Chapter 8

Genetic Engineering: Transcription, Translation, and Genetically Modified Organisms

1

8.1 Genetic Engineers

- **Genetic engineers** – ________________________
  ____
- The manipulation that they perform include changing a gene, changing how a gene is regulated (turn on or off), or moving a gene from one organism to another
- Genetic engineers in academia, government, and industry manipulate genes for both nonprofit and for-profit reasons

2

8.2 Protein Synthesis and Gene Expression

- In the early 1980s, genetic engineers at Monsanto® Company began producing **recombinant bovine growth hormone** (***r*****BGH**)
- Made by genetically engineered bacteria
- The bacteria were given DNA that carries instructions for making BGH

3

Protein Synthesis and Gene Expression

- Growth hormones act on organs to increase body size and milk production
- Before genetic engineering, the growth hormone was taken from the pituitary glands of the brains of slaughtered cows and then injected into live cows

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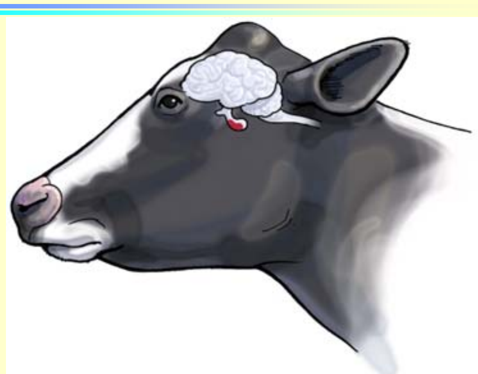

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Protein Synthesis and Gene Expression

- This can also be done to get human growth hormone from human cadavers
- When injected into humans with **pituitary dwarfism**, they grow in size
- Collecting growth hormone from dead animals is time consuming and results in relatively small amounts of hormone
- Genetic engineers wanted to produce BGH in large quantities in a lab to give to cows

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From Genes to Protein

- How are proteins made?
- A gene carries instructions for building a protein in a process called ______________________________
- Involves DNA and **RNA** (______________________________)

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From Genes to Protein: Basics

- __________: a polymer of nucleotides that make A to T and C to G chemical bonds
- __________: a sequence of DNA that encodes proteins (large amino acids molecules)
- Each protein has a unique function dictated by its particular structure – which results from how it folds due to the order of amino acids that comprise it

8

From Genes to Protein: Basics

- Instructions carried by the gene are copied before the protein can be built
- The gene copy is comprised of RNA, not DNA
- The differences between DNA and RNA are important

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DNA

- Double stranded
- Nucleotide made of the sugar **deoxyribose**, a phosphate group, and a nitrogen-containing base (**A, G, C, or T**)

RNA

- Single stranded
- Nucleotide made of the sugar **ribose**, a phosphate group, and a nitrogen-containing base (**A, G, C, or U**)

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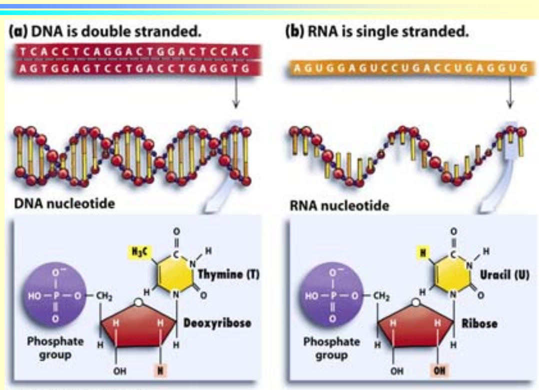

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From Genes to Protein

- When making a copy of the gene, the RNA nucleotides base pair with the DNA:
  - DNA T pairs with RNA A
  - DNA C pairs with RNA G
  - DNA G pairs with RNA C
  - DNA A pairs with RNA U

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From Genes to Protein

- The RNA copy is the blueprint that tells the cell which amino acids to join together to produce a protein
- Genetic information flow:

  DNA → RNA → protein

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From Genes to Protein

Flowing information from gene to protein takes two steps:

1. ___________________: involves producing the copy of the required gene (RNA synthesis)
2. ___________________: involves decoding the copied RNA sequence and producing the protein for which it codes (protein synthesis)

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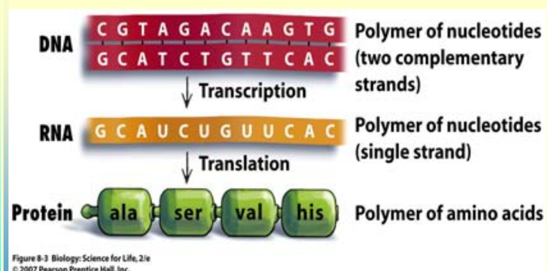

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Transcription

- Occurs in the nucleus
- An ___________________enzyme binds to the **promoter** (nucleotide sequence at the beginning of every gene) and makes a **mRNA** (**messenger RNA**) complementary to the DNA gene
  - Messenger RNA ___________________________

    ____

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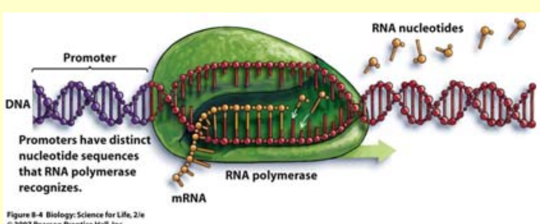

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Translation

- Occurs in the cytoplasm
- mRNA carries the code from the DNA
- Amino acids are assembled to synthesize proteins at _____________________
  - Ribosomes are subcellular, globular structures that are composed of another kind of RNA called **ribosomal RNA (rRNA)**, which is wrapped around many different proteins

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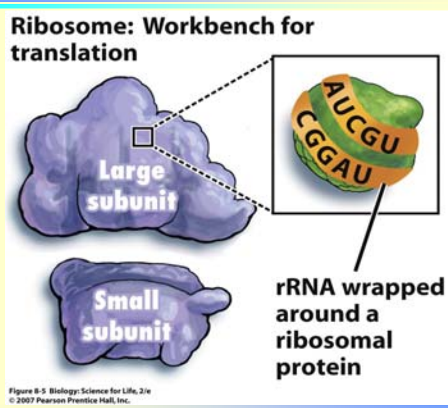
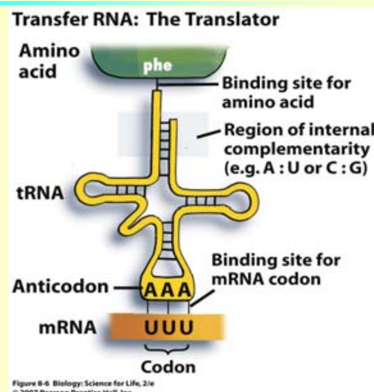


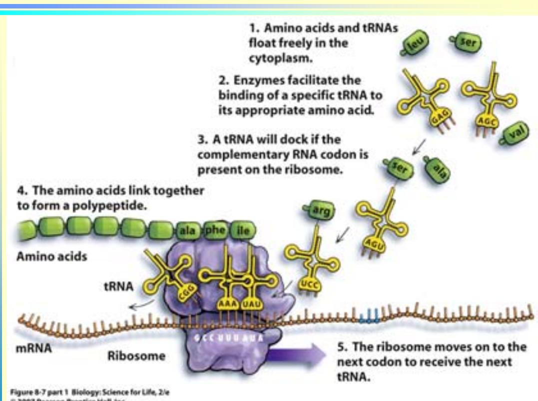
Translation

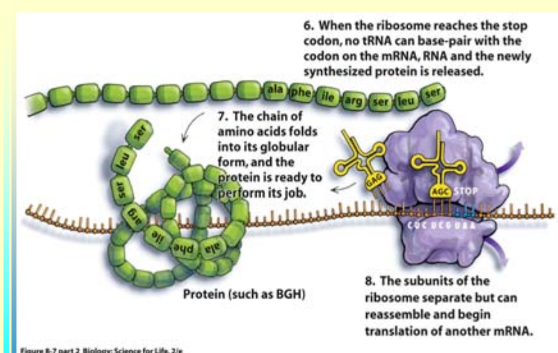
- **tRNA** (**transfer RNA**) – amino-acid-carrying RNA molecule with an anticodon that binds to an mRNA codon
- ____________ – sequence of mRNA that is 3 nucleotides long and encode an amino acid
- The codons on the mRNA match with the _____________ **–** region of tRNA that binds to a complementary mRNA codon

Translation

- That is how the correct tRNA brings in the correct amino acid
- The protein is built starting with a start codon on the RNA and is built until there is a stop codon on the RNA







Genetic Code

- The sequence from DNA dictates the order of amino acids in the proteins
- Scientists have figured out the **genetic code** and made a chart that tells what amino acid is coded for by what codon
  - Table showing which mRNA codons code for which amino acids

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Second base

First base	U	C	A	G	Third base
U	UUU, UUC Phenyl-alanine (phe); UUA, UUG Leucine (leu)	UCU, UCC, UCA, UCG Serine (ser)	UAU, UAC Tyrosine (tyr); UAA Stop codon; UAG Stop codon	UGU, UGC Cysteine (cys); UGA Stop codon; UGG Tryptophan (trp)	U C A G
C	CUU, CUC, CUA, CUG Leucine (leu)	CCU, CCC, CCA, CCG Proline (pro)	CAU, CAC Histidine (his); CAA, CAG Glutamine (gln)	CGU, CGC, CGA, CGG Arginine (arg)	U C A G
A	AUU, AUC, AUA Isoleucine (ile); AUG Methionine (met) Start codon	ACU, ACC, ACA, ACG Threonine (thr)	AAU, AAC Asparagine (asn); AAA, AAG Lysine (lys)	AGU, AGC Serine (ser); AGA, AGG Arginine (arg)	U C A G
G	GUU, GUC, GUA, GUG Valine (val)	GCU, GCC, GCA, GCG Alanine (ala)	GAU, GAC Aspartic acid (asp); GAA, GAG Glutamic acid (glu)	GGU, GGC, GGA, GGG Glycine (gly)	U C A G

Table 8-1 Biology: Science for Life, 2/e

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Genetic Code

- There are 64 codon combinations
- There are 3 codons (**stop condons**) that do not code for an amino acid – ________________
- There is one **start codon** – ______
- All proteins are built starting at the start codon, so all proteins begin with the same amino acid – Methionine (met)

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Genetic Code

- There are some codons that code for the same amino acids (called ____________________)
- But no one condon can call for more than one amino acid (no _________________)
- The genetic code is _______________ – it is used by all living organisms on the planet (different organisms typically decode the same gene to produce the same protein)

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Mutations

- **Mutations** – ___________________________
  - Can affect proteins (b/c the order of amino acids incorporated in the protein during translation is affected)
- Mutations to a gene can result in the production of different forms, or alleles, of a gene
  - Different alleles results from changes in the DNA that alter the amino acid order of the encoded protein
- The proteins produced due to a mutation can be different or nonfunctional

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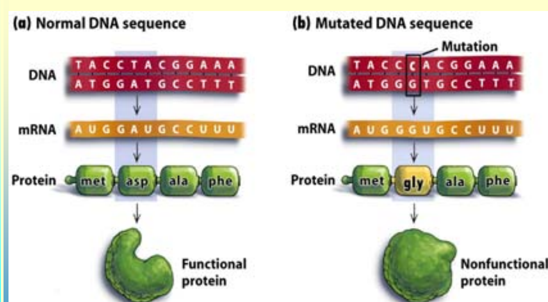

Figure 8-8 Biology: Science for Life, 2/e

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Mutations

- The change made in the protein can
  - Have ____________________
  - Have ____________________
  - Have ____________________

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Mutations

- No effect occurs when the mutation in the DNA does not change the amino acid that is called for – called a ______________________
- Mutation can result in the substitiution of one amino acid for another with similar chemical properties, which may have little or no effect on the protein – called a ________________

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Mutations

- If the mutation causes a change in the amino acid, the effects can be devastating, as in the case of sickle cell disease (glutamic acid to valine) – called a ______________________
- If a mutation turns the codon into a stop codon, the protein is abnormally shortened – called a ______________________

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Mutations

- In addition to changes in the DNA, sometimes bases are either added or deleted by mistake
  - ________________________: one or more pairs of nucleotides are inserted into a gene
  - ________________________: one or more pairs of nucleotides are removed form a gene
- This changes the groupins of nucleotides in every codon that follows – changing the triplet groupings is called altering the ____________________
- If one base is added or deleted, you get a ____________________________ – mutation where all nucleotides located after the insertion or deletion will be regrouped into different codons

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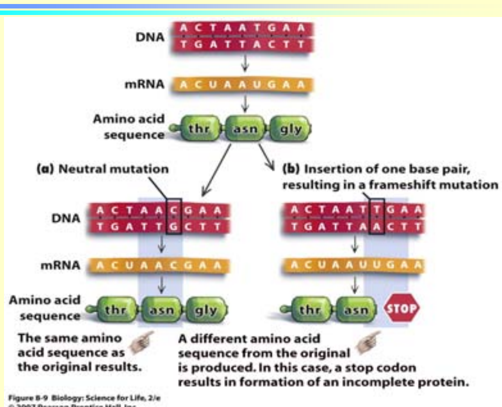

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Protein Synthesis by Cell Type

- All cells in all organisms undergo protein synthesis, but different cell types have somewhat different experiences:
  - In eukaryotic cells, transcription occurs in the nucleus and translation in the cytoplasm
  - Since prokaryotic cells have no nuclei, prokaryotic cell transcription and translation occur in the same location

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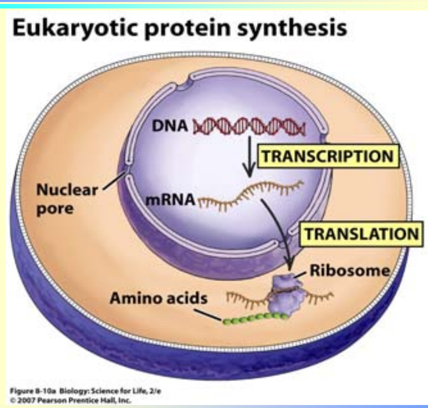

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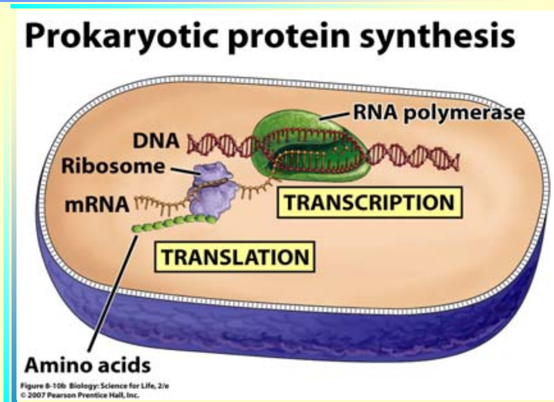

Regulating Gene Expression

- Different cells need different proteins made
- The cells all contain the entire DNA information, but only use the genes they need
  - Example: liver and pancreas perform specialized jobs, so the cells of your liver turn on or express one suite of genes and the cells of your pancreas, another
- Turning a gene on or off, or modulating it more subtly, is called ______________________________
- The expression of a given gene is regulated so that it is turned on and off in response to the cell's needs

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Regulating Gene Expression

- Different cell types regulate gene expression differently:
  - Prokaryotes keep gene expression turned off by blocking the promoter with a _______________ protein that prevents the RNA polymerase from binding
  - Eukaryotes regulate gene expression in any of 5 ways

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Regulation of Transcription

- Gene expression is most commonly regulated by controlling the rate of transcription
- There is a ________________ – a DNA sequence where the RNA polymerase enzyme attaches to begin transcription
- Sometimes cells can block transcription by having repressors covering the promoters – so the RNA polymerase can't bind and start transcription
- When the gene needs to be expressed, the repressor releases the promoter so RNA polymerase binds to begin translation

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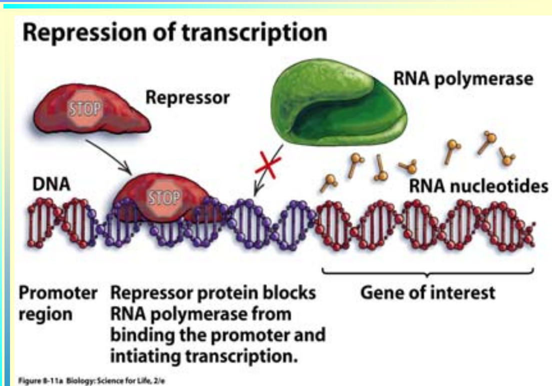

Regulation of Transcription

- Eukaryotic cells more commonly enhance gene expression using proteins called _______________ that help the RNA polymerase bind to the promoter, thus facilitating gene expression
- Other factors may affect transcription:
  - Alcohol in the liver may cause an increase in production of the gene involved in breaking down alcohol

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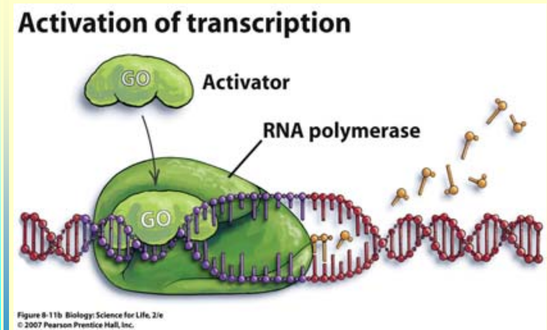

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Regulation of Chromosome Condensation

- Chromosomes can also be condensed so they are not accessible to RNA polymerase

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Regulation by mRNA Degradation

- Eukaryotic cells can also regulate the expression of a gene by regulating how long a mRNA is present in the cytoplasm
- Enzymes called _________________ roam the cytoplasm, cutting RNA molecules by binding to one end and breaking the bonds between nucleotides

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Regulation of Translation

- Regulating steps of translation is another form of the regulation of gene expression
- Steps in the process of translation are slowed down or accelerated

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Regulation of Protein Degradation

- Protein degradation can also affect gene expression
- Once a protein is synthesized, it will persist in the cell for a characteristic amount of time
- The life of a protein can be affected by enzymes inside the cell that degrade the protein
- Speeding up or slowing down the activities of these enzymes can change the amount of time a protein is able to be active inside a cell

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8.3 Producing Recombinant Proteins

- The first step in the production of *rBGH* protein is to transfer the *BGH* gene from the nucleus of a cow cell into a bacterial cell
- Making many copies of a gene is called _____________ a gene
- 3 steps are involved in moving a *BGH* gene into a bacterial cell

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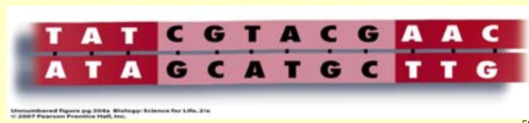
Cloning a Gene Using Bacteria

Step 1. Remove the Gene from the Cow Chromosome

- The cow gene is sliced out using **restriction enzymes**
- Restriction enzymes cut DNA only at specific sequences, called ___________________
  - The bottom middle sequence is the reverse of the top sequence



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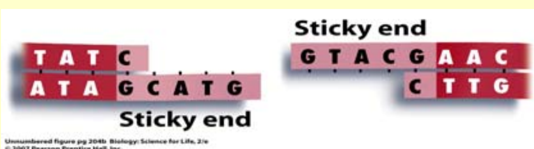
Cloning a Gene Using Bacteria

- Restriction enzymes cut the DNA in a staggered pattern, leaving "**sticky ends**"…



- The unpaired bases form bonds with any complementary bases with which they come into contact

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Cloning a Gene Using Bacteria

- The enzyme selected by the scientist cuts at both ends of the *BGH* gene, but not inside the gene

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Cloning a Gene Using Bacteria

- A _____________ is the entire suite of genes present in a particular organism

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Cloning a Gene Using Bacteria

Step 2. Insert the *BGH* Gene into the Bacterial Plasmid

- _____________ – a circular piece of DNA that normally exists separate from the bacterial chromosome and can replicate independently of the bacterial chromosome
- The bacterial plasmid is also cut with the restriction enzyme, leaving sticky ends
- Cutting both the plasmid and gene with the same enzyme allows the sticky ends that are generated to base-pair with each other

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Cloning a Gene Using Bacteria

- When the cut plasmid and the cut gene are placed together in a test tube, they reform into a circular plasmid with the extra gene incorporated
- This is now recombinant DNA
- The *BGH* gene is referred to as the *rBGH* gene, with the *r* indicating that this product is genetically engineered

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Cloning a Gene Using Bacteria

Step 3. Insert the Recombinant Plasmid into a Bacterial Cell

- The recombinant gene is then placed into bacterial cells
- Once inside the cell, the plasmids replicate themselves, as does the bacterial cell, making thousands of coupies of the *rBGH* gene

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Cloning a Gene Using Bacteria

- The bacteria produce the BGH protein
- This works, because bacteria use the same genetic code as cows (and all living things)

- Other proteins are made in this way:
  - ________________ for diabetics
  - _______________________ for hemophiliacs

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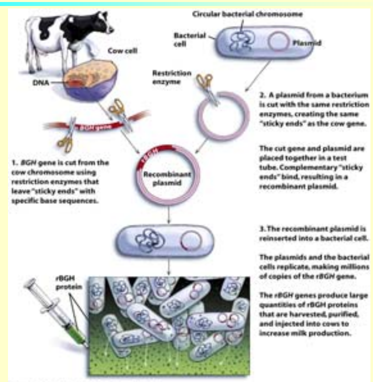

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FDA Regulations

- The Food and Drug Administration is the governmental organization responsible for ensuring the safety of all domestic and imported foods and food ingredients (except for meat and poultry, which are regulated by the US Department of Agriculture)
- Manufactures must get FDA approval for any food not ______________________________ (**GRAS**), including new genetically engineered food substances

- The FDA declared milk from *r*BGH cows safe for consumption in 1993

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Basic Versus Applied Research

- ____________________ seeks to answer questions for which there is no profit motive or direct commercial application – largely funded by taxpayers through government agencies such as the National Institutes of Health (NIH) and the National Science Foundation (NSF)
- ____________________ seeks to answer questions that will have an immediate and profitable application – generally privately funded

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8.4 Genetic Engineers Can Modify Foods

- Selective breeding techniques have affected foods for thousands of years
- Genetic engineering techniques (moving genes from one organism to another), however, allow the modification of food much more quickly

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Why Genetically Modify Crop Plants?

- Genetic modification of crops can increase their shelf life, yield, and nutritive value
- Tomatoes where the first genetically engineered fresh produce available in American grocery stores in 1994

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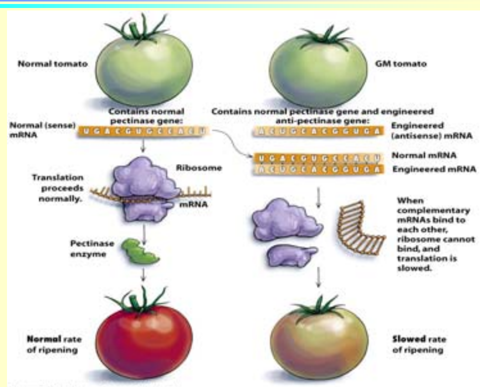

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Why Genetically Modify Crop Plants?

- Improving the yield of crop plants has been the driving force behind the vast majority of genetic engineering
- Yield can be increased when plants are engineered to be resistant to pesticides and herbicides, drought, and freezing
  - Ex.: a gene from an Artic fish has been transferred into a strawberry to help prevent frost damage

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Why Genetically Modify Crop Plants?

- Genetic engineers may also be able to increase the nutritive value of crops
  - Ex.: genetic engineers have increased the amount of β-carotene in rice
  - Scientists hope the rice will help decrease the number of people who become blind in underdeveloped nations, b/c cells require β-carotene in order to synthesize vitamin A, which is required for vision – eating this rice, called Golden Rice, increases a person's ability to synthesize vitamin A

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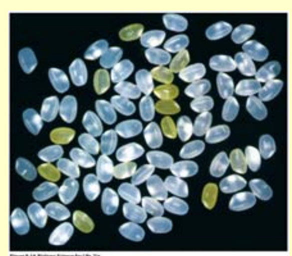
Why Genetically Modify Crop Plants?

- Pro-crop plant modification arguments include increasing farming yields, decreasing world hunger, and improving nutrition and health



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Modifying Crop Plants with the Ti Plasmid

- To modify crop plants, the gene must be able to gain access to the plant cell, which means it must be able to move through the plant's rigid, outer cell wall
- The vehicle for transferring genes into flowering plants is a naturally occurring plasmid of the bacterium *Agrobacterium tumefaciens*, which infects plants and causes tumors called **galls**
- The tumors are induced by a plasmid called **Ti plasmid** (for *T*umor *i*nducing)

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Gall caused by ***A tumefaciens***

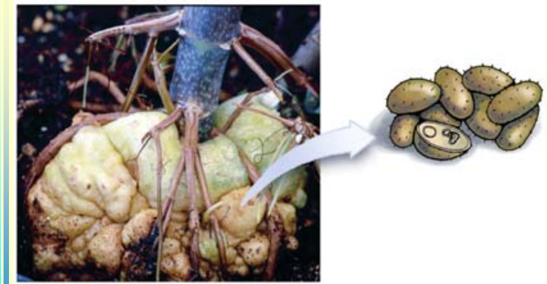

Figure 8-15a Biology: Science for Life, 2/e

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Using the Ti plasmid

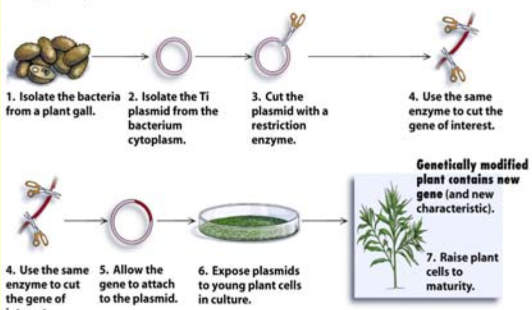

Figure 8-15b Biology: Science for Life, 2/e

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Modifying Crop Plants with the Gene Gun

- Transferring genes into other agricultural crops such as corn, barley, and rice can also be accomplished by using a device called a

  ____
  - Shoots DNA-coated pellets into plant cells

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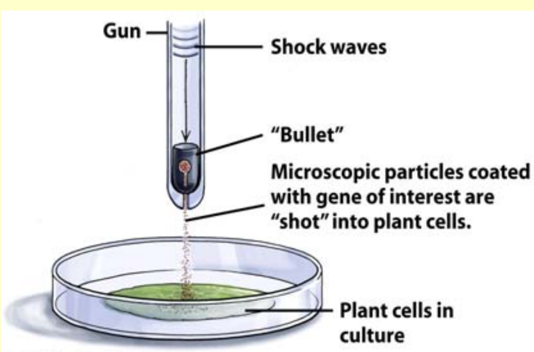

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Genetic Engineers Can Modify Food

- _________________________ are produced when a gene from one organism is incorporated into the genome of another
- The more popular term for transgenic organisms is **GMO**, for

  ____
- **GM** foods means genetically modified

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Genetic Engineers Can Modify Food

- Concerns about genetically modified (GM) crop plants include:
  - Large corporations that own many farms, called **agribusiness** corporations, profiting from GM crop production will put owners of family farms out of business
  - Other concerns focus on the impact of GMOs on human health and the environment

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Effect of GMOs on Health

- Much of the national debate on GMOs has centered on calls for labeling laws
  - Proponents say labels will enable consumers to make better decisions
  - Opponents counter that labeling is unnecessary in the absence of any proven health risk posed by GMOs

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Genetically Modified Foods in the U.S. Diet

- Like it or not, over half of all food in U.S. market contain at least some GM foods
  - Most soybeans grown are modified for herbicide resistance
  - GM corn – an ingredient in most processed foods – is common as well
  - GM canola and cottonseed oils are used in a huge range of food products

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How Are GM Foods Evaluated for Safety?

- The EPA must approve all GM crops
- GM foods can cause allergic reactions (8% of us are allergic to foods)
- Newly inserted genes may also encode proteins that prove to be toxins
- The FDA becomes involve in testing the GM crop only when the food the gene comes from has never been tested, or when there is reason to be concerned that the newly inserted gene may encode a protein that will prove to be a toxin or allergen

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How Are GM Foods Evaluated for Safety?

- If the gene being transferred from one organism to another is not known to be toxic or cause an allergic reaction, the FDA considers it to be substantially equivalent to GRAS
- If a modified crop contains a gene derived from a food that has been shown to cause a toxic or allergic reaction in humans, it must undergo testing prior to being marketed
  - ✓Ex.: a modified soybean carried a gene from the Brazil nut to increase its protein content; Brazil nuts are known to cause allergic reactions, so the modified soybeans were tested and found to cause an allergic reaction in susceptible people; so the product was withdrawn

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GM Crops and the Environment

- Concerns over GM crops extend beyond their impact on the human body:
  - ______________________________
    ____
  - ______________________________
    ____
  - ______________________________
    ____
  - ______________________________
    ____

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GM Crop Effects on Nontarget Organisms

- Plants are genetically engineered to resist pests
- This decreases the need for pesticides
- Corn has been genetically engineered to resist corn borers
  - Inserted gene from bacteria for toxin that is lethal to the corn borers but not to humans

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Corn plants have been engineered to kill the insects that eat them.

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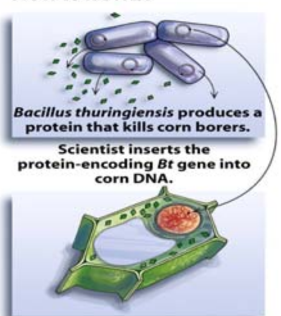
How it works:



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GM Crop Effects on Nontarget Organisms

- Bt corn can have an unintended severe impact on neighboring organisms
- Milkweed, the only food for Monarch, butterflies, grows near cornfields
- If some of the corn pollen lands on the milkweed, the milkweed may become lethal to the Monarch butterfly

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Pollen from Bt corn that dusts milkweed might unintentionally kill the butterfly larvae that eat the milkweed.

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Evolution of Resistant Pests & Transfer of Genetic Materials

- The evolution of the corn borers in response to Bt corn is another concern
- Also, can these GM crops transfer their genetically modified DNA to plants in the wild?

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Decreased Genetic Variation

- Genetic manipulation could lead to decreasing variation within a species
- GM varieties of most corn and soybean crops are nearly identical genetically
- An unforeseen disease or pest could sweep through these crops, devastating them and those who rely on them

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8.5 Genetic Engineers Can Modify Humans

The Human Genome Project

- Sequenced (determined) the nucleotide-base sequence (A,C,G, or T), of the entire human genome and the location of each of the 20,000-25,000 human genes
- Also sequenced were:
  - ________________
  - ________________
  - ________________
  - ________________

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The Human Genome Project

- These were used as ____________________ in genetic studies
- These model organisms contain genes that are the same as human genes
- Model organisms are easy to manipulate in genetic studies, and they help scientists understand human genes because they share genes with humans

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The Human Genome Project

- The human genome is very large
- It was sequenced using the technique of **chromosome walking** (Fig. 8.20, p. 216)
  - Scientists used overlapping fragments to figure out the entire chromosome

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Gene Therapy

- Once the genetics are worked out, gene therapy can be researched
- ____________________ – replacing defective genes (or their protein products) with functional ones
  - ______________ **gene therapy** – supplying an embryo with a normal version of a defective gene
    - Would ensure that the embryo and any cells produced by cell division would replicate the new, functional version of the gene
    - Most of the cells would have the corrected version of the gene
    - When these genetically modified individuals have children, they will pass on the corrected version of the gene

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Gene Therapy

- ______________ **gene therapy** – performed on body cells to fix or replace the defective protein in only the affected cells
  - Scientists introduce a functional version of a defective gene into an affected individual cell in the lab, allow the cell to reproduce, and then place the copies of the cell bearing the corrected gene into the diseased person

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Gene Therapy

- Genetic engineers have already successfully treated a genetic disorder called **Severe Combined Immunodeficiency Disorder (SCID)**
  - Disease caused by a genetic mutation resulting in the absence of an important enzyme, giving the individual a severely weakened immune system
  - People with SCID are incapable of fighting off any infection, and they often suffer severe brain damage from high temperatures associated with infection
  - Any exposure to infection can kill or disable someone with SCID, so most patients are kept inside their homes and often live inside protective bubbles that separate them from everyone, even family members

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Gene Therapy

- A non-disease causing virus is genetically engineered with the functioning gene that is needed in SCID patients
- Blood is removed from patient and the virus infects the immune cells and the immune cells get the functioning gene
- These cells are then injected back into the SCID patient

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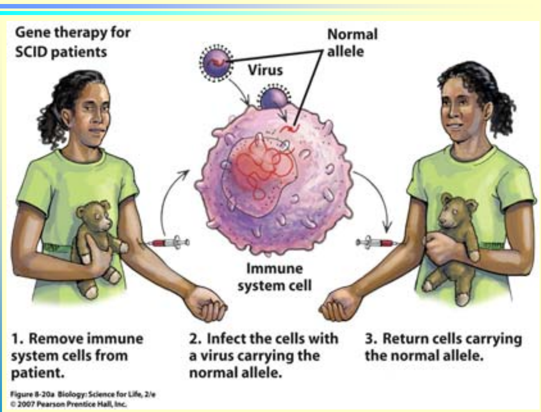

Gene Therapy

- Genetically engineering somatic cells requires repeated treatments
- Somatic cells have limited lifespans
- The condition may still be passed to offspring, because somatic cell gene therapy does not treat all the cells in the body (mainly not "fixing" the allele in the ovaries or testes)
- The only way to do this is to have germ line gene therapy

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Gene Therapy

- Current somatic gene therapy is not widely used
  - Only used for single gene disorders with cells that can be removed, engineered and then replaced in the body
- For gene therapy to be successful in curing more genetic diseases, it is necessary for scientists to:
  - Deliver the gene to the correct location
  - Make sure the gene is turned off and on at the proper times – the expression of the gene must be regulated (so, scientists must learn how to turn the right genes on in the right cell at the right time)

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Cloning Humans

- _______________ is the making of an exact copy of an entire organisms using genetic engineering
- Done in cattle, goats, mice, cats, pigs, rabbits, and sheep
- Dolly the sheep was the first animal to be cloned
  - Cells from a mammary gland were fused with an unfertilized egg cell that had had its nucleus removed

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Cloning Humans

- The treated egg was placed in the uterus of an adult ewe that had received hormone treatments to support pregnancy
- There were 277 failures before this ________________________ technique succeeded; Dolly was successfully born in 1997

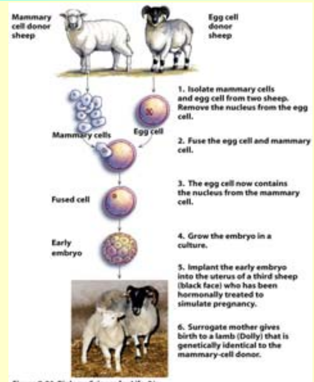

Figure 8-21 Biology: Science for Life, 2/e

Cloning Humans

- Dolly was put to sleep at the age of 6 in 2003
- She was suffering from arthritis and a progressive lung disease
- These are usually only seen in old sheep

Cloning Humans

- Is cloning safe?
- Are cloned animals aging prematurely?
- Are they showing signs of other health problems?

Cloning Humans

- Instead of cloning entire organisms, there is ______________________________
  - Involves the use of early embryos that can be induced to develop into particular tissues or organs to be used for transplants
  - This technique involves __________________ that can be induced to turn into specific tissue cells

Stem Cells

- Genetic engineers are trying to harness the healing powers of human stem cells
- Stem cells are ________________________ and, thus, can be pressed into service as many different cell types
- Tissues and organs grown from stem cells may someday be used to replace organs damaged in accidents or organs that are gradually failing due to **degenerative diseases**

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Stem Cells

- The use of embryonic stem cells in research fuels a heated national debate
- Embryonic stem cells are valued by researchers because they are ________________, or more able to become any other cell type
- In 2001, President Bush banned federal funding for researchers using embryonic stem cells

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Why the work of genetic engineers is important
• GM animals and crops may make farms more productive.
• GM crops may be made to taste better, last longer, or contain more nutrients.
• Genetic engineers hope to cure diseases and save lives.
Why the work of genetic engineers is controversial
• GM crops encourage agribusiness, which may close down some small farms.
• GM animals and crops may cause health problems in consumers.
• GM crops might have unexpected adverse effects on the environment.
• Present research might lead to the unethical genetic modification of humans.
• Lack of genetic diversity of GM crops could lead to destruction of food supply worldwide by pest or environmental change.

Table 8-2 Biology: Science for Life, 2/e

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