People & Ideas

Fumiyo Ikeda: Ubiquitin lines up for action

Ikeda is studying the biological significance of linear ubiquitin chains.

Modification with ubiquitin monomers or ubiquitin chains can regulate a protein's binding to appropriate partners, participation in signaling cascades, or targeting for degradation. But there are several different kinds of ubiquitin chains in cells that differ in which lysine residue is used to link each ubiquitin monomer to its neighbor. For example, linear ubiquitin polymers, which link together through their C-terminal lysines, are structurally distinct from lysine-63–linked chains and also exhibit different biological functions.

The diverse functional roles of different ubiquitin chains are still being teased out. Fumiyo Ikeda is working to pull linear ubiquitin chains free of the tangle. With a background in the mechanics of immune signaling (1, 2), Ikeda started studying ubiquitin biology as a postdoc in Ivan Dikic's laboratory (3–5). She soon demonstrated that linear ubiquitin chains regulate NF-κB signaling (4), a pathway critical to inflammatory processes. But, as she explained from her new lab at Vienna's Institute of Molecular Biotechnology (IMBA), that's probably not the end of the line for linear ubiquitin.

REACHING OUT

As a child, what kind of career did you hope for?

When I was a child I wanted to be a writer of children's books or a teacher because I loved to read. It sounds a little bit nerdy, but I was always reading. Whenever my parents or grandparents gave me any money, it all went toward buying books.

But your first degree is in dentistry…

I grew up in Japan, and there you have to choose at the end of high school what you want to study in university. When I was in high school, I was of course still reading a lot of books, including some by doctors, and I became very interested in how organisms cope with disease. I decided that I wanted to study either medicine or dentistry, and, when I passed the entrance exam to the Osaka School of Dentistry, that is where I decided to go.

I enjoyed my studies there, but interacting with patients was very difficult for me. It made me sad that patients never really want to see the dentist—kids especially don't like going to the dentist and get very unhappy about it! But once you have chosen a major at university it is very difficult to switch, so I had to continue my dentistry degree. Fortunately, around the same time I decided I didn't want to be a dentist, I took a three-month course in laboratory science. I really liked it, so I entered the PhD program in the same school. I really wanted to work on some disease-based project, focusing on molecular mechanisms, so I joined Dr. Toshiyuki Yoneda's laboratory to work on signaling pathways in osteoclasts, a type of immune cell that remodels bone.

> "I wanted to learn something new, but I also wanted to use my knowledge from my PhD."

Why did you go abroad for your postdoc?

When I was in high school, I spent a year as an exchange student with an American family in Cleveland, Ohio. Even though I had seen American movies and TV shows before I went there, it was a huge shock for me because it was so different from Japan and everything was so much more open. Ever after that, I knew that I wanted to spend more time abroad, to see places and cultures that were different from life in Japan.

Initially I wanted to return to the United States because it is one of the best countries to do research. I had a few interviews in Boston, but none of the laboratories felt like a good fit for me; one was too large, and the other was too narrowly focused on osteology. I wanted to branch out into new subjects, and I wanted to work in a smaller lab with a young PI, because I like the energy a young PI brings to their research.

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Fumiyo Ikeda

In the end, I found Ivan Dikic's lab in Frankfurt, Germany. Ivan had done a postdoc together with Riko Nishimura, who was my direct supervisor, in New York. When I heard he was looking for a postdoc, I went there to interview. Somehow, I liked his lab right from the start. They were studying a signaling pathway—ubiquitin signaling—rather than a specific biological function, which appealed to me.

UNTANGLING A SURPRISE

Was it your idea to work on ubiquitin signaling in immunology?

Yes, it was. I wanted to learn something new, but I also wanted to use my knowledge from my PhD. Dikic's lab studies ubiquitin signaling generally but, when I entered the lab, nobody was working on ubiquitin-mediated immunological signaling.

One of the focuses of the Dikic lab when I arrived was the study of proteins that have a ubiquitin-like domain—a region that folds similarly to ubiquitin. Based on studies of amino acid sequence, our collaborator, Kay Hofmann, had predicted that a protein called TBK-1 should have a ubiquitin-like domain. TBK-1 was known to help regulate the expression of

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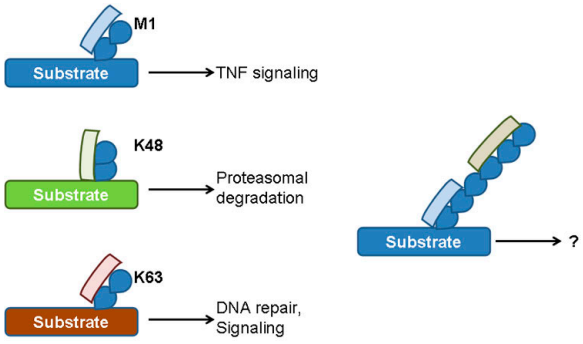

IMAGE COURTESY OF FUMIYO IKEDA

Linear ubiquitin chains (M1) have different biological roles than other chain arrangements (K48, K63 chains).

interferon-inducible genes, so this seemed like the perfect thing for me to start on. Right away I found that the TBK-1 ubiquitin-like domain is important for regulating downstream signaling because it is needed to help the neighboring TBK-1 kinase domain fold properly. My second project in the Dikic lab was more ambitious.

You're referring to your work on how ubiquitination regulates NEMO activity?

Yes. NEMO is a critical subunit of the IKK complex that regulates activation of NF-κB, a transcription factor that is important in many immunological contexts. It had been known for many years that NEMO has a ubiquitin-interacting domain, and I was already familiar with NEMO from my work on osteoclast signaling. But as a new postdoc, I wasn't ready to work on NF-κB signaling right away, which already had a lot of big labs working on it. So I left it for later.

The question we wanted to ask was: How does NEMO interact with ubiquitin? Many previous publications had shown that NEMO makes a complex with lysine-63–linked ubiquitin chains. Other labs were trying to co-crystallize the NEMO ubiquitin-binding domain with lysine-63 chains, but they were struggling to get good crystals.

Our collaborators, Soichi Wakatsuki's lab in Tsukuba, Japan, were using linear ubiquitin chains to co-crystallize with the ubiquitin-binding domain. They were doing this because linear ubiquitin and lysine-63 ubiquitin chains are structurally similar but linear ubiquitin chains are easier to synthesize in the lab. To all of our surprise, they got a beautiful structure, which showed that the two proteins interact in a very different way than we expected. But at the time, there wasn't any known biological function for linear ubiquitin chains, so we weren't sure this was a real interaction. What changed our mind about it was that the co-crystal structure showed which amino acid residues are critical for the interaction and, when I made mutations in these residues, they abolished downstream signaling. Also, I could easily detect a direct interaction of NEMO with linear diubiquitin but not with lysine-63 diubiquitin. So we had to change our minds—which was hard because it went against all the dogma in the field—but now it is accepted that NEMO interacts exclusively with linear ubiquitin and that this interaction is critical for the activation of NF-κB.

> "We had to change our minds—which was hard because it went against all the dogma in the field."

FOLLOWING THE THREAD

So linear ubiquitin is now accepted to be biologically relevant?

Around the same time, two other labs published papers about the importance of linear ubiquitin in the regulation of NF-κB signaling. One of these, Kazuhiro Iwai's lab in Japan, had also published studies about an enzyme complex, LUBAC, that generates linear ubiquitin chains in cells. In my last project in the Dikic lab, we identified a new subunit of this complex, SHARPIN, that regulates NF-κB signaling.

Now that I have started my own laboratory at IMBA, one of our aims is to understand how, enzymatically, linear ubiquitin chains are created. We know that LUBAC is responsible for this, but it's not yet clear how it achieves the ligation reaction. Another of our aims is to clarify what biological processes linear ubiquitin chains regulate.

Do you have that "new PI energy" you were looking for as a postdoc?

Yes! I have a small group right now because I only started my lab recently. But I plan to grow it gradually.

Are you still excited about living abroad?

Yes, although as I have gotten older I have become more interested in a stable life. I will probably have to move again in the future because I am not in a tenure-track position, but that is not something I am afraid of.

I think eventually I would like to return to Japan. After I left there, I started to appreciate the positive aspects of my country, and I miss it very much. I'm hoping that one day I will find a position where I can educate young people in Japan.

1. Ikeda, F., et al. 2007. *EMBO J.* 26:3451–3462.
2. Ikeda, F., and I. Dikic. 2008. *EMBO Rep.* 9:536–542.
3. Rahighi, S., et al. 2009. *Cell.* 136:1098–1109.
4. Ikeda, F., et al. 2010. *Cell.* 143:677–681.
5. Ikeda, F., et al. 2011. *Nature.* 471:637–641.

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Ikeda has just started her own lab at Vienna's IMBA.