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FIRST PERSON

First person – Rachel Margraf

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. Rachel Margraf is co-first author on 'Connexin 43 K63-polyubiquitylation on lysines 264 and 303 regulates gap junction internalization', published in Journal of Cell Science. Rachel recently graduated from the lab of Dr Matthias Falk at Lehigh University, Bethlehem, USA, where she studied molecular mechanisms of gap junction turnover, including phosphorylation and ubiquitylation.

Rachel Margraf

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

Gap junctions are protein channels that allow ions and small molecules to pass between cells. This molecular flow is vital for intercellular communication in processes as diverse as skeletal development and contraction of the heart. One way in which cells control the appropriate amount of communication is by removing and internalizing gap junction proteins when they are not needed. For the gap junction protein studied here, connexin 43, the cellular control of this removal process involves attaching chains of a protein called ubiquitin, which flags the protein for removal. This paper describes two amino acid locations on connexin 43, lysines 264 and 303, where a particular type of ubiquitin chain, K63-polyubiquitin, attaches during connexin 43 removal. This is the first time that these sites have been identified as sites of K63-polyubiquitylation, providing new insight into the molecular events that lead up to connexin 43 internalization.

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

One of the greatest challenges in this project was that although the impact of mutating the K264 and K303 sites on the half-life and phosphorylation of connexin 43 was easily observed, the amount of K63-polyubiquitylation on these sites is very small and therefore challenging to detect. Thus, we decided to show this K63-polyubiquitylation by using two methods – western blotting and immunofluorescence colocalization. Quantifying the immunofluorescence work was quite time-intensive; I manually circled over 2800 gap junction plaques to produce the data for Figure 6 alone, and I developed coding tools such as Fiji macros to efficiently save and measure these regions during my analysis.

Have you had any significant mentors who have helped you beyond supervision in the lab?

I took over this project from my graduate student mentor, Rachael Kells-Andrews, after her graduation. Rachael and I worked closely together during the first two of my three-and-a-half years as an undergraduate student in the Falk Lab, and her patience and guidance helped me to develop from a freshman who had never pipetted before to one of the best immunofluorescence microscopists in the lab. Rachael's departure required me to rely more on myself and on other mentors in the lab, including my research advisor Matthias Falk and another inspiring graduate student in the lab, Charles 'Chuck' Fisher. Chuck supported me during the evolution of this paper as both a great mentor and a great friend. As an experienced undergrad, I was challenged less by how to perform experiments and more by deciding which experiments to perform and what conclusions to draw from my data. Chuck taught me to question everything and to assume nothing, yet to be confident and assertive in my ideas – a balance I believe is challenging for many young scientists to learn.

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 pursued a career in science because I enjoy understanding the tiniest details about the world around me and using that knowledge to solve important problems. That fascination initially drew me to cellular/molecular biology, where I sought out microscopy-heavy projects, including my work on this paper. That drive also introduced me to another field, accelerator physics. I suppose

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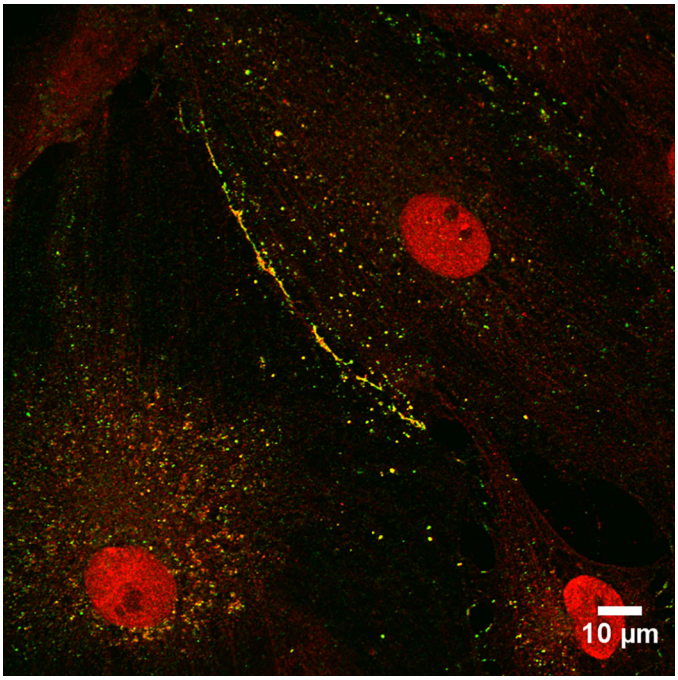

FK2 (mono and polyubiquitin) antibodies (red) colocalize strongly with endogenous connexin 43 gap junctions (green) in PAEC cells.

some of my most interesting moments in science so far have been interning at large particle accelerator research labs such as CERN and Fermilab, and explaining to physicists that I also study biology.

“Increasing dialog between fields can go a long way towards identifying problems that both fields face and finding interdisciplinary solutions.”

Based on your research experience in cellular biology and accelerator physics, how do you think scientists in these two different fields can learn from one another?

I think it is important for scientists to be at least a little bit rounded in scientific fields other than their own. Although science is a highly niched subject, it is mildly concerning to come across a physicist who doesn’t know what an amino acid is, or a biologist who won’t touch even a few basic lines of code. Each field has a specific set of tools, skills and mindsets that are used to approach problems, but at the core of each type of research I believe there are many more similarities than people expect. In my experience, having a 3 cm dish of HeLa cells die in an important biology experiment can be just as frustrating as having the power supply of a 3.4-m-long dipole magnet go down in the middle of a physics measurement. Increasing dialog between fields can go a long way towards identifying problems that both fields face and finding interdisciplinary solutions.

What’s next for you?

I completed my undergraduate dual degree in molecular biology and physics at Lehigh this spring and will be pursuing a PhD in applied physics at Stanford starting this fall. I plan to study accelerator physics at X-ray light sources such as SLAC National Laboratory’s Linac Coherent Light Source. While my graduate studies will focus mainly on physics, X-ray light sources are very relevant tools for molecular biology, providing protein structures through X-ray crystallography. In my future career I hope to further improve the capabilities of X-ray light sources and the information they can provide about biological systems.

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

In my free time, I enjoy writing science fiction. I like to joke that if I wasn’t constantly distracted by real science, I might actually finish a book…

Reference

Kells-Andrews, R. M., Margraf, R. A., Fisher, C. G. and Falk, M. M. (2018). Connexin 43 K63-polyubiquitylation on lysines 264 and 303 regulates gap junction internalization. *J. Cell Sci.* **131**, jcs204321.