MOLECULAR PATHOLOGY

** This course meets the 1 hr. Molecular Pathology requirement for Florida license renewal. **

RNA & DNA: A BASIC REVIEW

CREATED BY: US Department of Health and Human Services
National Institute of General Medical Science
National Institutes of Health

COURSE CODE:	MP009
CONTACT HOURS:	2.5
COURSE LEVEL:	Basic
ASCLS P.A.C.E. #:	511-774-17 **P.A.C.E. # Expiration:** June 30, 2019

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COURSE OBJECTIVES

At the end of this course the learner will be able to:

1. Describe the structure of DNA and recall how it replicates itself.
2. Describe the processes of meiosis, transcription, and translation.
3. Describe the structure of ribosomes and recall what they do.
4. Describe what a DNA microarray is and what it can do.
5. List the differences between RNA and DNA.
6. Define the terms microRNA, RNA interference, gene expression, chromatin, and imprinting.
7. Define what a telomere is and what its function is.
8. Describe what mitochondrial DNA is and where it comes from.

RIGHTSHOLDER:

Authors: Scientists from the NIGMS, NIH, and HHS

NIH Review Dates: Apr 2010, Oct 8, 2014, 2017

Original Article: The New Genetics * Note: We are using chapters 1 & 2 from the original booklet for this course *

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Q. How long will it take for my certificate to arrive if I'm sending my answers by mail?
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RNA & DNA: A BASIC REVIEW

Categories: Molecular Pathology | **Contact Hours:** 2 | **Course Code:** MP009

1.) DNA's building blocks, collectively called nucleotides, are:

- **A.** A (adenine), U (uricil), C (cytosine), and G (guanine)
- **B.** A (adenine), T (thymine), C (cytosine), and G (guanine)
- **C.** A (adenine), Tr (tyrosine), C (cytosine), and G (guanine)

2.) Long strings of nucleotides form genes, and groups of genes are packed tightly into structures called _____.

- **A.** a nucleus
- **B.** a base
- **C.** chromosomes

3.) Eggs and sperm are considered _____ cells because of the number of chromosome sets they have.

- **A.** haploid
- **B.** diploid
- **C.** triploid

4.) The first major step in making a protein is _____.

- **A.** splicing
- **B.** meiosis
- **C.** transcription

5.) According to Figure 11, the DNA segments that do contain protein-making instructions are known as introns (pictured as blue areas on the graphic).

- **A.** True
- **B.** False

6.) All states test newborns for PKU, the genetic disease known as phenylketonuria.

- **A.** True
- **B.** False

7.) Ribosomes are among the largest and most intricate structures in the cell, with human ribosomes having between _____ different proteins.

A. 15 and 20
B. 40 and 50
C. 70 and 80

8.) Science now has the ability to attach a piece of every gene in a genome to a postage stamp-sized glass slide. This ordered series of DNA spots is called a _____.

A. computerized DNA series
B. DNA microarray
C. serial gene display

9.) The chemical units of RNA are like those of DNA, except that RNA has the nucleotide uracil (U) instead of _____.

A. thymine (T)
B. adenine (A)
C. cytosine (C)

10.) Chromatin consists of long strings of DNA spooled around a compact assembly of proteins called _____.

A. protamines
B. cadherin
C. histones

11.) In human and mouse telomeres the nucleotide repeated sequence is _____.

A. TTGGGG
B. TTAGGG
C. TTAAAG

12.) Unlike chromosomal DNA, which is inherited from both parents, humans get all their mitochondrial DNA from their fathers.

A. True
B. False

******END OF QUIZ******

How Genes Work

People have known for many years that living things inherit traits from their parents. That common-sense observation led to agriculture, the purposeful breeding and cultivation of animals and plants for desirable characteristics. Firming up the details took quite some time, though. Researchers did not understand exactly how traits were passed to the next generation until the middle of the 20th century.

Now it is clear that **genes** are what carry our traits through generations and that genes are made of **deoxyribonucleic acid (DNA)**. But genes themselves don't do the actual work. Rather, they serve as instruction books for making functional molecules such as **ribonucleic acid (RNA)** and **proteins**, which perform the chemical reactions in our bodies.

Proteins do many other things, too. They provide the body's main building materials, forming the cell's architecture and structural components. But one thing proteins can't do is make copies of themselves. When a cell needs more proteins, it uses the manufacturing instructions coded in DNA.

The DNA code of a gene—the sequence of its individual DNA building blocks, labeled A (adenine), T (thymine), C (cytosine) and G (guanine) and collectively called **nucleotides**—spells out the exact order of a protein's building blocks, **amino acids**.

Occasionally, there is a kind of typographical error in a gene's DNA sequence. This mistake—which can be a change, gap or duplication—is called a **mutation**.

Genetics in the Garden

The monk Gregor Mendel first described how traits are inherited from one generation to the next.

In 1900, three European scientists independently discovered an obscure research paper that had been published nearly 35 years before. Written by Gregor Mendel, an Austrian monk who was also a scientist, the report described a series of breeding experiments performed with pea plants growing in his abbey garden.

Mendel had studied how pea plants inherited the two variant forms of easy-to-see traits. These included flower color (white or purple) and the texture of the peas (smooth or wrinkled). Mendel counted many generations of pea plant offspring and learned that these characteristics were passed on to the next generation in orderly, predictable ratios.

When he cross-bred purple-flowered pea plants with white-flowered ones, the next generation had only purple flowers. But directions for making white flowers were hidden somewhere in the peas of that generation, because when those purple-flowered

>>>Continued on botton of next page <<<

A mutation can cause a gene to encode a protein that works incorrectly or that doesn't work at all. Sometimes, the error means that no protein is made.

But not all DNA changes are harmful. Some mutations have no effect, and others produce new versions of proteins that may give a survival advantage to the organisms that have them. Over time, mutations supply the raw material from which new life forms evolve.

Beautiful DNA

Up until the 1950s, scientists knew a good deal about heredity, but they didn't have a clue what DNA looked like. In order to learn more about DNA and its structure, some scientists experimented with using X rays as a form of molecular photography.

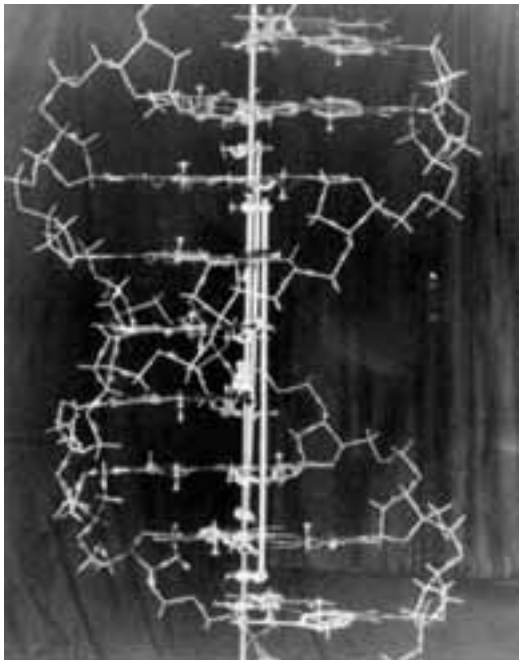
Rosalind Franklin, a physical chemist working with Maurice Wilkins at King's College in London, was among the first to use this method to analyze genetic material. Her experiments

plants were bred to each other, some of their offspring had white flowers. What's more, the second-generation plants displayed the colors in a predictable pattern. On average, 75 percent of the second-generation plants had purple flowers and 25 percent of the plants had white flowers. Those same ratios persisted, and were reproduced when the experiment was repeated many times over.

Trying to solve the mystery of the missing color blooms, Mendel imagined that the reproductive cells of his pea plants might contain discrete "factors," each of which specified a particular trait, such as white flowers. Mendel reasoned that the factors, whatever they were, must be physical material because they passed from parent to offspring in a mathematically orderly way. It wasn't until many years later, when the other scientists unearthed Mendel's report, that the factors were named genes.

Early geneticists quickly discovered that Mendel's mathematical rules of inheritance applied not just to peas, but also to all plants, animals and people. The discovery of a quantitative rule for inheritance was momentous. It revealed that a common, general principle governed the growth and development of all life on Earth.

FIGURE 2:



COLD SPRING HARBOR LABORATORY ARCHIVES

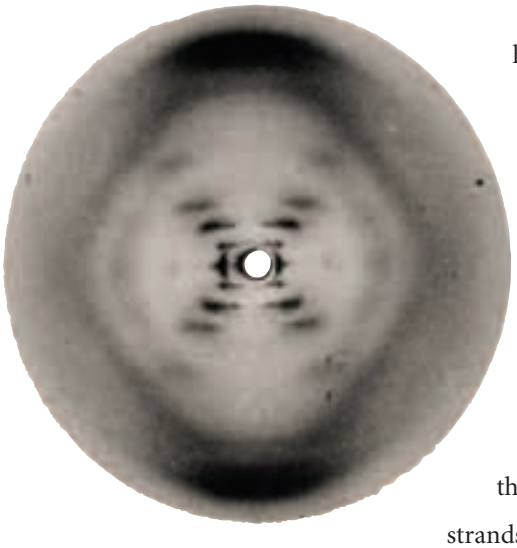
▲ In 1953, Watson and Crick created their historic model of the shape of DNA: the double helix.

produced what were referred to at the time as "the most beautiful X-ray photographs of any substance ever taken."

Other scientists, including zoologist James Watson and physicist Francis Crick, both working at Cambridge University in the United Kingdom, were trying to determine the shape of DNA too. Ultimately, this line of research revealed one of the most profound scientific discoveries of the 20th century: that DNA exists as a double helix.

The 1962 Nobel Prize in physiology or medicine was awarded to Watson, Crick and Wilkins for this work. Although Franklin did not earn a share of the prize due to her untimely death at age 38, she is widely recognized as having played a significant role in the discovery.

FIGURE 1:



▲ Rosalind Franklin's original X-ray diffraction photo revealed the physical structure of DNA.

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The spiral staircase-shaped double helix has attained global status as the symbol for DNA. But what is so beautiful about the discovery of the twisting ladder structure isn't just its good looks. Rather, the structure of DNA taught researchers a fundamental lesson about **genetics**. It taught them that the two connected strands—winding together like parallel handrails—were complementary to each other, and this unlocked the secret of how genetic information is stored, transferred and copied.

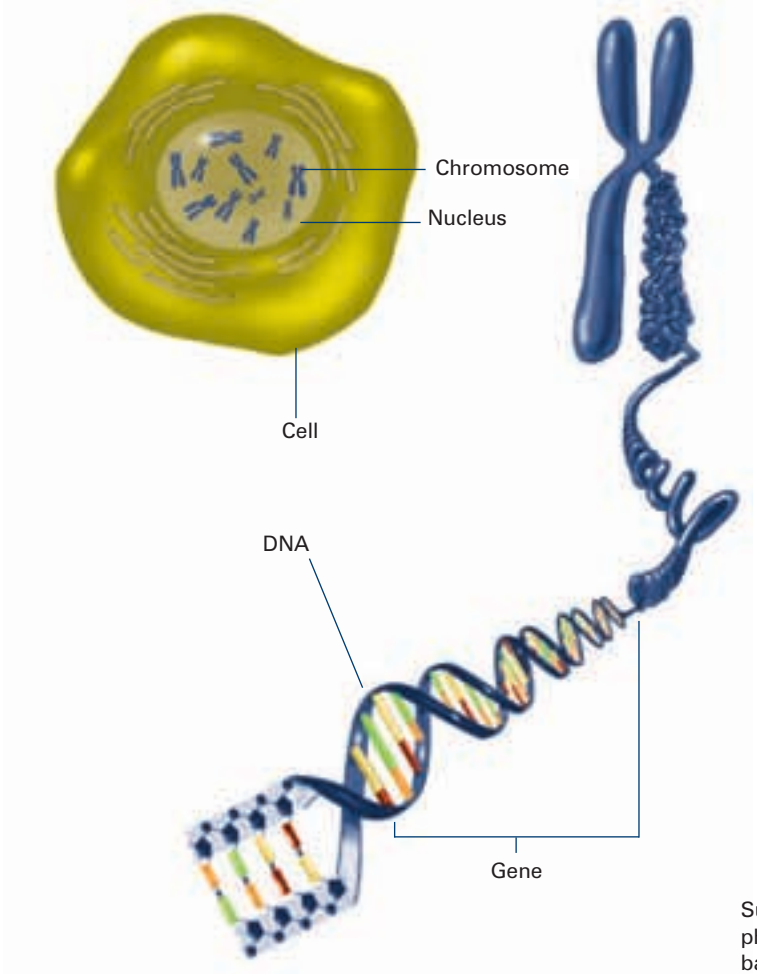
In genetics, complementary means that if you know the sequence of nucleotide building blocks on one strand, you know the sequence of nucleotide building blocks on the other strand: A always matches up with T and C always links to G (see next page).

Long strings of nucleotides form genes, and groups of genes are packaged tightly into structures called **chromosomes**. Every cell in your body except for eggs, sperm and red blood cells contains a full set of chromosomes in its **nucleus**.

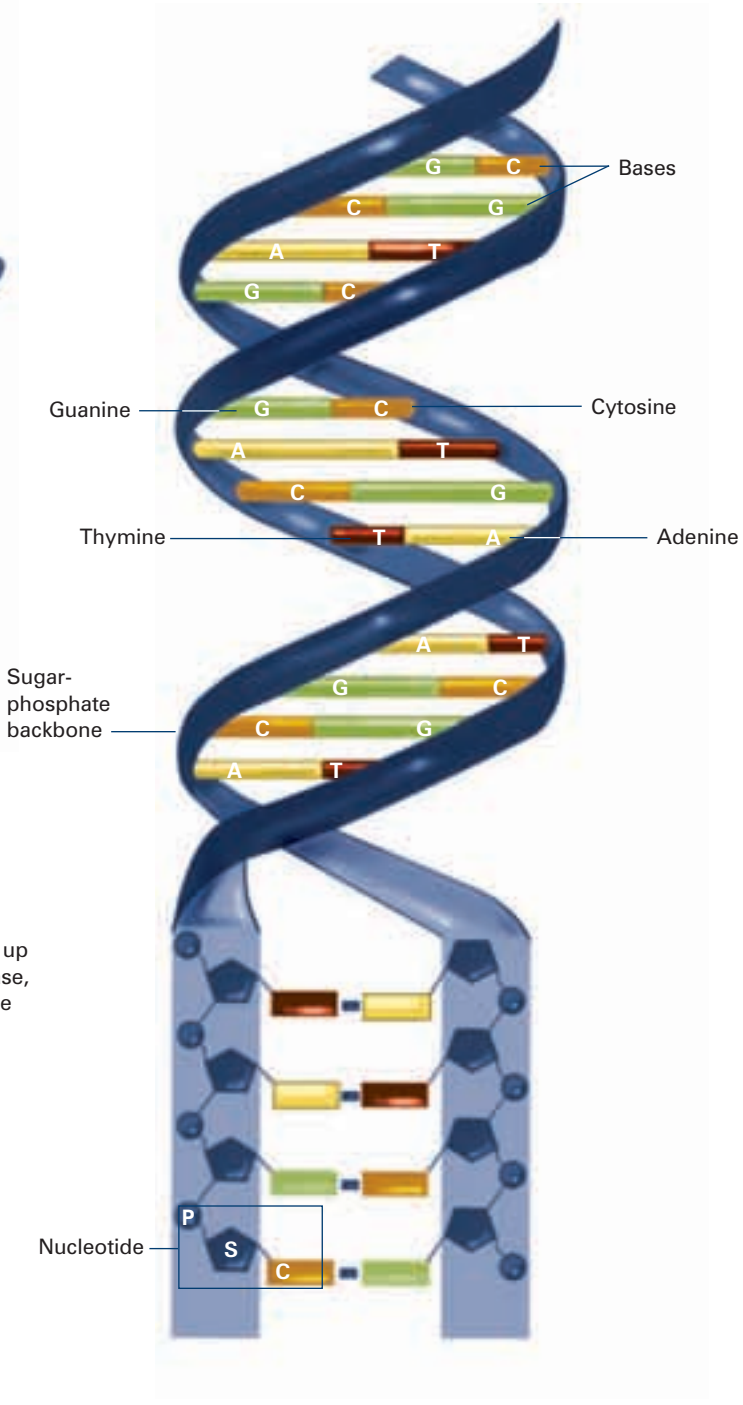
If the chromosomes in one of your cells were uncoiled and placed end to end, the DNA would be about 6 feet long. If all the DNA in your body were connected in this way, it would stretch approximately 67 billion miles! That's nearly 150,000 round trips to the Moon.

FIGURE 3:

DNA Structure



The long, stringy DNA that makes up genes is spooled within chromosomes inside the nucleus of a cell. (Note that a gene would actually be a much longer stretch of DNA than what is shown here.)



DNA consists of two long, twisted chains made up of nucleotides. Each nucleotide contains one base, one phosphate molecule and the sugar molecule deoxyribose. The bases in DNA nucleotides are adenine, thymine, cytosine and guanine.

FIGURE 5:

▲ Humans have 23 pairs of chromosomes. Male DNA (pictured here) contains an X and a Y chromosome, whereas female DNA contains two X chromosomes.

CYTOGENETICS LABORATORY, BRIGHAM AND WOMEN'S HOSPITAL

Copycat

It's astounding to think that your body consists of trillions of cells. But what's most amazing is that it all starts with one cell. How does this massive expansion take place?

As an embryo progresses through development, its cells must reproduce. But before a cell divides into two new, nearly identical cells, it must copy its DNA so there will be a complete set of genes to pass on to each of the new cells.

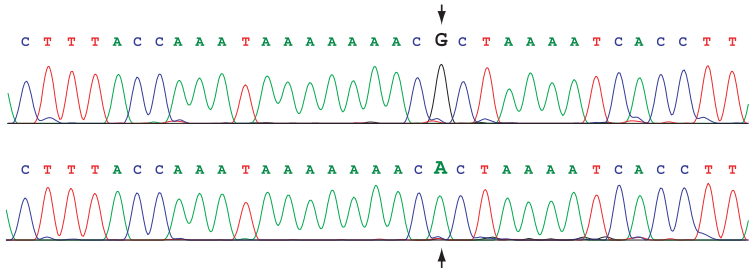
To make a copy of itself, the twisted, compacted double helix of DNA has to unwind and separate its two strands. Each strand becomes a pattern, or template, for making a new strand, so the two new DNA molecules have one new strand and one old strand.

The copy is courtesy of a cellular protein machine called **DNA polymerase**, which reads the template DNA strand and stitches together the complementary new strand. The process, called **replication**, is astonishingly fast and accurate, although occasional mistakes, such as deletions or duplications, occur. Fortunately, a cellular spell-checker catches and corrects nearly all of these errors.

Mistakes that are not corrected can lead to diseases such as cancer and certain genetic disorders. Some of these include Fanconi anemia, early aging diseases and other conditions in which people are extremely sensitive to sunlight and some chemicals.

DNA copying is not the only time when DNA damage can happen. Prolonged, unprotected sun exposure can cause DNA changes that lead to skin cancer, and toxins in cigarette smoke can cause lung cancer.

FIGURE 4:



▲ When DNA polymerase makes an error while copying a gene's DNA sequence, the mistake is called a mutation. In this example, the nucleotide G has been changed to an A.

It may seem ironic, then, that many drugs used to treat cancer work by attacking DNA. That's because these chemotherapy drugs disrupt the DNA copying process, which goes on much faster in rapidly dividing cancer cells than in other cells of the body. The trouble is that most of these drugs do affect normal cells that grow and divide frequently, such as cells of the immune system and hair cells.

Understanding DNA replication better could be a key to limiting a drug's action to cancer cells only.

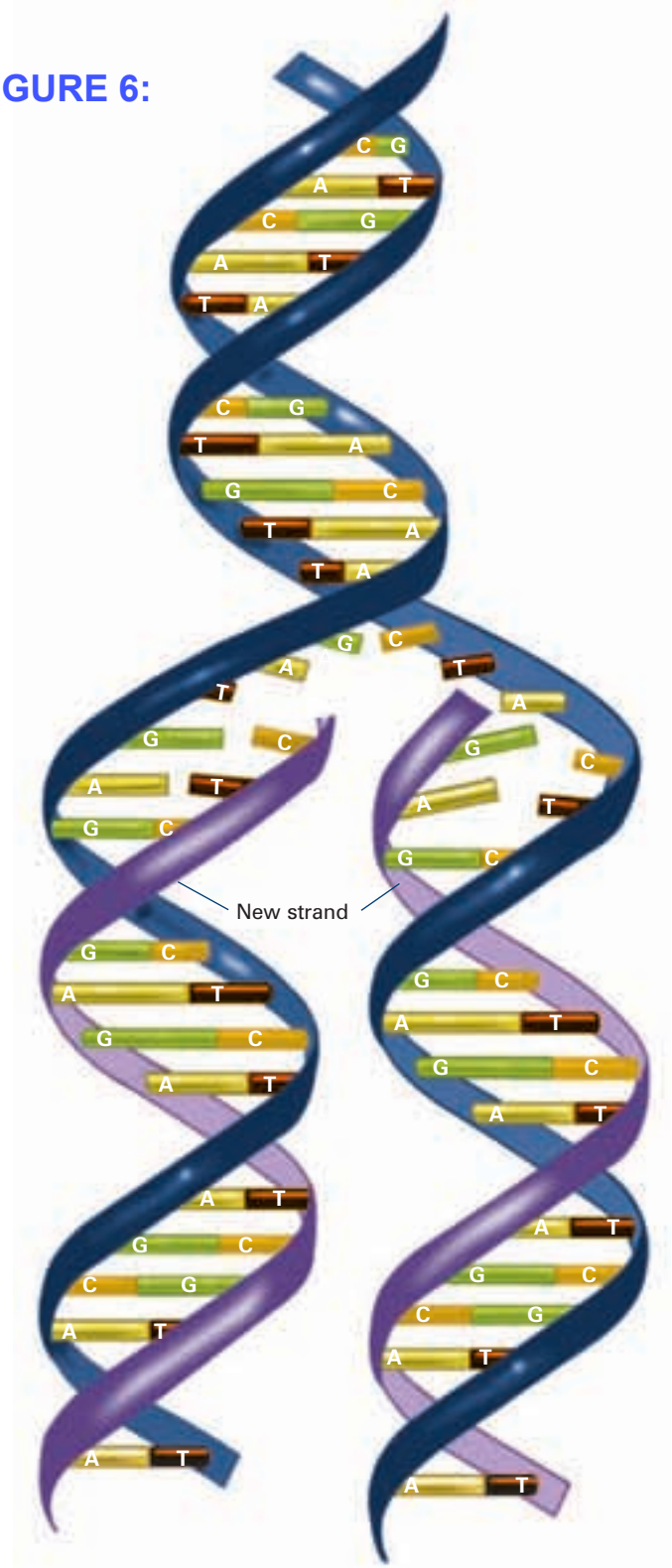
Let's Call It Even

After copying its DNA, a cell's next challenge is getting just the right amount of genetic material into each of its two offspring.

Most of your cells are called **diploid** ("di" means two, and "ploid" refers to sets of chromosomes) because they have two sets of chromosomes (23 pairs). Eggs and sperm are different; these are known as **haploid** cells. Each haploid cell has only one set of 23 chromosomes so that at fertilization the math will work out: A haploid egg cell will combine with a haploid sperm cell to form a diploid cell with the right number of chromosomes: 46.

Chromosomes are numbered 1 to 22, according to size, with 1 being the largest chromosome. The 23rd pair, known as the sex chromosomes, are called X and Y. In humans, abnormalities of chromosome number usually occur during **meiosis**, the time when a cell

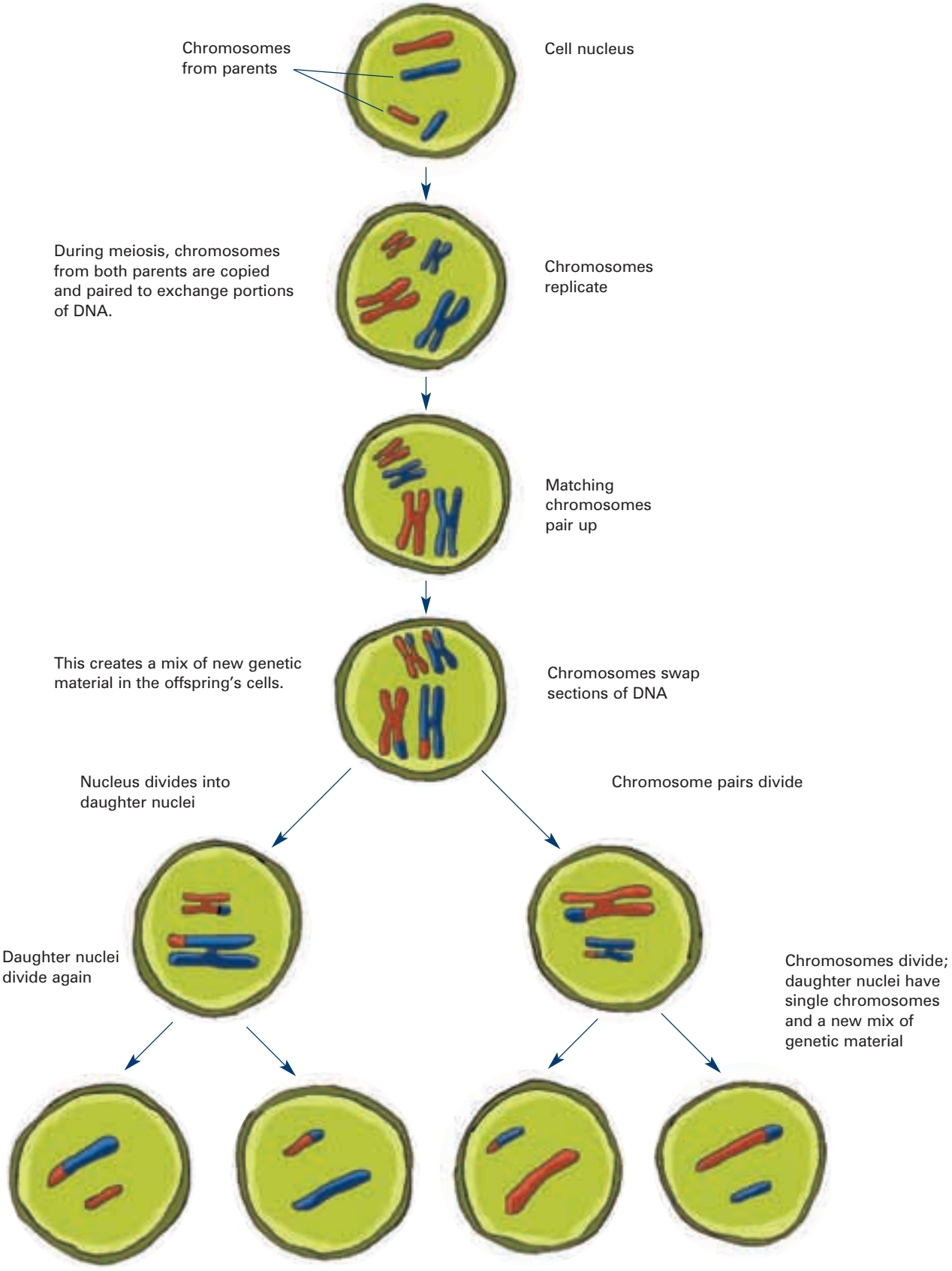
FIGURE 6:



▲ During DNA replication, each strand of the original molecule acts as a template for the synthesis of a new, complementary DNA strand.

FIGURE 7:

Meiosis



reduces its chromosomes from diploid to haploid in creating eggs or sperm.

What happens if an egg or a sperm cell gets the wrong number of chromosomes, and how often does this happen?

Molecular biologist Angelika Amon of the Massachusetts Institute of Technology in Cambridge says that mistakes in dividing DNA between daughter cells during meiosis are the leading cause of human birth defects and miscarriages. Current estimates are that 10 percent of all embryos have an incorrect chromosome number. Most of these don't go to full term and are miscarried.

In women, the likelihood that chromosomes won't be apportioned properly increases with age. One of every 18 babies born to women over 45 has three copies of chromosome 13, 18 or 21 instead of the normal two, and this improper balancing can cause trouble. For example, three copies of chromosome 21 lead to Down syndrome.

To make her work easier, Amon—like many other basic scientists—studies yeast cells, which separate their chromosomes almost exactly the same way human cells do, except that yeast do it much faster. A yeast cell copies its DNA and produces daughter cells in about $1\frac{1}{2}$ hours, compared to a whole day for human cells.

The yeast cells she uses are the same kind bakeries use to make bread and breweries use to make beer!

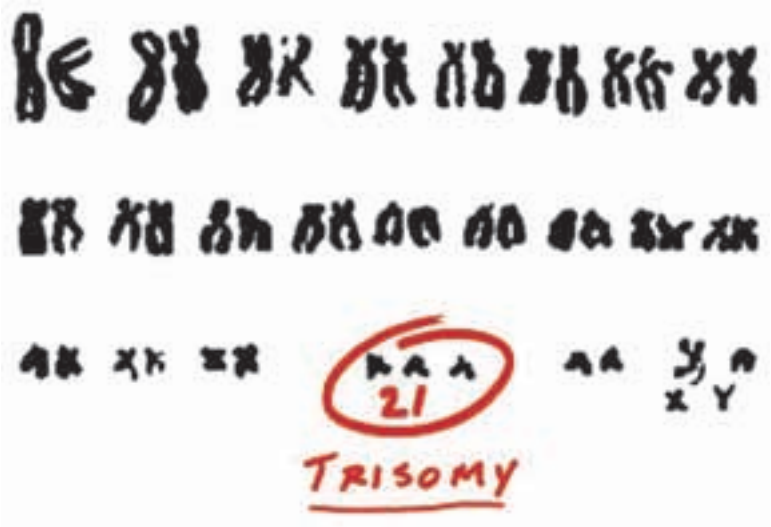
Amon has made major progress in understanding the details of meiosis. Her research shows how, in healthy cells, gluelike protein complexes called cohesins release pairs of chromosomes at exactly the right time. This allows the chromosomes to separate properly.

These findings have important implications for understanding and treating infertility, birth defects and cancer.

Getting the Message

So, we've described DNA—its basic properties and how our bodies make more of it. But how does DNA serve as the language of life? How do you get a protein from a gene?

FIGURE 8:



▲ Trisomy, the hallmark of Down syndrome, results when a baby is born with three copies of chromosome 21 instead of the usual two.

There are two major steps in making a protein. The first is **transcription**, where the information coded in DNA is copied into RNA. The RNA nucleotides are complementary to those on the DNA: a C on the RNA strand matches a G on the DNA strand.

The only difference is that RNA pairs a nucleotide called uracil (U), instead of a T, with an A on the DNA.

A protein machine called **RNA polymerase** reads the DNA and makes the RNA copy. This copy is called messenger RNA, or mRNA, because it delivers the gene's message to the protein-producing machinery.

At this point you may be wondering why all of the cells in the human body aren't exactly alike, since they all contain the same DNA. What makes a liver cell different from a brain cell? How do the cells in the heart make the organ contract, but those in skin allow us to sweat?

Cells can look and act differently, and do entirely different jobs, because each cell "turns on," or expresses, only the genes appropriate for what it needs to do.

That's because RNA polymerase does not work alone, but rather functions with the aid of many helper proteins. While the core part of RNA polymerase is the same in all cells, the helpers vary in different cell types throughout the body.

You'd think that for a process so essential to life, researchers would know a lot about how transcription works. While it's true that the basics are clear—biologists have been studying gene transcribing by RNA polymerases since these proteins were first discovered in 1960—some of the details are actually still murky.

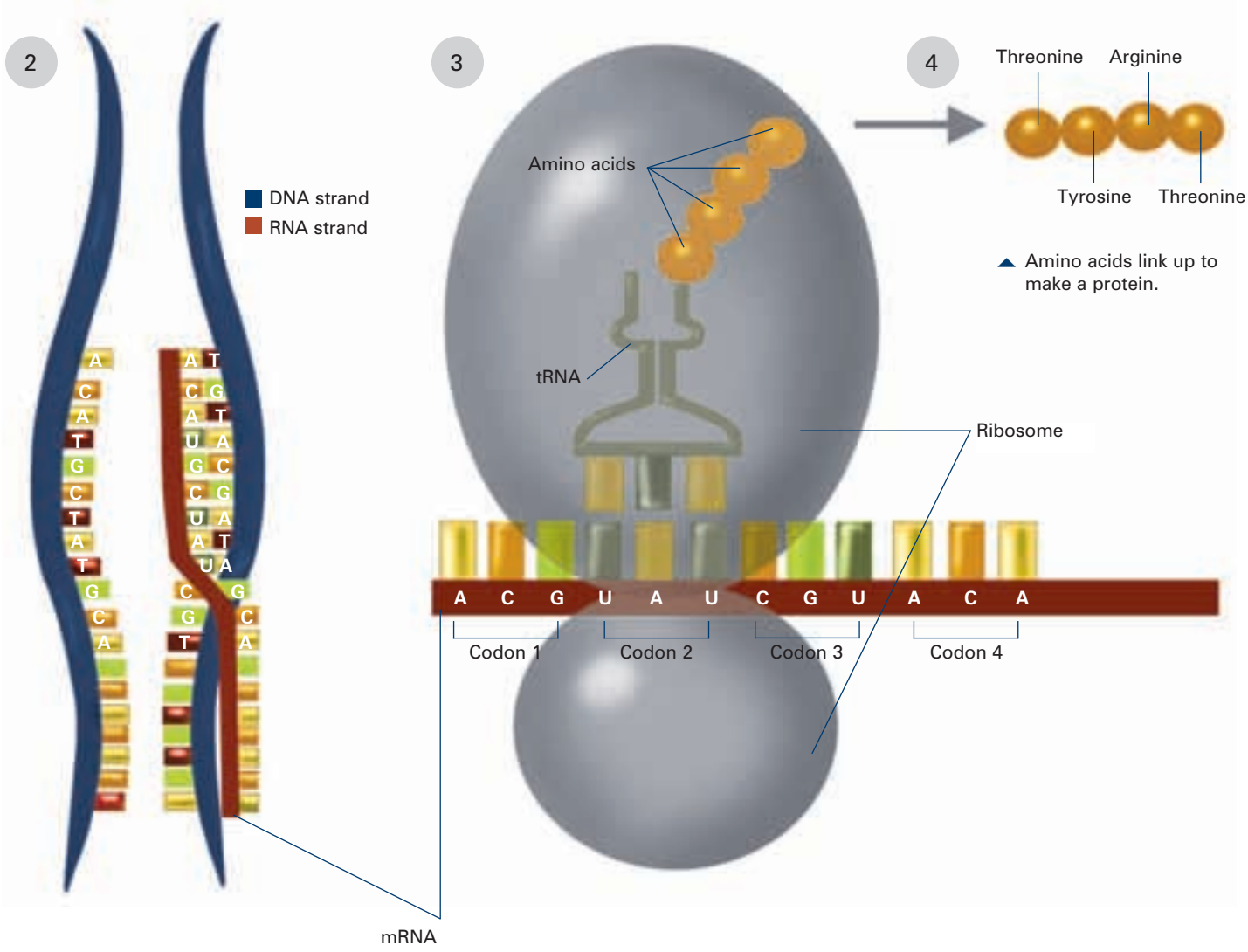
FIGURE 9:

▲ RNA polymerase transcribes DNA to make messenger RNA (mRNA).

The biggest obstacle to learning more has been a lack of tools. Until fairly recently, researchers were unable to get a picture at the atomic level of the giant RNA polymerase protein assemblies inside cells to understand how the many pieces of this amazing, living machine do what they do, and do it so well.

But our understanding is improving fast, thanks to spectacular technological advances. We have new X-ray pictures that are far more sophisticated than those that revealed the structure of DNA. Roger Kornberg of Stanford University in California used such methods to determine the structure of RNA polymerase. This work earned

FIGURE 9 (continued)

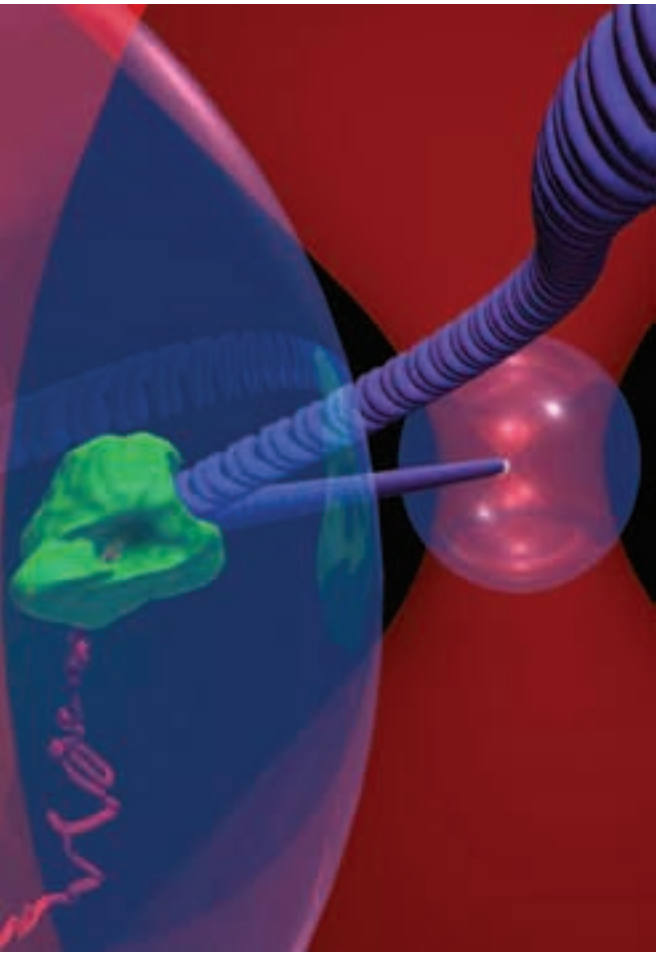


▲ The mRNA sequence (dark red strand) is complementary to the DNA sequence (blue strand).

▲ On ribosomes, transfer RNA (tRNA) helps convert mRNA into protein.

▲ Amino acids link up to make a protein.

FIGURE 10:



▲ RNA polymerase (green) and one end of a DNA strand (blue) are attached to clear beads pinned down in two optical traps. As RNA polymerase moves along the DNA, it creates an RNA copy of a gene, shown here as a pink strand.

STEVEN BLOCK

him the 2006 Nobel Prize in chemistry. In addition, very powerful microscopes and other tools that allow us to watch one molecule at a time provide a new look at RNA polymerase while it's at work reading DNA and producing RNA.

For example, Steven Block, also of Stanford, has used a physics technique called optical trapping to track RNA polymerase as it inches along DNA. Block and his team performed this work by designing a specialized microscope sensitive enough to watch the real-time motion of a single polymerase traveling down a gene on one chromosome.

The researchers discovered that molecules of RNA polymerase behave like battery-powered spiders as they crawl along the DNA ladder, adding nucleotides one at a time to the growing RNA strand. The **enzyme** works much like a motor, Block believes, powered by energy released during the chemical synthesis of RNA.

Nature's Cut-and-Paste Job

Several types of RNA play key roles in making a protein. The gene transcript (the mRNA) transfers information from DNA in the nucleus to the **ribosomes** that make protein. Ribosomal RNA forms about 60 percent of the ribosomes. Lastly, transfer RNA carries amino acids to the ribosomes. As you can see, all three types of cellular RNAs come together to produce new proteins.

But the journey from gene to protein isn't quite as simple as we've just made it out to be. After transcription, several things need to happen to mRNA before a protein can be made. For example, the genetic material of humans and other **eukaryotes** (organisms that have a nucleus) includes a lot of DNA that doesn't encode proteins. Some of this DNA is stuck right in the middle of genes.

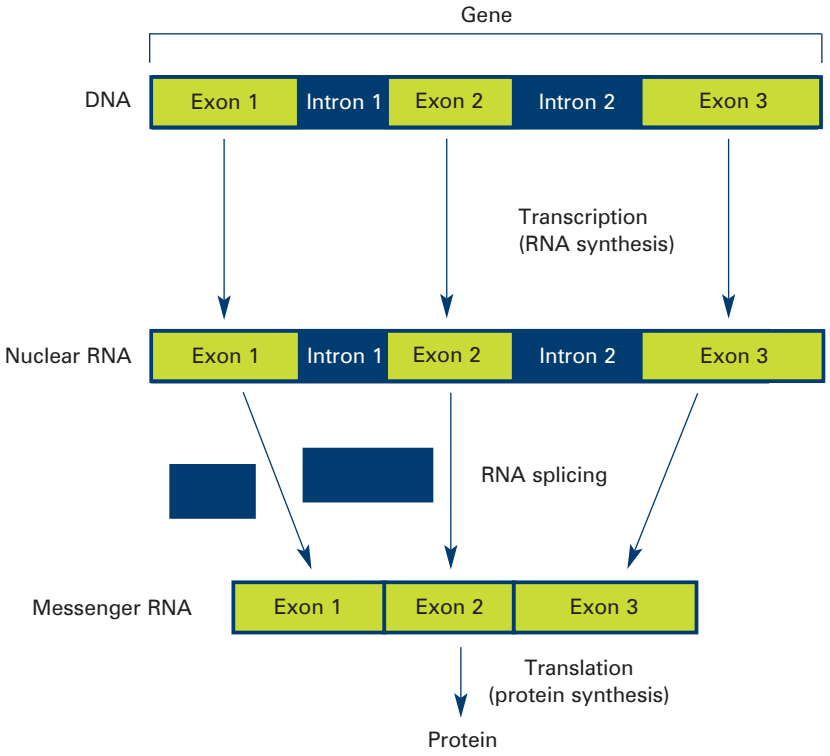
To distinguish the two types of DNA, scientists call the coding sequences of genes **exons** and the pieces in between **introns** (for intervening sequences).

If RNA polymerase were to transcribe DNA from the start of an intron-containing gene to the end, the RNA would be complementary to the introns as well as the exons.

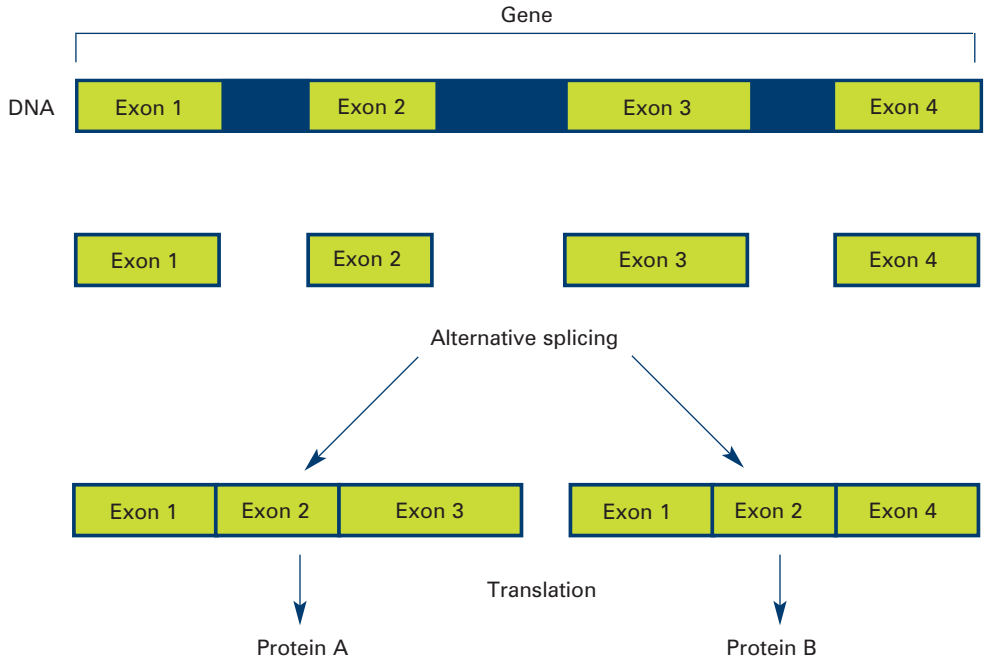
To get an mRNA molecule that yields a working protein, the cell needs to trim out the intron sections and then stitch only the exon pieces together (see drawing, next page). This process is called **RNA splicing.**

FIGURE 11:

RNA Splicing



Genes are often interrupted by stretches of DNA (introns, blue) that do not contain instructions for making a protein. The DNA segments that do contain protein-making instructions are known as exons (green).



Arranging exons in different patterns, called alternative splicing, enables cells to make different proteins from a single gene.

Splicing has to be extremely accurate. An error in the splicing process, even one that results in the deletion of just one nucleotide in an exon or the addition of just one nucleotide in an intron, will throw the whole sequence out of alignment. The result is usually an abnormal protein—or no protein at all. One form of Alzheimer's disease, for example, is caused by this kind of splicing error.

Molecular biologist Christine Guthrie of the University of California, San Francisco, wants to understand more fully the mechanism for removing intron RNA and find out how it stays so accurate.

She uses yeast cells for these experiments. Just like human DNA, yeast DNA has introns, but they are fewer and simpler in structure and are therefore easier to study. Guthrie can identify which genes are required for splicing by finding abnormal yeast cells that mangle splicing.

So why do introns exist, if they're just going to be chopped out? Without introns, cells wouldn't need to go through the splicing process and keep monitoring it to be sure it's working right.

As it turns out, splicing also makes it possible for cells to create more proteins.

Think about all the exons in a gene. If a cell stitches together exons 1, 2 and 4, leaving out exon 3, the mRNA will specify the production of a particular protein. But instead, if the cell stitches together exons 1, 2 and 3, this time leaving out exon 4, then the mRNA will be translated into a different protein (see drawing, page 15).

By cutting and pasting the exons in different patterns, which scientists call alternative splicing, a cell can create different proteins from a single gene. Alternative splicing is one of the reasons why human cells, which have about 20,000 genes, can make hundreds of thousands of different proteins.

All Together Now

Until recently, researchers looked at genes, and the proteins they encode, one at a time. Now, they can look at how large numbers of genes and proteins act, as well as how they interact. This gives them a much better picture of what goes on in a living organism.

Already, scientists can identify all of the genes that are transcribed in a cell—or in an organ, like the heart. And although researchers can't tell you, right now, what's going on in every cell of your body while you read a book or walk down the street, they can do this sort of "whole-body" scan for simpler, single-celled organisms like yeast.

Using a technique called genome-wide location analysis, Richard Young of the Massachusetts Institute of Technology unraveled a "regulatory code" of living yeast cells, which have more than 6,000 genes in their genome. Young's technique enabled him to determine the exact places where RNA polymerase's helper proteins sit on DNA and tell RNA polymerase to begin transcribing a gene.

Since he did the experiment with the yeast exposed to a variety of different conditions,

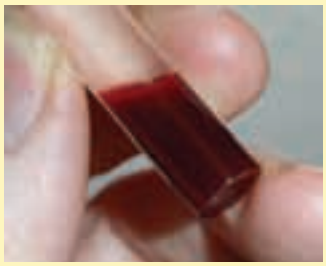
GENETICS AND YOU: *Nursery Genetics*

While most genetic research uses lab organisms, test tubes and petri dishes, the results have real consequences for people. Your first encounter with genetic analysis probably happened shortly after you were born, when a doctor or nurse took a drop of blood from the heel of your tiny foot.

Lab tests performed with that single drop of blood can diagnose certain rare genetic disorders as well as metabolic problems like phenylketonuria (PKU).

Screening newborns in this way began in the 1960s in Massachusetts with testing for PKU, a disease affecting 1 in 14,000 people. PKU is caused by an enzyme that doesn't work properly due to a genetic mutation. Those born with this disorder cannot metabolize the amino acid phenylalanine, which is present in many foods. Left untreated, PKU can lead to mental retardation and neurological damage, but a special diet can prevent these outcomes. Testing for this condition has made a huge difference in many lives.

Newborn screening is governed by individual states. This means that the state in which a baby is born determines the genetic conditions for which he or she will be screened. Currently, states test for between 28 and 54 conditions. All states test for PKU.



Although expanded screening for genetic diseases in newborns is advocated by some, others question the value of screening for conditions that are currently untreatable. Another issue is that some children with mild versions of certain genetic diseases may be treated needlessly.

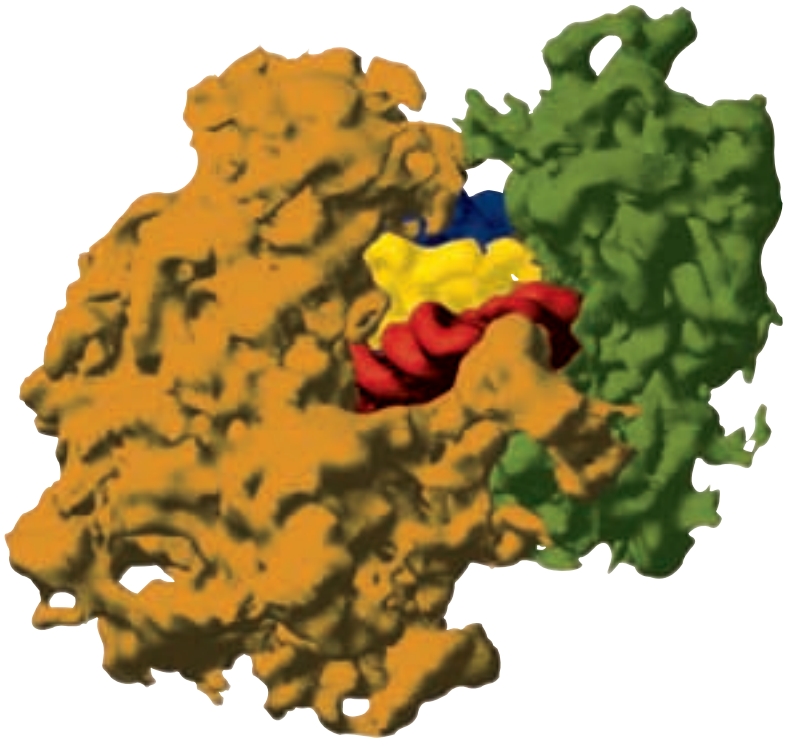
In 2006, the Advisory Committee on Heritable Disorders in Newborns and Children, which assists the Secretary of the U.S. Department of Health and Human Services, recommended a standard, national set of newborn tests for 29 conditions, ranging from relatively common hearing problems to very rare metabolic diseases.

Young was able to figure out how transcription patterns differ when the yeast cell is under stress (say, in a dry environment) or thriving in a sugary-rich nutrient solution. Done one gene at a time, using methods considered state-of-the-art just a few years ago, this kind of analysis would have taken hundreds of years.

After demonstrating that his technique worked in yeast, Young then took his research a step forward. He used a variation of the yeast method to scan the entire human genome in small samples of cells taken from the pancreases and livers of people with type 2 diabetes. He used the results to identify genes that aren't transcribed correctly in people with the disease.

This information provides researchers with an important tool for understanding how diabetes and other diseases are influenced by defective genes. By building models to predict how genes respond in diverse situations, researchers may be able to learn how to stop or jump-start genes on demand, change the course of a disease or prevent it from ever happening.

FIGURE 12:



▲ A ribosome consists of large and small protein subunits with transfer RNAs nestled in the middle.

RIBOSOME STRUCTURE COURTESY OF JAMIE CATE, MARAT YUSUPOV, GULNARA YUSUPOVA, THOMAS EARNEST AND HARRY NOLLER. GRAPHIC COURTESY OF ALBION BAUCOM, UNIVERSITY OF CALIFORNIA, SANTA CRUZ.

Found in Translation

After a gene has been read by RNA polymerase and the RNA is spliced, what happens next in the journey from gene to protein? The next step is reading the RNA information and fitting the building blocks of a protein together. This is called **translation**, and its principal actors are the ribosome and amino acids.

Ribosomes are among the biggest and most intricate structures in the cell. The ribosomes of bacteria contain not only huge amounts of RNA, but also more than 50 different proteins. Human ribosomes have even more RNA and between 70 and 80 different proteins!

Harry Noller of the University of California, Santa Cruz, has found that a ribosome performs several key jobs when it translates the genetic code of mRNA. As the messenger RNA threads through the ribosome protein machine, the

ribosome reads the mRNA sequence and helps recognize and recruit the correct amino acid-carrying transfer RNA to match the mRNA code. The ribosome also links each additional amino acid into a growing protein chain (see Figure 9).

For many years, researchers believed that even though RNAs formed a part of the ribosome, the protein portion of the ribosome did all of the work. Noller thought, instead, that maybe RNA, not proteins, performed the ribosome's job. His idea was not popular at first, because at that time it was thought that RNA could not perform such complex functions.

Some time later, however, the consensus changed. Sidney Altman of Yale University in New Haven, Connecticut, and Thomas Cech, who was then at the University of Colorado in Boulder, each discovered that RNA can perform work as complex as that done by protein enzymes. Their "RNA-as-an-enzyme" discovery turned the research world on its head and earned Cech and Altman the 1989 Nobel Prize in chemistry.

Noller and other researchers have continued the painstaking work of understanding ribosomes. In 1999, he showed how different parts of a bacterial ribosome interact with one another and how the ribosome interacts with molecules involved in protein synthesis. These studies provided near proof that the fundamental mechanism of translation is performed by RNA, not by the proteins of the ribosome.

FIGURE 13:

▲ Some first-aid ointments contain the antibiotic neomycin, which treats infections by attacking ribosomes in bacteria.

RNA Surprises

But which ribosomal RNAs are doing the work? Most scientists assumed that RNA nucleotides buried deep within the ribosome complex—the ones that have the same sequence in every species from bacteria to people—were the important ones for piecing the growing protein together.

However, recent research by Rachel Green, who worked with Noller before moving to Johns Hopkins University in Baltimore, Maryland, showed that this is not the case. Green discovered that those RNA nucleotides are not needed for assembling a protein. Instead, she found, the nucleotides do something else entirely: They help the growing protein slip off the ribosome once it's finished.

Noller, Green and hundreds of other scientists work with the ribosomes of bacteria. Why should you care about how bacteria create proteins from their genes?

One reason is that this knowledge is important for learning how to disrupt the actions of disease-causing microorganisms. For example, antibiotics like erythromycin and neomycin work by attacking the ribosomes of bacteria, which are different enough from human ribosomes that our cells are not affected by these drugs.

As researchers gain new information about bacterial translation, the knowledge may lead to more antibiotics for people.

New antibiotics are urgently needed because many bacteria have developed resistance to the current arsenal. This resistance is sometimes the result of changes in the bacteria's ribosomal RNA. It can be difficult to find those small, but critical, changes that may lead to resistance, so it is important to find completely new ways to block bacterial translation.

Green is working on that problem too. Her strategy is to make random mutations to the genes in a bacterium that affect its ribosomes. But what if the mutation disables the ribosome so much that it can't make proteins? Then the bacterium won't grow, and Green wouldn't find it.

Using clever molecular tricks, Green figured out a way to rescue some of the bacteria with defective ribosomes so they could grow. While some of the rescued bacteria have changes in their ribosomal RNA that make them resistant to certain antibiotics (and thus would not make good antibiotic targets) other RNA changes that don't affect resistance may point to promising ideas for new antibiotics.

An Interesting Development

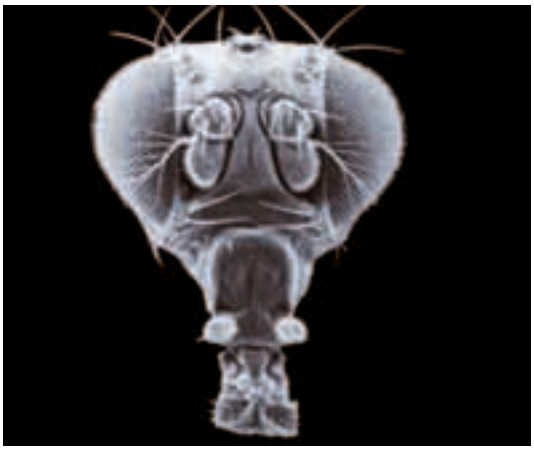
In the human body, one of the most important jobs for proteins is to control how embryos develop. Scientists discovered a hugely important set of proteins involved in development by studying mutations that cause bizarre malformations in fruit flies.

The most famous such abnormality is a fruit fly with a leg, rather than the usual antenna, growing out of its head (see page 21). According to Thomas C. Kaufman of Indiana University in Bloomington, the leg is perfectly normal—it's just growing in the wrong place.

In this type of mutation and many others, something goes wrong with the genetic program that directs some of the cells in an embryo to follow developmental pathways, which are a series of chemical reactions that occur in a specific order. In the antenna-into-leg problem, it is as if the cells growing from the fly's head, which normally would become an antenna, mistakenly believe that they are in the fly's thorax, and therefore ought to grow into a leg. And so they do.

Thinking about this odd situation taught scientists an important lesson—that the proteins made by some genes can act as switches. Switch genes are master controllers that provide each body part with a kind of identification card. If a protein that normally instructs cells to become an antenna is disrupted, cells can receive new instructions to become a leg instead.

FIGURE 14:



▲ Normal fruit fly head.

FLYBASE; R. TURNER

▲ Fruit fly head showing the effects of the *Antennapedia* gene. This fly has legs where its antennae should be.

Scientists determined that several different genes, each with a common sequence, provide these anatomical identification card instructions. Kaufman isolated and described one of these genes, which became known as *Antennapedia*, a word that means "antenna feet."

Kaufman then began looking a lot more closely at the molecular structure of the *Antennapedia* gene. In the early 1980s, he and other researchers made a discovery that has been fundamental to understanding evolution as well as developmental biology.

The scientists found a short sequence of DNA, now called the **homeobox**, that is present not only in *Antennapedia* but in the several genes next to it and in genes in many other organisms. When geneticists find very similar DNA sequences in the genes of different organisms, it's a good clue that these genes do something so important and useful that evolution uses the same sequence over and over and permits very few changes in its structure as new species evolve.

Researchers quickly discovered nearly identical versions of homeobox DNA in almost every non-bacterial cell they examined—from yeast to plants, frogs, worms, beetles, chickens, mice and people.

Hundreds of homeobox-containing genes have been identified, and the proteins they make turn out to be involved in the early stages of development of many species. For example, researchers have found that abnormalities in the homeobox genes can lead to extra fingers or toes in humans.

The Tools of Genetics: Mighty Microarrays

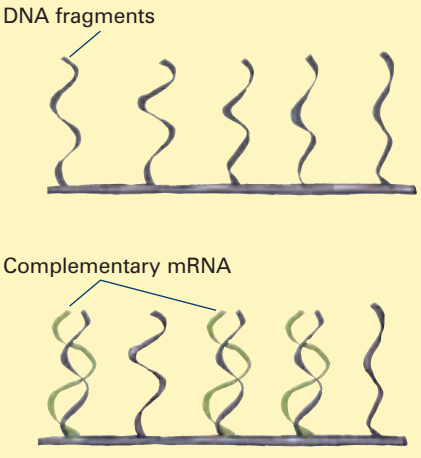
We now have the ability to attach a piece of every gene in a **genome** (all of an organism's genes) to a postage stamp-sized glass microscope slide. This ordered series of DNA spots is called a DNA **microarray**, a **gene chip** or a **DNA chip**.

Whichever name you prefer, the chip could also be called revolutionary. This technology has changed the way many geneticists do their work by making it possible to observe the activity of thousands of genes at once.

In recent years, microarrays have become standard equipment for modern biologists, but teachers and students are using them, too. The Genome Consortium for Active Teaching program (www.bio.davidson.edu/GCAT) provides resources and instructions for high school and college students to do gene-chip experiments in class.

Microarrays are used to get clues about which genes are expressed to control cell, tissue or organ function. By measuring the level of RNA production for every gene at the same time, researchers can learn the genetic programming that makes cell types different and diseased cells different from healthy ones.

The chips consist of large numbers of DNA fragments distributed in rows in a very small space. The arrays are laid out by robots that can



DNA fragments are attached to glass or plastic, then fluorescently tagged molecules are washed over the fragments.

Some molecules (green) bind to their complementary sequence. These molecules can be identified because they glow under fluorescent light.

▼ The resulting pattern of fluorescence indicates which genes are active.

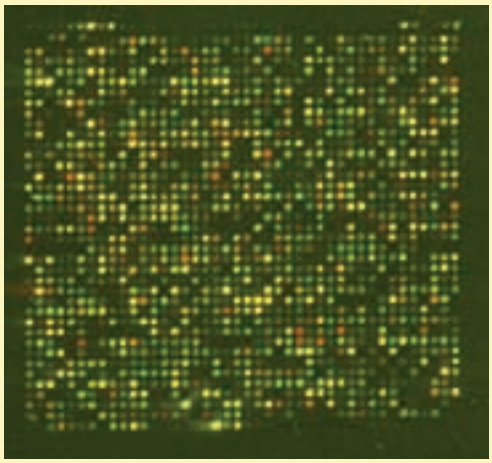
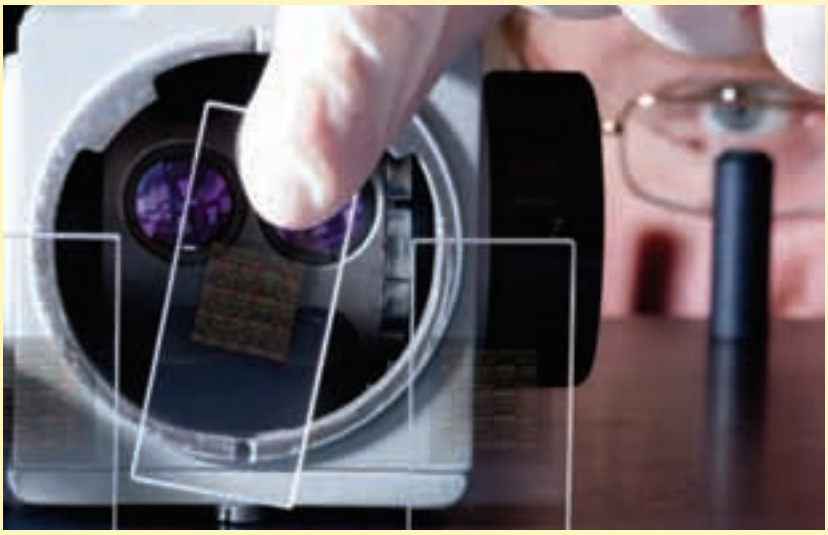


FIGURE 15:

FIGURE 16:



position DNA fragments so precisely that more than 20,000 of them can fit on one microscope slide.

Scientists isolate mRNA from cells grown under two conditions and tag the two sources of RNA with different colors of fluorescent molecules. The two colors of RNA are then placed on the chip, where they attach to complementary DNA fragments anchored to the chip's surface.

Next, a scanner measures the amount of fluorescence at each spot on the chip, revealing how active each gene was (how much mRNA each gene produced). A computer analyzes the patterns of gene activity, providing a snapshot of a genome under two conditions (*e.g.*, healthy or diseased).

In December 2004, the U.S. Food and Drug Administration cleared the first gene chip for medical use. The Amplichip CYP450™, made by Roche Molecular Systems Inc. of Pleasanton, California, analyzes variations in two genes that play a major role in the body's processing of many widely prescribed drugs. This information can help doctors choose the proper dose of certain medicines for an individual patient.

Got It?

Why are some infections hard to treat with antibiotics? What are some things researchers might do to solve this public health problem?

How does DNA work as a form of information storage?

How can 20,000 human genes provide the instructions for making hundreds of thousands of different proteins?

What newborn tests does your area hospital routinely do?

RNA and DNA Revealed: New Roles, New Rules

For many years, when scientists thought about heredity, DNA was the first thing to come to mind. It's true that DNA is the basic ingredient of our genes and, as such, it often steals the limelight from RNA, the other form of genetic material inside our cells.

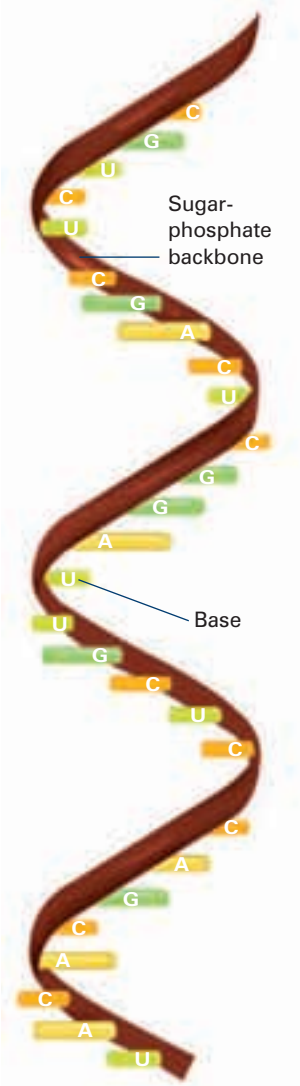
But, while they are both types of genetic material, RNA and DNA are rather different.

The chemical units of RNA are like those of DNA, except that RNA has the nucleotide uracil (U) instead of thymine (T). Unlike double-stranded DNA, RNA usually comes as only a single strand. And the nucleotides in RNA contain ribose sugar molecules in place of deoxyribose.

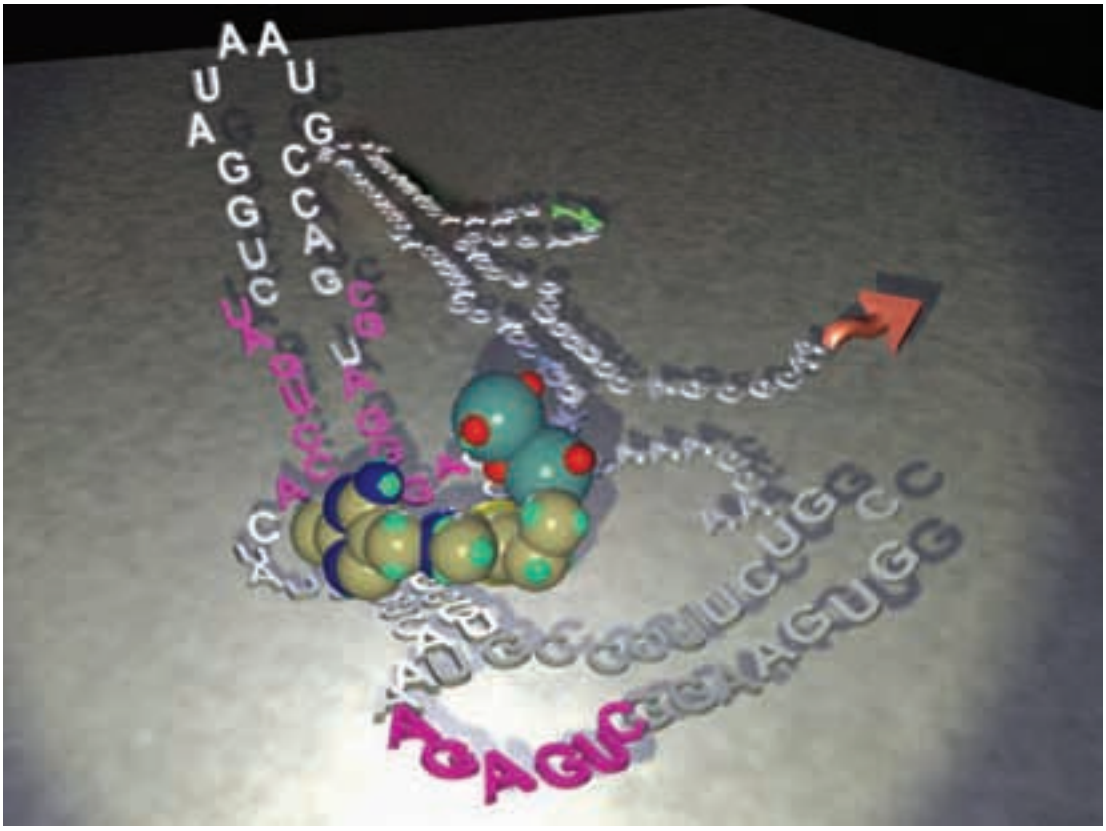
RNA is quite flexible—unlike DNA, which is a rigid, spiral-staircase molecule that is very stable. RNA can twist itself into a variety of complicated, three-dimensional shapes. RNA is also unstable in that cells constantly break it down and must continually make it fresh, while DNA is not broken down often. RNA's instability lets cells change their patterns of protein synthesis very quickly in response to what's going on around them.

Many textbooks still portray RNA as a passive molecule, simply a "middle step" in the cell's gene-reading activities. But that view is no longer accurate. Each year, researchers unlock new secrets about RNA. These discoveries reveal that it is truly a remarkable molecule and a multi - talented actor in heredity.

FIGURE 17:



▲ Ribonucleic acid (RNA) has the bases adenine (A), cytosine (C), guanine (G) and uracil (U).



RONALD BREAKER

FIGURE 18:

Riboswitches are RNA sequences that control gene activity. The riboswitch shown here bends into a special shape when it grips tightly onto a molecule called a metabolite (colored balls) that bacteria need to survive.

Today, many scientists believe that RNA evolved on the Earth long before DNA did. Researchers hypothesize—obviously, no one was around to write this down—that RNA was a major participant in the chemical reactions that ultimately spawned the first signs of life on the planet.

RNA World

At least two basic requirements exist for making a cell: the ability to hook molecules together and break them apart, and the ability to replicate, or copy itself, from existing information.

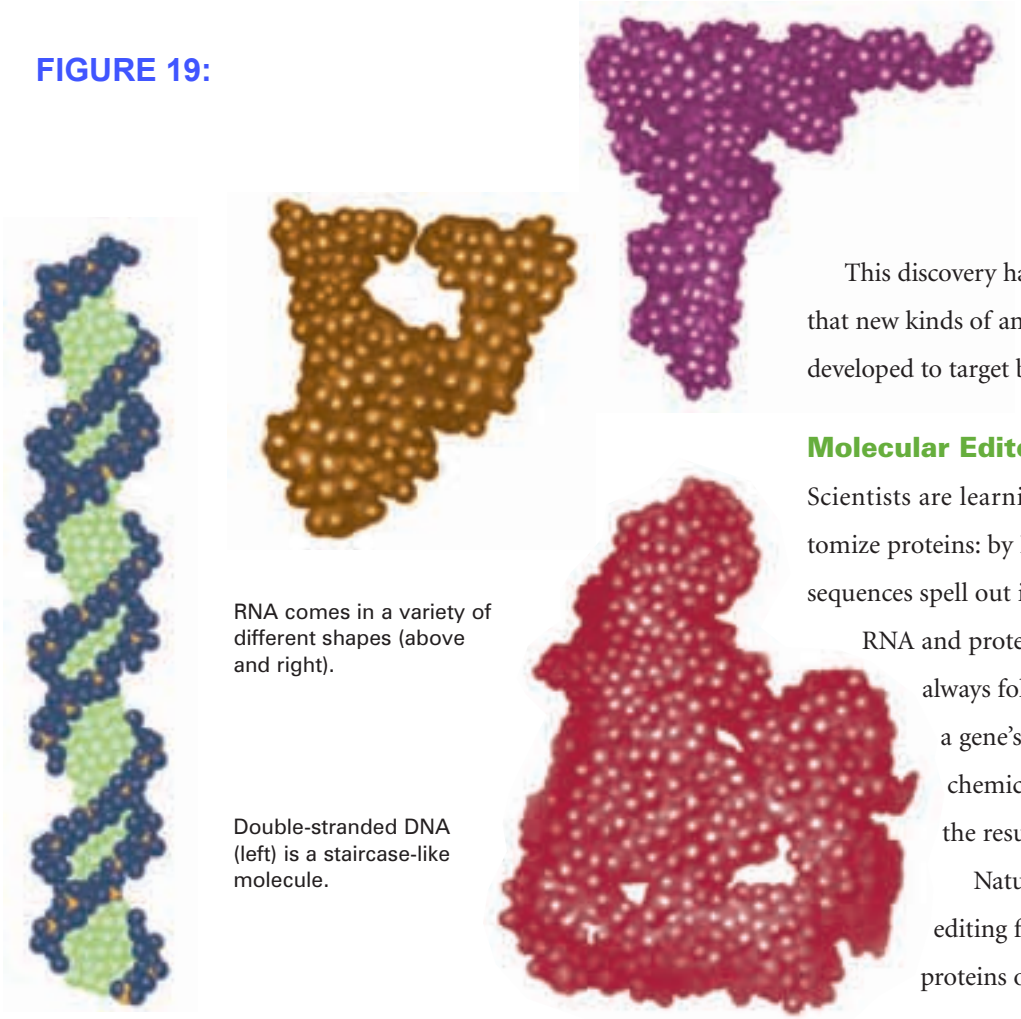
RNA probably helped to form the first cell. The first organic molecules, meaning molecules containing carbon, most likely arose out of random collisions of gases in the Earth's primitive atmosphere, energy from the Sun, and heat from naturally occurring radioactivity. Some scientists think that in this primitive world, RNA was a critical molecule because of its ability to lead a double life: to store information and to conduct chemical reactions. In other words, in this world, RNA served the functions of both DNA and proteins.

What does any of this have to do with human health? Plenty, it turns out.

Today's researchers are harnessing some of RNA's flexibility and power. For example, through a strategy he calls directed evolution, molecular engineer Ronald R. Breaker of Yale University is developing ways to create entirely new forms of RNA and DNA that both work as enzymes.

Breaker and others have also uncovered a hidden world of RNAs that play a major role in controlling gene activity, a job once thought to be performed exclusively by proteins. These RNAs, which the scientists named riboswitches, are found in a wide variety of bacteria and other organisms.

FIGURE 19:



RNA comes in a variety of different shapes (above and right).

Double-stranded DNA (left) is a staircase-like molecule.

This discovery has led Breaker to speculate that new kinds of antibiotic medicines could be developed to target bacterial riboswitches.

Molecular Editor

Scientists are learning of another way to customize proteins: by RNA editing. Although DNA sequences spell out instructions for producing RNA and proteins, these instructions aren't always followed precisely. Editing a gene's mRNA, even by a single chemical letter, can radically change the resulting protein's function.

Nature likely evolved the RNA editing function as a way to get more proteins out of the same number of

Small But Powerful

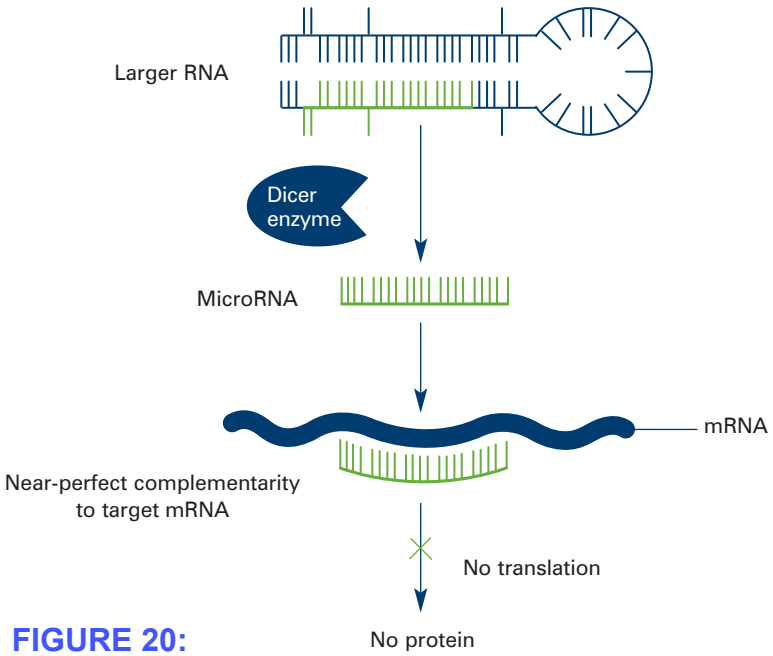


FIGURE 20:

The enzyme Dicer generates microRNAs by chopping larger RNA molecules into tiny Velcro®-like pieces. MicroRNAs stick to mRNA molecules and prevent the mRNAs from being made into proteins.

Recently, molecules called **microRNAs** have been found in organisms as diverse as plants, worms and people. The molecules are truly "micro," consisting of only a few dozen nucleotides, compared to typical human mRNAs that are a few thousand nucleotides long.

What's particularly interesting about microRNAs is that many of them arise from DNA that used to be considered merely filler material (see PDF page #'s 14-17 "Getting the Message" section).

How do these small but important RNA molecules do their work? They start out much bigger but get trimmed by cellular enzymes, including one aptly named Dicer. Like tiny pieces of

genes. For example, researchers have found that the mRNAs for certain proteins important for the proper functioning of the nervous system are particularly prone to editing. It may be that RNA editing gives certain brain cells the capacity to react quickly to a changing environment.

Which molecules serve as the editor and how does this happen? Brenda Bass of the University of Utah School of Medicine in Salt Lake City studies one particular class of editors called adenosine deaminases. These enzymes "retype" RNA letters at various places within an mRNA transcript.

They do their job by searching for characteristic RNA shapes. Telltale twists and bends in folded RNA molecules signal these enzymes to change the RNA sequence, which in turn changes the protein that gets made.

Bass' experiments show that RNA editing occurs in a variety of organisms, including people. Another interesting aspect of editing is that certain disease-causing microorganisms, such as some forms of parasites, use RNA editing to gain a survival edge when living in a human host. Understanding the details of this process is an important area of medical research.

FIGURE 21:

AMY PASQUINELLI

Worms with a mutated form of the microRNA let-7 (right) have severe growth problems, rupturing as they develop.

Velcro®, microRNAs stick to certain mRNA molecules and stop them from passing on their protein-making instructions.

First discovered in a roundworm model system (see *Living Laboratories*, page 49), some microRNAs help determine the organism's body plan. In their absence, very bad things can happen. For example, worms engineered to lack a microRNA called let-7 develop so abnormally that they often rupture and practically break in half as the worm grows.

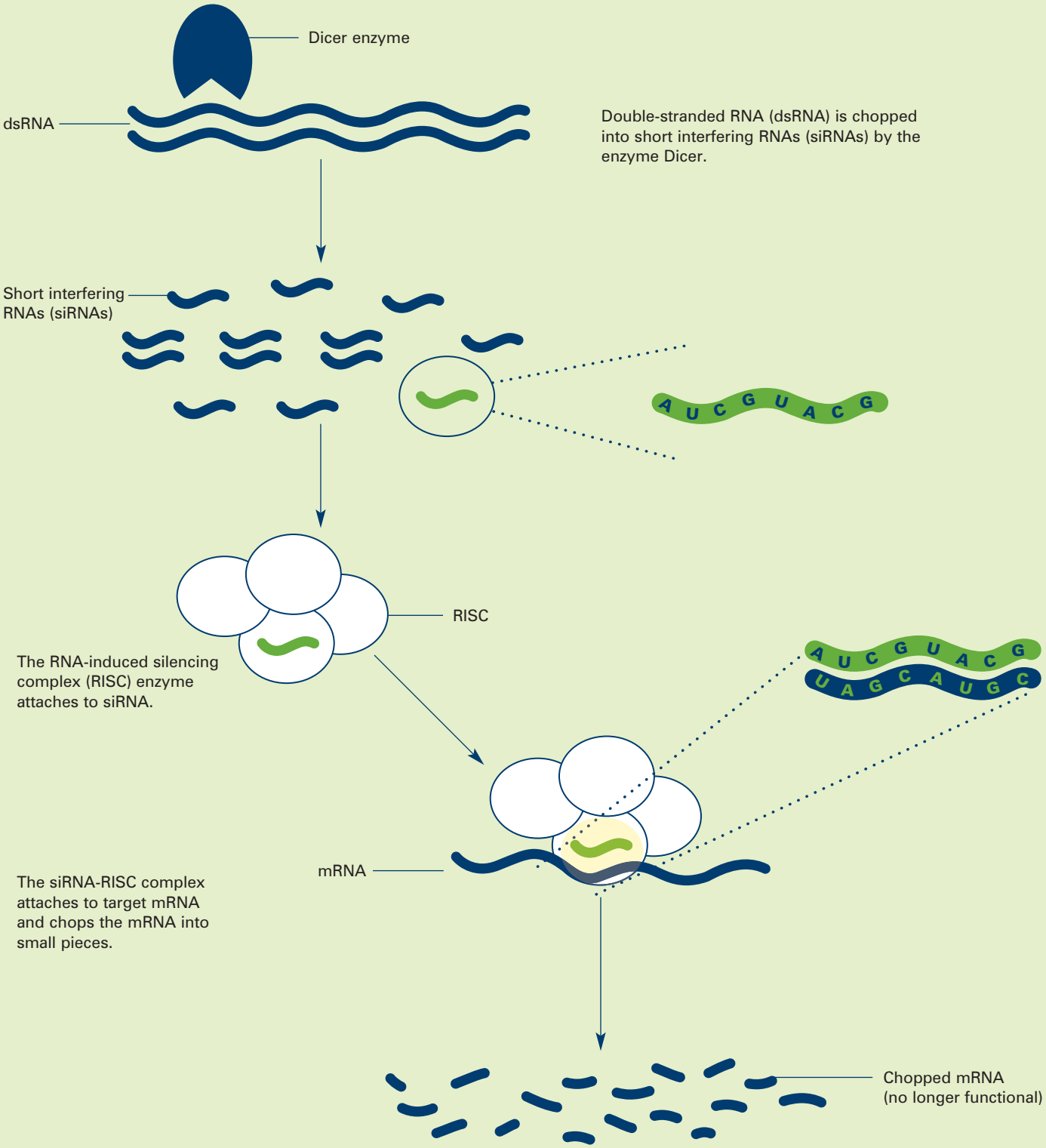
Perhaps it is not surprising that since microRNAs help specify the timing of an organism's developmental plan, the appearance of the microRNAs themselves is carefully timed inside a developing organism. Biologists, including Amy Pasquinelli of the University of California, San Diego, are currently figuring out how microRNAs are made and cut to size, as well as how they are produced at the proper time during development.

MicroRNA molecules also have been linked to cancer. For example, Gregory Hannon of the Cold Spring Harbor Laboratory on Long Island, New York, found that certain microRNAs are associated with the severity of the blood cancer B-cell lymphoma in mice.

Since the discovery of microRNAs in the first years of the 21st century, scientists have identified hundreds of them that likely exist as part of a large family with similar nucleotide sequences. New roles for these molecules are still being found.

FIGURE 22:

RNA Interference (RNAi)



Healthy Interference

RNA controls genes in a way that was only discovered recently: a process called **RNA interference**, or **RNAi**. Although scientists identified RNAi less than 10 years ago, they now know that organisms have been using this trick for millions of years.

Researchers believe that RNAi arose as a way to reduce the production of a gene's encoded protein for purposes of fine-tuning growth or self-defense. When viruses infect cells, for example, they command their host to produce specialized RNAs that allow the virus to survive and make copies of itself. Researchers believe that RNAi eliminates unwanted viral RNA, and some speculate that it may even play a role in human immunity.

Oddly enough, scientists discovered RNAi from a failed experiment! Researchers investigating genes involved in plant growth noticed something strange: When they tried to turn petunia flowers purple by adding an extra "purple" gene, the flowers bloomed white instead.

This result fascinated researchers, who could not understand how adding genetic material could somehow get rid of an inherited trait. The mystery remained unsolved until, a few years later, two geneticists studying development saw a similar thing happening in lab animals.

The researchers, Andrew Z. Fire, then of the Carnegie Institution of Washington in Baltimore and now at Stanford University, and Craig Mello of the University of Massachusetts Medical School in Worcester, were trying to block the expression of genes that affect cell growth and tissue formation in roundworms, using a molecular tool called antisense RNA.

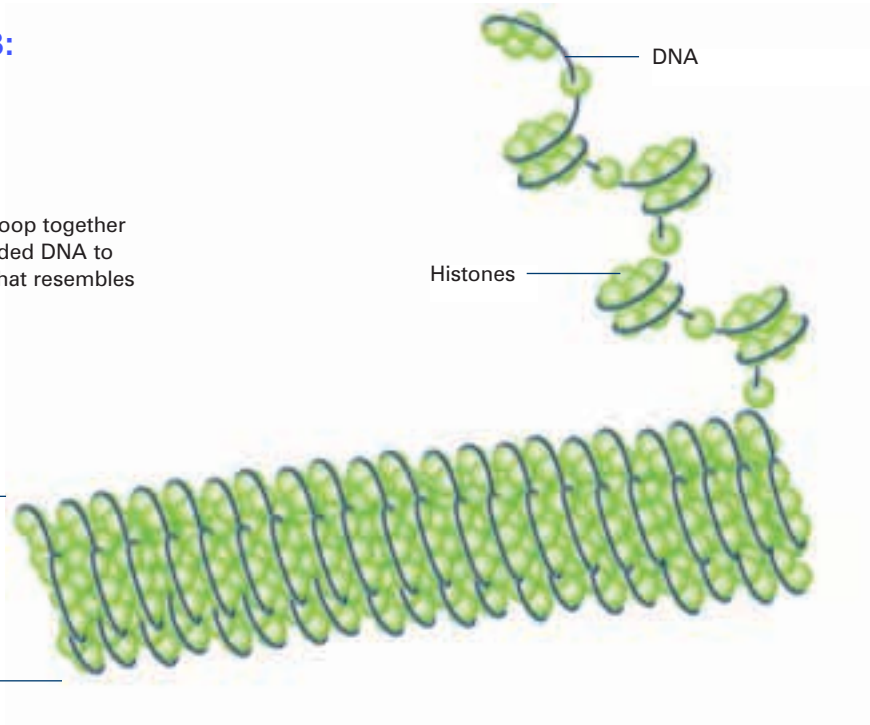
To their surprise, Mello and Fire found that their antisense RNA tool wasn't doing much at all. Rather, they determined, a double-stranded contaminant produced during the synthesis of the single-stranded antisense RNA interfered with **gene expression**. Mello and Fire named the process RNAi, and in 2006 were awarded the Nobel Prize in physiology or medicine for their discovery.

Further experiments revealed that the double-stranded RNA gets chopped up inside the cell into much smaller pieces that stick to mRNA and block its action, much like the microRNA pieces of Velcro discussed above (see drawing, page 28).

Today, scientists are taking a cue from nature and using RNAi to explore biology. They have learned, for example, that the process is not limited to worms and plants, but operates in humans too.

Medical researchers are currently testing new types of RNAi-based drugs for treating conditions such as macular degeneration, the leading cause of blindness, and various infections, including those caused by HIV and the herpes virus.

FIGURE 23:



Histone proteins loop together with double-stranded DNA to form a structure that resembles beads on a string.

Dynamic DNA

A good part of who we are is "written in our genes," inherited from Mom and Dad. Many traits, like red or brown hair, body shape and even some personality quirks, are passed on from parent to offspring.

But genes are not the whole story. Where we live, how much we exercise, what we eat: These and many other environmental factors can all affect how our genes get expressed.

You know that changes in DNA and RNA can produce changes in proteins. But additional control happens at the level of DNA, even though these changes do not alter DNA directly. Inherited factors that do not change the DNA sequence of nucleotides are called **epigenetic** changes, and they too help make each of us unique.

Epigenetic means, literally, "upon" or "over" genetics. It describes a type of chemical reaction that can alter the physical properties of DNA without changing its sequence. These changes make genes either more or less likely to be expressed (see Figure 24).

Currently, scientists are following an intriguing course of discovery to identify epigenetic factors that, along with diet and other environmental influences, affect who we are and what type of illnesses we might get.

Secret Code

DNA is spooled up compactly inside cells in an arrangement called **chromatin**. This packaging is critical for DNA to do its work. Chromatin consists of long strings of DNA spooled around a compact assembly of proteins called **histones**.

One of the key functions of chromatin is to control access to genes, since not all genes are turned on at the same time. Improper expression of growth-promoting genes, for example, can lead to cancer, birth defects or other health concerns.

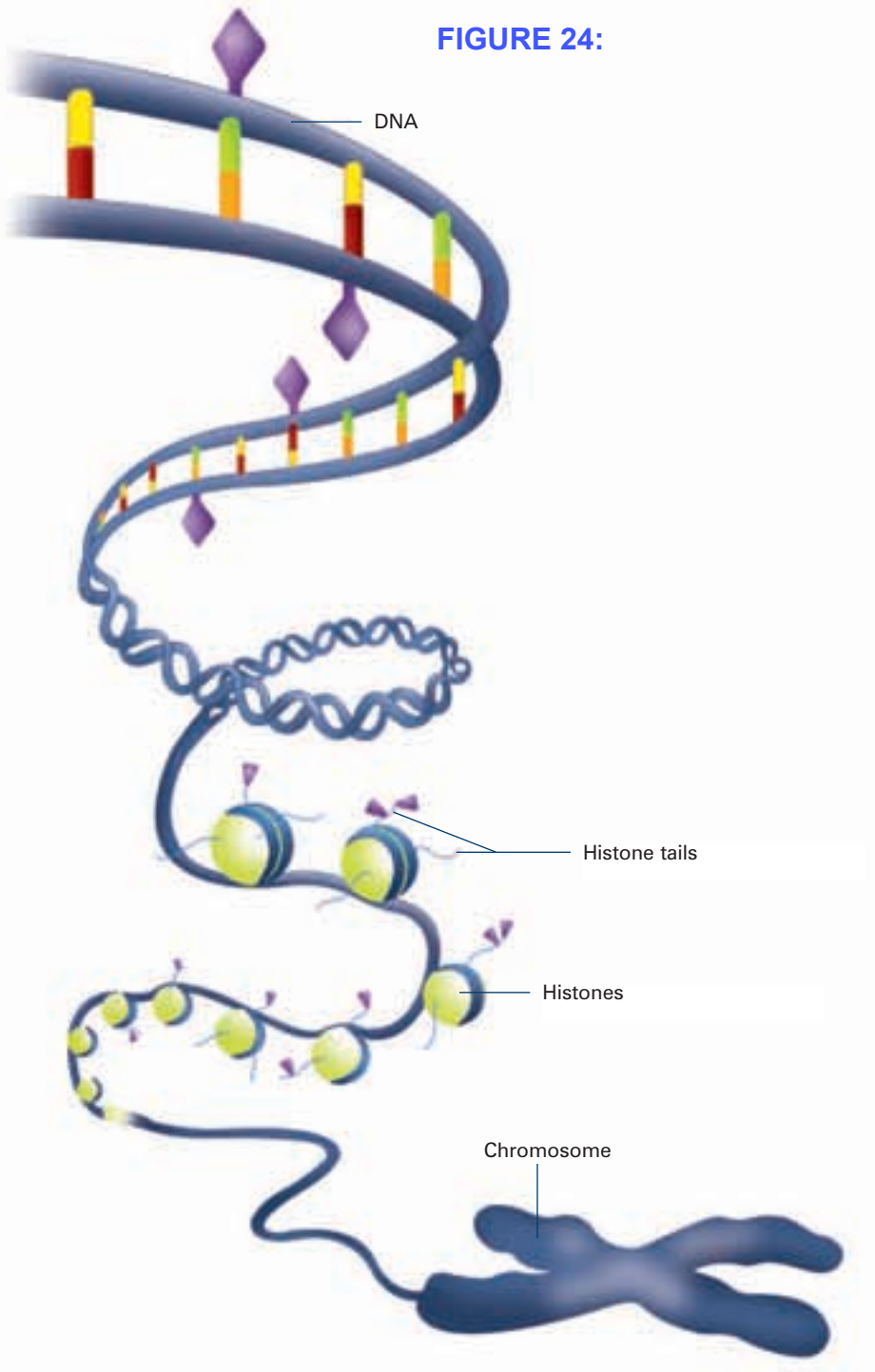
Many years after the structure of DNA was determined, researchers used a powerful device known as an electron microscope to take pictures of chromatin fibers. Upon viewing chromatin up close, the researchers described it as "beads on a string," an image still used today. The beads were the histone balls, and the string was DNA wrapped around the histones and connecting one bead to the next.

Decades of study eventually revealed that histones have special chemical tags that act like switches to control access to the DNA. Flipping these switches, called epigenetic markings, unwinds the spooled DNA so the genes can be transcribed.

The observation that a cell's gene-reading machinery tracks epigenetic markings led C. David Allis, who was then at the University of Virginia Health Sciences Center in Charlottesville and now works at the Rockefeller University in New York City, to coin a new phrase, the "histone code." He and others believe that the histone code plays a major role in determining which proteins get made in a cell.

Flaws in the histone code have been associated with several types of cancer, and researchers are actively pursuing the development of medicines to correct such errors.

FIGURE 24:



The "epigenetic code" controls gene activity with chemical tags that mark DNA (purple diamonds) and the "tails" of histone proteins (purple triangles). These markings help determine whether genes will be transcribed by RNA polymerase. Genes hidden from access to RNA polymerase are not expressed.

GENETICS AND YOU: *The Genetics of Anticipation*

Occasionally, unusual factors influence whether or not a child will be born with a genetic disease.

An example is the molecular error that causes Fragile X syndrome, a rare condition associated with mental retardation. The mutation leading to a fragile X chromosome is not a typical DNA typing mistake, in which nucleotides are switched around or dropped, or one of them is switched for another nucleotide. Instead, it is a kind of stutter by the DNA polymerase enzyme that copies DNA. This stutter creates a string of repeats of a DNA sequence that is composed of just three nucleotides, CGG.

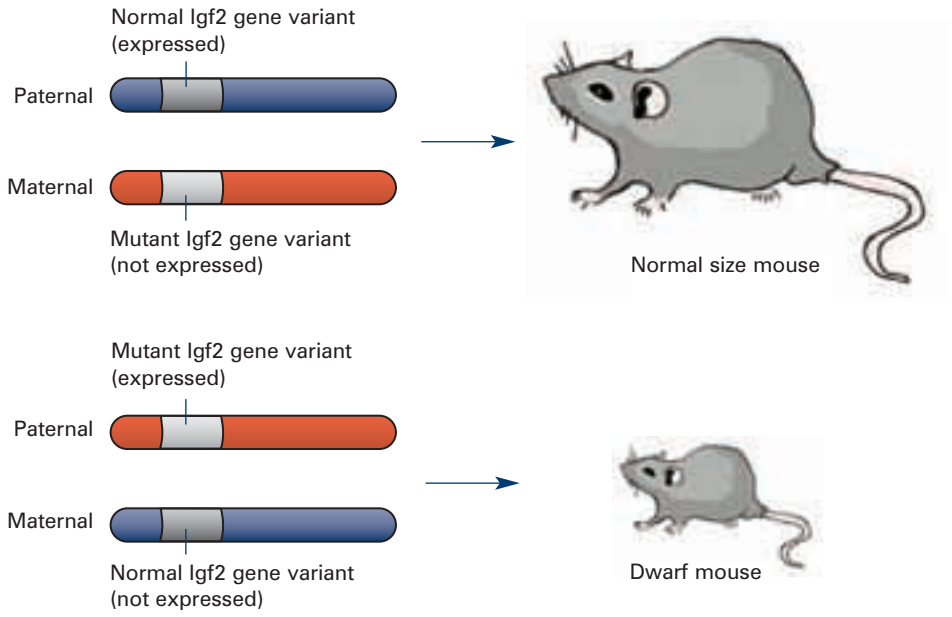
Some people have only one repeat of the CGG nucleotide triplet. Thus, they have two copies of the repeat in a gene, and the extra sequence reads CGGCGG. Others have more than a thousand copies of the repeat. These people are the most severely affected.

The number of triplet repeats seems to increase as the chromosome is passed down through several generations. Thus, the grandsons of a man with a fragile X chromosome, who is not himself affected, have a 40 percent risk of retardation if they inherit the repeat-containing chromosome. The risk for great-grandsons is even higher: 50 percent.

Intrigued by the evidence that triplet repeats can cause genetic disease, scientists have searched for other examples of disorders associated with the DNA expansions. To date, more than a dozen such disorders have been found, and all of them affect the nervous system.

Analysis of the rare families in which such diseases are common has revealed that expansion of the triplet repeats is linked to something called genetic **anticipation**, when a disease's symptoms appear earlier and more severely in each successive generation.

FIGURE 25:



Igf2 is an imprinted gene. A single copy of the abnormal, or mutant, form of the Igf2 gene (red) causes growth defects, but only if the abnormal gene variant is inherited from the father.

Battle of the Sexes

A process called **imprinting**, which occurs naturally in our cells, provides another example of how epigenetics affects gene activity.

With most genes, the two copies work exactly the same way. For some mammalian genes, however, only the mother's or the father's copy is switched on regardless of the child's gender. This is because the genes are chemically marked, or imprinted, during the process that generates eggs and sperm.

As a result, the embryo that emerges from the joining of egg and sperm can tell whether a gene copy came from Mom or Dad, so it knows which copy of the gene to shut off.

One example of an imprinted gene is insulin-like growth factor 2 (Igf2), a gene that helps a mammalian fetus grow. In this case, only the father's copy of Igf2 is expressed, and the mother's copy remains silent (is not expressed) throughout the life of the offspring.

Scientists have discovered that this selective silencing of Igf2 and many other imprinted genes occurs in all placental mammals (all except the platypus, echidna and marsupials) examined so far, but not in birds.

Why would nature tolerate a process that puts an organism at risk because only one of two copies of a gene is working? The likely reason, many researchers believe, is that mothers and fathers have competing interests, and the battlefield is DNA!

The scenario goes like this: It is in a father's interest for his embryos to get bigger faster, because that will improve his offspring's chances of survival after birth. The better an individual's

chance of surviving infancy, the better its chance of becoming an adult, mating and passing its genes on to the next generation.

Of course mothers want strong babies, but unlike fathers, mothers provide physical resources to embryos during pregnancy. Over her lifetime, a female is likely to be pregnant several times, so she needs to divide her resources among a number of embryos in different pregnancies.

Researchers have discovered over 200 imprinted genes in mammals since the first one was identified in 1991. We now know that imprinting controls some of the genes that have an important role in regulating embryonic and fetal growth and allocating maternal resources. Not surprisingly, mutations in these genes cause serious growth disorders.

Marisa Bartolomei of the University of Pennsylvania School of Medicine in Philadelphia is trying to figure out how Igf2 and other genes become imprinted and stay silent throughout the life of an individual. She has already identified sequences within genes that are essential for imprinting. Bartolomei and other researchers have shown that these sequences, called insulators, serve as "landing sites" for a protein that keeps the imprinted gene from being transcribed.

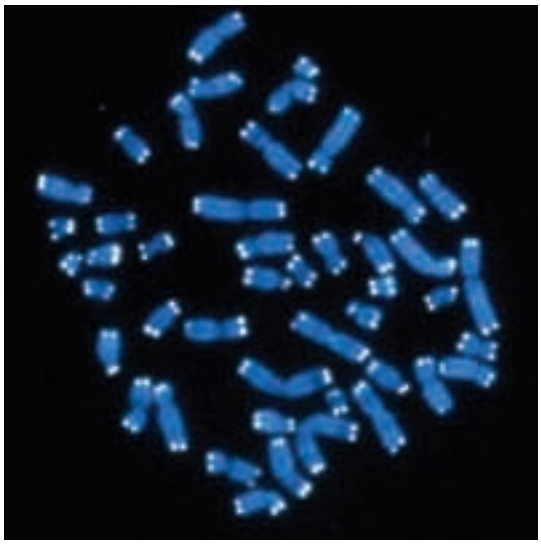
Starting at the End

When we think of DNA, we think of genes. However, some DNA sequences are different: They don't encode RNAs or proteins. Introns, described in Chapter 1, are in this category.

Another example is **telomeres**—the ends of chromosomes. There are no genes in telomeres, but they serve an essential function. Like shoelaces without their tips, chromosomes without telomeres unravel and fray. And without telomeres, chromosomes stick to each other and cause cells to undergo harmful changes like dividing abnormally.

Researchers know a good deal about telomeres, dating back to experiments performed in the 1970s by Elizabeth Blackburn, a basic researcher who was curious about some of the fundamental events that take place within cells.

FIGURE 26:



HESED PADILLA-NASH AND THOMAS RIED

Telomeres, repeated nucleotide sequences at the tips of chromosomes, appear white in this photo.

FIGURE 27:

CAROL GREIDER

Molecular biologist Carol Greider discovered the enzyme telomerase. This license plate, which was on her car when she worked at Cold Spring Harbor Laboratory on Long Island, New York, advertises her research interest!

At the time, Blackburn, now at the University of California, San Francisco, was working with Joseph Gall at Yale University. For her experimental system, she chose a single-celled, pond-dwelling organism named *Tetrahymena*. These tiny, pear-shaped creatures are covered with hairlike cilia that they use to propel themselves through the water as they devour bacteria and fungi.

Tetrahymena was a good organism for Blackburn's experiments because it has a large number of chromosomes—which means it has a lot of telomeres!

Her research was also perfectly timed, because methods for sequencing DNA were just being developed. Blackburn found that *Tetrahymena's* telomeres had an unusual nucleotide sequence: TTGGGG, repeated about 50 times per telomere.

Since then, scientists have discovered that the telomeres of almost all organisms have repeated sequences of DNA with lots of Ts and Gs. In human and mouse telomeres, for example, the repeated sequence is TTAGGG.

The number of telomere repeats varies enormously, not just from organism to organism but in different cells of the same organism and even within a single cell over time. Blackburn reasoned that the repeat number might vary if cells had an enzyme that added copies of the repeated sequence to the telomeres of some but not all chromosomes.

With her then-graduate student Carol Greider, now at Johns Hopkins University, Blackburn hunted for the enzyme. The team found it and Greider named it telomerase. Blackburn, Greider and Jack Szostak of Harvard Medical School in Boston shared the 2009 Nobel Prize in physiology or medicine for their discoveries about telomeres and telomerase.

As it turns out, the telomerase enzyme consists of a protein and an RNA component, which the enzyme uses as a template for copying the repeated DNA sequence.

What is the natural function of telomerase? As cells divide again and again, their telomeres get shorter. Most normal cells stop dividing when their telomeres wear down to a certain point, and eventually the cells die. Telomerase can counteract the shortening. By adding DNA to telomeres, telomerase rebuilds the telomere and resets the cell's molecular clock.

The discovery of telomerase triggered new ideas and literally thousands of new studies. Many researchers thought that the enzyme might play important roles in cancer and aging. Researchers were hoping to find ways to turn telomerase *on* so that cells would continue to divide (to grow extra cells for burn patients, for example), or *off* so that cells would stop dividing (to stop cancer, for instance).

So far, they have been unsuccessful. Although it is clear that telomerase and cellular aging are related, researchers do not know whether telomerase plays a role in the normal cellular aging process or in diseases like cancer.

Recently, however, Blackburn and a team of other scientists discovered that chronic stress and the perception that life is stressful affect telomere length and telomerase activity in the cells of healthy women. Blackburn and her coworkers are currently conducting a long-term, follow-up study to confirm these intriguing results.

The Other Human Genome

Before you think everything's been said about DNA, there's one little thing we didn't mention: Some of the DNA in every cell is quite different from the DNA that we've been talking about up to this point. This special DNA isn't in chromosomes—it isn't even inside the cell's nucleus where all the chromosomes are!

So where is this special DNA? It's inside **mitochondria**, the organelles in our cells that produce the energy-rich molecule adenosine triphosphate, or ATP. Mendel knew nothing of mitochondria, since they weren't discovered until late in the 19th century. And it wasn't until the 1960s that researchers discovered the mitochondrial genome, which is circular like the genomes of bacteria.

In human cells, mitochondrial DNA makes up less than 1 percent of the total DNA in each of our cells. The mitochondrial genome is very small—containing only about three dozen genes. These encode a few of the proteins that are in the mitochondrion, plus a set of ribosomal RNAs used for synthesizing proteins for the organelle.

Mitochondria need many more proteins though, and most of these are encoded by genes in the nucleus. Thus, the energy-producing capabilities of human mitochondria—a vital part of any cell's everyday health—depend on coordinated teamwork among hundreds of genes in two cellular neighborhoods: the nucleus and the mitochondrion.

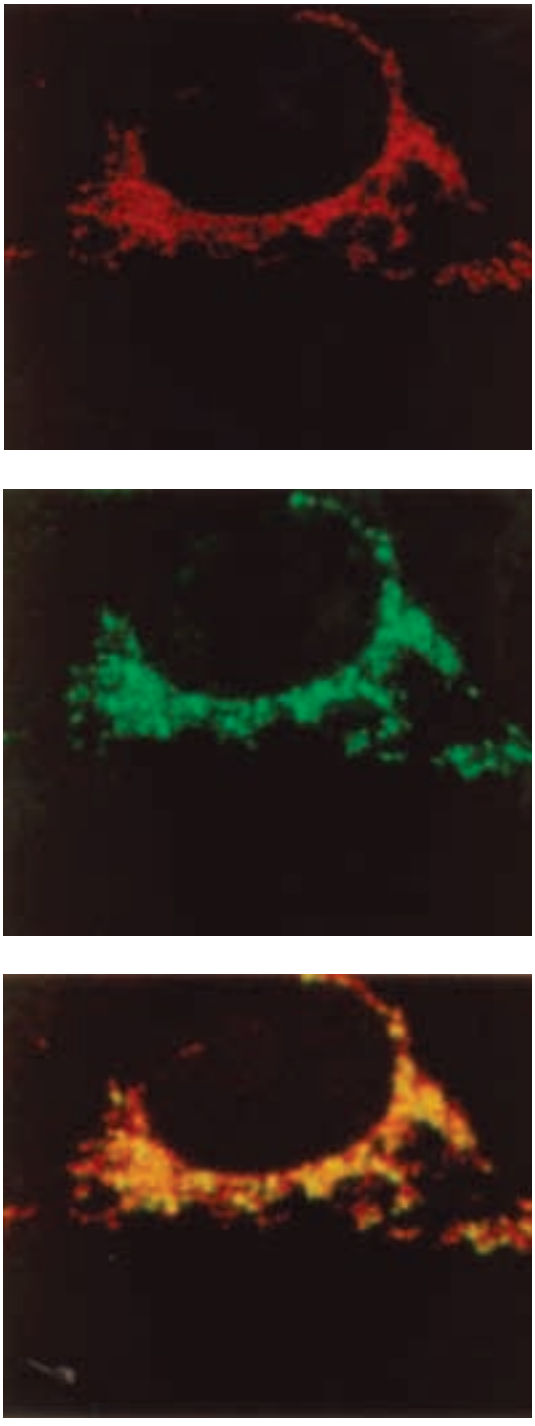
Mitochondrial DNA gets transcribed and the RNA is translated by enzymes that are very different from those that perform this job for genes in our chromosomes. Mitochondrial enzymes look and act much more like those from bacteria, which is not surprising because mitochondria are thought to have descended from free-living bacteria that were engulfed by another cell over a billion years ago.

Scientists have linked mitochondrial DNA defects with a wide range of age-related diseases including neurodegenerative disorders, some forms of heart disease, diabetes and various cancers. It is still unclear, though, whether damaged mitochondria are a symptom or a cause of these health conditions.

Scientists have studied mitochondrial DNA for another reason: to understand the history of the human race. Unlike our chromosomal DNA, which we inherit from both parents, we get all of our mitochondrial DNA from our mothers.

Thus, it is possible to deduce who our maternal ancestors were by tracking the inheritance of mutations in mitochondrial DNA. For reasons that are still not well understood, mutations accumulate in mitochondrial DNA more quickly than in chromosomal DNA. So, it's possible to trace your maternal ancestry way back beyond any relatives you may know by name—all the way back to "African Eve," the ancestor of us all!

FIGURE 28:



ALISON DAVIS

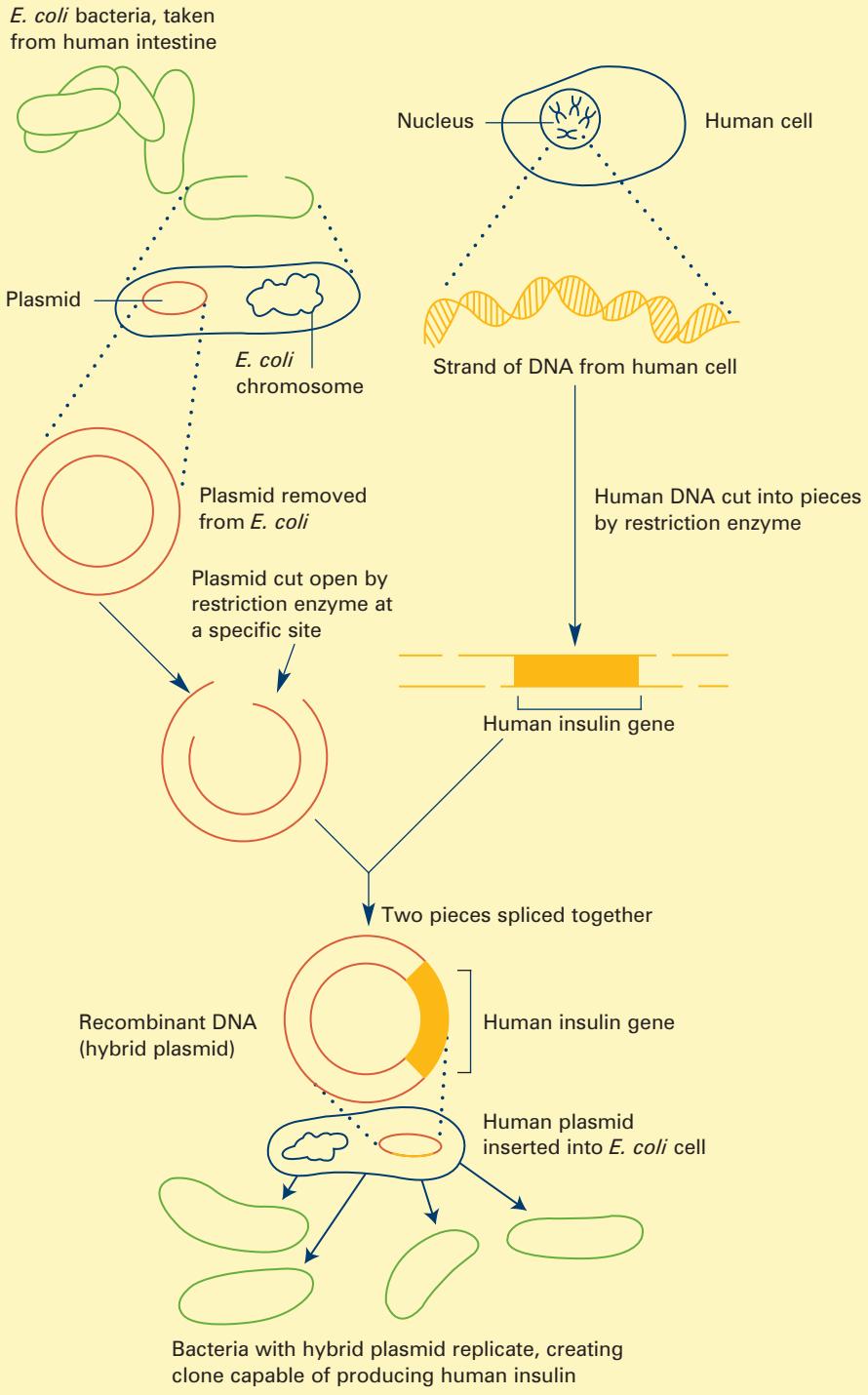
Mitochondria (labeled with a red dye) are scattered throughout the cytoplasm of this human cancer cell.

The cell has also been treated with a dye that colors the mitochondrial DNA green.

A computerized overlay of these two images of the same cell shows that mitochondria and its DNA appear together (yellow regions).

FIGURE 29:

The Tools of Genetics: Recombinant DNA and Cloning



Recombinant DNA. To splice a human gene (in this case, the one for insulin) into a plasmid, scientists take the plasmid out of an *E. coli* bacterium, cut the plasmid with a restriction enzyme and splice in insulin-making human DNA. The resulting hybrid plasmid can be inserted into another *E. coli* bacterium, where it multiplies along with the bacterium. There, it can produce large quantities of insulin.

FIGURE 30:

ROSLIN INSTITUTE, EDINBURGH

Scientists in Scotland were the first to clone an animal, this sheep named Dolly. She later gave birth to Bonnie, the lamb next to her.

In the early 1970s, scientists discovered that they could change an organism's genetic traits by putting genetic material from another organism into its cells. This discovery, which caused quite a stir, paved the way for many extraordinary accomplishments in medical research that have occurred over the past 35 years.

How do scientists move genes from one organism to another? The cutting and pasting gets done with chemical scissors: enzymes, to be specific. Take insulin, for example. Let's say a scientist wants to make large quantities of this protein to treat diabetes. She decides to transfer the human gene for insulin into a bacterium, *Escherichia coli*, or *E. coli*, which is commonly used for genetic research (see *Living Laboratories*, page 46). That's because *E. coli* reproduces really fast, so after one bacterium gets the human insulin gene, it doesn't take much time to grow millions of bacteria that contain the gene.

The first step is to cut the insulin gene out of a copied, or "cloned," version of the human DNA using a special bacterial enzyme from bacteria called a restriction endonuclease. (The normal role of these enzymes in bacteria is to chew up the DNA of viruses and other invaders.) Each restriction enzyme recognizes and cuts at a different nucleotide sequence, so it's possible to be very precise about DNA cutting by selecting one of several hundred of these enzymes that cuts at the desired sequence. Most restriction endonucleases make slightly staggered incisions, resulting in "sticky ends," out of which one strand protrudes.

The next step in this example is to splice, or paste, the human insulin gene into a circle of bacterial DNA called a plasmid. Attaching the cut ends together is done with a different enzyme (obtained from a virus), called DNA ligase. The sticky ends join back together kind of like jigsaw puzzle pieces. The result: a cut-and-pasted mixture of human and bacterial DNA.

The last step is putting the new, **recombinant DNA** back into *E. coli* and letting the bacteria reproduce in a petri dish. Now, the scientist has a great tool: a version of *E. coli* that produces lots of human insulin that can be used for treating people with diabetes.

So, what is cloning? Strictly speaking, it's making many copies. However, the term is more commonly used to refer to making many copies of a gene, as in the *E. coli* example above. Researchers can also **clone** entire organisms, like Dolly the sheep, which contained the identical genetic material of another sheep.

Got It?

Besides the sequence of nucleotides in genes, what are some other changes to DNA and RNA that can affect our health and who we are?

Can you imagine treat ments—other than vaccines and current medicines—crafted from genetic information and new molecular tools?

How is cloning a gene different from cloning an animal or a person? How do researchers use gene cloning to study health and disease?

Do you have any recurring illnesses in your extended family?