Weisswursts & Western Blots

by William Rivers

Photos by William Rivers

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There are lots of ways to get involved with research. You might have a burning question you want answered, or maybe you simply jump on board with a professor's existing project in a field in which you are interested. Personally, I have worked on a couple of professors' projects and I found them all in slightly different ways. The most unique one I found by combing through the database of fellowships that the Office of Fellowships and Scholar Programs maintains on their website. The program is called DAAD Rise, and it provides a stipend for students from the United States, Canada, or the United Kingdom to spend 10-12 weeks at a top tier German institution conducting research. The Deutscher Akademischer Austauschdienst (German Academic Exchange Program) provides a list of internships, delivers the students' applications to the graduate students in charge of the research, and ultimately picks which students receive internships and where. I applied to and was placed into the lab of Dr. Dejana Mokranjac in the Physiological Chemistry Department of Ludwig-Maximilian University in Munich to work with a doctoral student named Umut Gunsel. In a sentence, I joined their project to help biochemically and structurally characterize the TIM23 mitochondrial protein translocase.

The vast majority of mitochondrial proteins are synthesized in the cytosol and need to be imported into the organelle. TIM23 is a protein complex responsible for transferring proteins into the mitochondrial matrix or into the inner membrane. Because about 70% of mitochondrial proteins utilize this pathway to enter the mitochondria, it is essential for function of the mitochondria and its breakdown has severe negative effects on mitochondrial function and cell viability. Therefore, having a greater understanding of how the complex works not only provides a molecular insight into a fundamental cellular process but may also help understanding the cellular and biochemical basis of human disorders.

My role in the project was to analyze the dynamics and local environment of Tim23's N-terminal region during protein import. To do this we created 11 yeast mutants that each encoded a molecule called Benzoylphenylalanine (Bpa) in a different position of Tim23's N-terminal region. Bpa is a synthetic, photoreactive amino acid that covalently bonds nearby molecules when it is irradiated with UV light. The products of such reaction are called crosslinks. We then grew the yeasts, irradiated them, isolated the proteins from the cells, separated them by SDS-PAGE, and did a Western Blot analysis with Tim23 antibodies to visualize Tim23 and its crosslinks. SDS-PAGE separates proteins based on size, and the Western Blot tags Tim23 using Tim23-specific antibodies so we can see where on the gel it and its crosslinks are located.

The Tim23 versions with a Bpa incorporated at an amino acid position near another molecule appear at a different location on the gel from where Tim23 is normally located because the bond between the molecule and Tim23 combines their sizes. Therefore, the analysis allows us to identify which amino acid positions in Tim23 are near another molecule by looking for these shifts in protein size and identifying where we incorporated the Bpa for that mutant. The next step is identifying the molecules each mutant bound, since the identity and characteristics of these molecules may give insight into the mechanism of protein import.

Overall, the Bpa molecule was one of the most interesting things I learned about from this research. I hadn't ever worked with a synthetic amino acid before, considered how

they might be incorporated into a protein, or thought about them ever outside of class. After working with Bpa for a summer, it seems applicable to a lot of other research projects and really just expanded my knowledge of general techniques that can be used when studying proteins. As a side note, knowing about it also helped me on a test in which the professor asked us how a certain mechanism might be explored experimentally. I am also glad that I got the chance to learn more about protein analysis. At USC, I spent a lot of time manipulating E. coli and yeast genetics, but prior to this experiment was never able to work with the proteins we were creating due to issues in the mutation process. Understanding how to introduce mutant genes is useful, but mostly for the sake of producing protein mutants.

The whole process of going to Munich for research was a really cool experience not only for the research but also for the ability to travel around Germany and Europe. Most of my favorite experiences this summer came from those adventures and misadventures in the mountains around Munich and exploring the cities I found myself in. However, my all-time favorite moments were when I got the opportunity to spend time with the other students, graduate and undergraduate, in the department and German language course. I enjoyed learning more about them, where they came from, and their cultures. Since we were in Munich, several of these moments were in the world famous beer gardens which didn't hurt either.

Coming into the experience I was really nervous for a lot of different reasons. First was that I did not speak any German and I was concerned that it would make daily life difficult. I was also worried that I would not really understand a lot of the project or that I would mess up the research. These fears turned out to be relatively unfounded. A lot of the people in Germany, especially the younger population, speak English at least at a conversational level, and everyone in the Physiological Chemistry Department spoke fluently. The few times I faced a language barrier were when I was traveling to smaller, less touristy towns or outside of Germany. In terms of research, a lot of the work that I did was similar to work that I had already done here at USC and it was relatively easy to apply what I already understood to a new project. However, there were several new techniques and ideas to work with, especially since it was my first time working with protein analysis. While I took some time to really understand the new processes, my lab mates were really patient with me and helped walk me through everything that was new. Overall, it was a great experience and I would recommend anyone interested in research to reach out to the Office of Fellowships and Scholar Programs to talk about applying to DAAD Rise or at least to the Office of Undergraduate Research to start looking at getting involved in research here at USC.

Learn more about DAAD Rise