

## Rüdiger Klein: Reading the guideposts for axon guidance

Klein uses genetically modified mice to study how axons find the right targets to innervate.

In order for the nervous system to function properly, precise connections must be formed between individual neurons and the cells they synapse with. The growing ends of axons often travel long distances, integrating both attractive and repulsive cues along the way, before reaching their destinations. Rüdiger Klein has spent much of his career deciphering the guideposts axons use during their treks (1–4).

Klein's career didn't follow a straight path, though, as he traveled through several different countries and research subjects early in his career. He visited South America and spent time in the United States as both an undergraduate exchange student and as a postdoc (5). But ultimately he returned to his native Germany, where he is now Director of Molecular Neuroscience at the Max Planck Institute of Neurobiology in Martinsried. We reached him there to talk about his intellectual and physical travels.

### WANDERLUST

*How did you decide on a career in research?*

My biology teacher in high school was very influential. The way he taught us was very exciting, and I got excited about ecology. I enrolled in biology at university because of him.

At first, I wanted to become a political biologist, like one of those people who are in the Green Party, saving the environment. However, my father (who was in politics; he was the opposition leader in the German state of Rheinland-Pfalz, and the mayor of the town where I grew up) pointed out that there are very few jobs in that profession. I decided I wanted to become a parasitologist instead because I was excited about the complicated life cycles that parasites go through, and I also thought that by studying parasitology I could help people.

But then, before starting my PhD, I took a trip to South America and visited some hospitals while I was there. It was depressing. The working conditions were poor, and it was very hard to get money for research, so I decided to change my focus again. I studied virally encoded platelet-derived growth factor during my PhD.

*And then you did your postdoc with Mariano Barbacid?*

Yes. Mariano, who was at the National Cancer Institute in Frederick, Maryland, when I joined his lab, had just cloned an oncogene that he called Trk. It looked like a receptor, but they had no idea what it did or what its ligand was. So, my first job was to clone the mouse homologue of this human oncogene so that we could study its expression during mouse development and maybe find out its normal function. I started screening a mouse brain cDNA library with a human Trk probe, pulled out some clones and sequenced them. They looked similar to Trk, but they were not identical, so we knew we had a different gene; we called it TrkB, and started calling the original gene TrkA. But I wasn't able to pull out mouse TrkA—I only pulled out this new gene.

On the one hand, Mariano was happy that we had found this new gene, but on the other he was still unsatisfied because he wanted to have the original one. So another postdoc, Fabienne Lamballe, and I went back and this time screened the library with both the human

TrkA probe and the mouse TrkB probe to try to pull out mouse TrkA. Instead, we found a third Trk gene and called it TrkC [laughs].

In fact, we never pulled out mouse TrkA from this brain library. Later, we found out why: there is very little TrkA in the brain. TrkB and TrkC are mostly



Rüdiger Klein

PHOTO COURTESY OF GSNIMU

found in the brain, but TrkA is mostly found in the peripheral nervous system, in sensory neurons and so forth, where it is a receptor for NGF, which supports these neurons. So then we went on to use knockout technology to disrupt these genes in mice. It was exciting to see that when you knock out the TrkA gene all the neurons that express it—ones that innervate the skin and are responsible for sensation of heat and pain—die, so the mice basically have no sense of heat or pain.

### DECISIVE MOVE

*You continued to study Trk in your own lab at EMBL in Germany?*

For a little while, yes, but then I decided to move into a slightly different family of receptor tyrosine kinases. As a postdoc, I had been fortunate to learn how to make knockout mice—that was a new technology at the time, and it wasn't available in many places—and I wanted to use knockout technology to study orphan receptors.

I was intrigued by the interesting expression patterns of some novel orphan receptors called Eph receptors (or Ephs),

**"It looked like a receptor, but they had no idea what it did."**



IMAGE COURTESY OF DR. IRINA DUDANOVA

**A motor axon growth cone (green) approaching a cell expressing an ephrin (red).**

so we started making knockouts of the receptors. Right around this time, the ligands of these Ephs were found. It became clear that one of these ligands, which are now called ephrins, is an axon guidance cue. So then we knew what to look for in the mice, and it only took a few weeks to find that some of the major axon bundles in the brain were misguided in our Eph knockout mice.

One interesting thing that we've discovered since we started working on ephrin and Eph signaling is that it is bidirectional. For example, one group of ephrins, the B-type ephrins, look like little receptors; they have a transmembrane domain and an intracellular portion. Both we and Tony Pawson's lab in Toronto, with whom we were collaborating, independently found that B-type ephrins are transiently phosphorylated on an intracellular tyrosine residue when they bind to an Eph receptor. That was a very clear indication that the B-type ephrins could do reverse signaling, and now people have also found that the A-type ephrins (which do not have an intracellular domain) can as well, by interacting with other transmembrane proteins that serve as coreceptors.

### **What purpose do Eph receptor signaling and ephrin reverse signaling serve?**

You can find situations in which one cell is purely giving a signal but does not respond to a signal; it's like a guidepost cell. Let's take the example of the spinal cord midline, which expresses a B-type ephrin: it makes sure that axons that are not supposed to cross the midline stay on one side and find their synaptic partners on that side. If you remove the ephrin or its corresponding Eph genetically, those axons cross the midline and cause trouble. But, if you just truncate the B-type ephrin so that it cannot signal into the midline

cells, then you have no phenotype. Those midline cells present the ephrin, but they don't respond to an axon that binds to them. There are many situations like this where signaling is only required unidirectionally; it can occur either from the ephrins to the Ephs or the other way around.

On the other hand, Ephs and ephrins are also involved in the development of blood vessels. Here, it is believed that when a blood vessel wants to grow a new sprout both Ephs and ephrins need to become activated so that cells can lose contact, move away from one another, and start dividing to form a new sprout. The signaling is really bidirectional in this situation.

### **CURRENT DIRECTION**

#### ***In what direction are you now headed with this work?***

One of the questions we're interested in is how Ephs and ephrins mediate contact repulsion. The first thing that happens is ephrins binding to Ephs, which are high-affinity binding interactions. Normally such interactions mediate adhesion, but in this case they do the opposite.

What we've found is that you have to destroy or remove this ligand-receptor complex from the cells' surface for the cells to detach. There are two ways this

can happen: either through activating metalloproteases that cleave the ectodomains or by another rather aggressive method where cells actually eat a piece from the opposing cell and engulf—phagocytosis—the entire Eph-ephrin complex. We would really like to understand that pathway.

**"We want to understand how axons integrate attractive and repulsive cues."**

Another question that interests me is that, under certain conditions, Eph-ephrin signaling is attractive instead of repulsive. For example, when a neuron's axon arrives at its final target, Ephs and ephrins help to make synapses. How cells switch between repulsion and attraction is not understood. And, we're

examining how neurotrophic factors—which are often attractive for axons—might act as a counterpart to ephrins. We want to understand how axons integrate attractive and repulsive cues.

1. Henkemeyer, M., et al. 1996. *Cell*. 86:35–46.
2. Brückner, K., et al. 1997. *Science*. 275:1640–1643.
3. Kullander, K., et al. 2001. *Neuron*. 29:73–84.
4. Zimmer, M., et al. 2003. *Nat. Cell Biol.* 5:869–878.
5. Klein, R., et al. 1991. *Cell*. 65:189–197.

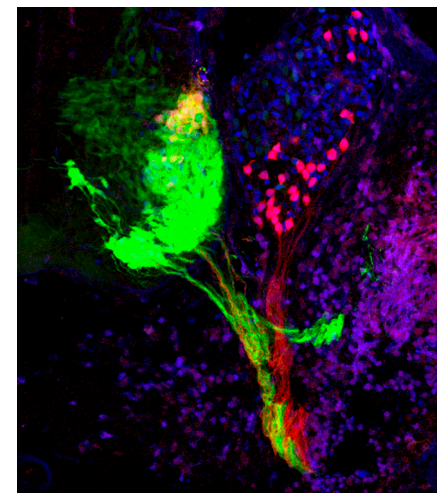


IMAGE COURTESY OF DR. IRINA DUDANOVA

**Section of a mouse transgenic embryo with motor (red + green) and sensory (red) neurons highlighted. Islet1 is shown in blue.**