New Gene, New Me

Gene therapy offers hope to people with debilitating genetic conditions

Courtesy Jennelle Stephenson

DRIVING QUESTIONS

1. What are mutations, what is their impact, and how do they occur?
2. How can genetic engineering be used to treat genetic diseases?
3. Are all mutations harmful?

For Jennelle Stephenson, a graduate student from Kissimmee, Florida, the pain had finally become unbearable. "Imagine there's an incredibly heavy object crushing you," she says. "You can't breathe, there's no air, and you feel like your bones are cracking from the pressure."

The pain comes on without warning, often beginning with a tingling sensation in her back. Then it spreads to her arms, her legs, and even her cheekbones.

When it's not the crushing sort of pain, it's sharp and localized—"like being stabbed over and over and over in same spot," she says.

During these pain crises, only the strongest narcotics will help. To obtain these, Jennelle must travel to the emergency room. But doctors and nurses there are typically reluctant to give her the medications, concerned she might be faking it. In the meantime, she must endure the excruciating pain.

Jennelle's experience, while dramatic, is all too typical for people with sickle cell disease. Pain is a constant threat, restricting their life choices.

And it's not just pain. There are also serious health dangers of the condition.

By the time Manny Johnson of Boston, Massachusetts, was 4 years old, he'd already had a stroke. A blood clot formed in a vessel in his brain, blocking the flow of oxygen. Since then, he's received a blood transfusion nearly every month to prevent a dangerous clot from occurring again. Like Jennelle, he has suffered pain his whole life.

Sickle cell disease is an inherited genetic disorder. It primarily affects people who can trace their ancestors to equatorial regions of the globe. In the United States, most sufferers of the condition are African American.

The disease gets its name from the characteristic shape of red blood cells in people with the disease. Red blood cells carry oxygen throughout the body—oxygen that is critical for cells to carry out cellular respiration (Chapter 6). Normally, a red blood cell is shaped like a jelly doughnut: round and squishy with a depression in the middle. In people with sickle cell disease, red blood cells become long and bent, like a sickle or a crescent moon.

Sickled red blood cells are less effective at carrying oxygen than normal red blood cells are, and they do not survive as long as normal blood cells. As a result, people with this condition often develop anemia, a shortage of red blood cells capable of delivering oxygen to tissues.

In addition, sickled cells tend to get stuck in the tiny blood vessels that feed tissues throughout the body, leading to blood clots and intense pain. The clots can also cause stroke, blockages in the lungs, organ failure, and, ultimately, death **(INFOGRAPHIC 9.1)**.

Few effective treatments for the disease exist. Narcotics and blood transfusions, given at the time of a pain crisis, can alleviate pain but will not prevent it. Drugs can help reduce the frequency of episodes, though these medicines don't work for everyone. The only real potential cure is a bone marrow transplant, a physically grueling and risky procedure. With such a transplant, a person's entire blood-making system is wiped out with toxic chemicals and then replaced with that of a genetically compatible donor—assuming one can be found.

But thanks to advances in genetic engineering, this dismal picture is starting to improve. Scientists at several medical centers in the United States are developing forms of **gene therapy** designed to fix the genetic mistake that these individuals are born with. Manny and Jennelle are two of the first people with sickle cell disease to receive these new therapies. Doctors and patients around the world are eagerly watching, with fingers crossed, to see if those treatments work.

GENE THERAPY
Correcting or replacing mutated genes as a treatment for a genetic disease.

Small Change, Big Effect

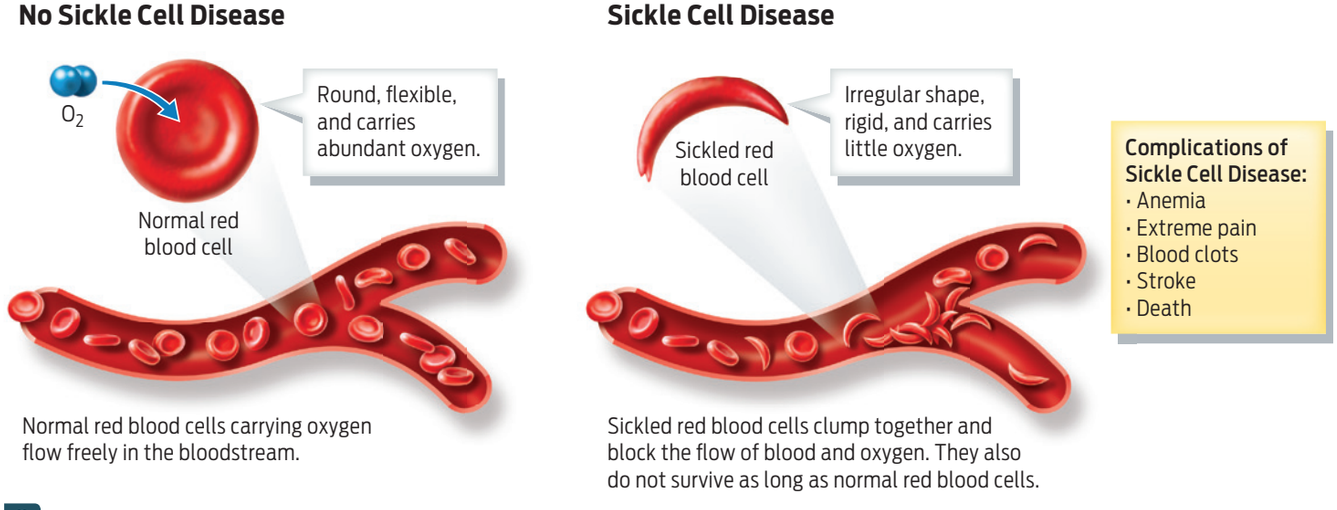
▸ Mutations and their consequences

Scientists have known since the 1950s that sickle cell disease is the result of a single change in the nucleotide sequence of one

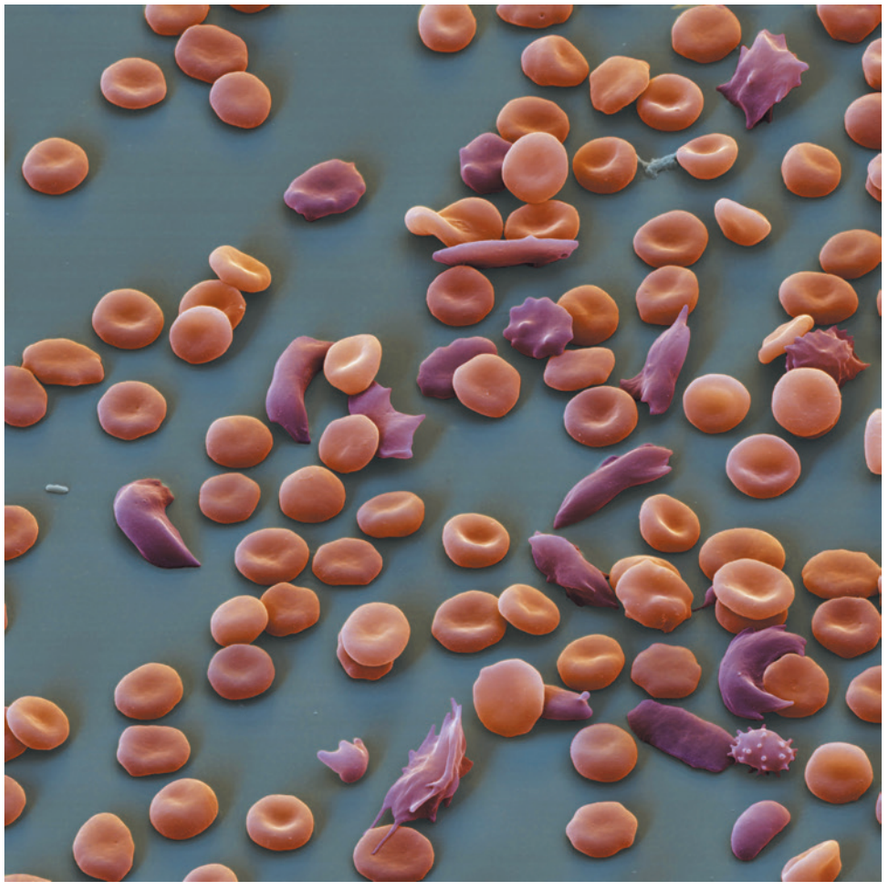
INFOGRAPHIC 9.1

Sickle Cell Disease Affects Red Blood Cells

Sickle cell disease is an inherited genetic disorder that causes red blood cells to take on a sickled shape and carry less oxygen. People with sickle cell disease experience anemia, episodes of severe pain, and other complications due to clots and blockages in blood vessels.



? How does the shape and function of red blood cells differ in people with and without sickle cell disease?



Eye of Science/Science Source

Normal and sickle cells from someone with sickle cell disease.

gene. That change alters the shape of **hemoglobin,** the oxygen-carrying molecule inside of red blood cells. Red blood cells are essentially bags of hemoglobin.

The particular gene affected encodes a protein called **beta-globin** that makes up one part of the hemoglobin molecule. What was an A-T base pair in the original version of the beta-globin gene is changed to a T-A base pair. A change in the nucleotide sequence of a DNA molecule is called a **mutation.**

Recall from Chapter 8 that during gene expression DNA is first transcribed into mRNA, which is then translated into protein. Groups of three mRNA nucleotides, called codons, specify particular amino acids according to the genetic code. In people with sickle cell disease, the original codon in the beta-globin mRNA, GAG, is changed to GUG. As a result, when the mRNA is translated into protein, glutamic acid (Glu) in the normal protein becomes a valine (Val). This amino acid change alters the physical shape and chemical properties of the protein.

Normally, hemoglobin is a compact protein molecule made up of four interacting subunits: two alpha-globin subunits and two beta-globin subunits. In sickle cell disease, the amino acid change alters the physical shape and chemical properties of the beta-globin subunit, causing it to link up with other beta-globin subunits in neighboring hemoglobin molecules. Since a protein's shape determines its function, hemoglobin molecules with these altered shapes are

HEMOGLOBIN
The oxygen-carrying protein in red blood cells.

BETA-GLOBIN
One of the proteins that makes up hemoglobin.

MUTATION
A change in the nucleotide sequence of a DNA molecule.

unable to effectively carry oxygen. Moreover, the long chains of hemoglobin molecules stretch the cell into its characteristic sickled form **(INFOGRAPHIC 9.2)**.

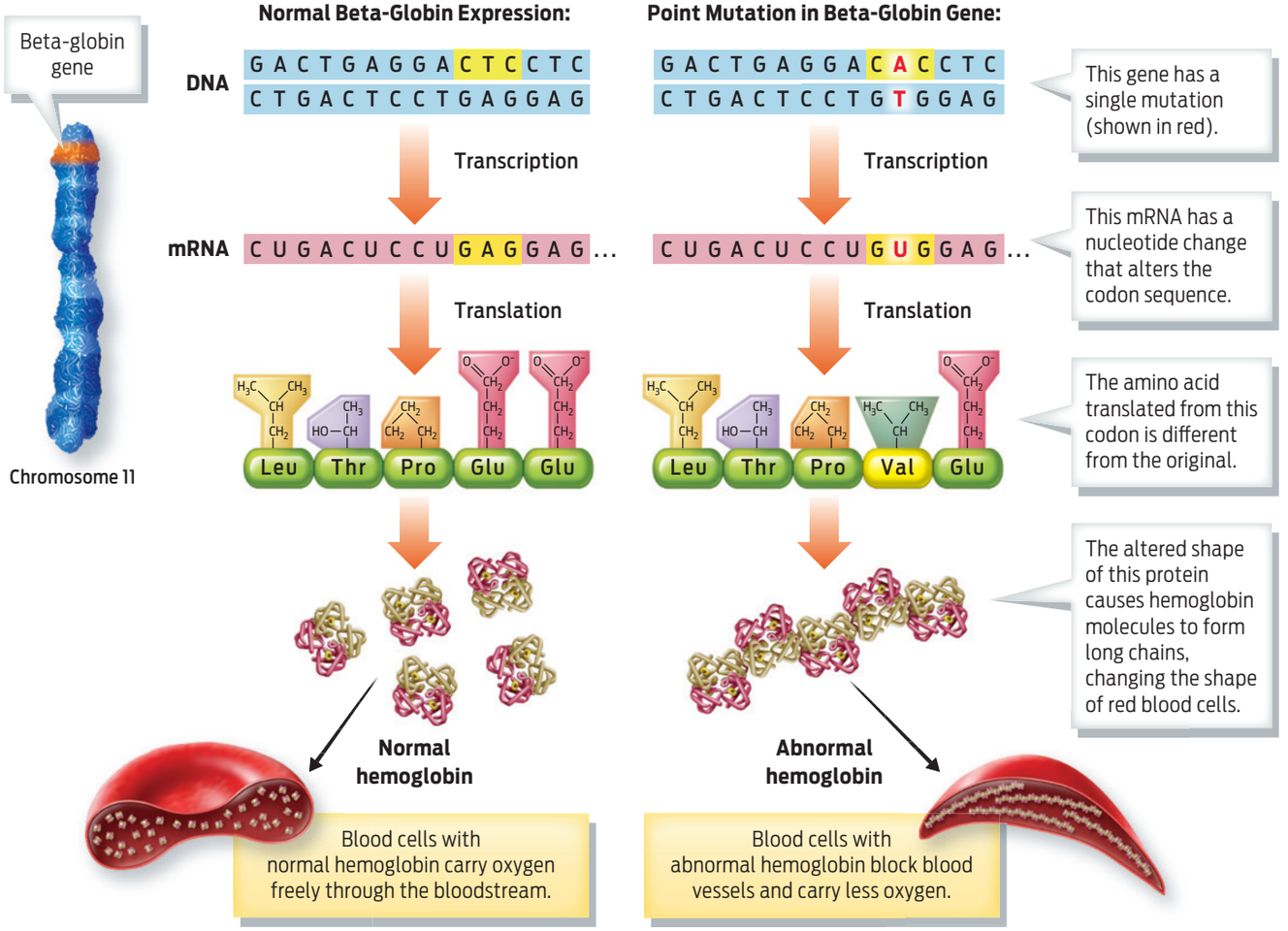
Sickle cell disease was the first inherited genetic disease to be understood on a molecular level. The discovery that a change in a single DNA nucleotide could wreak such havoc on the shape and function of a protein came as a surprise to most scientists at the time. But it provided a valuable lesson in understanding the importance of mutations in biology.

Mutations are central to both genetics and evolution—they are responsible for the diversity of traits that we see among

INFOGRAPHIC 9.2

Mutations Can Alter Protein Shape and Function

Mutations alter the nucleotide sequence of DNA. If a mutation changes the coding region of a gene, the resulting protein may have an altered structure and function. In this case, altered hemoglobin causes cells to take on a sickled shape, and interferes with the ability of red blood cells to carry oxygen to tissues.



? Which of the following molecules are altered due to the sickle cell mutation in the beta-globin gene: beta-globin DNA, beta-globin mRNA, or hemoglobin protein?

individuals and among species. Mutations come in a variety of forms and can have many different effects. The type of mutation that causes sickle cell disease is called a **point mutation;** it alters a single DNA nucleotide. Depending on where a point mutation occurs in a codon, it may—or may not—change the amino acid sequence of a protein. Point mutations that change the amino acid sequence of a protein are called **missense mutations;** those that do not change the protein sequence are called **silent mutations.**

In other cases, one or more DNA nucleotides may be inserted or deleted from genes, shifting the reading frame of that gene—that is, changing where a codon begins and ends. These types of mutations are known as **frameshift mutations.**

Whole blocks of DNA can be rearranged as a result of mutation. A segment of DNA can "flip" within its normal chromosomal location (a change called an inversion), or segments of DNA can trade places between different chromosomes (translocations). Large inversions and translocations can fuse portions of different genes together, creating new proteins with novel activity **(TABLE 9.1)**.

Ultimately, the impact of a mutation depends on how it affects the shape of a protein. In many cases, the shape of the protein is altered in a way that makes it nonfunctional. In other cases, the mutation changes the shape of the protein in a way that makes

POINT MUTATION
A mutation that alters a single DNA nucleotide.

MISSENSE MUTATION
A point mutation that changes the amino acid sequence of the encoded protein.

SILENT MUTATION
A point mutation that does not change the amino acid sequence of the encoded protein.

FRAMESHIFT MUTATION
A shift in the reading frame, such that codons start and end at an alternative position.

TABLE 9.1 Types of Mutations and Their Effects

Type of Mutation	Example	Effect on Protein Function
Original DNA sequence: No mutation	DNA HAS ALL YOU CAN ASK FOR	
Point Mutations		
Silent mutation: Change one nucleotide to another; no change in amino acid sequence	DNA HAS ALL YOO CAN ASK FOR	No change; normal function
Missense mutation: Change one nucleotide to another; different amino acid sequence in this location	DNA HAS ALL LOU CAN ASK FOR	Change in protein shape and function
Nonsense mutation: Change one nucleotide; introduces early stop codon	DNA HAS ALL YOU	Protein is too short and therefore not functional
Frameshift Mutations		
Insertion mutation: Insert one or more nucleotides; shifts reading frame of every codon after the insertion	DNA HAS ALL YYO UCA NAS KFO R	Severely modified sequence makes the protein not functional
Deletion mutation: Delete one or more nucleotides; shifts reading frame of every codon after the deletion	DNA HAS ALY OUC ANA SKF OR	Severely modified sequence makes the protein not functional
Rearranged DNA Mutations		
Inversion mutation: A group of DNA nucleotides are flipped to read in reverse order; different amino acid sequence in this location	DNA HAS ALL YOC UAN ASK FOR	Change in protein shape and function
Translocation mutation: Move segments of DNA from one chromosome to another, fusing portions of different genes together	DNA HAS ALL YOU CAN EAT THE DOG AND CAT ASK FOR	Significant change in protein shape and function

it overly active. In the case of sickle cell disease, the mutation causes the beta-globin subunits of hemoglobin to become "sticky" and attract the beta-globin subunits from other hemoglobin molecules.

This stickiness of hemoglobin molecules is what causes red blood cells to sickle. The degree of sickling depends on how much mutated hemoglobin is present in a red blood cell, relative to the amount of normal hemoglobin. As discussed in Chapter 7, we all have two copies of every gene—one copy from our biological father and one copy from our biological mother. People can have one, two, or no mutated beta-globin genes, depending on which versions they inherit from their parents. People with one copy of the mutated beta-globin gene and one copy of a normal beta-globin gene are carriers of the condition: they typically do not experience sickling of their cells and are said to have sickle cell trait. People with two mutated copies of the beta-globin gene, however, will have sickle cell disease.

Where do mutations like those that cause sickle cell disease come from? In the case of sickle cell disease, the mutations are inherited, meaning they are passed from parents to children and are present in every cell of the child's body at birth.

Other mutations occur during our lifetime. In this case, cells that develop a mutation will pass that mutation on to their daughter cells every time they divide (Chapter 10). As a result, only some cells in the body will have that mutation. One way that a cell can develop a new mutation is during DNA replication. Each time our cells replicate their DNA (Chapter 7), there is a small chance that a mistake will occur—say, an A nucleotide is paired with a G, instead of a T. If this mistake is not corrected, it will lead to a permanent change in the DNA sequence—a mutation. This mutation will then be passed on as the cell divides and reproduces.

MUTAGEN
Any chemical or physical agent that can damage DNA by changing its nucleotide sequence.

Our DNA is continually being bombarded by environmental factors that can also damage DNA and cause mutations. These factors include chemicals, ultraviolet light, radiation, and infectious agents like viruses. Physical or chemical agents that cause mutations are called **mutagens.**

Not all mutagens originate outside the body. For example, some of the reactions that occur in the mitochondria during cellular respiration (see Chapter 6) produce DNA-damaging molecules called free radicals. When cells attempt to repair this damage, the repairs may be carried out incorrectly, leading to a mutation **(INFOGRAPHIC 9.3)**.

One of the main existing treatments for sickle cell disease is a drug called hydroxyurea, which can reduce the amount of sickling that occurs in a person's body. Unfortunately, it doesn't work for everyone. Indeed, it didn't work for Jennelle or Manny. They also had no compatible sibling donor to provide bone marrow for a transplant. (The probability of finding a match in donor databases for African Americans is typically low.) So they needed another option.

The turning point for Jennelle came after a particularly bad pain crisis in August 2016. "I had just been discharged from the hospital and my boyfriend was encouraging me to do something because I was getting progressively worse," she says. "We had a long talk and he really convinced me that there's got to be something."

That's when she went online and discovered a number of clinical trials being offered for people like herself. She sent emails to 11 places, and heard back from one: the National Institutes of Health (NIH) in Bethesda, Maryland.

A Therapy Decades in the Making

Genetic engineering techniques

Gene therapy has been a goal of medical science since the 1970s. Ever since scientists learned how to cut and splice together pieces of DNA in the lab, they have been tantalized by the prospect of using such techniques to

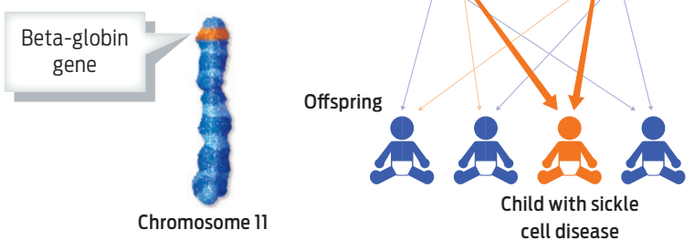
INFOGRAPHIC 9.3

What Causes Mutations?

Mutations are changes in the nucleotide sequence of DNA. There are several ways that a person can end up with a mutation: it may have been inherited; it may have occurred randomly during DNA replication; or it may have been the result of environmental insult.

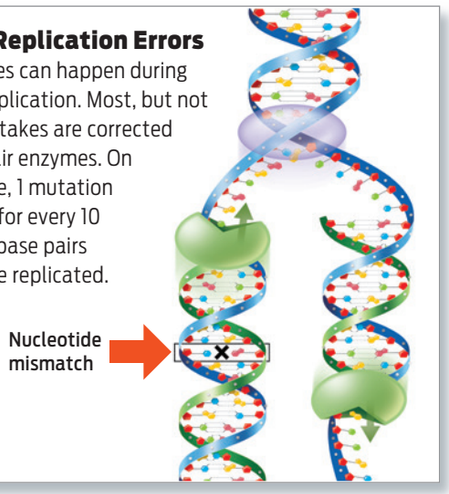
Inheritance

A mutation in the beta-globin gene can be inherited from either parent. If the mutation is inherited from both parents, then the offspring will have sickle cell disease.



DNA Replication Errors

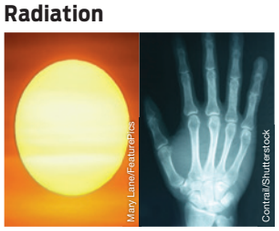
Mistakes can happen during DNA replication. Most, but not all, mistakes are corrected by repair enzymes. On average, 1 mutation occurs for every 10 billion base pairs that are replicated.



Mutagens

Many components of the environment, our food, and even our cells can cause mutations.

Radiation

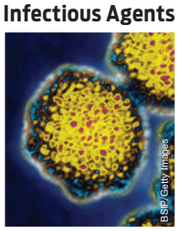


UV radiation X-rays

Chemicals

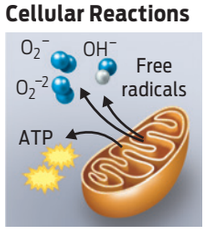
Pollution and pesticides | Smoking | Alcohol | Char (blackened bits) on meats cooked at high temperatures

Infectious Agents



Some viruses, like hepatitis C

Cellular Reactions



Cellular processes produce mutagenic free radicals

? List three things that you could do to decrease the risk of developing a cancer-causing mutation in a skin cell.

fix genetic errors and cure genetic diseases in people.

The first trials of gene therapy approaches in people were conducted in the early 1990s. A 4-year-old girl with a severe immune deficiency became the first person to receive successful gene therapy: she was cured of her disease.

Yet the field has had its share of failures as well. Most memorably, in 1999, a gene therapy trial ended in disaster when the patient, 18-year-old Jesse Gelsinger, died as a result of the procedure. The gene therapy was designed to fix a rare liver disease. Jesse developed a severe allergic reaction to the virus that was used to deliver the new gene to his liver cells. The scientist who led the trial admitted to not following his own procedures, and he shouldered some of the blame for the tragic result.

A few years later, in 2003, several people who received gene therapy developed cancer, and one died from the disease. In this case, the virus used to deliver the gene ended up landing next to a cancer gene, causing it to turn on abnormally. These deaths were a major setback for the field; it would be more than a decade before clinical trials started up again.

In the years since these deaths occurred, the field of gene therapy has become both better regulated and more scientifically advanced. There are still risks, but gene therapy has finally entered the medical mainstream. In 2017, the FDA approved two gene therapies for cancer, *Kymriah* and *Yescarta*. Gene therapies for other diseases are not far behind.

The NIH trial that Jennelle found involved using genetic engineering techniques to introduce a new gene into her blood cells. The new gene encodes a version of beta-globin that doesn't cause cells to sickle. The person still makes some abnormal hemoglobin from the mutated gene, but the engineered version of beta-globin is present in high enough amounts to prevent red blood cells from sickling.

The gene therapy the NIH team developed is similar to the process used to engineer yeast to produce spider silk (Chapter 8). As with the transgenic yeast, the scientists use a carrier molecule called a vector to deliver a new gene into the recipient cell. In this case, the vector is a genetically modified virus, and the gene is a beta-globin gene with the correct nucleotide sequence.

Recall that viruses are noncellular entities consisting of a protein "coat" surrounding genetic material (Chapter 1). Viruses make good vectors because they naturally inject genetic material—their own—into host cells as part of their normal life cycle. Specifically, viruses reproduce by infecting host cells and delivering viral genes into the infected cells. An infected host cell transcribes and translates these viral genes as if they are its own, and the virus then uses these components to make even more copies of itself.

Similarly, the engineered viral vector carrying the new beta-globin gene naturally infects host cells, delivering the beta-globin gene into those cells. The beta-globin gene is inserted into one of the host cell's chromosomes, where it is replicated and retained as a stable component of the host cell's genome.

Before a virus can be safely used in genetic engineering, it must be genetically altered so that it no longer contains the genes that allow it to replicate itself, kill cells, and cause illness. When that's done, all that's left are the tools it uses to insert DNA into the host chromosome, making it a handy gene delivery vehicle for gene therapy.

The NIH scientists expose the viral vector containing the beta-globin gene to blood-forming stem cells, called hematopoietic stem cells, obtained from a person's blood. The virus infects these cells, and delivers its beta-globin payload to the nucleus of these cells. The beta-globin gene is then stitched into one of the host cell's chromosomes. To avoid the problem of allergic reaction that caused Jesse Gelsinger's death, the virus is exposed to the stem cells outside of the body. Millions of copies of the genetically engineered stem cells are grown in the lab and then inserted back into the patient in the hope that they will take hold and begin producing normal hemoglobin **(INFOGRAPHIC 9.4)**.

Preliminary studies done in mice suggested that this approach had promise. Only with clinical trials in humans, however, could the scientists know whether it would be safe and effective in people.

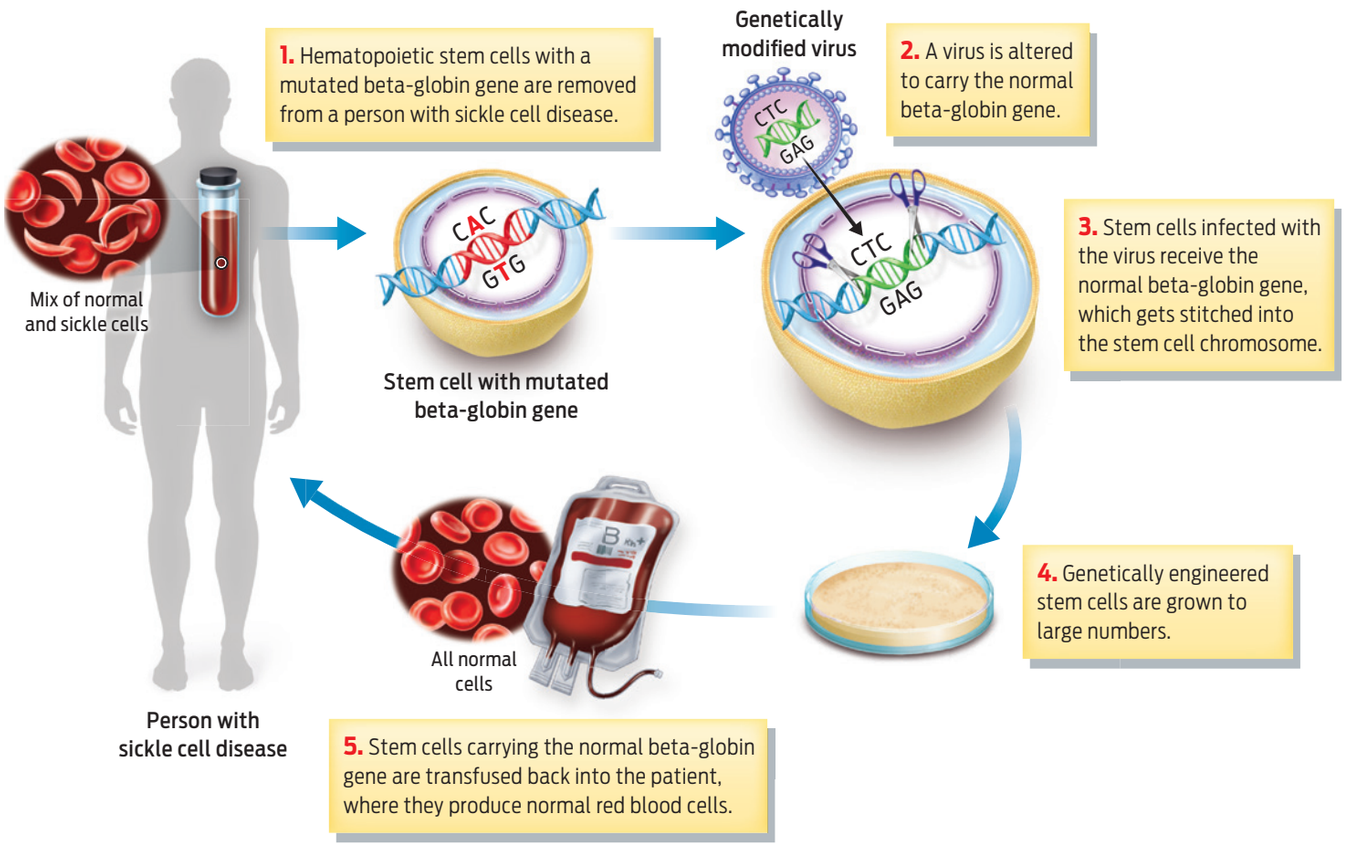
Gearing Up for the Big Day

Jennelle enrolled in the clinical trial in November 2016. Over the next few months, she received several rounds of a drug designed to boost the production of her blood stem cells. Then, in December 2017, doctors took a vial of Jennelle's blood and isolated some of her blood-forming stem cells. In a lab, they exposed these cells to the modified

INFOGRAPHIC 9.4

Gene Therapy for Sickle Cell Disease: One Approach

A modified virus serves as the vector to introduce a normal beta-globin gene to hematopoietic (blood-forming) stem cells isolated from a patient with sickle cell disease. Once stem cells have been obtained from the patient, they are infected with the engineered virus. The beta-globin gene carried by the virus is incorporated into a host cell's chromosome. The genetically modified cells are then grown in the lab, so that a large number of them can be introduced back into the patient, where they will produce normal beta-globin, which will be incorporated into normal hemoglobin in red blood cells that will not sickle.



? What is the role of the virus in this gene therapy approach?

virus vector containing the new hemoglobin gene. Then, they waited.

If all went as planned, the viral vector would infect Jennelle's blood stem cells and introduce its contents into her cells. The viral enzymes would then go to work cutting and pasting the new gene into Jennelle's DNA. After growing the cells into millions of copies, the doctors would introduce the genetically altered stem cells back into Jennelle's body.

To make room for all those new cells, doctors gave Jennelle a few rounds of intense chemotherapy to kill off her existing blood stem cells, which contained the mutated version of the hemoglobin. During this time, she says, she felt awful. She lost her hair, her mouth filled with sores, and she couldn't eat. But this step was necessary to ensure the new stem cells could take hold.

Jennelle received her infusion of cells on December 26, 2017. "It was the best Christmas present ever," she says. Both her dad and her brother were there by her side, along with her team of doctors and nurses. Everyone

held their breath as the bag began to drip its contents into her vein.

It would be a few months before she and her medical team would know whether the procedure worked.

"It was the best Christmas present ever."

—Jennelle Stephenson

Flipping a Genetic Switch

Changing gene expression

A few hundred miles away, in Boston, 20-year-old Manny Johnson was preparing to undergo a similar gene therapy procedure, this one pioneered by scientists at the Dana–Farber/Boston Children's Cancer and Blood Disorders Center. The approach that these doctors are using takes advantage of the fact that the human body is capable of making two different versions of hemoglobin over the course of its life—one during the fetal period and one during adulthood.

Both adult and fetal hemoglobin contain two alpha-globin subunits. But where adult hemoglobin has two beta-globin subunits, fetal hemoglobin contains two gamma-globin subunits. The gamma-globin subunits of fetal hemoglobin are expressed during prenatal development, with that expression beginning to taper off around the time of birth. Beta-globin expression starts a few months before birth and continues into infancy and adulthood. The result of this gene expression pattern is a gradual loss of fetal hemoglobin after birth, and its replacement by adult hemoglobin.

Erica Esrick, MD, and patient Manny Johnson.

A person with the sickle cell mutation in the beta-globin gene will make perfectly normal fetal hemoglobin, since fetal hemoglobin does not contain beta-globin subunits, and will display no symptoms as an infant. However, as beta-globin expression increases, the adult hemoglobin will be of the sickling type, and the individual will start to experience symptoms of the disease. The drug hydroxyurea—mentioned earlier as a treatment for sickle cell disease—works by increasing the amount of fetal hemoglobin that a person makes in adulthood, which helps offset the amount of abnormal hemoglobin produced.

The Dana–Farber/Boston Children's team's goal is to use genetic engineering techniques to turn on the expression of fetal hemoglobin and turn off the expression of adult hemoglobin. In essence, they are trying to flip a genetic switch so that Manny's normal fetal hemoglobin will be the only hemoglobin he produces **(INFOGRAPHIC 9.5)**.

David Williams, a physician-scientist at Dana–Farber/Boston Children's and the team's lead scientific investigator, says his group favors this "switching" approach because it takes advantage of a process that the body uses anyway. "Other trials are adding genes that encode fetal hemoglobin or corrected, non-sickling adult hemoglobin, without directly decreasing expression of the sickle hemoglobin gene," he says. "We predict our strategy is a more effective way to reduce or even eliminate the sickling of cells."

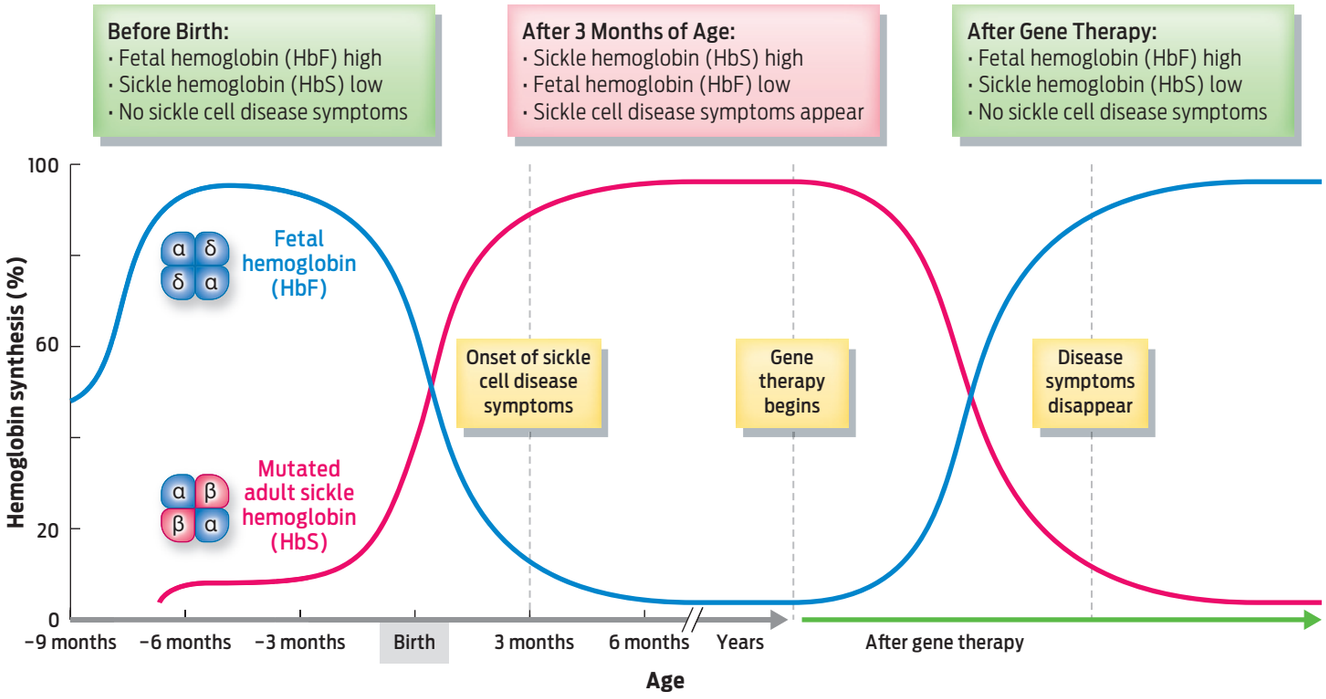
Manny was the first patient enrolled on the trial at Dana–Farber/Boston Children's. He was motivated to participate, he says, because his 7-year-old brother, Aiden,

INFOGRAPHIC 9.5

Using Gene Therapy to Switch Genes On or Off

Because fetal hemoglobin does not contain beta-globin subunits, it will not cause sickling, even in people with sickle cell disease. Genetic engineering approaches are being used to express fetal hemoglobin and shut down expression of sickle hemoglobin in people with the disease.

Types of Hemoglobin in a Person with Sickle Cell Disease, Before and After Gene Therapy



? **Which type of hemoglobin is expressed after this gene therapy? What subunits does it contain?**

also has sickle cell disease. "I wanted to do something to help him," Manny says. He has a tattoo of a sickle cell ribbon with Aiden's name in it.

Manny received his infusion of genetically modified blood stem cells in May 2018. "The day Manny received his cells back was a pretty emotional day for the whole team," says Erica Esrick, a pediatric hematologist-oncologist at Dana–Farber/Boston Children's and the co-principal investigator on the clinical trial. "It was the pinnacle of many, many years of many, many people's hard work."

Only time would tell if the massive effort would ultimately benefit Manny.

Correcting the Mistake

▸ Genome editing with CRISPR

Scientists sometimes make a distinction between gene therapy and **gene editing.** In gene therapy, entire genes are inserted or removed from a recipient cell. But what if it were possible to simply correct a genetic error in the original gene—for example, to edit out the mistaken "T" and replace it with the correct "A"? In fact, that goal may one day be possible thanks to the genome-editing tool CRISPR.

CRISPR (which stands for "clustered regularly interspaced short palindromic

GENE EDITING
A way to change the sequence of a gene.

CRISPR
A genome-editing tool based on a natural defense system in bacteria.

repeats") is a kind of molecular scissors that bacteria use to chop up viruses and thereby defend themselves from infection. Scientists have adapted this set of bacterial scissors to make it an exquisite tool for genetic engineering. CRISPR allows scientists to edit DNA at very specific regions of the genome. Compared to other tools for genome editing, it is relatively quick, easy, and cheap.

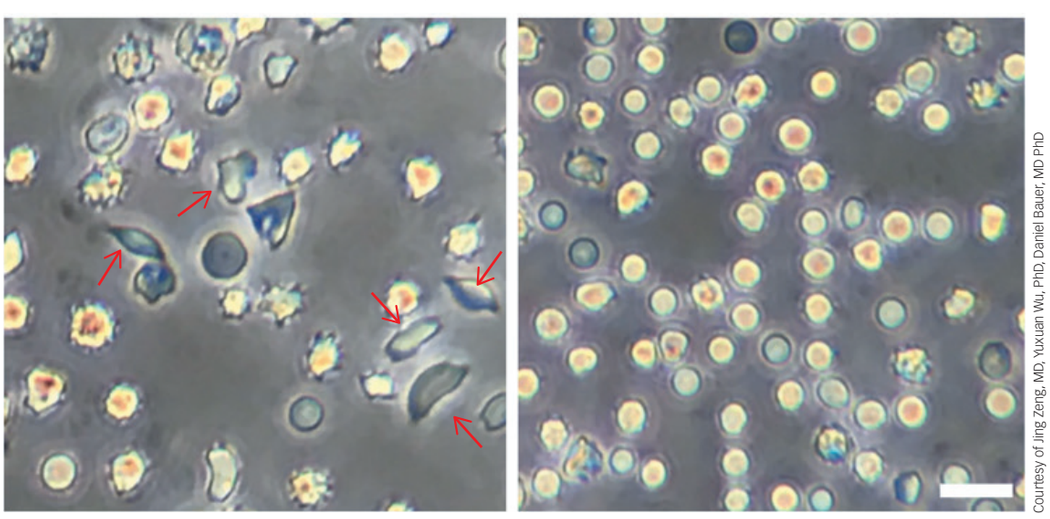
"The day Manny received his cells back was a pretty emotional day for the whole team."

—Erica Esrick

CRISPR has two main components: an enzyme that cuts DNA and a small piece of RNA that serves as a guide for the enzyme, directing it to a specific sequence of DNA. The RNA binds to DNA through complementary base pairing of the sort we have encountered before. Once the complementary match is found, the enzyme cuts the DNA in that precise location. The cell's DNA repair machinery then takes over to fill in the gap. If a supplementary piece of DNA is supplied along with the CRISPR enzyme, the cell will stitch this piece of DNA into the gap. Using CRISPR, scientists can introduce small deletions or additions at this cut site, or even replace one specific sequence of DNA with another.

In principle, CRISPR could enable scientists to correct the genetic mistake that causes sickle cell disease in the first place—by swapping in a small piece of DNA with the nonmutated sequence to replace the mutated version in a person's blood stem cells. The cells with the correct sequence would produce only healthy hemoglobin, so the person would no longer experience symptoms of the disease.

Correcting the genetic mistake with CRISPR is still a few years away. But at least one clinical trial now under way uses CRISPR to flip the switch to permit fetal hemoglobin expression in adults with sickle cell disease **(INFOGRAPHIC 9.6)**.



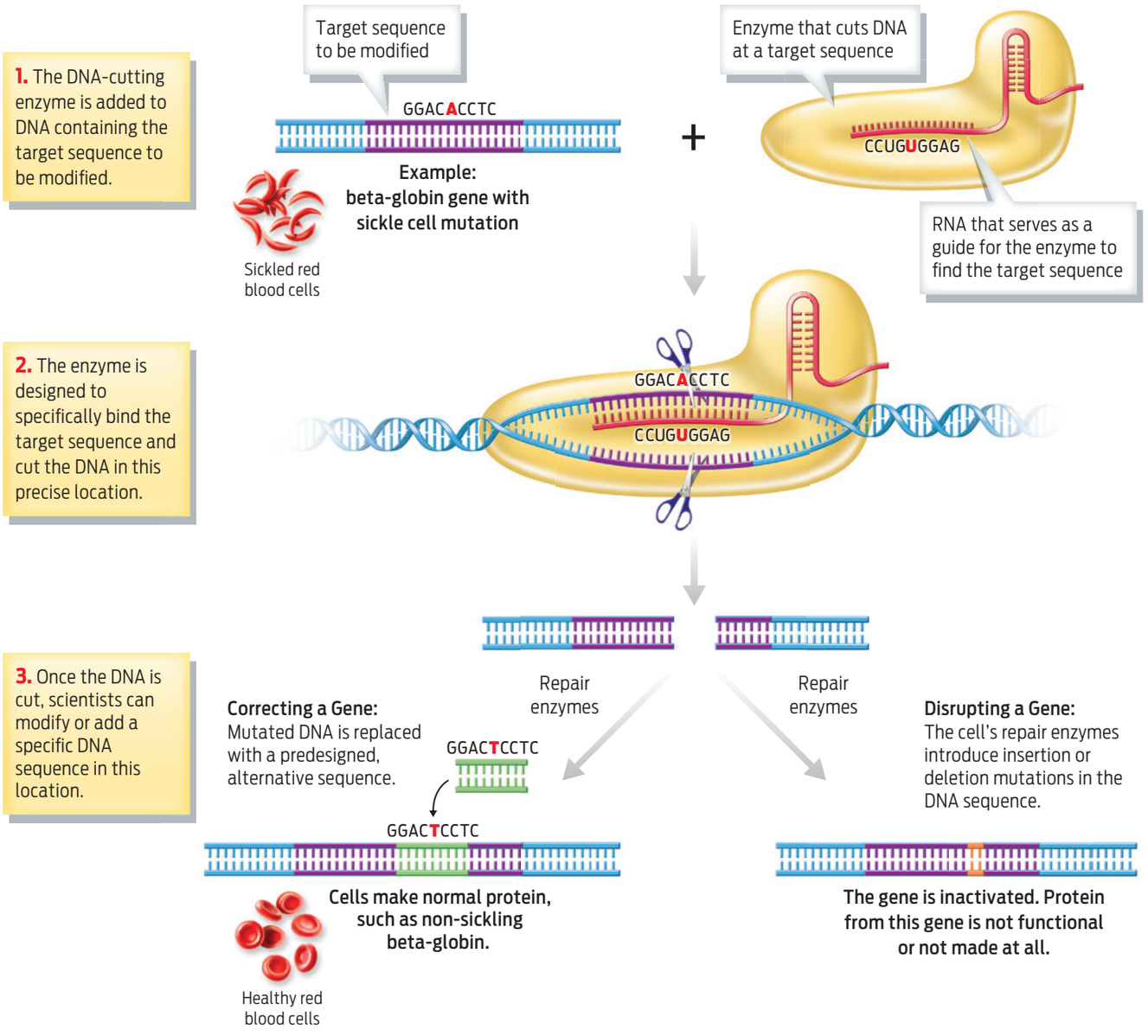
Courtesy of Jing Zeng, MD, Yuxuan Wu, PhD, Daniel Bauer, MD PhD

Left: In sickle cell disease, red blood cells sickle (red arrows). Blood stem cells from a person with sickle cell disease were edited with CRISPR with the goal of turning on fetal hemoglobin expression. Right: After genetic engineering, almost no red blood cells are sickled.

INFOGRAPHIC 9.6

CRISPR Adds Precision to Genetic Engineering

CRISPR is a genetic engineering method that can precisely modify specific gene sequences. Molecular tools target a specific DNA sequence, which can then be used to insert, delete, or alter a DNA sequence at that site.



? How does CRISPR target specific sites in the genome for modification?

A Permanent Fix?

Somatic versus germ-line editing

No matter how much Jennelle and Manny may personally benefit from their genetically engineered cells, they will not be free from all consequences of their genetic disease. That's because the changes are being made in only a subset of the **somatic cells** making up their body. They will still have the mutation

SOMATIC CELLS
Nonreproductive cells of the body.

in their **germ cells**—those that develop into sperm or eggs. That means they can still pass the mutation on to their children.

The only way to prevent that possibility would be to correct the genetic mistake in their germ cells or in an early embryo (changes in an early embryo will be present in virtually cells in the body, including germ cells). Such germ-line editing, while technically feasible, is quite controversial. That's because changing the DNA in germ cells affects not just one individual, but all of that person's descendants—and those future individuals cannot consent to having their genetic material altered.

Although the United States does not officially ban the editing of DNA in human embryos, the NIH, which funds most biomedical research in this country, currently prohibits it. In addition, the Food and Drug Administration (FDA) is not allowed (by law) to approve clinical trials involving genetically modified human embryos.

The situation is different in other countries. In 2018, a scientist in China announced that he had performed germ-line editing using CRISPR on two embryos that were subsequently implanted into a woman's uterus. The scientist edited a gene called *CCR5*, which makes a protein to which HIV binds to infect cells. The edited version of the gene shortens the CCR5 protein and makes cells more resistant to HIV infection. The woman later gave birth to the world's first CRISPR'd babies.

Scientists around the world condemned the Chinese scientist. Many said the experiment was reckless, given that we don't know enough about the edited gene to fully anticipate unintended consequences of its editing and that there are other ways to prevent HIV infection. CRISPR is such a new technology that there is not yet a lot of experience from which to learn. One concern with CRISPR technology is that it could make "off target" cuts in the genome, not just at the desired location. These unplanned changes could have serious consequences if they occur in genes that encode essential proteins.

GERM CELLS
Reproductive cells of the body.

These concerns have led some scientists to call for a complete moratorium on the editing of human embryos. The Chinese case, however, highlights the difficulty of preventing the technology from being misused **(INFOGRAPHIC 9.7)**.

Germ-line editing does have its supporters, many of whom are parents of children with disabling genetic conditions. If it's possible, through gene therapy, to spare future individuals the pain and anguish of a genetic disease like sickle cell disease, why wouldn't you? This is a question that bioethicists, parents, citizens, and lawmakers will likely have to address in the coming years.

One reason to be cautious about germ-line editing, from a strictly scientific standpoint, is that we don't always know what effect a mutation will have in different environments. Sickle cell disease provides a perfect example of a mutation whose impact on humans depends on the context.

Why Sickle Cell Disease Persists

Beneficial, neutral, and harmful mutations

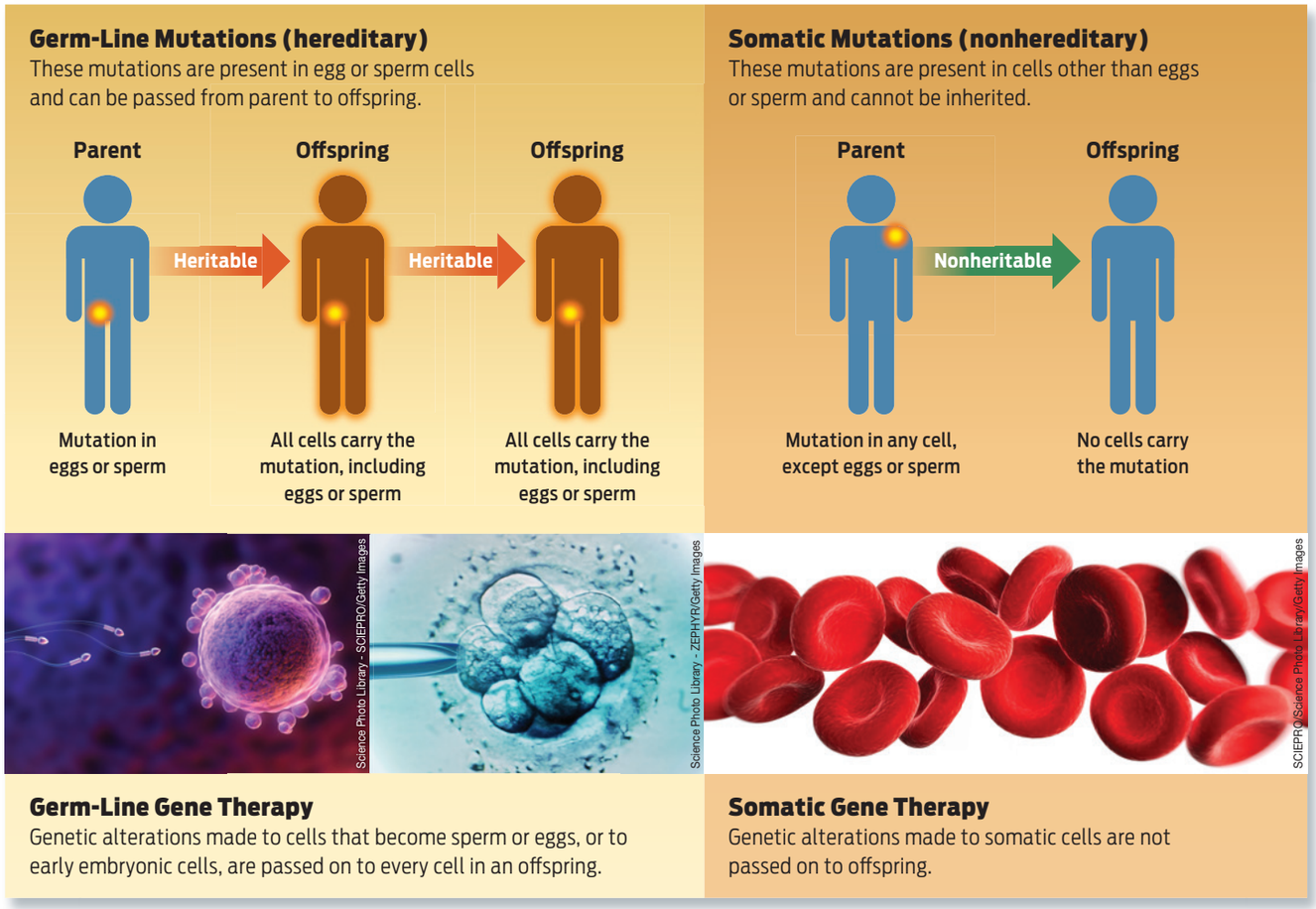
Sickle cell disease affects hundreds of thousands of people around the globe, primarily those living in sub-Saharan Africa, the Saudi Arabian peninsula, and central India—and those individuals who can trace their ancestry to these areas. In the United States, 90% of all patients with sickle cell disease are African American. About 1 out of every 365 African American babies will be born with the disease.

There is a reason why sickle cell disease is more common in individuals from certain geographic areas. In these environments, having one copy of the sickle cell mutation confers a benefit. The sickle cell mutation is found at high frequencies wherever the disease malaria is or has been common. Malaria is caused by a parasite that infects red blood

INFOGRAPHIC 9.7

Mutations Can Be Hereditary or Nonhereditary

Mutations that occur in sperm or egg cells are germ-line mutations that can be passed on to offspring. These inherited mutations are then found in every cell of the offspring, including egg or sperm cells, and can be passed on to subsequent generations. Somatic mutations are those that occur in any cell in the body other than sperm or egg cells. These mutations are not inherited but may cause disease in the individual that acquires them.



? Is the genetic alteration of hematopoietic stem cells a type of germ-line or somatic gene therapy? Explain your answer.

cells (see Milestone 5). In environments where malaria is common, having just one sickle cell mutation—the condition called sickle cell trait—provides protection against malaria infection. This is why the mutation continues to occur at high frequency in populations of people living in those areas.

In places without malaria, the mutation has no such protective effect. In addition, those who happen to inherit two copies of it suffer severe, painful consequences in the form of sickle cell disease.

In other words, whether a particular mutation provides a survival advantage, or is neutral or harmful, often depends on the environment where a person with that mutation lives. We'll have more to say about the relationship between mutation and

evolutionary fitness in Chapter 13. Without mutations, there would be no evolution at all **(INFOGRAPHIC 9.8)**.

Testing the Boundaries

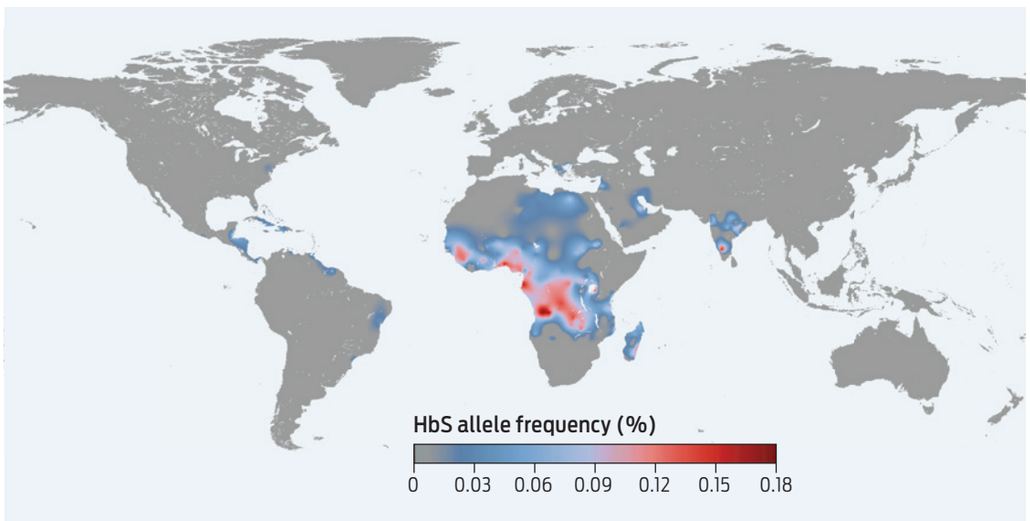
Although gene therapy is not new, only a relatively small number of people have been

INFOGRAPHIC 9.8

Sickle Cell Mutations Protect Against Malaria

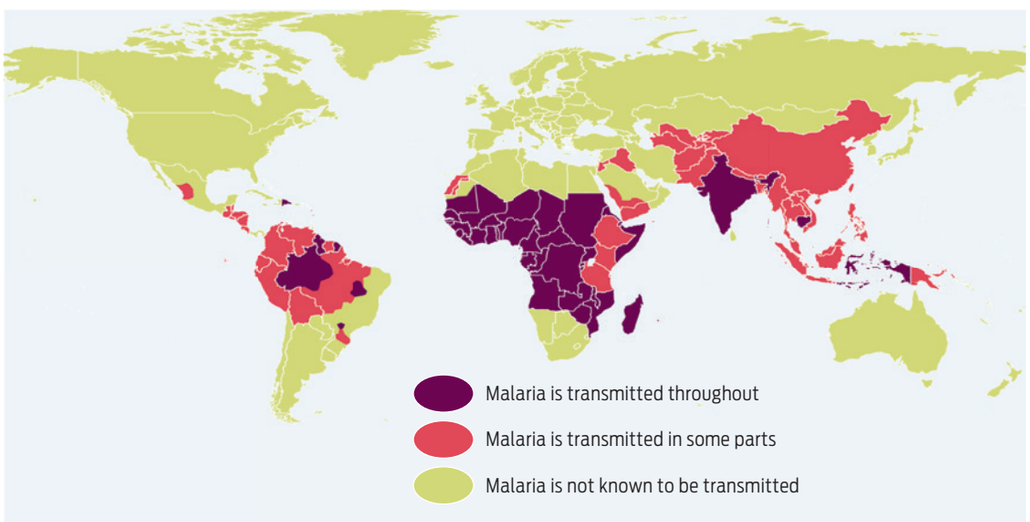
The frequency of the sickle cell mutation is higher in areas where malaria is or has been common. While having two copies of the sickle cell mutation is harmful, having one copy protects against malaria, which explains why this mutation is more frequent in certain parts of the world.

Frequency of Sickle Cell Mutation



Piel, F.B., Patil, A.P., et al., Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *The Lancet*, 2013; 381: 142–151.

Frequency of Malaria



Data from the CDC.

? Why is the sickle cell mutation present at high frequency in populations in Africa and South America?

treated, and their short- and long-term outcomes are being closely monitored. Given past mistakes, scientists are understandably cautious about moving too fast with these powerful techniques. That's why news about the experiences of people like Jennelle and Manny is so eagerly awaited.

So far, both gene therapy approaches seem to be working. More than a year after his treatment, Manny hasn't had any pain crises or needed a transfusion. Doctors can see that his blood is full of round, healthy red blood cells. You wouldn't be able to tell his blood from someone who doesn't have sickle cell disease. Now 21, Manny is thinking about applying to college. "I feel great, I'm looking great, I'm trying to be great," he says.

It's been more than two years since Jennelle received her infusion of genetically modified blood cells. She could tell something was different within a few months.

At first it was little things, like being able to run up stairs without getting completely out of breath, or sitting on a cold train and not being in pain. Over time, she began to notice a dramatic change in herself. "I really felt like I wanted to do something to test my boundaries," Jennelle says.

She landed on the martial art of jujitsu. She enjoys the confidence she gets from being able to kick and fall on the mat without worrying that she's going to be in pain. She also feels more in synch with herself: "In my head, I've always been kind of a bubbly, smiley, outgoing person. But my body, my actions, my physical health was never able to match that."

"Now everything matches," she says. "This is me. This is who I was supposed to be."

Courtesy Jennelle Stephenson

Jennelle Stephenson practicing jujitsu after gene therapy for sickle cell disease.

CHAPTER 9 SUMMARY

Driving Question 1 What are mutations, what is their impact, and how do they occur?

- Mutations are changes in the nucleotide sequence of DNA.
- The different types of mutations include point mutations, insertions and deletions, translocations, and inversions.
- The effect of a mutation depends on where it occurs in the genome, and whether it changes the amino acid sequence of a protein.
- Mutations can occur spontaneously during DNA replication. They can also be caused by environmental triggers such as tobacco, ultraviolet radiation, chemicals, and viruses, and by chemicals naturally produced by the body.

Driving Question 2 How can genetic engineering be used to treat genetic diseases?

- Viruses are useful tools in genetic engineering because they make good vectors.
- CRISPR is a genome-editing tool adapted from enzymes found in bacteria. It can be used to make changes to DNA at specific locations.
- Mutations that occur in body (somatic) cells will be found only in the descendants of that particular cell. Mutations that occur in germ cells (sperm and eggs) will be inherited by offspring and therefore will be present in all the cells of that offspring's body.
- Using CRISPR to modify germ cells is not a currently accepted therapy for genetic diseases.

Driving Question 3 Are all mutations harmful?

- The impact of a mutation may vary, depending on the environment in which it's found.
- Mutations can be beneficial, harmful, or neutral in terms of the effect they have on survival and reproduction.

More to Explore

- Sickle Cell Disease, National Heart, Lung, and Blood Institute: https://www.nhlbi.nih.gov/health-topics/education-and-awareness/sickle-cell
- NIH Director's Blog: https://directorsblog.nih.gov/2019/04/02/a-crispr-approach-to-treating-sickle-cell/
- Sickle Gene Therapy Timeline: https://vector.childrenshospital.org/2018/01/sickle-cell-gene-therapy-bcl11a-timeline/
- Pauling, L., et al. (1949). Sickle-cell anemia, a molecular disease. *Science* 110:543–548.
- Wailoo, Keith. (2001). *Dying in the City of Blues: Sickle Cell Anemia and the Politics of Race and Health.* Chapel Hill, NC: University of North Carolina Press.

CHAPTER 9 Test Your Knowledge

Driving Question 1 What are mutations, what is their impact, and how do they occur?

By answering the questions below and studying Infographics 9.1, 9.2, 9.3, and 9.7 and Table 9.1, you should be able to generate an answer for this broader Driving Question.

Know It

1. What is a mutation?
- **a.** a cause of a disease
- **b.** a change in the structure of a protein
- **c.** a change in the function of a protein
- **d.** a change in the nucleotide sequence of DNA

2. At the DNA level, what is the sickle cell disease mutation?
- **a.** a change of a single codon in the mRNA
- **b.** a change of a single base pair in the gene sequence
- **c.** a change of a single amino acid in the protein sequence
- **d.** all of the above

3. Which type of cells are impacted in sickle cell disease?
- **a.** white blood cells
- **b.** all blood cells
- **c.** red blood cells
- **d.** heart cells

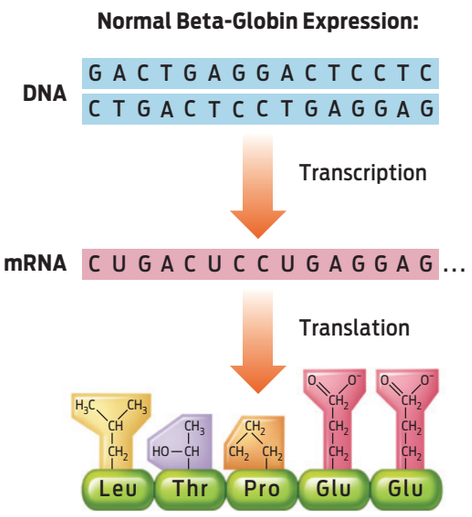
4. A mutation causes a substitution of one amino acid for another in the encoded protein. Which type of mutation is this?
- **a.** silent
- **b.** nonsense
- **c.** missense
- **d.** shift in reading frame caused by an insertion
- **e.** shift in reading frame caused by a deletion

5. Which of the following is a known mutagen?
- **a.** cigarette smoke
- **b.** sunlight
- **c.** charred meat cooked at high temperatures
- **d.** x-rays
- **e.** all of the above

Use It

6. How likely (very, potentially, not very) is each of the following forms of hemoglobin to cause symptoms of sickle cell disease? Explain your answer for each.
- **a.** hemoglobin A (2 alpha-globin subunits and 2 beta-globin subunits)
- **b.** hemoglobin S (2 alpha-globin subunits and 2 mutant beta-globin subunits)
- **c.** fetal hemoglobin (2 alpha-globin subunits and 2 gamma-globin subunits)

7. The mutation illustrated in Infographic 9.2 substituted a U for an A in the fourth codon of the mRNA shown.



Below is a short list of mutations of the normal mRNA sequence shown in this infographic. Use the genetic code (Infographic 8.8, p. 178) to match each of the following mutations with both its effect on the protein and the type of mutation. For each mutation, put a check mark next to the corresponding effect and type of mutation.

Mutation	Effect of Mutation	Type of Mutation
Substitution of a C for the U in the third codon	_____ The protein will have an incorrect amino acid in its sequence. _____ No impact on the protein _____ The protein will be shorter than normal.	_____ Nonsense _____ Missense _____ Silent
Substitution of a U for the first G in the fifth codon	_____ The protein will have an incorrect amino acid in its sequence. _____ No impact on the protein _____ The protein will be shorter than normal.	_____ Nonsense _____ Missense _____ Silent
Substitution of an A for the C in the second codon	_____ The protein will have an incorrect amino acid in its sequence. _____ No impact on the protein _____ The protein will be shorter than normal.	_____ Nonsense _____ Missense _____ Silent

8. A mutation in codon #42 of a gene encoding a 97-amino-acid protein causes a shift in the reading frame. The shift causes different amino acids to be encoded by codons #42 and #43 and then produces a stop codon at codon #44. Based on this information, describe

- **a.** the length (number of amino acids) of the mutant protein.
- **b.** the number of identical amino acids between the normal (unmutated) and mutant protein.
- **c.** how likely the mutant protein is to function properly.

Driving Question 2 How can genetic engineering be used to treat genetic diseases?

By answering the questions below and studying Infographics 9.4, 9.5, and 9.6, you should be able to generate an answer for this broader Driving Question.

Know It

9. Modifications of blood stem cells are

- **a.** somatic changes, so they can be passed on to offspring.
- **b.** somatic changes, so they will not be passed on to offspring.
- **c.** germ-line changes, so they can be passed on to offspring.
- **d.** germ-line changes, so they will not be passed on to offspring.

10. Why is a virus a good vector to deliver a new gene (like non-sickling beta-globin) into blood stem cells?

11. Manny's treatment uses CRISPR technology to edit the genome of blood stem cells, while Jennelle's treatment involves introducing a new beta-globin gene into her blood stem cells. What do their treatments have in common?

- **a.** Both rely on genetic engineering techniques.
- **b.** Both are designed to restore fetal hemoglobin production.
- **c.** Both are designed to precisely correct the original beta-globin mutation.
- **d.** Both cause permanent genetic alterations to germ-line cells.

Use It

12. Assume that Jennelle's treatment is successful (that the introduced gene continues to express a non-sickling form of beta-globin and she no longer has symptoms of sickle cell disease). If she were to have children in the future, would they inherit her mutated beta-globin and/or the therapeutic non-sickling beta-globin that she was treated with? Explain your answer.

Interpreting Data

Apply Your Knowledge

13. Hemophilia B is a genetic disease caused by a mutation in the gene (*F9*) encoding coagulation factor IX (FIX). People with hemophilia B have very low levels of FIX and are at high risk for episodes of spontaneous bleeding that can result in death. To maintain FIX levels of at least 1% of the normal level (the minimum needed to prevent spontaneous bleeding), patients must receive intravenous infusions of FIX every two to three days. A 2015 report estimated the cost of these infusions to be more than $270,000 per year for a single patient. Gene therapy (inserting a healthy *F9* gene) has been considered as a possible alternative treatment. Because the *F9* gene is expressed by liver cells, the *F9* gene must be introduced through a vector that can deliver it to liver cells.

One study of 10 patients used an "adeno-associated viral vector" known as AAV8. This viral vector has a DNA genome that is not inserted into the host cell's DNA.

All 10 patients stably produced FIX at levels between 1% and 6% of normal, and this production of FIX has been maintained for between 1.5 and 4.3 years (with patients continuing to be followed). Use the data below to determine whether this level of FIX expression is sufficient to make a substantial impact on their disease and its management. Explain your answer.

	1 year prior to gene therapy	1 year following gene therapy
Annual use of FIX (median units/kg per year)	2,613	206
Bleeding episodes (median number per year)	15.5	1.5

Data from Nathwani, A. C., et al. (2014). Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Engl J Med* 371:21.

Bring It Home

Apply Your Knowledge

14. The annual cost to treat a patient with sickle cell disease is estimated to be more than $10,000 per year for patients younger than 10, and more than $30,000 per year for patients who are 50–64 years old. By age 45, total lifetime costs approach $1 million. The cost of gene therapy is not yet determined, but is estimated to be between $500,000 and $700,000 (as a one-time expense). Make arguments to justify the coverage (or not) of gene therapy by insurance providers.

Driving Question 3 Are all mutations harmful?

By answering the questions below and studying Infographic 9.8, you should be able to generate an answer for this broader Driving Question.

Use It

15. What is sickle cell trait?

- **a.** a mild form of sickle cell disease
- **b.** having a single copy of the mutated beta-globin gene
- **c.** having sickle cell disease due to environmental rather than genetic traits
- **d.** having abnormally shaped red blood cells in the absence of sickle cell disease

16. What disease does sickle cell trait protect against?

- **a.** sickle cell disease
- **b.** anemia
- **c.** abnormal bleeding
- **d.** abnormal blood clotting
- **e.** malaria

Apply Your Knowledge

Mini Case

17. People who have two copies of a mutation in the *CCR5* gene are resistant to HIV infection—the virus cannot enter target cells when the protein encoded by the *CCR5* gene is mutated.

A 40-year-old male had been infected with HIV when he was 30. He had been treated with an advanced antiretroviral therapy drug cocktail for the past 4 years. He had not developed any AIDS-associated illnesses, and there was no HIV RNA detectable in his blood. It appeared that the anti-HIV therapy was successfully controlling the infection.

This patient developed a form of leukemia (a cancer of the blood). The treatment for most leukemias (as well as sickle cell disease) is a bone marrow transplant. In this case, several "matched" donors were identified. Having a matched donor minimizes the chances of transplant rejection. In this case, the transplant team was able to find a matched donor who also had two mutant copies of the *CCR5* gene. After the patient underwent chemotherapy to destroy his existing bone marrow, bone marrow stem cells from the donor were transfused into him. No anti-HIV therapy was used after the transplant, and the viral RNA levels remained undetectable (despite not taking the drug treatment).

a. Explain why this patient no longer needs anti-HIV therapy.

b. How practical is this approach as a general strategy for curing HIV?

18. When looking for mutations that protect against malaria, it made sense to look at populations living in regions where malaria is common.

a. Where should researchers look for mutations that protect against Ebola?

b. How should researchers look for mutations that increase life span (i.e., mutations that promote longevity)? (*Hint:* Think beyond geography; think about the types of populations researchers might study.)

c. How should researchers look for mutations that protect against type 2 diabetes in people who have risk factors for developing type 2 diabetes? (*Hint:* Think beyond geography; think about the types of populations researchers might study.)