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Sickle Cell Anemia and Genetics: Background Information

Background information to accompany the labs: *Allele Frequencies and Sickle Cell Anemia Lab* and *Sickle Cell Anemia: Diagnosis Using Restriction Analysis of DNA*

Genetics of Sickle Cell Anemia

Sickle cell anemia was the first genetic disease to be characterized at the molecular level. The mutation responsible for sickle cell anemia is small—just ONE nucleotide of DNA out of the three billion in each human cell. Yet it is enough to change the chemical properties of hemoglobin, the iron and protein complex that carries oxygen within red blood cells.

There are approximately 280 million hemoglobin molecules in each red blood cell (RBC). The protein portion of hemoglobin consists of four globin subunits: two alpha (α) and two beta (β). These two types of subunits are encoded by the α and β globin genes, respectively. While the binding of oxygen actually occurs at the iron sites, all four globin chains must work together in order for the process to function well.

Sickle cell anemia, also known as sickle cell disease, is caused by a point mutation in the β globin gene. As a result of this mutation, valine (a non-polar amino acid) is inserted into the β globin chain instead of glutamic acid (an electrically charged amino acid). The mutation causes the RBCs to become stiff and sometimes sickle-shaped when they release their load of oxygen. The sickle cell mutation produces a "sticky" patch on the surface of the β chains when they are not complexed with oxygen. Because other molecules of sickle cell hemoglobin also develop the sticky patch, they adhere to each other and polymerize into long fibers that distort the RBC into a sickle shape.

The sickled cells tend to get stuck in narrow blood vessels, blocking the flow of blood. As a result, those with the disease suffer painful "crises" in their joints and bones. They may also suffer strokes, blindness, or damage to the lungs, kidneys, or heart. They must often be hospitalized for blood transfusions and are at risk for a life-threatening complication called acute chest syndrome. Although many sufferers of sickle cell disease die before the age of 20, modern medical treatments can sometimes prolong these individuals' lives into their 40s and 50s.

There are two β globin alleles important for the inheritance of sickle cell anemia: A and S. Individuals with two normal A alleles (AA) have normal hemoglobin, and therefore normal RBCs. Those with two mutant S alleles (SS) develop sickle cell anemia. Those who are heterozygous for the sickle cell allele (AS) produce both normal and abnormal hemoglobin. Heterozygous individuals are usually healthy, but they may suffer some symptoms of sickle cell anemia under conditions of low blood oxygen, such as high elevation. Heterozygous (AS) individuals are said to be "carriers" of the sickle cell trait. Because both forms of hemoglobin are made in heterozygotes, the A and S alleles are codominant.

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About 2.5 million African-Americans (1 in 12) are carriers (AS) of the sickle cell trait. People who are carriers may not even be aware that they are carrying the S allele!

Sickle Cell Anemia and Malaria
In the United States, about 1 in 500 African-Americans develops sickle cell anemia. In Africa, about 1 in 100 individuals develops the disease. Why is the frequency of a potentially fatal disease so much higher in Africa?

The answer is related to another potentially fatal disease, malaria. Malaria is characterized by chills and fever, vomiting, and severe headaches. Anemia and death may result. Malaria is caused by a protozoan parasite (*Plasmodium*) that is transmitted to humans by the *Anopheles* mosquito.

When malarial parasites invade the bloodstream, the red cells that contain defective hemoglobin become sickled and die, trapping the parasites inside them and reducing infection.

Compared to AS heterozygotes, people with the AA genotype (normal hemoglobin) have a greater risk of dying from malaria. Death of AA homozygotes results in removal of A alleles from the gene pool. Individuals with the AS genotype do not develop sickle cell anemia and have less chance of contracting malaria. They are able to survive and reproduce in malaria-infected regions. Therefore, BOTH the A and S alleles of these people remain in the population. SS homozygotes have sickle cell anemia, which usually results in early death. In this way, S alleles are removed from the gene pool.

In a region where malaria is prevalent, the S allele confers a survival advantage on people who have one copy of the allele, and the otherwise harmful S allele is therefore maintained in the population at a relatively high frequency. This phenomenon will be examined in the **Allele Frequencies and Sickle Cell Anemia Lab**, which relates the change in allele frequency in a population to evolution.

The frequency of the S allele in malaria-infected regions of Africa is 16%. The sickle cell allele is also widespread in the Mediterranean and other areas where malaria is or used to be a major threat to life. In contrast, the S allele frequency is only 4% in the United States, where malaria has been virtually eliminated. Malaria was once common in the United States, but effective mosquito control caused the number of cases to drop. Recently, however, there has been an increase in the number of malarial cases because of increased travel, immigration, and resistance to medication. In Southern California there was a 1986 outbreak of nearly 30 cases of malaria transmitted by local mosquitos!

Sickle Cell Anemia and Current Research
The oxygen requirements of a fetus differ from those of an adult, and so perhaps not surprisingly, prenatal blood contains a special hemoglobin. Fetal hemoglobin contains two gamma (γ) globin polypeptide chains instead of two adult β chains. After

birth, the genes encoding γ globin switch off, and the ones encoding β globin switch on. Understanding how this genetic switch works could allow researchers to understand much about the control of genes in general and sickle cell anemia in particular.

Indian and Saudi Arabian people have a milder variation of sickle cell anemia, sometimes with no symptoms. In this population twenty-five percent of each person's hemoglobin is the fetal kind. Similarly, the blood of adults with an inherited condition called "hereditary persistence of fetal hemoglobin" also contains fetal hemoglobin and these individuals are healthy. Some people with this condition completely lack adult hemoglobin and still show no ill effects. Biochemical experiments have demonstrated that, in a test tube, fetal hemoglobin inhibits polymerization of sickle cell hemoglobin. These observations suggest that increasing fetal hemoglobin levels may be an effective treatment for sickle cell anemia. There are a number of lines of research related to activation of fetal hemoglobin as a therapy for sickle cell anemia:

• Some infants whose mothers suffered from diabetes during pregnancy have unusually high concentrations of the biochemical butyrate in their blood plasma. Butyrate is a natural fatty acid that stimulates RBCs to differentiate from their precursors (reticulocytes). Butyrate also prevents the γ globin gene from switching off and the β globin gene from switching on in these infants, who are healthy despite lacking adult hemoglobin. When butyrate is given to patients with sickle cell anemia, the γ globin mRNA levels in reticulocytes increase significantly. perhaps butyrate or other chemicals that stimulate fetal hemoglobin production could be used to treat sickle cell anemia.

• In 1983, a drug called hydroxyurea (HU) was first used on sickle cell patients to try to activate their fetal globin genes. By 1995, clinical trials had demonstrated that HU could increase fetal hemoglobin levels in patients' RBCs and prevent the cells from sickling. Patients treated with HU experienced less frequent and severe painful crises. However, hydroxyurea can be quite toxic when used continuously to maintain elevated levels of fetal hemoglobin and can increase the risk of leukemia.

• In 1992, it was found that alternating hydroxyurea with erythropoiten and providing dietary iron raised the percentage of RBCs with fetal hemoglobin and relieved the joint and bone pain of sickle cell disease. Erythropoiten is made in the kidneys and helps anemic patients replenish their RBCs. It can be manufactured for therapeutic use with recombinant DNA technology.

• Mice that have been genetically engineered to contain a defective human β globin gene have symptoms typical of sickle cell anemia, making them an ideal model for laboratory experimentation. In 2000, these mice were mated to another transgenic mouse line expressing human fetal hemoglobin. When compared to their sickle cell parents, the offspring had greatly reduced numbers of abnormal and sickled RBCs, increased numbers of RBCs overall (reduced anemia), and longer lifespans. These experiments established that only 9-16% of hemoglobin need be the fetal type in

order to ameliorate the sickle cell symptoms, and are an important first step in a gene therapy solution to sickle cell disease.

Disclaimer:

As with many "home-grown" resources teachers use in their classrooms, this background material was culled from a variety of sources and has been written, rewritten, and adapted by several people and then passed on to the next user. The exact, original source material is not at all clear, but some of the references below were used. We apologize if a source has been unwittingly plagiarized.

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Sickle Cell Anemia: Questions and Translation Practice Worksheet

Sickle Cell Background

After reading the handout *Sickle Cell Anemia and Genetics: Background Information*, answer the following questions.

1. Describe the symptoms of sickle cell anemia.

2. Describe the structure of hemoglobin. (How many chains are there? What types? Why is iron necessary for blood?)

3. How does sickle hemoglobin differ from normal hemoglobin?

Sickle Cell at the Molecular Level

In sickle cell anemia, there is a mutation in the gene that encodes the β chain of hemoglobin. Within this gene (located on Chromosome 11), ONE BASE in the DNA is replaced with another base, and this mutation causes the normal amino acid #6 to be replaced by another amino acid.

1. Making a Normal Beta Chain of Hemoglobin

The sequence below is the first part of the DNA sequence for the β chain of normal hemoglobin. Fill in the complementary DNA strand using the base-pairing rules for making DNA (A pairs with T, C pairs with G).

DNA: GTG CAC CTG ACT CCT GAG GAG

DNA:

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Now make the messenger RNA from the new, complementary strand of DNA that you just wrote down. Use the RNA base-pairing rules (same as DNA but use U instead of T).

mRNA:

Now, using the Genetic Code chart in your textbook, translate this mRNA into a sequence of amino acids.

Amino Acids:

2. Making Sickle Cell Hemoglobin
In sickle cell anemia, there is a mutation at the seventeenth nucleotide of DNA in this gene; the nucleotide is changed from A to T. Fill in the complementary DNA strand, mRNA, and amino acid sequence in the hemoglobin protein.

DNA: GTG CAC CTG ACT CCT GTG GAG

DNA:

mRNA:

Amino Acids:

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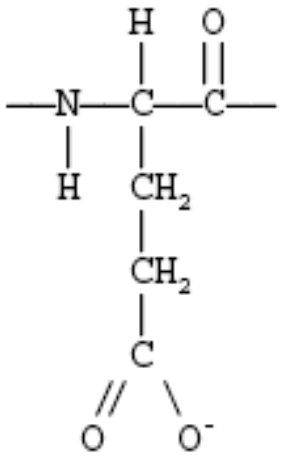
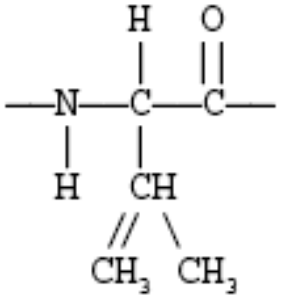
The Effect of Changing One Amino Acid

You can see that in normal hemoglobin, amino acid #6 is glutamic acid (Glu) and in sickle cell hemoglobin, amino acid #6 is valine (Val). Observe the two structural formulas for these amino acids:



glutamic acid



valine

Although the altered β globin has only one amino acid changed out of the total of 146, it's a crucial amino acid. When this new amino acid is at position #6 instead of the correct amino acid, the overall hemoglobin β chain becomes more hydrophobic. As a result, when the hemoglobin chains fold into their 3-dimensional shape and assemble together, the resulting molecules tend to STICK TOGETHER, forming long chains of hemoglobin. This altered hemoglobin deforms the normally rounded cell into the sickle shape. These red blood cells are destroyed at an increased rate, causing anemia. They are also prone to becoming stuck in capillaries, causing pain, organ damage, and often, premature death.

Summary

1. How does sickle cell hemoglobin differ from normal hemoglobin at the primary level of protein structure (order of amino acids)?

2. How does sickle cell hemoglobin differ from normal hemoglobin at the fourth level of protein structure (the sum of all the folded protein chains)?

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3. What is the effect on the red cell containing this altered hemoglobin?

Genetics review

Let A=allele for normal hemoglobin and S=allele for sickle hemoglobin.

1. What inheritance pattern does sickle cell anemia follow? (dominant, recessive, or other?)

2. If an individual with sickle cell anemia and a carrier have a child, what are the chances their child would have sickle cell anemia? Show using a Punnett square.

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