

It had been observed that the bacteria that cause tooth decay, a type of bacteria called *Streptococcus mutans* (*S. mutans*), could survive in acidic conditions that would normally harm other bacteria.

- The surface of your teeth is home to many different kinds of bacteria. Some form their own sticky environment, called **plaque**.
- In the **plaque**, bacteria multiply and compete for space, all of which requires energy. They get this energy by consuming and processing sugar.
- The energy stored in sugar is converted to a form energy that the bacteria can use. During this process, acid is produced as a waste product. This causes the **plaque** to become more **acidic**. The pH of the **plaque** drops.
- When the pH of the **plaque** drops the acid eats away at the tooth's surface, creating a cavity.
- When the pH of the **plaque** becomes very **acidic**, most bacteria can not continue to function properly. However, the bacteria *S. mutans* can survive these **acidic** conditions and continues to function, even as the pH of the **plaque** becomes so **acidic** that other bacteria die and the surface of the tooth decays.
- Because of its ability to survive **acidic** conditions, *S. mutans* is known to be a type of bacteria that causes cavities. Other bacteria, that would stop functioning at low pH, do not cause cavities to the same degree that *S. mutans* does.

How can *S. mutans* maintain a neutral internal pH when the pH of its environment changes?

- Cell membranes are semi-permeable. When the pH of the outside changes, the pH of the inside should also change
- In order for the bacteria to maintain a neutral internal pH (which is important for survival), it must have some way of removing acid from inside its cell membrane.
- The pH of the **plaque** can change over the course of a day, so *S. mutans* has to adjust to these changes...
- Here, we have shown *S. mutans* as using some kind of pump that is on when acid needs to be removed, and off when the pH of the cell is neutral. But what is this pump? And how does it know to be on or off?

Background Research: Where is the acid coming from?

- Bacteria use sugar to make energy... Sugar enters cells via a channel protein...
- Inside the cell, sugar is broken down by a number of enzymes in a process called RESPIRATION. In the case of *S. mutans*, sugar is processed into energy in the form of ATP, and produces lactic acid as a waste product.
- ATP is used in the cell as energy
- Lactic Acid (L.A.) is a waste product that needs to be removed from the cell. Click on either one to learn more and see how lactic acid is removed...

- Lactic Acid is excreted by the cell...
- Lactic acid can ionize - it gives up a hydrogen ion...and the **plaque** becomes more **acidic**.

Background Research: How does acid damage cells, like bacteria?

- Lactic acid produced during respiration can ionize - it gives up a hydrogen ion (which can cross the membrane)
- The lactate ion (that's what lactic acid is called once it has lost a proton) is too big to cross a semi-permeable membrane
- Hydrogen ions can interact with proteins and break down (denature) the three-dimensional protein structure
- Proper protein structure is necessary for proper function.
- When the pH inside the cell becomes too **acidic**, proteins involved in DNA replication and repair and even respiration will not work.

Background Research: What is a concentration gradient?

- The lactic acid (L.A.) that is produced during respiration is excreted outside of the cell.
- Lactic Acid is too big to cross a semi-permeable membrane, but if they ionize, the hydrogen ions can!
- There are more hydrogen ions outside of the cell than inside -- this is a concentration gradient
- Hydrogen ions will tend to move down their concentration gradient -- to the place where they are less concentrated
- The hydrogen ions will reach equilibrium, where there is an equal concentration of hydrogen ions inside and outside the cell
- Moving these hydrogen ions out into the **plaque** requires moving them against their concentration gradient. This will require energy (ATP)!

Background Research: How do bacteria remove acid?

- Moving hydrogen ions against their concentration gradient requires an active transporter called F-ATPase.
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- This enzyme uses the energy stored in ATP to move hydrogen ions out of the cell and into the **plaque**

Background Research: How do bacteria make more transporters?

- To maintain equilibrium, the bacteria has to adjust the speed at which it removes H⁺ depending on the pH of the cell and the **plaque**
- At neutral pH, there is no need to transport hydrogen ions out of the cell - the inside of the cell is already at pH=7!
- At low pH, the bacteria has to get rid of a few hydrogen ions. It can not get rid of too many though - that would increase the pH of the cell!
- At even lower pH, the bacteria now has to get rid of more hydrogen ions, in the same amount of time!
- This may involve making more transporters...
- Transporter proteins are made when the gene that codes for the transporter (the F-ATPase gene) is transcribed and translated. Click on these words above to see how this works...

- If the bacteria needs more transporters, it can increase the transcription of the gene and make more mRNAs
- Having more transporters means that the bacteria can move more hydrogen ions...

When pH becomes **acidic**, *S. mutans* increases its transcription of the F-ATPase gene.

- Scientists at the University of Rochester believed that if they compared the amount of F-ATPase RNA made by *S. mutans* grown at pH7 versus pH5, that there should be more F-ATPase RNA at pH 5

Grow *S. mutans* at different pHs and measure the amount of F-ATPase RNA that the bacteria produce...

Independent Variable:

- *S. mutans* is grown in a solution that contains nutrients. This is known as a liquid culture. The liquid culture is split into two cultures - the pH of one of them is changed to a more **acidic** pH (pH=5)

Dependent Variable:

- In order to test the hypothesis, it is necessary to measure the amount of F-ATPase RNA each group of bacteria produces
- Cells are broken apart using proteases (Enzymes that break down proteins) and detergent
- (disrupts cell membranes)
- This releases the nucleic acids (DNA and RNA)
- After breaking apart the cells and removing cell debris and proteins, all that is left is DNA and RNA
- To isolate the RNA from the bacteria (called Total RNA), the DNA and RNA mix is treated with DNase - an enzyme that breaks down DNA
- TOTAL RNA includes all the RNA in the cell. Looking at total RNA does not tell you how much F-ATPase RNA is present, which is what you really want to know
- Probe: An RNA that is complementary to the RNA you are interested in (the F-ATPase RNA!)
- If you "probe" for the F-ATPase RNA, that is the only RNA you will see.
- One of the techniques that is used for probing specific RNA's (like the F-ATPase RNA) is called a "slot blot."
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- This involves placing each RNA sample onto a piece of absorbent paper
- The RNA sticks to the absorbent paper (in the shape of a slot). In order to see the F-ATPase RNA, the probe is placed on top of the slots...
- The probe will only attach to complementary RNA's (the F-ATPase RNA), so that when the extra probe is removed there will only be probe attached to the paper if there is F-ATPase RNA present
- The more F-ATPase RNA that is present, the more probe will attach. The probe is visible - the more probe, the more visible the slot.
- You measure the amount of F-ATPase RNA by measuring the color of the slot...

Constant:

- If you want to compare the amounts of F-ATPase RNA in two different total RNA samples, you need to make sure you are comparing the same amount of total RNA
- It is up to the researcher to decide how much RNA to use, she just has to make sure she compares the same amount of RNA (10mg vs. 10mg or 5mg vs. 5mg)

Positive and Negative Control

- Positive and negative controls help you to interpret your results correctly. Sometimes you may come to an incorrect conclusion because your experiment didn't work and you didn't know it!
- Positive Control: This should be something that you KNOW should work. In this case, a sample that you know has F-ATPase RNA
- F-ATPase is such a necessary protein, that any total RNA sample you isolate from bacteria is going to have F-ATPase, so it is not necessary to create a sample that has F-ATPase. If you don't see probe binding to the RNA samples, something is wrong...
- But just as an example: If you wanted to know if *S. mutans* transcribed a gene that is normally only expressed in humans, your positive control would be human RNA
- Negative Control: This should be something that you KNOW should not work. In this case, a sample that you know has no F-ATPase RNA
- It was just mentioned that any RNA sample from bacteria would have F-ATPase RNA, so would one get a sample of RNA that did not have F-ATPase RNA?
- What scientists can do is to treat the RNA samples with RNase - an enzyme that breaks down RNA...
- If there is no RNA, no F-ATPase probe should attach to the RNA in the slot blot.

Slot blots of RNA prepared from *S. mutans* grown at pH 5 or 7. Total RNA samples were probed with an F-ATPase specific probe.

- At pH 5, the slot is darker, indicating that more F-ATPase RNA is present, compared to pH 7. This suggests that *S. mutans* does increase transcription of the F-ATPase gene at low pH.
- Therefore, an appropriate conclusion is that *S. mutans* maintains homeostasis during changing environmental pH by altering the amount of F-ATPase transcription. However, it is important to realize that this does not mean this is the ONLY way *S. mutans* maintains homeostasis.
- The next step to this research is to determine HOW changing pH alters transcription. Is there something that is affected by low pH that increases the number of times RNA polymerase transcribes the gene? If that was true, maybe blocking that thing would prevent cavities...