How Do Some Cells Affect Mouse Color?

Activity
Educator Materials

OVERVIEW

In this phenomenon-driven activity, students investigate how cells are signaled to make melanin and explain how mutations in melanin pathway proteins affect the coat color of various organisms. This activity is based on content covered in the BioInteractive short film *The Making of the Fittest: Natural Selection and Adaptation*. Instead of frontloading information from the film, the activity guides students through "figuring out" the key concepts first — including the mechanisms that lead from genotype to phenotype and how natural selection influences those phenotypes.

This activity is meant to be given to students in separate parts, so that they can focus on figuring out one part before receiving the next and coherently build on their understanding. It is not recommended to provide all parts of the "Student Handout" as a packet together.

- In **Part 1**, students make predictions based on the rock pocket mouse fur color phenomenon. Then, they observe a model of the MC1R protein in the membrane of a melanocyte, which shows how this protein receives a signal from the hormone MSH to produce the dark pigment eumelanin.
- In **Part 2**, they observe a second model that shows portions of the amino acid sequences of the extracellular and intracellular portions of the MC1R of both the light phenotype and the dark phenotype of the rock pocket mouse. By comparing amino acid sequences, students identify the locations and types of mutations responsible for the fur-color change described in the film.
- In **Part 3**, students observe a diagram of the pathway for the biosynthesis of melanin in melanocytes.
- In **Part 4**, students transfer what they have learned about melanin production in rock pocket mice to explain how variants of the proteins in the melanin pathway affect the fur color of lab mice.
- In **Part 5**, they transfer what they have learned to fur color in Labrador retrievers.

The educator document contains multiple resources for implementing this case study with students, including the following (select links to go directly to each section in the document):

- teaching tips for this resource
- suggested procedures for each part of the "Student Handout"
- assessment guidance for the questions in the handout
- appendix with more information on the melanin pathway

Additional information can be found on this resource's webpage, including suggested audience, estimated time, and curriculum connections.

KEY CONCEPTS

- Cells receive signals from outside the cell (reception) and convert those to intracellular signals (transduction) for making a particular protein (response).
- The environment affects whether a mutation is advantageous, deleterious, or neutral.
- Fur color is an example of a polygenic trait, meaning that it is affected by multiple genes.
- Mutations in different genes in a biosynthetic pathway can result in varied phenotypes.

STUDENT LEARNING TARGETS

- Use a model to illustrate how changes in a biosynthetic pathway can lead to different phenotypes.

- Develop and use a model to make connections between changes in amino acid sequences and the resulting phenotype.
- Analyze and interpret a visual representation to determine how mutations affect a phenotype.

PRIOR KNOWLEDGE

Students should be familiar with:

- genes, proteins, and the connection between genes and proteins
- the inheritance of genes
- (Part 2) basic structure of an animal cell, particularly the cell membrane and cytoplasm
- (Parts 3–5) interpreting simple genotypes of one to three genes
- (Part 4) some concepts and terms associated with Mendelian genetics
- (Parts 4–5) using Punnett squares

MATERIALS

- copies of the "Student Handout"
- colored version of Figure 1 in the handout (can be projected, printed, or distributed digitally)

TEACHING TIPS

- It is *not* recommended to show the short film *The Making of the Fittest: Natural Selection and Adaptation* in its entirety until students have worked through at least Parts 1 and 2 of this activity.
- Distribute each part of the "Student Handout" separately. Students should receive the next part only after they have completed each previous part. It is not recommended to give students all parts of the handout at once.
- Students may work on the handout individually or in small groups.
- To navigate from one part of the activity to the next, consider facilitating a whole-group discussion after students have had ample time to work. You may use this discussion to surface student ideas that they will be able to use to continue explaining the phenomenon.
- If time is limited, you can skip or select specific parts of this activity to use, providing scaffolding as needed. Parts 4 and/or 5 can be done individually as homework or as a formative assessment to gauge understanding.
- In **Part 1**, students need a colored version of Figure 1 in the "Student Handout." If color printing is limited or unavailable, you can project the figure in class or print a few color copies for students to share (which can be laminated for reuse). You can also distribute the handout to students digitally.

PROCEDURE

PART 1: Making Observations

Students first observe Figure 1 in the "Student Handout," which shows dark-colored and light-colored rock pocket mice on light-colored and dark-colored substrates. Allow them to make observations of the images so that they can engage in sensemaking prior to viewing any associated film clips. The goal is for students to try to figure out the phenomenon without frontloading information.

- The predators of rock pocket mice include birds of prey, such as hawks, that search for them from the air. The more contrast between the mice and their background, the more visible the mice are to these predators.
- It is important to stress that light-colored mice are still found on dark-colored backgrounds, and dark-colored mice are still found on light-colored backgrounds. However, mice that blend in will survive to reproduce more often and leave more offspring that will also blend into the background.

The second half of Part 1 introduces cell signaling. Students learn about a simple signaling pathway of reception, transduction, and response that involves the protein MC1R, which affects fur color through pigment production. More information about MC1R signaling is provided in the appendix. In brief:

- The hormone **MSH** acts as a signal to produce **eumelanin**, a dark pigment. The receptor for MSH is **MC1R**, which is found in the cell membrane of **melanocytes** (a type of cell that produces pigments).
- To explain this pathway, it may help to use a "light switch" analogy in which MC1R is the switch.
  - The switch is *on* if MC1R is functional. In this case, when MSH binds to MC1R, the receptor receives a "message" and sends a signal to the interior of the cell. This signal prompts the cell to make the dark-colored pigment eumelanin, which results in dark-colored fur.
  - The switch is *off* if part of MC1R is nonfunctional. In this case, the cell does not receive a signal to make eumelanin. However, melanocytes can still make a light-colored pigment called **phaeomelanin**, which results in light-colored fur.

Students should read the information about the MC1R signaling pathway in the handout and then observe Figure 2, which shows the MC1R protein in the cell membrane both before and after it is signaled by MSH.

Later parts of the activity introduce other proteins involved in melanin production. In this part of the activity, we are just focusing on MC1R, so we assume that all of the other proteins in the melanin pathway are functional.

PART 2: Analyzing and Interpreting Data

Students examine the partial amino acid sequences in Figure 3 of the "Student Handout," which represent the extracellular domain and the intracellular domain of MC1R for both the light phenotype (light-colored mice) and the dark phenotype (dark-colored mice).

- Note that this figure shows only the parts of the amino acid sequence that contain mutations associated with fur color. The full *MC1R* gene is much longer than what is represented in Figure 3, as it codes for the entire amino acid sequence of the intracellular, transmembrane, and extracellular domains of the protein.
- If students wonder what a protein "domain" is, tell them it is a part of a protein that has a specific function. A single protein can have different parts, each with its own function.

Students compare the sequences of the light and dark phenotypes and note the amino acid differences in Question 7. This activity does not ask students about the effects of these amino acid differences. However, you may want them to know that different amino acids have different properties that may affect a protein's structure. For example, the amino acid arginine (Arg) is positively charged and polar, whereas tryptophan (Trp) is nonpolar. Changing Arg to Trp in a protein's amino acid sequence would likely change the way the protein folds and influence its shape.

PART 3: Using Models to Develop Explanations

Students are introduced to the melanin biosynthetic pathway through Figure 4 in the "Student Handout." More information about this pathway is provided in the appendix of this document.

Students use sensemaking to figure out how certain proteins in Figure 4 affect the biosynthesis of different types of melanin. Give students time to try to figure out the pathway and answer the questions in the handout.

PART 4: Arguing from Evidence

Using Figure 4 again, students interpret genotypes of three proteins (MC1R, TYR, and TYRP1) involved in melanin production. They list the corresponding phenotypes for these genotypes in Table 2 of the "Student Handout."

- The left side of Table 2 has dihybrid genotypes, and the right side has trihybrid genotypes.

- If a protein is omitted from a genotype in Table 2, assume that protein is functional. For example, the genotype PPBB means that both TYR and TYRP1 are functional; we assume that MC1R, which is not included in this genotype, is functional as well. Based on Figure 4, a mouse with all three functional proteins would have a phenotype of black fur.

Students are then given a scenario in which a researcher mates two mice, and they must work through a dihybrid genetics problem. They will discover that, because of epistasis, two of the categories of a typical dihybrid phenotypic ratio are combined into one, giving a 9:3:4 ratio (because any combination of “pp” codes for an albino mouse).

PART 5: Transfer Task with Labrador Retriever Phenotypes

Students now transfer what they have learned to a new scenario. They apply the principles behind the biosynthesis of fur color that they learned in mice to breeding Labrador retrievers (“labs”). This part could be given as a group or individual formative assessment, or as homework. Students could also whiteboard their answers, then have a class discussion in which groups justify their answers.

Part 5 focuses on the effects of the proteins MC1R and TYRP1 on lab fur color:

- A yellow lab has a nonfunctional MC1R.
- A chocolate (brown) lab has a functional MC1R but a nonfunctional TYRP1.
- A black lab has a functional MC1R and a functional TYRP1.

In the Part 5 scenario, students are asked to determine the possible genotypes of two labs.

- The yellow male must be either rrBB or rrBb, and the chocolate female could be RRbb or Rrbb. Rrbb is unlikely, though, since there have never been any yellow labs born in the chocolate lab’s family.
- In order to have all black puppies, it is likely that the male is rrBB and the female is RRbb, but other genotypes are also possible.
- The genotype rrbb is not possible for the yellow lab, because one parent must give a “B” for the puppies to be black (able to make eumelanin). Since the chocolate lab only has “b,” the yellow lab must contribute “B.”

To get chocolate puppies (Question 16c), the breeder must breed dogs with “bb” and at least “Rr.” If the dogs are Rrbb and Rrbb, however, it is possible that they could have a yellow rrbb puppy. To get only chocolate puppies, the breeder should either breed Rrbb with RRbb or RRbb with RRbb.

Students may be uncomfortable that there isn’t one right answer for some of the questions, but let them struggle through this and justify their answers.

ASSESSMENT GUIDANCE

PART 1: Making Observations

1. Which of the mice appear to have a better chance of surviving in their environments?
   The mice that have fur colors matching the colors of their environment will have a better chance of survival. These are Mouse A (light-colored fur in a light-colored environment) and Mouse B (dark-colored fur in a dark-colored environment).
2. Provide evidence for why you think the mice you picked in Question 1 have a better chance of survival.
   Because these mice blend into their environments, they are less likely to be seen and killed by predators.
3. Mouse C was born with a different fur color than Mouse A. Why do you think this is?
   Mouse C must have inherited gene copies from its parents or had a new mutation that caused dark-colored fur.

(Make sure that students understand that even though light-colored mice are more likely to survive in light-colored environments — and may thus become more common in those environments over time — there is still a chance that a dark-colored mouse could be born there due to genetic variation in the population.)

4. The hormone MSH acts as the signaling molecule for MC1R. Using Figure 2, explain what you observe and what you think it means.
Figure 2 shows that MSH binds/"fits into" part of the MC1R protein outside of the cell. When MSH binds to MC1R (reception), it sends a message that signals the cell to do something.

5. Based on the phenomenon of the rock pocket mice that you observed in Figure 1:
   a. If MC1R receives the signal from MSH, what do you think will happen inside the cell?
   Any reasonable responses are acceptable here. One potential reply may include that the signal will be carried across the membrane (transduction) to the interior of the cell, and the mouse will be dark colored (response).
   b. If no signal is received from MSH, what do you think will happen inside the cell?
   Any reasonable responses are acceptable here. One potential reply may include that, if no signal is received, the mouse will be light colored.

PART 2: Analyzing and Interpreting Data

6. Using Figure 3, compare the amino acid sequence of the light phenotype with that of the dark phenotype. Note the differences between them.
Four of the amino acids are different, two in the extracellular domains and two in the intracellular domains.

7. Complete the following table comparing the amino acids that differ between the light-phenotype and dark-phenotype MC1R proteins. For each amino acid difference, record the number of the position where the difference occurs, the amino acid in the light-phenotype MC1R, and the amino acid in the dark-phenotype MC1R.

Position Number	**Amino acid in the *light*-phenotype MC1R**	**Amino acid in the *dark*-phenotype MC1R**
18	***Arg***	***Cys***
109	***Arg***	***Trp***
160	***Arg***	***Trp***
233	***Gln***	***His***

8. Based on what you have learned about MC1R and cell signaling:
   a. How does the MC1R protein function in determining fur color in a rock pocket mouse?
   The MC1R receives the MSH (signal), which binds to the receptor site. This causes the transduction of the signal across the membrane and causes a response inside the cell. This determines the fur color of the mouse.

b. What could happen if a mutation changes the shape of the extracellular (outside the cell) or intracellular (inside the cell) portion of the MC1R protein?
A mutation in the extracellular portion may affect whether MSH can bind to MC1R. A mutation in the intracellular portion may affect whether the message can be relayed to the interior of the cell.

c. How could these changes affect whether a mouse survives in its environment?
If these mutations affect the mouse's fur color, they will affect how well the mouse blends into its environment. If the mouse has a fur color that doesn't match the color of its environment, it will be more visible to its predators and have a lower chance of survival.

PART 3: Using Models to Develop Explanations

9. Based on Figure 4 and Table 1, what might happen to fur color if:

a. MC1R and TYRP1 are functional but TYR is not
The fur will be white. TYR turns tyrosine into dopaquinone, which is the starting material for making melanin. If TYR is nonfunctional, there is no dopaquinone, so neither type of melanin can be made. Assuming that melanin is the main pigment that determines fur color, no melanin means that the mammal's fur has "no color" (is white/albino).

b. MC1R and TYR are functional but TYRP1 is not
The fur will be brown. Since TYR is functional, dopaquinone (and thus melanin) can be made. Since MC1R is functional, it will signal the cell to make eumelanin. But since TYRP1 isn't functional, the eumelanin will be brown instead of black.

c. TYR and TYRP1 are functional but MC1R is not
The fur will be light red/yellow. Since TYR is functional, dopaquinone (and thus melanin) can be made. But since MC1R is nonfunctional, the cell will make phaeomelanin (a light red/yellow pigment) instead of eumelanin.

PART 4: Arguing from Evidence

10. Using Figure 4 and Table 1 from Part 3 of this activity, practice reading the genotypes in Table 2 and figuring out what the phenotype of each mouse would be. Record the phenotype (black, brown, light, or albino) for each genotype in the "Phenotype" columns.
Answers are provided in the table below. In general:

- ***Nonfunctional TYR (pp) results in an <u>albino</u> phenotype.***
- ***Functional TYR (not pp) and nonfunctional MC1R (rr) results in a <u>light</u> phenotype.***
- ***Functional TYR (not pp) and functional MC1R (not rr) result in a <u>black</u> phenotype with functional TYRP1 (not bb) or a <u>brown</u> phenotype with nonfunctional TYRP1 (bb).***

Table 2. Possible genotypes and phenotypes for lab mouse fur colors. If the genotype for a protein is omitted, assume that protein is functional.

Genotype	Phenotype (black, brown, light, or albino)
PPBB	***black***
Ppbb	***brown***

Genotype	Phenotype (black, brown, light, or albino)
RrPpBb	***black***
RrppBb	albino

ppBB	albino	rrPPBB	***light***
rrBb	***light***	RRPpbb	***brown***
RRbb	***brown***	RRppbb	***albino***
RrBb	***black***	rrppbb	***albino***

11. A researcher mates two lab mice that each have heterozygous genotypes for both TYR and TYRP1.
Make sure students assume that MC1R is functional. This would mean that if TYRP1 is also functional (not bb), the fur will be black.
   a. What are the genotypes of these mice?
   PpBb for both
   b. What are the phenotypes of these mice?
   black
   c. What are the possible gamete genotypes for these mice? Recall that each gamete genotype will have only one letter for each protein (e.g., PB).
   PB, Pb, pB, pb

12. Consider the possible offspring of the two lab mice in Question 11.
   a. Complete the following Punnett square with a dihybrid cross of the two mice. List the possible gamete genotypes of each mouse along the top and left side of the Punnett square. List their offspring's possible genotype combinations, and the resulting phenotypes, inside the square.

	PB	***Pb***	***pB***	***pb***
PB	***PPBB (black)***	***PPBb (black)***	***PpBB (black)***	***PpBb (black)***
Pb	***PPBb (black)***	***PPbb (brown)***	***PpBb (black)***	***Ppbb (brown)***
pB	***PpBB (black)***	***PpBb (black)***	*ppBB (albino)*	*ppBb (albino)*
pb	***PpBb (black)***	***Ppbb (brown)***	*ppBb (albino)*	*ppbb (albino)*

   b. What are the ratios of the offspring phenotypes?
   9 black: 3 brown: 4 albino (any combination of "pp" will result in albino mice)

PART 5: Transfer Task with Labrador Retrievers

13. A dog breeder wants to produce chocolate lab puppies. She plans to mate a male yellow lab with a female chocolate lab.
   a. List *all* possible genotypes of the yellow lab.
   rrbb, rrBb, rrBB

b. List *all* possible genotypes of the chocolate lab.
RRbb, Rrbb

14. The male yellow lab's mother was black, and his father was chocolate. With this new information, what can you figure out about the genotypes of the yellow lab's parents?
Each parent must give one "r" in order for their offspring to be yellow. The mother, which was black, could be RrBB or RrBb. The father, which was chocolate, could only be Rrbb.

15. The female chocolate lab comes from a long line of chocolate labs. No yellow or black labs have ever been born in this line of dogs. With this new information, what can you figure out about the genotypes of the female chocolate lab's parents?
The parents of the female chocolate lab are most likely to be RRbb. If this line of dogs had any individuals that were Rrbb, it would be possible to have a yellow puppy eventually, but there have been no yellow puppies so far.

16. When the puppies of these two labs were born, the breeder discovered they were all black labs!
    a. What is the genotype for the puppies?
    RrBb
    b. What are the probable genotypes of the yellow lab and the chocolate lab?
    rrBB for the yellow lab and RRbb for the chocolate lab
    c. Which genotype(s) of labs could the breeder mate together to get *only* chocolate labs?
    Rrbb x RRbb or RRbb x RRbb

17. Applying what you now know about cell signaling from this activity, construct an explanation of the process that led to black lab puppies from the pairing discussed above. Be sure to start with the cell signal and include the major steps outlined in the melanin pathway (Figure 4, reshown below) that lead to the black phenotype.
A possible explanation is provided below. Note that students may also draw on Figure 4 to show their thinking.

    The male yellow lab gave the puppies his "b" allele and one of his "R" alleles. The female chocolate lab gave her "r" allele and one of her "B" alleles. So the puppies each have one "R" allele and one "B" allele.

    With at least one "R" allele, the MC1R receptor is functional. This means it can receive a signal from MSH and relay a message to the interior of the cell, which causes the cell to produce eumelanin. With at least one "B" allele, TYRP1 is also functional, which causes the cell to make black eumelanin instead of brown. This results in the black fur phenotype.

REFERENCES

Barsh, Gregory S. 2003. "What Controls Variation in Human Skin Color?" *PLoS Biology* 1, 1: e27. https://doi.org/10.1371/journal.pbio.0000027.

D'Mello, Stacy A. N., Graeme J. Finlay, Bruce C. Baguley, and Marjan E. Askarian-Amiri. 2016. "Signaling Pathways in Melanogenesis." *International Journal of Molecular Sciences* 17, 7: 1144. https://doi.org/10.3390/ijms17071144.

Hoekstra, Hopi E., and Michael W. Nachman. 2006. “Coat Color Variation in Rock Pocket Mice (*Chaetodipus intermedius*): from Genotype to Phenotype.” In *Mammalian Diversification: From Chromosomes to Phylogeography*, 79–99. https://doi.org/10.1525/california/9780520098534.003.0003.

Lamoreux, M. Lynn, Kazumasa Wakamatsu, and Shosuke Ito. 2001. “Interaction of major coat color gene functions in mice as studied by chemical analysis of eumelanin and pheomelanin.” *Pigment Cell Research* 14, 1: 23–31. https://doi.org/10.1034/j.1600-0749.2001.140105.x.

Nachman, Michael W., Hopi E. Hoekstra, and Susan L. D’Agostino. 2003. “The genetic basis of adaptive melanism in pocket mice.” *Proceedings of the National Academy of Sciences* 100, 9: 5268–5273. https://doi.org/10.1073/pnas.0431157100.

Templeton, J. W., A. P. Stewart, and W. S. Fletcher. 1977. “Coat color genetics in the Labrador retriever.” *Journal of Heredity* 68, 2: 134–136. https://doi.org/10.1093/oxfordjournals.jhered.a108792.

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APPENDIX: Background on the Melanin Pathway

Animal coloration involves many proteins in complex biosynthetic pathways. This activity focuses on a few of the proteins involved in production of **melanin**: a group of pigments that affect the colors of many organisms. Melanins are produced by **melanocytes**, cells in the lower skin layers of animals. The activity discusses two types of melanin:

- **eumelanin:** a dark brown/brown pigment that leads to dark-colored fur/hair
- **phaeomelanin:** a light red/yellow pigment that leads to light-colored fur/hair

Figure 4 and Table 1 from the Student Handout (reshown below) summarize the main steps of melanin production. They highlight three key proteins in this pathway: TYR, MC1R, and TYRP1.

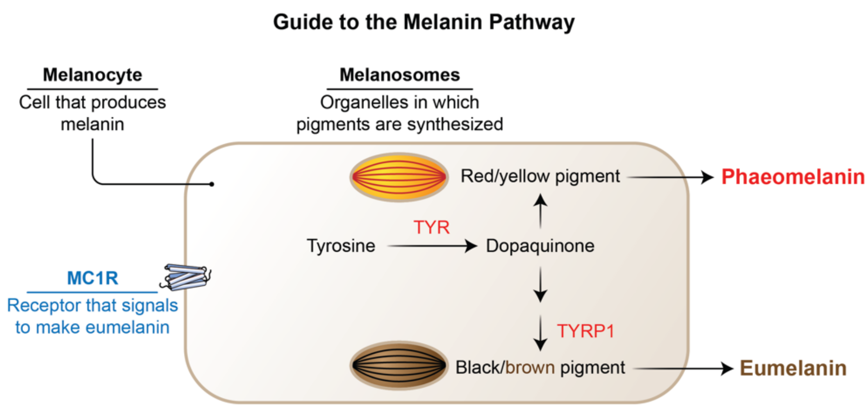

Figure 4. A diagram of the melanin pathway.

Table 1. Genotypes and phenotypes of MC1R, TYR, and TYRP1.

Protein	Genotypes	Phenotype/Effect
MC1R	RR or Rr	Signals cell to make eumelanin (dark brown/black pigment).
	rr	Phaeomelanin (light red/yellow pigment) is made.
TYR	PP or Pp	Pigment is made.
	pp	No pigment is made.
TYRP1	BB or Bb	Black eumelanin is made.
	bb	Dark brown eumelanin is made.

TYR

TYR (tyrosinase) is an enzyme that converts the amino acid tyrosine to dopaquinone. We will designate the dominant allele for TYR as “P” for “pigment.”

- If TYR is *functional* (genotype PP or Pp), dopaquinone can be converted to melanin — either phaeomelanin or eumelanin based on the function of MC1R.

- If TYR is *nonfunctional* (genotype pp), dopaquinone cannot be converted, so the animal will have no melanin at all. The absence of melanin often results in white fur/hair (an "albino" phenotype). This is an example of **epistasis**: a phenomenon in which one gene can "mask" the effects of other genes. Even if the other proteins in the melanin pathway are functional, as long as TYR is nonfunctional, the animal will still be albino (as there is no product for the other proteins to work on).

MC1R

MC1R (melanocortin 1 receptor) is a cell signaling protein found in the cell membrane of melanocytes. It is the receptor for the hormone **MSH** (melanocyte-stimulating hormone), which is secreted from the pituitary gland in mammals.

When MSH binds to MC1R, the receptor sends a signal to the interior of the cell to ultimately convert dopaquinone to eumelanin instead of phaeomelanin. We will designate the dominant allele for MC1R as "R" for "receptor."

- If MC1R is *functional* (genotype RR or Rr), it can bind to MSH and send a signal to convert dopaquinone into intermediates that ultimately give rise to eumelanin. (This is done by the enzyme TYRP2, which is not labeled on the diagram. The first arrow below dopaquinone represents the effect of TYRP2.) Since eumelanin is dark colored, this results in dark-colored fur/hair. Whether the eumelanin is brown or black depends on TYRP1.
- If MC1R is *nonfunctional* (genotype rr), dopaquinone is ultimately converted to phaeomelanin instead.

TYRP1

TYRP1 (tyrosinase-related protein 1) is an enzyme that affects whether the cell produces brown or black eumelanin. We will designate the dominant allele for TYRP1 as "B" for "black."

- If TYRP1 is *functional* (genotype BB or Bb), the cell produces black eumelanin.
- If TYRP1 is *nonfunctional* (genotype bb), the cell produces brown eumelanin