JCB Journal of Cell Biology

PEOPLE & IDEAS

Lena Ho: Micropeptides under the spotlight

Lucia Morgado-Palacin

Lena Ho studies small ORF-encoded peptides (SEPs; also known as micropeptides), with a particular focus on mitochondrial SEPs, and their role in vascular biology and immunometabolism.

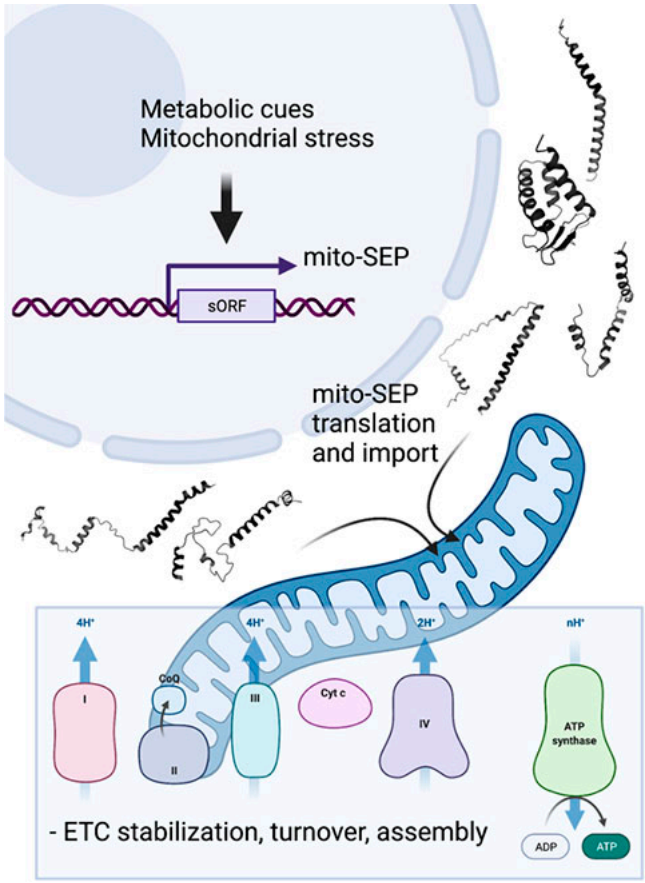
"Mom, I want to be an actress"—sadly for the entertainment industry and luckily for science, the teenage dream of Lena Ho of performing theater arts did not materialize. Although, to be fair, there were few chances of her becoming a Broadway star: Lena used to devour Shakespeare books but was kind of deadpan in her mannerisms, and she hated rote learning, so memorizing lines wouldn't have panned out well for her. She preferred to apply logic instead, as she thinks that "even in the seeming chaos of the biological world, everything is objectively based on a set of predictable ground rules." So, over time, her love for science grew while that for the performing arts faded. But, besides its rational aspects, science had always been fun for Lena. She has vivid memories of the little science lab in her primary school—while simple in appearance, the sprouting bean or the moldy bread experiment was effective in prompting kids to ask questions, make observations, and draw conclusions. Lena originally contemplated the idea of pursuing medicine after high school and even got accepted into the only medical school in Singapore, the National University of Singapore (NUS). However, she was—in her own words—"deadly afraid of cadavers, no pun intended, and had a mental block about needing to attend anatomy lessons, which is literally the first class they make you do." Fortunately, at the same time, she was awarded a scholarship from the government, who invested in sending students overseas to train and return with new ideas and skills, to study biochemistry and microbiology at the University of Wisconsin-Madison in the U.S. Lena accepted the challenge immediately, without even being entirely sure of which country Wisconsin was in!

During her undergraduate, Lena sequenced bacterial genomes to find determinants of *Escherichia coli* pathogenicity in Nicole Perna's lab and, under the supervision of Michael Cox, investigated what makes *Deinococcus radiodurans* resistant to radiation—"I was really fascinated how bacteria could be resistant to γ-irradiation when we were so vulnerable, something I thought about every time I walked past the nuclear reactor on campus." Because of her obsession with microorganisms, one could think that Lena would stay in the field of microbiology for the rest of her scientific trajectory—but that was far from reality. Inspired by an introduction to immunology course given by Gary Splitter, Lena switched gears and enrolled in an immunology PhD program at Stanford University, where she worked with Gerald Crabtree, known for defining NFAT functions in immune activation. In the Crabtree lab, Lena focused on how chromatin remodeling by the SWI/SNF-like BAF complex regulates myeloid cell fate determination and developmental transitions in embryonic stem cells (ESCs). After 10 yr abroad, Lena returned to Singapore for her postdoc. Not afraid to tackle new topics, she decided to join the lab of Bruno Reversade at the Agency for Science, Technology and Research (A*STAR) to characterize the role of an unknown long non-coding RNA, *AK052978*, in the growth of the early human embryo. Lena discovered, to everyone's surprise, a potential ORF in *AK052978* that encoded a very small protein

Lena Ho. Photo by Lena Ho.

of 54 amino acids—which they named ELABELA—and was highly conserved throughout vertebrate evolution. The path to show that the small ORF-encoded peptide (SEP; a.k.a. micropeptide) but not the RNA was indeed the relevant biological entity was arduous, but it finally paid off when they found a receptor for ELABELA, the apelin receptor, and uncovered that this newly discovered signaling axis sustained human ESC self-renewal (1) and regulated cardiovascular development in zebrafish and mice (2, 3). ELABELA was a small bite of the micropeptide world that Lena was about to discover. In 2017, she established her independent lab at Duke-NUS/A*STAR aimed to portray the full landscape of micropeptides.

lmorgado@rockefeller.edu.



Schematic representation of mito-SEPs and their cellular functions. Image provided by Lena Ho and created with Biorender.

Lena has recently been selected as an European Molecular Biology Organization Young Investigator (2021). We contacted her to learn more about her current and future research—despite her admitting in the interview that she would like to run a coffee shop and a pizzeria in a parallel universe, her excitement when talking about science says otherwise.

What interested you about mitochondrial SEPs and immunometabolism, and what are you currently working on?

After discovering ELABELA in the Reversade lab, I was eager again to venture out into new territory, so I started my own group with the goal of identifying new SEPs in mind. To achieve this, I followed Isaac Newton's advice that "truth is ever to be found in simplicity," so I first asked where else in the cell are SEPs working? To answer this question, I did a very simple analysis of the size distribution of proteins in various cellular compartments. The results were striking. The mitochondria were by far the organelles that had the highest number of small proteins under 100 amino acids, which I used as the cutoff for SEPs. This prompted us to develop a platform to use ribosome profiling data to uncover and predict which peptides localize to the mitochondria, which we call "mito-SEPs". We chose the top 250 candidates to experimentally verify and ended with about 20 that were bona fide mito-SEPs (4)—we've been studying these ever since. While examining mito-SEP candidates computationally, we came across a striking anti-correlation of mito-SEPs with signatures of inflammation, so we went on to screen for mito-SEPs that are integral to regulating the outcomes of inflammation. This led us to find a bifunctional gene, *C15ORF48*, that encodes both a mito-SEP, MOCCI, and a microRNA that work in tandem to resolve inflammation in cardiovascular cells by modulating the electron transport chain (5). Because we don't have a hypothesis a priori when studying SEPs, we are reliant on empirical observations and where they lead us. I think this is the best way to make discoveries.

We are currently trying to better understand how and why small peptides are so well-positioned to function in the mitochondria, along with the exact mechanism of the novel mito-SEPs that we have discovered. We are also eager to see if any of them can be targeted for clinical value.

What kind of approach do you bring to your work?

My motto is "be true, be curious, be unwavering." Working in SEP deorphanization, being tenacious and perseverant is key, as sometimes you feel like you are working in a dark for a long time before a breakthrough happens. Be curious because that provides the drive that fuels what we do, i.e., to characterize a gene whose function was oblivious to the world and which was thought of not even existing. Sometimes people wonder why I study things that don't seem to have any perceived importance or phenotype. I usually reply that every gene has a phenotype, but you just need to know how and where to look. Being true is essential—one of my favorite refrains in the lab is "it is what it is." When someone brings a piece of data that does not support our hypothesis, we accept what it tells us, as long as it is clean data. We just need to get better at asking the right question because we are working on unpaved grounds, without any prior background knowledge about a given peptide. One tiny mistake could set us on the wrong course for a long way, so it's better to be realistic and let go of your favorite hypothesis rather than waste time barking up the wrong tree.

What did you learn during your PhD and postdoc that helped prepare you for being a group leader? Is there anything you were unprepared for?

I had the freedom during both my PhD and postdoc to craft all my projects and manuscripts from start to end, so I got a very good sense of how to ask a good question and tell a nice story that people will be interested to hear about. I think that's very important part of science—to design and communicate effectively.

However, I was unprepared for working simultaneously on 10 projects, now that I lead a team. I prefer to work on one project at a time, but that is obviously not tenable when there are students and postdocs who all want to develop their own projects. I was also unprepared to be the perpetual cheerleader for my trainees even when I'm having

The Ho lab having dinner, pre-pandemic. Photo by Lena Ho.

a bad day. When you are the PI [principal investigator], you must be strong and encouraging, the steady hand that guides the ship, no matter how much stress you are fielding. I sometimes feel lonely because I think PIs are expected to be larger than life, and able to do a million things well and still keep it together at all times without complaining. I think it's a much tougher job than most people think it is.

Lastly, I was unprepared for how harsh editors at big journals are to newly minted assistant professors, now devoid of the support of having the name of their postdoc supervisor as a senior author. The first time I ever had a paper rejected without review in my career was the first paper we published reporting our discovery of the preponderance of mito-SEPs (4). It's by far, I feel, the best work of my career, but clearly, editors at the major journals did not think so. I was surprised, because I have confidence in my acumen. After its publication, I got a lot of compliments on the paper, the kind that I did not receive for previous papers that were published at much higher-impact journals. So go figure!

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

In my 4.5 years being a PI, I would say it is having built a niche and research program from scratch. The mito-SEP work literally started when I got to my new office. We didn't know we were going to be working on mitochondria. Now everyone in the building comes to us for their mitochondria questions. We've generated a lot of meaningful research leads that will keep us going for many years, if the funding gods are willing.

Another major accomplishment is to help trainees reach their desired career and life goals. One of my postdocs has already transitioned to an independent faculty position. My first student is also in his final year and has grown so much scientifically since he first started. Knowing that you've personally made a big positive difference in someone's life trajectory is very gratifying, more so than having papers or citations.

And the biggest challenge in your career so far?

Geographical isolation from the rest of the scientific world is a problem here in Singapore. We are an island physically, and also scientifically. During the pandemic, this was much more poignant when we could not go to meetings, and we are in the wrong time zone to participate productively in virtual conferences—think 12 a.m.–6 a.m. I tried but failed many times... Being isolated also poses challenges to recruiting. The pool of local scientists and technicians in Singapore is very limited because of our small population. But few would want to move here to be a trainee because it's a faraway and expensive city.

You've been clear about how the challenge of starting a niche from scratch became a big accomplishment for you. If you could turn any challenge in academia into an opportunity, what would it be?

I like the European model where postdocs don't always have to feel that they are trainees in transition to a "permanent" job. We should fund talented researchers to stay in academia even if not at the level of a PI, but rather at the level of a scientist, to continue contributing to scientific progress. Funding bodies should recognize this as a viable alternative and accord funding to accommodate these alternate career paths, as long as they stay productive. There are obviously not enough PI positions for all our trainees to become PIs, but that doesn't mean they have to exit academic research if they don't want to.

The current funding scheme seems like a drawback to secure these permanent "scientist" positions in academia. And the limited funding is often an obstacle too for early-career investigators to fully develop their ideas. So, in the hypothetical case that you had unlimited funding, which crazy ideas you would be working on?

Well...more like if I had unlimited talent and passion. But if I had unlimited funding, I would like to develop in silico methods that predict within 80% accuracy the molecular function of a SEP without any experimentation, leveraging on existing data in the public domain—we are reaching a stage where 90% of a paper is done in silico, so experiments will no longer be exploratory but rather confirmatory. A bit of a long-term goal—if there are any readers out there who think you can help, please get in touch with me!

And unrelated to my current line of work, I would like to recapitulate the positive effects of exercise on the skeletal, cardiovascular, and neurological systems, preferentially in a pill [laughs]. I hate exercising but force myself to do it. Let's be honest that many of us would welcome such a pill!

To finish this interview on a more personal note, what has been your biggest accomplishment outside of the lab?

Nurturing my child who was born at 28 wk of gestation and helping her overcome all the challenges that ensued. And learning to play the violin in midlife!

References

1. Ho, L., et al. 2015. *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2015.09.018
2. Chng, S.C., et al. 2013. *Dev. Cell*. https://doi.org/10.1016/j.devcel.2013.11.002
3. Ho, L., et al. 2017. *Science*. https://doi.org/10.1126/science.aam6607
4. Zhang, S., et al. 2020. *Nat. Commun*. https://doi.org/10.1038/s41467-020-14999-2
5. Lee, C.Q.E., et al. 2021. *Nat. Commun*. https://doi.org/10.1038/s41467-021-22397-5