Genetics of Circadian Rhythms

The Negative Feedback Loop of the *Drosophila* Circadian Clock

What Are Proteins?

Each protein molecule has very specific role in the functioning of a cell. It's as if the cell consists of several machines that make the system work, except instead of machines made of metal, the machines are made up of proteins. Some people refer to proteins as "nano-machines" they are so tiny (*nano* comes from Greek and means *extremely small*) and because they are responsible for cranking out important products necessary for cell and organism survival. Proteins are nano-machines because they produce:

- enzymes; they control the speed of chemical reactions
- structural components of the body: muscle, cartilage, skin, hair
- carrier molecules: such as hemoglobin
- immune system components: antibodies
- hormones: insulin, adrenaline
- transcription factors: control the switching on and off of genes

For our purposes, we will focus upon transcription factors. The transcription factors control when the production of all the other substances begins. This switching mechanism controls the molecular processes that explain the function of the Drosophila clock. We begin by explaining how proteins themselves are created.

Transcribing and Translating

Genes are sections of DNA that are the recipes for proteins. Each gene contains the code for making one specific protein. These genes are situated inside the cell nucleus, but the **ribosomes** that assemble the proteins are located outside the nucleus, within the cytoplasm. Therefore, the genes must pass the protein recipes from inside the nucleus to the ribosomes in the cytoplasm. This is accomplished by messenger RNA (**mRNA**). The recipe for a protein is decoded from the DNA within the cell by messenger RNA (or mRNA). Basically mRNA copies a particular segment of the DNA sequence (this is called **transcription**) so that the short and specific recipe for a protein can be transported outside of the nucleus to a ribosome. You can think of the DNA has a giant cookbook, the genes as the recipes, and the mRNA as the scrap of paper that the recipe is copied into and then taken away to be used.

Now that the mRNA has transcribed a particular protein recipe, it carries that information (in the form of a genetic code) for a particular protein to a ribosome in the cytoplasm. The ribosome "reads" the recipe and assembles the protein by making a sequence from the available amino acid "ingredients" that the recipe calls for. This production of a protein by the ribosome is called **translation**. When the mRNA carries a different recipe the ribosome uses different amino acids in a different sequence. In this way, thousands of kinds of proteins can be created with just a handful of ingredients.

Every time that a cell creates a protein, the process had to begin with this process. First, a mRNA molecule transcribes the gene from inside the nucleus and transports that information to a ribosome, The ribosome then translates that information in order to string together the appropriate amino acids in the correct order to manufacture a protein molecule. This entire process is called **protein synthesis**. The amount of protein that is created, and whether it is being synthesized or not, is controlled by this process.

Controlling the Synthesis of Proteins

It seems reasonable to expect that proteins would be synthesized at some times while at other times there may be no need for a protein to be synthesized at all. The control of protein synthesis is through a feedback loop. A **negative feedback loop** is one where as the amount of protein made increases, the system actually slows down the amount that is being made. When there is enough of a protein, the system is shut down – which only makes sense because making more is unnecessary. A **positive feedback loop** would describe a system that goes faster and faster – imagine a factory that is completely out of control.

In the presentation you would have seen that an automatic icemaker is an example of a negative feedback loop. The amount of ice builds up to the point that it turns off the icemaker. Another example is the thermostat in a home. Warm air (the product) accumulates and eventually shuts off the heater. Similarly, the end product of protein synthesis accumulates so that it eventually turn off its own production — this is negative feedback.

If the product in these examples is gradually removed, then the synthesis process would begin again. Take enough ice out of the freezer and the icemaker starts making more. When the air temperature in a room drops below a certain level, then the heater begins producing warm air. And at the submicroscopic level, this is what occurs inside cells with protein synthesis.

We could measure the rise and fall of ice in the freezer, the temperature in a room, or even the concentration of a protein inside a cell. When graphing this data, we would see a cycle of rising and falling amounts over time. Measuring this rise and fall of certain molecules is one way that scientists explore biological clocks. For our purpose, we will be examining a negative feedback loop that explains the control of the *Drosophila* circadian clock.

Negative Feedback of *Drosophila*

Scientists studying the circadian rhythms of *Drosophila* found that the levels of two different proteins, called PER and TIM, would rise and fall over time in a pattern that matched with the fly's activities. It seemed reasonable to predict that the changing levels PER and TIM proteins (like the rise and fall of ice in the freezer) were under the control of a negative feedback loop. This is an example of the relationship between evidence and explanation.

Scientists have noticed the changes in protein concentrations (evidence) and used that to propose a connection to the cycle of activity of the flies (an explanation). Knowing that each protein is synthesized from a single gene, the scientists used that knowledge to examine the *per* gene (which has the code for PER protein molecules) and the *tim* gene (which codes for TIM protein molecules). Examining the activity of these two genes would potentially lead scientists to understand the negative feedback loop.

Recall that one function of proteins is to act as transcription factors. These molecules regulate protein synthesis by "turning on" certain genes. Two such transcription factors are involved in the *Drosophila* clock: CLK (clock) and CYC (cycle). These protein molecules, CLK and CYC, activate the *per* and *tim* genes within the cell nucleus. When the CLK/CYC "turn on" *per* and *tim* genes, the transcription process begins. CLK and CYC cause the mRNA to start transcribing the codes for PER and TIM. As the mRNA is translated by the ribosomes, the amount of PER and TIM protein molecules begins to increase within the cytoplasm.

As the PER and TIM protein molecules accumulate, begin to pair up. For some reason, any PER or TIM proteins that are not paired up likely to be destroyed by another protein called DBT (doubletime). So while some PER and TIM proteins are safe once they bond with each other, others are destroyed by DBT. Gradually, the PER/TIM pairs begin to migrate — into the cell nucleus.

Once inside the nucleus, the PER/TIM pairs attach to CLK/CYC. Since CLK/CYC are responsible for turning on the *per* and *tim* genes, when PER/TIM pairs interact with the CLK/CYC, the *per* and *tim* genes are no longer being transcribed. Consequently the recipe for PER and TIM is not being read, so those proteins are not being manufactured.

This provides a somewhat complicated example of negative feedback. The products of the assembly line (PER and TIM proteins) accumulated and eventually turned off their own production (by turning off the *per* and *tim* genes). However, while we have *negative feedback*, we don't yet have a *loop*. If we stopped here, the *per* and *tim* genes would be permanently turned off. In order for this system to constantly cycle and create a circadian rhythm, we need *per* and *tim* to eventually get turned back on. Interestingly this is accomplished by sunlight.

Light striking the cell activates a type of protein called a **cytochrome**. When activated, this cytochrome destroys the TIM proteins. Once TIM has been destroyed, PER is left unpaired and vulnerable to destruction by DBT. Therefore, the presence of sunlight leads to the destruction of both TIM and PER proteins.

Once the TIM/PER pairs are removed from the nucleus, the CLK/CYC transcription factors are once again free to turn on the *per* and *tim* genes. This causes the transcription of *per* and *tim* mRNA to begin again and the process of protein synthesis resumes. We now have a loop that cycles the protein synthesis of PER and TIM.

Entrainment to the Day/Night Cycle

From the online Explore activity, you found that light plays an important role in the biological clock of *Drosophila*. Scientists have similarly noticed the influence of light on circadian rhythms in many different organisms. Remember the shift in activity for the human in the cave experiment? This "setting" of a circadian rhythm by some signal, in these examples it was a light source, is said to "entrain" the organism. Its internal clock is entrained so that it is synchronized with the natural day/night pattern.

Apparently light is responsible for entraining circadian rhythms in a wide variety of life forms, Because of this, those who study circadian rhythms expect that light has a role in most daily cycles. However, scientists are still open to new explanations based on new evidence. No matter how well scientists have explained their observations about biological clocks so far, new evidence may surface that result in new explanations. This shouldn't suggest that scientists are unsure – but they acknowledge that as good as their current explanations are, there may be an even better one that someone will propose in the future. In this way, we can claim that scientific knowledge is durable.

The tentative yet durable nature of science is evident in how scientists use what they know. They rely upon their understandings of *Drosophila* biological clocks to better understand human circadian rhythms. Scientists are aware of many kinds of circadian rhythms within humans. This observation has led them to study how each of these clocks are entrained. What these scientists already know about light entrainment in *Drosophila* will shape how they explore human clocks.

Summary

Up until this point, you have probably been aware that there are many examples of circadian rhythms. But now you should begin to recognize that there have been biochemical explanations for circadian rhythms. Through this reading you have learned that there is a chemical basis to behaviors that change in response to night and day, and that there are also genetic components.