Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include 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 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.

Over 50 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

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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Cyclin-dependent kinases 1 and 2 control β-cell metabolism and insulin secretion

By

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Chapter 6

For a general audience

There are many reasons why communication is an important part of the scientific method. First, scientific research is not performed by a single person. As the adage goes, “we stand on the shoulders of giants.” For science to advance, we need to learn from the published studies that came before ours, and to document our own research sufficiently so that our peers can rigorously review and build upon our findings. Second, as taxpayer dollars fund a lot of biochemical research, I believe our findings should be easily accessible to everyone. As not everyone is trained to read and understand scientific literature, scientists have an important responsibility to educate the general public about our research, by putting our findings into context that is easily understandable. Also, I spent a long time in graduate school because I loved what I did! I want to share my excitement about the work I did while I was here with the people I care about. So, I have written this chapter to explain my research to a broad, non-scientific audience. Here, I discuss one vignette of my thesis that identified new connections between cell division, metabolism, and insulin secretion.

Thanks to the Wisconsin Initiative for Science Literacy at UW-Madison, especially Professor Bassam Shakhashiri, Elizabeth Reynolds, and Cayce Osborne, for providing this platform, and for sponsoring and supporting the creation of this chapter. Thank you to Dylan Plaskon, Isabella Sdao, and Angelina Sdao, who also provided valuable feedback while writing this chapter.

Introduction: Connecting insulin to cell division

Diabetes, β-cells, and insulin

When people ask me what I study I usually start with something like “I study **diabetes**!” which is mostly accurate. Diabetes is a complex disorder that affects too many

people (1 in 10 Americans!). Almost everyone I talk to has been touched by diabetes in some way, either personally or through family or friends. Our goal in the Merrins lab is to (eventually) cure diabetes by learning more about the cells in our body that underlie the disease, the **β-cells** (pronounced “beta cells”).

When we eat, our blood sugar goes up, since our food contains sugar (Figure 1A). The job of the β-cell is to sense this increase in blood sugar after eating, and release an amount of **insulin** that is proportional to how much we ate (Figure 1B). In other words, eating a whole meal will increase the blood sugar a lot more than a small snack. The β-cell will thus secrete more insulin after a large meal than it would after the snack.

Insulin is a signaling molecule that tells other organs that it’s a good time to take up sugar out of the blood stream. In this way, insulin lowers blood sugar. It’s very important that insulin levels are tightly controlled. Too much insulin is deadly, since it leads to very low blood sugar (**hypoglycemia**), and your brain can’t survive very long without blood sugar. Too little insulin over a long period of time leads to high blood sugar (**hyperglycemia**).

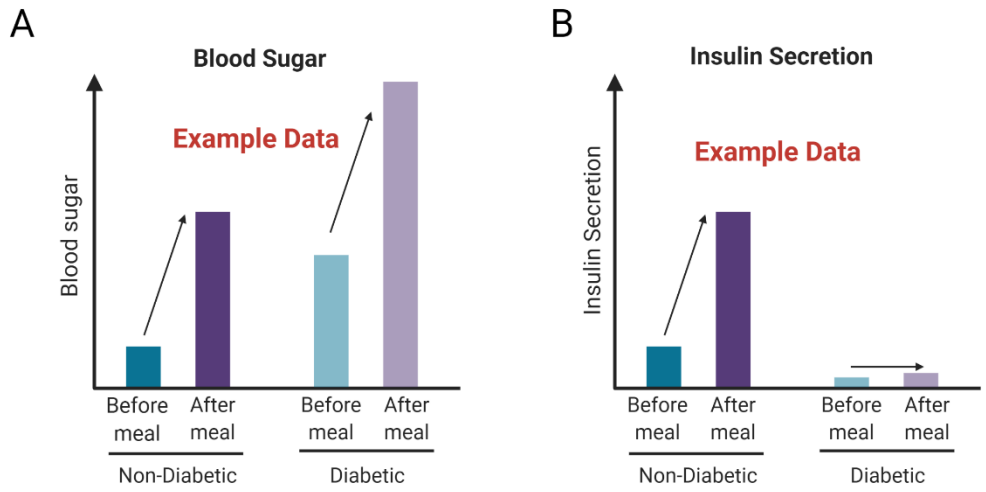

Figure 1. Blood sugar and insulin secretion while fasting (before meal) and after a meal. (A) In non-diabetic individuals (dark colors), blood sugar is relatively low while fasting (before a meal), and increases after a meal. In diabetic individuals (light colors), blood sugar is higher both while fasting and after a meal than non-diabetic individuals. **(B)** In non-diabetic individuals, insulin secretion is low while fasting when blood sugar is low, and increases after a meal when blood sugar increases. In diabetic individuals, insulin secretion is low while fasting and after a meal, due to a failure of the β-cells.

There are a few different types of diabetes. Our work is motivated by Type 2 diabetes, which is the most common form. Type 2 diabetes is caused by constant hyperglycemia because of too little insulin. Insulin insufficiency is caused in part by a failure of the β-cells to release an appropriate amount of insulin. This is why insulin injections are a common treatment for Type 2 diabetes. The problems with that are: (i) It's very difficult for people to monitor and control blood glucose using only insulin. Even the most attentive patient isn't as good as a healthy β-cell population at controlling blood glucose with insulin. And (ii) In Type 2 diabetes, another problem is that other organs in your body that actually remove the sugar become resistant to insulin, so it takes more

and more insulin to get the same effect. In diabetes, other organs develop a higher and higher “tolerance” for insulin, like what happens with some other drugs like alcohol or opiates. Adding more insulin without addressing the underlying conditions is pouring fuel on the fire, since it’ll keep raising the tolerance for insulin. This is why diet and exercise regimens and other medications are also typically prescribed alongside insulin injections. Type 2 diabetes researchers are always on the lookout for new treatments to help lower blood sugar. Some researchers (including us) focus on understanding more about the β-cells so that we can see how they do such a good job at sensing and lowering blood sugar, and to see if we can protect them from failing during diabetes.

Even after 50 years of intense research, it’s still not 100% understood how exactly β-cells sense blood sugar and secrete a proportionate amount of insulin. Furthering our understanding of this process is the goal of the Merrins lab.

The motivation for my research: what we already knew about β-cells

Obviously, this is not going to be an exhaustive chapter of everything we’ve ever learned about β-cells. We’d be here forever. Instead, I want to introduce a little about what we know about how β-cells respond to increases in blood sugar, and how that connects to the research I did.

It’s important that the β-cell be very flexible in its job so it can respond to changes in blood sugar quickly. The β-cell can adapt to increases in blood sugar in a few ways. In the short term, like right after a meal when blood sugar spikes, the β-cell population increases its insulin output per cell. In other words, each β-cell works a little bit harder to secrete more insulin. In the longer term, like in prediabetes where blood sugar is constantly higher than healthy ranges, β-cells have two options (and often use both): (1)

they can shift their sensitivity to sugar so that they secrete more insulin at a lower sugar level. If that's not enough (and sometimes in untreated prediabetes, it isn't), (2) the β-cell population can grow. Most new β-cells are created by **cell division** of existing β-cells to keep up with blood sugar demands.

Usually, β-cells are reluctant to divide, and will only do so as a "last resort" in response to long-term hyperglycemia. They don't really need to, and you don't really want them to. Imagine if you didn't have a β-cell population with a fixed number of cells. What a nightmare it would be if every time you ate something and your blood sugar spiked, you got new β-cells. Sure, it would definitely increase insulin levels, but now it's much harder to keep track of how much insulin each individual β-cell should be secreting. It's like if you had a group of coworkers, and each person was responsible for an exact fraction of the work. They all get really good at working at an optimal pace for how much work they're assigned. Then you suddenly hire a new coworker and that really changes the dynamic of the team. Who's work is it going to take, and what happens to that coworker? Do you re-divide all the work so it's fair to everyone? Are they going to distract everyone else with their water cooler breaks? Etc…

Regulating insulin levels would be much harder, and more dangerous, with a population of β-cells that are eager to divide. It's definitely more advantageous to have β-cells that are reluctant to divide and risk temporary hyperglycemia while the team of β-cells catches up, than to have β-cells that are too eager to divide and risk hypoglycemia after a blood sugar spike (remember, your brain ***needs*** sugar!)

A problem with that is, in diabetes your β-cells don't work as well, and you *want* them to divide. As a physician, my grandpa described Type 2 diabetes as "outgrowing

your pancreas," since β-cells are found in the pancreas. I might revise that to "outgrowing your β-cells" since other cells in the pancreas work just as well in diabetic people, if not better, than in healthy people (although it's not as catchy). Anyway, what we're both saying is, if high blood sugar is not managed, your β-cells basically burn out after a while. They did their best, but they just don't work as well as they used to. Which is understandable if you consider the stress they've been under. You'd burn out if you had to work 24/7/365 too. In the very bad cases, they start to die. Here, an intervention that would cause β-cells to divide would be really helpful for disease management. More β-cells around to help out means that each β-cell can take a load off, relax a little, and reduce burnout. To understand what that intervention might look like, we need to know a lot more about β-cell division, what regulates it, and what happens to insulin secretion during it, so we can design the most effective treatments. As you can imagine, playing around with mis-regulated cell division can lead to nasty diseases like cancer, which is essentially defined by too much cell growth.

Cell Division

Cell division is another tightly controlled process, also for good reason (re: cancer). It's a good thing that there are so many molecules and signaling pathways dedicated to regulating cell division. It also makes studying cell division, especially in a cell that rarely divides, extremely difficult. For the past 6 years I've studied the molecular players that are about as "close to the source" as you can get to the mechanisms that drive cell division. These molecules are called **cyclin-dependent kinases (CDKs)** and all the roads to cell division lead to CDKs, so to speak.

Cell division can be broken up into four stages that happen sequentially to make two cells from one. CDKs are like the managers of these stages. Each one is responsible for making sure the cellular changes that have to happen during the stages are happening on time and correctly- kind of like filling out and compiling the necessary paperwork before the cell is allowed to transition to the next stage. They also help the cell transition from stage-to-stage.

Cell division is a high-stakes game. It takes a lot of resources, time, and energy for the cell to complete one division. The cell needs to be really (*really*) sure that it's able to commit the resources it needs for division before it actually goes through with it. That's what the first stage is all about- the cell basically checks itself before it wrecks itself. It makes sure the answers to questions like: Is it safe? Do I have enough resources and energy for division? are a resounding *yes* before CDKs will allow the cell to transition to the second stage. The first stage is also about preparing for a *yes* by starting to make the building blocks to build a new cell. This means diverting resources and energy to processes that make the building blocks- essentially, the cell starts bulking. That's really interesting to me, and motivated my research- before explaining why, we need to take a quick detour back to insulin secretion.

Are there any connections between insulin secretion and cell division?

The β-cell is basically a nutrient sensor. It needs to know exactly how much sugar is out there in the blood so it can fine-tune how much insulin to make and release. Unlike other cells, which sometimes have a limit to how much sugar they'll take in, β-cells have basically an open door policy for sugar, so they can really accurately sense how much sugar is actually out there. The β-cell is just like a lot of other cell types in other ways: it

needs to break sugar down (**metabolize**) into energy to keep it functioning. The way that it determines how much insulin to secrete is essentially by sensing the amount of energy it's producing from the sugar it's metabolizing. This is how the β-cell conveniently connects how much sugar the β-cell sees to how much insulin is released. The β-cell basically back-calculates how high blood sugar is by how much energy the cell got out of metabolizing sugar.

Because insulin secretion is so tightly tied to cellular energy status and metabolism, anything that changes the cell's metabolism might change insulin secretion. During cell division, building a new cell takes a lot of energy and resources. That's the subject of my project: I wanted to understand if and how a CDK that manages the first stage of the cell cycle (specifically, **CDK2**) also controls insulin secretion. Especially since most β-cells just exist in the first stage pretty much their whole lives without advancing (see: reluctance to divide).

Experimental Procedures: What I actually did

The Merrins lab uses mice as a model organism since they pretty closely resemble human physiologically, and it's much easier to acquire mouse pancreatic tissue than human pancreatic tissue. Plus using mice allows us to perform experiments that wouldn't be possible in humans, like the ones below.

One way we can ask what CDK2 is doing in β-cells is to delete the **gene**†† that encodes for CDK2 (i.e., "**knockout**" CDK2) and watch what happens when there's no CDK2 around. Since CDK2 is a very important molecule to all sorts of different cells, we

†† A **gene** is a part of the animal's DNA that contains instructions for making a particular **protein**. Proteins have incredibly diverse functions in cells and each one is different in DNA encoding sequence. One of my favorite proteins is CDK2.

wanted to specialize our mouse model in a few ways: (i) we wanted to knockout CDK2 only in β-cells and no other cell types, (ii) we wanted to be able to induce the knockout of CDK2 after the mice reach adulthood, which is when we'd study them. If β-cells never saw CDK2, there's a chance the β-cells wouldn't develop normally, since CDK2 is heavily involved in organ development. There's also a chance the cell has compensation mechanisms that would be fully in place by the time the mice reached adulthood. We wanted to know if the jobs that CDK2 performs control insulin secretion. If there wasn't any CDK2 around for a long time, the cell might find other co-workers that could take over CDK2's jobs. By completely knocking out CDK2 from the start, it wouldn't be guaranteed that we were studying what CDK2 does in adult β-cells, which is what we really wanted to do.

We used a few fancy genetics techniques to selectively breed mice that lack CDK2 only in their β-cells, and only after we induce the knockout (**CDK2-KO** mice). Then we measured β-cell metabolism and insulin secretion, and compared these to β-cells from animals who have all their CDK2 (**control** mice). With current scientific methods, it's really difficult to measure these things in live animals, although technology for that is improving all the time. For now, we can get a more accurate look at β-cell function by removing the β-cells from the mouse, keeping the β-cells alive by keeping (**culturing**) them in similar conditions as the live pancreas, then performing experiments on them after they've recovered from the isolation process (Figure 2).

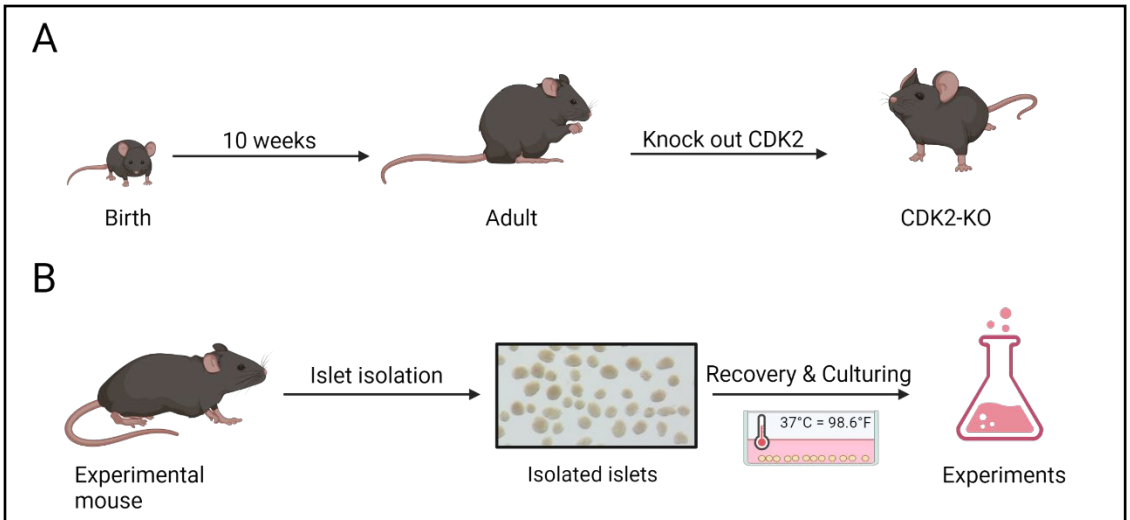

Figure 2. Using mice as a model to understand β-cells. (A) Mice are grown to 10 weeks of age, which is the mouse equivalent of young adulthood. In some mice we can knock out CDK2 at this point. These mice are "CDK2-KO." Some mice we can't knock out CDK2. These mice are the "controls" that we use to compare to the CDK2-KO. **(B)** To study β-cells from CDK2-KO mice, we euthanize the mouse, isolate the islets from the pancreas, and keep the islets alive in an incubator that mimics the environment in the mouse.

A quick detour: How we actually do science in the Merrins lab

When we isolate β-cells from mice, we euthanize the mouse, take the pancreas out, break up some of the pancreatic tissue, and then extract cell clusters called **islets of Langerhans** or **islets**. Islets are made of many different cell types, many of which also secrete hormones that control blood sugar levels. For example, **α-cells** (pronounced "alpha cells") produce and secrete another heavily studied hormone called **glucagon**, which has the opposite effect on blood sugar as insulin does. Really exciting research is currently being done to investigate how the different cells in the islets talk to each other to influence each other's function.

Sometimes it makes sense to break up the islet into single cells to measure individual cell function. In our case, we decided to keep the islet together to keep the β-cell as close to its native pancreatic environment as possible.

To measure insulin secretion, we put isolated islets in **media** (AKA, solutions) that have either low **glucose** (a fancy word for sugar) or high glucose, and no insulin. We keep them there for 45 minutes and let them do their job- secrete insulin. Then we measure the insulin in the media, which should only have insulin that's been secreted from the islets that we put in there. As you would expect, we measure more insulin secreted from islets kept at high glucose than islets kept at low glucose (Figure 3). We can also compare between experimental groups (like comparing our CDK2-KO to controls).

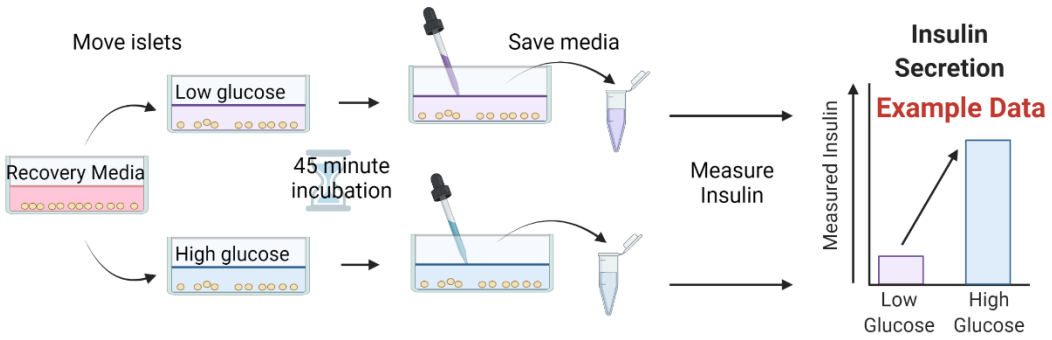

Figure 3. Measuring glucose-stimulated insulin secretion from isolated islets. After recovery, islets are moved into media with either low glucose or high glucose. We put them back in the incubator, and wait 45 minutes for them to secrete insulin into the media. Then we collect the media into tubes, and measure the insulin in the tubes. We expect islets put into high glucose to secrete more insulin than islets put into low glucose.

Measuring β-cell metabolism is more complicated. There are lots of different **metabolic pathways** that the cell uses to break down sugar for energy. You can think of metabolic pathways kind of like a road. Some are two-way, some are one-way, and all of

them carry "*car"*go toward a destination. Some roads end in insulin secretion. Some roads end in creating building blocks for new cells, and some end in breaking down those building blocks for energy. Some roads end in storing energy in a spare-change jar for a rainy day, and some end in digging into the spare-change jar on a rainy day. All of these roads can be traveled at any time by any cars- in this analogy, cars are nutrients. The cell's activity depends on the balance of the cars on each of the roads.

The cell carries nutrient cargo through metabolic pathways, creating energy along the way. Just like roads carry cars from stop-to-stop, metabolic pathways carry nutrients and their breakdown products from stop-to-stop. The cell determines how much insulin to secrete by sensing the flow through all the different metabolic pathways.

Ideally, we want to measure the activity of all the different pathways so we can see what (if anything) changes in our CDK2-KO. We can't quite get there yet with current technology (but again, technology is improving all the time). What we can do is, we can put markers (AKA, **biomarkers**) for different metabolic pathway "stops" in β-cells to report how activity through the pathways change. This is kind of like putting a traffic camera at intersections, except we take pictures of every car that comes through the intersection, not just the ones that speed through red lights. If we see a lot of cars through the traffic camera, then we know that the road is especially busy. We can measure the biomarkers to compare the traffic in the CDK2-KO to controls.

The biomarkers we use are **fluorescent**. That means that when we hit them with light, they'll **fluoresce**, which means they'll emit light back. We can take a picture of the emitted light with a really high-quality camera at a really high magnification with the help of a **microscope** (Figure 4). The fluorescence properties of the biomarkers change in

response to changing activity through the pathway they report. For example, some change the intensity of the emitted light. Some change which color light they'll emit, or which color light will trigger fluorescence. We can measure those changes with our microscope.

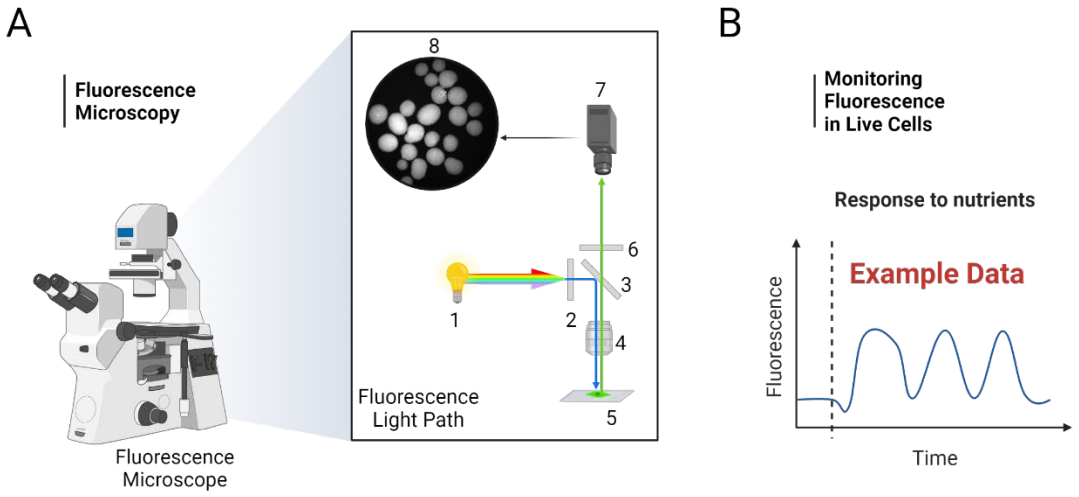

Figure 4. Fluorescence and fluorescence microscopy. (A) *Left:* We use a fluorescence microscope to collect fluorescence from β-cells in islets. *Right:* The light path through a fluorescence microscope starts with a light source (1) which is typically an LED. Light gets filtered through an excitation filter (2) that blocks every color except a very specific one that we want to use to excite our sample. The excitation light in this diagram is blue. After passing through the excitation filter, excitation light is bounced off of a dichroic mirror (3) that mirrors some colors of light, and lets other colors of light pass right through. The dichroic mirror in this diagram mirrors blue light, but allows green light to pass through. The dichroic mirror bounces the excitation light through a high-magnification objective lens (4) to the sample (5), where it excites the fluorescent biomarkers inside the β-cells. The biomarkers emit a different color of fluorescence: in this diagram, the fluorescent light is green. Fluorescence from the sample passes through the dichroic mirror and through an emission filter (6) before hitting the camera (7). The digital image that the camera produces (8) can be viewed and the intensity of light can be quantified using a computer. **(B)** Taking many pictures in a row over time and quantifying the intensity of the fluorescence, we can generate a graph that tracks the intensity of the biosensor fluorescence over time. We can measure the response of a specific biosensor to different nutrients or drugs to probe different “roads” inside the cell. In the example graph, nutrients were added at the time corresponding to the dashed line, and the response of a biosensor was recorded over time.

Luckily for us, islets are, for all intents and purposes, transparent. That means that we can put fluorescent biomarkers inside β-cells (installing the traffic cameras), then easily measure the fluorescence through the cells (monitoring the traffic cameras). Then we can compare our CDK2-KO to controls.

Results: CDK2 controls β-cell metabolism and insulin secretion

CDK2 inhibits insulin secretion in β-cells

We found that both CDK2-KO and control islets secreted more insulin at high glucose than low glucose, as expected. We were very excited to see that CDK2-KO islets secreted even more insulin than controls did at high glucose. Because we saw *more* insulin secretion in our *knockout* (i.e., *more* secretion with *less* CDK2), this indicates that CDK2 normally *slows down* or *inhibits* insulin secretion in β-cells.

CDK2 rewires metabolism in β-cells away from insulin secretion pathways and toward cellular growth pathways

Our hypothesis was that, as part of its role in the cell cycle, CDK2 might redirect the β-cell's metabolism toward pathways that help build a new cell. Conveniently, scientists who study cancer have already developed a deep understanding of those particular building and growth pathways, since they're all about understanding exactly what happens during (uncontrolled) cell division. We know from them that it takes a lot of energy for the cell to build another cell. We know from β-cell biologists that it also takes a lot of energy to secrete insulin. Considering that the cell has a finite amount of energy, we hypothesized that the cell has to at some point make a choice to either do one or the other. As mentioned above, cell division is a high-stakes game, so committing to cell division means diverting a lot of resources to cell division. Maybe that means that when

the β-cell chooses to divide, it needs to divert all its energy and attention to division, and maybe that doesn't leave a whole lot left for secretion.

There's some evidence to support this concept in the β-cell literature already. We know that β-cells that are dividing are not as good at connecting how much glucose is available to how much insulin to secrete- *kind of like they're a little distracted*. We thought that CDK2 might be coordinating this, so this was our hypothesis: maybe CDK2 limits insulin secretion to conserve energy for the really energy-expensive pathways that build new cells. To test our hypothesis, we needed to measure the pathways that control insulin secretion, and the pathways that build new cells.

Using a few different fluorescent biomarkers for insulin secretion pathways, we determined that pathways that controlled insulin secretion were more active in our CDK2-KO β-cells compared to controls. This matched really well with our direct measurement of insulin secretion, and again indicated to us that the ability of the CDK2-KO β-cells to sense glucose was improved compared to the controls. This told us that when CDK2 is *gone*, insulin secretion pathways are *more active*, so CDK2 must be restricting insulin secretion.

"Why'd you have to go and make things so complicated?"

Up until now I've been referring to the "metabolic pathways" we measured as a simplified roadway that starts at glucose breakdown and ends at insulin secretion. To explain the next part of the study, I need to complicate that definition a little bit.

When cells break down sugar, they produce energy in the form of a molecule called **ATP**. This is the cell's energy "currency." You might have heard that "the mitochondria are the powerhouses of the cell" in school. That's because the mitochondria are the

largest ATP producers in the cell. Like other cells, β-cells break down sugar into ATP, mostly through the mitochondria. Unlike other cells, β-cells use ATP for insulin secretion. When ATP levels are high, that tells the cell that it's breaking down a lot of sugar, so there must be high blood sugar, so it's time to secrete insulin. One metabolic pathway that controls insulin secretion is really responsive to ATP levels. We call it the **triggering pathway** because it "triggers" insulin secretion in response to high ATP levels. We think about it kind of like an on/off light switch that turns on insulin secretion when ATP levels are high. In the experiments above, we measured the triggering pathway and showed that the triggering pathway was more active when CDK2 was lost in β-cells.

The triggering pathway isn't the only pathway that controls insulin secretion. If the triggering pathway is the on/off light switch, **amplifying pathways** act as a dimmer switch that gives the β-cell control of how much insulin is secreted when the light is turned on. While the triggering pathway is really well-studied, the amplifying pathways are still somewhat of a mystery to β-cell biologists. We do know that some of the amplifying pathways are the same metabolic pathways that build building blocks for new cells during cell division. To go back to the roadway analogy, when glucose enters the β-cell, there's a few different roads it can take (Figure 5A). Most end in increasing ATP, which is what turns on the triggering pathway. Some roads are amplifying pathways, which fine-tune how much insulin is secreted when the triggering pathway is turned on, and builds building blocks for new cells along the way. Glucose takes both the triggering and amplifying roads, and a lot of neat research in the Merrins lab is currently trying to figure out how it decides which road to take.

We found that the CDK2-KO had lower amplifying pathway activity. This told us that when CDK2 levels are ***low***, there's ***less*** amplification of insulin secretion. So, CDK2 must ***activate*** the amplifying pathways in β-cells. We were really excited about this too, since, as a molecule that promotes cell division, we expected CDK2 to be important for building new cell parts.

Conclusions: So What?

So, putting this all together, we found that CDK2 controls insulin secretion in two ways (Figure 5B). First, by decreasing the triggering pathway, CDK2 shuts off insulin secretion. We also found that CDK2 promotes the amplifying pathways, which are important for building new cells. Insulin secretion is ultimately determined by the balance between the two pathways, and we found that CDK2 inhibition of the triggering pathway outweighed its activation of the amplifying pathways. So why would it be advantageous for CDK2 to shut off insulin secretion?

One possible answer is that it takes a lot of energy to secrete insulin. It also takes a lot of energy to build new cells. By shutting off the triggering pathway of insulin secretion, we think that CDK2 might conserve energy to be used for cell division. It also shunts glucose off the triggering pathway road and onto amplifying pathway roads, which also help cell division by building new cell parts.

Understanding what happens when β-cells divide is important if we want to design good diabetes therapeutics that build more β-cells. If we stimulate proliferation willy-nilly, we might end up with unintended consequences. We showed here that activating CDK2, which would also activate β-cell division, might actually shut down insulin secretion. More β-cells with no insulin secretion are pretty much useless. When developing any

therapeutics that include activating CDK2, we should keep in mind that we might also need to find a way to counteract CDK2-induced shutdown of insulin secretion.

Figure 5. How CDK2 controls β-cell metabolism and insulin secretion (we think).

(A) This is what we knew before: when glucose enters the β-cell, it can be broken down to produce ATP and trigger insulin secretion through the triggering pathway (turning the light switch "on"). Glucose can also be broken down to make building blocks to build a new cell, which amplifies insulin secretion (turning the dimmer switch "up"). **(B)** This is how we've added to what we already knew. We found that CDK2 can put a brake on insulin secretion through the triggering pathway (turning the light switch "off"). CDK2 can also turn the amplifying pathways up (turning the dimmer switch "up") in order to make building blocks for new cells.