Communicating Research to the General Public

The WISL Award for Communicating PhD Research to the Public launched in 2010, and since then over 100 Ph.D. degree recipients have successfully included a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance—as well as their excitement for and journey through their area of study—to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere, through the cooperation of PhD candidates, their mentors, and departments. WISL offers awards of $250 for UW-Madison Ph.D. candidates in science and engineering. Candidates from other institutions may participate, but are not eligible for the cash award. WISL strongly encourages other institutions to launch similar programs.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

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Coordination of ion channel delivery and dendrite growth in Drosophila sensory neurons

By
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Chapter 4: Studying proteins in fruit fly neurons: Communicating my thesis research to the public through the Wisconsin Initiative for Science Literacy

Contributions: I wrote this chapter in collaboration with the Wisconsin Initiative for Science Literacy, with edits and suggestions from Elizabeth Reynolds, to communicate my thesis research to a broad, non-science audience.

Why I wrote this chapter

I wrote this chapter because my personal and professional goal is to make science exciting and accessible to everyone. As an educator, I achieve this goal through teaching and involving undergraduates in the process of science. As a scientist, I strive to get others excited about science by distilling my research into terms that can be broadly understood by a general audience with minimal to no scientific background. I went to graduate school to further my scientific education to pursue a career in teaching undergraduate students. In graduate school, I gained experience asking scientific questions, testing hypotheses, and disseminating my research discoveries to contribute to the progression of scientific knowledge. Not everyone is trained to understand primary scientific literature, which is full of jargon and requires prior knowledge. Therefore, in this chapter, my hope is that I can share the exciting journey I have been on for the last 5 years studying a protein in fruit fly neurons.

Early in graduate school, I was introduced to the concept of the Wisconsin Idea, which states that “education should influence people’s lives beyond the boundaries of the classroom.” This Idea has been central to my teaching and science outreach efforts. I believe that scientific knowledge must be shared effectively and widely, especially in today’s environment, in which misinformation spreads like wildfire on social media platforms. Additionally, I believe that anyone can be a scientist and that we need more voices in Science, Technology, Engineering, and Mathematics (STEM) fields. The mission of the Wisconsin Initiative for Science Literacy (WISL) is “to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.” As a scientist and

educator, I am inspired by this mission, and I strive to work towards these goals. I am so thankful that Emeritus Chemistry Professor Bassam Z. Shakhashiri, Elizabeth Reynolds, Cayce Osborne, and other members of WISL value effective science communication and provided this platform for me to communicate my research to the public.

Our senses help us navigate the world

Imagine eating an ice cream cone outside the Chocolate Shoppe on State Street in Madison, WI. You might **taste** the sweetness of the ice cream and **smell** the earthy summer breeze drifting across Lake Mendota. You might **see** people riding bikes and skateboards along State Street and **hear** the music at the Terrace as you **feel** the bench you are sitting on and the warm sun on your skin. Your senses allow you to experience the joy of this moment. Most humans have six primary senses: touch, sight, hearing, smell, taste, and proprioception (balance and spatial awareness). Senses allow us to experience joyful moments and they also protect us from potential dangers. Imagine hitting your finger with a hammer. You may feel a sharp pain and immediately stop and jerk your hand away. How quickly did you feel the pain? How quickly did you react? How did your brain know that your finger was experiencing pain? In both the ice cream and hammer scenarios, sensory neurons allow you to perceive external stimuli. But what exactly is a sensory neuron?

What is a sensory neuron?

Sensory neurons are special cells that sense external stimuli and generate messages that are communicated to the brain. There are sensory neurons dedicated to

processing all the senses listed above. In my research, I study a specific type of sensory neuron that allows an organism to feel painful stimuli (imagine the hammer example). These types of sensory neurons, called nociceptors, are embedded in our skin and they allow us to feel potentially harmful stimuli. But how does a cell (the neuron) “feel” an external input?

The structure of a sensory neuron is important for how it functions. Neurons are composed of 3 main compartments: dendrites, the cell body, and the axon. The structure of a neuron is like a tree. So much so, that after spending 5 years looking at sensory neurons, every time I look up at a tree, I am reminded of a neuron. In this analogy, the dendrites are like the branches, the cell body is like the top of the trunk where the branches all meet, and the axon and axon terminals are like the length of the trunk and roots of the tree (Figure 1).

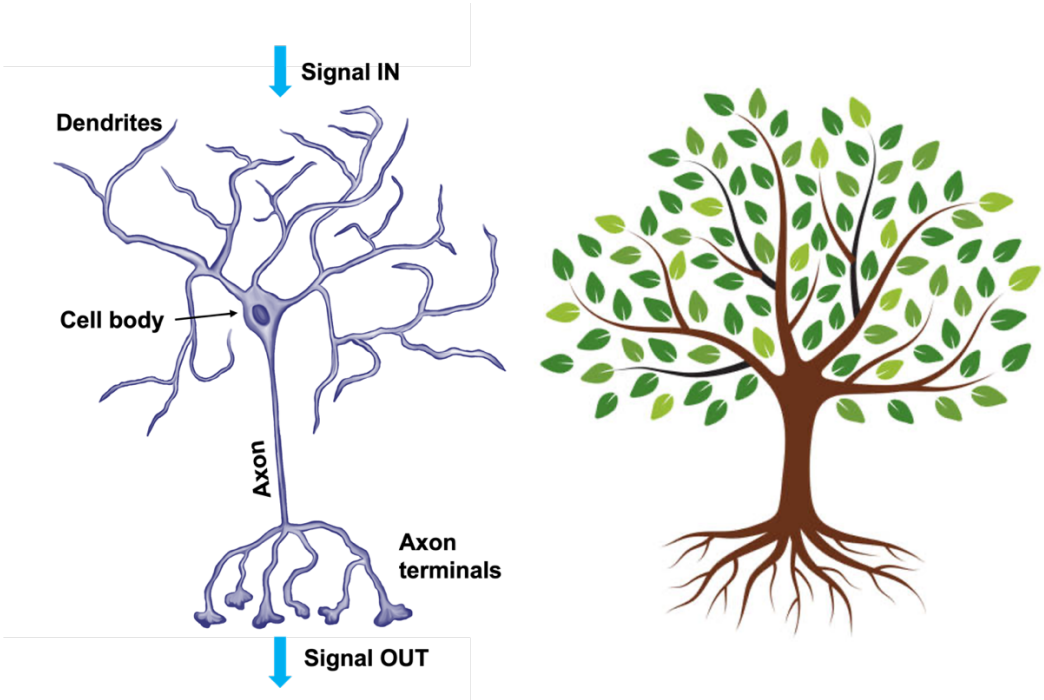

Figure 1. The shape of a neuron is similar to a tree. The dendrites (branches) receive signals. The cell body (top of the trunk) houses the nucleus, which contains the DNA. The axon and axon terminals (length of trunk and roots) send electrical signals to other neurons. Tree image: https://www.istockphoto.com/photos/tree-roots-branches

Dendrites (the branches) receive inputs from other neurons, or in the case of a sensory neuron, dendrites receive external stimuli. The cell body (the top of the trunk) houses the nucleus, which contains DNA. The axon and axon terminals (length of the trunk and roots) sends electrical messages to the next neuron in the circuit, and ultimately to the brain. Formation of these 3 distinct compartments is important for how the neuron functions, but how does each part of the neuron (dendrites, cell body, axon) perform its distinct functions?

Each compartment of the neuron contains a unique mix of proteins that perform specialized functions. In my research, I study a protein called **Pickpocket** (nicknamed Ppk1), which has the specific function of acting as a "door" into the dendrites (Figure 2). These "doors" are called ion channels, and just like their name implies, they are channels into the cell that allow ions to pass through.

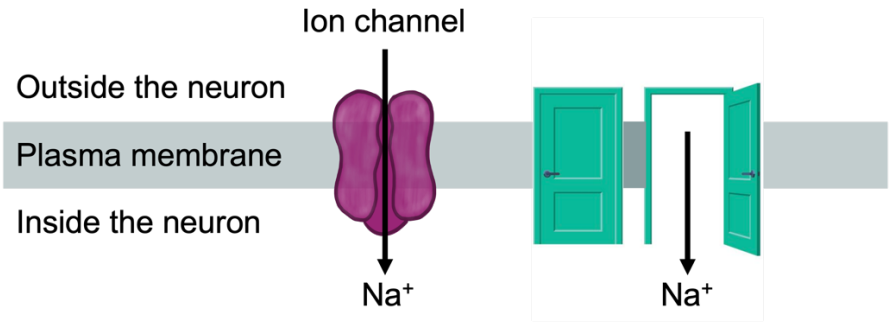

Figure 2. Ion channels function as doorways into the neuron. Just like a door, ion channels can open and close. Ion channels, such as the Pickpocket ion channel that I study, allow ions to pass into the cell through the plasma membrane (grey bar). In this example, the sodium (Na^+) ion is moving into the neuron through the ion channel (colored magenta).

You may be wondering, what is an ion? Consider table salt (sodium chloride, or NaCl). When NaCl is dissolved in water, it forms positively charged sodium (Na^+) and

negatively charged chloride (Cl^-) ions. Ions are what create the electrical messages processed and sent by neurons. The Pickpocket ion channel is found in the plasma membrane (outer cell boundary) of dendrites (the branches) in fruit fly sensory neurons. But why am I studying an ion channel in the sensory neurons of the pesky fly that loves to inhabit your fruit bowl?

Why do I study sensory neurons in fruit flies?

The human nervous system is extremely complex, and it is challenging to experimentally access the inner workings of human neurons. To better understand biological processes, researchers study model organisms, including mice, fruit flies, worms, fish, bacteria, and yeast. Even though humans look very different from all these organisms, we share much of the same DNA that codes for important proteins in cells. A quick refresher on how our DNA codes for proteins: DNA stores our genetic information and is what we inherit from our parents. DNA can be transcribed into RNA and then RNA is translated into proteins. Proteins are microscopic functional machines that perform important jobs in the cell (Figure 3).

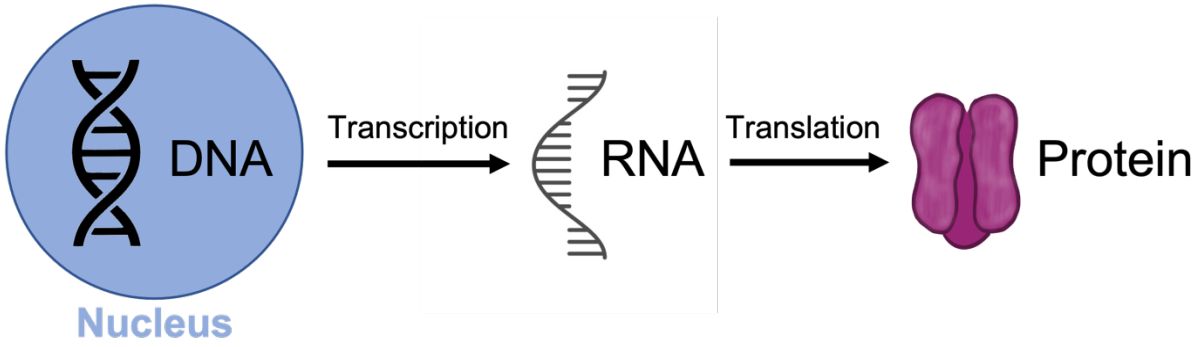

Figure 3. DNA codes for protein machines that perform jobs in the cell. The "Central Dogma" of biology describes the flow of information from DNA to RNA to proteins. Genetic information is stored in DNA. Transcription involves decoding the DNA into RNA messages. Translation involves reading the RNA message and creating a protein. Proteins perform various jobs throughout the cell.

This conservation (similarity) of DNA across animals means that a protein in a model organism often performs similar functions in humans. By studying proteins in model organisms, we can learn about biological processes that are similar in humans and ultimately apply our discoveries to human health and disease.

I was first introduced to model organisms in my genetics lab in college. Under the mentorship of two excellent genetics professors at Grand Valley State University, Bruce Ostrow and Georgette Sass, we studied how traits are passed down from parents to offspring in fruit flies. I learned how to cross male and female fruit flies and count the offspring that inherited certain traits from their parents. This was my first introduction to application of Mendelian inheritance (in class I had learned about Gregor Mendel's discoveries regarding inheritance in pea plants), and I believe that these experiences got me hooked on the power of the tiny fruit fly for exploring important scientific questions.

In my thesis research, I study sensory neurons in fruit flies, or *Drosophila melanogaster*. There are many practical advantages to studying biological processes in fruit flies. They are inexpensive to maintain, and they have a short lifespan. It takes about 10 days for a fruit fly egg to progress through developmental larval and pupal stages and then hatch into an adult fly, making it efficient for us to do experiments in a short amount of time. I study sensory neurons in the fruit fly larvae, which is about the size of a grain of rice. Larvae are transparent (see-through), and the sensory neurons I study are located right underneath the skin (cuticle). These fly sensory neurons are similar to the human nociceptor neurons I mentioned earlier that are embedded in our skin and allow us to feel painful stimuli. Because of their superficial location, I can use a

microscope to see the sensory neuron in a living larva and study how proteins get to the right place in the neuron.

Motivation for my research: How does Pickpocket get to the right place in the neuron?

The central question of my research is: What regulates the localization of the Pickpocket ion channel in sensory neurons? To understand this question, I will first describe what I mean by “regulation” and “localization.” Remember that neurons are complex cells that contain specific compartments that perform unique functions. So how does the DNA, which is housed in the nucleus in the cell body (trunk), generate a protein that performs its job far away in the dendrites (branches)? To think about the significance of this challenge, first consider an epithelial (skin) cell. These cells are cube-shaped and there are shorter, simpler routes for a protein to be transported to the site where it functions than in a neuron. Now, consider the tree-shaped neuron. Proteins must be transported over longer distances and navigate a more complicated cell shape. So how do proteins move within a neuron?

The movement of proteins within a neuron is a fascinating process that is similar to cars on a highway. There are specific proteins that act as cars (these are fittingly called motor proteins, and more specifically named kinesin-1 and dynein) and the cars drive along highways. The cellular highways are made up of other proteins that form the cytoskeleton. Much like the bones of our own skeleton, the cytoskeleton also provides structural support to cells. The cytoskeleton is composed of many copies of protein building blocks called tubulin that link together to form microtubules. The motor protein

cars carry cargo (other proteins that need to get to a specific place in the neuron) along the cytoskeleton highways (Figure 4).

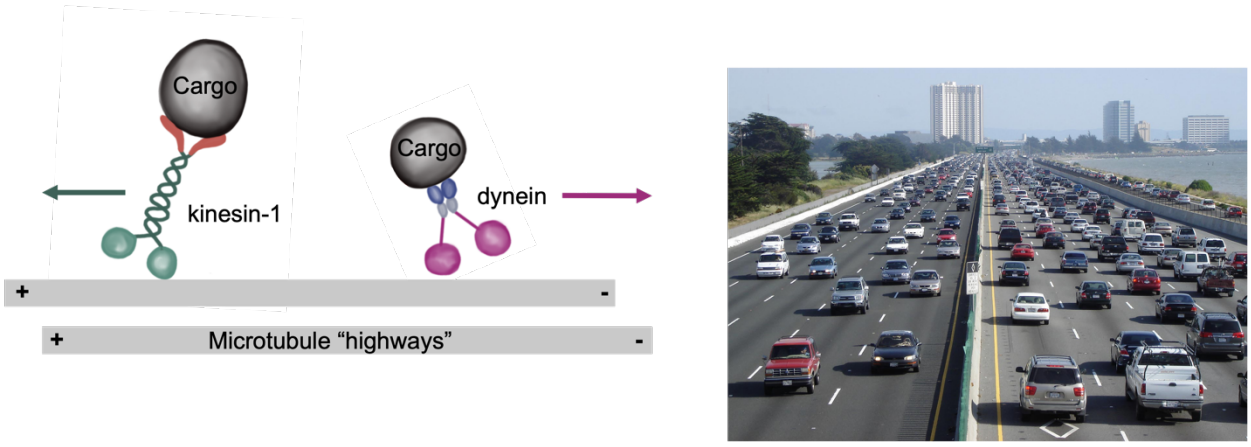

Figure 4. Motor protein “cars” carry cargos along microtubule “highways.” Kinesin-1 and dynein are motors proteins that act as cars to carry neuronal cargos along microtubule highways. Microtubules have structural directionality (plus and minus ends). Kinesin-1 carries cargos towards the “plus” (+) end and dynein carries cargos towards the “minus” (-) end. Car image: wikipedia.org

During my 5-week rotation in the Wildonger lab, I was immediately hooked when I could see this motor-protein transport occurring in a living neuron. I peered through the eye pieces of the microscope into a live fruit fly sensory neuron and could see tiny bright dots moving back and forth in the axon (just like cars on the highway!). A quick side note about the “bright dots” – by attaching fluorescent tags to the protein we are studying, we can visualize its location and movement. One of these fluorescent tags is green fluorescent protein (GFP), which was initially discovered in glowing jellyfish. So how does the neuron regulate this traffic to deliver (localize) the right proteins to the right place? I set off to study this question by trying to understand how the Pickpocket ion channel localizes to the dendritic plasma membrane.

Experimental procedures: What I did day-to-day in the lab

To study Pickpocket localization in dendrites, I first needed a way to see and track its localization. To do this, I tagged the Pickpocket protein with GFP, which allows me to study where Pickpocket is and how it gets there. To tag Pickpocket, I used CRISPR-Cas9 genome engineering to add the DNA sequence of GFP onto the end of the DNA sequence for *pickpocket*. The CRISPR-Cas9 system acts as molecular scissors and allowed me to cut a specific site in the DNA near the *pickpocket* gene and add the GFP sequence. When this *pickpocket*-GFP DNA sequence is translated into a protein, the GFP fluorescence allows me to visualize where Pickpocket is and to study how it gets there. But how do I actually "see" Pickpocket in the microscopic neuron?

I use a confocal microscope to zoom-in and visualize Pickpocket in sensory neurons. Using a laser with a specific wavelength of light, I can excite the GFP fluorophore tag, which causes it to emit light and glow. Then, this glowing signal is collected by a detector to create an image, similar to how a camera captures a picture. Using the tagged Pickpocket and confocal microscopy, I studied how Pickpocket localized in sensory neurons of live fruit fly larvae. To image Pickpocket in live larvae, I immobilized the larvae between two pieces of glass and imaged directly through the transparent larval cuticle (Figure 5). Studying the processes occurring in live neurons has many advantages because we are peering into what is happening in real time in a living organism (*in vivo*) rather than drawing conclusions from what happens in a "test-tube" outside of an organism (*in vitro*).

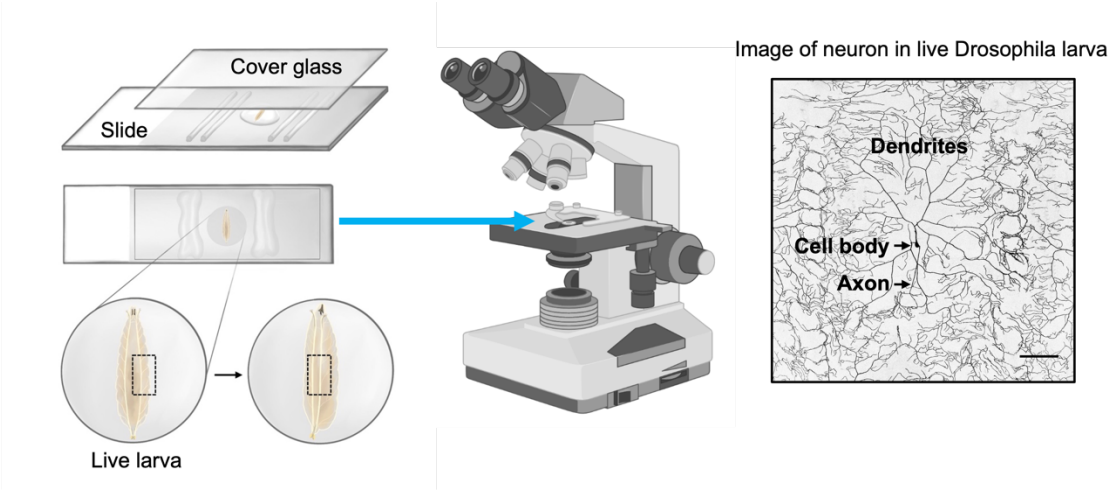

Figure 5. Method for imaging sensory neurons in live fruit fly larvae. Individual live larvae are stabilized between two thin pieces of glass (cover glass and slide). Then, the slide is placed on a confocal microscope to magnify and image the sensory neurons. The sensory neurons have many dendrite branches responsible for sensing external stimuli (rightmost image). Image of microscope: Biorender.com

Many of my family and friends may recall me talking about maintaining and "flipping" my fruit flies. Every other week, to maintain fruit flies for my experiments, I had to "flip" the adults from the old vial of food to a new, fresh vial of food. Fruit flies can't be frozen; they must be maintained in live colonies at room temperature. On average, I maintained ~200 stocks in the lab throughout graduate school and kept 2 copies of each stock. My quick calculations estimate that I "flipped" ~52,000 vials during graduate school (and this is an underestimate!). Much of my day-to-day work in the lab consisted of keeping my flies alive and setting up crosses to build stocks with unique genetic combinations to use in experiments.

What did we discover?

I started my research project with very little knowledge about how Pickpocket localizes in sensory neurons. From previous research done by other labs, we knew that

Pickpocket (Ppk1) was found in the class IV sensory neurons in Drosophila (the specific sensory neuron that I study that lies right beneath the larval cuticle) and that it formed an ion channel with its partner protein subunit Pickpocket 26 (Ppk26). We also knew that Ppk1 is important for larvae to feel a sharp painful poke by functioning as an ion channel to transduce external stimuli into electrical messages within the neuron. We knew that Ppk1 localizes to the plasma membrane of the dendrites, however, it was not known how it gets there. Is Ppk1 carried by specific motor protein “cars?” Is there a limit to how much Ppk1 gets transported to the dendritic membrane? What other proteins regulate where and when Ppk1 is inserted into the dendritic membrane?

Science and the process of discovery is a group effort, and it takes many hands and many experiments to understand biological processes. Through collaborative research with a talented undergraduate student, Jessica Liang, an exceptional research technician, Ipek Midillioglu, and my fearless advisor, Jill Wildonger, we discovered that the localization of the Pickpocket protein is coordinated with growth of the dendritic plasma membrane. Many of my experiments in fruit flies throughout grad school were “fruitless” (pun intended), but many experiments ultimately led us to this discovery.

In the lab, we use mutations in genes to disrupt processes and then study that effect on our protein of interest. Mutations are changes in the DNA that create broken protein machines that do not perform their job properly. If a mutation causes a change in the levels or location of the protein of interest, then that mutant protein is likely normally important for regulating the protein levels or localization. In my third year of grad school, we realized that there are very few mutants that disrupt the ability of Ppk1 to localize to the dendritic membrane. In other words, no matter what we did to disrupt

important trafficking processes, if there was dendrite present, then Ppk1 was present in the dendrite membrane. It was almost as if Ppk1 was an integral part of the membrane. This was surprising to us, and we designed experiments to study this idea further. When we tested a mutant that disrupts the dynein motor (one of the cars that delivers cargo), we expected that Ppk1 delivery to the dendrites would be decreased since dynein is the primary motor protein that delivers cargo to dendrites. However, Ppk1 was still present at normal levels in the dendrites! I remember many days and many experiments that resulted in feelings of frustration and confusion. When will I find *the* mutant(s) that will tell us all the biological secrets that control Ppk1 localization? However, in my fourth year of grad school, shortly after returning to the lab from COVID-19 stay-at-home orders, several experiments began to shed light on what was really happening.

Since Ppk1 was always in the dendritic membrane, we began to ask the question: Is Ppk1 *part* of the growing dendritic membrane? When the neuron grows, lipid building blocks (like tiny Legos) form the exterior boundary of the neuron called the plasma membrane. Remember that Ppk1 is an ion channel “door” that allows ions to pass from the outside to the inside of the neuron through the plasma membrane (Figure 2). Using a confocal microscope, we can watch as the neuron grows its dendrites. I remember how excited I was when I imaged a growing dendrite tip and saw that Ppk1 was present in the actively growing tip. This experiment showed us that Ppk1 is an integral part of the Lego building blocks that forms new dendritic membrane. So, membrane growth and Ppk1 localization are intertwined.

We also found that when we decrease the amount of dendritic membrane available (using mutants that reduce dendrite growth), Ppk1 density scales in proportion

to the dendritic membrane that is present. In other words, Ppk1 does not become more concentrated when dendrite length is decreased. Imagine a suitcase with 20 items of clothing. The airline tells you that you must use a smaller suitcase that is half the size of your original. If you squeeze all 20 items of clothing into a smaller suitcase, the density increases. If you reduce the number of clothing items to 10 to fit the smaller suitcase, you have essentially kept the same density of clothing in your suitcase. What we found is that the sensory neuron regulates the levels of Ppk1 in the dendritic membrane so that it maintains a consistent density. This was interesting to us because it suggests that even if the neuron is smaller (less dendrites to receive signals), the density of Pickpocket ion channel gates remains the same. This could have important implications in how the neuron is equipped to sense external stimuli.

Finally, we discovered one specific protein that is important for Ppk1 localization to the dendritic membrane: a small protein machine called Rab11. Rab11 is important for recycling proteins back to the plasma membrane. What exactly does “recycling” mean in the context of a neuron? Membrane proteins, like ion channels, are often removed from the plasma membrane when they aren’t needed and re-inserted when it is their time to shine. Rab11 is kind of like the recycling center: it takes your old plastics and recycles them into new plastic items. Rab11 is involved in recycling membrane proteins, but it can also be involved in the initial trafficking of proteins to the plasma membrane (you could think of this like Rab11 driving the brand-new plastic bottles to the store). We found that Rab11 is important for delivering Ppk1 to the dendritic membrane.

So what? What's the significance of these discoveries?

Finally, the punchline everyone has been waiting for! This is the question I get asked the most by friends, family, and others who are interested in my research. Why did I spend 5 years in graduate school studying a protein in fruit fly neurons? What is the significance of these discoveries in the big picture?

When I began my thesis research, we did not know how ion channels are localized to sensory dendrites. We knew that ion channels are the doors that allow ions to pass into the cell and transduce an external stimuli into an electrical message; however, how those ion channels get to the sites where they perform this job was unknown. In research, we call this the "Gap of Knowledge": what is unknown about the processes you are studying. As you could imagine, scientific research thrives on curiosity, asking measurable questions, and filling these gaps of knowledge.

Our discoveries about Ppk1 localization gave us three key takeaways: (1) Ppk1 is part of the membrane that grows dendrites, (2) Ppk1 dendritic levels scale proportionally to maintain consistent density when dendrite length is reduced, and (3) Rab11 helps carry Ppk1 to the dendritic plasma membrane. Overall, these discoveries tell us that the functional unit of the neuron (the Pickpocket ion channel) is an integral part of the structure of the neuron (the plasma membrane). As the neuron grows and establishes its structure, it is simultaneously establishing its function: to perceive external stimuli. This is important because as soon as the dendrites develop, they are equipped with the ability to sense external stimuli. My research in fruit fly sensory neurons may help us eventually understand more complex processes of ion channel trafficking in dendrites of human neurons. Broadly, this could lead to a better

understanding of how painful external stimuli are felt. Even more broadly, this field of sensory neuron research could lead to new discoveries about how we can treat chronic pain (without using severely addictive narcotics like opioids).

Those last two broad significances may seem a bit of a stretch to some, but this is how scientific knowledge progresses. Fundamental discoveries made through countless hours of experiments at the bench build on each other and lead to future discoveries. In graduate school, I focused on a very specific scientific question, but in the process I learned broad skills that I will continue to apply in my research and teaching. As I continue my scientific career as a professor at a liberal arts college, I will help mentor the next generation of career scientists and citizen scientists (because I believe everyone can be a scientist). I will strive to inspire my students to be curious as they explore the mysteries of biochemistry and encourage them to share their discoveries with others.