Biology 1002B Cycle 8: Control of Gene Expression 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

Intro to Stem Cells

→ **Stem cells** are powerful cells that have many useful properties in medicine and beyond. To get started with stem cells it is important to note the different types and examples of where they come from.

Totipotent: Can differentiate into embryonic AND extraembryonic cells. Can form an entire viable organism! Only the zygote is considered to be totipotent.

Pluripotent: Can differentiate into nearly all cells of the body. An example is the inner mass cells of a blastocyst.

Multipotent: Can differentiate into a closely related family of cells. Includes adult stem cells.

Unipotent: Can only differentiate into one type of cell. An example is an epidermal stem cell.

Stem Cell Mechanics

The function of adult stem cells is to replenish tissue. As a human, you will have stem cell **niches** in areas like the small intestine or bone marrow. These cells are in a **quiescent** state until they receive signals from neighbouring cells that tell them to enter G1 and divide. There 3 ways stem cells can divide:

Symmetric self-renewal: 1 stem cell divides into 2 stem cells. This allows the stem cell to maintain **count** while having an extra stem cell that can either remain in the niche or go an differentiate.

Asymmetric self-renewal: 1 stem cell divides into 1 stem cell and 1 **progenitor** cell. This allows the stem cell to maintain count while the progenitor is committed to differentiation.

Symmetric differentiation: 1 stem cell divides into 2 progenitors. To maintain count, a neighbouring stem cell will undergo symmetric self-renewal.

Molecular/Physiological Basis of Stem Cells

Examples of adult stem cells in action are stem cells in the **crypt** of the villi in the small intestine. If new cells are needed at the tip of the villi, neighbouring cells will release the **transcription factors** Wnt, Notch and EGF, telling the stem cell to enter G1 and migrate out of the crypt. Another example are unipotent epidermal cells; they reside in the basal layer of the skin and migrate/differentiate upon receiving a signal.

A useful skill when working with stem cells is to know how to test for their **potency**. To do this, you would **fluorescently label** your sample of stem cell, inject them into the inner cell mass of a mouse blastocyst and implant it into a pseudopregnant mouse. Then you would scan the offspring and see where there are fluorescently labelled cells. If they are in a wide variety of tissue, it is likely your sample is pluripotent as they were able to differentiate into many types of cell. If you only see fluorescence in one specific tissue, your sample is likely multipotent.

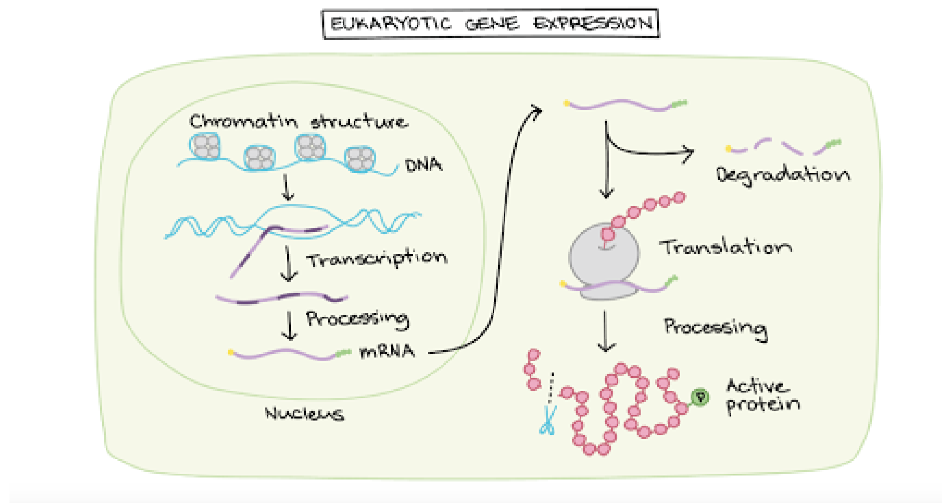
A Note on Induced Pluripotent Cells

Induced pluripotent stem cells (iPS) are fibroblasts that have been reprogrammed by adding the **Yamanaka factors**, 4 transcription factors that tell a cell to revert back to a stem cell. There are incredible therapeutic and research applications of iPS cells including treating **macular degeneration**, burn victims, heart attack patients, or reconstructing tissues in vitro. However, there are still many unknowns about the uses of iPS cells, as they may become cancerous after transplantation.

Gene Expression

Remember that every somatic cell in the body shares an identical genome in principle. **The genome is the same, it is the expression of these genes that dictates the protein products/functionality of cells.** Unused genes still retain the ability to be expressed, under the right conditions.

This might be a good time to go back to your lecture slides and look at the big graphic that depicted how all the steps of gene expression can be regulated. Different aspects of these regulation mechanisms have consistently popped up on term tests, so make sure you know this graphic in and out! Since we cannot show the exact graphic in this breakdown, here is another image that presents the same information.



Khan Academy

→ **Some things to ask yourself…**

- How does each step directly affect transcription/translation abundance and rate?

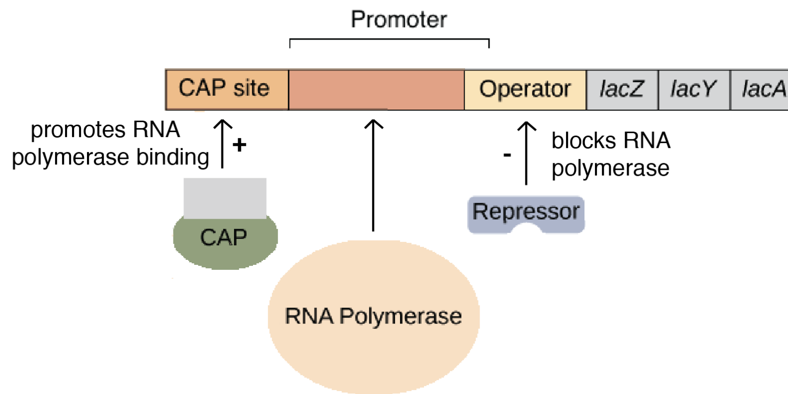
- What molecular components are required for each step to occur? How would these regulation mechanisms behave without these components? *(i.e: pre-mRNA requires specific splice signals at the border of introns/exons. These are necessary for splicing to occur).*

- What implications does this have on the phenotype of the individual, depending on what the gene/protein product is?

- What is the exact mechanism that each regulation pathway employs (base-pair binding, protein binding domains, protein-protein interactions, protein and DNA/RNA binding, etc.)

The lac Operon

The diagrams from class look pretty complicated, but there's only a few details you need to worry about. Remember that **only prokaryotes have operons**, and eukaryotes do not. This is a common caveat found in multiple-choice options of test questions, so read carefully.



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→ **From left to right on a lac operon, we have:**

- **DNA regulatory genes (i.e: lacI):** gene that codes for some regulatory mechanism that affects the actual promoter/operator of the operon. In lac, this is what codes for the repressor.
- **Promoter (lacP):** Region at the beginning of the gene that binds RNA polymerase and facilitates transcription. **This is usually a site of regulation for DNA-binding proteins,** although for the lac operon in particular the repressor does not bind here.
- **Operator (lacO):** Region that regulates gene transcription. This is where the lac repressor binds to inhibit the lac operon. Regulation here **affects every gene in the operon.**
- **Transcription initiation site:** the start of transcription, downstream of the operator and upstream of the first operon gene.
- **lacZ gene:** codes for β-Galactosidase enzyme (metabolism of lactose).
- **lacY gene:** codes for permease enzyme (a lactose influx transporter).
- **lacA gene:** codes for transacetylase enzyme. This is not that relevant on the coming term test.

→ **Notice that lacZ and lacY have very intertwined functions (both involved in processing of lactose).** Since these two genes would always be expressed together anyway, it makes sense for the bacteria to regulate them within the same operon (Think about it: why is it important to keep the genome as efficient/compact as possible for bacteria?)

Mechanics of lac Operon

If no lactose is present in the cell/cell environment: **lac operon has very low/no expression…**

No lactose → lac repressor is active by default → binds to the lac operator (lacO) → inhibits ability of RNA polymerase to transcribe gene → no genes on the operon are transcribed into mRNA

If lactose is present in the cell/cell environment: **lac operon expression is induced (suppression lifted)...**

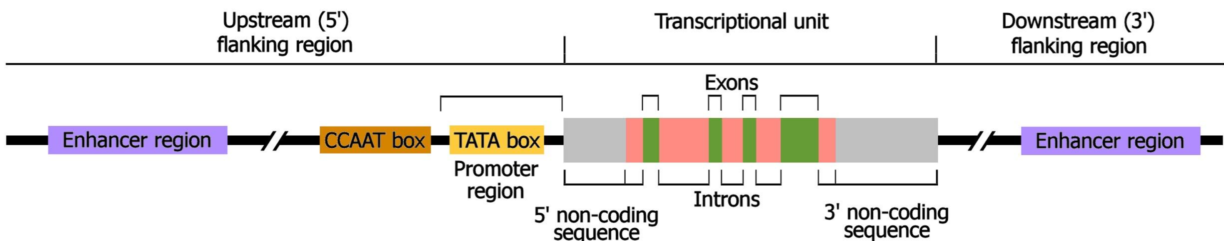
Lactose present in cell → processed into allolactose via lactose metabolism pathway (don't worry about the pathway itself) → allolactose binds to the lac repressor, inactivating it → the lac repressor + allolactose complex is unable to bind to the operator → RNA polymerase is able to transcribe genes of lac operon

Instead of memorizing these pathways, try to understand how the steps together interact and produce the expression/lack of expression of the operon. You may be given hypothetical situations, or novel circumstances where one or more of the steps in these regulation pathways are affected to some degree (i.e: aberrant increased/decreased function or abundance)

Transcription in Eukaryotes

Eukaryotes tend to have **genes with their own regulation mechanisms**, instead of placing them all together into multi-gene complexes like prokaryotes do with operons. They also have versatile ways of manipulating their genome/mRNA/proteins that allows for a larger diversity of products stemming from the same gene (i.e: splicing).

Below is a brief structure of the eukaryotic genome:



Memorial University of Newfoundland

→ **Some of the important components of this diagram and their respective functions are as follows:**

- **Enhancer:** regulatory region, could code for DNA binding proteins
- **Promoter proximal elements (Not shown):** regulatory sequences that can alter the affinity of the promoter towards RNA polymerase
- **Promoter:** binding site for the RNA polymerase. This is what transcription factors (TFs) bind to to provide a mechanism of gene regulation.
- **TATA box:** sequence of AT rich nucleotides bound to by **TATA box binding proteins (TBPs),** which allows RNA polymerase to bind. Found within the promoter.
- **5' UTR (non-coding sequence):** untranslated region on the **5' end of the mRNA molecule**. Since complementary strands are antiparallel, this means that it is actually on the **3' side of the DNA molecule**. UTRs are untranslated, but are still transcribed into mRNA.
- **3' UTR (non-coding sequence):** untranslated region on the **3' end of the mRNA molecule.** Same idea as with the 5' UTR; the 3' UTR will be on the **5' side of the DNA molecule.**
- **Introns:** region of the gene that gets spliced out from pre-mRNA, forming mature mRNA afterwards. Transcribed, but not translated. Ends of intron contain **consensus sequences** for splicing.
- **Exons:** "coding" regions of the gene. Found in the final mature mRNA, and specify the amino-acid sequence of the protein product through codons.

A Note on Splicing

Splicing requires the presence of both the 5' and 3' intronic **splice site consensus sequences**. In class, the example consensus sequence given was "**NCAG"**, where N can be any RNA nucleotide (A, U, G, C).

A protein-RNA complex called a **spliceosome** is also required, which contains RNA in the form of **snRNA** (don't worry about the specifics of these details too much).

→ **So… how does splicing work?** It's actually pretty simple. **Splicing requires a 5' consensus sequence signal and a 3' consensus signal, and will splice everything in between.** You can also consider the spliceosome to be "reading" from 5' → 3'. This means it will start every cut at a 5', and end it (by cutting again) at the next *available* 3'.

(a) Wild-type splicing

Rowlands et al., 2019

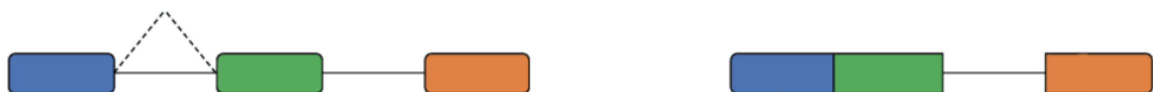
→ **What if we lose a 3' consensus sequence?** As mentioned earlier, the spliceosome will still read the pre-mRNA indifferently. In the diagram above, we see that the first intron is unaffected, as its splice signals are still present. **However,** the second intron has lost its 3' consensus, and so the spliceosome will cut from the 5' of intron 2 to the next available 3', which happens to be at the end of intron 3. **As such**, an entire extra exon is spliced out in a process called **exon skipping.**

(b) Exon skipping

Rowlands et al., 2019

→ **What if we lose a 5' consensus sequence?** The second intron now has no starting signal to cut at, and so it is skipped entirely. The next "cut-start site" won't be until intron 3, and so the entire intron 2 is kept in the final mRNA product. This is called **intron inclusion/retention**.

(e) Intron retention



Rowlands et al., 2019

There are some good questions for the implications of intron/exon splicing at the end of the cycle from the class notes. These are very good "big-picture" examples for you to use in synthesizing your knowledge.

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 here