

HOW DO FIBERS FORM?

OVERVIEW

This hands-on activity serves as an extension to the Howard Hughes Medical Institute's short film [The Making of the Fittest: Natural Selection in Humans](http://www.hhmi.org/biointeractive/making-fittest-natural-selection-humans) (<http://www.hhmi.org/biointeractive/making-fittest-natural-selection-humans>). Students construct models of sickle cell hemoglobin inside red blood cells to illustrate how changes in the structure of a protein can affect cell shape.

KEY CONCEPTS AND LEARNING OBJECTIVES

- Mutation of a single DNA base can result in a significant difference in the chemical structure and behavior of a protein,
- The substitution of the amino acid valine for glutamic acid in the beta chains of hemoglobin molecules results in sickle-shaped cells,
- In areas where the malaria parasite is present, individuals who are heterozygous for the sickle cell allele are at a selective advantage because they are protected against malaria; under most conditions these individuals do not have sickle-shaped cells.

Students will be able to

- use models of sickle cell hemoglobin (HbS) and normal adult hemoglobin (HbA) to explain why fibers form in some red blood cells (RBCs),

CURRICULUM CONNECTIONS

Curriculum	Standards
NGSS (April 2013)	HS-LS1-1, HS-LS3-1, HS-LS3-2, HS-LS4-2, HS-LS4-4 HS-LS1.A, HS-LS3.B, HS-LS4.B, HS-LS4.C
Common Core (2010)	CCSS.ELA-Literacy.RST.9-10.2, CCSS.ELA-Literacy.RST.11-12.4, CCSS.ELA-Literacy.W.9-10.2, CCSS.ELA-Literacy.WHST.9-10.2, CCSS.ELA-Literacy.WHST.11-12.2
AP Biology (2012–13)	3.A.1, 3.C.1, 3.C.2, 4.A.1, 4.B.1
IB Biology (2009)	4.1, 4.3, 5.4, 6.2, 7.5

KEY TERMS

amino acid, autosomal, dominant, evolution, HbA, HbS, hemoglobin, heterozygous, homozygous, malaria, messenger RNA (mRNA), mutation, natural selection, recessive, sickle cell anemia, sickle cell disease, sickling, trait

TIME REQUIREMENTS

This activity was designed to be completed within two to three 50-minute class periods, including watching the film.

SUGGESTED AUDIENCE

This activity is appropriate for high school biology (all levels including AP and IB) and introductory college biology.

PRIOR KNOWLEDGE

Students should have watched the film [The Making of the Fittest: Natural Selection in Humans](http://www.hhmi.org/biointeractive/making-fittest-natural-selection-humans) before doing this hands-on activity. The film provides necessary background information regarding the relationship between sickle cell disease and malaria. This activity covers a wide range of biological topics including biochemistry, genetics, physiology, and protein structure. We recommend that you use this activity toward the end of the year when students have been exposed to these topics.



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**HANDS-ON ACTIVITY
TEACHER MATERIALS**

MATERIALS

- scissors
- tape
- copies of the four types of Hb molecules (You may wish to laminate and pre-cut the hemoglobin molecules for future use. You may also want to print the four different hemoglobins in color and put them in appropriately labeled resealable snack bags.)
- eight mosquito netting circles, each with an 8-centimeter diameter, per setup (You can purchase mosquito netting at any craft store). For each student setup, cut eight 8-centimeter-diameter circles. These can be used multiple times.)
- four sheets of blank copy paper per setup

TEACHING TIPS

- Students should first watch the film [The Making of the Fittest: Natural Selection in Humans](#). It is important for them to know that individuals heterozygous for the sickle cell allele have some protection against the most virulent forms of malaria.
- You may want to review the function of RBCs and the role of hemoglobin in oxygen transport.
- The cytoplasm of RBCs is rich in hemoglobin. Oxygen binds to the heme component of the hemoglobin molecule, forming oxyhemoglobin. In humans, the binding process takes place in the capillaries of the alveoli in the lungs. The RBCs transport oxygen to the cells of the body, where it is dropped off and used in cellular respiration. Deoxyhemoglobin forms when oxygen is released. The RBCs containing deoxyhemoglobin are transported back to the lungs, where they again pick up oxygen.
- You may choose to use this hands-on activity in multiple lessons. Provide the first page of the student handout but with the amino acids whited out. Have students translate the mRNA of the small portion of hemoglobin protein. Next, have them highlight the differences in colored pencil and discuss how the one amino acid change could affect protein structure and function. Revisit the rest of the activity after students have covered protein structures.
- With AP Biology students, you may wish to have your students study the protein hemoglobin on the PDB-101 Molecule of the Month “Hemoglobin” web page at <http://www.rcsb.org/pdb/101/motm.do?momID=41>. Have them compare oxyhemoglobin to deoxyhemoglobin and normal hemoglobin to sickle cell hemoglobin as three-dimensional structures.
- Students can work individually or in teams of two. Tell students that they should use most of their Hb molecules. Individual, free-floating Hb molecules can overlap.

ANSWER KEY

PROCEDURE

Students should develop four model RBCs, one each of the following molecules:

- Oxygenated HbA
- Deoxygenated HbA
- Oxygenated HbS
- Deoxygenated HbS

They should demonstrate that only deoxygenated HbS molecules form fibers. They should show how the fibers cause the RBC to take on a different two-dimensional shape.

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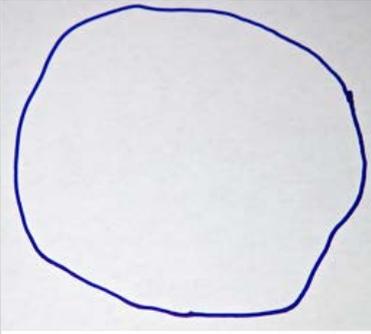
STEP 3

Is the shape of the model RBC containing oxyhemoglobin A normal or sickled?

Normal



Oxyhemoglobin A



Trace of Oxyhemoglobin A

STEP 4

Is the shape of the model RBC containing deoxyhemoglobin A normal or sickled?

Normal



Deoxyhemoglobin A

STEP 5

Is the shape of the model RBC containing oxyhemoglobin S normal or sickled?

Normal



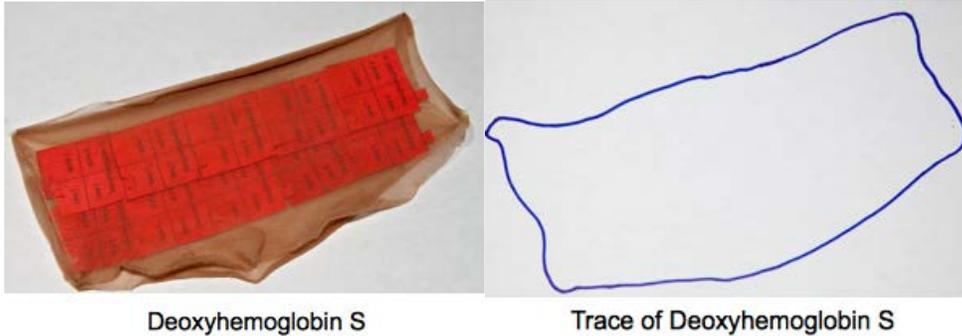
Oxyhemoglobin S

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STEP 6

Is the shape of the model RBC containing oxyhemoglobin S normal or sickled?

Sickled



QUESTIONS

1. Explain why fibers do not form in oxygen-rich or oxygen-poor environments in individuals who have HbA molecules.

In an oxygen-rich environment, such as the lung, the HbA molecules are not attracted to one another and are freely distributed throughout the RBC. In an oxygen-poor environment, deoxygenated HbA molecules have the hydrophobic pocket, but they do not have the mutant valine to act as a donor. There is no interaction between the hemoglobin molecules, and the HbA molecules are freely distributed throughout the RBCs.

2. Explain why fibers do not form in oxygen-rich conditions in individuals who have HbS molecules.

HbS molecules have a mutant valine that looks like a hydrophobic protrusion on the surface of the hemoglobin molecule. However, in oxygen-rich conditions, the HbS molecules do not interact with each other, because there are no hydrophobic pockets for the valine to interact with.

3. Explain why fibers do form in oxygen-poor conditions in individuals who have HbS molecules.

HbS molecules have a mutant valine that looks like a hydrophobic protrusion on the surface of the hemoglobin molecule. In an oxygen-poor condition, the HbS molecule also has a hydrophobic pocket exposed. The valine protrusion of one HbS molecule interacts with the hydrophobic pocket of another HbS molecule, and they begin to form long fibers.

4. Explain how the formation of fibers results in the sickling of RBCs.

The cell membranes of the RBCs stretch to conform to the shape of these fibers. These fibers cause the cell membrane to become more rigid, and the RBC takes on a sickle shape.

5. Read the passage below and answer Questions 5 and 6.

a. Explain why heterozygous individuals might have less severe malaria infections.

One explanation for why heterozygous individuals might have less severe malaria infections is that some of their RBCs sickle when infected with the malaria parasite. The deformed cells are marked for destruction. When the RBCs are destroyed, so are the parasites.

However, scientists have proposed a number of other mechanisms to explain protection. Although we do not expect students to know this, several studies have shown that *Plasmodium* does not infect or grow as well in RBCs that contain HbS (Pasvol, G., D. J. Weatherall, and R. J. Wilson. "Cellular Mechanism for the Protective Effect of Haemoglobin S Against *P. falciparum* Malaria." *Nature* 274 (1978): 701–703). Other studies have shown that HbS increases the destruction of infected RBCs (Ayi, Kodjo, Franco Turrini, Antonio Piga, and Paolo Arese. "Enhanced Phagocytosis of Ring-Parasitized Mutant Erythrocytes: A Common Mechanism That



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**HANDS-ON ACTIVITY
TEACHER MATERIALS**

May Explain Protection against *falciparum* Malaria in Sickle Trait and Beta-Thalassemia Trait.” *Blood* 104 (2004): 3364–3371). Recent studies have also shown that HbS causes free heme to accumulate in the plasma. Free heme is damaging to cells, so RBCs produce heme oxygenase-1 (HO-1) to counteract the effect of free heme. HO-1 production protects against many immune diseases, including infection, and might be the reason why people with the HbS allele don’t have as severe symptoms of malaria (Ferreira, A., I. Marguti, I. Bechmann, V. Jeney, A. Chora, N. R. Palha, S. Rebelo, A. Henri, Y. Beuzard, and M. P. Soares. “Sickle Hemoglobin Confers Tolerance to *Plasmodium* Infection.” *Cell* 145 (2011): 398–409).

In summary, many mechanisms of protection have been proposed, but it remains to be established whether any of them actually account for protection. Students should understand that the mechanism of protection is very complex and far from clear.

b. Explain why the same responses that provide some protection to a heterozygous individual would be very harmful in an individual who is homozygous for sickle cell hemoglobin.

Individuals with sickle cell disease have a large number of RBCs that are sickle shaped. Many of these cells will be destroyed. If these individuals are infected with the parasite that causes malaria, many parasites will be destroyed along with RBCs, but the individual will have major problems due to a lack of blood cells. In addition, RBCs infected with the parasite will stick to the walls of capillaries and block blood flow to vital organs.

6. Now that you have examined the mechanism by which fibers form and cause cells to sickle, explain how the formation of sickled cells leads to anemia.

Sickle-shaped RBCs are not flexible and clog up blood vessels, preventing enough oxygen-rich blood from being delivered to different parts of the body.

In addition, the immune system marks sickled RBCs for destruction. When the body removes RBCs from circulation faster than it produces new RBCs, anemia results.

Although students are not expected to know this, you may want to tell them that at any one time, there are approximately 20 trillion to 30 trillion RBCs present in the human body. About 2 million RBCs form each second in a normal, healthy adult. The life expectancy of a typical RBC is between 100 and 120 days. In the case of individuals with sickle cell disease, the rate of RBC production cannot keep up with the rate of RBC destruction.

REFERENCES

- Allison, Anthony C. “The Discovery of Resistance to Malaria of Sickle-Cell Zygotes.” Mini-Series: Significant Contributions to Biological Chemistry Over the Past 125 Years. *Biochemistry and Molecular Biology Education* 30 (2002): 279–287. <http://onlinelibrary.wiley.com/doi/10.1002/bmb.2002.494030050108/abstract>.
- . “Two Lessons from the Interface of Genetics and Medicine.” Perspectives: Anecdotal, Historical and Critical Commentaries on Genetics. Edited by James F. Crow and William F. Dove. *Genetics* 166: 1591–1599, 2004. <http://www.genetics.org/content/166/4/1591.full>.
- Blouin, Marie-José, Hugues Beauchemin, Adrian Wright, Monique De Paepe, Martin Sorette, Anne-Marie Bleau, Betty Nakamoto, Ching-Nan Ou, Georges Stamatoyannopoulos, and Marie Trudel. “Genetic Correction of Sickle Cell Disease: Insights Using Transgenic Mouse Models.” *Nature Medicine* 6 (2000): 177–182. http://www.nature.com/nm/journal/v6/n2/abs/nm0200_177.html.
- Campbell, Neil, and Jane Reece. *Biology*. 8th ed. San Francisco: Pearson Benjamin Cummings, 2007.
- Costanzo, Linda S. *Physiology*. Hagerstown, MD: Lippincott Williams & Wilkins, 2007.
- Khan, Amina. “Sickle Cell Anemia as Malaria Defense.” *Los Angeles Times*. November 25, 2011. <http://articles.latimes.com/2011/nov/25/science/la-sci-sickle-cell-malaria-20111126>.
- Mukerji, Ishita. “Fiber Formation.” About Sickle Cell Disease. http://www.sicklecellinfo.net/fiber_formation.htm.



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EXTENSION ACTIVITY

Students may want to investigate the following questions.

1. How are scientists using stem cells to better understand and potentially treat sickle cell disease?

There are numerous online references students can use to answer this question.

The article “Stem Cell Transplant for Sickle Cell Disease Subject of Clinical Trial” by Beth Miller, published on July 11, 2008, describes how researchers at Washington University School of Medicine in St. Louis treat children with severe sickle cell disease with blood stem cells. To read the article, go to <http://news.wustl.edu/news/Pages/12009.aspx>.

The article “Scientists Cure Mice of Sickle Cell Using Stem Cell Technique” by Rick Weiss, published in the *Washington Post* on December 7, 2007, describes how transgenic mice with sickle cell disease were cured using induced pluripotent stem cells. To read the article, go to <http://www.washingtonpost.com/wp-dyn/content/article/2007/12/06/AR2007120602444.html>.

The article “Stem Cell and Gene Therapy for Sickle Cell and Other Genetic Diseases” by Jeffrey Norris, published on December 13, 2011, discusses the use of stem cells in treating sickle cell anemia. To read the article, go to <http://www.ucsf.edu/news/2011/12/11123/stem-cell-and-gene-therapy-sickle-cell-and-other-genetic-diseases>.

2. Are there other genetic diseases besides sickle cell that protect against malaria? If so, what are they and where are they most commonly found? Do they also involve the formation of fibers?

Thalassemia provides some protection against malaria. There are two main types of thalassemia: alpha thalassemia occurs when genes regulating the production of the alpha amino acid chains of a hemoglobin molecule are mutated; beta thalassemia occurs when similar gene mutations affect the production of the beta amino acid chains. Alpha and beta thalassemia both occur in two forms: thalassemia major and thalassemia minor. Defective genes must be inherited from both parents to develop thalassemia major. Thalassemia minor occurs if a defective gene is inherited from only one parent. As is the case with sickle cell trait, individuals with thalassemia minor are carriers and usually do not have symptoms of disease.

Scientists do not clearly understand how thalassemia provides some resistance to malaria. Fibers don't form in the RBCs. Protection may have something to do with the higher levels of fetal hemoglobin (HbF) present in the blood of carriers. The malaria parasite does not thrive when high levels of HbF are present. It is also thought that thalassemic RBCs infected with parasites are quickly marked and removed from circulation. When the RBCs are destroyed, so are the parasites they contain. Just as with sickle cell, carriers of thalassemia can contract malaria. However, the infections are less severe and more individuals survive.

Glucose-6-Phosphate Dehydrogenase Deficiency is a sex-linked enzyme deficiency. It causes a life-threatening form of anemia in which red blood cells burst. However, it develops under very specific conditions. It can be triggered by certain infections and stress as well as by ingesting some foods (such as fava beans), inhaling specific types of pollen, or taking certain medications. Studies of African children have shown that fewer heterozygous females and hemizygous males for G6PD deficiency have severe malaria than children lacking the mutant allele. This suggests that the mutant allele protects against severe malaria.

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