

## DRIVING QUESTIONS

1. What determines the shape of a protein molecule?
2. What are the steps of gene expression, and where in the cell do they occur?
3. How can organisms be genetically modified to produce recombinant proteins?
4. What are some pros and cons of genetically modified organisms?

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# BULLET PROOF

Scientists hope to spin spider silk into the next indestructible superfiber

**P**ETER PARKER'S SPIDEY SENSE is tingling from the latest news out of biotech: genetically engineered spider silk, produced from spider DNA but assembled inside an entirely different organism.

For more than two decades, scientists have sought ways to harness the unique properties of spider silk—a near-miracle fiber that is pound for pound stronger than steel but also lightweight and flexible. Yet harvesting silk from spiders is challenging.

“Spiders don’t like neighbors,” says David Kaplan, a professor of biomedical engineering at Tufts University. “They are territorial and cannibalistic.”

This makes them much harder to work with than, say, silkworms—another source of silk fibers. “If I put a hundred silkworms together on a table with enough food, I can come back in 30 days and find a hundred cocoons,” Kaplan explains. “If I put a hundred spiders together with enough food and come back 30 days later, there’d be only one spider.”

The other approach—collecting spider silk from webs in the wild—is exceedingly tedious. It would take about 1 million spider webs to make a single spider silk garment. Which is why, recently, scientists have turned to putting spider DNA into other, more congenial organisms.

The potential applications of genetically engineered spider silk are vast. They include more-durable, environmentally friendly clothing; safer medical products such as biocompatible bone screws and sutures; and lightweight military vests that deflect bullets better than Kevlar. While some of these products are years away, others debuted in 2016.

## Stronger than Steel

If you think about it, a spider’s web is, in essence, a device for stopping a speeding projectile—such as a flying insect. It’s no wonder, then, that the fibers making up the web are uncommonly strong.

“On an equal weight basis, spider silk has a higher toughness than steel and Kevlar,”

says Kaplan, whose lab is using genetically engineered spider silks to improve medical technologies. But it’s also flexible and elastic, which allows it to absorb the energy of whatever hits it. A rope of spider silk the diameter of a pencil could theoretically stop a jet landing on an aircraft carrier, though no one has tried that yet.

“On an equal weight basis, spider silk has a higher toughness than steel and Kevlar.”

—David Kaplan

The physical properties of spider silk reflect the structure of the **proteins** making it up. Recall from Chapter 2 that proteins are one of the four main macromolecules that make up cells, along with carbohydrates, nucleic acids, and

lipids. Proteins are the cell’s workhorse molecules. They perform myriad functions inside cells, and in turn help our bodies perform countless tasks—everything from contracting our muscles and sensing light to regulating blood sugar and fighting infections. Spiders use their silk proteins to build webs for trapping prey, sacs to protect eggs, and bungee cord-like draglines that catch them when they fall.

All proteins are made of the same building blocks, which are called **amino acids**.

### PROTEIN

A macromolecule made up of repeating subunits called amino acids, which determine the shape and function of a protein. Proteins play many critical roles in living organisms.

### AMINO ACIDS

The building blocks of proteins. There are 20 different amino acids.

## Mechanical properties of spider silk

Spider silk exhibits a unique combination of strength and elasticity, enabling silk fibers to absorb a lot of energy before breaking (toughness).

Material	Strength (MPa) Spider silk is as strong as high-grade alloy steel and about half as strong as Kevlar.	Extensibility (%) Silk is extremely elastic, and some silks are able to stretch up to four times their relaxed length.	Toughness (MJ/m <sup>3</sup> ) The combination of strength and extensibility make silks tougher than many engineered polymers like Kevlar.
Spider dragline silk	880–1500	21%–27%	136–194
Silkworm silk	600	18%	70
Nylon	950	18%	80
Kevlar 49 fiber	3600	2.7%	50
High-tensile steel	1500	0.8%	6

Data from Anna Rising and Jan Johansson. 2015. *Nature Chem Biol*. 11:309–315. Lin Römer and Thomas Scheibel. 2008. *Prion* 2(4):154–161.

## Spider silk characteristics and applications

Spider silk is tough, flexible, and elastic, making it well suited for military and industrial uses. It is also biocompatible, making it ideal for a variety of surgical applications, and better for the environment, too.



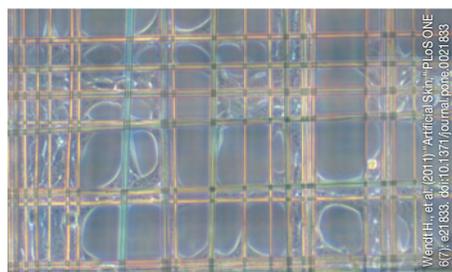
Bulletproof vests



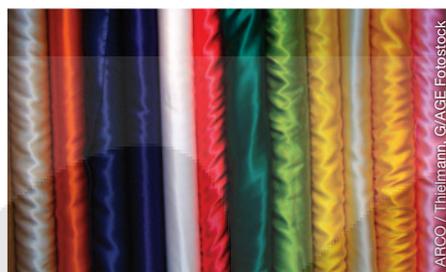
Strong, elastic airbags



Biocompatible and biodegradable screws and sutures



Stronger skin grafts



Durable ecotextiles



Scaffolds for growing tissues

There are 20 different amino acids in cells. All amino acids have the same basic core structure, but each of the 20 also has a unique chemical side chain that distinguishes it from all the others. To form proteins, amino acids link together in linear chains. Spider silk proteins, called spidroins, are chains of about 3,500 amino acids. This is longer than many animal proteins, which average around 400 amino acids in length. But proteins can be much longer or much shorter. The longest human protein, titin (involved in muscle contraction), is a single chain of 34,350 amino acids. Insulin, a protein that helps regulate blood sugar, has only 51.

In cells, the amino acid chain of a protein folds into a distinct three-dimensional shape, or conformation, that underlies a protein's function. Some proteins, like insulin, are made up of just one folded chain. Other proteins, such as the antibodies of our immune system or the hemoglobin that carries oxygen in our red blood cells, are made up of multiple folded amino acid chains bound together. A spider silk thread is made up of

many spidroin proteins linked together into a fiber.

The particular sequence of amino acids in a chain determines how the chain will fold. Interactions between amino acid side chains, and between these side chains and the surrounding water, influence the precise folding pattern. Hydrophobic amino acid side chains tend to clump together, away from water, while hydrophilic amino acids face out toward water. The distinct three-dimensional shape that forms as a result is what ultimately determines how a protein functions.

According to Kaplan, the first thing you'd notice about the amino acid sequence of a spider silk protein is its repetitive nature. Most of the protein—roughly 90%—is composed of repeated sequences of relatively hydrophobic amino acids. It's these repetitive sequences that are responsible for the toughness of spider silks. Regions rich in the hydrophobic amino acid alanine pack closely together, away from water, and form flat, interlocking structures called beta sheets (a folded structure found in many proteins but

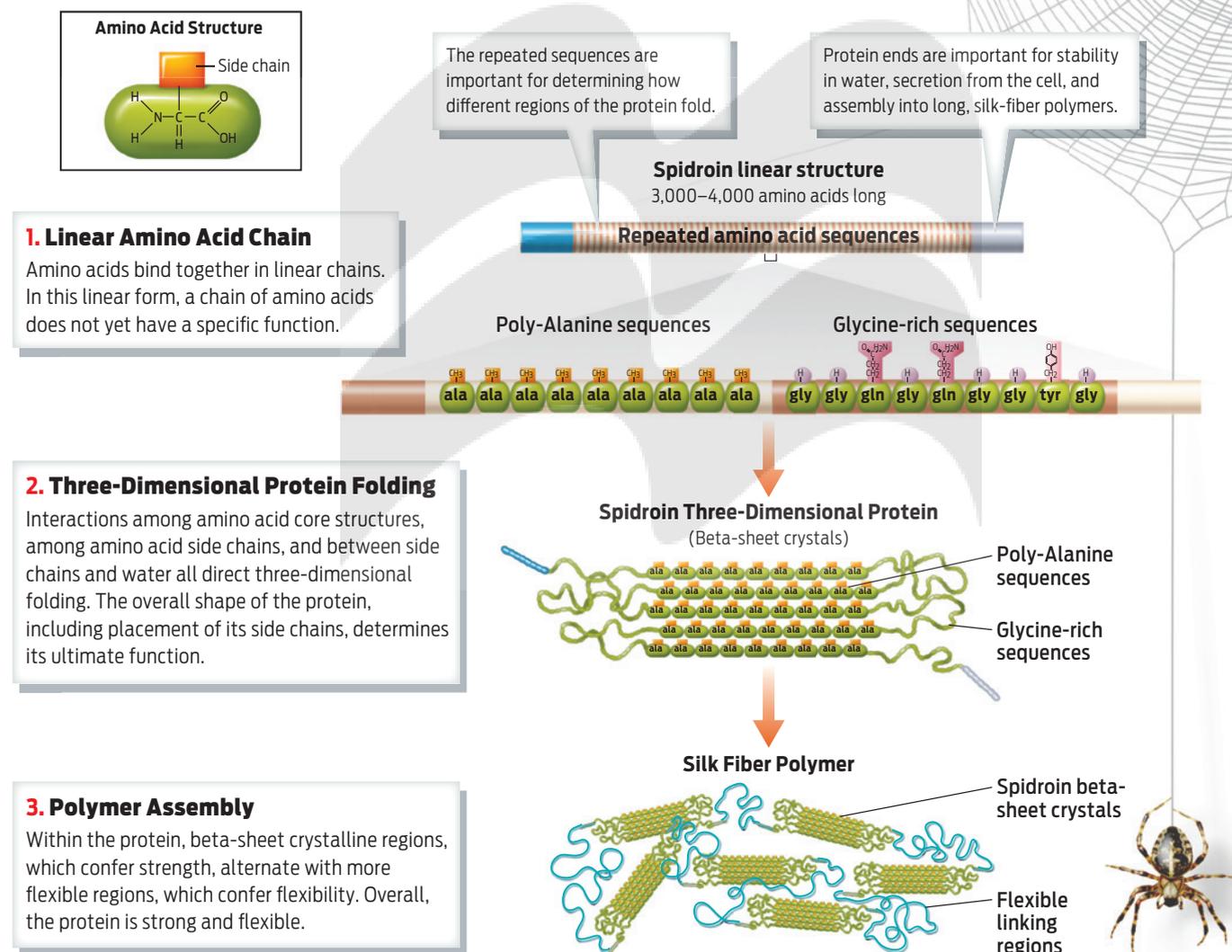
at a much higher frequency in silk). These regions impart strength to the protein. Other regions, rich in the amino acid glycine, form more flexible parts of the protein, which confer elasticity. Nonrepetitive regions that flank these core repeated sequences are made of charged amino acids. These hydrophilic end regions keep the spidroin proteins dissolved in the silk gland's watery environment

and prevent silk proteins from crystallizing spontaneously into fibers inside the spider, which would kill the animal (**INFOGRAPHIC 8.1**).

Silk proteins lack the structural complexity you would see in an enzyme or an antibody, in which every region has a different function or activity. In that sense, Kaplan says, "They're very much like synthetic polymers." A polymer is a large molecule made up

## INFOGRAPHIC 8.1 Amino Acid Sequence Determines Protein Shape and Function

➔ The sequence of amino acids determines how a chain will fold into a three-dimensional shape and potentially interact with other chains to establish the final shape (and function) of that protein.



? Based on their side chains, are alanine and glycine hydrophobic or hydrophilic? How will this characteristic influence their tendency to interact with water?

of many repeating subunits. Natural polymers include spider silk, as well as other fiber-like proteins in cells, such as those making up the filaments of the cytoskeleton.

Where do spiders get the information to build such unique proteins? As with all organisms, the instructions to make proteins are encoded in the DNA, in genes. A **gene** is a sequence of DNA that provides instructions for making one or more proteins. These instructions come in the form of the particular DNA nucleotide sequence making up the gene. Genes are found along

the length of chromosomes, with each specific chromosome carrying a unique set of genes.

The synthesis of a protein from the information encoded in a gene is called **gene expression**. When a cell makes the protein encoded by that gene, the gene is said to be “expressed” (**INFOGRAPHIC 8.2**).

Silk proteins are made in the spiders’ silk glands. Each silk gland is connected to a microscopic “spigot” that protrudes from a larger structure called a spinneret on the rear end of the spider. Most spiders have three

#### GENE

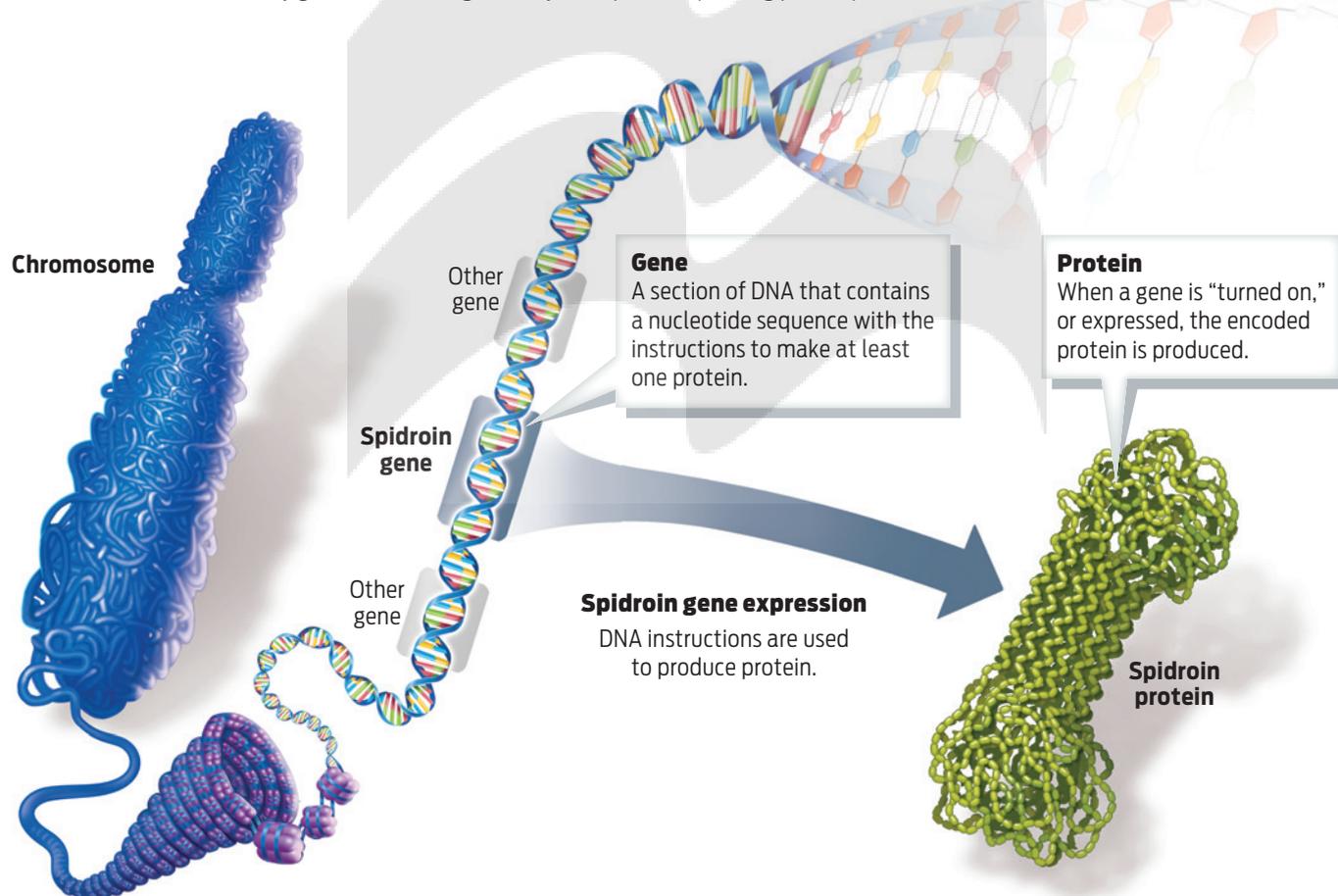
A sequence of DNA that contains the information to make at least one protein.

#### GENE EXPRESSION

The process of using DNA instructions to make proteins.

## INFOGRAPHIC 8.2 Chromosomes Include Gene Sequences That Code for Proteins

➔ Chromosomes have many genes along their length. Each gene contains instructions to make at least one protein. Depending on the needs of the cell at any given time, each gene may be expressed (making protein) or silenced.



? Why is spidroin protein being produced, but not the proteins encoded by the other genes?



**A colored scanning electron micrograph (SEM) of silk fibers emerging from a spider's spinneret.**

spinnerets, each with multiple spigots that extrude silk protein polymers.

Silk starts as a protein-rich liquid made by cells in the silk gland. As the liquid travels through the gland, water is gradually removed and the remaining solution containing the dissolved silk proteins is dowsed in an acid bath, which precipitates the silk proteins into a solid form. During “spinning,” or thread formation, the silk proteins link up and pack together tightly, forming a silk protein polymer. This polymer is squeezed like toothpaste from the spigot as a fiber about 50 micrometers thick—roughly half the width of a human hair. Spiders can extrude silk fibers from multiple spigots simultaneously, lending additional strength to the webs and draglines they produce.

## Back to Nature

Humans have used silk for thousands of years to make lightweight yet durable textiles. The silk in these textiles comes primarily from silkworms—the larvae of silk moths. During

their development, silkworms produce protective cocoons made of silk, which is harvestable by humans.

In addition to gowns and neckties, silk makes good medical bandages and suturing thread because it is sturdy yet biocompatible. Until World War II, silk was also used to make military products like parachutes and flak vests. But the scarcity of the material during wartime opened the way for new materials to move into silk territory, beginning with the synthetic petroleum-based polymer called nylon.

Cheap, sturdy, and elastic, nylon is a nearly miracle fiber. DuPont introduced the fiber in 1939, and it is now found in everything from women’s stockings (“nylons”) to parachutes, tires, and toothbrushes. But for all its versatility, it has two very large drawbacks: (1) it’s not biodegradable, which means it sticks around in the environment for a long time; (2) producing it requires nonrenewable resources like petroleum, the extraction of which contributes to climate change. The same is true of other synthetic fibers made from petroleum, such as Lycra, polyester, and acrylic.

“Manmade fabrics like nylon and polyester have transformed the fashion industry, for better and for worse,” says Dan Widmaier, CEO of Bolt Threads, a San Francisco Bay Area company that is developing clothing made from genetically engineered spider silk. But, he says, “The use of hydrocarbon polymers in these textiles has created a lingering toxic problem for the environment.” Although garments with synthetic fibers can be recycled, roughly 90% still end up in the waste stream. Spider silks, by contrast, are biodegradable.

A former graduate student in chemical biology at the University of California, San Francisco, Widmaier started Bolt Threads in 2009 with the help of three friends and fellow scientists. Their original goal was to make lightweight bulletproof vests that would appeal to the defense industry. But Widmaier—whose wife is a fashion designer at Old Navy—eventually decided to shift the company’s focus to the consumer textile

industry, which represents a much larger market and has a much greater impact on the environment.

Besides Bolt Threads, several other biotech companies are also hoping to snag a piece of the spider silk action, including Spiber Inc. in Japan and Kraig Biocraft Laboratories in Ann Arbor, Michigan.

## Spider Silk Factories

Scientists have tried making genetically engineered spider silk in a variety of different organisms, including bacteria, insects, plants—even goats. (The goats can be engineered to produce the silk in their milk.) But each of these organisms has drawbacks. Silks are large proteins and can be tricky to produce in the prokaryotic cells of bacteria. Animals like goats reproduce slowly and take up a lot of space and resources. And it's hard to scale up from insects like silkworms.

Bolt Threads seeks to overcome these hurdles by relying on a different unicellular organism: yeast. Better known for its role in making bread and beer, yeasts have several attributes that make them good silk producers. For one, they are eukaryotic cells, which means their protein production machinery is much like that of a spider's (another eukaryote). But they are easier to house and cheaper to feed than more complex multicellular animals like silkworms and goats. When yeasts are fed a simple diet of sugar, they grow and divide plentifully. In the process, they synthesize new proteins, including—if they have been engineered to contain the silk gene—an abundance of silk. By conducting the entire process in large industrial vats, scientists can make literally tons of silk protein this way.

The process of making genetically engineered silk begins with isolating spider DNA from spider cells or synthesizing it from scratch. This bit of DNA is then inserted into a cell of a new organism, which is then coaxed to make the protein encoded by the spider gene. Organisms that have been genetically

modified to contain genes from other species are called **transgenic** (“trans” means “across”—in this case, across species, from one to another).

The method that scientists use to make a transgenic organism relies on the fact that every gene has two parts: a regulatory sequence and a coding sequence. **Regulatory sequences** are like on-off switches for genes: they determine when, where, and how much protein is produced from a gene. **Coding sequences** determine the identity of a protein: they specify the order, or sequence, of amino acids (**INFOGRAPHIC 8.3**).

By combining the regulatory sequence of one species with the coding sequence of another, scientists can coax an unrelated organism to make the desired protein. To make a transgenic yeast, for example, scientists first fuse the coding sequence of a spider silk gene to the regulatory sequence of a yeast gene. The combination is called a **recombinant gene**, since it mixes and matches segments of genes that weren't naturally found together. Next, using **genetic engineering** techniques, which manipulate DNA, scientists insert the recombinant gene into a piece of

### TRANSGENIC

Refers to an organism that carries one or more genes from a different species.

### REGULATORY SEQUENCE

The part of a gene that determines the timing, amount, and location of protein production.

### CODING SEQUENCE

The part of a gene that specifies the amino acid sequence of a protein. Coding sequences determine the identity, shape, and function of proteins.

### RECOMBINANT GENE

A genetically engineered gene that contains portions of genes not naturally found together.

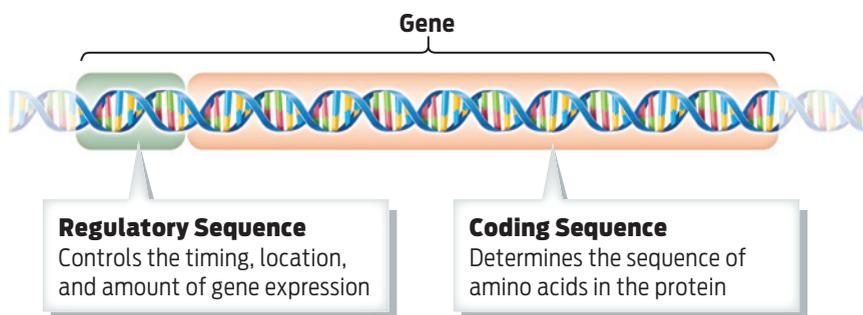
### GENETIC ENGINEERING

Altering or manipulating the DNA of organisms by modern laboratory techniques.

## INFOGRAPHIC 8.3 The Two Parts of a Gene



Genes are organized into two parts. Regulatory sequences determine when a protein is made from a gene and in which cells, and how much protein a gene makes. Coding sequences determine the amino acid sequence of the encoded protein, which determines its shape and function.



**?** Why does a milk protein gene (expressed in mammary glands) have both a different regulatory sequence and a different coding sequence from the insulin gene (expressed in the pancreas)?

**VECTOR**

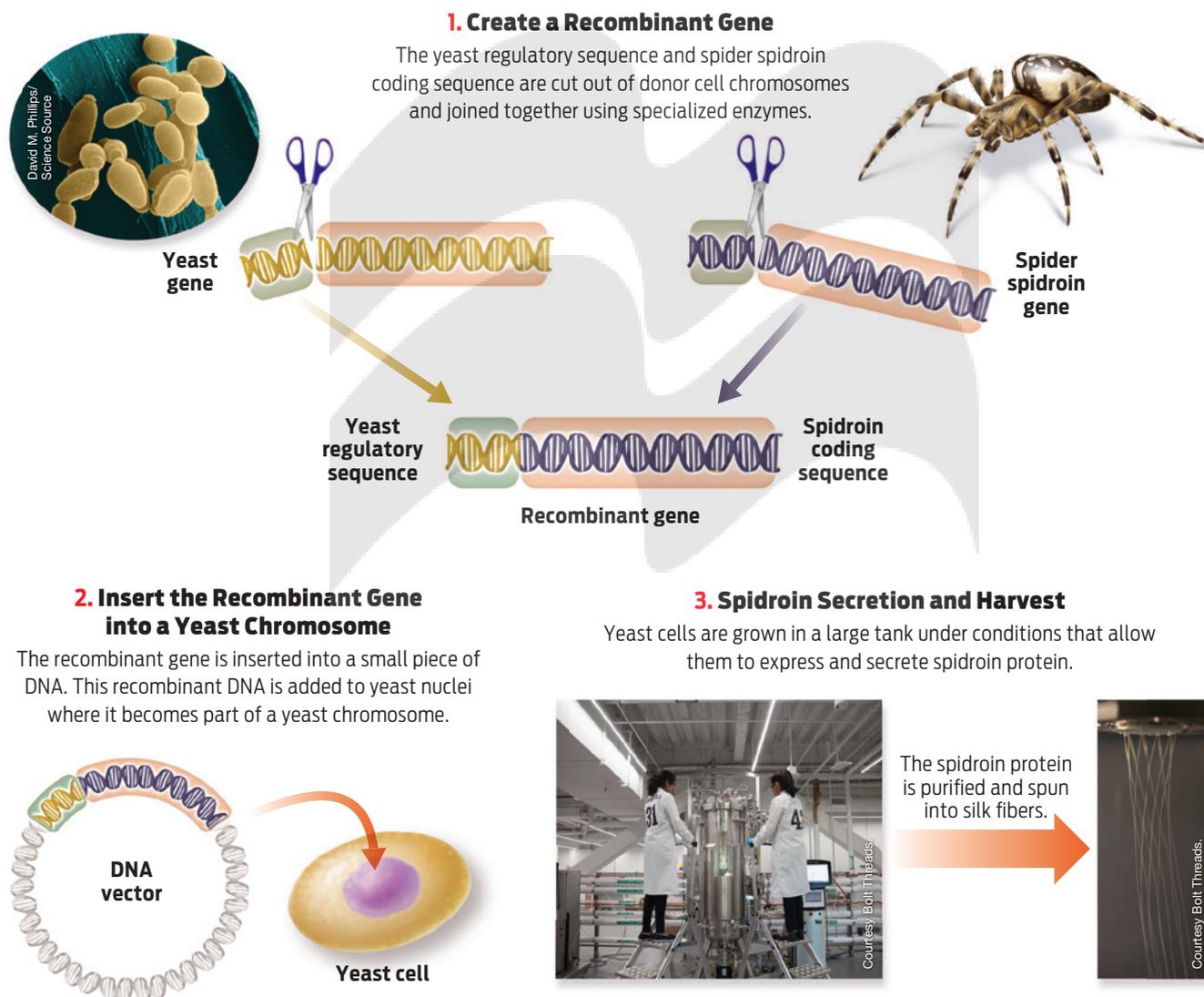
A DNA molecule used to deliver a recombinant gene to a host cell.

DNA that can carry the recombinant gene into the yeast cell, and ultimately into a yeast chromosome. The carrier DNA molecule is called a **vector**. The final step is gene expression, when the yeast protein machinery “reads” the instructions in the recombinant gene and synthesizes spider silk protein (**INFOGRAPHIC 8.4**).

As the yeast grow, they make abundant quantities of silk protein, which they secrete into the surrounding culture media. The scientists then harvest these proteins. Once the silk proteins are in hand, the next step in the manufacturing process is spinning them into fibers, using a process that mimics what happens naturally inside spiders. The wet silk

## INFOGRAPHIC 8.4 Making a Transgenic Organism

➔ Transgenic organisms contain genes from other organisms. In order for the foreign gene to be expressed in the new host, it needs to be modified. The modified recombinant gene contains a regulatory sequence from the host organism and the coding sequence from the gene of interest. The recombinant gene is then inserted into and expressed in the host.



? Why did the scientists give the recombinant spidroin gene a yeast regulatory sequence?

protein solution produced by yeast is squeezed through the small holes of a metal container into a liquid acid bath that transforms the liquid proteins into solid fibers. The fibers are then collected and can be woven together to make fabrics.

## Making Proteins, or How Genes Are Expressed

Whether produced inside a spider's abdomen or inside yeast grown in steel vats, all spider

silk proteins are made from silk genes in essentially the same way.

In order to get from a gene to a protein, cells carry out two major steps: transcription and translation. **Transcription** is the process of using DNA to make a **messenger RNA (mRNA)** copy of the gene. **Translation** is the process of using this mRNA copy as a set of instructions to assemble amino acids into a protein (**INFOGRAPHIC 8.5**).

Why two separate steps? As the names "transcription" and "translation" imply, the

### TRANSCRIPTION

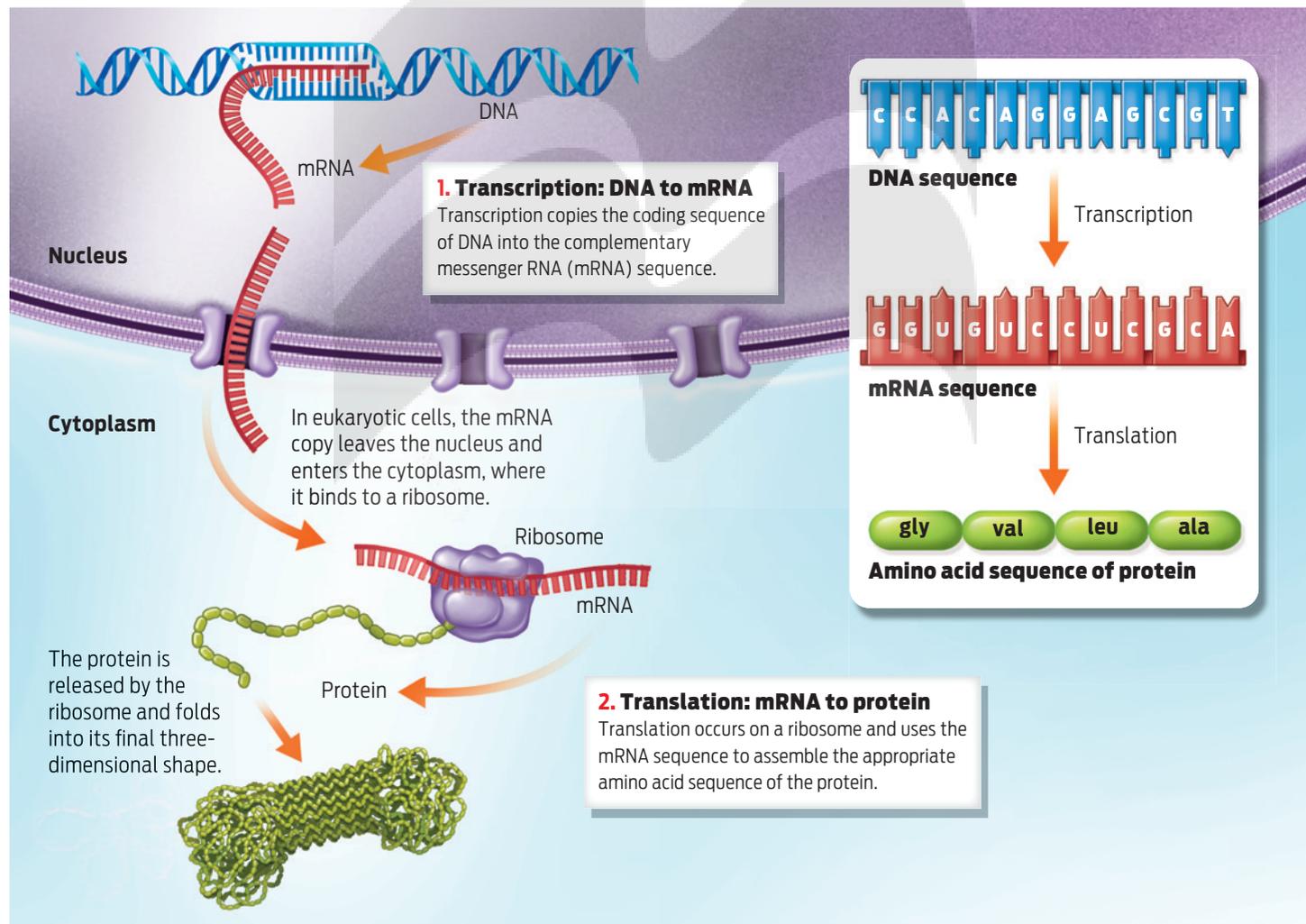
The first stage of gene expression, during which cells produce molecules of messenger RNA (mRNA) from the instructions encoded within genes in DNA.

### MESSENGER RNA (mRNA)

The RNA copy of an original DNA sequence made during transcription.

## INFOGRAPHIC 8.5 Gene Expression: An Overview

➔ Gene expression is the process of converting the genetic information of DNA into the amino acid sequence of a protein. Gene expression has two main steps: transcription and translation.



? What are the products of transcription and translation?

**TRANSLATION**

The second stage of gene expression, during which mRNA sequences are used to assemble the corresponding amino acids to make a protein.

**RNA POLYMERASE**

The enzyme that carries out transcription. RNA polymerase copies a strand of DNA into a complementary strand of mRNA.

process of gene expression is like copying a text and then converting it into another language. In this case, the text to be translated is a valuable, one-of-a-kind document: DNA. Just as you would be forbidden to borrow a rare manuscript from the library at school and would instead have to copy the text into your notebook or laptop, the cell cannot take DNA out of its “library”—the nucleus. It must first make a copy—the mRNA. The cell can then take this mRNA copy into the cytoplasm, where it is translated into a new language: protein.

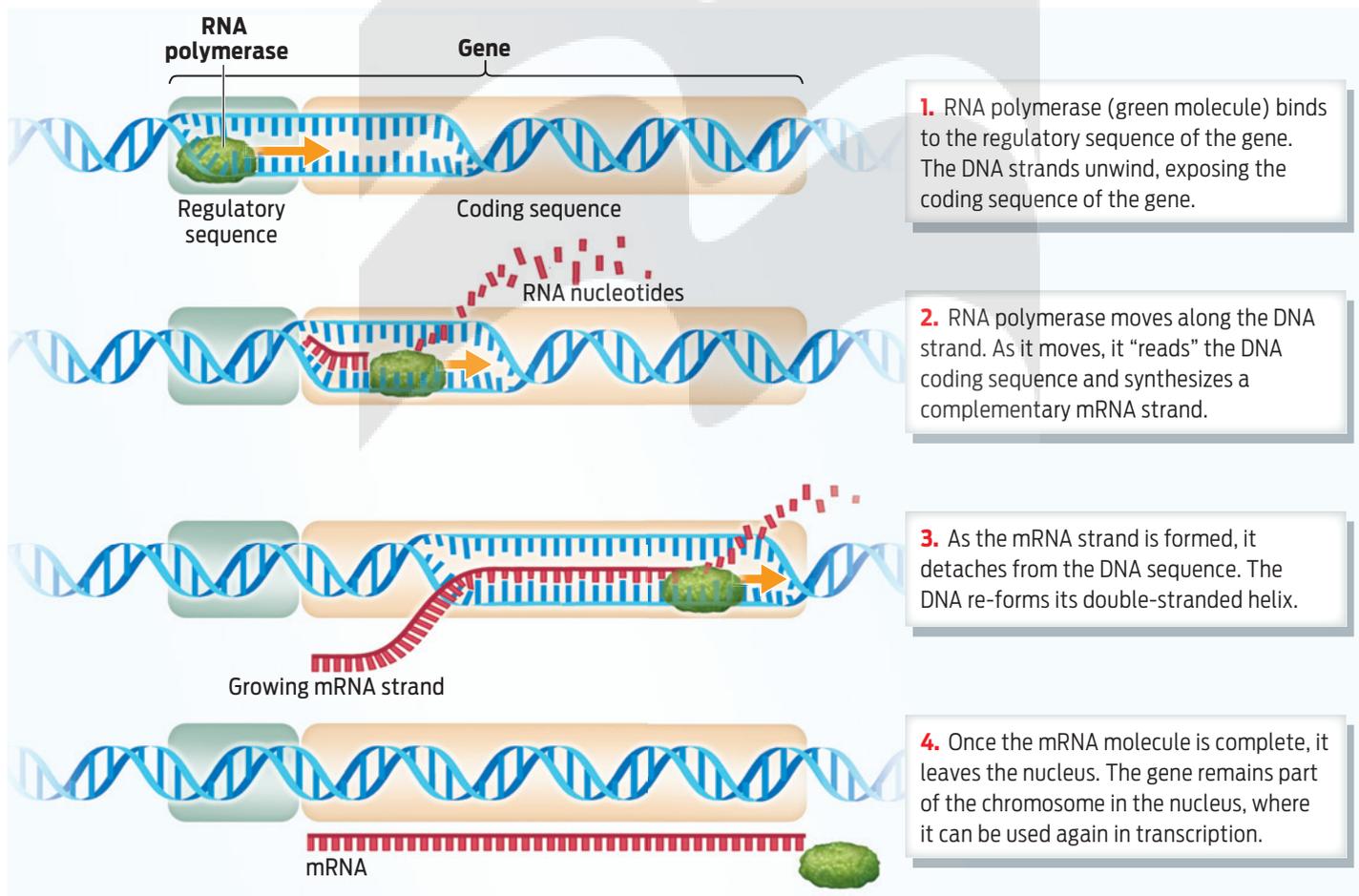
Transcription begins in the nucleus of a cell when an enzyme called **RNA polymerase** binds to DNA at a gene’s regulatory

sequence, located just ahead of the coding sequence. At that site, RNA polymerase separates the two strands of the DNA double helix and begins moving along one DNA strand. As it moves, the RNA polymerase reads the DNA sequence and synthesizes a complementary mRNA strand according to the rules of base pairing. The same rules that govern DNA base pairing apply here, with one difference: RNA nucleotides are made with the base uracil (U) instead of thymine (T). So the complementary base pairs are C with G and A with U (**INFOGRAPHIC 8.6**).

As its name implies, messenger RNA serves to relay information. Once the mRNA

**INFOGRAPHIC 8.6 Transcription: A Closer Look**

➔ In eukaryotic cells, transcription occurs in the nucleus and copies a DNA sequence into a corresponding mRNA sequence. RNA polymerase is the key enzyme involved. In prokaryotic cells, transcription occurs in the cytoplasm, where DNA is located.



? What enzyme is responsible for transcription, and what, specifically, does it do?

copy, or transcript, is made, it leaves the nucleus and attaches to a complex piece of cellular machinery in the cytoplasm called the ribosome. This is the start of translation.

During translation, the **ribosome** reads the mRNA transcript and translates it into a chain of amino acids. The sequence of nucleotides in the mRNA transcript specifies which amino acids should be joined together in the newly forming protein chain. Each of the 20 amino acids that occur in proteins is specified by a group of three mRNA nucleotides called a **codon** that functions like a word: for example, the codon GGU specifies the amino acid glycine.

The actual building blocks of proteins—amino acids—are physically delivered to the

ribosome by another type of RNA, called **transfer RNA (tRNA)**. Each tRNA molecule serves as a kind of adaptor, with one end binding to a specific amino acid and the other end binding to the mRNA codon for that particular amino acid. The part that binds mRNA is called the **anticodon** because it base-pairs in a complementary fashion with the mRNA codon. When the amino acid-toting tRNA finds its mRNA codon match, it releases the amino acid to the ribosome, which adds it to the growing protein chain (**INFOGRAPHIC 8.7**).

The vast majority of mRNA codons specify a specific amino acid, but there are a few with other functions. The “start codon,” which in eukaryotes codes for methionine, is the first

#### RIBOSOME

The cellular machinery that assembles proteins during translation.

#### CODON

A sequence of three mRNA nucleotides that specifies a particular amino acid.

#### TRANSFER RNA (tRNA)

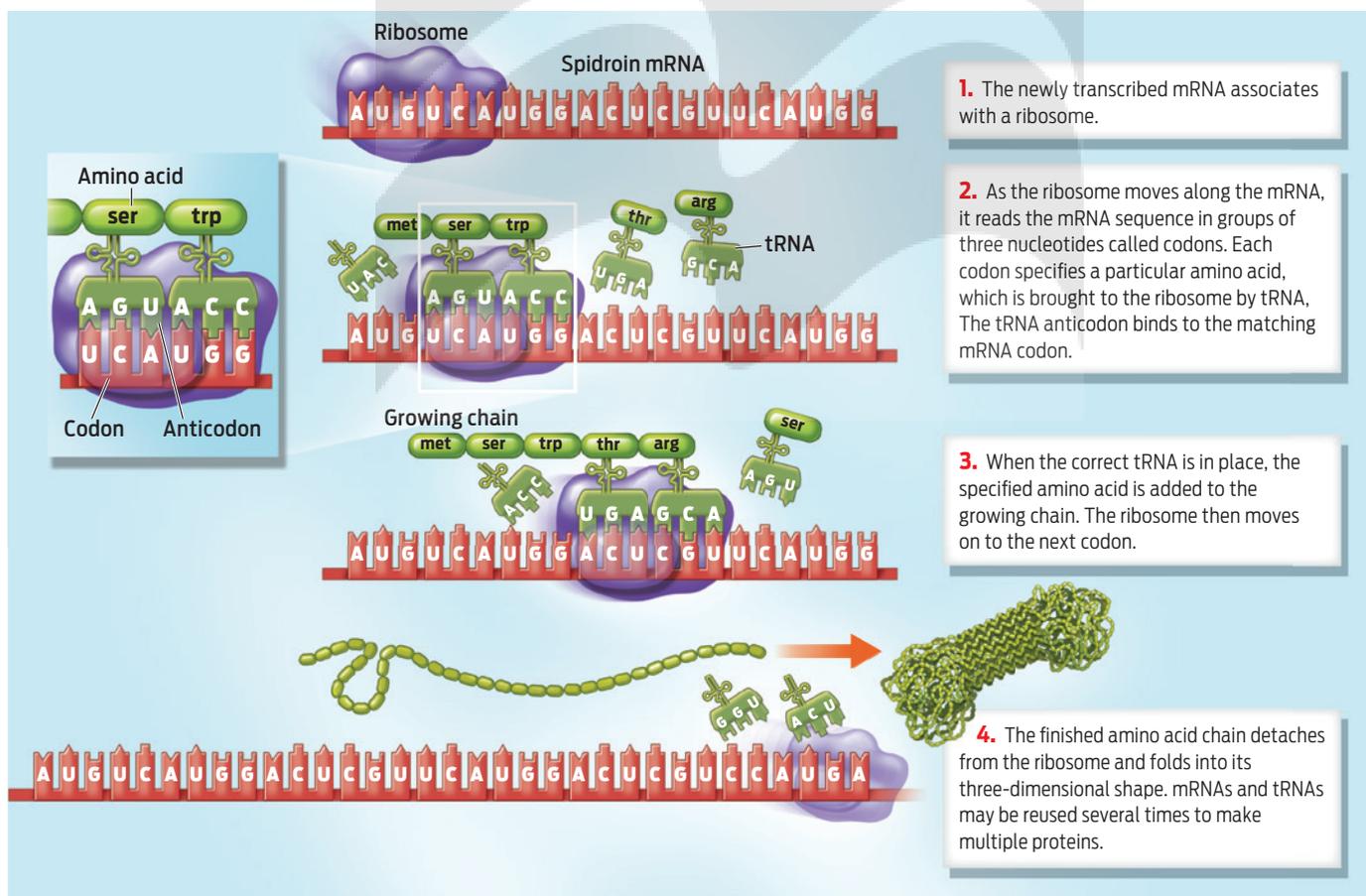
A type of RNA that transports amino acids to the ribosome during translation.

#### ANTICODON

The part of a tRNA molecule that binds to a complementary mRNA codon.

## INFOGRAPHIC 8.7 Translation: A Closer Look

➔ In the cytoplasm, the ribosome reads the mRNA sequence and translates it into a chain of amino acids to make a protein.



? What molecule reads the mRNA codons and brings amino acids to the growing protein? How does it do this?

**GENETIC CODE**

The set of rules relating particular mRNA codons to particular amino acids.

codon of a coding sequence; it tells the ribosome to start translating and begin adding amino acids. “Stop codons” (there are three) tell the ribosome to stop translating and not add any more amino acids to the growing chain.

Although the human genome encodes many thousands of different proteins, each one is pieced together from the starting set of just 20 amino acids. In the same way that the 26 letters in our alphabet can spell hundreds of thousands of words, the basic set of amino acids can make hundreds of thousands of proteins. The set of rules dictating which mRNA codons specify which amino acid is called the **genetic code**. Scientists have pieced together this code by systematically studying how changes to the letters of a codon alter the

specified amino acid, so that we now know what amino acid each codon stands for. Each codon specifies one and only amino acid.

Two additional features of the genetic code stand out. (1) The code is redundant: multiple codons specify the same amino acid. In many cases, a codon will differ at the third nucleotide position without changing the amino acid that is specified. (Note that while the code is redundant it is not ambiguous—the same codon will not specify more than one amino acid.) (2) The genetic code is universal, which means that it is the same in all living organisms. It is because the code is universal that a yeast cell carrying a spider gene can express that gene and produce spider silk (**INFOGRAPHIC 8.8**).

## INFOGRAPHIC 8.8 The Genetic Code Is Universal

➔ Codons are three-nucleotide sequences within chains of mRNA. Most codons specify a particular amino acid. One codon specifies where to start translation (start codon) and others specify where to end (stop codons). There is redundancy in the genetic code, as 64 possible codons code for only 20 different amino acids. Since the genetic code is universal, the same gene will be transcribed and translated into the same protein in virtually all cells and organisms.

		Second letter							
		U	C	A	G				
First letter U	UUU ]	Phenylalanine (Phe)	UCU ]	Serine (Ser)	UAU ]	Tyrosine (Tyr)	UGU ]	Cysteine (Cys)	U
	UUC ]		UCC ]		UAC ]		UGC ]		C
	UUA ]		UCA ]		UAA ]		UGA ]		A
	UUG ]		UCG ]		UAG ]		UGG ]		G
First letter C	CUU ]	Leucine (Leu)	CCU ]	Proline (Pro)	CAU ]	Histidine (His)	CGU ]	Arginine (Arg)	U
	CUC ]		CCC ]		CAC ]		CGC ]		C
	CUA ]		CCA ]		CAA ]		CGA ]		A
	CUG ]		CCG ]		CAG ]		CGG ]		G
First letter A	AUU ]	Isoleucine (Ile)	ACU ]	Threonine (Thr)	AAU ]	Asparagine (Asn)	AGU ]	Serine (Ser)	U
	AUC ]		ACC ]		AAC ]		AGC ]		C
	AUA ]		ACA ]		AAA ]		AGA ]		A
	AUG ]		ACG ]		AAG ]		AGG ]		G
First letter G	GUU ]	Valine (Val)	GCU ]	Alanine (Ala)	GAU ]	Aspartic acid (Asp)	GGU ]	Glycine (Gly)	U
	GUC ]		GCC ]		GAC ]		GGC ]		C
	GUA ]		GCA ]		GAA ]		GGA ]		A
	GUG ]		GCG ]		GAG ]		GGG ]		G

? What is the sequence of the start codon, and what amino acid does it specify?

By tweaking the specific sequence of nucleotides in the spider genes they introduce into yeast, scientists can produce spider proteins with unique amino acid sequences and therefore unique properties—perhaps sturdier than the native form, or stickier, or more elastic. With genetic engineering techniques, the possibilities to produce designer proteins are nearly endless.

## Brave New World?

Bolt Threads announced in May 2016 that it was partnering with clothing manufacture Patagonia to develop a new line of eco-friendly clothing, which it plans to bring to market in the coming years. The Japan-based company Spiber, which has partnered with North Face, unveiled a winter parka in 2016, making it the first clothing made from genetically engineered spider silk to be sold in stores. (It currently costs \$1,000).

Yet these are just the tip of the iceberg when it comes to spider silk products in development. “Nature provides a great starting point and then once we’re outside of the spider we can expand the set of materials and uses,” says bioengineer Kaplan. His lab at Tufts University is focused on medical applications—like genetically engineered spider fibers that can substitute for bone and ligaments.

Other researchers have their eyes set on industrial uses, like a less bruising material for car airbags. Eventually, the goal is to make superstrength products that substitute spider silk for Kevlar, such as in bulletproof vests. Even superhuman tissues might not be out of the question. In 2013, Dutch researchers grew human skin cells together with spider silk proteins to make what they call “bulletproof skin,” which can stop a bullet traveling at half the normal speed of a typical bullet.

Genetically engineered spider silks capture headlines, but other uses of genetic

engineering are already common in daily life. Much of the corn we eat today is transgenic, as are the soybeans that we feed to farm animals. Transgenic organisms are examples of **genetically modified organisms (GMOs)**—organisms whose genomes have been altered through modern genetic engineering techniques, sometimes to contain new genes. Transgenic crops such as corn and soybeans usually contain genes for natural pesticides, which help the plants fight pests and reduce the amount of pesticide a farmer must use. Other varieties of GM crops contain herbicide-resistance genes, allowing farmers to spray herbicides on fields to kill weeds without at the same time killing the crops (see Chapter 24).

Genetic engineering also has important medical applications. The drug insulin, used for treating diabetes, is commonly produced inside a genetically engineered bacterium—one into which the (human) insulin gene has been inserted. Through **gene therapy**—replacing a defective human gene with a healthy one—scientists hope to one day be able to treat, cure, or even prevent several inherited genetic disorders, including cystic fibrosis, Huntington’s disease, and hemophilia.

Despite the many actual and potential benefits of genetic engineering, the practice inspires debate among scientists, environmentalists, and the general public alike. Some groups object to humans’ meddling with the biology of organisms that have evolved naturally because they are afraid that eating GMOs might have negative effects on health. Others worry about the consequences to our environment if, for example, pesticide genes were to spread in natural populations. And for many, the idea of tampering with human genes to build “better” people raises the specter of eugenics—the early twentieth century practice of trying to weed out the “unfit” from society.

The process of gene expression is like copying a text and then converting it into another language.

### GENETICALLY MODIFIED ORGANISM (GMO)

An organism whose genome has been altered through genetic engineering techniques, often to contain a gene from another species.

### GENE THERAPY

A treatment that aims to cure, treat, or prevent human disease by replacing defective genes with functional ones.



Spiber, Inc.

**Moon Parka™, a prototype by Spiber and The North Face Japan, is the first apparel product to integrate synthetic spider silk with existing industrial manufacturing technology, and is made using Spiber's Qmonos™ fiber.**

Those who may find nothing ethically troubling about using gene therapy to treat human diseases might nonetheless find the prospect of using it to change physical appearance or psychological traits like sexual orientation

much more problematic. Even in the case of treating disease, using gene therapy to modify human embryos or germ cells is more troubling to some than using it to treat adults with a medical condition. That's because an adult person can consent to the procedure, which will affect only his or her cells; changes to an embryo or germ cell will be inherited, affecting future generations who did not consent.

Disquieting or not, genetic engineering appears to be speeding ahead. In 2015, a group of researchers in China reported that they had used a new genetic engineering technique called CRISPR (see Chapter 24) to edit the genome of a developing human embryo. The group used the technique, which can very precisely change DNA sequences, to repair a faulty gene that causes a rare blood disorder. They were roundly criticized by scientists in the West, who have urged caution when applying such gene editing techniques to humans. Although the United States does not officially ban the editing of DNA in human embryos, the National Institutes of Health, which funds most biomedical research in this country, currently prohibits it.

Charged with both hope and fear, debates about the ethical use of genetic engineering are unlikely to go away any time soon. And, given the rapid progress being made in the related field of bioengineering, opportunities to remake our world—perhaps even ourselves—are sure to proliferate. But it will be at least a few years before Spiderman has any real competition. ■

# CHAPTER 8 SUMMARY

- Proteins are folded chains of amino acids that perform many functions in cells, such as transmitting signals, catalyzing chemical reactions, and generating force for movement.
- The order and identity of amino acids in a protein chain determine the shape and function of the protein.
- Genes provide instructions to make proteins. The process of using the information in genes to make proteins is called gene expression.
- Every gene has two parts: a coding sequence and a regulatory sequence. The coding sequence determines the identity of a protein; the regulatory sequence determines where, when, and how much of the protein is produced.
- Gene expression occurs in two stages, transcription and translation, which take place in separate compartments in eukaryotic cells.
- Transcription is the first step of gene expression, copying the information stored in DNA into mRNA. Transcription occurs in the nucleus.
- Translation, the second step of gene expression, uses the information carried in mRNA to assemble a protein. Translation occurs in the cytoplasm.
- Proteins are assembled by ribosomes with the help of tRNA, which delivers amino acids to the ribosome.
- The genetic code is the set of rules by which mRNA sequences are translated into protein sequences; the code is redundant and universal—shared by all living organisms.
- Through genetic engineering, genes from one species of organism can be inserted into the genome of another species of organism to make a transgenic organism.
- Transgenic organisms have numerous uses in biotechnology and health.

## MORE TO EXPLORE

- Hayashi, C. (2010) TED Talk: The magnificence of spider silk: [https://www.ted.com/talks/cheryl\\_hayashi\\_the\\_magnificence\\_of\\_spider\\_silk](https://www.ted.com/talks/cheryl_hayashi_the_magnificence_of_spider_silk)
- Tokareva, O., et al. (2013) Recombinant DNA production of spider silk proteins. *Microbial Biotechnology* 6(6), 651–663.
- Center for Genetics and Society: <http://www.geneticsandsociety.org>
- *Nature* Special Report (2015): CRISPR: The Good, the Bad and the Unknown. <http://www.nature.com/news/crispr-1.17547>
- Kevles, D. J. (1995) *In the Name of Eugenics: Genetics and the Uses of Human Heredity*. Cambridge: Harvard University Press.

# CHAPTER 8 Test Your Knowledge

**DRIVING QUESTION 1** What determines the shape of a protein molecule?

By answering the questions below and studying Infographic 8.1, you should be able to generate an answer for the broader Driving Question above.

## KNOW IT

- 1 A protein is made up of a chain of \_\_\_\_.
- a. nucleotides
  - b. amino acids
  - c. lipids
  - d. fatty acids
  - e. simple sugars

**2 What determines a protein's function?**

- the sequence of amino acids
- the three-dimensional shape of the folded protein
- the location of its gene on the chromosome
- all of the above
- a and b

**USE IT**

**3 Spidroin proteins are in an unfolded state in the spider's silk gland before they are extruded through the spinneret. In their unfolded state, will they have the same properties as spider fibers in a web? Explain your answer.**

**4 If the repeated alanines in spidroin were changed to amino acids with hydrophilic side chains, would they still cluster together away from water? Explain your answer.**

**DRIVING QUESTION 2** What are the steps of gene expression, and where in the cell do they occur?

By answering the questions below and studying Infographics 8.2, 8.3, 8.5, 8.6, 8.7 and 8.8, you should be able to generate an answer for the broader Driving Question above.

**KNOW IT**

**5 "A gene contains many chromosomes. Each chromosome encodes a protein." Is this statement accurate? If not, explain why not, and rewrite the statement to make it correct.**

**6 What is the final product of gene expression?**

- |                    |                  |
|--------------------|------------------|
| a. a DNA molecule  | d. a ribosome    |
| b. an RNA molecule | e. an amino acid |
| c. a protein       |                  |

**7 For each structure or enzyme listed, indicate by N (nucleus) or C (cytoplasm) where it acts in the process of gene expression in a eukaryotic cell.**

- \_\_\_\_\_ RNA polymerase
- \_\_\_\_\_ Ribosome
- \_\_\_\_\_ tRNA
- \_\_\_\_\_ mRNA

**8 What is encoded by a single codon?**

- a single protein
- an RNA nucleotide
- a DNA nucleotide
- an amino acid
- any of the above, depending on the organism

**9 A gene has the sequence ATCGATTG. What is the sequence of the complementary RNA?**

- |             |             |
|-------------|-------------|
| a. ATCGATTG | d. UAGCUAAC |
| b. AGCTAAC  | e. CAAUCGAU |
| c. GTTAGCTA |             |

**USE IT**

**10 If a spider wasn't making the normal amount of its spidroin protein, would you suspect a problem in the regulatory or coding sequence of the spidroin gene? Explain your answer.**

**11 If you wanted to try to increase the amount of spidroin protein a spider produces, would you modify the regulatory sequence or the coding sequence? Explain your answer.**

**12 A change in DNA sequence can affect gene expression and protein function. What would be the impact of each of the following changes? How, specifically, would each change affect protein or mRNA structure, function, and levels?**

- a change that prevents RNA polymerase from binding to a gene's regulatory sequence
- a change in the coding sequence that changes the amino acid sequence of the protein
- a change in the regulatory sequence that allows transcription to occur at much higher levels
- a combination of the changes in b and c

**13 The insulin gene is normally expressed in specific cells in the pancreas, but not in a type of immune cell known as a B cell. On the other hand, B cells express large amounts of antibody proteins. What would you have to do to get a B cell to express insulin? (Hint: Remember that all cells in an organism have the same set of chromosomes and associated genes.)**

**DRIVING QUESTION 3** How can organisms be genetically modified to produce recombinant proteins?

By answering the questions below and studying Infographic 8.4, you should be able to generate an answer for the broader Driving Question above.

**KNOW IT**

**14 Why is recombinant protein production in yeast an efficient strategy?**

- because yeast can easily be grown in large quantities
- because yeast can secrete large amounts of recombinant proteins into their growth medium
- because yeast are multicellular, so have a variety of cell types for recombinant gene expression
- all of the above
- a and b

- 15** What is the purpose of the vector in generating a transgenic organism?
- 16** Describe the recombinant gene that would be needed to create a transgenic spider that produces a yeast protein in its silk glands.

### USE IT

- 17** Why is it important that the transgenic yeast expressing recombinant spidroin proteins secrete the protein into their culture (growth) medium? (Hint: What has to happen to spidroin to convert it into actual silk?)
- 18** Melanin is a pigment expressed in skin cells; melanin gives skin its color. If you wanted to express a different gene in skin cells, which part of the melanin gene would you use? Why? If you wanted to produce melanin in yeast cells, what part of the melanin gene would you use? Why?
- 19** Lysozyme is a protein secreted in tears and saliva in all mammals. Amylase is a protein secreted in mammalian saliva.
- Describe the recombinant gene that you would assemble to express recombinant human lysozyme in the tears of goats.
  - Describe the recombinant gene that you would assemble to express recombinant human amylase in goat saliva.

**DRIVING QUESTION 4** What are some pros and cons of genetically modified organisms?

By answering the questions below and studying Infographic 8.4, you should be able to generate an answer for the broader Driving Question above.

### KNOW IT

- 20** Why is transgenic technology needed to produce large quantities of spider silk?
- 21** Why is spider silk such a valuable product?

### USE IT

- 22** Type 1 diabetes results from a loss of insulin production from the pancreas. People with diabetes take recombinant human insulin expressed in bacteria.
- Describe the gene construct necessary for expression of human insulin in bacteria.
  - Describe the gene construct necessary to produce human insulin in goat's milk.
  - If you were to attempt gene therapy (genetically modifying the human's genome so that insulin would be produced in the human's pancreas), would you need a recombinant form of the insulin gene? Explain your answer.

### INTERPRETING DATA

*apply YOUR KNOWLEDGE*

- 23** A biotechnology company has created a number of strains of transgenic yeast with a recombinant spidroin gene. Each strain is grown in 1 L of culture medium. The cells are separated from the culture medium. All of the spidroin protein present in the culture medium is isolated and quantified. Similarly, all the cells are lysed (broken open) and all the spidroin present within the cells is quantified. The results are shown in the table below.

Strain	Spidroin protein isolated from cells (mg)	Spidroin protein present in culture medium (mg)
Nontransgenic yeast	0	0
Transgenic strain 1	80	0
Transgenic strain 2	40	40
Transgenic strain 3	0	80
Transgenic strain 4	60	20

- How much protein (total) is being produced by each strain in 1 L of culture?
- What are the differences in spidroin production in the different strains?
- Which strain should the company use to commercialize spidroin production? Explain your answer,

*apply YOUR KNOWLEDGE*

### MINI CASE

- 24** A physician is stationed in a military hospital in Iraq. She often has to deal with severe wounds caused by sniper shots. Infection is always a concern, and current bandages are not always flexible enough to permit movement of the affected area as the wound heals. Often, the treated soldiers need to return to duty before their stitches are ready to be removed.
- Given the scenario described, what case could a spider silk biotechnology company representative make to the army to support spider silk research?

*apply YOUR KNOWLEDGE*

### BRING IT HOME

- 25** A number of concerns have been expressed about GMOs. Search the Internet for reliable sources about a particular GMO that you have heard of or in which you are interested (e.g., Golden Rice or genetically modified salmon). List what you consider to be the pros and cons of at least two GMOs. Has what you have read in this chapter about other genetically modified organisms and the transgenic yeast changed your opinion about GMOs? What restrictions (if any) would you place on GMOs?