Chapter 20

Biotechnology

Lecture Outline

Overview: The DNA Toolbox

- In 1995, researchers sequenced the entire genome of a free-living organism, the bacterium *Haemophilus influenzae*.
- A mere 12 years later, genome sequencing was under way for more than 2,000 species.
- By 2007, researchers had completely sequenced hundreds of prokaryotic genomes and dozens of eukaryotic ones, including all 3 billion base pairs of the human genome.
- Rapid advances in DNA technology—methods of working with and manipulating DNA—had their roots in the 1970s.
- A key accomplishment was the invention of techniques for making **recombinant DNA**, DNA molecules formed when segments of DNA from two different sources—often different species—are combined *in vitro*.
- Scientists also have powerful techniques for analyzing genes and gene expression.
- Human lives are greatly affected by **biotechnology**, the manipulation of organisms or their components to make useful products.
  - Biotechnology includes such early practices as selective breeding of farm animals and the use of microorganisms to make wine and cheese.
  - Today, biotechnology also encompasses **genetic engineering**, the direct manipulation of genes for practical purposes.
- DNA technology is now applied in areas ranging from agriculture to criminal law to medical diagnosis, but many of its most important achievements are in basic research.

Concept 20.1 DNA cloning yiels multiple copies of a gene or other DNA segment.

- To study a particular gene, scientists needed to develop methods to isolate the small, well-defined portion of a chromosome that contains the gene of interest.
- Techniques for *DNA cloning* enable scientists to prepare multiple identical copies of well-defined segments of DNA.
- One common approach to cloning pieces of DNA uses bacteria, usually *Escherichia coli*, whose chromosome is a large circular DNA molecule.
- In addition, bacteria have **plasmids**, small circular DNA molecules with a small number of genes that replicate independently from the chromosome.
- One basic cloning technique begins with the insertion of a "foreign" gene into a bacterial plasmid to produce a recombinant DNA molecule.

- The plasmid is returned to a bacterial cell, producing a *recombinant bacterium,* which reproduces to form a clone of genetically identical cells.
- Every time the bacterium reproduces, the recombinant plasmid is replicated as well.
- The production of multiple copies of a single gene is called **gene cloning**.
- Gene cloning is useful for two basic purposes: to make many copies of a particular gene and to create a protein product.
  - Isolated copies of a cloned gene may enable scientists to determine the gene's nucleotide sequence or provide an organism with a new metabolic capability, such as pest resistance.
  - Alternatively, a protein with medical uses, such as human growth hormone, can be harvested in large quantities from cultures of bacteria carrying the cloned gene for the protein.
  - Most protein-coding genes exist in only one copy per genome, so the ability to clone rare DNA fragments is very valuable.

Restriction enzymes are used to make recombinant DNA.

- Gene cloning and genetic engineering were made possible by the discovery of **restriction enzymes** that cut DNA molecules at specific locations.
- In nature, bacteria use restriction enzymes to cut foreign DNA, to protect themselves against phages or other bacteria.
- Restriction enzymes are very specific, recognizing short DNA nucleotide sequences, or **restriction sites**, and cutting both DNA strands at specific points within these sequences.
  - Bacteria protect their own DNA by methylating the sequences recognized by these enzymes.
  - Each restriction enzyme cleaves a specific sequence of bases.
  - Because the target sequence usually occurs (by chance) many times on a long DNA molecule, an enzyme makes many cuts.
  - A given restriction enzyme yields the same set of **restriction fragments** when it cuts a specific DNA molecule.
- Restriction enzymes cut the covalent sugar-phosphate backbones of both strands, often in a staggered way that creates single-stranded **sticky ends.**
- These extensions can form hydrogen-bonded base pairs with complementary single-stranded stretches (sticky ends) on other DNA molecules cut with the same restriction enzyme.
- These DNA fusions can be made permanent by **DNA ligase**, which seals the strand by catalyzing the formation of covalent bonds to close up the sugar-phosphate backbone.
- Restriction enzymes and DNA ligase can be used to make a stable recombinant DNA molecule, with DNA that has been spliced together from two different organisms.

Eukaryotic genes can be cloned in bacterial plasmids.

- Recombinant plasmids are produced when restriction fragments from foreign DNA are spliced into plasmids.
- The original plasmid used to produce recombinant DNA is called a **cloning vector**, defined as a DNA molecule that can carry foreign DNA into a cell and replicate there.
- Bacterial plasmids are widely used as cloning vectors because they can easily be isolated from bacteria, manipulated to form recombinant plasmids by *in vitro* insertion of foreign DNA, and then reintroduced into bacterial cells.
- Bacterial cells that carry the recombinant plasmid reproduce rapidly, replicating the inserted foreign DNA.

- Imagine that researchers are interested in studying the β-globin gene in a particular species of hummingbird to see whether this oxygen-carrying protein is different from its counterpart in less metabolically active species.
- The first step is the isolation of hummingbird genomic DNA, which contains the β-globin gene, from hummingbird cells. Researchers also isolate the chosen vector, a particular bacterial plasmid from *E. coli* cells.
- The plasmid carries two useful genes, amp^R, which confers resistance to the antibiotic ampicillin, and *lacZ*, which encodes the enzyme ß-galactosidase that catalyzes the hydrolysis of lactose.
- ß-galactosidase can also hydrolyze a synthetic mimic of lactose called X-gal to form a blue product.
- The plasmid has a single recognition sequence, within the *lacZ* gene, for the restriction enzyme used.
- Both the plasmid and the hummingbird DNA are digested with the same restriction enzyme.
- The fragments are mixed together, allowing base pairing between their complementary sticky ends.
- DNA ligase is added to permanently join the base-paired fragments.
- Some of the resulting recombinant plasmids contain hummingbird DNA fragments; one fragment carries the β-globin gene.
    - This step also generates other products, such as plasmids containing several hummingbird DNA fragments, a combination of two plasmids, or a rejoined, nonrecombinant version of the original plasmid.
- The DNA mixture is mixed with bacteria that have a mutation in the *lacZ* gene on their own chromosome, making them unable to hydrolyze lactose or X-gal.
- The bacteria take up foreign DNA by transformation.
    - Some cells acquire a recombinant plasmid carrying a gene, while others may take up a nonrecombinant plasmid, a hummingbird DNA fragment, or nothing at all.
- The transformed bacteria are plated on a solid nutrient medium containing ampicillin and X-gal.
- Only bacteria that have the ampicillin-resistance (amp^R) plasmid grow.
- Each reproducing bacterium forms a clone by repeating cell divisions, thus generating a *colony* of cells on the agar.
- The X-gal in the medium is used to identify plasmids that carry foreign DNA.
    - Bacteria with plasmids lacking foreign DNA stain blue when ß-galactosidase from the intact *lacZ* gene hydrolyzes X-gal.
    - Bacteria with plasmids containing foreign DNA inserted into the *lacZ* gene are white because they lack ß-galactosidase.
- In the final step, thousands of bacterial colonies with foreign DNA are sorted to find those that contain the gene of interest.

Cloned genes are stored in DNA libraries.

- In the "shotgun" cloning approach described above, a mixture of fragments from the entire genome is included in thousands of different recombinant plasmids.
- A complete set of recombinant plasmid clones, each carrying copies of a particular segment from the initial genome, forms a **genomic library**.
- In addition to plasmids, certain bacteriophages are common cloning vectors for making genomic libraries.

- Fragments of foreign DNA can be spliced into a phage genome using a restriction enzyme and DNA ligase.
- An advantage of using phage as vectors is that phage can carry larger DNA inserts than plasmids can.
- The normal infection process allows the production of many new phage particles, each carrying the foreign DNA.
- A genomic library made using phage is stored as a collection of phage clones.

- Because restriction enzymes do not recognize gene boundaries, some genes in either of these types of genomic library are cut and divided up among two or more clones.
- **Bacterial artificial chromosomes (BAC)** are used as vectors for library construction.
- BACs are large plasmids containing only the genes necessary to ensure replication and capable of carrying inserts of 100–300 kb.
- The very large insert size minimizes the number of clones that are needed to make up the genomic library, but it makes them more difficult to work with.
  - BAC clones are usually stored in multiwelled plastic plates, with one clone per well.
- A more limited kind of gene library can be developed by starting with mRNA extracted from cells.
  - The enzyme reverse transcriptase is used *in vitro* to make single-stranded DNA transcripts of the mRNA molecules.
  - The mRNA is enzymatically digested, and a second DNA strand complementary to the first is synthesized by DNA polymerase.
  - This double-stranded DNA is called **complementary DNA** (**cDNA**).
  - For creating a library, cDNA is modified by the addition of restriction sites at each end and then inserted into vector DNA.
  - A **cDNA library** represents that part of a cell's genome that was transcribed in the starting cell from which the mRNA was isolated.
- If a researcher wants to clone a gene but is unsure in what cell type it is expressed or unable to obtain that cell type, a genomic library will likely contain the gene.
- A researcher interested in the regulatory sequences or introns associated with a gene needs to obtain the gene from a genomic library.
  - These sequences are missing from the processed mRNAs used in making a cDNA library.
- A researcher interested in only the coding sequence of a gene can obtain a stripped-down version of the gene from a cDNA library.
  - This is an advantage if a researcher wants to study the genes responsible for the specialized functions of a particular kind of cell.
  - By making cDNA libraries from cells of the same type at different times in the life of an organism, one can trace changes in the patterns of gene expression.
- The researcher screens all the colonies with recombinant plasmids for a clone of cells containing the hummingbird β-globin gene.
- One technique, **nucleic acid hybridization**, depends on base pairing between the gene and a complementary sequence on a short, single-stranded nucleic acid, a **nucleic acid probe**.
- Identifying the sequence of the RNA or DNA probe depends on knowledge of at least part of the sequence of the gene of interest.

- A radioactive or fluorescent tag is used to label the probe, which hydrogen-bonds specifically to complementary single strands of the desired gene.
- The clones in the hummingbird genomic library have been stored in a multiwell plate.
- If a few cells from each well are transferred to a defined location on a membrane made of nylon or nitrocellulose, a large number of clones can be screened simultaneously for the presence of DNA complementary to the DNA probe.
- Once the location of a clone carrying the β-globin gene has been identified, cells from that colony can be grown in order to isolate large amounts of the β-globin gene.
  - The cloned gene itself can be used as a probe to identify similar or identical genes in DNA from other sources, such as other species of birds.

Eukaryote genes can be expressed in bacterial host cells.

- The protein product of a cloned gene can be created in either bacterial or eukaryotic cells, for research purposes or for practical applications.
- Inducing a cloned eukaryotic gene to function in bacterial host cells can be difficult because certain aspects of gene expression are different in eukaryotes and bacteria.
- One way around this is to insert an **expression vector**, a cloning vector containing a highly active bacterial promoter, upstream of the restriction site.
- The bacterial host cell recognizes the promoter and proceeds to express the foreign gene that has been linked to it.
  - Such expression vectors allow the synthesis of many eukaryotic proteins in bacterial cells.
- The presence of noncoding introns in eukaryotic genes may prevent the correct expression of these genes in bacteria, which lack RNA-splicing machinery.
- This problem can be surmounted by using a cDNA form of the gene, which includes only the exons.
- Molecular biologists can avoid incompatibility problems by using eukaryotic cells as hosts for cloning and expressing eukaryotic genes.
- Yeast cells, single-celled fungi, are as easy to grow as bacteria and, unlike most eukaryotes, have plasmids.
- Scientists have constructed recombinant plasmids that combine yeast and bacterial DNA and can replicate in either type of cell.
- Scientists have produced **yeast artificial chromosomes** (**YACs**) that combine the essentials of a eukaryotic chromosome (an origin site for replication, a centromere, and two telomeres) with foreign DNA.
- These chromosome-like vectors behave normally in mitosis and can carry more DNA than a plasmid vector.
- Another advantage of eukaryotic hosts is that they are capable of providing the post-translational modifications that many proteins require.
  - Such modifications may include adding carbohydrates or lipids.
  - For some mammalian proteins, the host must be an animal cell to perform the necessary modifications.
- Experimental techniques facilitate the entry of foreign DNA into eukaryotic cells.
  - In **electroporation,** brief electrical pulses create a temporary hole in the plasma membrane through which DNA can enter.

- Alternatively, scientists can inject DNA into individual cells using microscopically thin needles.
  - To get DNA into plant cells, the soil bacterium *Agrobacterium* can be used.
- Once inside the cell, the DNA is incorporated into the cell's DNA by natural genetic recombination.

***The polymerase chain reaction (PCR) amplifies DNA* in vitro.**

- DNA cloning in cells remains the best method for preparing large quantities of a particular gene or other DNA sequence.
- When the source of DNA is scanty or impure, however, the **polymerase chain reaction (PCR)** is quicker and more selective.
- This technique can quickly amplify any piece of DNA without using cells.
  - PCR can make billions of copies of a targeted DNA segment in a few hours.
  - This process is faster than cloning via recombinant bacteria.
  - In fact, PCR is being used increasingly to make enough of a specific DNA fragment to insert it directly into a vector, skipping the steps of making and screening a library.
- In PCR, a three-step cycle—heating, cooling, and replication—brings about a chain reaction that produces an exponentially growing population of identical DNA molecules.
  - The reaction mixture is heated to denature (separate) the DNA strands.
  - The mixture is cooled to allow annealing (hydrogen bonding) of short, single-stranded DNA primers complementary to sequences on opposite sides at each end of the target sequence.
  - A heat-stable DNA polymerase extends the primers in the 5′→3′ direction.
- If a standard DNA polymerase were used, the protein would be denatured along with the DNA during the first heating step.
- The key to easy PCR automation was the discovery of an unusual DNA polymerase, isolated from a bacterium living in hot springs, that can withstand the heat needed to separate the DNA strands at the start of each cycle.
- Just as impressive as the speed of PCR is its specificity.
- The key to this high specificity is the primers, which hydrogen-bond only to sequences at opposite ends of the target segment.
- Only minute amounts of DNA need be present in the starting material, as long as a few molecules contain the complete target sequence.
  - The DNA can be in a partially degraded state.
- By the end of the third cycle, one-fourth of the molecules are identical to the target segment, with both strands the appropriate length.
- With each successive cycle, the number of target segment molecules of the correct length doubles, soon greatly outnumbering all other DNA molecules in the reaction.
- Despite its speed and specificity, PCR amplification cannot substitute for gene cloning in cells when large amounts of a gene are desired.
  - Occasional errors during PCR replication impose limits on the number of good copies that can be made.
  - When PCR is used to provide the specific DNA fragment for cloning, the resulting clones are sequenced to select clones with error-free inserts.
- Devised in 1985, PCR has had a major impact on biological research and technology.

- PCR has amplified DNA from a variety of sources: fragments of ancient DNA from a 40,000-year-old frozen woolly mammoth; DNA from footprints or tiny amounts of blood or semen found at the scenes of violent crimes; DNA from single embryonic cells for the rapid prenatal diagnosis of genetic disorders; and DNA of viral genes from cells infected with HIV.

Concept 20.2 DNA technology allows us to study the sequence, expression, and function of a gene.

- Once scientists have prepared homogeneous samples of DNA, each containing a large number of identical segments, they can ask some interesting questions about specific genes and their functions.
  - Does the sequence of the hummingbird β-globin gene code for a protein structure that can carry oxygen more efficiently than its counterpart in less metabolically active species?
  - Does a particular human gene differ from person to person?
  - Are certain alleles of that gene associated with a hereditary disorder?
  - Where in the body and when during development is a given gene expressed?
  - What role does a certain gene play in an organism?
- To answer these questions, researchers need to know the nucleotide sequence of the gene and its counterparts in other individuals and species, as well as its expression pattern.

One method of rapidly analyzing and comparing genomes is gel electrophoresis.

- **Gel electrophoresis** separates macromolecules—nucleic acids or proteins—on the basis of their rate of movement through a polymer gel in an electrical field.
  - The rate of movement of each molecule depends on its size, electrical charge, and other physical properties.
- In *restriction fragment analysis*, the DNA fragments produced by restriction enzyme digestion of a DNA molecule are sorted by gel electrophoresis.
  - When the mixture of restriction fragments from a particular DNA molecule undergoes electrophoresis, it yields a band pattern characteristic of the starting molecule and the restriction enzyme used.
  - The relatively small DNA molecules of viruses and plasmids can be identified simply by their restriction fragment patterns.
- The separated fragments can be recovered undamaged from gels, providing pure samples of individual fragments.
- Scientists can use restriction fragment analysis to compare two different DNA molecules representing, for example, different alleles of a gene.
  - Because the two alleles differ slightly in DNA sequence, they may differ in one or more restriction sites.
  - If the alleles do differ in restriction sites, each produces different-sized fragments when digested by the same restriction enzyme.
  - In gel electrophoresis, the restriction fragments from the two alleles produce different band patterns, allowing researchers to distinguish the two alleles.
- Restriction fragment analysis is sensitive enough to distinguish between two alleles of a gene that differ by only one base pair in a restriction site, such as the normal and sickle-cell alleles of the β-globin gene.

- A method called **Southern blotting** combines gel electrophoresis with nucleic acid hybridization.
  - One of this method's many applications is to identify heterozygous carriers of mutant alleles associated with genetic disease, although more rapid methods involving PCR amplification are currently used for this.
  - Because gel electrophoresis yields too many bands to distinguish individually, scientists use nucleic acid hybridization with a specific probe to label discrete bands that derive from the gene of interest.
  - The probe is a radioactive, single-stranded DNA molecule that is complementary to the gene of interest (for example, the β-globin gene).

The entire nucleotide sequence of a gene can be determined.

- Gene sequencing has been automated based on a technique called the *dideoxyribonucleotide* (or *dideoxy*, for short) *chain termination method.*
- Knowing the entire nucleotide sequence of a gene allows researchers to compare it to genes in other species, whose function may be known.
- If two genes from different species are similar in sequence, their gene products likely perform similar functions.

Other experimental approaches analyze when and where a gene is expressed.

- Labeled nucleic acid probes that hybridize with mRNAs can provide information about the time or place in the organism at which a gene is transcribed.
- For example, there are two ways to find out how the expression of the β-globin gene changes during the embryonic development of the hummingbird.
- In the first method, called **Northern blotting**, scientists carry out gel electrophoresis on samples of mRNA from hummingbird embryos at different stages of development, transfer the samples to a nitrocellulose membrane, and then allow the mRNAs on the membrane to hybridize with a labeled probe that recognizes β-globin mRNA.
  - If the mRNA band is seen at a particular stage, scientists can hypothesize that the protein functions during events taking place at that stage.
- A new and more sensitive method is called the **reverse transcriptase-polymerase chain reaction**, or **RT-PCR**.
  - Analysis of hummingbird β-globin gene expression with RT-PCR begins similarly to Northern blotting, with the isolation of mRNAs from different developmental stages of hummingbird embryos.
  - Next, reverse transcriptase is added to make cDNA, which then serves as a template for PCR amplification using primers from the β-globin gene.
  - When the products are run on a gel, copies of the amplified region are observed as bands only in samples that originally contained the β-globin mRNA.
  - In the case of hummingbird β-globin, scientists might expect to see a band appear at the stage when red blood cells begin forming, with all subsequent stages showing the same band.
- RT-PCR can also be carried out with mRNAs collected from different tissues at one time to discover which tissue is producing a specific mRNA.
- Specific mRNAs can be identified by using labeled probes in place, or *in situ,* in the intact organism.
  - This technique, called ***in situ* hybridization**, is most often carried out with probes labeled by the attachment of fluorescent dyes.

- When the entire genomes of a number of organisms have been sequenced, it is possible to study the expression of large groups of genes, to study how genes act together to produce and maintain a functioning organism.
- Researchers use genome sequences as probes to investigate which genes are transcribed in different situations, such as in different tissues or at different stages of development.
- Researchers also look for groups of genes that are expressed in a coordinated manner, with the aim of identifying networks of gene expression across an entire genome.
- The basic strategy in such global (genome-wide) expression studies is to isolate the mRNAs made in particular cells, use these molecules as templates for making the corresponding cDNAs by reverse transcription, and then employ nucleic acid hybridization to compare this set of cDNAs with a collection of DNA fragments representing all or part of the genome.
- The results identify the subset of genes in the genome that are being expressed at a given time or under certain conditions.
- Genome-wide expression studies are made possible by the use of **DNA microarray assays**.
  - A DNA microarray consists of tiny amounts of a large number of single-stranded DNA fragments representing different genes fixed to a glass slide in a tightly spaced array, or grid, also called a *DNA chip*.
  - Ideally, these fragments represent all the genes of an organism.
  - The DNA fragments on a microarray are tested for hybridization with cDNA molecules that have been prepared from the mRNAs in particular cells of interest and labeled with fluorescent dyes.
- In one example of a global expression study, researchers performed DNA microarray assays on more than 90% of the genes of the nematode *Caenorhabditis elegans* during every stage of its life cycle.
- The expression of nearly 60% of the genes changed dramatically during development. Many genes were expressed in a sex-specific pattern.
- In addition to uncovering gene interactions and providing clues to gene function, DNA microarray assays may contribute to a better understanding of certain diseases and suggest new diagnostic techniques or therapies.
  - Comparing patterns of gene expression in breast cancer tumors and noncancerous breast tissue, for example, has already resulted in more informed and effective treatment protocols.

Scientists disable genes and observe the consequences to determine the function of the genes.

- In an application called ***in vitro* mutagenesis**, specific mutations are introduced into the sequence of a cloned gene, and then the mutated gene is returned to a cell in such a way that it disables ("knocks out") the normal cellular copies of the same gene.
- If the introduced mutations alter or destroy the function of the gene product, the phenotype of the mutant cell may help reveal the function of the missing normal protein.
- Researchers can even put such a mutated gene into cells from the early embryo of a multicellular organism (such as a mouse) to study the role of the gene in the development and functioning of the whole organism.
- In 2006, government agencies in the United States, Canada, China, and the European Union announced several large-scale projects whose aim is to disable every one of the 25,000 genes in the mouse genome.
- A newer method for silencing the expression of selected genes exploits the phenomenon of **RNA interference (RNAi).**

- This approach uses synthetic, double-stranded RNA molecules that match the sequence of a particular gene to trigger the breakdown of the gene's messenger RNA or to block its translation.
  - To date, the RNAi technique has been used successfully to reduce ("knock down") the expression of specific genes in mammalian cells, including human cells in culture, and plans are under way to try using RNAi for the treatment of human disorders such as macular degeneration of the eye.
  - In one study, RNAi was used to prevent the expression of 86% of the genes in early nematode embryos, one gene at a time.
  - Analysis of the phenotypes of the worms that developed from these embryos enabled the researchers to classify most of the genes into a small number of groups by function.

Concept 20.3 Cloning organisms may lead to the production of stem cells for research and other applications.

- Scientists can now clone multicellular organisms from single cells.
  - This is called *organismal cloning* to distinguish it from gene cloning and cell cloning, the division of an asexually reproducing cell into a collection of genetically identical cells.
- Organismal cloning has the potential to generate stem cells, which can develop into many different tissues.
- The cloning of plants and animals was first attempted more than 50 years ago in experiments designed to determine whether all the cells of an organism have the same genes (a concept called *genomic equivalence*) or whether cells lose genes during the process of differentiation.

Whole plants have been cloned from single differentiated cells since the 1950s.

- Successful attempts to clone whole plants from single differentiated cells were made during the 1950s by F. C. Steward of Cornell University.
- Steward and his students found that cultured, differentiated root cells could grow into normal adult plants, each genetically identical to the parent plant, thus demonstrating that differentiation does not necessarily involve irreversible changes in the DNA.
- In plants, mature cells can dedifferentiate and then give rise to all the specialized cell types of the organism. Any cell with this potential is said to be **totipotent**.
- Plant cloning is now used extensively in agriculture to reproduce plants that have valuable characteristics, such as the ability to resist a plant pathogen.

Nuclear transplantation was used in animal experiments.

- Differentiated cells from animals do not readily divide in culture.
- Early researchers investigating whether differentiated animal cells can be totipotent removed the nucleus of an unfertilized frog's egg or zygote and replaced it with the nucleus of a differentiated cell from a tadpole, a procedure called *nuclear transplantation*.
- If the nucleus from the differentiated donor cell retains its full genetic capability, then it should be able to direct development of the recipient egg into all the tissues and organs of an organism.
- The transplanted nucleus was often able to support normal development of the egg into a tadpole.
- However, the potential of a transplanted nucleus to direct normal development was inversely related to the age of the donor: The older the donor nucleus, the lower the percentage of normally developing tadpoles.

- Clearly, something in the nucleus changed as the tadpole's cells differentiated.
  - In frogs and most other animals, nuclear potential tends to be increasingly restricted as embryonic development and cell differentiation progress.
- In 1997, Scottish researchers captured newspaper headlines when they announced the birth of Dolly, a lamb cloned from an adult sheep by nuclear transplantation from a differentiated cell.
- These researchers cultured donor mammary cells in a nutrient-poor medium and then fused these cells with enucleated sheep eggs.
- The resulting diploid cells divided to form early embryos, which were implanted into surrogate mothers.
  - Out of several hundred implanted embryos, one successfully completed normal development, and Dolly was born.
- Later analyses showed that Dolly's chromosomal DNA was identical to that of the nucleus donor.
- Dolly's mitochondrial DNA came from the egg donor.
- In 2003, at age 6, Dolly suffered complications from a lung disease usually seen in only much older sheep, and she was euthanized.
- Dolly's premature death led to speculation that her cells were not quite as "healthy" as those of a normal sheep, possibly reflecting incomplete reprogramming of the original transplanted nucleus.
- Since 1997, *reproductive cloning*—the production of new individuals—has been demonstrated in many other mammals, including mice, cats, cows, horses, mules, pigs, and dogs.
- Cloned animals of the same species do *not* always look or behave identically.
  - In a herd of cows cloned from the same line of cultured cells, certain cows are dominant and others are more submissive.
  - The first cloned cat, named CC for Carbon Copy has a calico coat, like her single female parent, but the color and pattern are different because of random X chromosome inactivation, which is a normal occurrence during embryonic development.
  - Identical human twins, naturally occurring "clones," always differ somewhat.
  - Environmental influences and random phenomena can play a significant role during development.
- The successful cloning of so many mammals has led to speculation about the cloning of humans.
- Nuclei from differentiated human cells have been transplanted into unfertilized enucleated eggs, and the eggs stimulated to divide.
- In 2001, a research group in Massachusetts observed a few early cell divisions in such an experiment.

There are problems associated with animal cloning.

- In most nuclear transplantation studies, very few cloned embryos develop normally to birth and many of those exhibit defects.
  - Even cloned animals that appear normal likely have subtle defects.
- In the nuclei of fully differentiated cells, the expression of many genes is repressed as a result of epigenetic changes in chromatin, such as acetylation of histones or methylation of DNA.
- During the nuclear transfer procedure, many of these changes must be reversed in the later-stage nucleus from a donor animal in order for genes to be expressed or repressed appropriately in early stages of development.

- DNA in embryonic cells from cloned embryos, like DNA of differentiated cells, tends to have more methyl groups than DNA in equivalent cells from uncloned embryos of the same species.
- This suggests that the reprogramming of donor nuclei requires chromatin restructuring, which does not occur fully during cloning procedures.
- Because DNA methylation helps regulate gene expression, misplaced methyl groups in the DNA of donor nuclei may interfere with the pattern of gene expression necessary for normal embryonic development.

Stem cells can be used to treat human diseases.

- Scientists' major aim in cloning human embryos is the production of stem cells for treating human diseases.
- A **stem cell** is a relatively unspecialized cell that can both reproduce itself indefinitely and, under appropriate conditions, differentiate into specialized cells of one or more types.
- Thus, stem cells are able both to replenish their own population and to generate cells that travel down specific differentiation pathways.
- Many early animal embryos contain stem cells capable of giving rise to any type of differentiated embryonic cells.
  - *Embryonic stem cells* can be isolated from early embryos at the blastula or blastocyst stage.
- The adult body also has *adult stem cells*, which are able to give rise to many, but not all, cell types.
  - For example, bone marrow contains several types of stem cells, including one that can generate all the different kinds of blood cells and another that can differentiate into bone, cartilage, fat, muscle, and the linings of blood vessels.
  - Even the adult brain contains stem cells that produce certain kinds of nerve cells.
- Scientists are learning to identify and isolate stem cells from various tissues and, in some cases, to grow them in culture.
- With the addition of specific growth factors, cultured stem cells from adult animals have been made to differentiate into multiple types of specialized cells.
- This technology may provide cells for the repair of damaged or diseased organs, such as insulin-producing pancreatic cells for people with diabetes or certain kinds of brain cells for people with Parkinson's disease or Huntington's disease.
- Embryonic stem cells offer more potential than adult stem cells for medical applications, at least for now.
- However, deriving embryonic stem cells from human embryos raises ethical and political issues.
  - Embryonic stem cells are currently obtained from embryos donated by patients undergoing infertility treatment or from long-term cell cultures originally established from donated embryos.
  - If scientists can clone human embryos to the blastocyst stage, they might be able to use such clones as the source of embryonic stem cells.
  - A donor nucleus from a patient with a particular disease could allow the production of embryonic stem cells for treatment that match the patient and are not rejected by his or her immune system.
- Although most people believe that the reproductive cloning of humans is unethical, opinions vary about the morality of *therapeutic cloning*, whose major aim is to produce embryonic stem cells to treat disease.

- Some believe it is wrong to create embryos that will be destroyed, whereas others, in the words of the researcher who created Dolly, believe that "cloning promises such great benefits that it would be immoral not to do it."

Concept 20.4 The practical applications of DNA technology affect our lives in many ways.

DNA technology is reshaping medicine and the pharmaceutical industry.

- Modern biotechnology is making enormous contributions both to the diagnosis of diseases and in the development of pharmaceutical products.
- The identification of genes whose mutations are responsible for genetic diseases may lead to ways to diagnose, treat, or even prevent these conditions.
- Susceptibility to many "nongenetic" diseases, from arthritis to AIDS, is influenced by a person's genes.
  - Diseases of all sorts involve changes in gene expression within the affected genes and within the patient's immune system.
  - Using DNA microarray assays and other techniques to compare gene expressions in healthy and diseased tissue can identify these changes and lead to the development of targets for prevention or therapy.
- PCR and labeled nucleic acid probes can track down the pathogens responsible for infectious diseases.
  - For example, RT-PCR can amplify and thus detect HIV DNA in blood and tissue samples, detecting an otherwise elusive infection.
- Medical scientists can use DNA technology to identify individuals who have genetic diseases before the onset of symptoms, even before birth.
- Genetic disorders are diagnosed by using PCR and primers corresponding to cloned disease genes, and then sequencing the amplified product to look for the disease-causing mutation.
  - Cloned disease genes include those for sickle-cell disease, hemophilia, cystic fibrosis, Huntington's disease, and Duchenne muscular dystrophy.
- It is possible to detect abnormal allelic forms of genes even in cases in which the gene has not yet been cloned.
- The presence of an abnormal allele can be diagnosed with reasonable accuracy if a closely linked *genetic marker* has been found.
  - A genetic marker is a DNA sequence that varies in a population; in a gene, such sequence variation is the basis of different alleles.
- Just like coding sequences, noncoding DNA at a specific place or locus on a chromosome may exhibit small nucleotide differences among individuals, or *polymorphisms*.
- Single base-pair variations in the genomes of the human population serve as useful genetic markers.
- A single base-pair site where variation is found in at least 1% of the population is called a **single nucleotide polymorphism** (**SNP**).
  - SNPs occur on average about once in 100 to 300 base pairs in the human genome, in both coding and noncoding sequences.

- Some SNPs alter the sequence recognized by a restriction enzyme, changing the lengths of the restriction fragments formed by digestion with that enzyme.
  - This type of sequence change is called a **restriction fragment length polymorphism** (**RFLP**, pronounced "Rif-lip").
- Today, SNPs can be detected by very sensitive microarray analysis or by PCR.
- The presence of an abnormal allele that causes a genetic disorder can be diagnosed with reasonable accuracy if a closely linked SNP marker has been found.
  - Alleles for Huntington's disease and a number of other genetic diseases were first detected by means of RFLPs in this indirect way.
  - If the marker and the gene itself are close enough, crossing over between the marker and the gene is very unlikely to occur during gamete formation, and the two regions are almost always inherited together.

Human gene therapy holds great potential.

- **Gene therapy**—introducing genes into an afflicted individual for therapeutic purposes—holds great potential for treating genetic disorders caused by a single gene.
- In theory, a normal allele of the defective gene could be inserted into dividing somatic cells of the tissue affected by the disorder, such as bone marrow cells.
  - Bone marrow cells, which include the stem cells that give rise to all the cells of the blood and immune system, are prime candidates for gene therapy.
- Severe combined immunodeficiency (SCID), caused by a single defective gene, has been treated in gene therapy trials.
  - If successful, gene therapy cures the patient, whose bone marrow cells then produce the missing protein.
  - In 2000, ten young children with SCID were treated by gene therapy.
  - After two years, nine of these patients showed significant improvement. However, three of the patients subsequently developed leukemia and one died.
  - The retroviral vector used to carry the normal allele into bone marrow cells had inserted near a gene involved in the proliferation and development of blood cells, which may have caused leukemia.
  - In a recent experiment on mice, a replacement of the mouse version of the same gene led to a high incidence of lymphoma, a blood cancer.
  - Perhaps an unknown function of the gene itself may be responsible.
- Gene therapy also raises technical questions.
  - How can the activity of the transferred gene be controlled so that cells make appropriate amounts of the gene product at the right time and in the right place?
  - How can scientists be sure that the insertion of the therapeutic gene does not harm some other necessary cell function?
- In addition to these technical challenges, gene therapy raises ethical questions.
- Some critics believe that tampering with human genes in any way is immoral.
- Others see no difference between the transplantation of genes into somatic cells and the transplantation of organs.
- The treatment of human germ-line cells in the hope of correcting a defect in future generations raises ethical questions.

- Under what circumstances, if any, should we alter the genomes of human germ lines?
- Would this inevitably lead to the practice of eugenics, a deliberate effort to control the genetic makeup of human populations?

- From a biological perspective, the elimination of unwanted alleles from the gene pool could be problematic because genetic variation is a necessary ingredient for the survival of a species as environmental conditions change with time.
  - Genes that are damaging under some conditions may be advantageous under other conditions.
  - Do we have the right to make genetic changes that could be detrimental to the survival of our species in the future?

The pharmaceutical industry uses biotechnology to develop new drugs.

- Small molecules can be tailored to combat certain cancers by blocking the function of a protein crucial for the tumor cells' survival.
- One drug, called Gleevec (or imatinib), is a small molecule that inhibits a specific receptor tyrosine kinase.
- The overexpression of this receptor, resulting from a chromosomal translocation, is instrumental in causing chronic myelogenous leukemia (CML).
- Patients in the early stages of CML who have been treated with Gleevec have exhibited nearly complete, sustained remission from the cancer.
- Similar drugs have also been used to treat a few types of lung and breast cancers, whose molecular basis is fairly well understood.

Proteins are produced in cell cultures.

- DNA cloning and gene expression systems can produce large quantities of proteins that are present naturally in only minute amounts.
- The host cells used in these expression systems can be engineered to secrete a protein as it is made, thus removing the need for its purification.
- Among the first pharmaceutical products "manufactured" in this way were human insulin and human growth hormone (HGH).
  - Some 2 million people with diabetes in the United States require daily insulin injections.
  - Human growth hormone has been used to treat children born with a form of dwarfism caused by inadequate amounts of HGH.
- Another important pharmaceutical product created by genetic engineering is tissue plasminogen activator (TPA).
  - If administered shortly after a heart attack, TPA helps dissolve blood clots and reduces the risk of subsequent heart attacks.
- Cells in culture can also be used to produce vaccines, which stimulate the immune system to defend against specific pathogens.
- Alternatively, genetic engineering can be used to modify a pathogen's genome, resulting in a weakened pathogen that can then serve as a vaccine.

Proteins are produced by "pharm" animals and plants.

- In some cases, whole animals are used to produce proteins, instead of cell systems.
- Using experimental methods, scientists introduce a gene from one animal into the genome of another animal, which is then called a **transgenic** animal.

- To do this, scientists first remove eggs from a female and fertilize them *in vitro*.
- They then inject the cloned DNA from another organism directly into the nuclei of the fertilized eggs.
- Some of the cells integrate the foreign DNA, the *transgene*, into their genomes and are able to express the foreign gene.
- The engineered embryos are then surgically implanted into a surrogate mother.
- If an embryo develops successfully, the result is a transgenic animal, containing a gene from a third "parent."
- Assuming the introduced gene encodes a protein desired in large quantities, these transgenic animals can act as pharmaceutical "factories."
    - For example, a transgene for a human blood protein such as antithrombin has been inserted into the genome of a goat so that antithrombin is secreted in the animal's milk.
    - The protein can then be purified from the milk.
    - Researchers have also engineered transgenic chickens that express large amounts of the transgene's product in eggs.
- The human proteins produced by farm animals may differ in some ways from the corresponding natural human proteins, so they must be tested carefully to ensure that they will not cause allergic reactions or other adverse effects in patients who receive them.
- In a novel twist, the pharmaceutical industry is beginning to develop "pharm" plants, analogous to "pharm" animals.
- "Pharm" plants make human proteins for medical use and viral proteins for use as vaccines.
    - In 2007, the U.S. Food and Drug Administration approved a plan to plant more than 3,000 acres in Kansas with rice containing genes for milk proteins, used to treat infant diarrhea.

Genetic profiles may be important forensic evidence.

- Body fluids or small pieces of tissue may be left at the scene of a violent crime or on the clothes of the victim or assailant.
- If enough blood, semen, or tissue is available, forensic laboratories can determine the blood type or tissue type by using antibodies to detect specific cell-surface proteins.
- Such tests require fairly fresh samples in relatively large amounts, however, and can serve only to exclude a particular suspect.
- DNA testing, in contrast, can identify the guilty individual with a high degree of certainty because the DNA sequence of every person is unique (except for identical twins).
- Genetic markers that vary in the population can be analyzed for a given person to determine that individual's unique set of genetic markers, or **genetic profile**.
- The FBI started applying DNA technology in forensics in 1988, using RFLP analysis by Southern blotting to detect similarities and differences in DNA samples.
    - This method required much smaller samples of blood or tissue than earlier methods—only about 1,000 cells.
- Today, in place of RFLPs, forensic scientists usually use an even more sensitive method, which takes advantage of genetic markers called **short tandem repeats (STRs).**
- STRs are variations in the lengths of certain repeated base sequences in specific regions of the genome.

- The number of repeats present in these regions is highly polymorphic, varying from person to person.
  - For example, one individual may have the sequence ACAT repeated 30 times at one genome locus, whereas another individual may have 18 repeats at this locus.
- PCR is used to amplify particular STRs using sets of primers that are labeled with different colored fluorescent tags; the length of the region, and thus the number of repeats, can then be determined by electrophoresis.
  - Since Southern blotting is not required, this method is quicker than RFLP analysis.
- The PCR step allows the method to be used even when the DNA is in poor condition or available in only minute quantities.
  - A tissue sample containing as few as 20 cells can be sufficient for PCR amplification.
  - In a murder case, for example, this method can be used to compare DNA samples from the suspect, the victim, and a small amount of blood found at the crime scene.
- Forensic scientists test only a few selected portions of the DNA—about 13 STR markers.
- The probability that two people (who are not identical twins) would have exactly the same set of STR markers is vanishingly small.
- The Innocence Project, a nonprofit organization dedicated to overturning wrongful convictions, uses STR analysis of archived samples from crime scenes to revisit old cases.
  - As of 2006, 18 innocent people had been released from prison as a result of forensic and legal work by this group.
- In another example of genetic profiling, a comparison of the DNA of a mother, her child, and the purported father can conclusively settle a question of paternity.
  - Sometimes paternity is of historical interest: Genetic profiles provided strong evidence that Thomas Jefferson or one of his close male relatives fathered at least one of the children of his slave, Sally Hemings.
- Analyzing genetic profiles can also identify victims of mass casualties.
  - After the World Trade Center attack in 2001, more than 10,000 samples of victims' remains were compared with DNA traces on personal items provided by families.
  - These comparisons led to the identification of nearly 3,000 victims.
- Just how reliable is a genetic profile?
- The greater the number of markers examined in a DNA sample, the more likely it is that the profile is unique to one individual.
- In forensic cases using STR analysis with 13 markers, the probability of two people having identical DNA profiles is somewhere between one chance in 10 billion and one in several trillion.
- The exact probability depends on the frequency of those markers in the general population.
  - Information on how common various markers are in different ethnic groups is critical because these marker frequencies may vary considerably among ethnic groups and between a particular ethnic group and the population as a whole.
- With the increasing availability of frequency data, forensic scientists can make extremely accurate statistical calculations.
- Genetic profiles are now accepted as compelling evidence by legal experts and scientists alike.

Microorganisms can be used for environmental cleanup.

- As a group, microorganisms have a remarkable ability to transform chemicals.

- Many bacteria can extract heavy metals, such as copper, lead, and nickel, from their environments and incorporate the metals into compounds such as copper sulfate or lead sulfate, which are readily recoverable.
- Genetically engineered microbes may become important in both mining minerals (especially as ore reserves are depleted) and cleaning up highly toxic mining wastes.
- Biotechnologists are also trying to engineer microbes that can be used in wastewater treatment plants to degrade chlorinated hydrocarbon.

Biofuels can supplement or replace fossil fuels.

- Biofuels from crops such as corn, soybeans, and cassava have been proposed as a supplement or even replacement for fossil fuels.
  - To produce "bioethanol," starch is converted to sugar and then fermented by microorganisms; a similar process can produce "biodiesel" from plant oils. Either product can be mixed with gasoline or used alone to power vehicles.
- Critics charge that the environmental effects of growing these crops makes their total impact greater than that of fossil fuels.

DNA technology has agricultural applications.

- The selective breeding of livestock, or animal husbandry, has exploited naturally occurring mutations and genetic recombination for centuries.
- DNA technology permits the genetic engineering of transgenic animals, which speeds up the selective breeding process.
  - The goals of creating a transgenic animal are often the same as the goals of traditional breeding—for instance, to make a sheep with better quality wool, a pig with leaner meat, or a cow that will mature in a shorter time.
  - Scientists might, for example, identify and clone a gene that leads to the development of larger muscles (muscles make up most of the meat we eat) in one variety of cattle and transfer it to other cattle or even to sheep.
  - This manipulation may cause low fertility or increased susceptibility to disease, however.
- Plants are easier to genetically engineer than most animals because a single tissue cell grown in culture and genetically manipulated can give rise to an adult plant with new traits.
- The most commonly used vector for introducing new genes into plant cells is a plasmid, called the **Ti plasmid**, from the soil bacterium *Agrobacterium tumefaciens*.
  - This plasmid integrates a segment of its DNA, known as T DNA, into the chromosomal DNA of its host plant cells.
  - For vector purposes, researchers work with versions of the plasmid that do not cause disease.
- Scientists can introduce recombinant Ti plasmids into plant cells by electroporation or by infecting plant cells in culture with bacteria containing the recombinant plasmid.
- Alternatively, the recombinant plasmid can be put back into *Agrobacterium*; susceptible plants or plant cells growing in culture are then infected with bacteria that contain the recombinant plasmid.
- Genetic engineering is rapidly replacing traditional plant-breeding programs for simple genetic traits such as herbicide or pest resistance.
  - Genetically engineered crops that can resist destructive insects have reduced the need for chemical insecticides.

- In India, the insertion of a salinity-resistance gene from a coastal mangrove plant into the genomes of several rice varieties has resulted in rice plants that can grow in water three times as salty as seawater.
  - The research foundation that carried out this genetic engineering estimates that one-third of all irrigated land has high salinity owing to over-irrigation and intensive use of chemical fertilizers, which represents a serious threat to the food supply. Salinity-resistant crop plants would be enormously valuable worldwide.
- Genetic engineering also has great potential for improving the nutritional value of crop plants.
  - For instance, scientists have developed transgenic rice plants that produce yellow rice grains containing beta-carotene, which our body uses to make vitamin A.
  - This "golden" rice could help prevent vitamin A deficiency in the half of the world's population that depends on rice as a staple food.

DNA technology raises some safety and ethical concerns.

- Early concerns about potential dangers associated with recombinant DNA technology focused on the possibility that hazardous new pathogens might be created.
  - What might happen, for instance, if cancer cell genes were transferred into bacteria or viruses?
- To guard against such rogue microbes, scientists developed strict laboratory procedures designed to protect researchers from infection by engineered microbes and to prevent the microbes from accidentally leaving the laboratory.
- Strains of microorganisms to be used in recombinant DNA experiments are genetically crippled to ensure that they cannot survive outside the laboratory, and certain obviously dangerous experiments have been banned.
- Today, most public concern about possible hazards centers not on recombinant microbes but on **genetically modified (GM)** organisms used as food.
- A GM organism is one that has acquired by artificial means one or more genes from another species or even from another variety of the same species.
  - Salmon, for example, have been genetically modified by the addition of a more active salmon growth hormone gene.
- GM crops are widespread in the United States, Argentina, and Brazil; together these countries account for more than 80% of the world's acreage devoted to GM crops.
  - In the United States, the majority of corn, soybeans, and canola are GM crops, and GM products are not required to be labeled.
- GM crops are a subject of ongoing controversy in Europe, where the GM revolution has met with strong opposition from countries concerned about the safety of GM foods and possible environmental consequences of growing GM plants.
  - Although a small number of GM crops have been grown on European soil, their products have generally failed in local markets, and the future of GM crops in Europe is uncertain.
- Early in 2000, negotiators from 130 countries (including the United States) agreed on a Biosafety Protocol that requires exporters to identify GM organisms present in bulk food shipments and allows importing countries to decide whether the products pose environmental or health risks.
- Advocates of a cautious approach toward GM crops fear that transgenic plants might pass their new genes on to close relatives in nearby wild areas.
  - If crop plants carrying genes for resistance to herbicides, diseases, or insect pests pollinated wild plants, the offspring might become "super weeds" that are very difficult to control.

- Another possible hazard, suggested by one laboratory-based study, is that a transgene encoding a pesticide-type protein might cause plants to produce pollen toxic to butterflies.
- However, scientists with the Agricultural Research Service concluded from a two-year study that butterflies are unlikely to be exposed to toxic levels of pollen.
- Some people fear that the protein products of transgenes might lead to allergic reactions, although advocates suggest that advance tests would prevent the production of such proteins.
- In the United States, new applications of biotechnology are evaluated for potential risks by various regulatory agencies.
- Advances in biotechnology have enabled scientists to obtain complete genome sequences for humans and many other species, providing a vast treasure trove of information about genes.
- The increasing speed and decreasing cost with which genome sequences of individual people can be determined are raising significant ethical questions.
  - Who should have the right to examine someone else's genetic information?
  - How should a person's genetic information be used?
  - Should a person's genome be a factor in suitability for a job or eligibility for insurance?
- The power of DNA technology and genetic engineering—our ability to profoundly and rapidly alter species that have been evolving for millennia—demands that we proceed with humility and caution.