Marko Kaksonen: Tracking the inner secrets of endocytosis

Marko Kaksonen has always been interested in the natural world and in science, from childhood explorations of the forest paths near his home to adult explorations of the natural labyrinth that is cell biology. But, he says, it took him awhile to find the subject he was most passionate about—and to select the path his career would ultimately follow.

As an undergraduate and then doctoral candidate studying syndecan-3 in Heikki Rauvala’s laboratory at the University of Helsinki, Kaksonen explored the byways of cell culture and fixed-cell immunofluorescence, and behavioral neuroscience and electrophysiology (1, 2). During the course of these studies he encountered GFP tags and live-cell microscopy—and everything finally came into focus. Joining David Drubin’s laboratory in Berkeley for his postdoctoral work, Kaksonen sought to capitalize on this new technology to explore the cell cytoskeleton. Combining live-cell microscopy with yeast genetics, he probed the cellular purpose of mysterious structures known as actin patches, and showed that they are endocytic sites (3, 4).

Kaksonen’s studies with Drubin yielded several important insights about the composition and regulation of the endocytic machinery in yeast. But there’s still so much more to learn, says Kaksonen. Now starting his own laboratory at the European Molecular Biology Laboratory (EMBL), he says he’s dedicating much of his energy to further studies on endocytosis (5). We tracked him down to talk about his past work and what he sees as the way forward.

**SEARCHING FOR A CLEAR PATH**

**When did you first become interested in science?**

I think I was interested in science and nature since I was a very little kid. My family lived in a small town in Finland, and as a child I spent a lot of time with friends exploring the woods around where we lived, just for fun. I became interested in all the animals and plants and little creatures we would dig up there. Then, I studied genetics as an undergraduate at the University of Helsinki. While still an undergraduate I joined Heikki Rauvala’s neurobiology laboratory, where we studied syndecans—a type of transmembrane proteoglycan—and their ligands. Syndecans are involved in neuronal development and plasticity (that is, memory and learning processes), and in axon guidance. I got interested in this subject and stayed on there to do my PhD.

**What was your PhD project while in Heikki’s laboratory?**

My project was to make a syndecan-3 knockout mouse. This protein is expressed at high levels in different areas of the brain during development, so we’d expected a knockout animal might have an interesting phenotype. But when I had made the mouse, it turned out that the mice seemed quite normal. I did lots of experiments that showed that there was no difference between the knockout and the wild-type animals, and it wasn’t really clear what to do next. Finally, as a result of collaborations with other groups, we did find the phenotype of these animals. A group at Harvard had made a mouse that overexpressed syndecan-1 that had abnormal feeding behavior—but syndecan-1 is not expressed in the parts of the brain that regulate feeding behavior, whereas syndecan-3 is expressed there. They had heard we were making a knockout mouse, so they contacted us, and together we investigated this and eventually published a paper showing defects in feeding behavior.

In the meantime though, I started working with cell cultures, trying to understand the link between transmembrane proteoglycans and the cytoskeleton, because there was lots of evidence to suggest that they regulated cell adhesion—important for neurite outgrowth. This got me more and more interested in the cell biological aspects of these proteins.

**FORK IN THE ROAD**

**Was there a particular point where you realized you were shifting your emphasis?**

We had a sabbatical visitor in the laboratory, Benjamin Peng, who at that time was a professor at Chapel Hill. He was interested in the same proteoglycans that our laboratory was studying, and when he visited he brought with him a GFP cDNA, which at that time was still relatively new technology. Benjamin knew how to do live cell imaging, and we made a GFP fusion plasmid for cortactin, a cytoskeletal remodeling protein, and transfected it into cells. I had been looking at the cytoskeleton in fixed cells for quite a while, trying to figure out what was going on—it all seemed kind of abstract to me. But then suddenly, with the GFP fusions, I saw these things come alive! It was very exciting.

**And that spurred you to switch fields for your postdoc?**

Yes. While it was very interesting to study the nervous system, especially in
such a complex organism as the mouse, it was also somewhat frustrating, because it’s quite laborious and takes a long time to make a knockout. The cycle from hypothesis to experimental result to new hypothesis is very long. I wanted to work with some other model organism that is simpler and easier to use, and of course yeast is much simpler and also one of the best genetic model systems. Also, the cytoskeleton, which is where my interest was now focused, is quite accessible in yeast.

So you joined David Drubin’s laboratory at Berkeley—did you know specifically what you wanted to study there? I had read some papers about actin patches, and I thought they were probably the simplest cytoskeletal structures that exist; they’re just tiny spots you see on the surface of the cells without any underlying structure. Around that time I saw a paper from Fred Chang’s laboratory where they had used live cell imaging and quantitation to visualize actin patches and get some quantitative data about them, describing how they move, form, and disappear. I thought if I could combine yeast genetics with this kind of quantitation, it would be a great system to study the actin cytoskeleton. I didn’t really have any more specific ideas in my mind; at the beginning I just thought, “Okay, this is a nice thing to look at, a very simple actin structure.”

Taking in the Sights
But it turned out that these actin patches are not simple at all. At that time it wasn’t really clear what these actin patches were, so the first thing I did was to further characterize them. We found that they’re really endocytic sites. This wasn’t totally unexpected because there were data that suggested that they might be involved in endocytosis, but how they were involved wasn’t clear. When we looked more closely at them, we saw that there are really quite a large number of proteins that localize to these tiny actin patches. So, we explored what proteins are recruited, and what the timing of their association with actin patches can tell us about how endocytosis proceeds.

What parts of this work have you taken with you to your own laboratory at EMBL?
I’m still working on endocytosis. We’re focusing mainly on the early part of the pathway, trying to figure out how the endocytic site forms, looking at proteins such as clathrin and adaptor proteins that we see going to the actin patches at the beginning. We want to understand what triggers the formation of the endocytic site. When and how is the location of endocytic sites determined? The timing and location of endocytosis is clearly regulated in yeast cells, and is somehow linked to the cell cycle, but it’s not really very well understood how this happens.

Another process that most likely happens during the assembly of the endocytic machinery is the recruitment of cargo, which also is tightly regulated in yeast. For example, there’s evidence that changes in the culture medium can trigger endocytosis of certain cargo proteins, and this is something that we’re working on now. Eventually, we also want to look at other membrane trafficking processes, like exocytosis, using similar approaches as in our other work: live cell imaging combined with genetics. We have so many questions about these processes. It’ll be fun to chase down the answers. JCB