my PhD advisor Professor Urs Greber for his help and support throughout my time in his lab. I particularly admire the independence I had during my research time in his lab, to ask myself questions that were interesting for me. I am also thankful to Dr Maarit Suomalainen for the collaborative work in the present paper and discussions on research and topics outside lab. When I was stuck on a scientific question, the discussions we had were valuable in finding the way through.

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?

Every day is a new day in the life of a scientist. Every experiment offers a new glimpse on the biological processes still unknown to science, provided we pay attention. Finding something new in experiments drives me to try new techniques and methods.

Who are your role models in science? Why?

I seek scientific inspiration from Emeritus Professor Ari Helenius, ETH Zürich. As a member of my PhD committee, I got many opportunities to interact with him about science and his experiences on everything surrounding it. His opinions and suggestions on biological and academic problems are invaluable. The way he successfully managed his research group across two continents and four countries is beyond imagination.

What's next for you?

After finishing my postdoctoral training, I want to have an academic career and my own research group. Using viruses as active tools to understand gaps in the current knowledge in cell biology and molecular virology processes will be my main aim. There is still so much left to find out, so I am excited.

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

I have an avid interest in photography and videography. I love making photo and video blogs of the cities that I visit while mounting my GoPro camera on my bike or helmet. My imagination in capturing a photo or a video has similarities to what I want to portray in my research.

Reference

Suomalainen, M., Prasad, V., Kannan, A. and Greber, U. F. (2021). Cell-to-cell and genome-to-genome variability of adenovirus transcription tuned by the cell cycle. *J. Cell Sci.* **134**, jcs252544. doi:10.1242/jcs.252544