THE BIOCHEMISTRY AND CELL SIGNALING PATHWAY OF MC1R

OVERVIEW

This lesson serves as an extension to the short film *The Making of the Fittest: Natural Selection and Adaptation,* which can be downloaded at http://www.hhmi.org/biointeractive/making-fittest-natural-selection-and-adaptation. Students use amino acid sequences from the rock pocket mouse genome to illustrate the different levels of protein structure and the relationship between a protein's structure and function. In addition, students analyze the MC1R signaling pathway in both light- and dark-colored rock pocket mouse populations. Through their analyses, students come to understand the concepts of cell signaling. Finally, students hypothesize how the change in sequence and thus protein function might directly affect the coat-color cell signaling pathway of the rock pocket mouse populations.

KEY CONCEPTS AND LEARNING OBJECTIVES

- Proteins fold into three-dimensional structures of varying levels of complexity.
- A protein's function is dependent on its three-dimensional structure, which is determined by the sequence of amino acids in the protein.
- Cell signaling pathways mediate the sensing and processing of stimuli. They involve signal reception, transduction, and cellular response.
- In melanocytes, the MC1R signaling pathway is involved in determining a rock pocket mouse's coat color.

Students will be able to

- use an amino acid class chart to determine whether an amino acid in a protein sequence is nonpolar, polar, acidic, or basic;
- analyze amino acid data to hypothesize how changes in the sequence of the MC1R protein domains might affect the function of the protein; and
- analyze amino acid data to hypothesize how the change in sequence and thus protein function might affect the coat color of rock pocket mouse populations.

CURRICULUM CONNECTIONS

Curriculum	Standards
NGSS (April 2013)	HS-LS1-1, HS-LS3-1 HS.LS1.A, HS.LS4.B, HS.LS4.C
Common Core (2010)	CCSS.ELA-Literacy.RST.9-10.3, CCSS.ELA-Literacy.RST.9-10.4, CCSS.ELA-Literacy.RST.9-10.7
AP Biology (2012–13)	3.D.1, 3.D.3, 4.A.1, 4.B.1
IB Biology (2009)	2.4, 3.5, 4.1, 4.3, 7.3, 7.4, 7.5, C.1, G.1

KEY TERMS

adaptation, evolution, mutation, natural selection, trait, variation

TIME REQUIREMENTS

This lesson was designed to be completed within one 50-minute class period; additional time for the analysis questions might be required depending on students' pace.

SUGGESTED AUDIENCE

This lesson is appropriate for high school biology (AP, IB) and introductory college biology.

PRIOR KNOWLEDGE

Students should understand the relationship between protein structure and function and should be comfortable with the processes of transcription and translation. Students should also be familiar with the basics of cell signaling, including the function of ligands, reception, transduction pathways, second messengers, and response.

MATERIALS

amino acid class chart

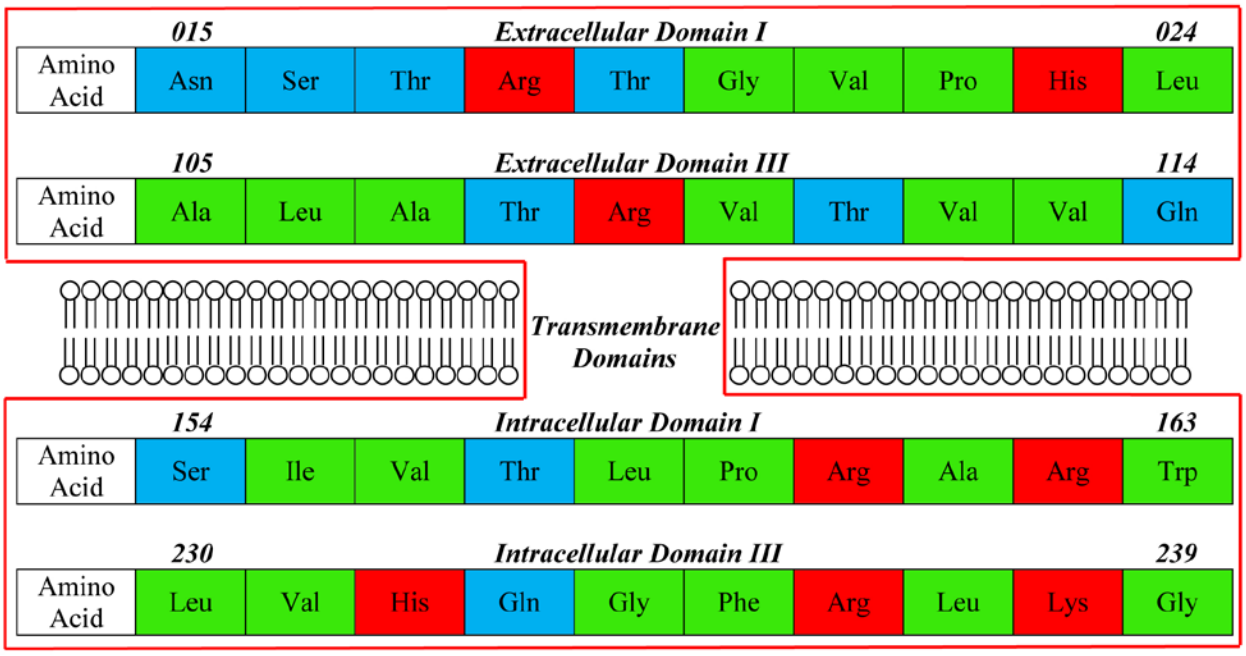
blue, red, green, and yellow colored pencils

TEACHING TIPS

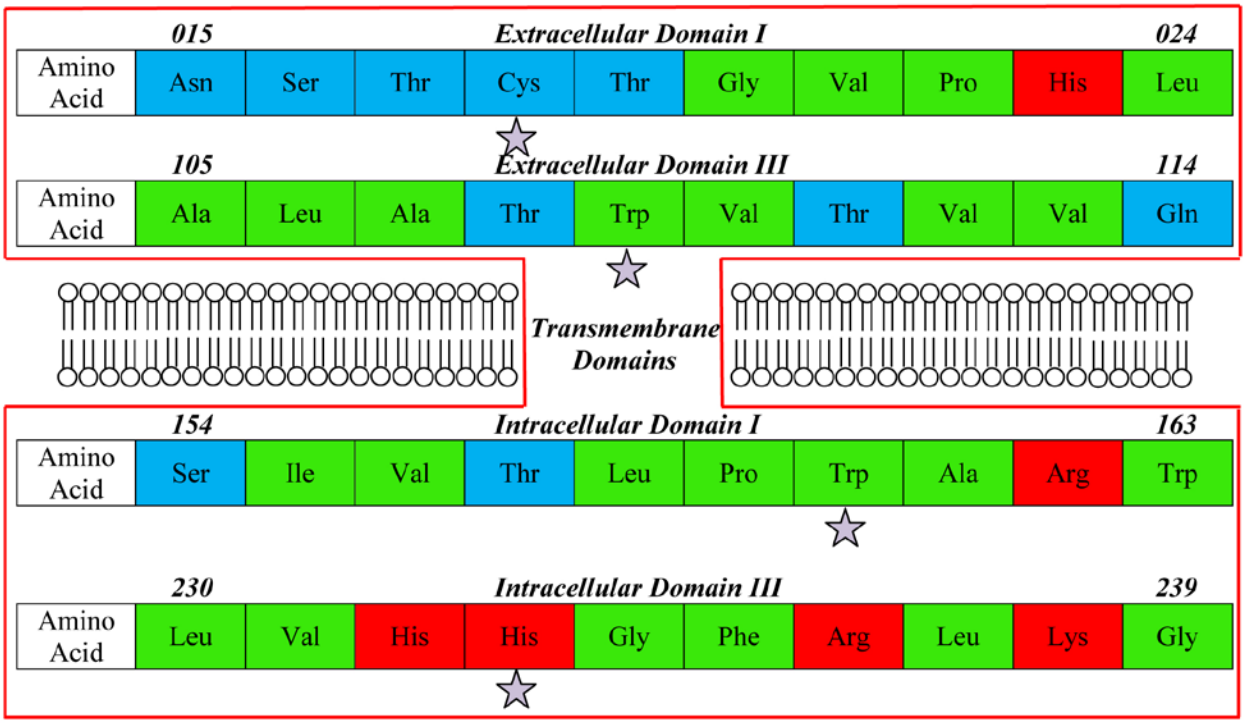
- Although this lesson can be completed as a stand-alone lesson, we recommend that students first complete the related lesson "Molecular Genetics of Color Mutations in Rock Pocket Mice," which can be downloaded at http://www.hhmi.org/biointeractive/molecular-genetics-color-mutations-rock-pocket-mice.
- Students can complete the procedure individually or in small groups. Analysis questions are designed to be challenging and should be completed by groups of three or four students to encourage discussion and help identify misconceptions.
- Students use color to classify amino acid types in Step 3 of the procedure. If you do not have access to a color printer, you should compare student work to the answer key (on page 3 of this lesson) on your computer screen.

ANSWER KEY

Wild-type *Mc1r* gene (light phenotype)



Mutant *Mc1r* gene (dark phenotype)



QUESTIONS

1. Where is the melanocortin 1 receptor located, and what is its role in the cell?

The melanocortin 1 receptor is embedded in the cell membranes of specialized cells called melanocytes. It plays a role in the determination of coat color.

2. *a. What does the following shape on the gene tables represent?*

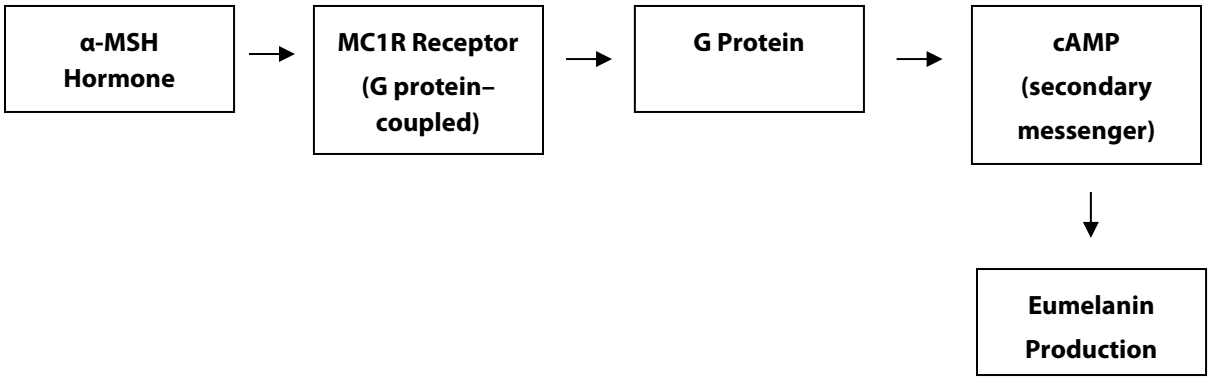
The shape represents a phospholipid molecule, which is found in all cell membranes. Phospholipids make up the phospholipid bilayer structure of membranes.

b. Why is the phospholipid membrane included in the figure with respect to the receptor's location and three-dimensional structure? (Hint: Refer to the introduction and Question 1 above.)

The receptor is located within the membrane. The MC1R protein is a transmembrane protein with extracellular domains, transmembrane domains, and intracellular domains. Inclusion of the phospholipid shapes on the gene tables helps visualize the portion of the protein extending out of the cell (extracellular) and the portion extending into the cytoplasm (intracellular).

3. Using the information provided in the introduction, create a simple flowchart depicting the MC1R pathway. There should be a minimum *of five steps in the pathway. Be sure to include reception, a portion of the transduction pathway, and the cellular response.*

Sample flowchart:



4. Complete the table below comparing the chemistry of amino acids in the wild-type MC1R protein and the mutant MC1R protein.

Comparison Table

Amino Acid Mutation Position Number	Wild-Type MC1R Amino Acid Chemistry	Mutated MC1R Amino Acid Chemistry
Example 1	*Polar (hydrophilic), neutrally charged*	*Electrically charged, negative (acidic)*
018	**Electrically charged, positive (basic)**	**Polar (hydrophilic), neutrally charged**
109	**Electrically charged, positive (basic)**	**Nonpolar (hydrophobic), neutrally charged**
160	**Electrically charged, positive (basic)**	**Nonpolar (hydrophobic), neutrally charged**
233	**Polar (hydrophilic), neutrally charged**	**Electrically charged, positive (basic)**

5. The wild-type (normal) Mc1r *gene results in the light coat-color phenotype, while the mutated* Mc1r *gene results in the dark coat-color phenotype. Based on your knowledge of the MC1R signaling pathway (Question 3), cell signaling, and the chemistry of the amino acid changes (Question 4), write a hypothesis for each of the following questions.*

a. How could the two extracellular mutations lead to the dark phenotype? (Hint: Think about the chemistry of the amino acids, particularly their charge.)

The extracellular mutations at positions 018 and 109 both change a positively charged arginine amino acid to a neutrally charged amino acid (polar cysteine and nonpolar tryptophan, respectively). This change in electrical charge in the extracellular domains of the receptor protein could increase the affinity of the ligand for the receptor, therefore amplifying the pathway signal and thus producing more eumelanin. The change in charge could also decrease the effect of an antagonist from another gene, which would also lead to the production of more eumelanin and a dark coat color.

b. How could the two intracellular mutations lead to the dark phenotype? (Hint: Think about the chemistry of the amino acids, particularly their charge.)

The missense mutation found at position 160 changes a positively charged arginine to a neutral, nonpolar tryptophan, and the mutation at position 233 replaces glutamine, which is neutral and polar, with a positively charged and basic histidine. These changes will ultimately change the shape of the intracellular domains of the protein, perhaps producing an overly active receptor, which would lead to the production of eumelanin. In addition, this change could increase the activation of the G protein without the need of a ligand. This increase in activation would amplify the levels of cAMP, thus increasing eumelanin production.

c. How does the wild-type Mc1r *gene result in the light phenotype? (Hint: It might be helpful to think of it as not resulting in the dark phenotype.)*

The normal receptor conformation requires a ligand for activation of the G protein. If the ligand is not readily available, the production of eumelanin will decrease, resulting in a light coat color. Another hypothesis might be the presence of an antagonist from another gene in the rock pocket mouse that, when expressed, interferes with the MC1R pathway, thus reducing eumelanin production.

Although we do not expect students to know this, a second gene is known to be involved in the coat color pathway, namely the *agouti* gene. This gene produces a protein antagonist to the MC1R pathway. The presence of this antagonist decreases the production of eumelanin and increases the production of pheomelanin. Obviously, the mice with light-colored coats contain the normal MC1R receptor and related pathway, and they also contain the *agouti* gene. During the hair development cycle, there is a pulse of *agouti* expression, producing the antagonist that results in the deposition of pheomelanin in the hair. This results in the light coat-color phenotype. Preliminary results indicate that the mice with the dark *Mc1r* alleles have hyperactive MC1R receptors that increase the levels of cAMP, thus producing eumelanin in the melanocytes and resulting in the dark phenotype, even though these mice also contain the *agouti* gene and the protein antagonist it produces.

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