Biology 1002B Cycle 1: Light and Life Breakdown

Introduction

Dear Student,

Thank you for opening this cycle breakdown for Bio 1002B. This resource has been created by the Education Team at WebStraw. The Education Team consists of students that have previously taken and/or students that are currently taking Bio 1002B.

Purpose

This resource focuses on key concepts that are important for students to understand to succeed within this course. This resource was created by students for other students. Our goal is to help students (1) further develop their understanding of course content and (2) achieve greater academic success. (3) Our resource is also open access meaning there are no financial or legal barriers to students who wish to access and use our resource.

Instructions

To maximize the benefits of this resource, we recommend that you read carefully through the cycle breakdown with specific focus on bolded terms and the “Think about it” paragraphs. Then, try applying your knowledge with some of our custom-made questions at the end of this document. Make sure you already have a good understanding of course content before using this resource, as it will not cover all testable content!

Disclaimer

This resource is supplementary to your course content and is not meant to (1) replace any of the resources provided to you by your instructor nor is it meant to (2) be used as a tool to learn the course material from scratch. We assume that students who use this resource will have a basic understanding of the course content. This resource does not contain everything you need to know for your evaluations. Please refer to the course material provided by your instructors if there are any discrepancies between our resource and your course content.

We wish you the best of luck on your exams!

- The WebStraw Team

Note to Instructors:
If this resource has been created for your course and you would like to collaborate with us, please email us at team@webstraw.ca

Chlamydomonas

- What characteristics does Chlamy have that makes it a "model system" for experimentation?
- Differences among photosynthetic organisms...prokaryote vs eukaryotes....generation time, chloroplast/ genome size ORF...density of ORF within genome
- Analysis of 7, 476 Chlamy proteins...what processes underlie the proteins common to only Chlamy/Humans, Chlamy/Arabidopsis, and Chlamy/Humans/Arabidopsis
- Chlamy life cycle: why organisms in stressful environments undergo sexual reproduction.

Chlamydomonas is a eukaryote that is a photosynthetic green algae. It has a flagella, chloroplast, eyespot and mitochondria. Additionally, it is single-celled and has a short **generation time**, making it easy to grow and manipulate in the lab. These factors make it a useful **model system** to study phenomena such as photosynthesis, motility and phototaxis.

If we compare Chlamydomonas' **genome size** and **ORFs** to other photosynthetic model systems (Synechocystis and Arabidopsis), we can appreciate the fact that as genome size increases, so does the number of ORFs, while the density of ORFs in a genome decreases. For a given gene on double stranded DNA, there are 6 possible starting positions for an ORF. It is also important to know that Synechocystis is a bacteria that is photosynthetic, meaning that a chloroplast is NOT required to perform photosynthesis!

Upon analysis of 7,476 Chlamydomonas proteins, there are proteins that Chlamy shares with humans, Arabidopsis, or with humans only or Arabidopsis only, or no one.

Think about it; Which proteins would Chlamydomonas share or not share with humans and/or Arabidopsis? Chlamy would likely share its photosynthesis and phototactic associated proteins with only Arabidopsis, while it would likely share some flagella associated proteins with humans only. Proteins associated with functions such as DNA replication, cell respiration are shared with all three.

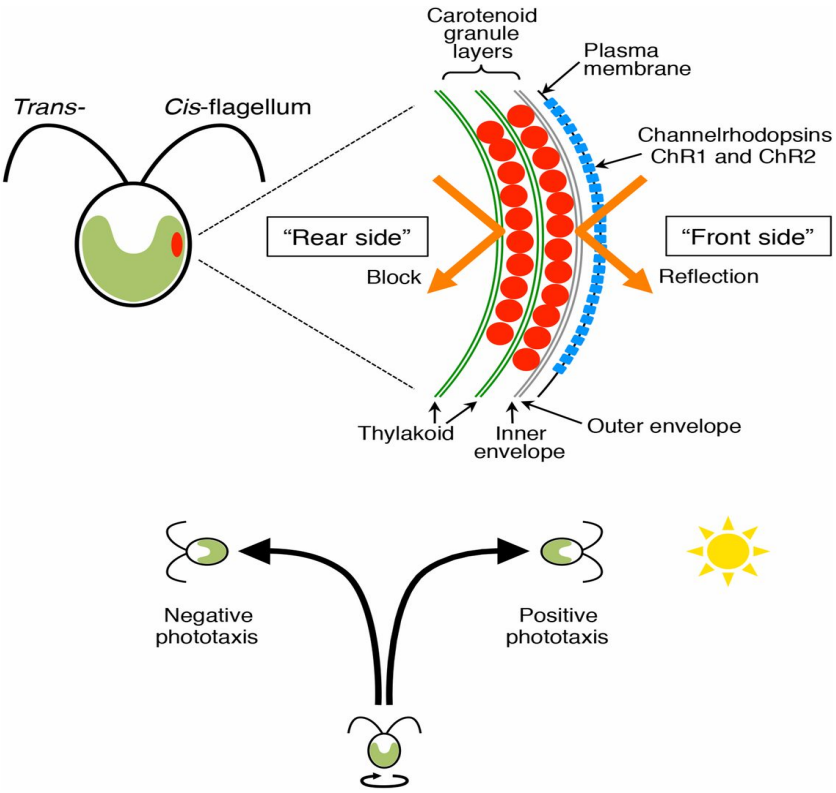
Chlamydomonas can undergo **sexual** and **asexual** reproduction. Under normal conditions, chlamy undergoes asexual reproductions producing copies of itself. Under stressful conditions, chlamy undergoes sexual reproduction.

Think about it; Why would it be beneficial for chlamy to undergo sexual reproduction under stressful conditions? This enables **recombination** to occur through meiosis. This results in more **variation** and an increased likelihood for **adaptation** in stressful environments and reduces the chance that the whole population gets wiped out.

Chlamydomonas, like humans, have an **eyespot** that provides information to them about their environment and a **flagella** which enables them to move. The eyespot contains channelrhodopsin (a protein) which **transduces** (converts light into an electrical message) light and relays information to the flagella. Channelrhodopsin in chlamy and rhodopsin in humans are **not homologous**. Flagella in chlamy and flagella in humans are **homologous**

Eyespot

- Basic organization and functional features of the eyespot.
- Structure & function of channelrhodopsin.
- Key similarities and differences between function of channelrhodopsin in the eyespot and rhodopsin in the human eye.



Ueki et al., 2016

The **eyespot** is what allows Chlamydomonas to “see” and respond to light in its environment. It consists of about 200 proteins. Here are some of the important ones you need to know.

- **Channelrhodopsin:** these are photoreceptor proteins that sit on the plasma membrane. Channelrhodopsins are composed of a **retinal pigment molecule** (which absorbs the actual light) and an **opsin protein** (essentially just a protein that’s attached to the retinal and facilitates the transduction of a chemical signal when exposed to light).
- **Carotenoids:** we don’t need to know too much about the structure of these, but we just need to keep in mind that they are found at the back of the eyespot and act like a “shield” against peripheral light.

 **Think about it; if light from any direction could hit channelrhodopsin and generate a signal, how could the cell tell which direction it’s coming from?** You can think about the carotenoids as giving the cell “tunnel vision”, allowing it to only respond to light from one direction (this is what you want, as being able to identify where light comes from allows **phototaxis**). Not having this would be like you trying to process all visual cues in your full surroundings simultaneously.

The last thing we need to understand about the channelrhodopsin in eyespots is their relationship to rhodopsin in human eyes. To begin, we know they’re structurally and functionally similar in many ways- a table comparing the two is given below.

Channelrhodopsin Function (Chlamy)	Similarities between Chlamy channelrhodopsin and human rhodopsin	Rhodopsin Function (Humans)
• Photons change the conformation from Trans-Cis • Directly activates change in ion movement • Ion channel • Protein and Pigment • Embedded within the chloroplast membrane	• Photon causes changes in conformation • Both use light for information • 7 transmembrane domains • Both bind retinal • Retinal changes shape upon light absorption	• Photons change the conformation from Cis-Trans • Not an ion channel • Indirectly activates change in ion movement through activation of a signaling pathway • Protein only • More complex • Located inside the rods and cones

We can see that they share many similarities AND differences. So how can we tell if they are homologues? It turns out that this cannot be done by a simple analysis of their physical characteristics, and we must instead compare the similarity of their associated gene sequences.
We can do this through a process called **BLAST** (discussed more in the next cycle), which reveals that **the channelrhodopsin in chlamydomonas eyespots and the rhodopsin in human eyes are not homologous**, and evolved independently of one another.

Remember that just because the eyespot is more simple does not mean it is more primitive.

How Chlamydomonas Uses Light

- Mechanism by which the signal is transduced from eyespot to flagella.
- Mechanism of photoisomerization of retinal and its consequences
- Distinction between photochemistry as it occurs in eyes and eyespots compared to how it occurs within a photosystem in photosynthesis
- Basic understanding of optogenetics: what is it, why is it useful, and the basics of what one does to a neutron to make optogenetic experiments possible.

How does the eyespot actually respond to light and send a signal? Recall that the "signal" we are referring to is an **action potential** (depolarizing→ repolarizing → hyperpolarizing → stabilizing) that travels along the membrane, activating different components of the cell that allow it to respond accordingly.

Here is a summary of the full mechanism:

1. Light enters the eyespot, hits the carotenoid back layer, and is reflected towards the channelrhodopsin proteins at the front of the eyespot. **Note that channelrhodopsins are not activated by light hitting them from the environment directly.**
2. Photons cause **photoisomerization** (explained later) of retinal pigments. This forces the opsin protein to change shape, which activates the ion gates along the plasma membrane.
3. As ions flow across the membrane, this generates an action potential which essentially acts as an electrochemical signal for the cell. Gates opening near the eyespot causes adjacent gates to open in a chain reaction.
4. This signal travels along the membrane in a process called **signal transduction** (or phototransduction) and reaches the flagella, which allows the cell to move and exhibit phototaxis.

A couple of things to note **(that are very testable!)** are the few similarities and differences between the photochemistry in eyespot channelrhodopsin and chlorophyll in a photosystem.

- Both photoisomerization and photosynthesis are light-driven processes. Without the energy from photons, neither of these processes are possible.
- Photoisomerization involves a structural change in the opsin protein by energizing electrons. There is, however, no net movement of electrons in channelrhodopsin.
- Photosynthesis involves the excitation and eventual ionization of an electron. There is a net movement of electrons from the chlorophyll molecule, but there are no structural changes.

The field of **optogenetics** has used channelrhodopsin to allow neurons to be activated by light. This was done by expressing channelrhodopsin in neurons. Since channelrhodopsins are channels that are activated by light, they can perform the same functions as the K+/Na+ ion channels involved in traditional action potentials by blocking or allowing ions to cross into the neuron. This means you can shine a light on a neuron with channelrhodopsin and cause it to fire an action potential.

Gene Regulation

- Aspects of gene expression: transcription, translation, mRNA and protein breakdown (decay)
- Regulatory importance for why mRNA degrades relatively rapidly
- Distinction between constitutive, induced and repressed gene/protein expression
- Understanding of how genes influence biochemical pathways (e.g. retinal, hormone biosynthesis)

Gene expression can be studied by focusing on a single aspect of the **central dogma**. For example, transcriptomics analyzes the **transcript abundance** of a certain gene. This focuses on the transcription rate of that gene as well as the degradation rate of its mRNA. It's important to take both rates into account when measuring transcription abundance.

 **Think about it; why would analysis of transcript abundance be more useful for studying gene expression than genomics or proteomics?** The answer to this question is centred around the fact that mRNA is unstable and can be easily degraded. This means if we wanted to highly express a certain gene, we could make many mRNA transcripts from the 1 DNA sequence, translate it, and then degrade it all after. This is important because we don't want mRNA to hang around for too long. Genomic analysis would not be useful since it only measures how much of the DNA sequence is present (which should stay the same). Proteomics can also measure gene expression, but proteins can hang around for longer and won't give as accurate analyses as transcriptomics.

Genes in organisms are constantly being expressed at different levels. For example, **housekeeping genes** are usually always expressed at the same level. This is useful in experimentation as a baseline control, as the expression of that gene should stay the same no matter what conditions you impose on the organism. This is called **constitutive expression**.

The expression of other genes can be induced. For example, a protein that helps the organism survive in a cold environment would only be highly expressed when the organism is in a cold environment; you would not expect the gene to be expressed in a warm environment. This is called an **induced/repressed expression**.

 **Think about it; Make a list of genes in Chlamydomonas that you would expect to be constitutively expressed and explain why.** Your list should include genes that are critical to its survival. Some examples could be: Genes that make proteins associated with oxidative phosphorylation, photosynthesis, the eyespot, flagella, etc.

In class, Dr Maxwell talked about how the **Foles9 mutation** decreases the amount of functional rhodopsin because the mutation influences an enzyme that is required in the biochemical pathway for synthesizing **retinal**. It is important to understand the big picture of what can happen if a gene is destroyed, or under/overexpressed and what it can do to the resulting protein and the pathways it's involved in.

Apply Your Knowledge

Consider the following (not multiple choice, these are designed to allow you to think more freely about the testable concepts):

1. There is a mutation in the gene that codes for the opsin protein which requires it to absorb a higher intensity of light in order to undergo structural change. What will the resulting effect be on *Chlamydomonas'* ability to undergo phototaxis?

2. Recall channelrhodopsin can generate an action potential similar to the one in neurons. Describe an action potential generated by channelrhodopsin and how it differs from one generated by a neuron. Include how the neuron ion channel and channelrhodopsins are activated, which ions move in/out, and the overall objective of each action potential.

3. A scientist is trying to determine the regulation mechanism for a gene that codes for an enzyme that plays a role in the biosynthesis of heat shock proteins. The scientist is unsure of whether it is the transcription of this gene which regulates the abundance of the enzyme product, or if it is the stability/longevity of the transcribed mRNA. What experiment can the scientist carry out to determine the regulation mechanism?

Congratulations for making it through the entire breakdown. Remember to continually reinforce your understanding over as long a period of time as possible in order to maximize your performance. Best of luck in your studies! Here are some links that might interest you.

Want to learn more about WebStraw? Check out our website at www.webstraw.ca