Bao-Liang Song: Loving biology in the time of cholesterol

Marie Anne O'Donnell

Song studies the trafficking and biological activities of cholesterol.

Bao-Liang Song's parents raised him in a small and relatively impoverished town in central China. Like many others, his parents believed education was the best way to a better life and did their utmost to send Song and his sisters to school. Driven by his parents' unconditional love and support, Song studied hard and quickly became the top student in class. He loved mathematics and physics by nature and excelled at them, winning first and second prize in the Chinese Physics and Mathematics Olympiads. Song applied to Nanjing University with the hope of training in either of these subjects; however, he was enrolled as a biology student because neither the math nor physics department was recruiting from his region that year. As he says of this time, "Though somewhat disappointed at the very beginning, I soon found out biology could be cool to learn as well. I liked spending time in the library where I could read and think deeply." The unexpected redirection of Song's studies toward biology has led to a successful research career investigating how organelles control where cholesterol goes in the cell and what it does en route.

We contacted Song to find out more.

Where did you study before starting your own laboratory?

After graduation, I went to the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, and did my PhD with Dr. Bo-Liang Li, the director of the institute back then. The subject of my research was ACAT2 (acyl-CoA:cholesterol acyltransferase 2), an ER-resident enzyme that catalyzes the formation of cholesteryl esters. Dr. Li is not only a great mentor who introduced me to the cholesterol field, but also a generous colleague to work with when I became an independent principal investigator. We recently published a paper together in *Nature Cell Biology* showing that Cys277 of ACAT2 was competitively regulated by ubiquitination and oxidation in response to varying lipid levels (1).

In 2001, one year before I got my PhD, Dr. Joseph Goldstein and Dr. Michael Brown paid their first visit to China, which was quite a hit. I met these two Nobel Laureates at the seminar and had a short scientific talk with them afterwards. I never imagined this meeting would totally change my life. I was later offered a postdoc position in their laboratory at the University of Texas Southwestern Medical Center in Dallas. I still remember how thrilled I was when I first heard the news. During my three and half years in their laboratory, I collaborated with Dr. Russell DeBose-Boyd and characterized lanosterol and 24,25-dihydrolanosterol as physiological regulators that could trigger degradation of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), the rate-limiting enzyme in cholesterol biosynthesis (2). I also found that the ubiquitin ligase gp78 was mandatory for sterol-regulated HMGCR degradation (3). These experiences reinforced my confidence in continuing to research cholesterol metabolism as an independent scientist.

"One of the questions that intrigue me most is how cholesterol . . . travels between intracellular compartments"

What drew you to study cholesterol in particular?

I started working on cholesterol because Dr. Li's laboratory is a cholesterol laboratory and I wanted to be one of his students [Laughs]. It turned out to be a great choice and I got more and more obsessed with this ancient, simple molecule. As we all know, cholesterol is an essential component of eukaryotic membranes as well as a precursor to steroid hormones, bile acids, and vitamin D. In the past century, intense efforts were made to address how elevated cholesterol levels contribute to cardiovascular diseases. More recently, there is increasing appreciation that disturbed cholesterol homeostasis also correlates with many neurodegenerative diseases, such as Alzheimer's. Very recently, we discovered that cholesterol can mediate hedgehog (Hh) signal transduction by covalently linking to Smoothened (Smo), a second cholesterol-modified protein (4). Our understanding of cholesterol keeps moving forward with the help of rapid advances in technology. It is exciting to embrace the unknowns.

Bao-Liang Song. PHOTO COURTESY OF BAO-LIANG SONG.

What is your laboratory actively working on?

One of the questions that intrigue me most is how cholesterol, particularly that from low-density lipoprotein (LDL) particles, travels between intracellular compartments. We recently demonstrated that LDL-derived free cholesterol, after being inserted into the lysosomal membrane by Niemann Pick type C1 (NPC1) and NPC2 proteins, can translocate to peroxisomes through the lysosome–peroxisome membrane contact (5). Interestingly, we observed robust cholesterol accumulation in lysosomes well in advance of the manifestation of neurological phenotypes in mice with X-linked adrenoleukodystrophy, the most common peroxisomal disorder (5), implying a contribution of cholesterol transport blockage to these diseases. Inspired by these exciting results, we are interested in understanding the detailed mechanisms regulating lysosome–peroxisome membrane contact formation as well as how

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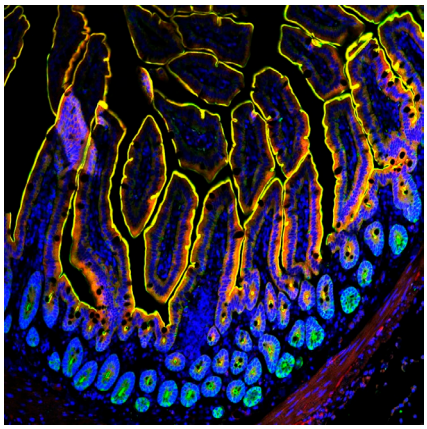


Mouse small intestine labeled with the antibodies against NPC1L1 (green) and Villin (red). Nuclei are shown in blue. IMAGE COURTESY OF YING-YU ZHANG.

cholesterol transfers at this interface. We are also trying to figure out the next destination of cholesterol upon arriving at the peroxisome. Additionally, we are actively investigating cholesterol transport at other places, for example, between the ER and the plasma membrane.

Another question that fascinates us is how exactly cholesterol modification occurs on Smo. We previously used a synthesized cholesterol probe in combination with click chemistry reaction and identified Smo as a second cholesterol-modified protein in addition to Hh (4). SMO cholesterylation is essential for Hh signaling and embryonic development and we are interested in understanding the underlying mechanisms but are also curious to find out if there are additional proteins modified by cholesterol.

What kind of approach do you bring to your work?

I received professional and well-rounded training as a biochemist in Dr. Goldstein and Dr. Brown's laboratory, for which I have been so grateful. And I continued with the classic "biochemical" style—black and white immunoblots and quantitative curves—for quite a long time after I established my own laboratory. One day when I was preparing slides for a talk, I felt bored all of a sudden and realized that I needed to bring something new into my research to make the data look much prettier, or at least more colorful and diverse [Laughs]. So I started using other approaches in my work, such as microscope-based imaging, animal physiology studies, and human genetic analyses. This led to the pattern we now use to dissect a scientific question, as you can tell from our recent papers. We usually begin with a cell-based screen to search for phenotype-causing candidate genes. After several rounds of verification to narrow down the candidate list, we focus on one and further examine the underlying mechanisms using biochemical and cell biological methods. Next, we look at its pathophysiological function in animal models as well as determining if there is any correlation with human disease. Eventually, based on what we learn from these approaches, we aim to develop novel and effective strategies to alleviate or rescue the phenotype. Although screening is the first and foremost step of the whole project, we always try to combine this with the latest concepts and techniques, for example, CRISPR/Cas9, lncRNA, Click Chemistry, and so on, so that the final story won't look outdated when it's done.

"The reason we keep driving beyond tradition is simple: to discover something truly exciting"

What did you learn during your PhD and postdoc that helped prepare you for being a group leader?

Being a group leader is never an easy thing. My current way of running a laboratory is a combination of what I learned during my training with Goldstein and Brown and my own experience over recent years. At UT Southwestern, I personally benefited a lot from the cell culture support team they had, which saved substantial amounts of time on passaging and plating cells. I think this highly efficient workflow also enhances consistency of the work, at least the cell culture part, produced by different trainees. So I passed down this tradition when I set up my first laboratory in China and we currently have a cell culture technician, an animal husbandry technician, and another technician that takes care of daily routine work. It is definitely expensive but worth every penny. I also try my best to share the valuable lessons I learned from my postdoctoral training, such as how to design experiments, how to explore the optimal conditions, and how to evaluate the data critically, with my students. I spend time writing step-by-step instructions for students, especially the beginners, before they start experiments so that they execute my ideas precisely and efficiently. With more experienced students, I instead encourage them to propose alternative approaches to those I planned. Honestly, working with students can be very exhausting; however, it seems to be the best way to maintain high standards of research. Plus, it is rewarding to see these young people developing into future stars.

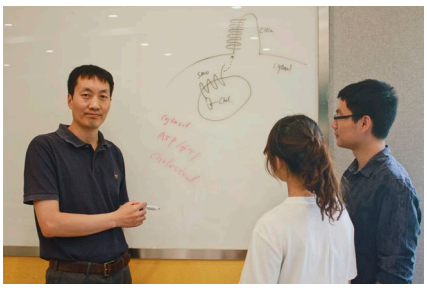
What has been the biggest challenge in your career so far?

My biggest challenge has always been to step out of my comfort zone and do something new and risky. In fact, my research focus has evolved a lot since I established my laboratory in 2005. In the first two years, I continued to follow my postdoctoral project and identified Ufd1 as a cofactor of gp78 in regulating HMGCR stability. I then spent another seven years defining the mechanisms by which the Niemann Pick C1-Like 1 (NPC1L1) protein mediates intestinal cholesterol absorption (6). More recently, we moved into the cholesterol trafficking field and try to understand how cholesterol is dynamically transported within the cells. We are also expanding our research to determine the functional consequences of cholesterylation during development and cancer. I must admit that there have been enormous efforts, countless failures, and, of course, high risks behind each of these transitions. However, the reason we keep driving beyond tradition is simple: to discover something truly exciting.

Any tips for a successful research career?

Follow the questions that really interest you. Set up reliable research systems. Keep the standards high. Be alert to new techniques and never be afraid of challenges. Last but not least, enjoy the beauty of science.

1. Wang, Y.J., et al. 2017. *Nat. Cell Biol.* 19:808–819. https://doi.org/10.1038/ncb3551
2. Song, B.L., et al. 2005. *Cell Metab.* 1:179–189. https://doi.org/10.1016/j.cmet.2005.01.001
3. Song, B.L., et al. 2005. *Mol. Cell.* 19:829–840. https://doi.org/10.1016/j.molcel.2005.08.009
4. Xiao, X., et al. 2017. *Mol. Cell.* 66:154–162. https://doi.org/10.1016/j.molcel.2017.02.015
5. Chu, B.B., et al. 2015. *Cell.* 161:291–306. https://doi.org/10.1016/j.cell.2015.02.019
6. Ge, L., et al. 2008. *Cell Metab.* 7:508–519. https://doi.org/10.1016/j.cmet.2008.04.001



Song (left) discussing experiments with students. PHOTO COURTESY OF DAN LIANG.