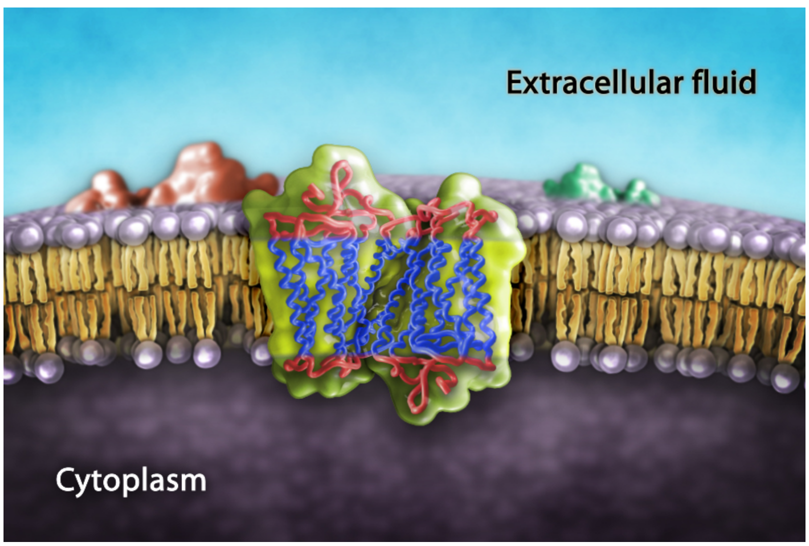
THE BIOCHEMISTRY AND CELL SIGNALING PATHWAY OF MC1R

INTRODUCTION

THE ROCK POCKET MOUSE

The rock pocket mouse, *Chaetodipus intermedius*, is a small, nocturnal animal found in the deserts of the southwestern United States. Most rock pocket mice have a sandy, light-colored coat that enables them to blend in with the light color of the desert rocks and sand on which they live. However, populations of primarily dark-colored rock pocket mice have been found living in areas where the ground is covered in a dark rock called basalt, which was formed by geologic lava flows thousands of years ago. Scientists have collected data from a population of primarily dark-colored mice living in an area of basalt in Arizona called the Pinacate lava flow, as well as from a light-colored population living nearby. Researchers analyzed the data from these two populations in search of the genetic mutation responsible for the dark coat color. Their analyses led to the discovery of a mutation in the *Mc1r* gene, which is involved in coat-color determination.



Model of a transmembrane protein

THE MC1R PATHWAY

The *Mc1r* gene encodes a protein called melanocortin 1 receptor (MC1R). It is found in specialized cells called melanocytes that are responsible for producing pigments that affect the rock pocket mouse's coat color. MC1R is a transmembrane protein (see figure), meaning it is embedded in the cell membrane and has a portion of its structure projecting out of the cell (an extracellular portion), a portion projecting into the cell (an intracellular portion), and a portion embedded within the phospholipid bilayer of the cell membrane (a transmembrane portion).

In rock pocket mice with the wild-type *Mc1r* gene, which have the light-colored coat, melanocytes decrease the production of the dark-colored pigment called **eumelanin** and increase the production of **pheomelanin**, a light-colored pigment. In mice with the mutant version of the *Mc1r* gene, there is an increase in the production of eumelanin from the melanocytes, resulting in the dark coat-color phenotype.

A typical cell communication pathway involves reception, transduction, and response. MC1R is a G protein–coupled (linked) receptor. This type of receptor contains an extracellular binding site for a ligand (signal molecule) and an intracellular binding site for a G protein. For MC1R, the signal molecule is a hormone called alpha-melanocyte-stimulating hormone (α-MSH). When α-MSH binds to MC1R, MC1R changes its shape and its intracellular portion binds inactive G protein. The G protein becomes activated by this process and triggers the first step in the transduction pathway. Transduction is a series of intracellular reactions that convert a signal from outside the cell to a form that can bring about a specific cellular response. In melanocytes, activation of MC1R-coupled G protein results in increased levels of cyclic adenosine monophosphate, or cAMP— a small, nonprotein molecule that relays the message within the cytoplasm of the cell—which then lead to increased eumelanin production.

MATERIALS

amino acid class chart (see page 6 of this handout or an advanced biology textbook)

blue, red, green, and yellow colored pencils

PROCEDURE

1. The gene tables that follow show partial amino acid sequences from two extracellular domains and two intracellular domains encoded by the wild-type and mutant *Mc1r* gene. (Note: The amino acid data included here are the same as those collected in the lesson titled "Molecular Genetics of Color Mutations in Rock Pocket Mice," available at http://www.hhmi.org/biointeractive/molecular-genetics-color-mutations-rock-pocket-mice.)

2. In the mutant gene tables, place a star below each of the four boxes containing the missense mutations in the mutant MC1R protein amino acid sequence. (Hint: You will need to compare the amino acid sequence of the wild-type receptor with that of the mutant one.)

3. Use an amino acid class chart (see page 6 of this handout or an advanced biology textbook) to determine the class of each amino acid in both the wild-type and mutant proteins. Color in each box of the two tables according to the color key below.

Nonpolar (hydrophobic), neutrally charged amino acids: **Green**

Polar (hydrophilic), neutrally charged amino acids: **Blue**

Electrically charged, positive (basic) amino acids: **Red**

Electrically charged, negative (acidic) amino acids: **Yellow**

Example:

Amino Acid	**Ser**	**Val**	**His**
	(Blue)	(Green)	(Red)

4. Answer the questions starting on page 4 of this handout by using the introduction on page 1 and your knowledge of biochemistry and cell signaling.

GENE TABLES

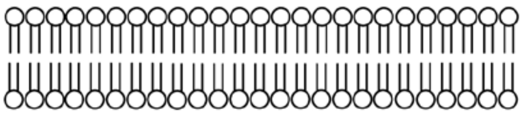
Wild-type *Mc1r* gene (light phenotype)

015 — Extracellular Domain I — 024

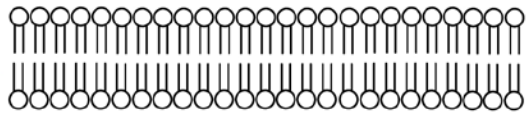
Amino Acid	Asn	Ser	Thr	Arg	Thr	Gly	Val	Pro	His	Leu

105 — Extracellular Domain III — 114

Amino Acid	Ala	Leu	Ala	Thr	Arg	Val	Thr	Val	Val	Gln



Transmembrane Domains



154 — Intracellular Domain I — 163

Amino Acid	Ser	Ile	Val	Thr	Leu	Pro	Arg	Ala	Arg	Trp

230 — Intracellular Domain III — 239

Amino Acid	Leu	Val	His	Gln	Gly	Phe	Arg	Leu	Lys	Gly

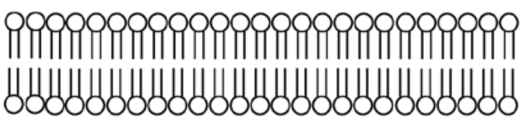
Mutant *Mc1r* gene (dark phenotype)

015 — Extracellular Domain I — 024

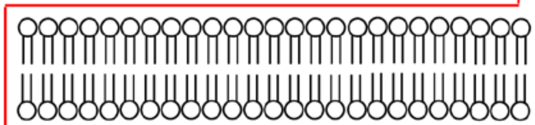
Amino Acid	Asn	Ser	Thr	Cys	Thr	Gly	Val	Pro	His	Leu

105 — Extracellular Domain III — 114

Amino Acid	Ala	Leu	Ala	Thr	Trp	Val	Thr	Val	Val	Gln



Transmembrane Domains



154 — Intracellular Domain I — 163

Amino Acid	Ser	Ile	Val	Thr	Leu	Pro	Trp	Ala	Arg	Trp

230 — Intracellular Domain III — 239

Amino Acid	Leu	Val	His	His	Gly	Phe	Arg	Leu	Lys	Gly

QUESTIONS

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

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2.

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

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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.)

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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.

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**	**Mutant MC1R Amino Acid Chemistry**
Example 1	Polar (hydrophilic), neutrally charged	Electrically charged, negative (acidic)

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.)

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

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.)

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Amino acid class chart