Case Study 1: The Role of Insulin Resistance in Type-2 Diabetes

Adapted from Case studies by Univ. of Buffalo NY, Nature magazine, Medicinenet, Joselin Diabetes Research Center

Part I

Tania is an Undergraduate student from Germany and is visiting Dr. Wen's lab under the International Visiting Scholars program. She will be working with Dr. Wen for the summer trying to get some practical research experience which will help her decide between applying to graduate school or medical school. Dr. Wen works primarily on Type-2 diabetes and Tania is interested in his work on trying to understand the role of insulin and cellular signaling in diabetes. She first got interested in this topic because her Uncle has Type – 2 diabetes. She has a list of questions which she hopes she will be able to answer by the end of her project with Dr. Wen. Here is her list and some information to help her understand how cell signaling works

Q1) Cellular signaling is very important in a cell, but what does it have to do with diabetes?

Hint: Cellular signaling controls our response to the environment, like changes in the temperature or responses to eating. It helps our bodies maintain homeostasis. Many medications alter cellular signaling in order to treat diseases like cancer, allergies, and diabetes.

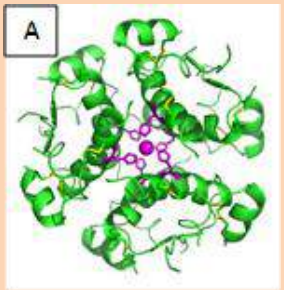
Q2) How can understanding cellular signaling help us understand how diabetes occurs and how to treat it?

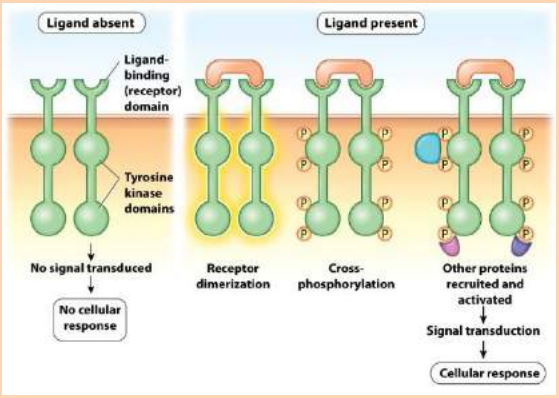
Hint: In cellular signaling each pathway involves many proteins, thus carrying out messages can be very complicated for cells. Understanding cellular signaling in general will help to understand what role it plays in diabetes. Refer to slides from Lecture 4 and revise the details of signal transduction pathways

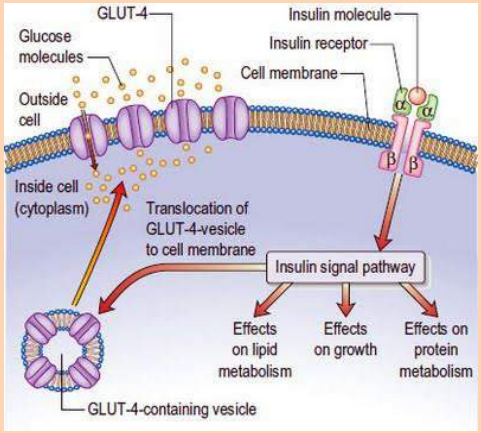
<u>Knowledge Clip 1:</u>

What is Cell signaling?

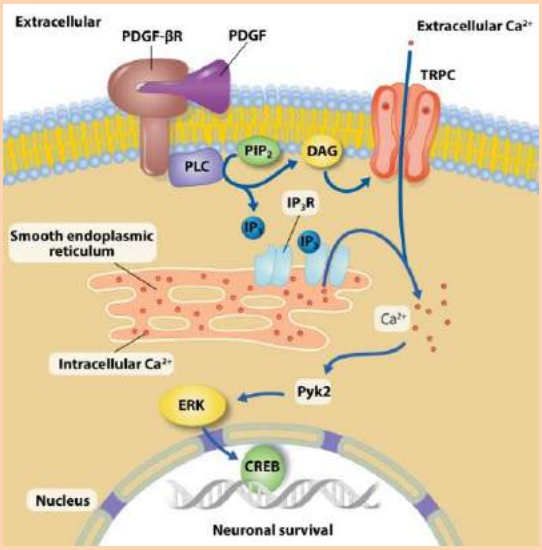
A signaling pathway has four essential components: (1) the initial signal, (2) the receptor that binds the signal, (3) the signaling molecule or molecules that transmit the message, and (4) the effector or effectors that result in a short-term or long-term cellular change. The initial signal can range in size and composition from a small molecule like nitric oxide (NO), a hormone like estrogen, or a protein like insulin (Figure A). The type of signal determines if the receptor signal-binding domain can be intracellular or extracellular. For example, estrogen is hydrophobic and can readily pass through the plasma membrane, so its receptor is intracellular. Other signaling molecules like the protein insulin are hydrophilic and too large to pass through the plasma membrane so the insulin receptor is an integral membrane protein with an extracellular signal-binding domain (made of an outer-membrane component alpha and an inner membrane component beta). Once the signal (which is insulin in this case) binds to the receptor, the receptor changes its shape or conformation. This conformation change might include the opening of an ion channel allowing ions to travel into the cell (like the Na+/K+ channel) , or it might include changing the organization of domains like the extracellular domain of a receptor tyrosine kinase (Fig B). A receptor conformation change causes the associated signaling molecule(s) to transition from inactive to active. The signaling molecule(s) can carry the message through many different mechanisms. The activated signaling molecule then influences the effector(s) that cause the short-term or long-term cellular change. A short-term change can be stimulating cellular movement or changing the activation state of an enzyme going from inactive to active or active to inactive. This happens for instance when activating an enzyme to increase sugar metabolism. Long-term cellular changes are generally the result of changes in DNA transcription. For example, a protein could be made to begin cellular replication by activating the cell cycle.

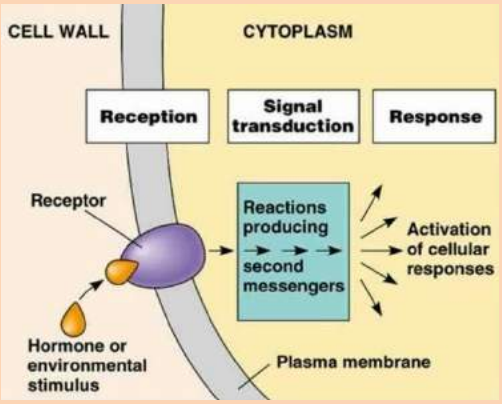




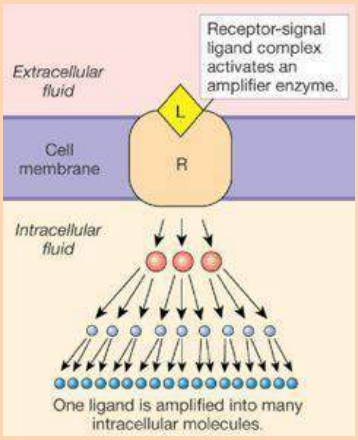


The sites phosphorylated by the previous kinase activate the next kinase, but another site of phosphorylation on the same kinase could turn it off. The activity of each kinase in the cascade can be regulated in this manner. One common mode of regulation is called feed-back inhibition (Figure 2B). This occurs when some downstream effector (or result of the cellular response) inhibits an earlier step in signal transduction. Thus, the dynamics of speed and magnitude of response can be fine tuned or stopped entirely. This negative regulation is reversible. In the example in Figure 2B, another enzyme called a phosphatase could remove the phosphate group from the kinase, allowing it to be activated again. Another common mechanism for multi-protein signal transduction is the activation of a second messenger (Figure 3). A second messenger is generally a small molecule that can travel freely through the cytoplasm or the membrane. Some examples of second messengers are cyclic-AMP, Ca2+ ions, phosphoinositides (PIP3, PIP2, etc.), and diacylglycerol (DAG). These second messengers are either released from intracellular stores (like Ca2+ ions) or created through enzymatic action (like cyclic-AMP). Once released, second messengers can interact with many targets throughout the cell simultaneously. Thus, second messengers lead to signal amplification and increased speed in signal transduction.





Signal transduction cascade



Signal transduction amplification

After reading this information, Tania has a fair idea of how signaling works. But she has some more questions:

Q3) Does the kinase cascade and second messenger signaling where lots of proteins are activated require a lot of energy to make all those extra proteins? Why couldn't the signal be transmitted with just one signaling molecule?

Hint: The above figures show that in the kinase cascade, with each additional kinase activated, more of the next kinase is activated thus growing exponentially. This is called as Signal amplification. Signal amplification can lead to greater cellular changes, and it also speeds up the cellular response. It works the same with second messenger pathways too, with the small molecules activating lots of signaling proteins. Each new signaling molecule also provides another opportunity for the body to regulate the signaling.

Answer Questions for Part I in the Case Study I Question sheet

Part II

Now that Tania understands the basics of signaling and its mechanisms, she is ready to understand why Dr. Wen's lab studies cellular signaling. Tania's Uncle's life is adversely affected by Diabetes. He has to be careful what he eats and he goes for walks most days. He also has to monitor his blood glucose level at regular times and he gives

himself injections before most meals to keep his glucose levels balanced; it can't be too high or too low. For instance, after people eat their blood glucose generally goes up. This causes the pancreas to release a signal known as insulin into the blood stream. In diabetics, the cellular signaling is messed up so it doesn't work as well. So her uncle injects himself with insulin or an insulin analogue. Insulin is a protein that controls cellular signaling of various types. By controlling insulin changing signaling, the adverse effects of diabetes can be managed.

Q1) What are the symptoms of Diabetes?

Knowledge Clip 2:

Symptoms of Diabetes:

Hunger and fatigue: Your body converts the food you eat into glucose that your cells use for energy. But your cells need insulin to bring the glucose in. If your body doesn't make enough or any insulin, or if your cells resist the insulin your body makes, the glucose can't get into them and you have no energy. This can make you more hungry and tired than usual.

Peeing more often and being thirstier: The average person usually has to pee between four and seven times in 24 hours, but people with diabetes may go a lot more.
Why? Normally your body reabsorbs glucose as it passes through your kidneys. But when diabetes pushes your blood sugar up, your body may not be able to bring it all back in. It will try to get rid of the extra by making more urine, and that takes fluids. You'll have to go more often. You might pee out more, too. Because you're peeing so much, you can get very thirsty. When you drink more, you'll also pee more.

Dry mouth and itchy skin. Because your body is using fluids to make pee, there's less moisture for other things. You could get dehydrated, and your mouth may feel dry. Dry skin can make you itchy.

Blurred vision. Changing fluid levels in your body could make the lenses in your eyes swell up. They change shape and lose their ability to focus.

Yeast infections: Both men and women with diabetes can get these. Yeast is a fungus that feeds on glucose, so having plenty around makes it thrive. Infections can grow in any warm, moist fold of skin, including:

- Between fingers and toes
- Under breasts
- In or around sex organs

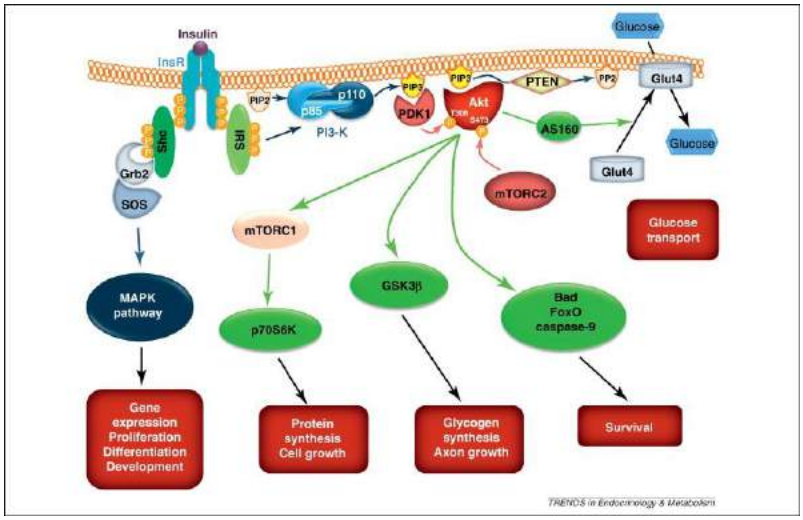
Slow-healing sores or cuts: Over time, high blood sugar can affect your blood flow and cause nerve damage that makes it hard for your body to heal wounds.

Pain or numbness in your feet or legs: This is another result of nerve damage.

From information given by Dr. Wen, Tania now understands that the symptoms of Diabetes occur due to the presence of high glucose concentrations in the blood and very little of the glucose from the blood getting into the cell. Since glucose is an energy source and it needs to get into the cell to be used, the cells need to use something else for energy. In the absence of glucose, or in the case of diabetes, due to the inability to uptake glucose, proteins or fats are used as energy sources. When the cells start using proteins, it leads to a buildup of ketoacids. Being acids, they lower pH of the blood. This lower pH can damage a lot of tissues, causing the symptoms listed above. Another problem with diabetics is that they lose feeling in their feet, so if they get a blister on their foot they may not feel it. Then it may get infected because diabetics have poor wound healing, and if the infection isn't noticed it may lead to amputation of the foot or leg
Now that Tania understands what the symptom of Diabetes are and what causes them, she is still unclear about how cell signaling is involved in this whole scenario?

Q2) What are the steps involved in the insulin signaling pathway?
Dr. Wen gives her this video to understand the concepts of insulin signaling.
https://www.youtube.com/watch?v=FkkK5lTmBYQ

After watching the video, it is pretty clear to her how insulin signaling happens in the cell and how glucose enters the cell. But Tania realizes that Insulin not only affects Glucose uptake by the cell, but also plays an important part in Fatty acid production, protein synthesis and Glycogen synthesis by joining of multiple glucose molecules for storage. So how does it do that? Dr. Wen draws out this simple map to explain some of the other pathways that insulin affects. He explains that the insulin signaling that causes uptake of glucose via the GLUT-4 molecule is a short term change caused by insulin signaling. The other signaling cascades can cause long term effects like:
1) Gene expression and cell division via the MAPK pathway
2) Protein synthesis and cell growth by the AKT-mTORC pathway
3) Glycogen synthesis by the AKT-GSK3 pathway
4) Fatty acid synthesis by AKT-FOXO pathway



Dr. Wen goes on to explain that insulin does not cause the same long-term and short-term effects in different kinds of tissues in your body, like they are different in your muscle and liver. Although, insulin is released into the blood stream so it could bind to receptors on all the different tissues, Insulin binding to the insulin receptor doesn't have the same effect in the different cell types in our body. Insulin is released into the blood stream, but the amount of a receptor or any downstream signaling effector could affect the short-term and long-term effect. Different cells have the same set of DNA, but the accessibility of that DNA is changed in different cell types. The insulin receptor DNA might not be expressed as much in different tissues because of the DNA packing or a variety of other reasons.

Answer Questions for Part II in the Case Study I Question sheet

Part III

The primary cause of Type-2 diabetes is insulin resistance. This means that even through insulin is present in the blood stream, the cells don't respond as robustly. Type-2 diabetes occurs as a result of continuous insulin signaling due to genetics, poor diet, obesity, and lack of exercise. This continuous over stimulation of insulin signalin g alters how the insulin receptor and its down-stream signaling pathways will respond to insulin. There have been lots of possible changes to insulin signaling proposed as the key mechanisms responsible for insulin resistance, but the reality is that insulin resistance isn't understood. Here are a few examples and already known pathways of insulin resistance

Knowledge Clip 3:

Causes and types of insulin resistance:

Insulin resistance results from inherited and acquired influences. Hereditary causes include mutations of insulin receptor, glucose transporter, and signaling proteins, although the common forms are largely unidentified. Acquired causes include physical inactivity, diet, medications, hyperglycemia (glucose toxicity), increased free fatty acids, and the aging process

Classification of prereceptor, receptor, and postreceptor causes:

The underlying causes of insulin-resistant states may also be categorized according to whether their primary effect is before, at, or after the insulin receptor (see below).

Prereceptor causes of insulin resistance include the following:

- Abnormal insulin (mutations)
- Anti-insulin antibodies

Receptor causes include the following:

- Decreased number of receptors (mainly, failure to activate tyrosine kinase)
- Reduced binding of insulin
- Insulin receptor mutations
- Insulin receptor–blocking antibodies

Postreceptor causes include the following:

- Defective signal transduction
- Mutations of *GLUT4* (In theory, these mutations could cause insulin resistance, but polymorphisms in the *GLUT4* gene are rare.)

Combinations of causes are common. For example, obesity, the most common cause of insulin resistance, is associated mainly with postreceptor abnormality but is also associated with a decreased number of insulin receptors.

Specific causes of insulin resistance

Specific conditions and agents that may cause insulin resistance include the following:

- **Aging**: This may cause insulin resistance through a decreased production of GLUT-4.
- **Increased production of insulin inhibitors**: A number of disorders are associated with this effect, such as Cushing syndrome, acromegaly, and stress states, such as trauma, surgery, diabetes ketoacidosis, severe infection, uremia, and liver cirrhosis.
- **Medications**: Agents associated with insulin resistance syndrome include glucocorticoids (Cushing syndrome), cyclosporine, niacin, and protease inhibitors. Glucocorticoid therapy is a common cause of glucose intolerance; impairment of glucose tolerance may occur even at low doses when administered long term.
- **Sodium**: High sodium intake has been associated with increased glucocorticoid production and insulin resistance.
- **Anti-HIV therapy**
- **Insulin therapy**: Antibodies are proteins produced by our immune system to neutralize or destroy foreign substances in our body, Antibodies against insulin have been found in most patients who receive insulin. Rarely, the antibodies result in significant prereceptor insulin resistance. Patients with a history of interrupted exposure to beef insulin treatment are particularly prone to this resistance. Clinically significant resistance usually occurs in patients with preexisting, significant tissue insensitivity to insulin.