HC 70A
Winter 2003
Professor Bob Goldberg

Learning Unit #2
What are Genes & How do they Work?

THEMES / CONCEPTS

1.5 lecture with 45' discussion for pg 31

pts 1-8 / 2 hr lecture to pg 57 pts 9-11

1. Diverse organisms & Gene Numbers
2. The Scientific Method / Hypothesis Testing
3. Properties of the Genetic Material
4. Testing Hypothesis that DNA is the Genetic Material
5. Origins of TRANSformation
6. TRANSforming Higher Organisms - DNA → Phenotype
7. What is a gene?
8. Structure of DNA

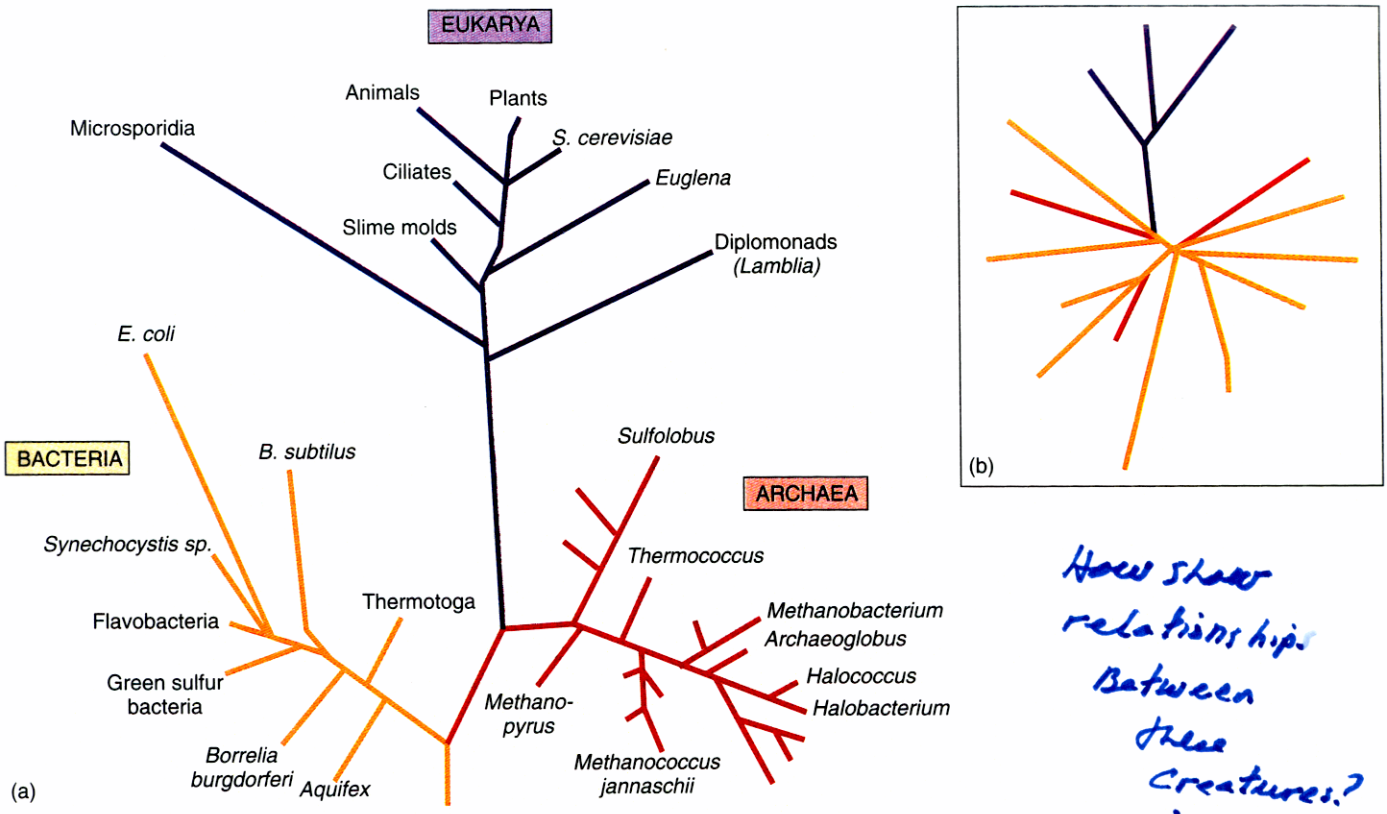
STOP 1/14/03

9. Gene Activity in Prokaryotes & Eukaryotes
10. DNA Replication & PCR
11. Mutations & Cancer

12. Bacterial Exp.
13. DNA Cloning / Fingerprinting

STOP 1/21/03

Diverse Organisms Use Same Molecules



How show relationships Between these Creatures? Prediction?

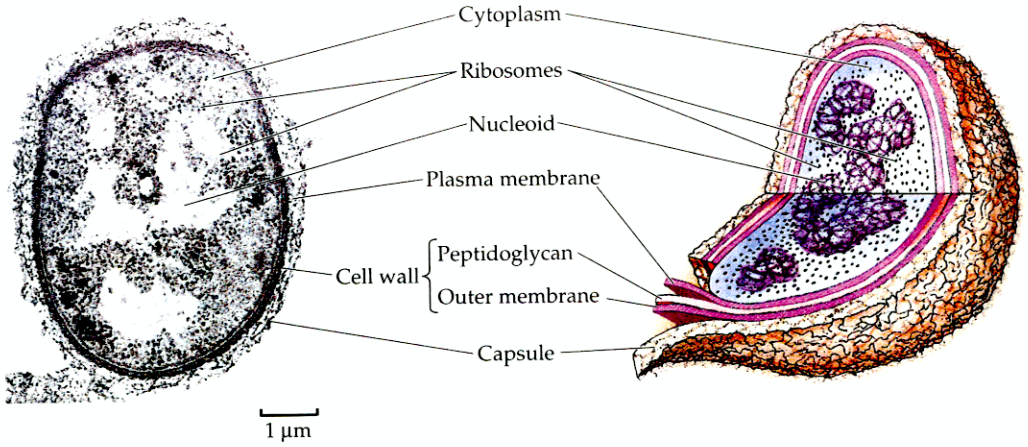
FIGURE 4.15
The three domains of life. The kingdoms Archaebacteria and Eubacteria are as different from each other as from eukaryotes, so biologists have assigned them a higher category, a "domain." (*a*) A three-domain tree of life based on ribosomal RNA consists of the Eukarya, Bacteria, and Archaea. (*b*) New analyses of complete genome sequences contradict the rRNA tree, and suggest other arrangements such as this one, which splits the Archaea. Apparently genes hopped from branch to branch as early organisms either stole genes from their food or swapped DNA with their neighbors, even distantly related ones.

Table 3.1 Macromolecules

Macromolecule	Subunit	Function	Example
PROTEINS			
Globular	Amino acids	Catalysis; transport	Hemoglobin
Structural	Amino acids	Support	Hair; silk
NUCLEIC ACIDS			
DNA	Nucleotides	Encodes genes	Chromosomes
RNA	Nucleotides	Needed for gene expression	Messenger RNA
LIPIDS			
Fats	Glycerol and three fatty acids	Energy storage	Butter; corn oil; soap
Phospholipids	Glycerol, two fatty acids, phosphate, and polar R groups	Cell membranes	Lecithin
Prostaglandins	Five-carbon rings with two nonpolar tails	Chemical messengers	Prostaglandin E (PGE)
Steroids	Four fused carbon rings	Membranes; hormones	Cholesterol; estrogen
Terpenes	Long carbon chains	Pigments; structural	Carotene; rubber
CARBOHYDRATES			
Starch, glycogen	Glucose	Energy storage	Potatoes
Cellulose	Glucose	Cell walls	Paper; strings of celery
Chitin	Modified glucose	Structural support	Crab shells

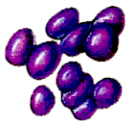
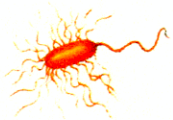
Propose Hypothesis to Explain common Molecules?

Bacteria are Highly Evolved & very diverse



4.4 A Prokaryotic Cell
The bacterium *Pseudomonas aeruginosa* illustrates typical prokaryotic cell structures. The electron micrograph on the left is magnified about 80,000 times. Note the existence of several protective structures external to the plasma membrane.

Table 34.1 Bacteria

Major Group	Typical Examples		Key Characteristics
			ARCHAEBACTERIA
Archaebacteria	Methanogens, thermophiles, halophiles		Bacteria that are not members of the kingdom Eubacteria. Mostly anaerobic with unusual cell walls. Some produce methane. Others reduce sulfur.
			EUBACTERIA
Actinomycetes	*Streptomyces*, *Actinomyces*		Gram-positive bacteria. Form branching filaments and produce spores; often mistaken for fungi. Produce many commonly used antibiotics, including streptomycin and tetracycline. One of the most common types of soil bacteria; also common in dental plaque.
Chemoautotrophs	Sulfur bacteria, *Nitrobacter*, *Nitrosomonas*		Bacteria able to obtain their energy from inorganic chemicals. Most extract chemical energy from reduced gases such as H_2S (hydrogen sulfide), NH_3 (ammonia), and CH_4 (methane). Play a key role in the nitrogen cycle.
Cyanobacteria	*Anabaena*, *Nostoc*		A form of photosynthetic bacteria common in both marine and freshwater environments. Deeply pigmented; often responsible for "blooms" in polluted waters.
Enterobacteria	*Escherichia coli*, *Salmonella*, *Vibrio*		Gram-negative, rod-shaped bacteria. Do not form spores; usually aerobic heterotrophs; cause many important diseases, including bubonic plague and cholera.
Gliding and budding bacteria	Myxobacteria, *Chondromyces*		Gram-negative bacteria. Exhibit gliding motility by secreting slimy polysaccharides over which masses of cells glide; some groups form upright multicellular structures carrying spores called fruiting bodies.
Pseudomonads	*Pseudomonas*		Gram-negative heterotrophic rods with polar flagella. Very common form of soil bacteria; also contain many important plant pathogens.
Rickettsias and chlamydias	*Rickettsia*, *Chlamydia*		Small, gram-negative intracellular parasites. *Rickettsia* life cycle involves both mammals and arthropods such as fleas and ticks; *Rickettsia* are responsible for many fatal human diseases, including typhus (*Rickettsia prowazekii*) and Rocky Mountain spotted fever. Chlamydial infections are one of the most common sexually transmitted diseases.
Spirochaetes	*Treponema*		Long, coil-shaped cells. Common in aquatic environments; a parasitic form is responsible for the disease syphilis.

] Factories for Gene Engineering

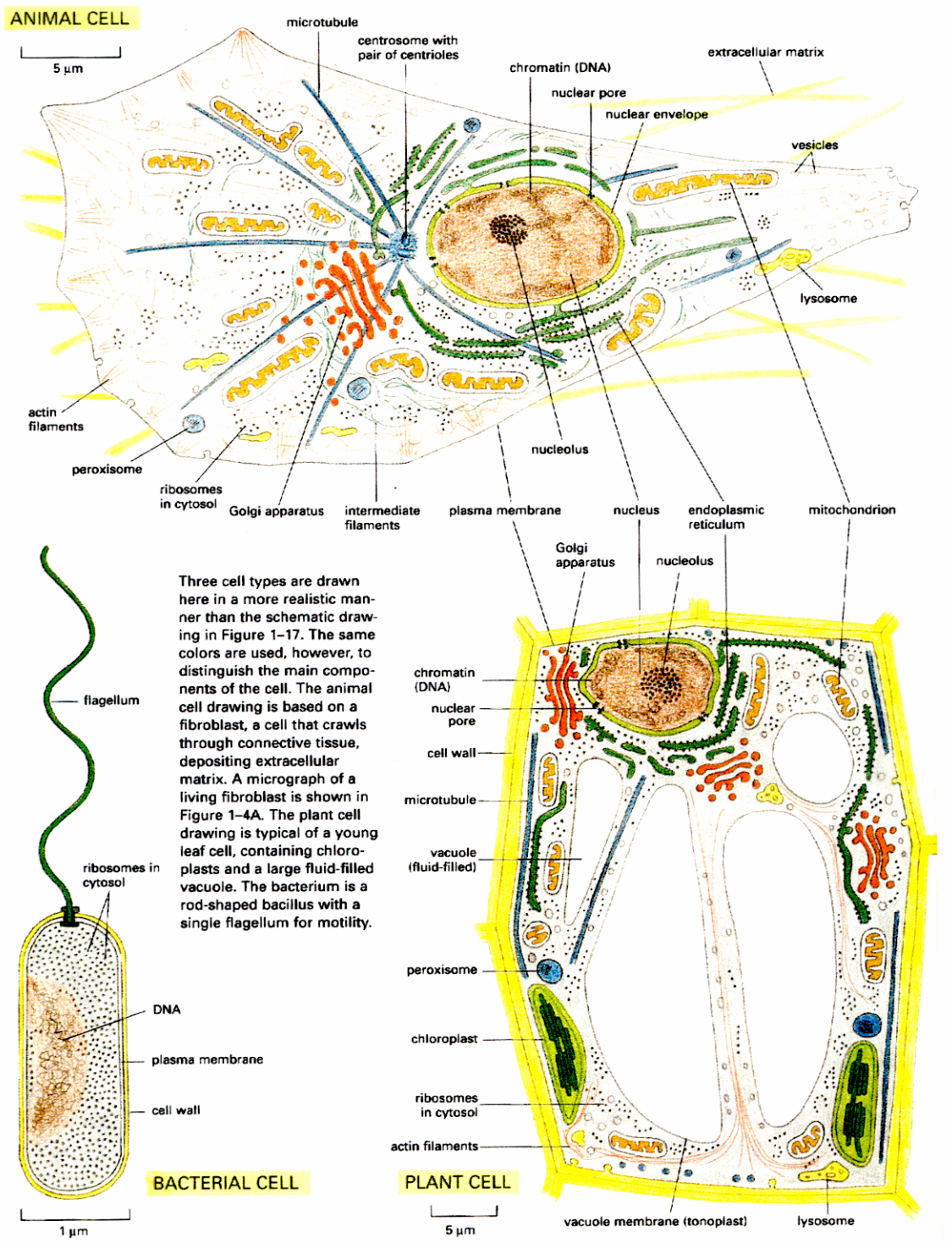
Bacteria are among the most lethal organisms on Earth

Table 34.2 Important Human Bacterial Diseases

Disease	Pathogen	Vector/Reservoir	Epidemiology
Anthrax	*Bacillus anthracis*	Animals, including processed skins	Bacterial infection that can be transmitted through contact or ingested. Rare except in sporadic outbreaks. May be fatal.
Botulism	*Clostridium botulinum*	Improperly prepared food	Contracted through ingestion or contact with wound. Produces acute toxic poison; can be fatal.
Chlamydia	*Chlamydia trachomatis*	Humans, STD	Urogenital infections with possible spread to eyes and respiratory tract. Occurs worldwide; increasingly common over past 20 years.
Cholera	*Vibrio cholerae*	Human feces, plankton	Causes severe diarrhea that can lead to death by dehydration; 50% peak mortality if the disease goes untreated. A major killer in times of crowding and poor sanitation; over 100,000 died in Rwanda in 1994 during a cholera outbreak.
Dental caries	*Streptococcus*	Humans	A dense collection of this bacteria on the surface of teeth leads to secretion of acids that destroy minerals in tooth enamel—sugar alone will not cause caries.
Diphtheria	*Corynebacterium diphtheriae*	Humans	Acute inflammation and lesions of mucous membranes. Spread through contact with infected individual. Vaccine available.
Gonorrhea	*Neisseria gonorrhoeae*	Humans only	STD, on the increase worldwide. Usually not fatal.
Hansen's disease (leprosy)	*Mycobacterium leprae*	Humans, feral armadillos	Chronic infection of the skin; worldwide incidence about 10–12 million, especially in Southeast Asia. Spread through contact with infected individuals.
Lyme disease	*Borrelia bergdorferi*	Ticks, deer, small rodents	Spread through bite of infected tick. Lesion followed by malaise, fever, fatigue, pain, stiff neck, and headache.
Peptic ulcers	*Helicobacter pylori*	Humans	Originally thought to be caused by stress or diet, most peptic ulcers now appear to be caused by this bacterium; good news for ulcer sufferers as it can be treated with antibiotics.
Plague	*Yersinia pestis*	Fleas of wild rodents: rats and squirrels	Killed ¼ of the population of Europe in the 14th century; endemic in wild rodent populations of the western U.S. today.
Pneumonia	*Streptococcus, Mycoplasma, Chlamydia*	Humans	Acute infection of the lungs, often fatal without treatment
Tuberculosis	*Mycobacterium tuberculosis*	Humans	An acute bacterial infection of the lungs, lymph, and meninges. Its incidence is on the rise, complicated by the development of new strains of the bacteria that are resistant to antibiotics.
Typhoid fever	*Salmonella typhi*	Humans	A systemic bacterial disease of worldwide incidence. Less than 500 cases a year are reported in the U.S. The disease is spread through contaminated water or foods (such as improperly washed fruits and vegetables). Vaccines are available for travelers.
Typhus	*Rickettsia typhi*	Lice, rat fleas, humans	Historically a major killer in times of crowding and poor sanitation; transmitted from human to human through the bite of infected lice and fleas. Typhus has a peak untreated mortality rate of 70%.

what you learn with E. coli generally is true for other bacteria!

Eukaryotic Cells are More Complex than Bacterial Cells



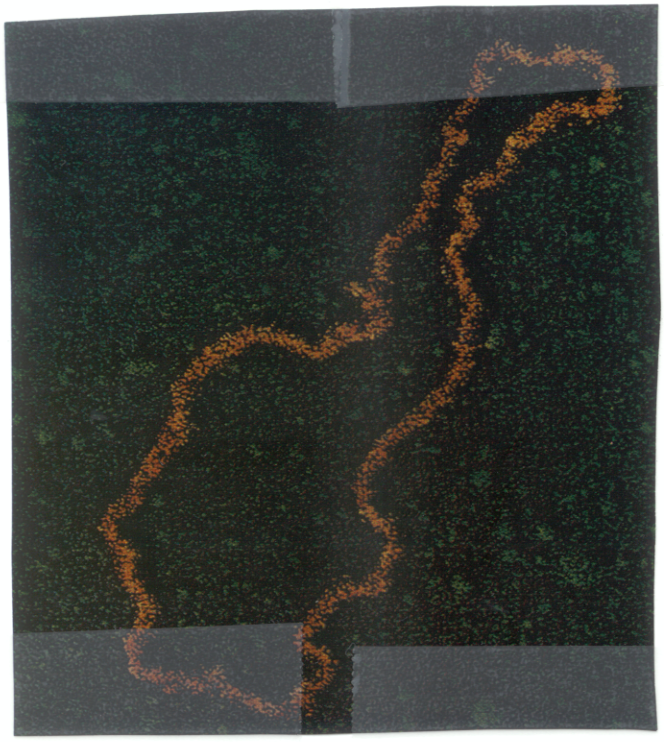
Three cell types are drawn here in a more realistic manner than the schematic drawing in Figure 1–17. The same colors are used, however, to distinguish the main components of the cell. The animal cell drawing is based on a fibroblast, a cell that crawls through connective tissue, depositing extracellular matrix. A micrograph of a living fibroblast is shown in Figure 1–4A. The plant cell drawing is typical of a young leaf cell, containing chloroplasts and a large fluid-filled vacuole. The bacterium is a rod-shaped bacillus with a single flagellum for motility.

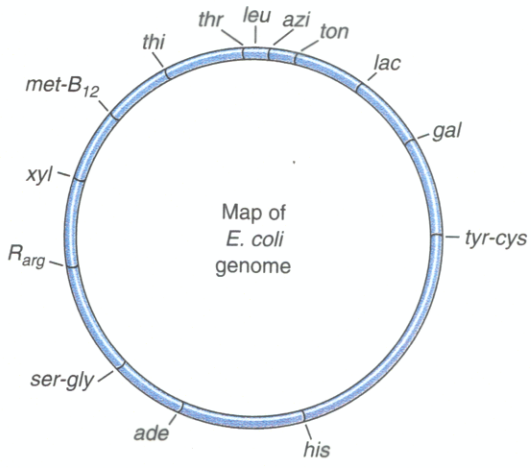
18 **Panel 1–2 Cells: the principal features of animal, plant, and bacterial cells.**

What allows these cells to be larger?

1 µm = 1×10^{-6} meters

Bacterial Chromosomes & Eukaryotic Chromosomes Differ





(1) Size (2) Shape (3) Number

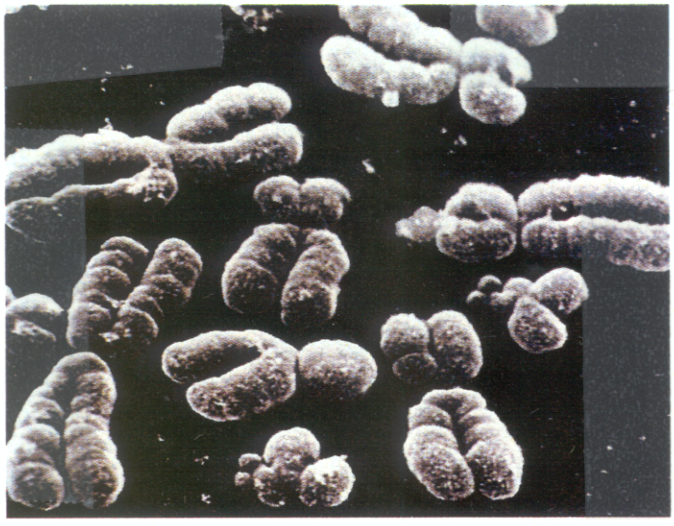


FIGURE 11.4
Human chromosomes. This photograph (950×) shows human chromosomes as they appear immediately before nuclear division. Each DNA molecule has already replicated, forming identical copies held together by a constriction called the centromere.

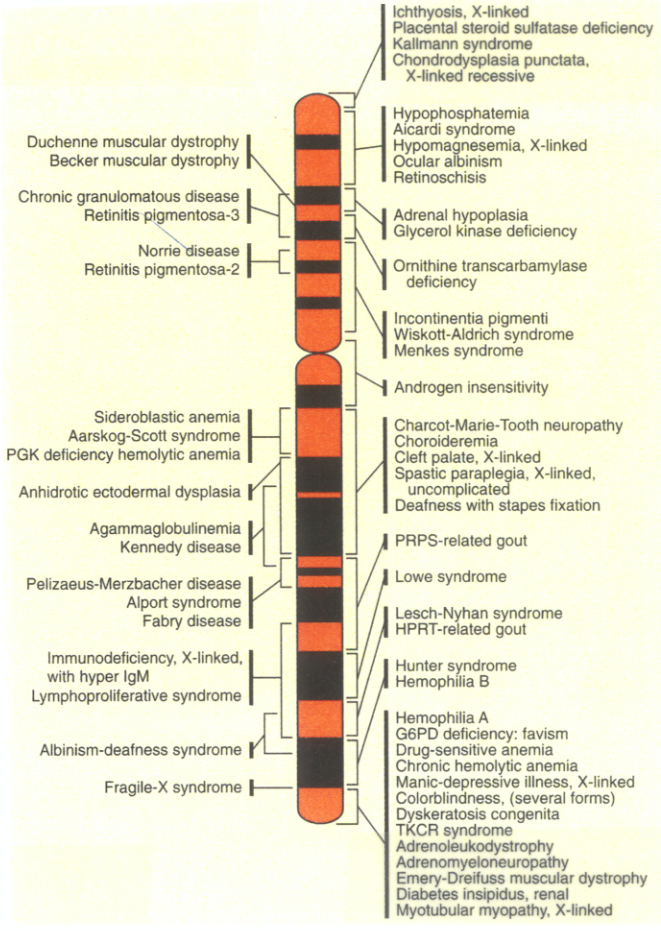
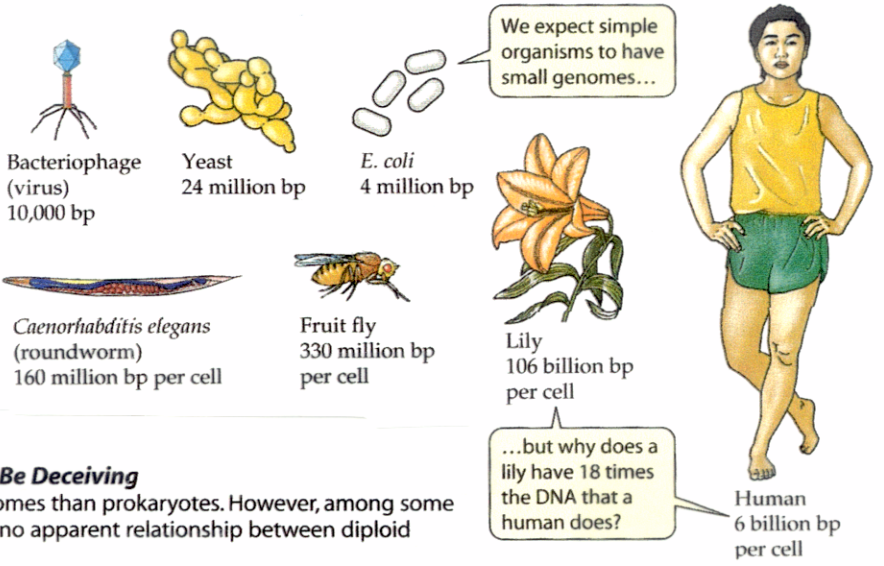


FIGURE 13.34
The human X chromosome gene map. Over 59 diseases have been traced to specific segments of the X chromosome. Many of these disorders are also influenced by genes on other chromosomes.

(5)

Gene # increases as organism complexity increases

Genome size



14.1 Amounts of Genomic DNA Can Be Deceiving
Eukaryotes have more DNA in their genomes than prokaryotes. However, among some eukaryotes—especially plants—there is no apparent relationship between diploid genome size and organism complexity.

Gene # from sequencing projects

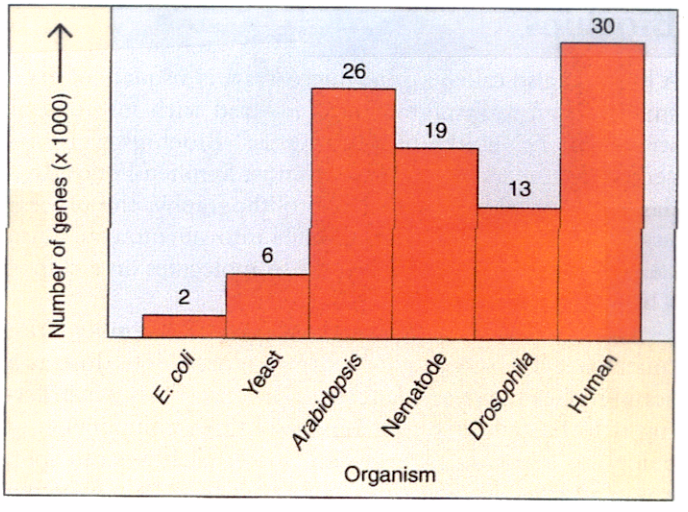
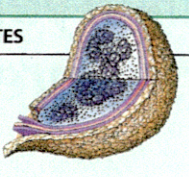


FIGURE 19.15
What the human genome is like. The human genome has an unexpectedly small number of genes, some 30,000. This is not many more than the plant *Arabidopsis*, and only a third more than nematode worms.

14.1 A Comparison of Prokaryotic and Eukaryotic Genes and Genomes



CHARACTERISTIC	PROKARYOTES	EUKARYOTES
Genome size (base pairs)	10^4–10^7	10^8–10^{11}
Repeated sequences	Few	Many
Noncoding DNA within coding sequences	Rare	Common
Transcription and translation separated in cell	No	Yes
DNA segregated within a nucleus	No	Yes
DNA bound to proteins	Some	Extensive
Promoter	Yes	Yes
Enhancer/silencer	Rare	Common
Capping and tailing of mRNA	No	Yes
RNA splicing required	Rare	Common
Number of chromosomes in genome	One	Many

What are the functions of a Gene?

Review

Genotype = Collection of Genes

Phenotype

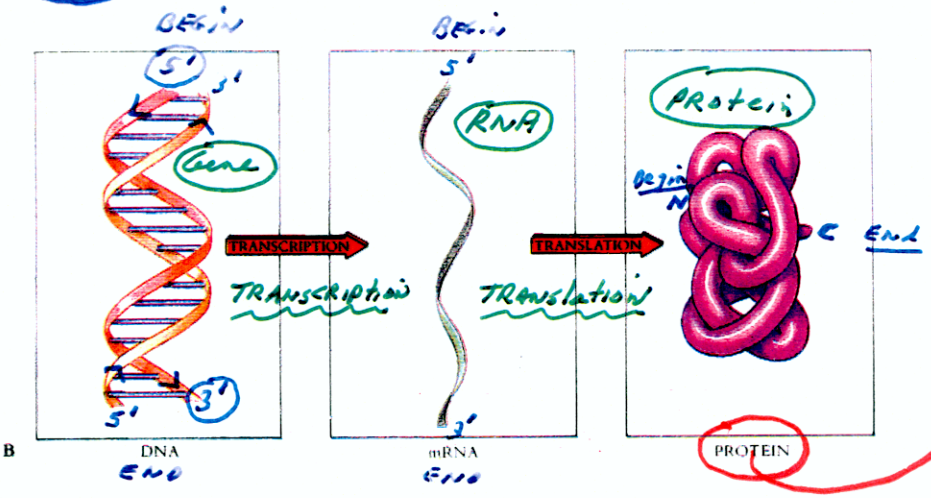
Cell Function

(2) Gene Action

(1) Gene Replication

Gene → Protein → Cell Function

Define gene by Sequence of 5'→3' Strand



Gene → Protein → Cell Function

Change by Genetic Engineering

Also Change by Mutation OR change gene chemistry

Change

Change

Altered or Engineered Cell

GENETIC Engineering by changing Genotype Alters Cell Function

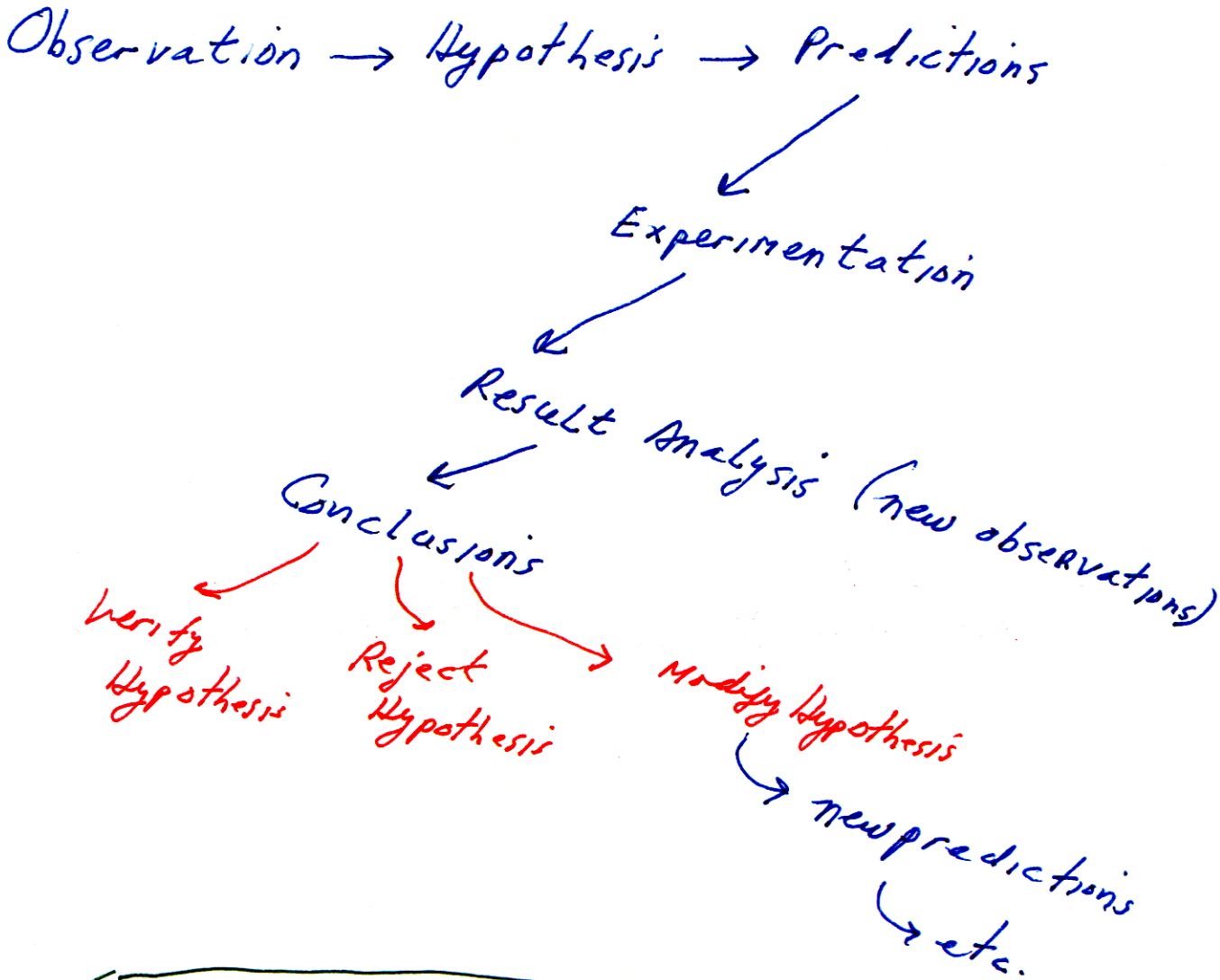
What ARE THE PROPERTIES OF A GENE?

1. Replication
2. Stability (Mutations?)
3. Universality
   (a) all cells
   (b) all organisms
4. Direct Cell Function / Phenotype

How can these properties be predicted experimentally? Scientific method hypothesis testing? If → Then

How SHOW THAT DNA is The Genetic Material?

How is Science carried out?



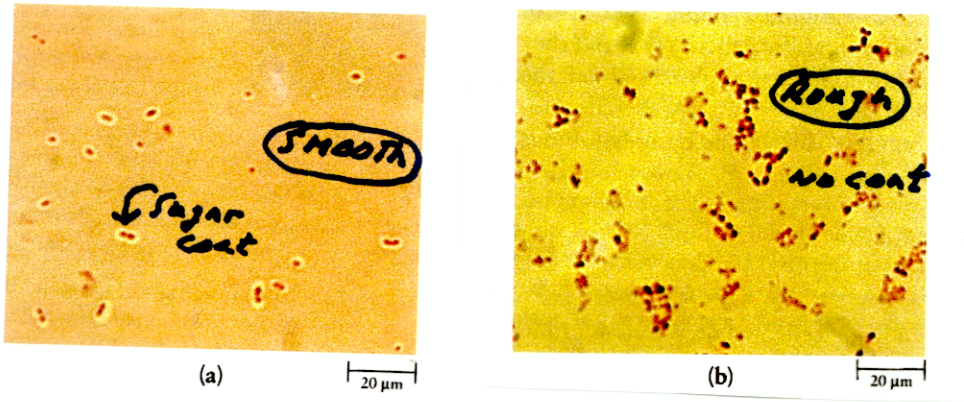
Science is very precise & is carried out by observation, hypothesis, & experiments

Scientists analyze results by saying —
"What did I miss!" "What about that?"
They look critically for new horizons!

What ARE GENES MADE OF?

Hypothesis Testing

Pneumonia Bacteria → Streptococcus pneumoniae



14–2 (a) *Encapsulated and* (b) *nonencapsulated forms of pneumococci. The capsule is made up of polysaccharides deposited outside the cell wall. The encapsulated form, which is resistant to phagocytosis by white blood cells, produces pneumonia; the mutant, nonencapsulated form is harmless.*

SMOOTH/VIRULENT STRAIN → MUTATION → ROUGH/AVIRULENT STRAIN

~1927 Griffith Experiment

THE FIRST TRANSFORMATION EXPERIMENT

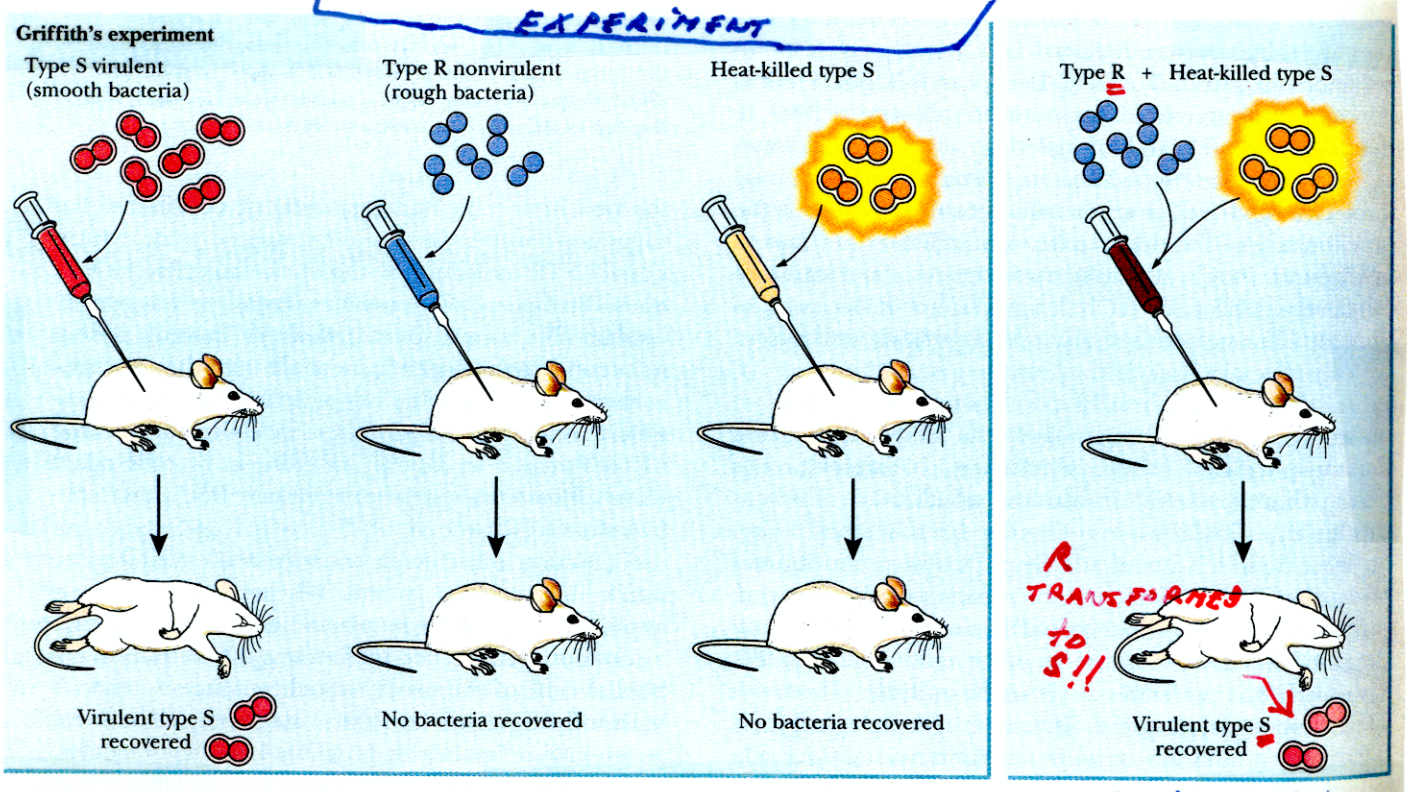


Figure 12-8 The experiment of Frederick Griffith, showing that an extract of a virulent strain of pneumococcus can "transform" a nonvirulent strain.

What is the TRANSFORMING PRINCIPLE?

HOW TO SHOW THE TRANSFORMING PRINCIPLE is DNA?

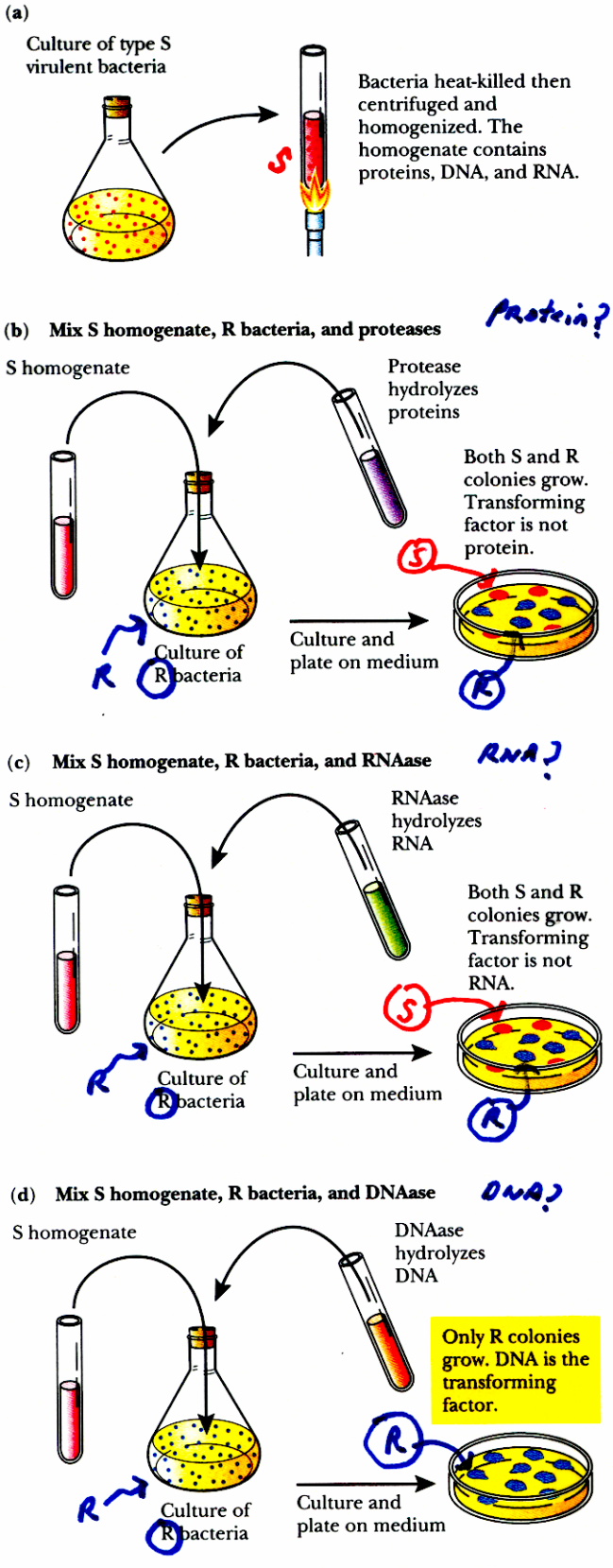


Figure 12-9 Identification of the "transforming factor" as DNA. **(a)** Extraction of proteins, DNA, and RNA from virulent (S) bacteria; **(b)** protease treatment does not prevent transformation, showing that the transforming factor is not a protein; **(c)** RNAase treatment does not prevent transformation, showing that the transforming factor is not RNA; **(d)** DNAase treatment prevents transformation, showing that the transforming factor is DNA.

① Hypothesis - DNA is the Genetic Material

② Prediction? If DNA, then ??

③ Experiment?

④ Result? → Verify? yes/no?

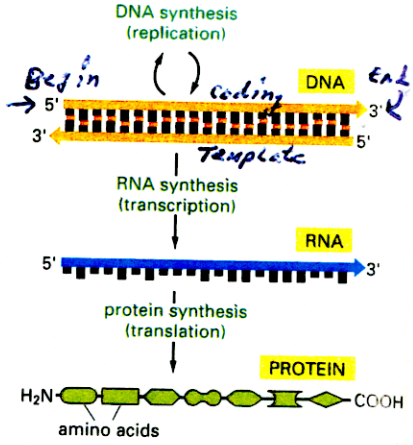
← Avery experiment ~1945

The "first" Gene Engineering Experiment!

How Did Avery's Experiment Verify the Hypothesis that DNA is the Genetic Material?

Predictions **Results**

1. Replication? yes
2. Phenotype? yes
3. Stability? yes
   - ↳ How obtain R cells?



S DNA taken up by R cell

S "gene"

↓

S "RNA"

↓

S "protein"

↳ Smooth S virulent cell

Avery + Griffith's Experiments Enabled Genetic Engineering to become possible because they Established that DNA is the Genetic Material

Transformation? Ability of cells to be transformed by DNA – A trait transformed!

TRANSFORMATION USED AS A Genetic Engineering PROCESS to Present day!

BACTERIA CAN BE TRANSFORMED WITH ANTIBIOTIC RESISTANCE GENES/DNA

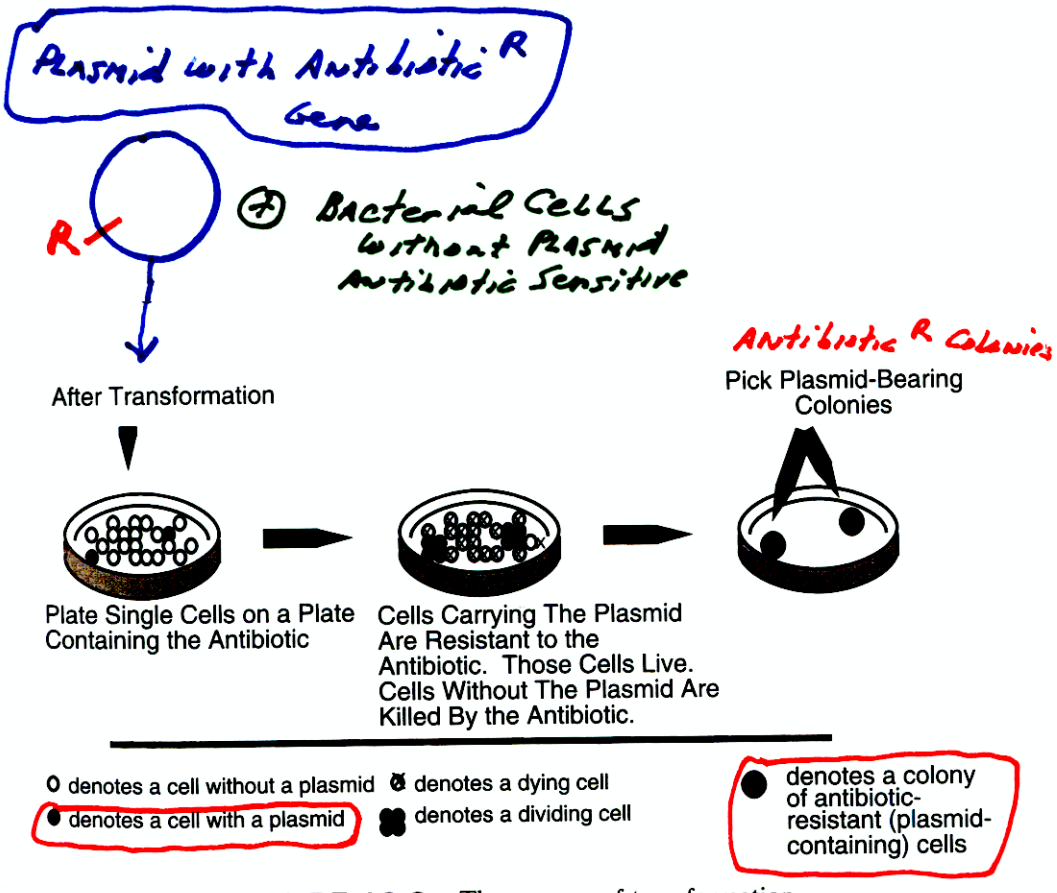


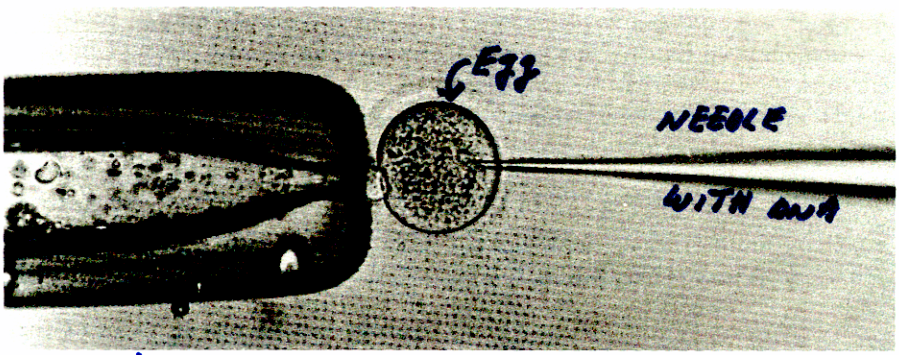
FIGURE 12.3 The process of transformation.

ANTIBIOTIC Sensitive CELLS TRANSFORMED → Antibiotic Resistant by Antibiotic Resistant Gene/DNA!

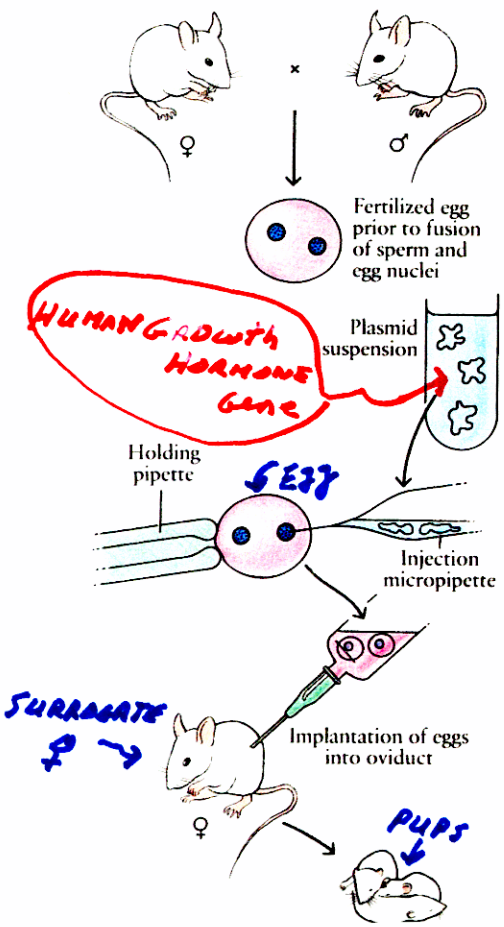
DNA → Phenotype (Antibiotic Resistance!)

Replicates

CAN HIGHER ORGANISMS BE TRANSFORMED/ENGINEERED WITH DNA?



HOLDING PIPET (b)



18–23 (a) *The procedure by which Gordon and Ruddle inserted the gene for rabbit beta globin into mice. The gene was spliced into plasmids, which were then injected into fertilized eggs before the egg and sperm nuclei fused. After injection, the fertilized eggs were implanted in a female mouse, who gave birth. The rabbit beta-globin polypeptide was present in the red blood cells of the offspring, and hybridization techniques revealed that the gene had been incorporated into their DNA.*

(b) *Injection of the plasmid suspension into a fertilized egg. The diameter of the micropipette tip is only about 0.5 micrometer, but Gordon says that the injection "is equivalent to your being speared by a telephone pole." Nevertheless, a significant number of the eggs survive.*

Mighty Mouse

CAN HUMAN GENES TRANSFORM A MOUSE?

18–24 *The two female mice shown here are litter mates, approximately 24 weeks old. The fertilized egg from which the mouse on the left developed was injected with a gene consisting of the promoter and regulator sequences of a mouse gene combined with the structural gene for human growth hormone. Following integration of the new gene into the genome of the female mouse, it is passed on to her offspring. On the average, mice that express the new gene grow two to three times as fast as mice lacking the gene and, as adults, they are twice the normal size. (Those unable to see the utility of a giant mouse may be interested to learn that a similar procedure has now been successfully performed in fish.)*

Human Growth Hormone Gene Engineered to be active at high level in Mouse

A HUMAN GROWTH HORMONE GENE in a MOUSE → Mighty Mouse!

How does this experiment show that DNA is the genetic material?

∴ DNA is the Genetic Material of ALL Living Cells!!! Prokaryote + Eukaryote

AND ALL CELLS CAN BE Engineered / TRANSFORMED!

Genetic Engineering Involves INCORPORATING Engineered DNA or Genes in Chromsomes of Different Organisms

Engineered DNA/gene Must —:

1. Enter Target cell
2. Use Target cell Enzymes to become incorporated into a chromosome — Either replaces similar gene or Inserted at Random sites (>99% of the time) OR DNA Replicates independently (e.g., plasmid, chromosome).
3. Replicate with Target cell Chromsomes (& cell!)
4. Use Target cell Protein Synthesis Machinary to make a new protein &/: a new phenotype.

→ ∴ cell Genetically Engineered with a New Trait

Cell Recognizes DNA as its own — why?

Engineered Gene Can Be —:

1. FROM Same organism as Target cell
2. FROM Different organism as Target cell
3. FROM A combination of organisms either different and/or Same as Target cell

Inference: Genetic Processes similar

What is a Gene?

Example! How show gene is DNA & DNA the genetic Material!

5' Begin

CCCTGTGGAGCCACACCCTAGGGTTGGCCA
ATCTACTCCCAGGAGCAGGGAGGGCAGGAG
CCAGGGCTGGGCATAAAAGTCAGGGCAGAG
CCATCTATTGCTTACATTTGCTTCTGACAC
AACTGTGTTCACTAGCAACTCAAACAGACA
CCATGGTGCACCTGACTCCTGAGGAGAAGT
CTGCCGTTACTGCCCTGTGGGGCAAGGTGA
ACGTGGATGAAGTTGGTGGTGAGGCCCTGG
GCAGGTTGGTATCAAGGTTACAAGACAGGT
TTAAGGAGACCAATAGAAACTGGGCATGTG
GAGACAGAGAAGACTCTTGGGTTTCTGATA
GGCACTGACTCTCTCTGCCTATTGGTCTAT
TTTCCCACCCTTAGGCTGCTGGTGGTCTAC
CCTTGGACCCAGAGGTTCTTTGAGTCCTTT
GGGGATCTGTCCACTCCTGATGCTGTTATG
GGCAACCCTAAGGTGAAGGCTCATGGCAAG
AAAGTGCTCGGTGCCTTTAGTGATGGCCTG
GCTCACCTGGACAACCTCAAGGGCACCTTT
GCCACACTGAGTGAGCTGCACTGTGACAAG
CTGCACGTGGATCCTGAGAACTTCAGGGTG
AGTCTATGGGACCCTTGATGTTTTCTTTCC
CCTTCTTTTCTATGGTTAAGTTCATGTCAT
AGGAAGGGGAGAAGTAACAGGGTACAGTTT
AGAATGGGAAACAGACGAATGATTGCATCA
GTGTGGAAGTCTCAGGATCGTTTTAGTTTC
TTTTATTTGCTGTTCATAACAATTGTTTTC
TTTTGTTTAATTCTTGCTTTCTTTTTTTTT
CTTCTCCGCAATTTTTACTATTATACTTAA
TGCCTTAACATTGTGTATAACAAAAGGAAA
TATCTCTGAGATACATTAAGTAACTTAAAA
AAAAACTTTACACAGTCTGCCTAGTACATT
ACTATTTGGAATATATGTGTGCTTATTTGC
ATATTCATAATCTCCCTACTTTATTTTCTT
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ATATTTATGGGTTAAAGTGTAATGTTTTAA
TATGTGTACACATATTGACCAAATCAGGGT
AATTTTGCATTTGTAATTTTAAAAAATGCT
TTCTTCTTTTAATATACTTTTTTGTTTATC
TTATTTCTAATACTTTCCCTAATCTCTTTC
TTTCAGGGCAATAATGATACAATGTATCAT
GCCTCTTTGCACCATTCTAAAGAATAACAG
TGATAATTTCTGGGTTAAGGCAATAGCAAT
ATTTCTGCATATAAATATTTCTGCATATAA
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TGCTTTTATTTTATGGTTGGGATAAGGCTG
GATTATTCTGAGTCCAAGCTAGGCCCTTTT
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CACCCCACCAGTGCAGGCTGCCTATCAGAA
AGTGGTGGCTGGTGTGGCTAATGCCCTGGC
CCACAAGTATCACTAAGCTCGCTTTCTTGC
TGTCCAATTTCTATTAAAGGTTCCTTTGTT
CCCTAAGTCCAACTACTAAACTGGGGGATA
TTATGAAGGGCCTTGAGCATCTGGATTCTG
CCTAATAAAAAACATTTATTTTCATTGCAA
TGATGTATTTAAATTATTTCTGAATATTTT
ACTAAAAAGGGAATGTGGGAGGTCAGTGCA
TTTAAAACATAAAGAAATGATGAGCTGTTC
AAACCTTGGGAAAATACACTATATCTTAAA
CTCCATGAAAGAAGGTGAGGCTGCAACCAG
CTAATGCACATTGGCAACAGCCCCTGATGC
CTATGCCTTATTCATCCCTCAGAAAAGGAT
TCTTGTAGAGGCTTGATTTGCAGGTTAAAG
TTTTGCTATGCTGTATTTTACATTACTTAT
TGTTTTAGCTGTCCTCATGAATGTCTTTTC

End 3'

The Human β-globin gene

What is a Gene?

UNIQUE!! SEQUENCE OF NUCLEOTIDES IN FUNCTION

What if this Sequence Changed?

SEQUENCE IN BIOLOGY!

17

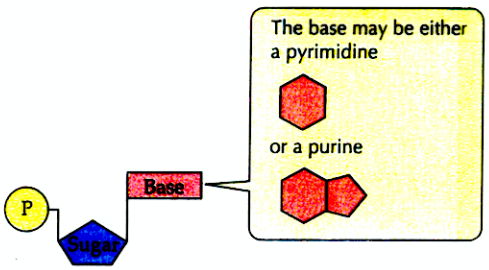
→ globin protein

Oxygen transporter from lungs in humans to tissues

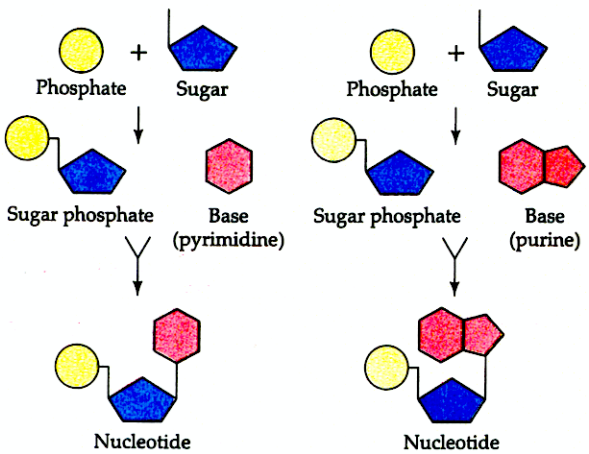
Respiration

Energy!

DNA AND GENES CONSIST OF NUCLEOTIDES JOINED TOGETHER BY PHOSPHODIESTER BONDS



A nucleotide consists of a phosphate, a pentose sugar, and a nitrogen-containing base.



3.21 Nucleotides Have Three Components A nucleotide consists of a phosphate group, a pentose sugar, and a nitrogen-containing base—all linked together by covalent bonds. The nitrogenous bases fall into two categories: Purines have two fused rings, and the smaller pyrimidines have a single ring.

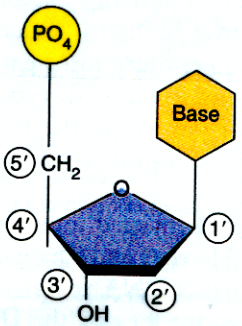
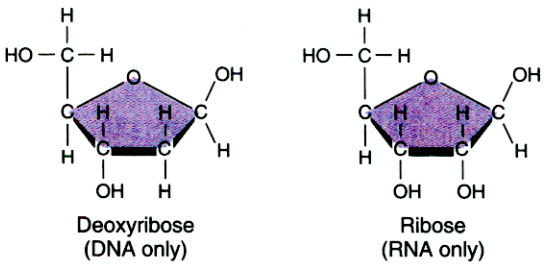


FIGURE 14.7
Numbering the carbon atoms in a nucleotide. The carbon atoms in the sugar of the nucleotide are numbered 1′ to 5′, proceeding clockwise from the oxygen atom. The "prime" symbol (′) indicates that the carbon belongs to the sugar rather than the base.



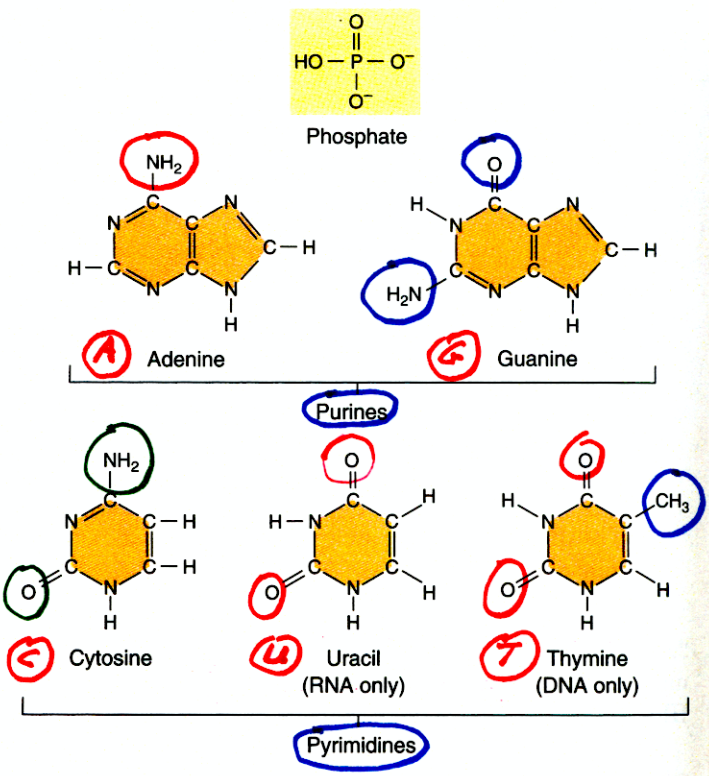


FIGURE 14.6
Nucleotide subunits of DNA and RNA. The nucleotide subunits of DNA and RNA are composed of three elements: a five-carbon sugar (deoxyribose in DNA and ribose in RNA), a phosphate group, and a nitrogenous base (either a purine or a pyrimidine).

Chemistry → Biology

ORDER OF NUCEOTIDES → BIOLOGY

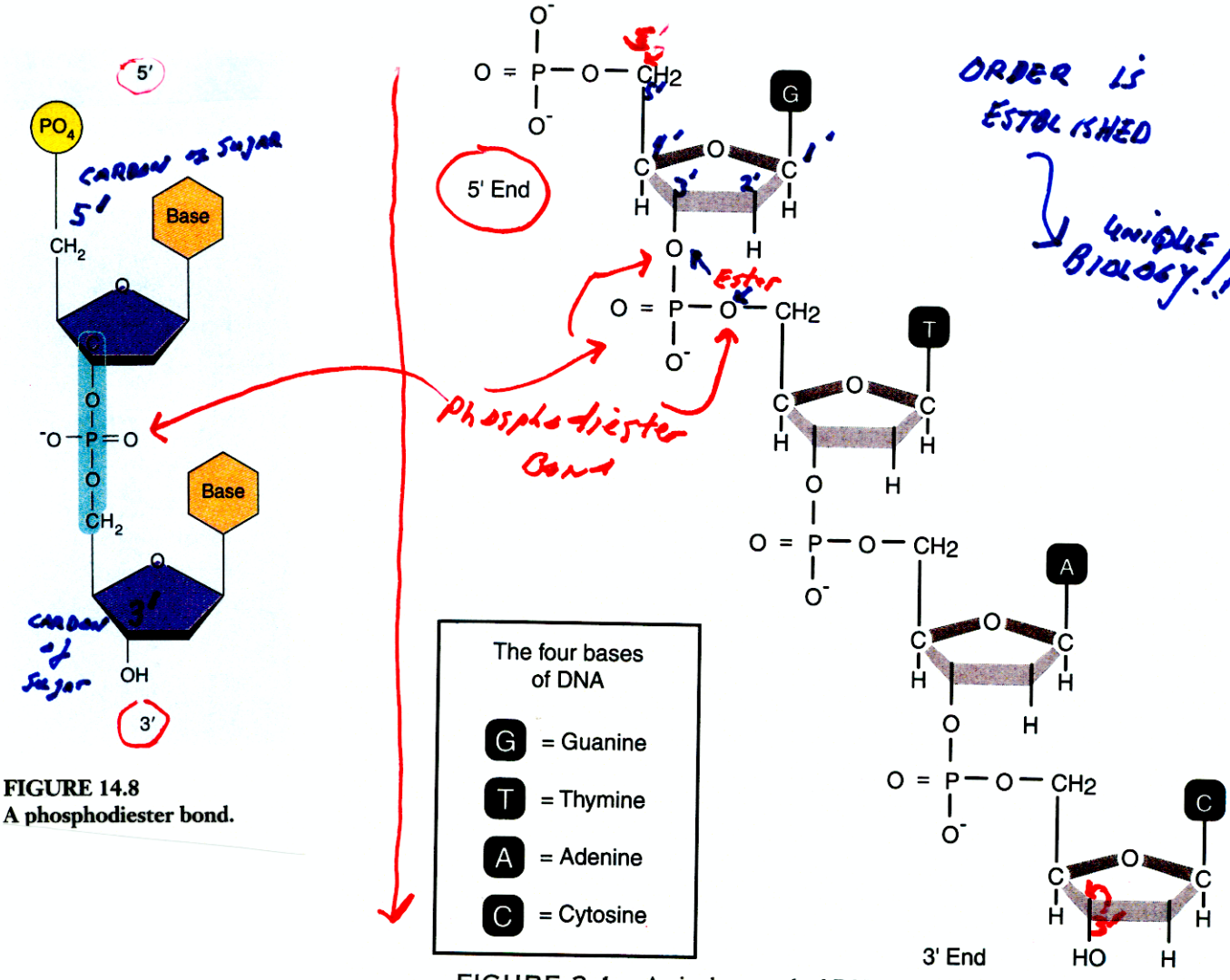


FIGURE 14.8
A phosphodiester bond.

FIGURE 3.1 A single strand of DNA composed of four nucleotides.

ORDER MAINTAINED in Each ROUND OF REPLICATION

DNA CONTAINS TWO COMPLENTARY CHAINS OF DNA

A = T G ≡ C ∴ order always specified each Replication!

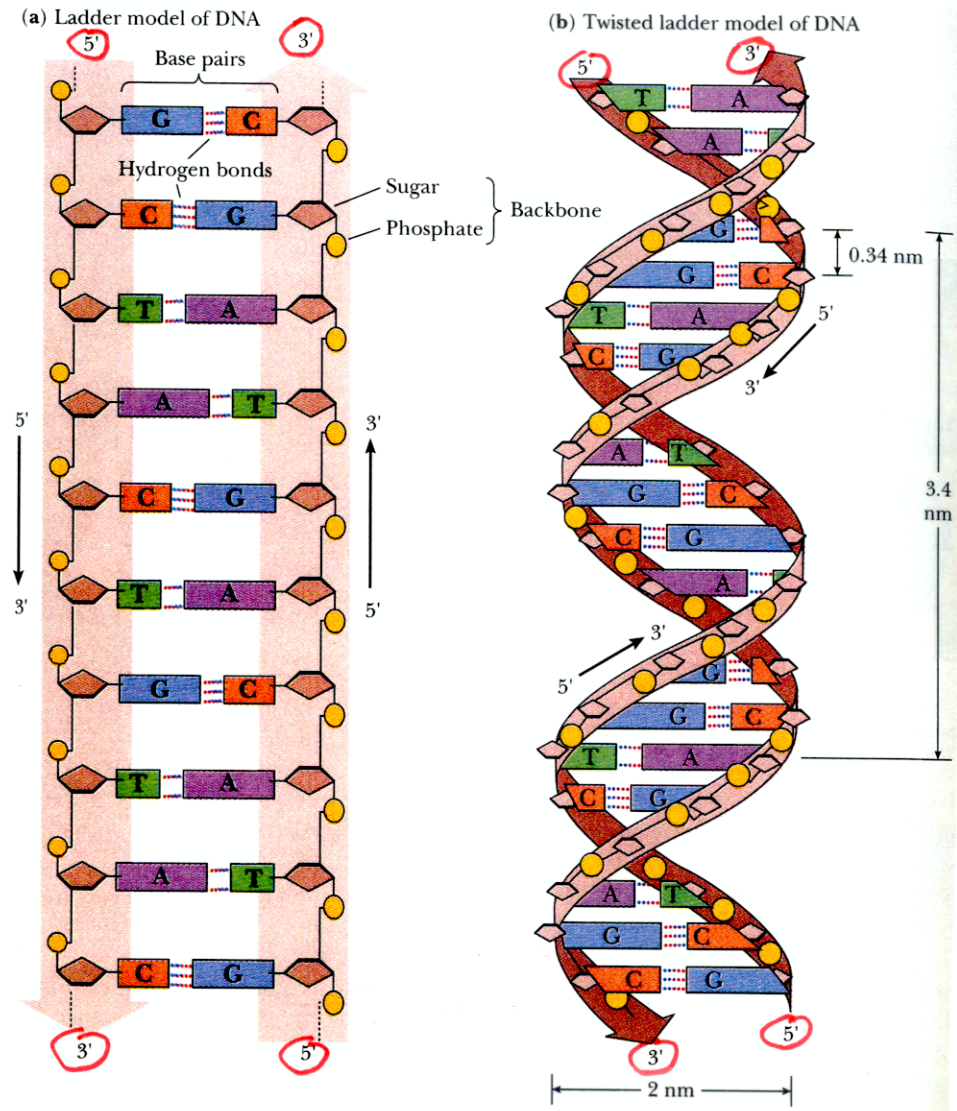


Figure 13-5 DNA is a twisted ladder. **(a)** An untwisted ladder with two antiparallel and complementary polynucleotide chains; **(b)** a twisted ladder.

Chains move in opposite directions

Why? To fit into a double Helix

Watson - Crick Double Helix

BASIS OF ALL BIOLOGY + LIFE!!

A Chromosome Contains One (or Two!) Linear DNA Molecules from Beginning to End

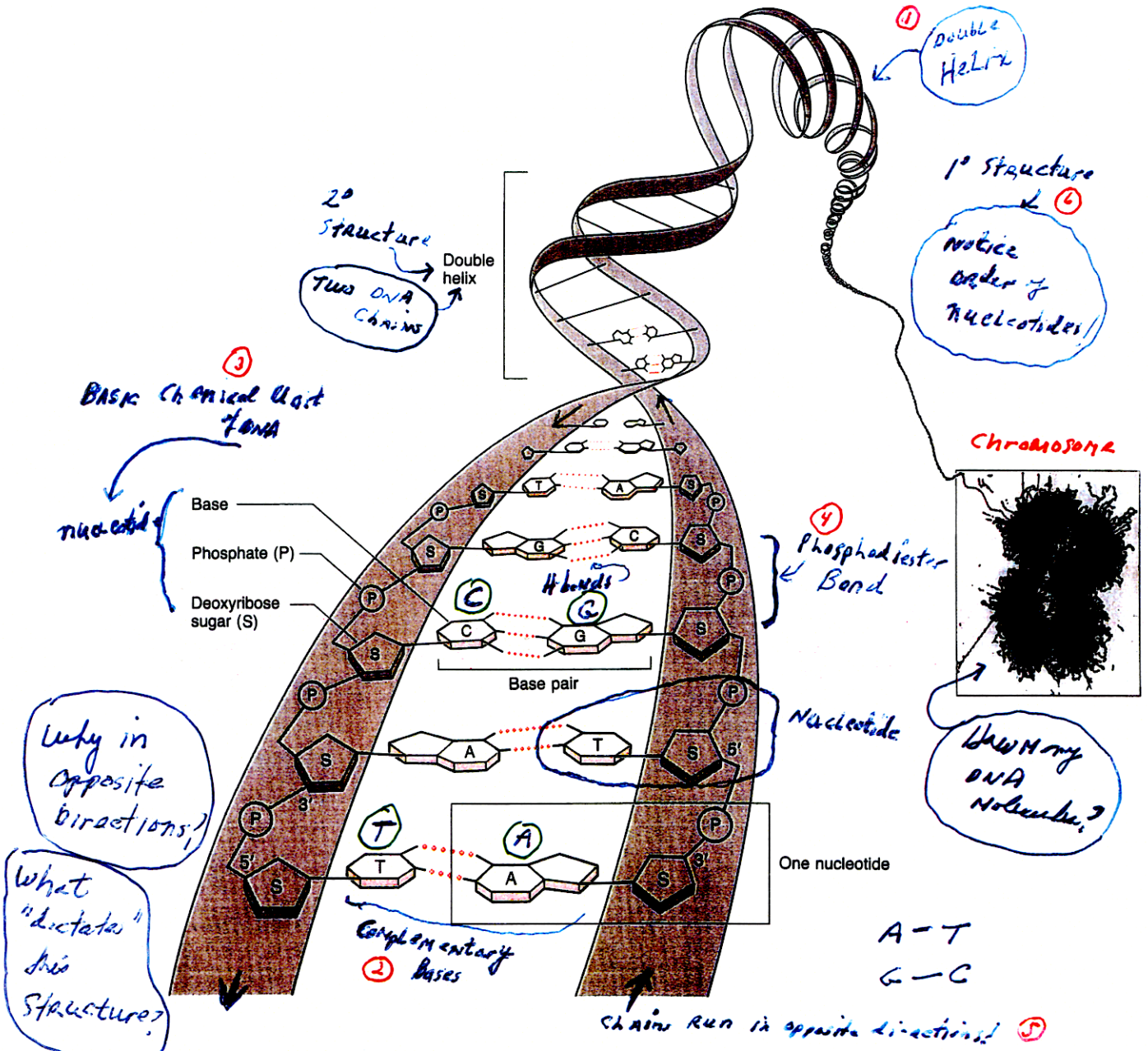


Figure 2.5 The arrangement and association of nucleotides in the DNA double helix.

DNA is a Double Helix Consisting of Two DNA Strands Bound Together by Hydrogen Bonds

The DNA Molecule

(1) Four different kinds of nucleotides — Adenine, Thymine, Cytosine, Guanine bases.

(2) Nucleotides linked together by phosphodiester bonds — order 5' → 3'. Sugar-Phosphate backbone.

(3) Two polynucleotide chains in antiparallel direction — one chain 5' → 3' : other 3' → 5'.

(4) Polynucleotide chains complementary to each other — A-T & G-C base pairs held together by H-bonds → Replication & Expression/Function

(5) Sugar-Phosphate backbone & complimentary bases to interior.

(6) No contraint on order of nucleotides : 4^n = # different combinations.

(7) Complementary bases → How genes replicate & direct cell processes.

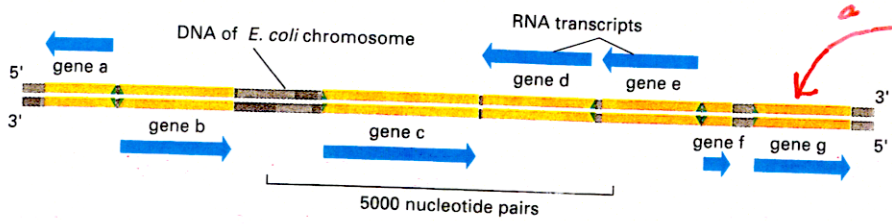
(8) DNA molecule has dimensions — 3.4Å/bp & 20Å diameter & 10 base pairs/turn

Know length — predict # bp

A Chromosome is A CONTINUOUS "STRETCH" OF DNA

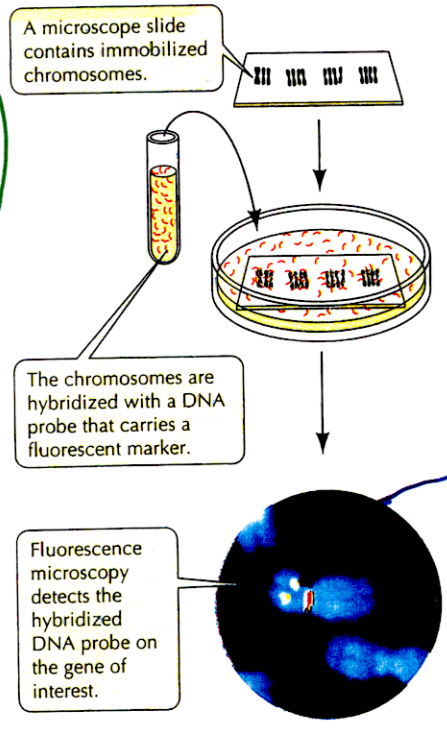
What Distinguishes ONE Gene FROM the Next ONE OR its Neighbors?

a LOCUS for gene g



Genes Reside at Specific Positions on chromosomes Called Loci

Genes Act As Independent Units!



TRANSformation Experiment Shows that!

one gene in a position of a human chromosome

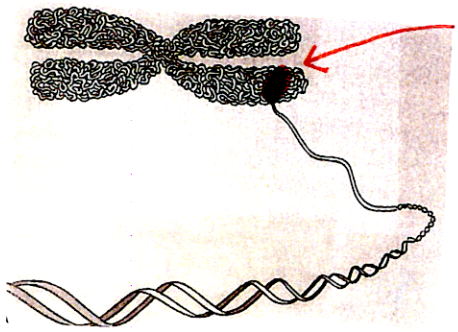
Can detect using A PROBE!

↓

a DNA or RNA coding for a known gene!

17.19 Mapping a DNA Sequence by Fluorescence *in situ* Hybridization In the FISH technique, banding patterns are used to locate a gene using a fluorescent probe. (The red band is a marker for the centromere of the X chromosome, further specifying the location of the gene.)

A Chromosome Contains Many Genes



Position of Genes 1, 2, & 3 in Chromosome

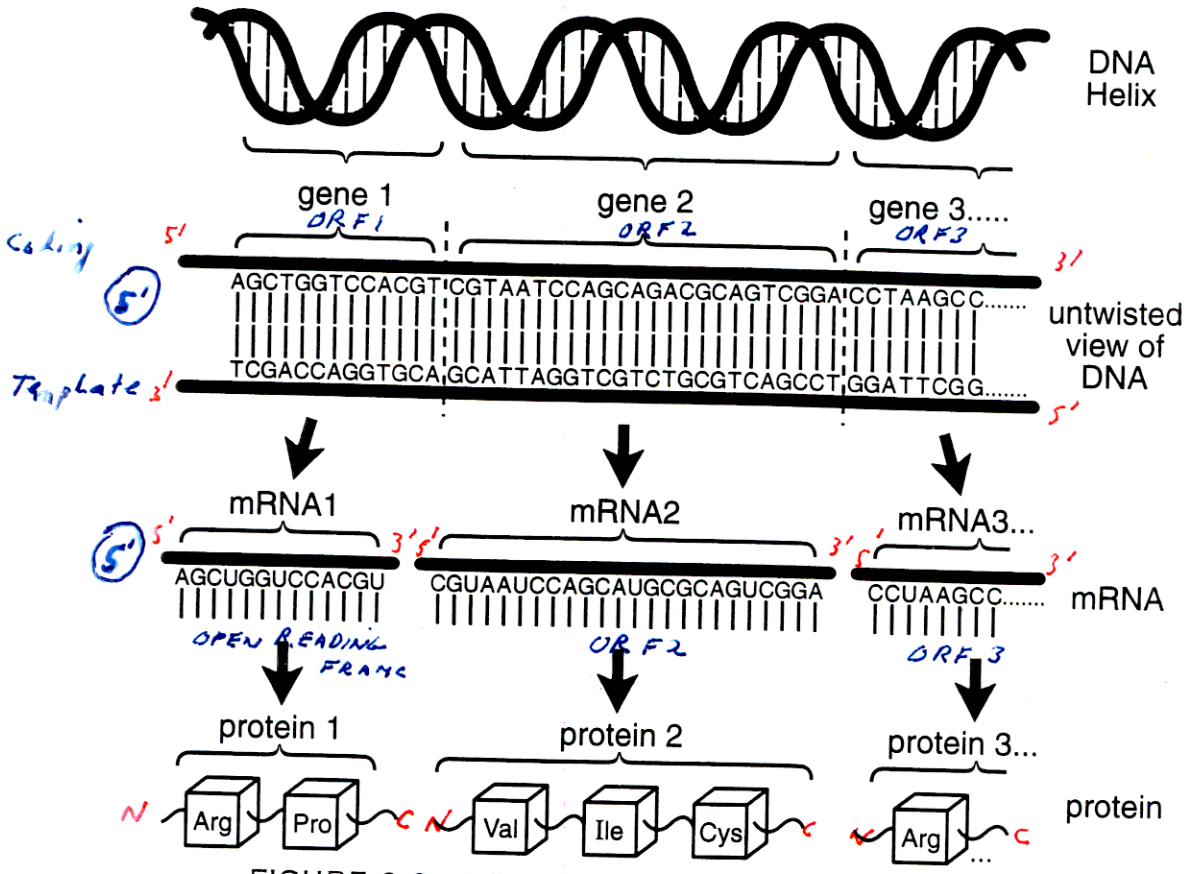


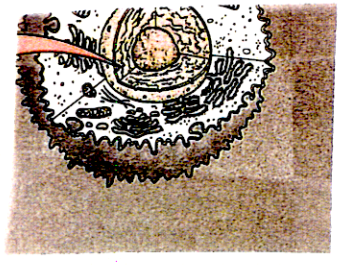
FIGURE 2.6 Adjacent sets of base pairs comprising different genes.

What delineate each gene?

Notice Sequence of each gene

Function 1 | Function 2 | Function 3

Notice Sequence of each protein



Central Dogma

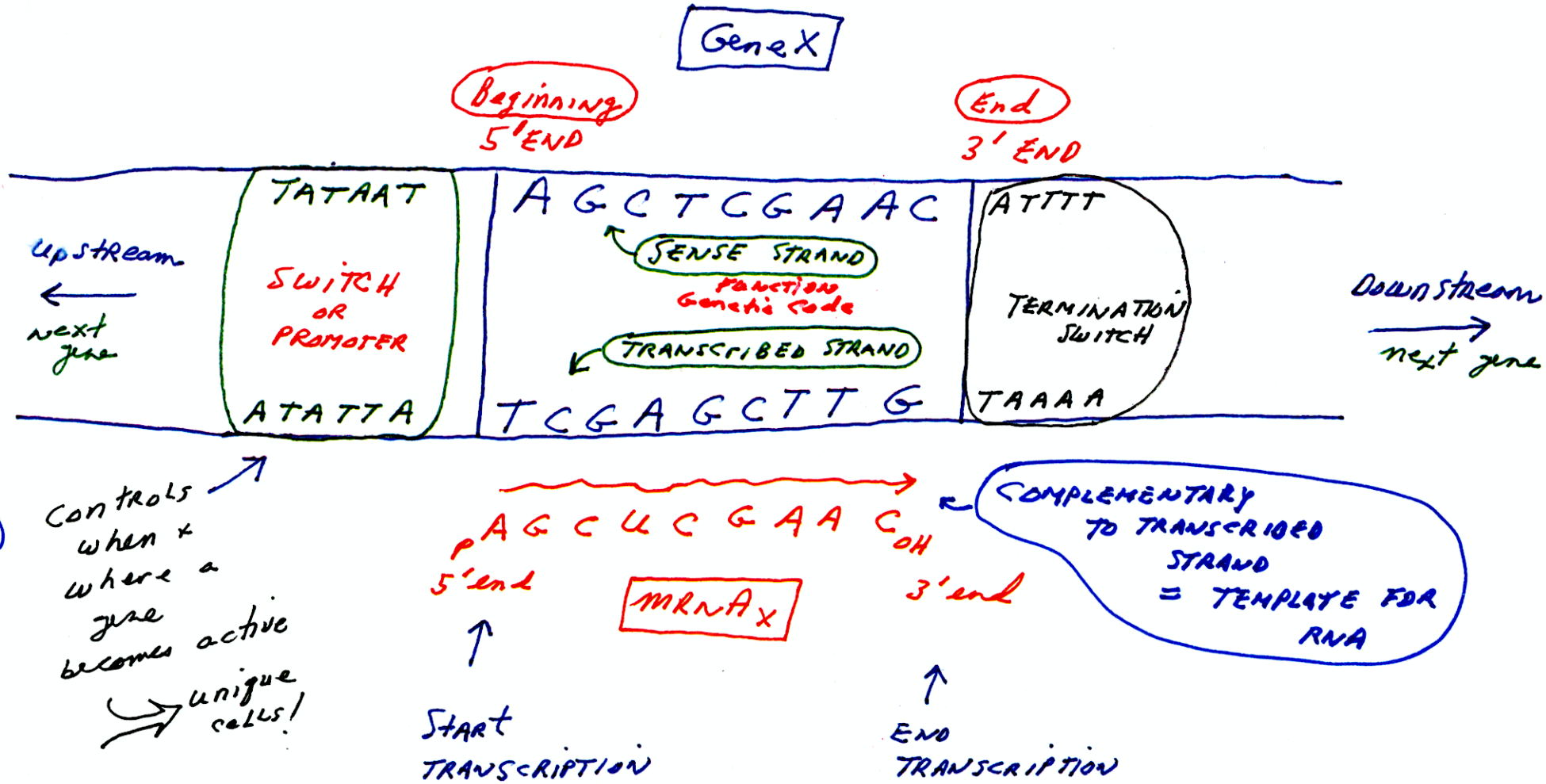
∴ Genes → Functions in Cells via Proteins

Cells duplicate & stay the same → DNA Replication

Notice – Each gene, mRNA, & protein has a unique order / Sequence of Monomeric Units

A Simple Gene = A DOUBLE HELIX

ONLY ONE STRAND TRANSCRIBED



NOTICE: Specific Sequences Specify Beginning & End of Gene & CONTROL ITS ACTIVITY!

NOTICE: mRNA Sequence = SEQUENCE OF SENSE STRAND

Control Switches Are Unique DNA Sequences & CAN be Cloned!

AND USED TO Re-ENGINEER ORGANISMS!! Switches ACT independently of Gene!!

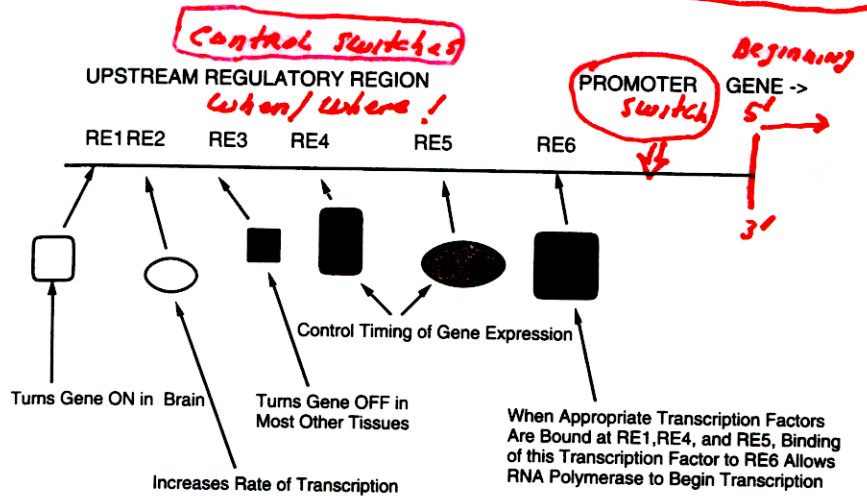


FIGURE 3.13 Enhancers and transcription factors in eukaryotic cells. A schematic diagram of the upstream regulatory region for a brain specific transcript is provided.

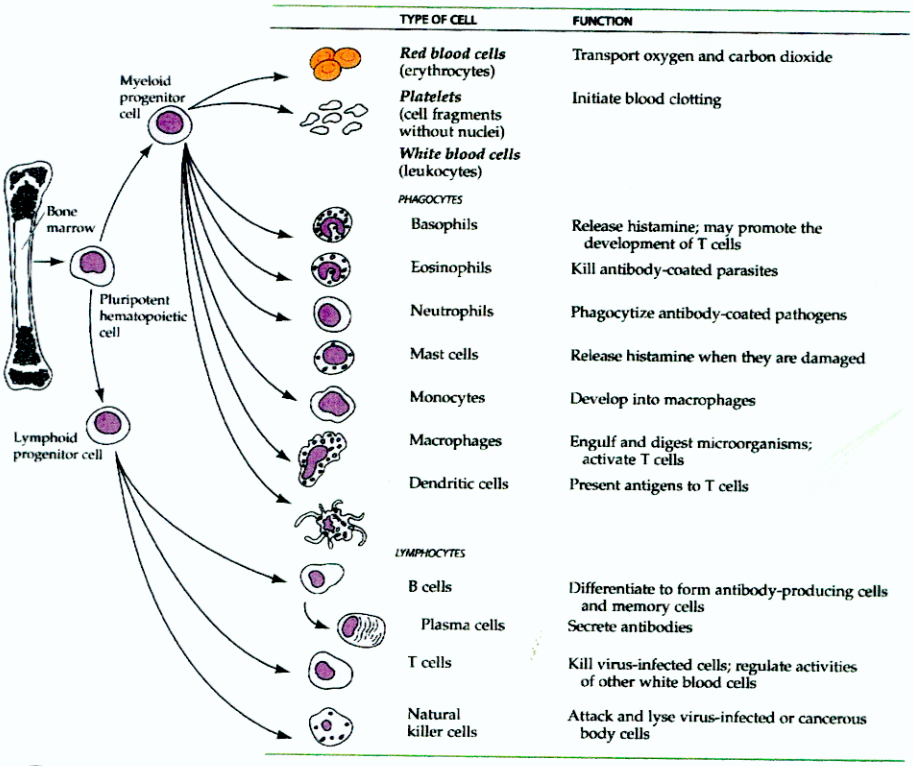
Each Switch = Unique DNA Sequence!

Genome Projects Reveal BOTH the Genes & the Logic that Controls them!

RULE! SEQUENCE → BIOLOGY!!

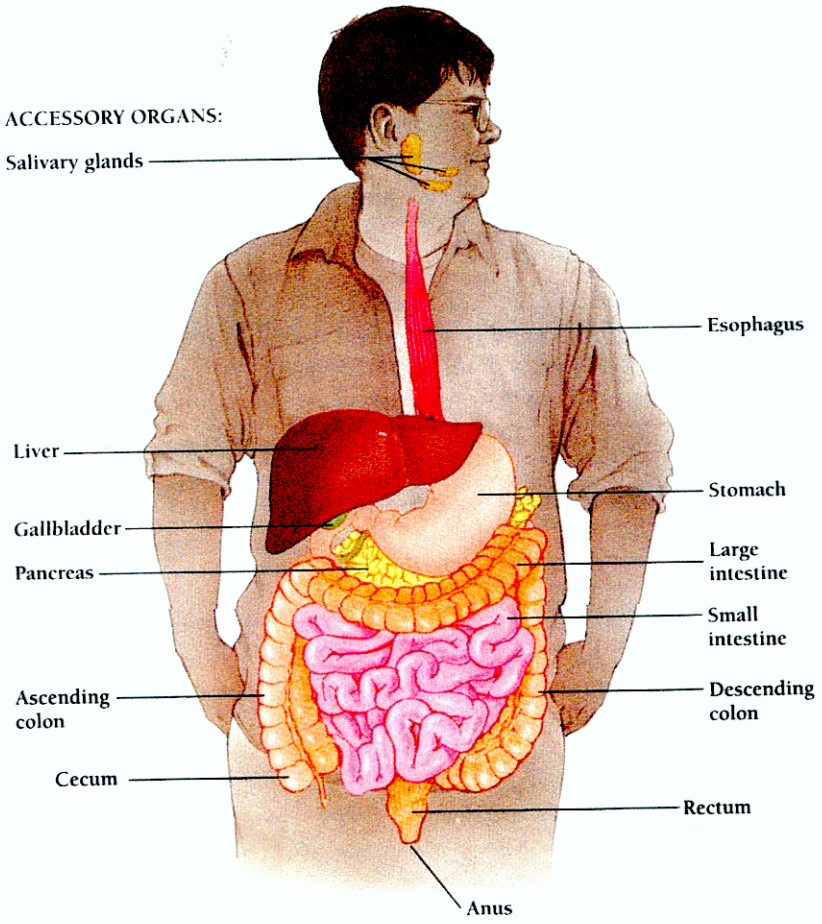
NO "HOCUS POCUS"

SWITCHES CONTROL WHERE & WHEN A GENE IS ACTIVE → UNIQUE FUNCTIONS → UNIQUE CELLS!



TYPE OF CELL	FUNCTION
Red blood cells (erythrocytes)	Transport oxygen and carbon dioxide
Platelets (cell fragments without nuclei)	Initiate blood clotting
White blood cells (leukocytes)	
PHAGOCYTES	
Basophils	Release histamine; may promote the development of T cells
Eosinophils	Kill antibody-coated parasites
Neutrophils	Phagocytize antibody-coated pathogens
Mast cells	Release histamine when they are damaged
Monocytes	Develop into macrophages
Macrophages	Engulf and digest microorganisms; activate T cells
Dendritic cells	Present antigens to T cells
LYMPHOCYTES	
B cells	Differentiate to form antibody-producing cells and memory cells
Plasma cells	Secrete antibodies
T cells	Kill virus-infected cells; regulate activities of other white blood cells
Natural killer cells	Attack and lyse virus-infected or cancerous body cells

19.2 Blood Cells
Pluripotent stem cells in the bone marrow can differentiate into red blood cells, platelets, and the various types of white blood cells.



THE GENE AND SWITCHES ARE UNIQUE DNA SEQUENCES

They CAN BE Cloned & "Shuffled" & Engineered

① CREATING **new** Genes that have no counterparts in nature ⟹ Genetic Engineering

② These new genes CAN be transcribed in New cell types (switch change) &/or organisms &/or Both (e.g., human genes in plant leaves)

→ human gene ⊕ plant leaf switch

③ ALL genes are regulated & controlled by Switches. The Genome Projects reveal both the genes & the switches & WIRING together of all switches in gene

→ Program of life from birth to death

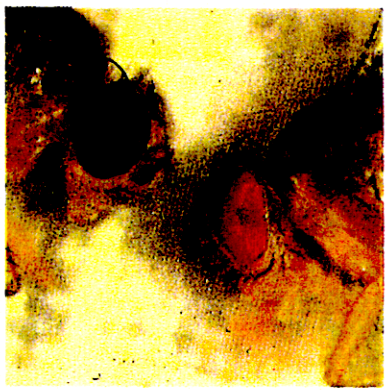
An Eye of a fly can be Produced at other Places on the Fly's Body by Genetic Engineering

① Control Genes Activate Switches of other Genes

② These genes can Specify Proteins that tell cells to develop into complex organs (e.g., eye!)

∴ genes that do the "work" are connected to genes that control them → regulatory circuits / logic

CAN USE Switches to Engineer where/ when Gene Active in an organism → Controls ON/OFF



18–25 *The red-eyed fruit fly at the right is the offspring of the brown-eyed fly at the left.* Drosophila *transposons bearing a gene for red eyes were injected into the brown-eyed fly when it was an early embryo. Transposons with the gene for red eyes were incorporated into chromosomes of the cells that ultimately formed its gametes. The gene for red eyes was therefore passed on to its offspring.*

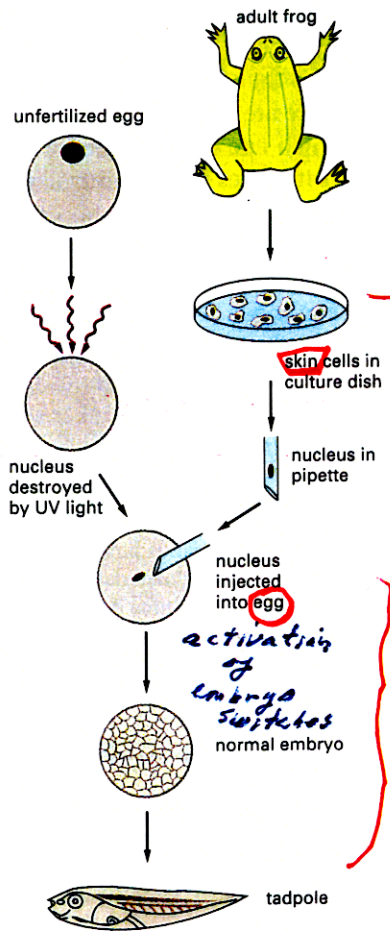
Use the Appropriate Switch with a Master Control Gene that Switches on other Switches to activate genes needed to Make an eye!

CLONING AN ANIMAL FROM a DIFFERENTIATED CELL NUCLEUS SHOWS THAT Gene Switches Contain....

"The Logic" for all of life is contained in the Genome!

Prediction?

Experiment?



What is Hypothesis Being tested?

Skin cells express specific genes due to their skin cell switches

Development of an organism from a fertilized egg requires all switches of genes to work at correct times to allow animal to form!

∴ all genes & switches present in skin cell!!

THE LOGIC TO PROGRAM ALL OF development!

If the Logic of how switches are connected is understood → life can be programmed!

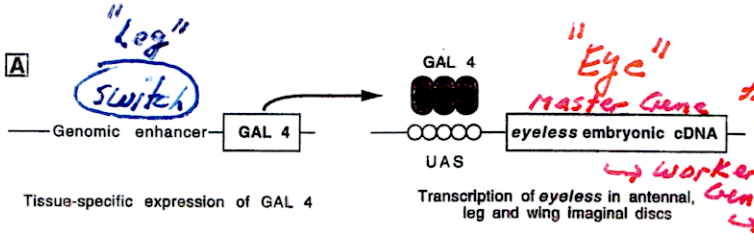
Age of Developmental Engineering is Beginning

This is the ultimate outcome of the Genome Projects! Unraveling our Developmental Gene Networks!

New Genes → Master Genes → Direct Cells → Organs

ALL in the DNA Sequences

Engineered Master Gene to Be Activated in Different Body Parts



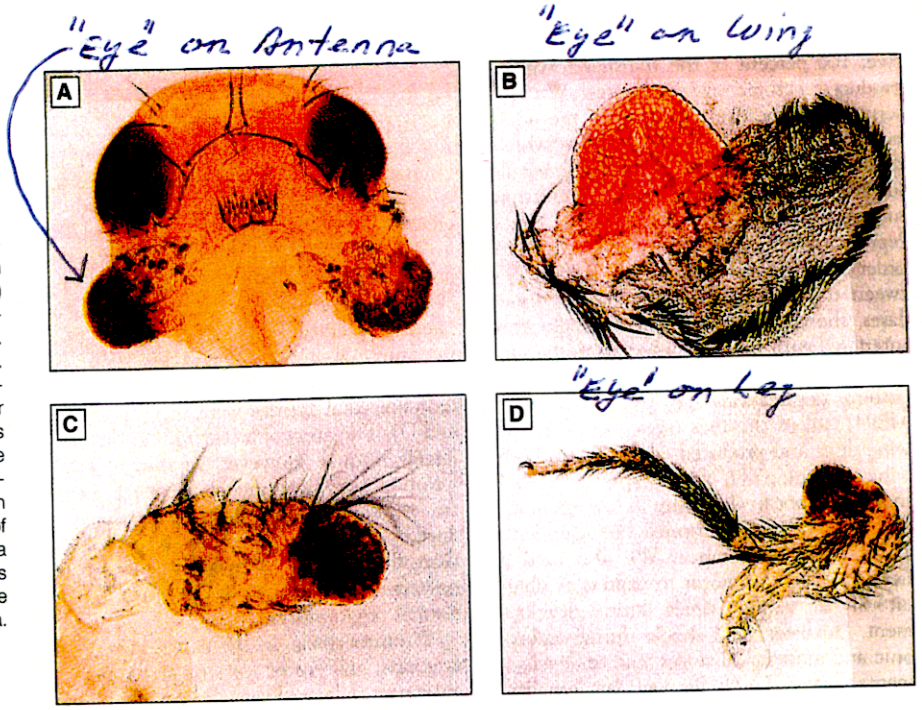


Fig. 2. GAL4 driven ectopic expression of *ey* induces the formation of eye structures in various tissues. The sites at which ectopic eyes form correspond to the regions in the imaginal discs, in which GAL4 is expressed as assayed by the activation of a *lacZ* reporter construct (Fig. 1, B, C, and D). The ectopic eye structures show ommatidial arrays, interommatidial bristles, and red pigmentation (29). (**A**) Cuticle of an adult head in which both antennae formed eye structures. (**B**) Dissected wing with a large outgrowth of eye tissue. The ectopic eye contains about 350 facets. Many interommatidial bristles are also apparent. The normal eye contains approximately 800 ommatidia. The wing is reduced in size. The anterior margin with its characteristic triple row of bristles occupies most of the circumference, whereas the more posterior structures are absent and replaced by eye tissue. The characteristic venation pattern of the wing is disturbed by the formation of the ectopic eye structures. (**C**) Dissected antenna in which most of the third antennal segment is replaced by eye structures. (**D**) Dissected middle leg with an eye-outgrowth on the base of the tibia.

BIG IMPLICATIONS

SHOWS function of eyeless Gene

where does this lead to?
organ growth in culture?

Transplants?
organ design?
Organism design?

30

How does a gene lead to a specific protein/phenotype?

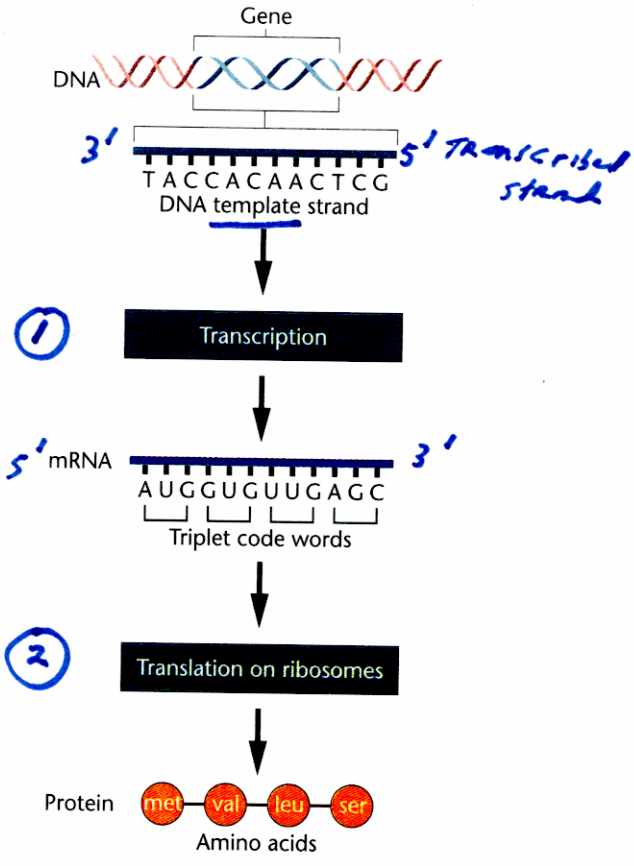


FIGURE 13.1 An overview of the concept of the flow of genetic information encoded in DNA to messenger RNA to protein.

note –

1. mRNA complementary to transcribed (nonsense) strand!
2. mRNA same sequence as sense strand
3. Sequence of Gene → sequence of mRNA → sequence of protein

Single genes can program specific phenotypes in engineered cells – How?

1. Genes work individually
2. Switches work individually
3. Other genes – regulatory genes – Activate switches but these are present in the engineered cells – e.g.; genes to activate bacterial switches!

Step By Step Approach

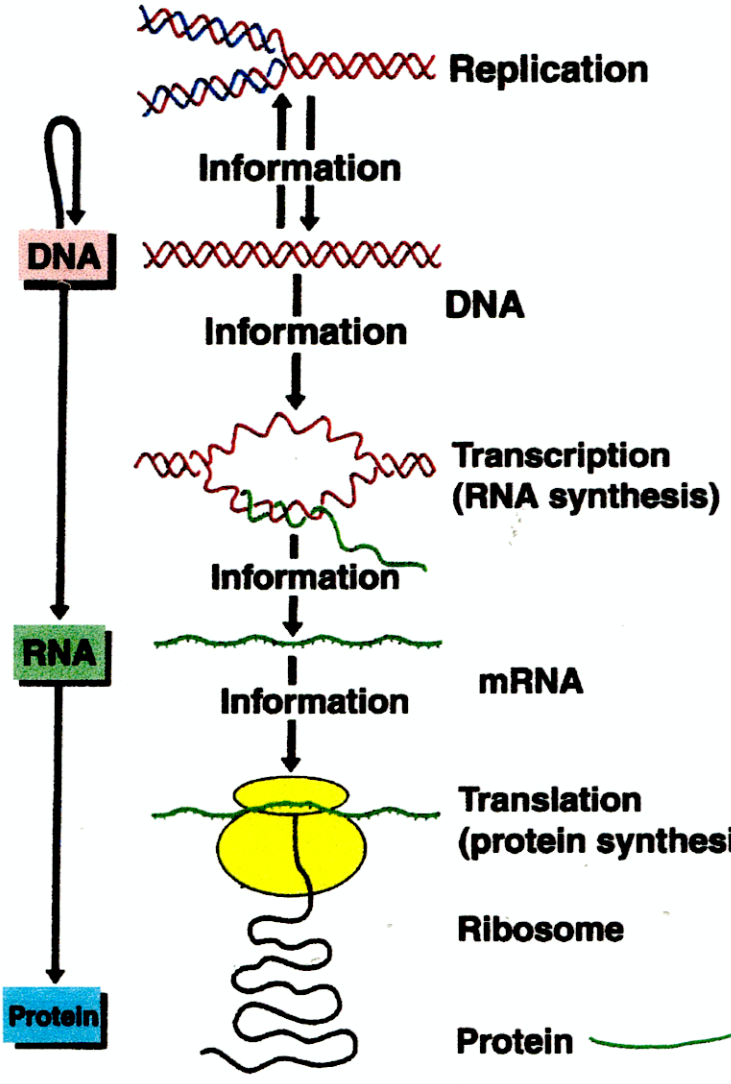
1. TRANSCRIPTION
2. Translation → protein → phenotype

The Process of Gene to Trait is the "SAME" in all organisms!

THE MAIN REASON THAT ALLOWS GENES TO BE ENGINEERED

Translating The Genetic Code Into Proteins is a Conserved Process

The reason "why" genetic engineering is possible



CAN INTERVENE in this Process in Living Cells

Combine switches + genes

What is the "Big" Implication of "this" for Biology?

TRAIT (e.g. eye color)

ALL ORGANISMS USE THE SAME PROCESSES and "RULES" to Generate TRAITS!!
And the SAME MOLECULES/CHEMISTRY is involved!

DO Classical Breeding + Gene Engineering use same Molecular Processes in end!?

TRANSCRIPTION OF A GENE INTO Complementary RNA

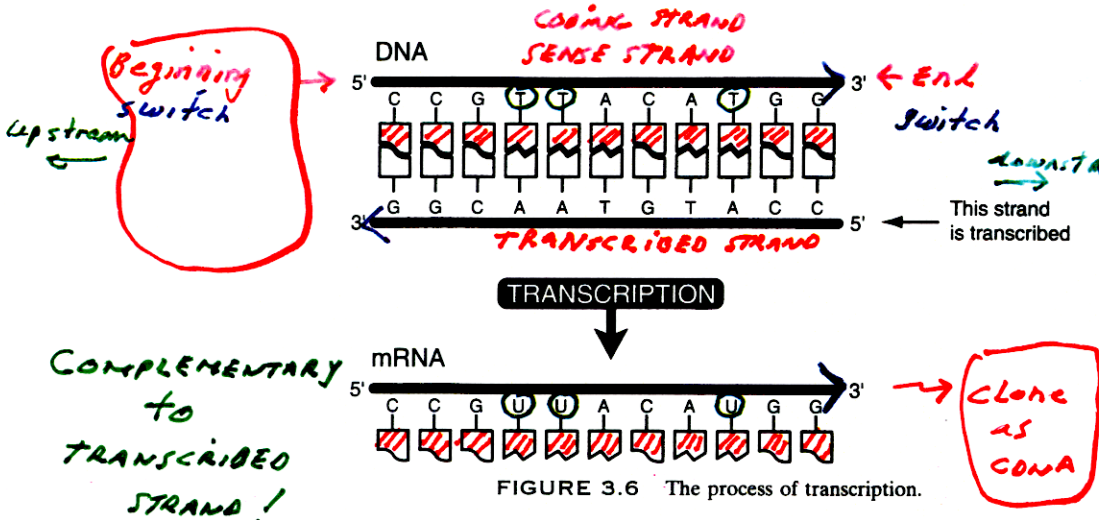


FIGURE 3.6 The process of transcription.

COMPLEMENTARY to TRANSCRIBED STRAND! (opposite polarity)

clone as cDNA

NOTE!

1. one gene strand TRANSCRIBED
2. RNA has same sequence as SENSE STRAND
3. RNA is complementary to TRANSCRIBED strand
4. Uracil takes the place of Thymine in RNA!

PROCESS OF TRANSCRIPTION

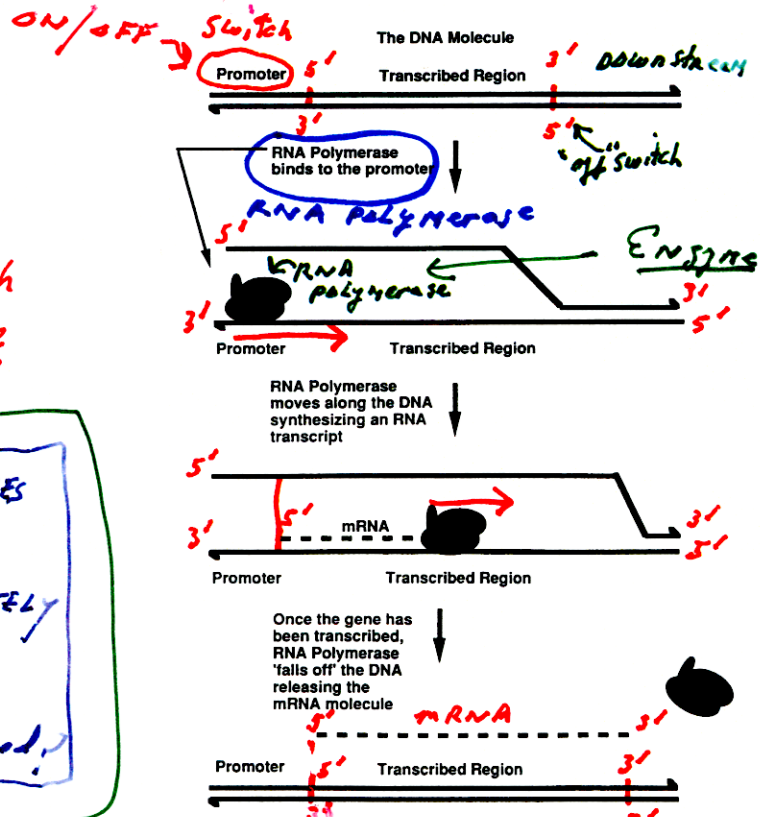


FIGURE 3.11 Transcription made simple.

NOTE!

The switch TURNS the Gene on!

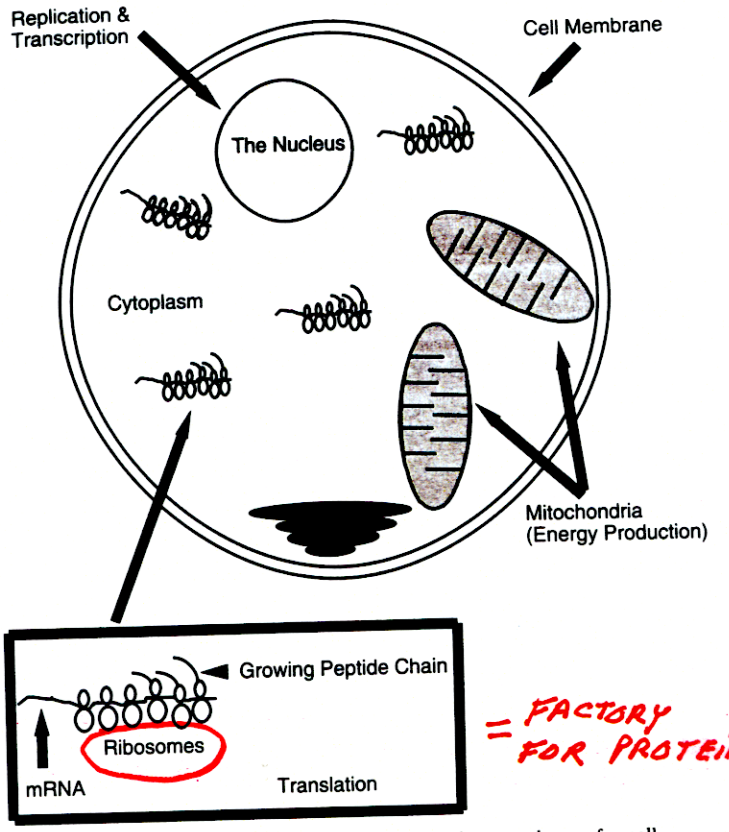
CAN SWITCHES + GENES BE SEPARATELY CLONED & ENGINEERED?

QUESTIONS?!

ARE SWITCHES Gene Specific?

ARE switches ORGANISM or KINGDOM Specific?

PROTEINS ARE synthesized in the cytoplasm of ALL cells.....



= FACTORY FOR PROTEIN synthesis

FIGURE 3.5 Structures and events in the cytoplasm of a cell.

Using factories called ribosomes .....

And 3 RNAs called.....

Table 7–1 Types of RNA Produced in Cells	
Type of RNA	**Function**
mRNAs	codes for proteins
rRNAs	forms part of the structure of the ribosome and participates in protein synthesis
tRNAs	used in protein synthesis as an adaptor between mRNA and amino acids

(1) Instructions

(2) Ribosome structure → Peptide bond Formation

(3) CARRY amino acids

A Protein is Synthesized by TRANSLATING THE GENETIC CODE of the Gene/mRNA into the Amino Acids of a specific Protein

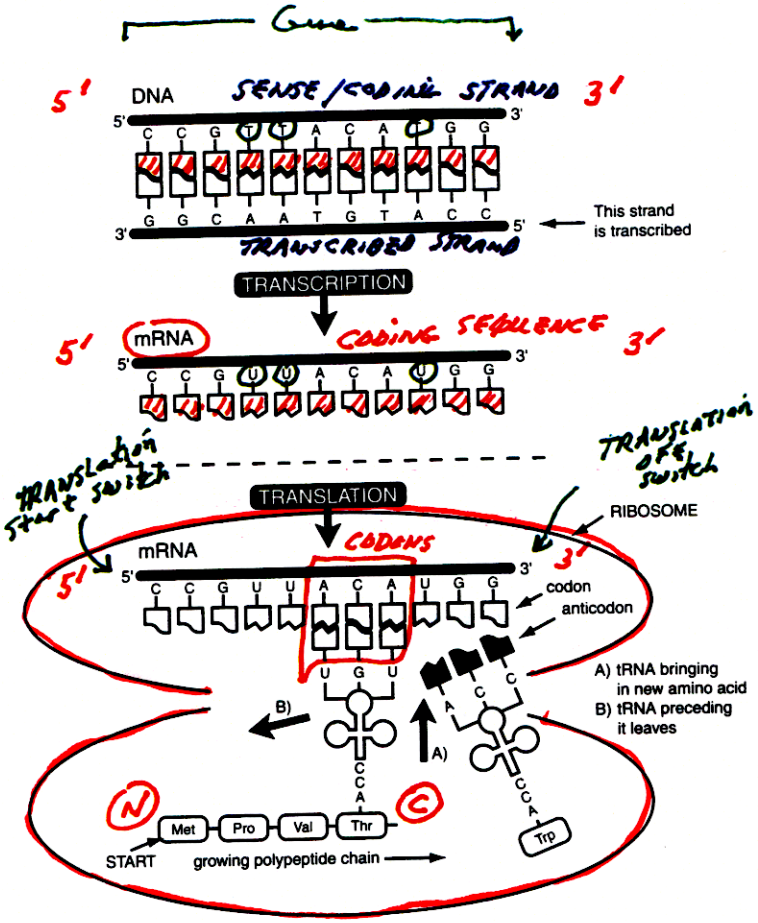


FIGURE 3.7 The process of translation.

Note!
1. Gene has unique 5'→3' sequence
2. mRNA has unique 5'→3' sequence
3. protein has unique aa sequence
4. gene sequence "translated" into protein sequence!
5. mRNA has signals or sequences to tell ribosome ① to bind or ② fall off! & where start of translation is!

Colinearity

DNA Sequence → mRNA Sequence → Protein Sequence → Trait

RIBOSOME FACTORY for protein PRODUCTION

CODONS ON the mRNA Specify Specific Amino Acids

The Genetic Code is Universal

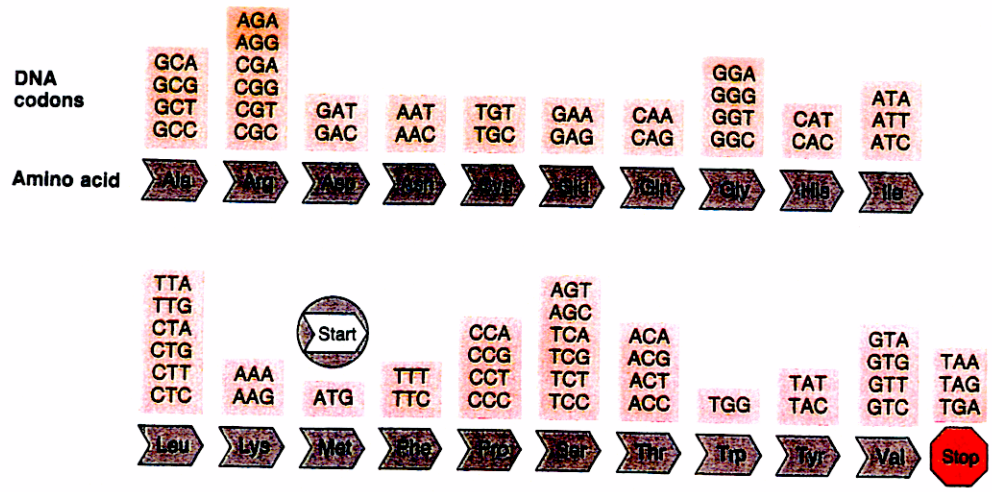


Figure 3.3 The genetic code. The codons shown for each amino acid are those for DNA. For RNA, the Ts are replaced by Us.

Converts Nucleic Acid Information
↳ Protein Information

How know universal?
Design an Experiment!

12.5 The Universal Genetic Code
Genetic information is encoded in mRNA in three-letter units—codons—made up of the bases uracil (U), cytosine (C), adenine (A), and guanine (G). To decode a codon, find its first letter in the left column, then read across the top to its second letter, then read down the right column to its third letter. The amino acid the codon specifies is given in the corresponding row. For example, AUG codes for methionine, and GUA codes for valine.

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phenylalanine	UCU, UCC, UCA, UCG: Serine	UAU, UAC: Tyrosine	UGU, UGC: Cysteine	U C A G
	UUA, UUG: Leucine		UAA: Stop codon; UAG: Stop codon	UGA: Stop codon; UGG: Tryptophan	
C	CUU, CUC, CUA, CUG: Leucine	CCU, CCC, CCA, CCG: Proline	CAU, CAC: Histidine; CAA, CAG: Glutamine	CGU, CGC, CGA, CGG: Arginine	U C A G
A	AUU, AUC, AUA: Isoleucine	ACU, ACC, ACA, ACG: Threonine	AAU, AAC: Asparagine; AAA, AAG: Lysine	AGU, AGC: Serine; AGA, AGG: Arginine	U C A G
	AUG: Methionine; start codon				
G	GUU, GUC, GUA, GUG: Valine	GCU, GCC, GCA, GCG: Alanine	GAU, GAC: Aspartic acid; GAA, GAG: Glutamic acid	GGU, GGC, GGA, GGG: Glycine	U C A G

What's Implication for Genetic Engineering?

USE SPECIFIC CODONS + PUNCTUATION CODES TO DESIGN PROTEINS / GENES!

PROPERTIES

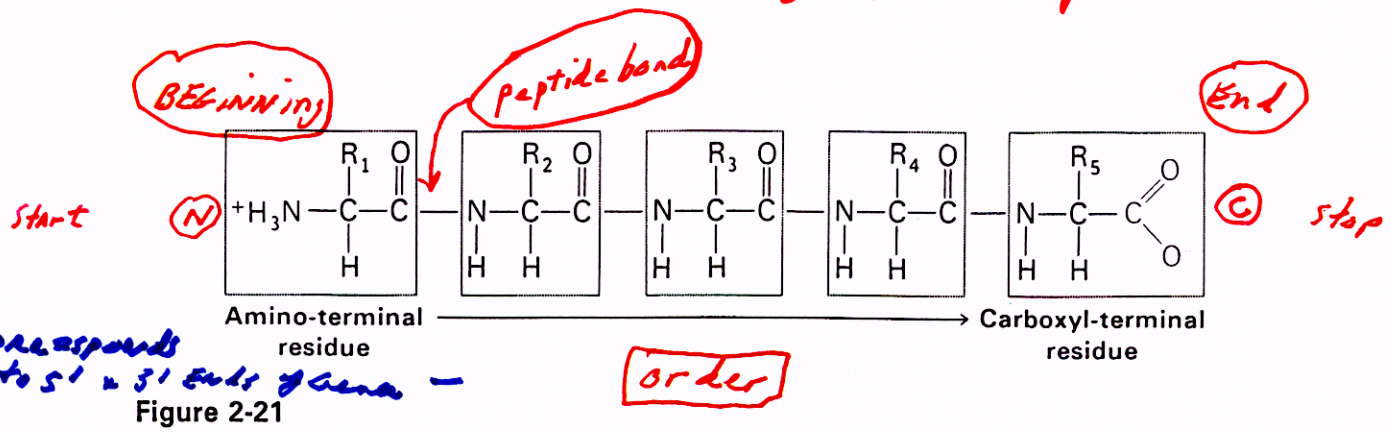
Genetic Dictionary

Properties

1. Universal
2. Degenerate → 1 codon/aa
3. Punctuation
   - Start / Stop
4. 3 Nucleotides / codon

Proteins Consist of Amino Acids Linked By Peptide Bonds

order → unique protein → function!



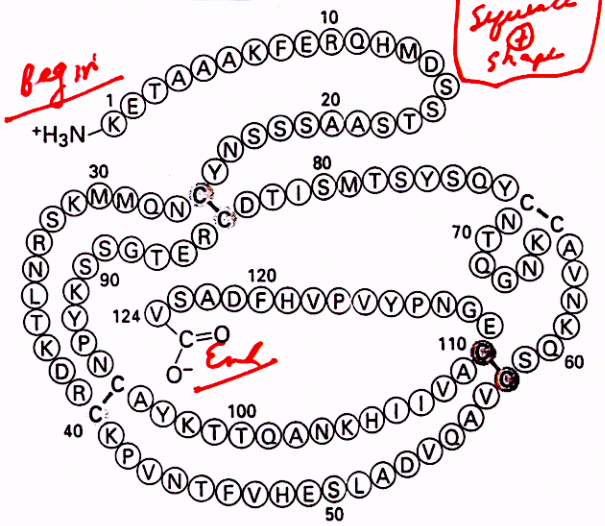
Corresponds to 5' & 3' ends of Gene

Figure 2-21
A pentapeptide. The constituent amino acid residues are outlined. The chain starts at the amino end.

Unique amino acids + unique Sequence → unique protein → unique function → unique gene expression

(1) Could you identify the RNase Gene from this Sequence?

Sequence & shape



(2) How obtain?

MUTATIONS? How Disrupt Function?

amino acid order → function → gene expression

Figure 2-50
Amino acid sequence of bovine ribonuclease. The four disulfide bonds are shown in color. [After C.H.W. Hirs, S. Moore, and W.H. Stein. *J. Biol. Chem.* 235(1960):633.]

aa order → Specific protein → Specific function → Phenotype - Gene Expression

PROTEINS CARRY OUT DIVERSE CELL FUNCTIONS AND ARE UNIQUE BECAUSE OF SEQUENCE!

Table 3.2 The Many Functions of Proteins

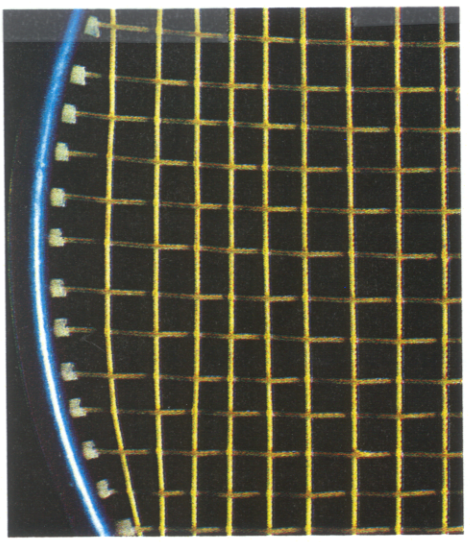
Function	Class of Protein	Examples	Use
Metabolism (Catalysis)	Enzymes	Hydrolytic enzymes	Cleave polysaccharides
		Proteases	Break down proteins
For Gene Engineer →	DNA Polymerase, Reverse Trans. Terminal Transferase, Restriction Enzymes	Polymerases	Produce nucleic acids
		Kinases	Phosphorylate sugars and proteins
Defense	Immunoglobulins	Antibodies	Mark foreign proteins for elimination
	Toxins	Snake venom	Block nerve function
Cell recognition	Cell surface antigens	MHC proteins	"Self" recognition
Transport throughout body	Globins	Hemoglobin	Carries O_2 and CO_2 in blood
		Myoglobin	Carries O_2 and CO_2 in muscle
		Cytochromes	Electron transport
Membrane transport	Transporters	Sodium-potassium pump	Excitable membranes
		Proton pump	Chemiosmosis
		Anion channels	Transport Cl^- ions
Structure/Support	Fibers	Collagen	Cartilage
		Keratin	Hair, nails
		Fibrin	Blood clot
Motion	Muscle	Actin	Contraction of muscle fibers
		Myosin	Contraction of muscle fibers
Osmotic regulation	Albumin	Serum albumin	Maintains osmotic concentration of blood
Regulation of gene action → regulate switches!	Repressors	*lac* repressor	Regulates transcription
Regulation of body functions	Hormones	Insulin	Controls blood glucose levels
		Vasopressin	Increases water retention by kidneys
		Oxytocin	Regulates uterine contractions and milk production
Storage	Ion binding	Ferritin	Stores iron, especially in spleen
		Casein	Stores ions in milk
		Calmodulin	Binds calcium ions

Mutate Gene → Mutate Protein → Defective function

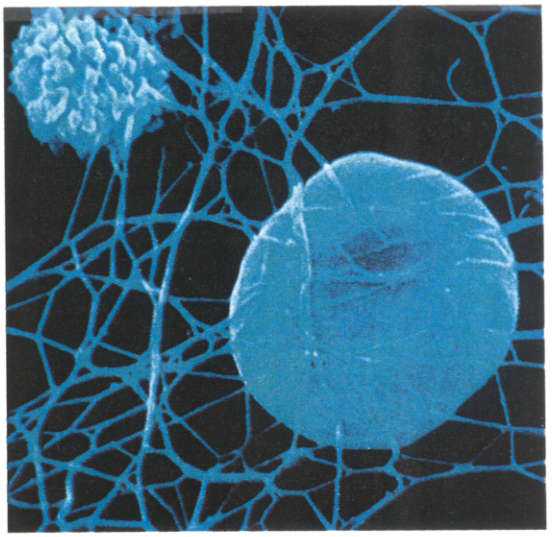
UNIQUE PROTEINS → UNIQUE FUNCTION

Engineer cells by adding a new gene
∴ new protein ∴ new function

collagen

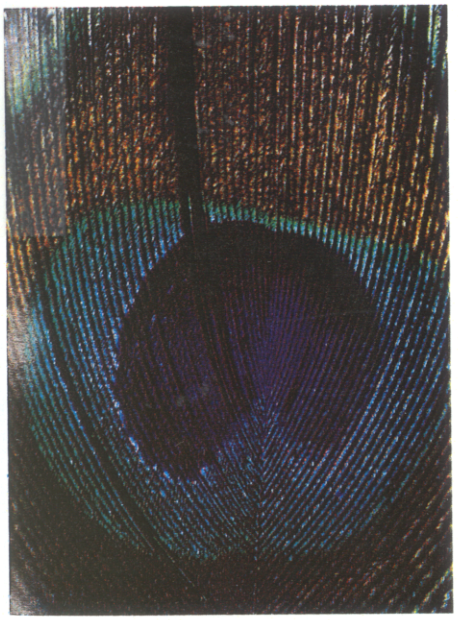


(a)



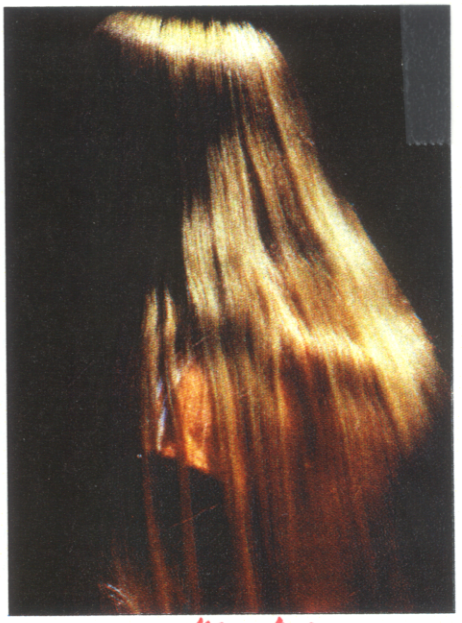
(b)

blood clot (protein) etc.



(c) keratin (feather)

(d) e.g. spider silk protein in plants/goat milk!

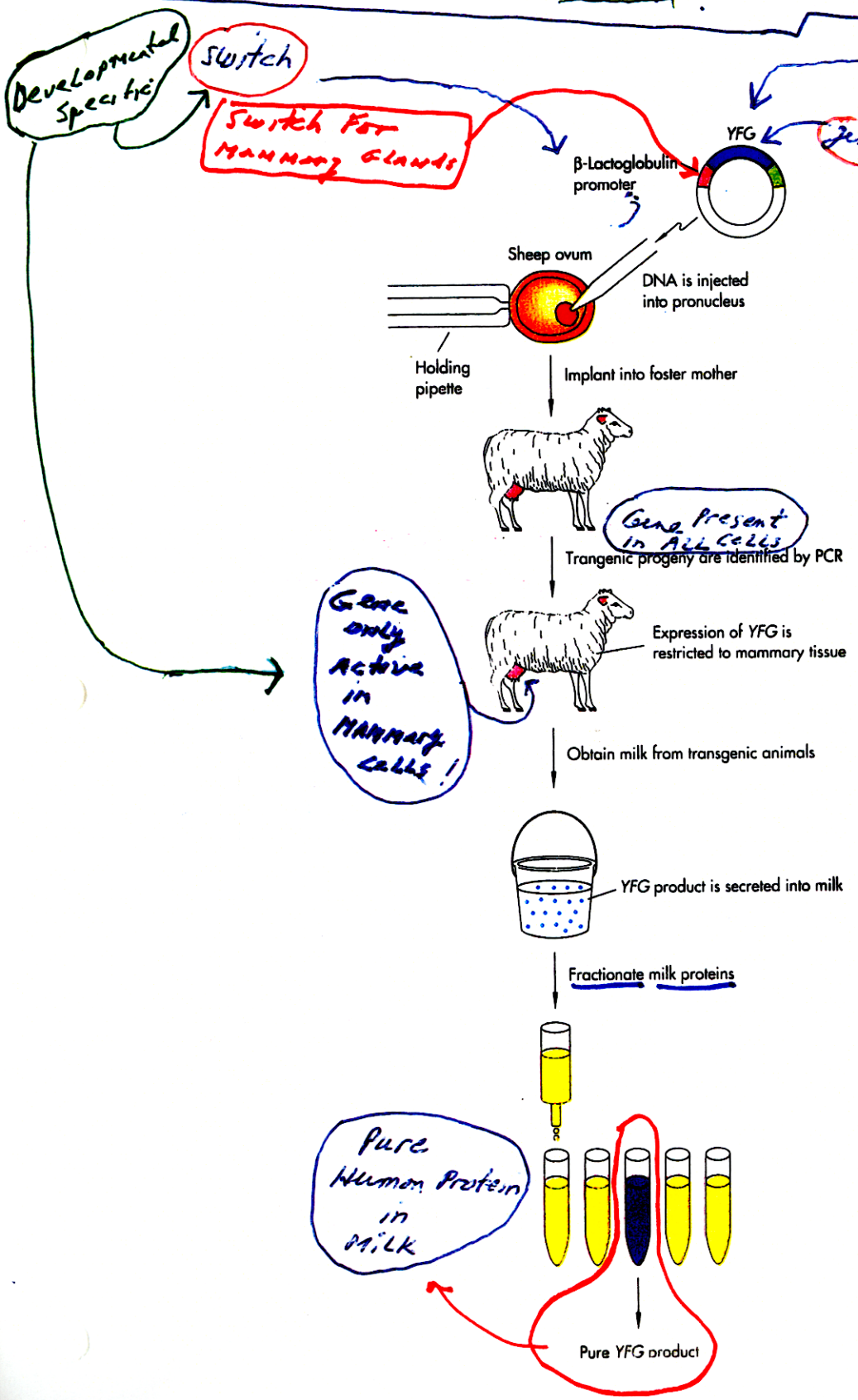


(e) keratin (hair)

FIGURE 3.4
Some of the more common structural proteins. (*a*) Collagen: strings of a tennis racket from gut tissue; (*b*) fibrin: scanning electron micrograph of a blood clot (3000×); (*c*) keratin: a peacock feather; (*d*) silk: a spider's web; (*e*) keratin: human hair.

DNA → RNA → Protein is A universal process!

CAN HUMAN GENES WORK in Sheep?



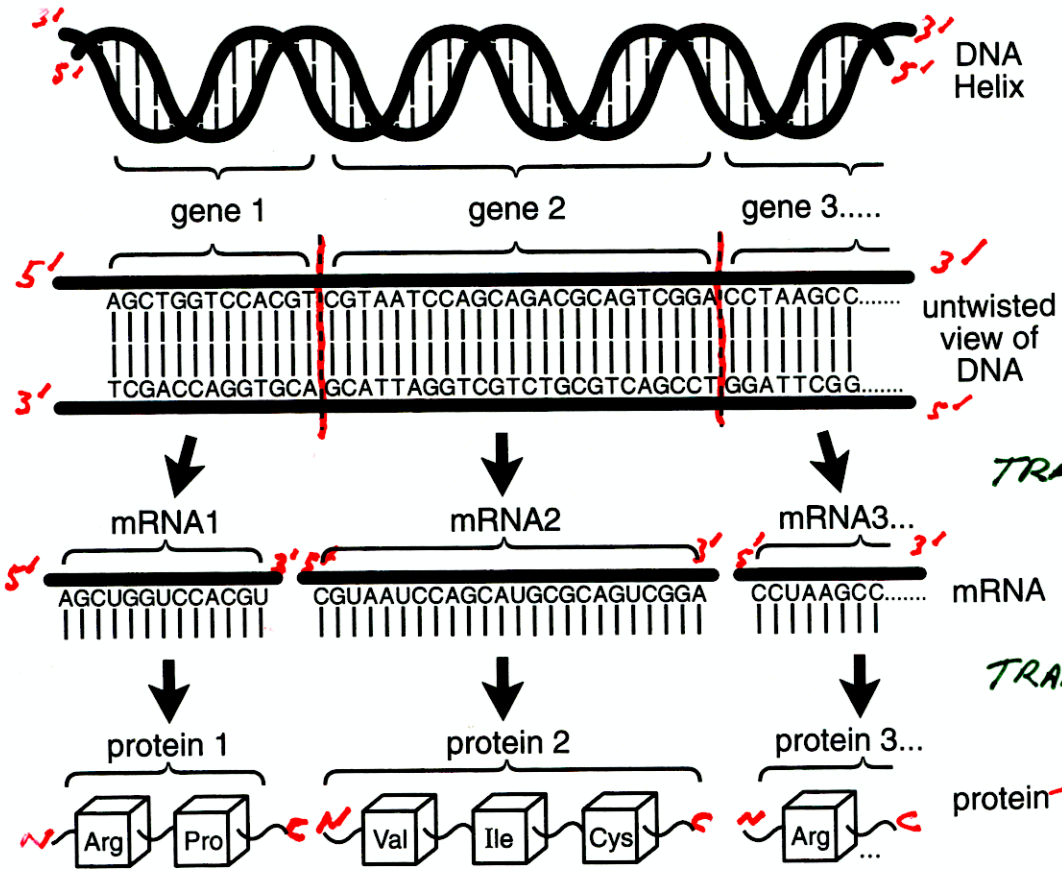
Questions?

1. How identify presence of gene in Sheep?
2. How Engineer to be Active only in Mammary Cells?
3. How do human gene work in sheep?
4. How does this experiment show DNA is genetic material?

Human Gene Engineered to be Active in Sheep Mammary Gland!

HOW DOES A SPECIFIC DNA SEQUENCE OR GENE SPECIFY THE Phenotype ?

COLINEARITY BETWEEN NUCLEOTIDE SEQUENCE OF A GENE AND AMINO ACID SEQUENCE OF PROTEIN



TRANSCRIPTION

TRANSLATION of Genetic CODE

Trait → Phenotype

FIGURE 2.6 Adjacent sets of base pairs comprising different genes.

Specific TRAIT 1 → FUNCTION 1

Specific TRAIT 2 → Function 2

Specific Trait 3 → FUNCTION 3

ONE GENE → ONE PROTEIN → ONE FUNCTION

in simplest form

EUKARYOTIC & PROKARYOTIC Gene Expression Processes differ Slightly

Genes Differ
Switches/RNA Polymerases Differ } Because cells & life cycles differ
Genetic Code the SAME
General Processes the SAME

but..... .....

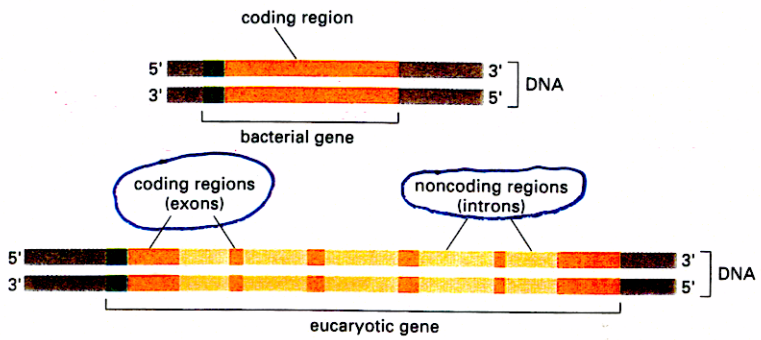


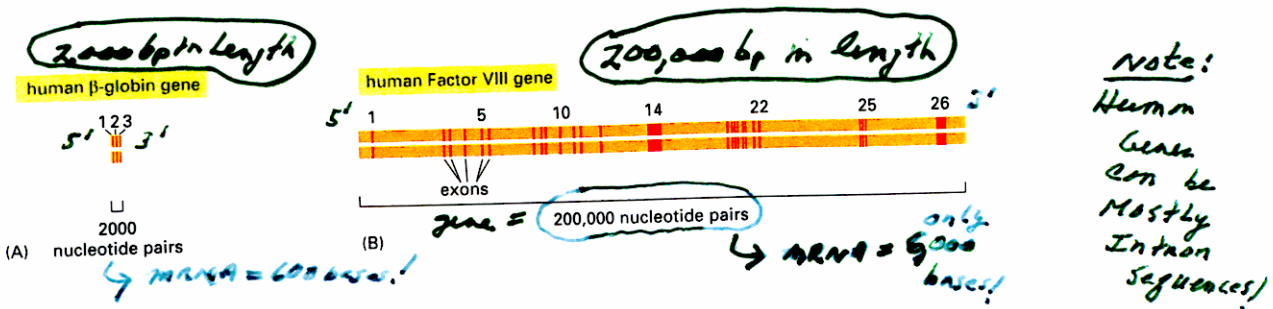
Figure 7–13 Comparison of a bacterial gene with a eucaryotic gene. The bacterial gene consists of a single stretch of uninterrupted nucleotide sequence that encodes the amino acid sequence of a protein. In contrast, the coding sequences of most eucaryotic genes (*exons*) are interrupted by noncoding sequences (*introns*). Promoters for transcription are indicated in *green*.

SWITCHES UNIQUE TO BACTERIA & TO PLANTS/ANIMALS

Eukaryotic Genes (CAN) have NON-coding regions "Stuck" in coding regions

Prokaryotic Genes only have coding regions!

Thus: Eukaryotic cells must remove non-coding regions in mRNA BEFORE genetic code can be translated CONTINUOUSLY!



Note: Human Genes can be Mostly Intron Sequences!

NOTE! Gene CAN Be Huge!

EUKARYOTIC GENES HAVE INTRONS OR NON-CODING DNA INTERSPERSED IN CODING SEQUENCES OR EXONS

INTRONS ARE TRANSCRIBED BUT MUST BE SPLICED OUT IN NUCLEUS TO MAKE MRNA WITH CONTINUOUS GENETIC CODE!!

GENE → RNA → SPLICING → MRNA

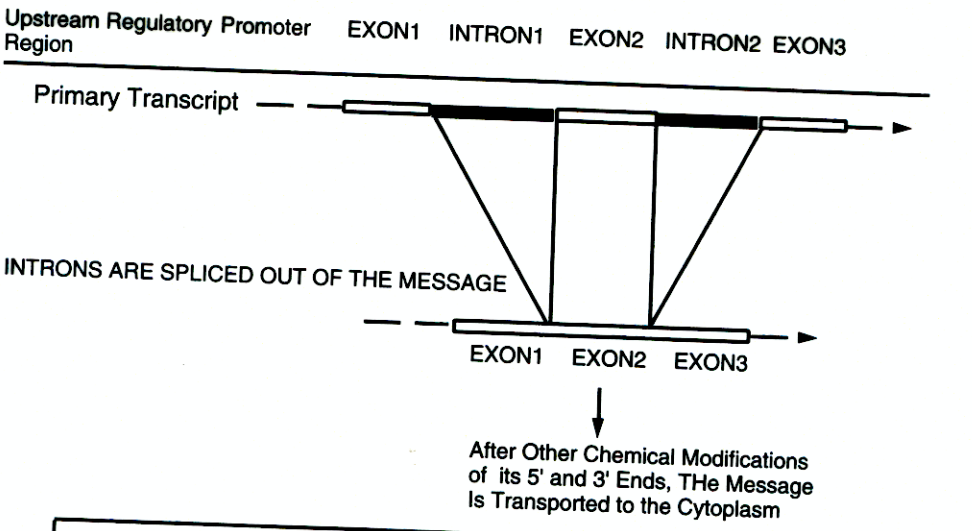


FIGURE 3.14 Exons, introns, and splicing.

Implication for Engineering Eukaryotic genes in Bacteria?

BACTERIAL GENES DO NOT HAVE INTRONS & DO NOT PROCESS EUKARYOTIC RNAS!

Eukaryotic Gene TRANSCRIPTS Are Processed By Splicing in the Nucleus to Form mRNAS

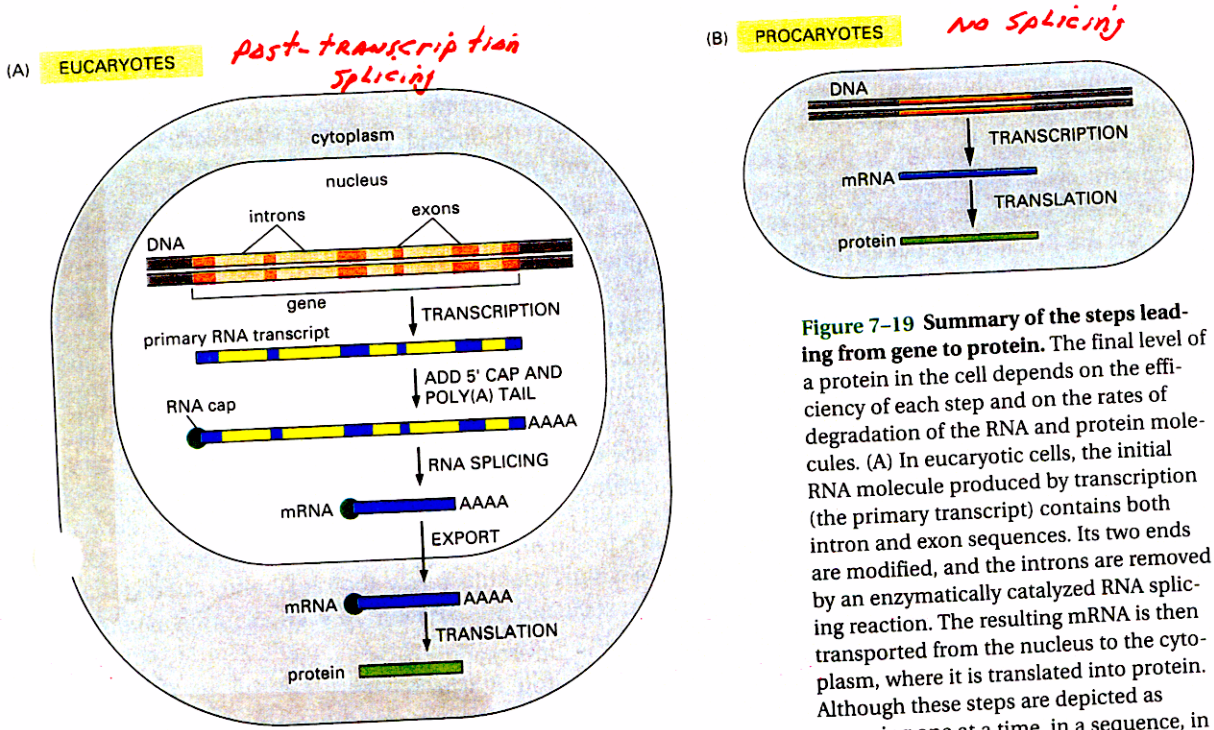


Figure 7–19 Summary of the steps leading from gene to protein. The final level of a protein in the cell depends on the efficiency of each step and on the rates of degradation of the RNA and protein molecules. (A) In eucaryotic cells, the initial RNA molecule produced by transcription (the primary transcript) contains both intron and exon sequences. Its two ends are modified, and the introns are removed by an enzymatically catalyzed RNA splicing reaction. The resulting mRNA is then transported from the nucleus to the cytoplasm, where it is translated into protein. Although these steps are depicted as occurring one at a time, in a sequence, in reality they often occur simultaneously. For example, the RNA cap is typically added and splicing typically begins before the primary transcript has been completed. (B) In procaryotes, the production of mRNA molecules is simpler. The 5′ end of an mRNA molecule is produced by the initiation of transcription by RNA polymerase, and the 3′ end is produced by the termination of transcription. Since procaryotic cells lack a nucleus, transcription and translation take place in a common compartment. In fact, translation of a bacterial mRNA often begins before its synthesis has been completed.

What are Consequences For Expressing a Human Gene with Introns in a Bacterial Cell???

Engineer mRNA not gene!!

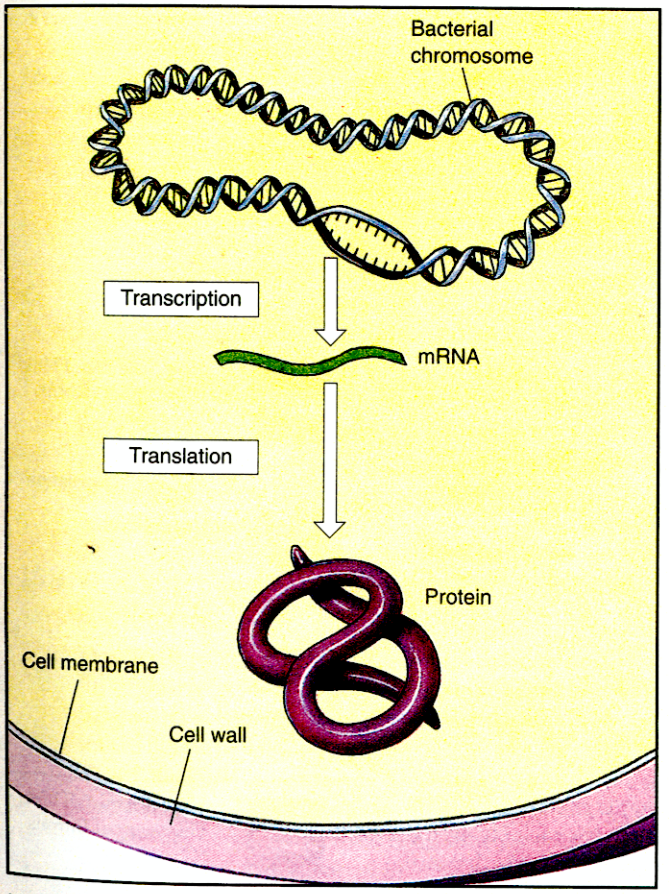
GENE ACTIVITY in PROKARYOTES + EUKARYOTES

Differences between Bacterial and Eukaryotic Gene Expression

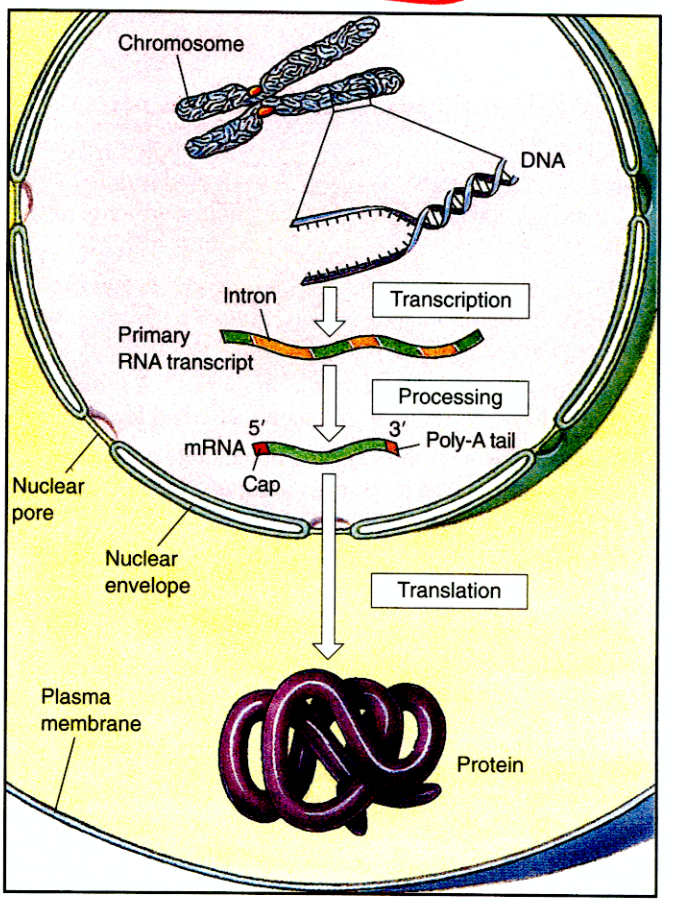
1. Most eukaryotic genes possess introns. With the exception of a few genes in the Archaebacteria, prokaryotic genes lack introns (figure 15.17).
2. Individual bacterial mRNA molecules often contain transcripts of several genes. By placing genes with related functions on the same mRNA, bacteria coordinate the regulation of those functions. Eukaryotic mRNA molecules rarely contain transcripts of more than one gene. Regulation of eukaryotic gene expression is achieved in other ways.
3. Because eukaryotes possess a nucleus, their mRNA molecules must be completely formed and must pass across the nuclear membrane before they are translated. Bacteria, which lack nuclei, often begin translation of an mRNA molecule before its transcription is completed.
4. In bacteria, translation begins at an AUG codon preceded by a special nucleotide sequence. In eukaryotic cells, mRNA molecules are modified at the 5′ leading end after transcription, adding a 5′ cap, a methylated guanosine triphosphate. The cap initiates translation by binding the mRNA, usually at the first AUG, to the small ribosomal subunit.
5. Eukaryotic mRNA molecules are modified before they are translated: introns are cut out, and the remaining exons are spliced together; a 5′ cap is added; and a 3′ poly-A tail consisting of some 200 adenine (A) nucleotides is added. These modifications can delay the destruction of the mRNA by cellular enzymes.
6. The ribosomes of eukaryotes are a little larger than those of bacteria.

Gene expression is broadly similar in bacteria and eukaryotes, although it differs in some details.

What are implications for Gene Engineering?



(a)



(b)

FIGURE 15.17
Gene information is processed differently in prokaryotes and eukaryotes. (*a*) Bacterial genes are transcribed into mRNA, which is translated immediately. Hence, the sequence of DNA nucleotides corresponds exactly to the sequence of amino acids in the encoded polypeptide. (*b*) Eukaryotic genes are typically different, containing long stretches of nucleotides called introns that do not correspond to amino acids within the encoded polypeptide. Introns are removed from the primary RNA transcript of the gene and a 5' cap and 3' poly-A tail are added before the mRNA directs the synthesis of the polypeptide.

Engineering Genes Requires:

1. The Gene & its Sequence
2. A Roadmap of where Coding Sequence & ALL switches located (What's the roadmap?)
3. TRANSCRIPTION Start & Stop Switches
4. Coding Part of Gene / Genetic Code Part
5. TRANSLATION Start & Stop Switches
6. Kingdom-Specific Switches/Signals

Note! The general process of gene → protein is the same in all organisms but the Specific switches & enzymes (e.g., RNA polymerase) differ in kingdoms!!

PLANT TRANSCRIPTION ON SWITCH + HUMAN CODING SEQUENCE + Plant TRANSCRIPTION off Switch

7. It's that easy Because living cells use SAME overall genetic processes! → human protein in plant

8. There are NO LIMITS! The Limit?

CAN ANIMAL GENES BE ENGINEERED TO WORK IN PLANTS?

Which kingdom switch?

note)

plant switch

developmental specific

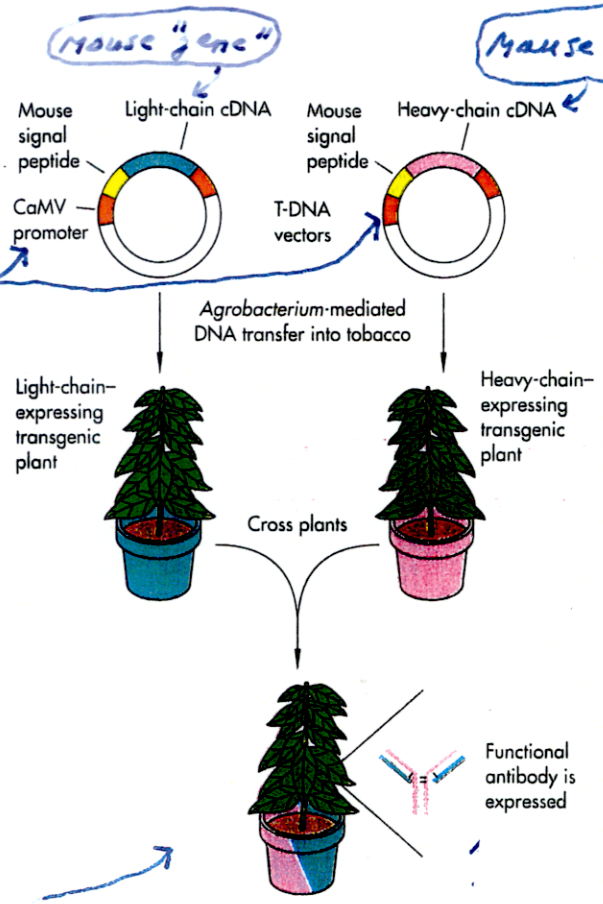


FIGURE 24-5
Plants as bioreactors to produce antibodies. Cloned cDNAs encoding the light and heavy chains from a mouse monoclonal antibody were ligated into separate T-DNA vectors and placed under control of a constitutive CaMV promoter. The plasmids were transferred separately into tobacco plants by *Agrobacterium* infection. Transgenic plants containing the light- and heavy-chain genes were sexually crossed to produce progeny plants that contained both genes. Examination of protein extracted from leaves demonstrated the expression of functional antibody molecules in these progeny plants. Other experiments showed that the presence of a signal sequence was necessary for high-level expression. These results suggest that the plant secretion machinery can recognize the mouse signal peptide.

Mouse protein in Leaves of PLANT!

QUESTIONS?

① How identify gene in plant?

② How Engineer to be active in Leaves of plant?

③ What does this experiment tell us about genetic processes → gene → protein synthesis?

IMPORTANT CONCEPT!

Genes ARE Replicated
DURING CELL DIVISION
AND EACH DAUGHTER CELL
RECEIVES THE SAME
COLLECTION of Genes

Property of DNA as Genetic Material

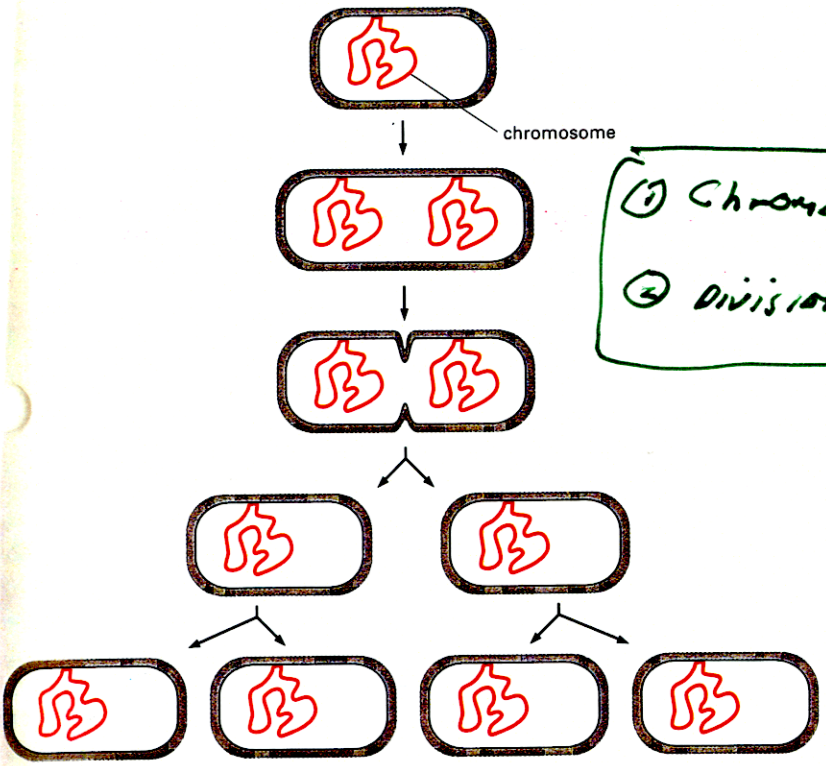


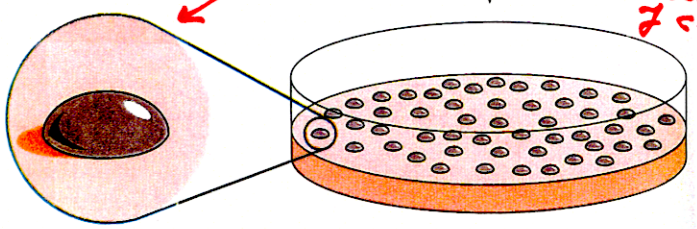
Figure 9–1 Duplication of bacterial cells. The division of one bacterium into two takes 20–25 minutes under ideal growth conditions.

① Chromosome / DNA Replication / Genome - Genes
② Division of Cell / Cytoplasm

MUST Be Precise / DNA is Stable!

Identical Cells in a Colony ∴ CLONES

Note - Each Clump of Bacteria Contain Clones of Cells



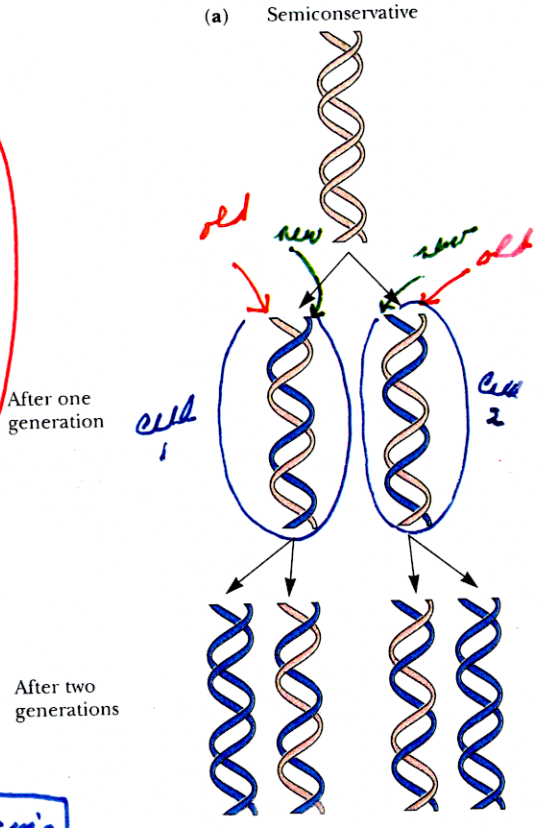
~10^6 cells per colony ~50 colonies per dish

A BACTERIAL COLONY Represents Many Copies of the SAME CELL or CLONES

CLONE = Genetically IDENTICAL cells / organism

DNA REPLICATION OCCURS IN A SEMI-CONSERVATIVE MANNER

DNA STRUCTURE ALLOWS Replication → Complementary BASES

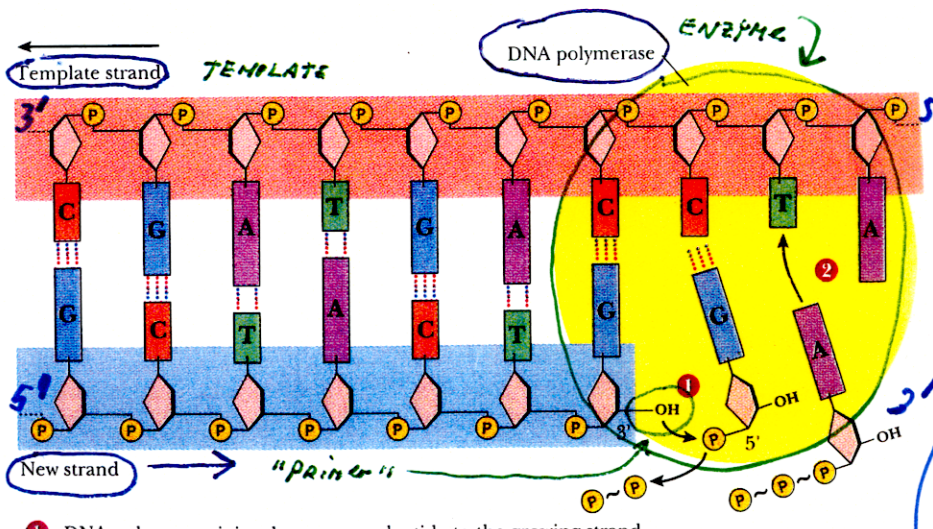


① Each STRAND Serves as a template for the Synthesis of A complementary Strand

② NEW DNA Molecules are precise copies of the parental DNA with one template strand ⊕ one complementary new strand!

For Genetic Engineering also used in CONACloning

DNA Polymerase is the enzyme that "copies" the template STRAND. It binds to the ORI!



1. DNA polymerase joins the a new nucleotide to the growing strand.
2. Another free nucleotide pairs up with its complement on the template strand.

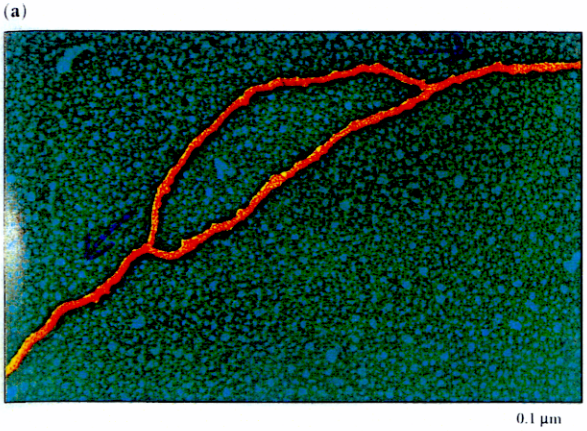
Figure 13-10 The formation of a p[illegible]hodiester bond. Here dGTP a[illegible] opposite a C, to the 3′-end of a growing DNA strand.

5'→3' Synthesis ALWAYS!

DNA POLYMERASE & ORI ARE kingdom-specific

Vectors ARE Needed to Replicate Genes in Specific Cells

DNA in the Process of Being Replicated



Because!

① ORIgins of Replication are SPECIFIC DNA Sequences

② DNA Polymerases are Kingdom-Specific to Recognize ORI Sequences specific to that Kingdom —

CONSEQUENCES to GENETIC ENGINEERING?

∴ Need BACTERIAL ORI to clone a human gene in a bacterial cell & have it replicated!

HOW Clone an ORI?

Question?

Vice Versa

THE Polymerase Chain Reaction OR PCR is a Molecular Xerox Machine in a Test Tube!

PCR allows specific DNA sequences to be SELECTIVELY "copied" from "tiny" amounts of DNA!!

e.g.; globin gene amplified directly from blood cells!

USE DNA template + DNA polymerase in test tube.

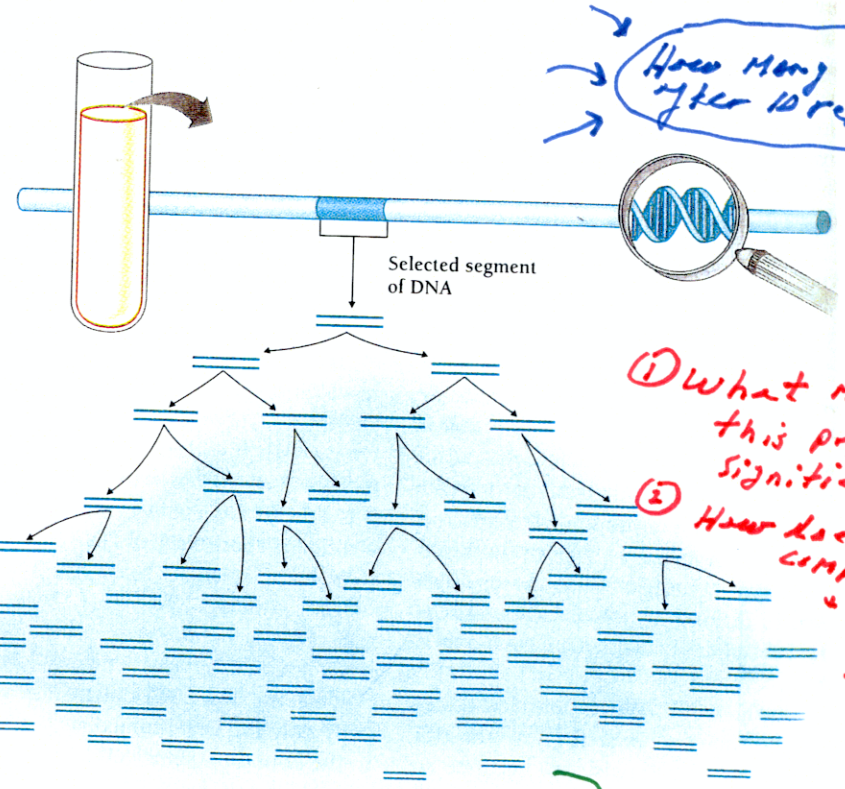


Figure 13-2 PCR is a simple, powerful technique for multiplying specific sequences of DNA. A. When DNA is heated, the two strands uncoil. They are then cooled and replicated. The cycle of heating, cooling, replicating, and then heating again is repeated until millions or billions of copies of the sequence are obtained. B. Short segments of single-stranded DNA called oligonucleotides act as primers and allow researchers to replicate a particular sequence, not just any DNA. The 20 or so bases of the oligonucleotide pair with the correct segment of the DNA and initiate replication.

① What makes this procedure significant?

② How does it complement & substitute for bacterial cloning?

SIGNIFICANCE?

TO: Gene/mRNA "cloning"?

TO: LAW/Forensics?

TO: Anthropology?

TO: Evolutionary studies?

TO: DNA Identification? Diseases? Pathology?

TO: Gene Expression Studies

Amplify & amount of Specific DNA Sequence — FROM ANY Source — (e.g., 3 exons!) —

PCR Has Revolutionized Genetic Engineering Methodology

MUST KNOW DNA Sequence in Advance!

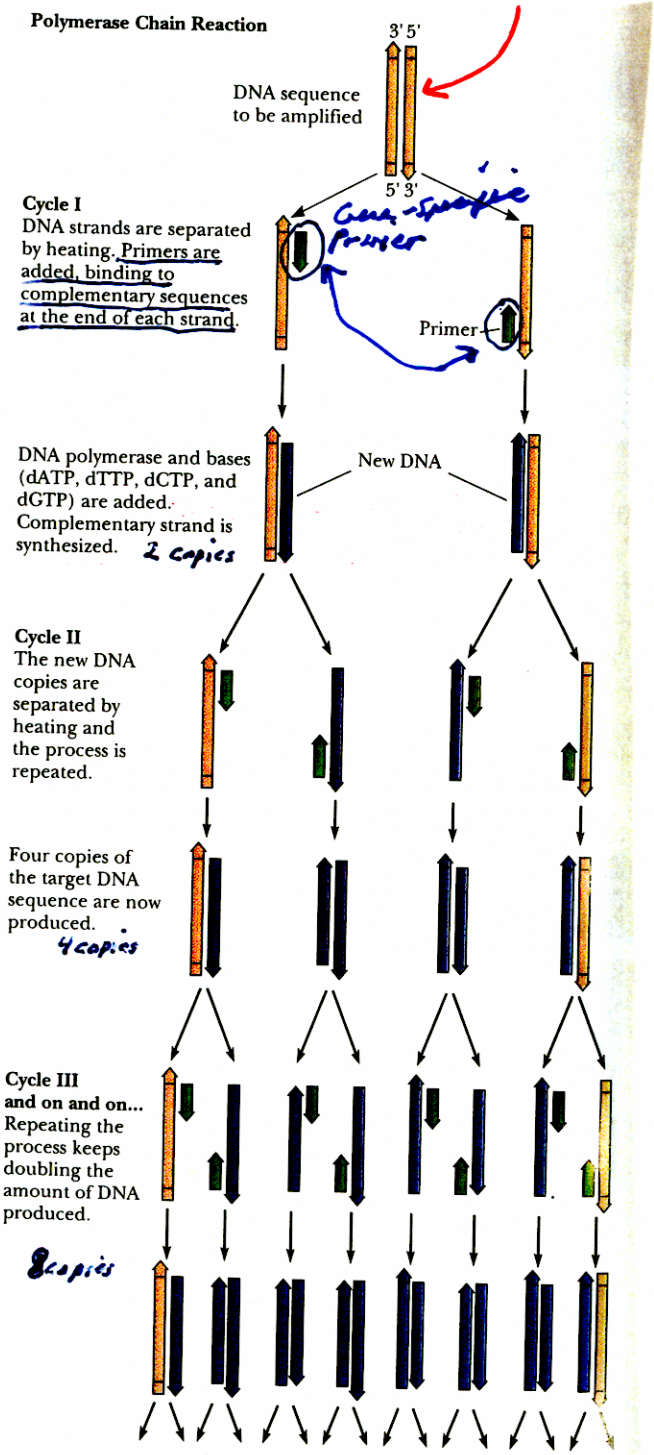


Figure 13-16 The polymerase chain reaction (PCR).

Requires

1. Knowledge of a specific DNA sequence
2. DNA Polymerase (Heat stable)
3. Programable Machine to separate DNA strands when formed & allow separated new strands to "re-form"
4. Primers to "recognize" specific gene & start DNA synthesis on both DNA strands

AN ∞ amount of a specific gene can be synthesized from as little as one DNA molecule!

Uses:

1. gene identity
2. forensics
3. mRNA detection
4. ancient DNA
5. Diagnosis of disease gene - e.g., CANCER! → oncogene
6. "Cloning"

USING PCR TO DETERMINE Genotypes in Single Human Embryo & Sperm Cells

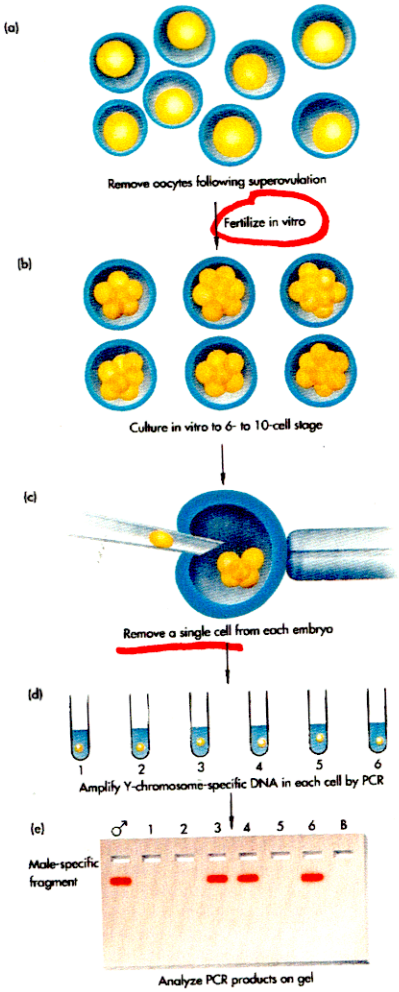


FIGURE 6-11
Determining sex of fetuses at risk for X-linked inherited disorders. (a) Oocytes are removed from the mother following superovulation and fertilized in vitro. (b) The oocytes that are fertilized successfully are cultured in vitro until there are 6 to 10 cells in each embryo. (c) A hole is made in the zona pellucida and a single cell removed from each embryo. (d) Amplification of the DYZ1 sequence is attempted. (e) Only in DNA from males is the male-specific DYZ1 sequence amplified by PCR, giving rise to a 149-bp, male-specific fragment. The lane marked with the male symbol is a positive control showing the expected fragment; the lane marked B (for "Blank") is from a PCR that included all the reagents but no DNA and is used to detect any contamination. Female embryos are negative (lanes 1, 2, and 5) and are implanted into the mothers.

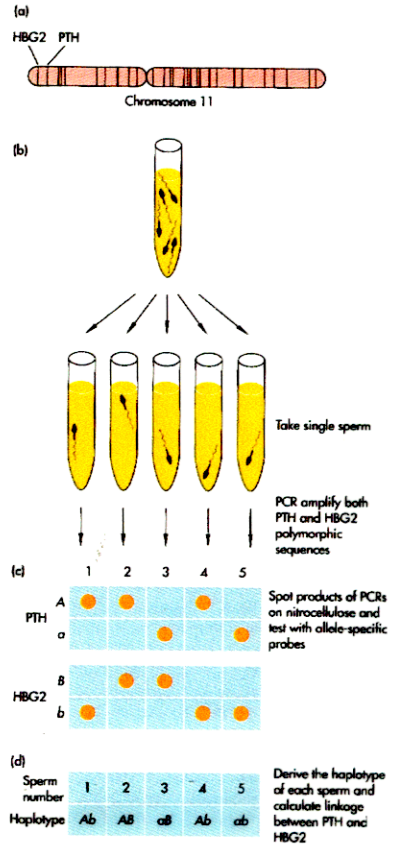


FIGURE 6-13
Gene linkage analysis using single sperm. (a) The positions of the parathyroid hormone gene locus (PTH) and $^{G}\gamma$-globin gene locus (HBG2) are shown on the short arm of human chromosome 11. (b) Single sperm are transferred to tubes, and amplification of the PTH and HBG2 loci is carried out simultaneously using two different sets of primers. (c) The products of the PCR are spotted onto a nitrocellulose filter and tested with probes specific for each of the four possible alleles, *A* and *a* for PTH and *B* and *b* for HBG2. The hybridization pattern is revealed after exposing the filter to x-ray film. (d) The haplotype of each sperm for these alleles can be read from the autoradiograph, and the recombination fraction between the loci can be calculated; hence the genetic distance between the two loci can be determined.

Sperm Cells

Single Cell
From 8 Cell Embryo (Blastomere)

Blastomere Analysis Before Implantation = BABI Test

DNA Replication is Precise But Mistakes Happen!

1 human gene mutation every 10 divisions

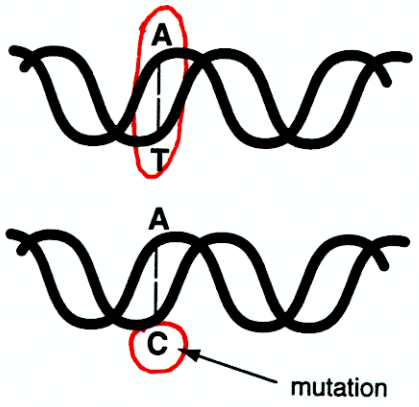
$\frac{1}{\sim 10^6}$ divisions

3000 nts human DNA change per division!

MISTAKES CHANGE GENETIC CODE → ∴ Protein → ∴ Lead to Phenotypic change → must = this!

Gene A

Random Mispairing at Replication



ORIGINAL BASE PAIR

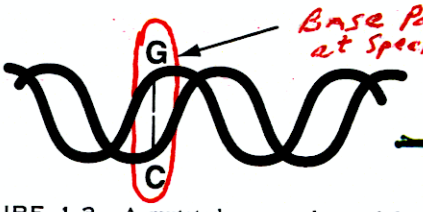
MUTATION DURING REPLICATION

① Coding Sequence or ② switch Sequence

Gene A'

Variant of SAME gene or allele

Base Pair Change at Specific Location in gene



RESULTING DEFECT

"See" As a change in phenotype!

FIGURE 1.3 A mutated gene makes a defective protein that leads to an illness in an individual.

MISTAKES CAN LEAD TO CHANGES in A CODON → CHANGING PROTEIN → ALTERING FUNCTION

A MISSENSE MUTATION

..... GGUCACUGGCGGUUCUUAAUGAAA.....
gly his trp arg phe leu met lys

..... GGUCUCUGGCGGUUCUUAAUGAAA.....
gly leu trp arg phe leu met lys

A single base change in the mRNA, due to a base change mutation in the DNA, results in the incoproration of a different amino acid.

FIGURE 3.8 A missense mutation.

A NONSENSE MUTATION

..... GGUCACUGGCGGUUCUUAAUGAAA.....
gly his trp arg phe leu met lys

..... GGUCACUAGCGGUUCUUAAUGAAA.....
gly his STOP

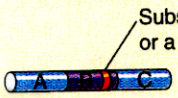
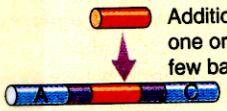
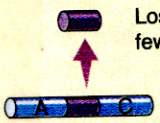
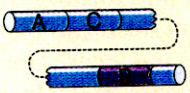
A single base change in the mRNA, due to a base change mutation in the DNA, creates a STOP codon.

FIGURE 3.9 A nonsense mutant.

→ Changed Protein Sequence → Function

HOW DO MUTATIONS OCCUR?

Table 18.1 Types of Mutation

Mutation	Example result
NO MUTATION	
	Normal B protein is produced by the *B* gene.
POINT MUTATION	
Base substitution	
	B protein is inactive because changed amino acid disrupts function.
Insertion	
	B protein is inactive because inserted material disrupts proper shape.
Deletion	
	B protein is inactive because portion of protein is missing.
CHANGES IN GENE POSITION	
Transposition	
	*B* gene or B protein may be regulated differently because of change in gene position.
Chromosomal rearrangement	
	*B* gene may be inactivated or regulated differently in its new location on chromosome.

(1) BASE CHANGE

(2) BASES INSERTED
e.g. cloning into TetR gene

(3) BASES DELETED

(4) GENE POSITION CHANGES
∴ Switches change!

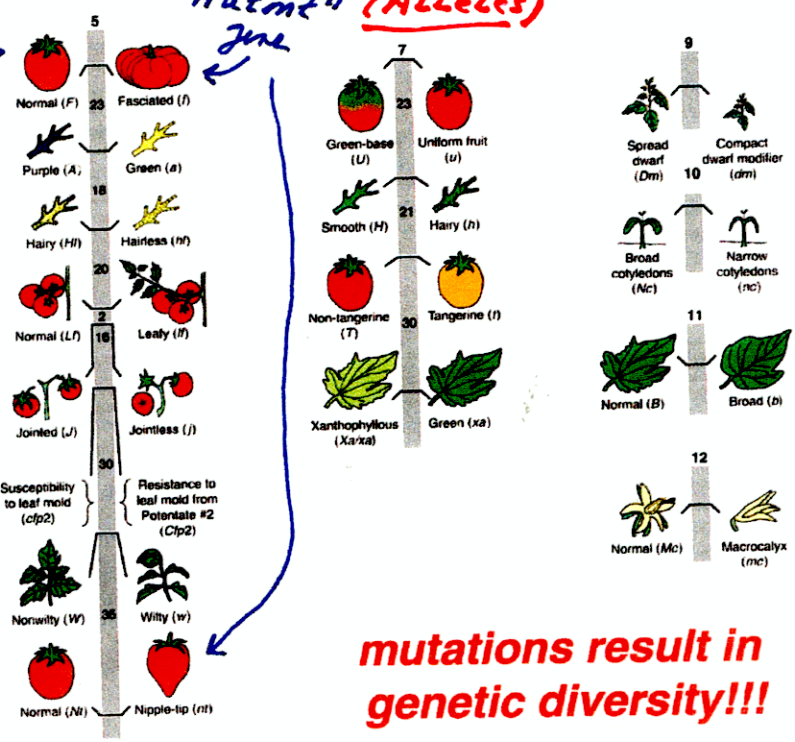
CAN CREATE
MANY alternative
FORMS OF SAME GENE (ALLELES)
→ Genetic / phenotypic diversity

GENE DIVERSITY THAT WAS USED TO "Engineer" Crop Plants

Alternative Forms of the Same Gene Lead to Genetic Diversity

"normal gene"

"Mutant" Gene (ALLELES)



12 Chromosomes Different Genes

mutations result in genetic diversity!!!

What is the Relationship Between the Mutant & Normal Gene?

How does Breeding / Molecular Genetic Engineering Accomplish SAME Goals?

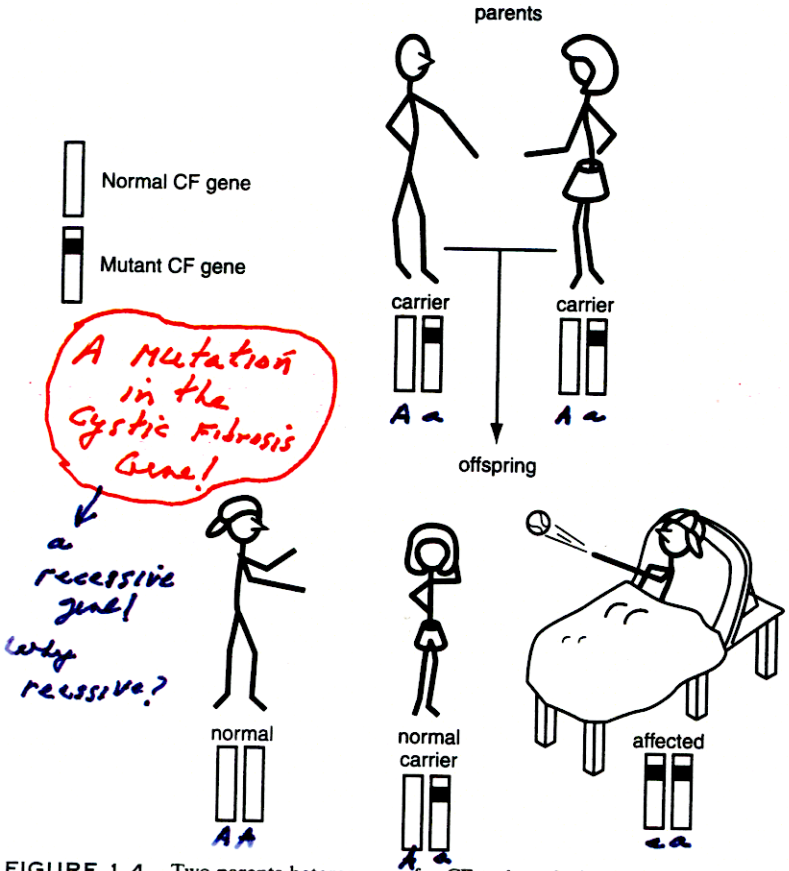
SAME Process → Diversity of Human Genes

MUTATIONS OCCUR INFREQUENTLY BUT ARE Inherited

Because change DNA Sequence!

2 genes per somatic cell

1 gene per gamete



A x a differ by a bp change

How detect differences + Follow inheritance?

Prediction? Exp?

FIGURE 1.4 Two parents heterozygous for CF and producing normal or affected kids.

How ARE MUTATIONS in Genes detected at DNA Level?

What Changes & How CAN These Changes be Recognized?

HUMAN GENETIC DISORDERS ARE RARE BUT ARE INHERITED WITHIN FAMILIES

Table 13.2 Some Important Genetic Disorders

Disorder	Symptom	Defect	Dominant/ Recessive	Frequency among Human Births
Cystic fibrosis	Mucus clogs lungs, liver, and pancreas	Failure of chloride ion transport mechanism	Recessive	1/2500 (Caucasians)
Sickle cell anemia	Poor blood circulation	Abnormal hemoglobin molecules	Recessive	1/625 (African Americans)
Tay-Sachs disease	Deterioration of central nervous system in infancy	Defective enzyme (hexosaminidase A)	Recessive	1/3500 (Ashkenazi Jews)
Phenylketonuria	Brain fails to develop in infancy	Defective enzyme (phenylalanine hydroxylase)	Recessive	1/12,000
Hemophilia	Blood fails to clot	Defective blood clotting factor VIII	Sex-linked recessive	1/10,000 (Caucasian males)
Huntington's disease	Brain tissue gradually deteriorates in middle age	Production of an inhibitor of brain cell metabolism	Dominant	1/24,000
Muscular dystrophy (Duchenne)	Muscles waste away	Degradation of myelin coating of nerves stimulating muscles	Sex-linked recessive	1/3700 (males)
Hypercholesterolemia	Excessive cholesterol levels in blood, leading to heart disease	Abnormal form of cholesterol cell surface receptor	Dominant	1/500

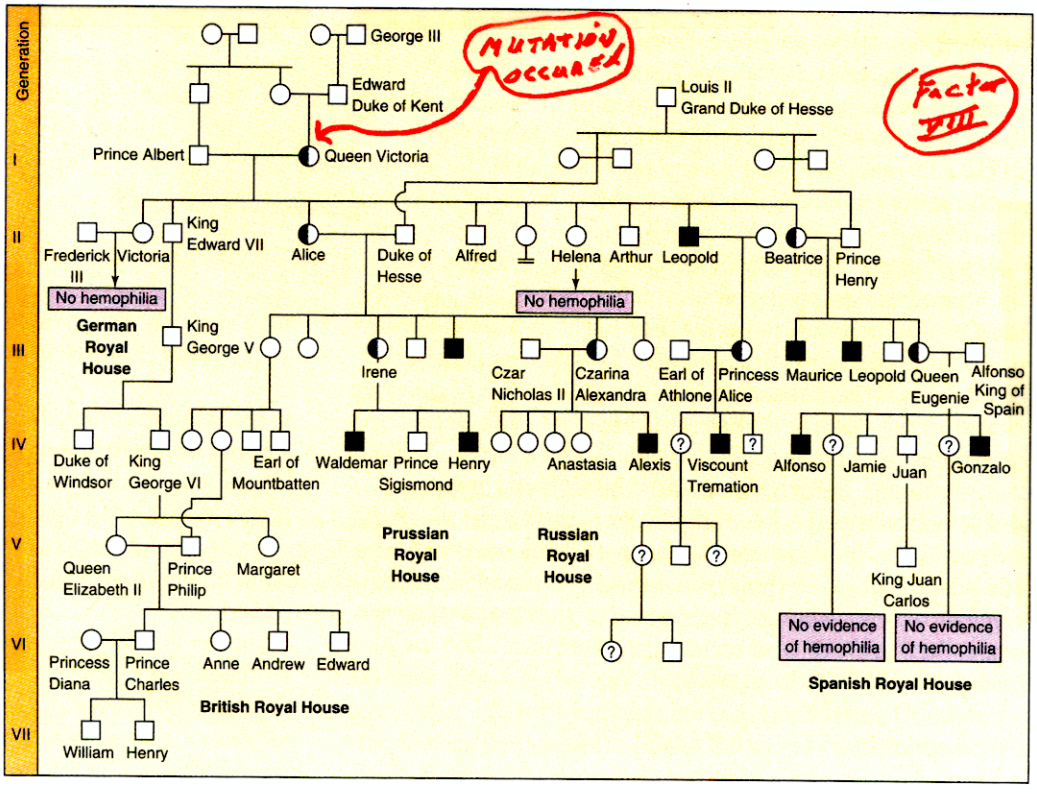


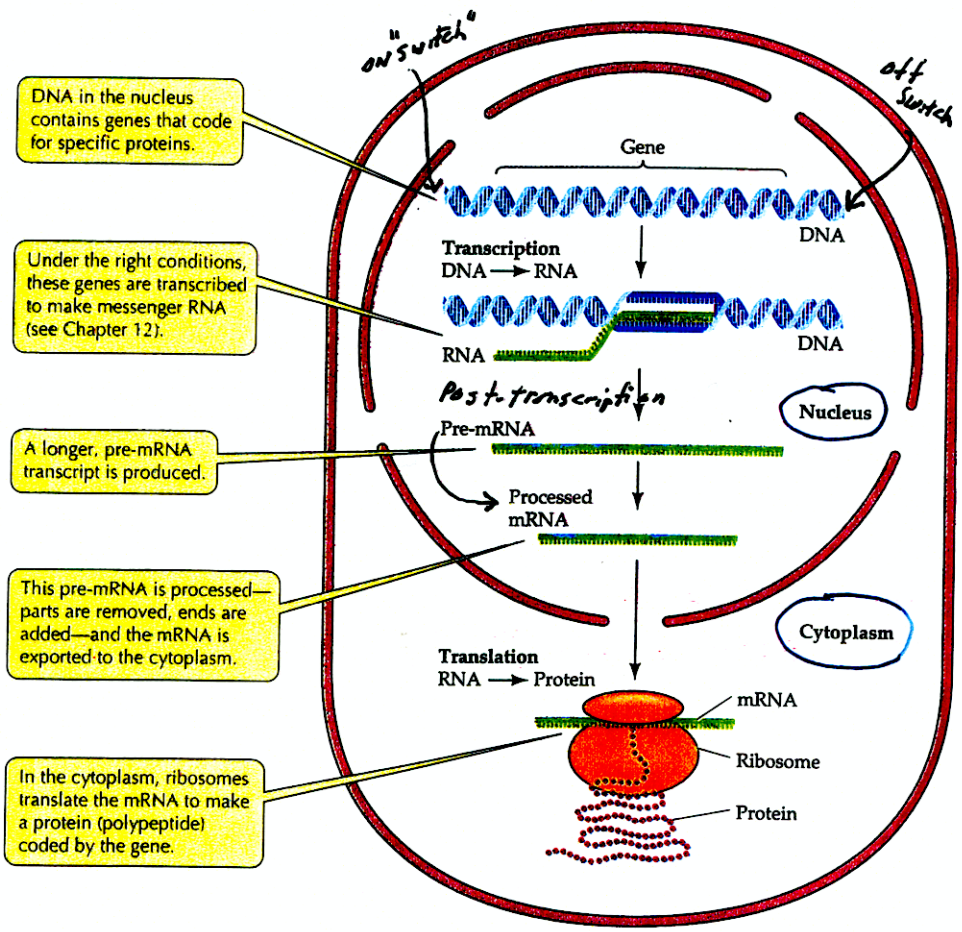
FIGURE 13.26
The Royal hemophilia pedigree. Queen Victoria's daughter Alice introduced hemophilia into the Russian and Austrian royal houses, and Victoria's daughter Beatrice introduced it into the Spanish royal house. Victoria's son Leopold, himself a victim, also transmitted the disorder in a third line of descent. Half-shaded symbols represent carriers with one normal allele and one defective allele; fully shaded symbols represent affected individuals.

CAN USE PEDIGREES TO FOLLOW Defective Gene in HUMAN FAMILIES?

HOW FOLLOW USING MOLECULAR BIOLOGY? What would you predict?

Gene Action in a Human Cell

What Happens if Gene Required for Cell Division Mutates FROM OFF to ON?



TRANSCRIPTION in NUCLEUS

TRANSLATION in CYTOPLASM

14.1 Eukaryotic mRNA Is Processed in the Nucleus and Exported to the Cytoplasm Compare this "road map" to Figure 12.3.

DNA Replication Occurs during Cell Division

An Inherited Disease can be caused By a defect at Any Level of Gene Action → from "switch" to translation!

NOTE:

Mutation – Any change in Any Sequence (coding / switch) that changes production of functional protein!

e.g.; ① switch ② processing mRNA ③ codons

MUTATIONS CHANGE Specific Restriction Enzyme Recognition Sites

CAN Be Used As Diagnostic tools! FOR DNA TESTING

A allele

EcoRI site

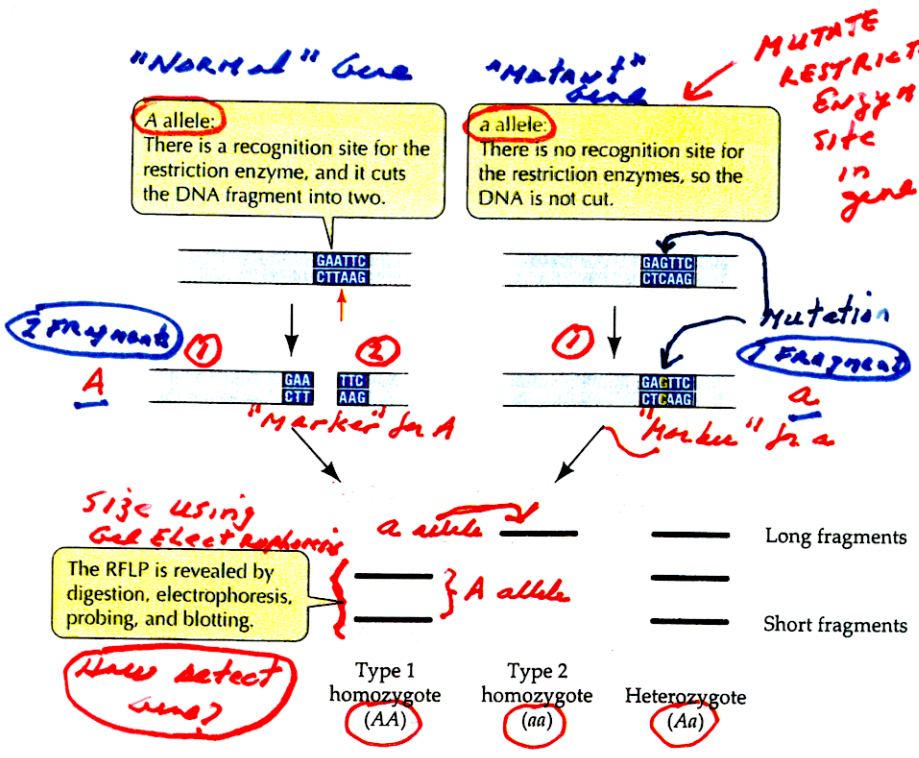
5' GAATTC 3'
3' CTTAAG 5'

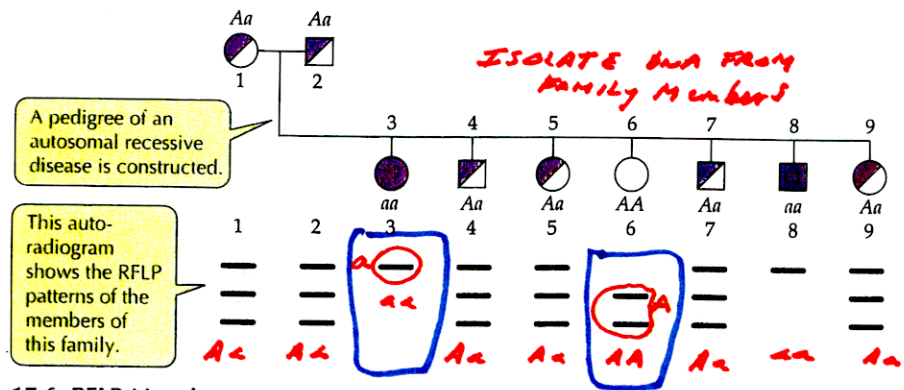
EcoRI Bonds & Cuts

Fingerprint

MARKER Like red vs. green cabbage

Humans are Diploid 2 chromosomes EACH except for egg/sperm





17.6 RFLP Mapping Restriction fragment length polymorphisms are differences in DNA sequences that serve as genetic markers. More than 1,000 such markers have been described for the human genome.

a allele

Mutant EcoRI site

5' GAGTTC 3'
3' CTCAAG 5'

EcoRI does not bind or cut

DNA Testing

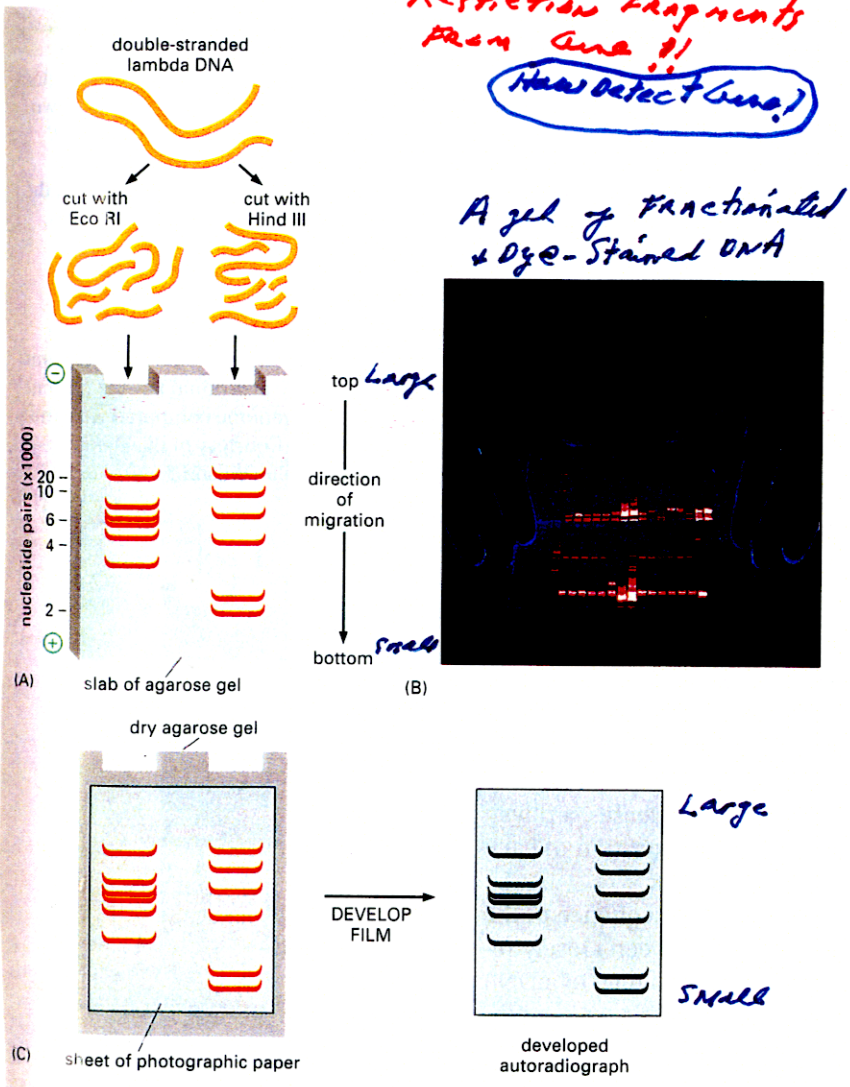
2 Copies of gene per somatic cell!

Differences in DNA Sequence CAN Be Revealed by Restriction Enzyme Digestion → Reveals genetic variability or changes in genes!

Gel Electrophoresis SEPARATES DNA (+RNA) Fragments (Molecules) By Size

∴ Can use to detect size differences in Restriction Fragments from Gene #1

How detect Gene?



A gel of fractionated + Dye-Stained DNA

top Large

bottom Small

Large

Small

Figure 10–3 Separation and detection of DNA molecules by size using gel electrophoresis. (A) This schematic illustration compares the results of cutting the same DNA molecule (in this case the genome of the bacteriophage lambda; see Figure 9–15) with two different restriction nucleases—Eco RI (*left*) and Hind III (*right*). The fragments are then separated by gel electrophoresis. The mixture of DNA fragments obtained from treatment with the enzyme is placed at the top of a thin gel slab, and under the influence of an electric field, the fragments move through the gel toward the positive electrode. Larger fragments migrate more slowly than smaller fragments, and thus the fragments in the mixture become separated by size. For example, the two lowermost bands in the lane on the right correspond to the two smallest DNA fragments produced by Hind III digestion. To visualize the DNA bands, the gel has been soaked in a dye that binds to DNA and fluoresces brightly under ultraviolet light (B). (C) An alternative method for visualizing the DNA bands is autoradiography. Prior to cleavage with restriction enzymes, the DNA has been "labeled" with the radioisotope ^{32}P by substituting ^{32}P for some of the nonradioactive phosphorus atoms. This could be done, for example, by replicating the virus in the presence of ^{32}P. Since the β particles emitted from ^{32}P will expose photographic film, a sheet of film placed flat on top of the agarose gel will, when developed, show the position of all the DNA bands. (B, courtesy of J. C. Revy, Science Photo Library.)

How detect a Specific Gene or RNA in a Population of DNA or RNA Fragments?

HC 70 A

Winter 2003

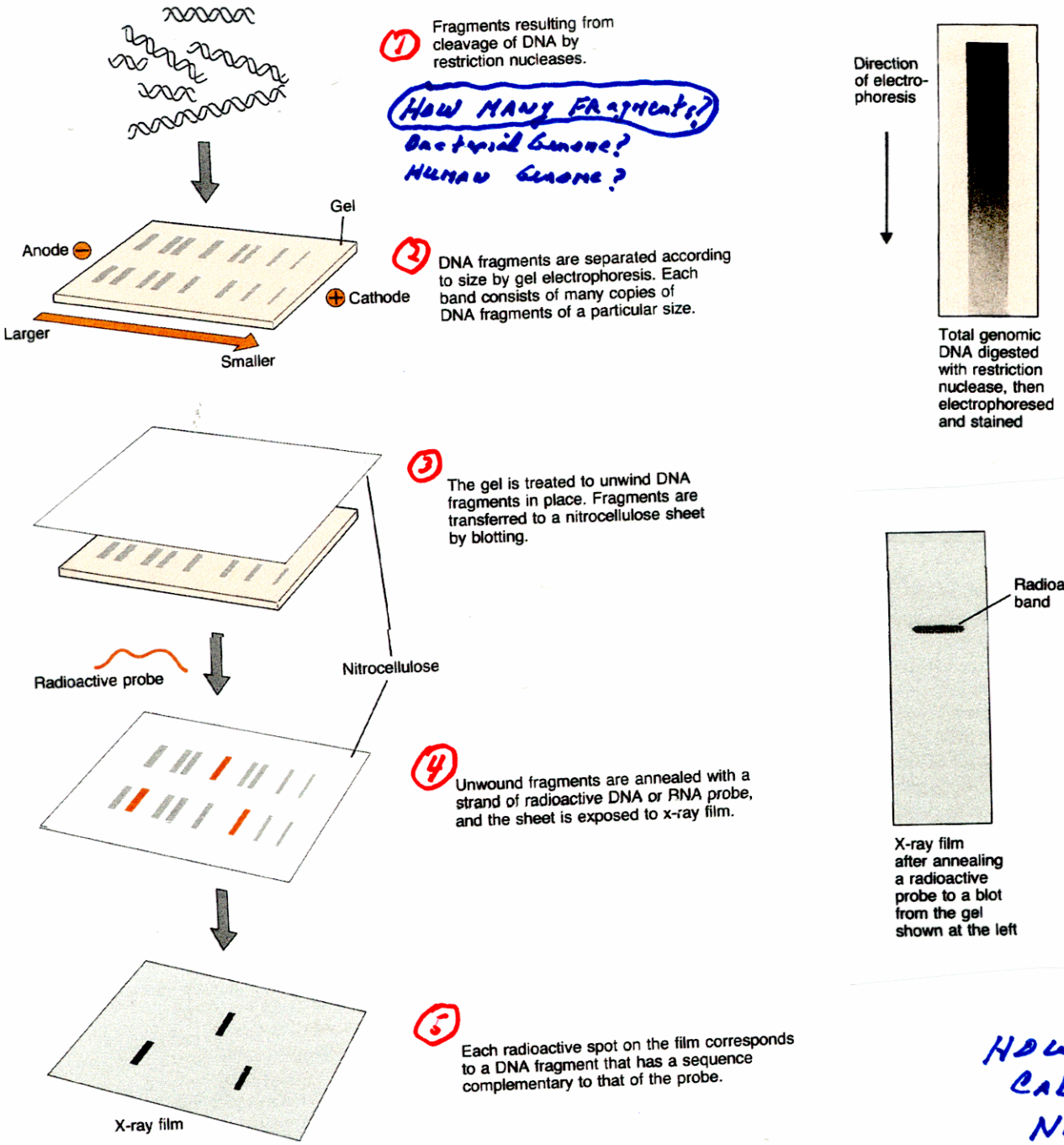
Professor Bob Goldberg

Supplement to Learning Unit #2

DNA Blots & DNA Finger prints

DNA Gel Blots Detect SPECIFIC DNA Sequences/Genes in a Population of DNA Fragments (Genome)

Used for DNA Fingerprinting



HOW MANY Fragments? Bacterial Genome? Human Genome?

1 How many different fragments??

1 Why only one detected??

Figure 5.7 DNA blotting.

HOW Calculate Number of different Restriction fragments?

Detect mutations in DNA or Genetic variability

DETECTING MUTATIONS IN HUMAN GENES BY DNA FINGERPRINTS

Figure 14.2

The beginning of the β-globin gene, mRNA, and polypeptide showing the normal Hb-A sequences and the mutant Hb-S sequences. The sequence differences between Hb-A and Hb-S are shown in red. The mutation alters a *Dde*I site (boxed in the Hb-A DNA).

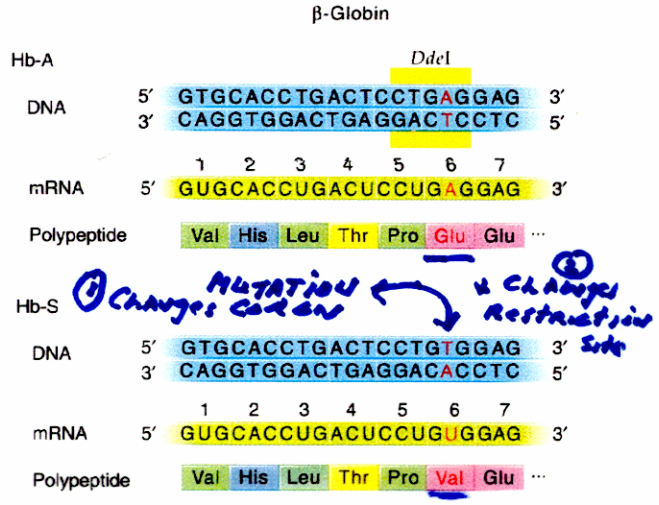
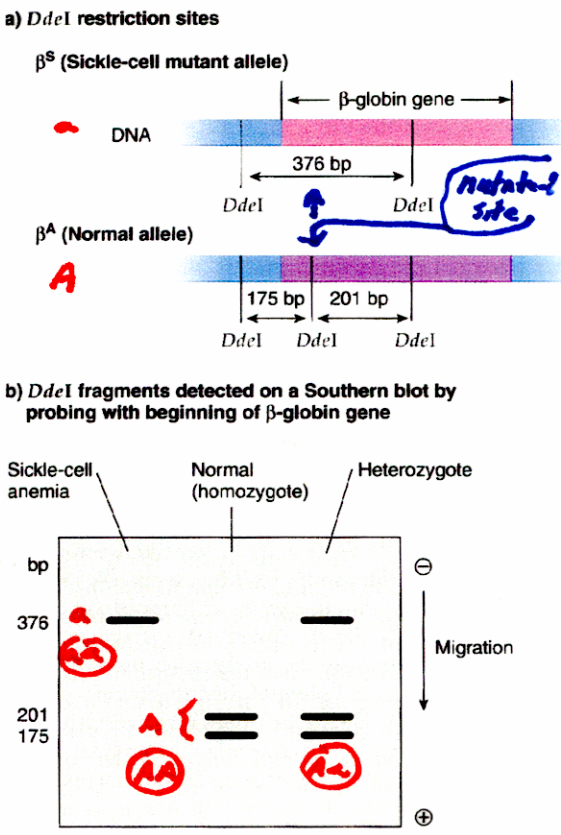


Figure 14.3

Detection of sickle-cell gene by the *Dde*I restriction fragment length polymorphism. **(a)** DNA segments showing the *Dde*I restriction sites. **(b)** Results of analysis of DNA cut with *Dde*I, subjected to gel electrophoresis, blotted, and probed with a β-globin probe.



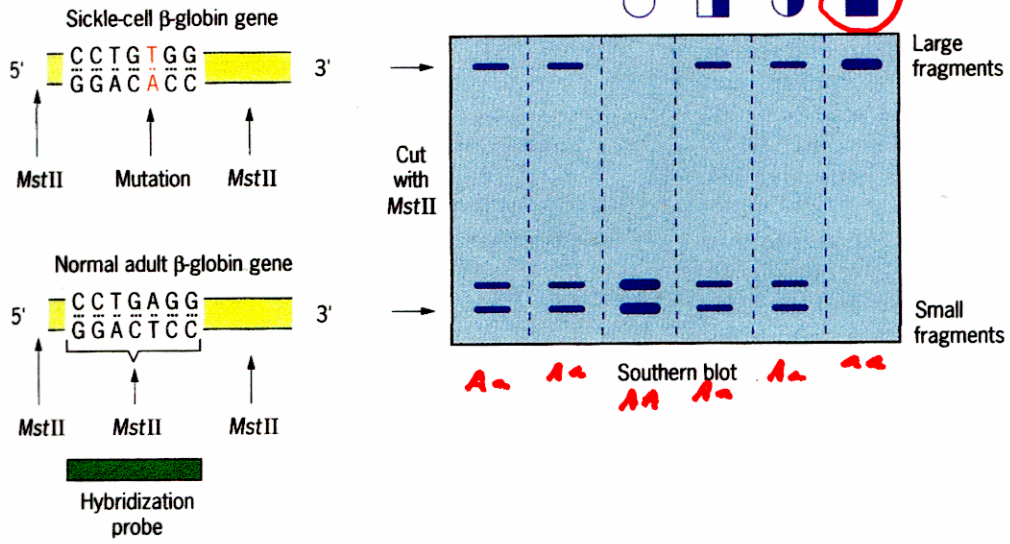
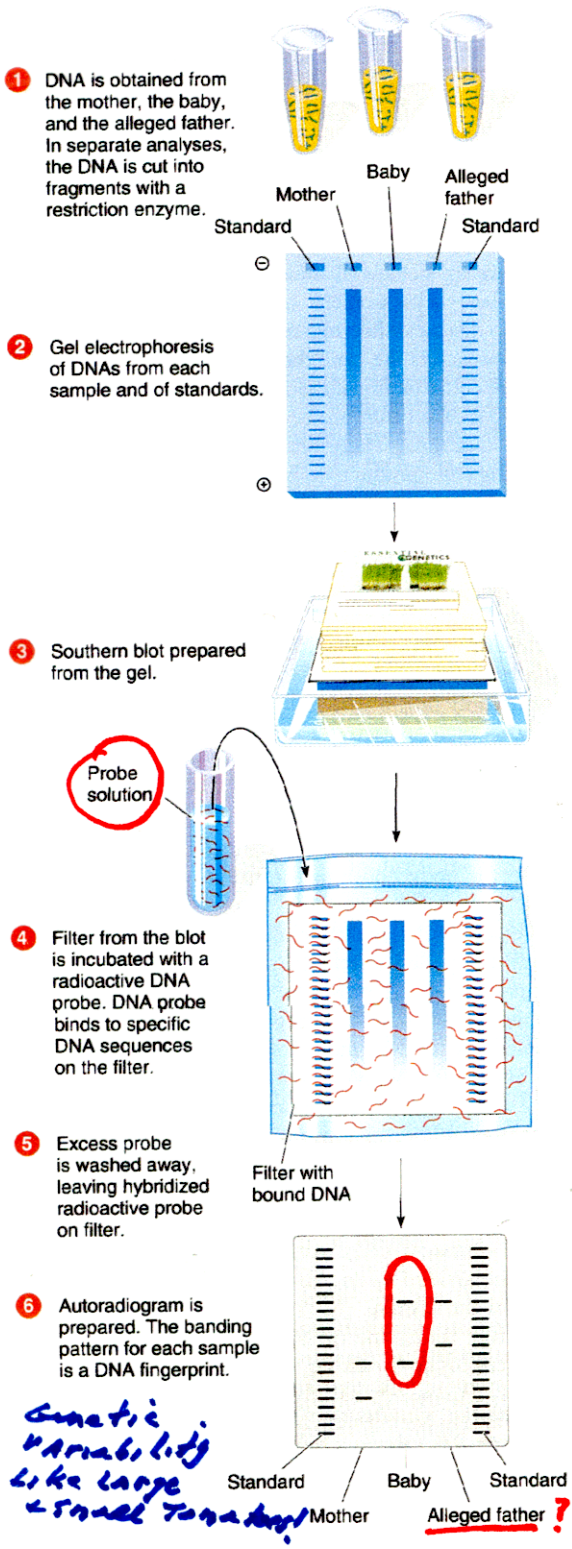


Figure 22.7 Detection of the sickle-cell hemoglobin mutation by Southern blot analysis of genomic DNAs cut with restriction enzyme *Mst*II.

DNA Gel Blots can be used to fingerprint/Genotype my DNA using specific and probes obtained by cloning



Genetic variability like large & small Tandem Repeats

Figure 14.6
DNA typing as used in a paternity case.

clusions are now widely accepted by U.S. courts. In our paternity case, we would probably be more persuaded that the accused was the father of the child if the data for each of five different monomorphic probes indicated that he contributed a particular allele to the child.

Other Applications of DNA Typing

There are many uses of DNA typing. Here we list just a few examples to illustrate the scope of usefulness of DNA typing for human testing and for tests involving other organisms.

1. *Forensic analysis in murder, rape, and other violent crimes.* Typically, DNA samples are taken during criminal investigations and compared with those of victims and suspects. In a murder case, DNA can be isolated from blood or hair at the scene; in a rape case, DNA can be taken from a semen sample. If only minuscule amounts of DNA can be collected, PCR is used to amplify the DNA for typing experiments.
2. *Conservation biology studies.* DNA typing can be used in studies of endangered species to determine genetic variability in those species.
3. *Forensic analysis in wildlife crimes.* Wild animals sometimes are killed illegally, and DNA typing is increasingly helping to solve the crimes. For example, a set of six STR markers was used in a poaching investigation in Wyoming involving pronghorn antelope. Six headless pronghorn antelope carcasses were discovered and reported to authorities. An investigation turned up a suspect who had a skull with horns. DNA samples were taken from the skull and compared with DNA samples from carcass samples, and a match was found. At the trial, the suspect was convicted on six counts of wanton destruction of big or trophy game.
4. *Testing for pathogens in food.* PCR using strain-specific primers can test for the presence of pathogenic *E. coli* strains in foods such as hamburger meat.
5. *Detection of genetically modified organisms (GMOs).* GMOs have been introduced widely into agriculture in the United States. Genetically modified crops typically contain genes that were introduced in the development of the new crop. PCR primers based on those sequences or the promoters that control them can be used to test for the presence of those genes. We can do these tests with the plants themselves or with processed foods. One current estimate is that between 50 and 75 percent of produce and processed foods in a supermarket are genetically modified or contain GMOs.

CANCER – AN EXAMPLE OF MUTATIONS IN CELL DIVISION CONTROL GENES

What Is Cancer?

Cancer is a growth disorder of cells. It starts when an apparently normal cell begins to grow in an uncontrolled and invasive way (figure 18.8). The result is a cluster of cells, called a **tumor,** that constantly expands in size. Cells that leave the tumor and spread throughout the body, forming new tumors at distant sites, are called **metastases** (figure 18.9). Cancer is perhaps the most pernicious disease. Of the children born in 1999, one-third will contract cancer at some time during their lives; one-fourth of the male children and one-third of the female children will someday die of cancer. Most of us have had family or friends affected by the disease. In 2000, 552,200 Americans died of cancer.

Not surprisingly, researchers are expending a great deal of effort to learn the cause of this disease. Scientists have made a great deal of progress in the last 20 years using molecular biological techniques, and the rough outlines of understanding are now emerging. We now know that cancer is a gene disorder of somatic tissue, in which damaged genes fail to properly control cell proliferation. The cell division cycle is regulated by a sophisticated group of proteins described in chapter 11. Cancer results from the mutation of the genes encoding these proteins.

Cancer can be caused by chemicals that mutate DNA or in some instances by viruses that circumvent the cell's normal proliferation controls. Whatever the immediate cause, however, all cancers are characterized by unrestrained growth and division. Cell division never stops in a cancerous line of cells. Cancer cells are virtually immortal—until the body in which they reside dies.

Cancer is unrestrained cell proliferation caused by damage to genes regulating the cell division cycle.

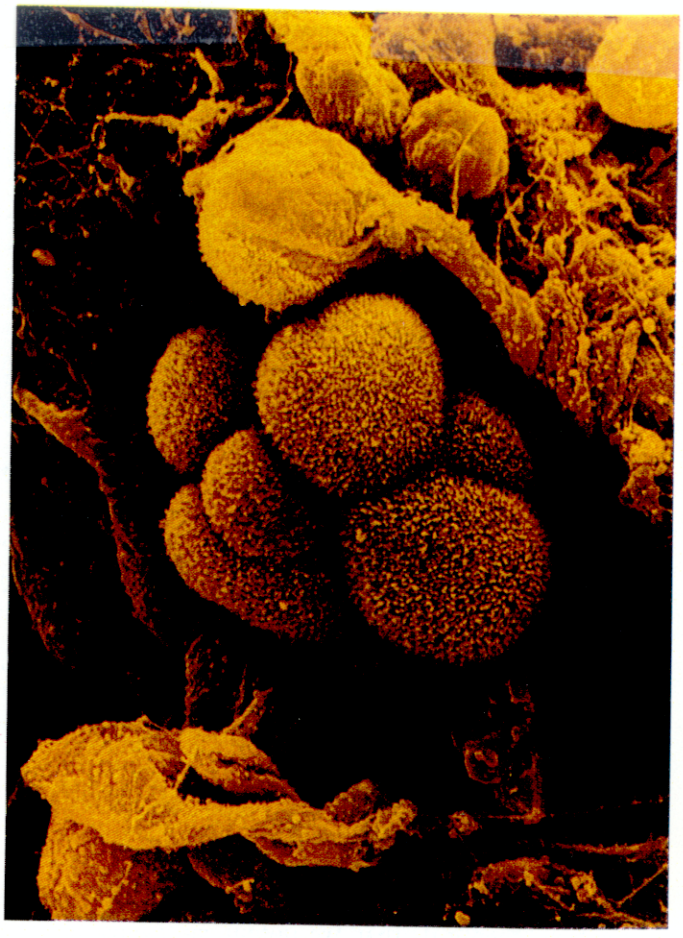


FIGURE 18.8
Lung cancer cells (530×). These cells are from a tumor located in the alveolus (air sac) of a lung.

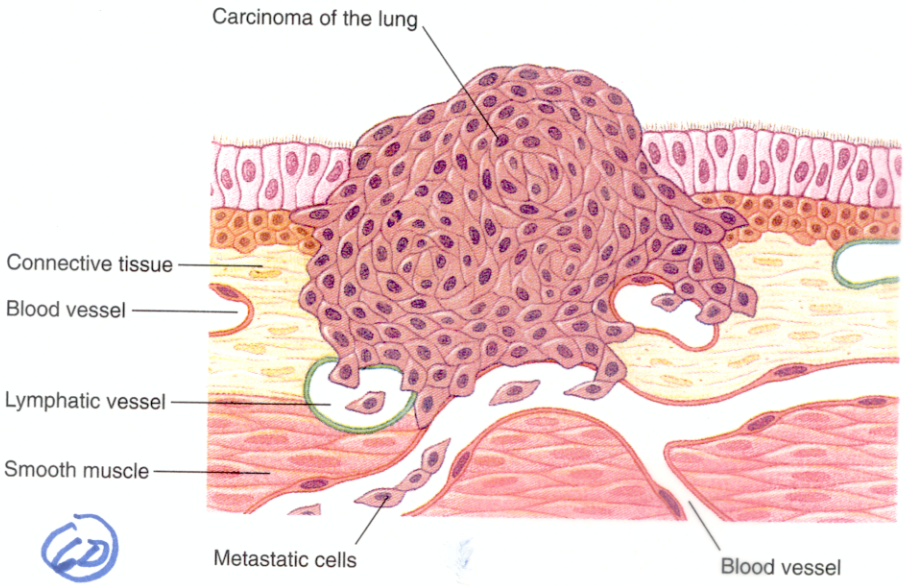
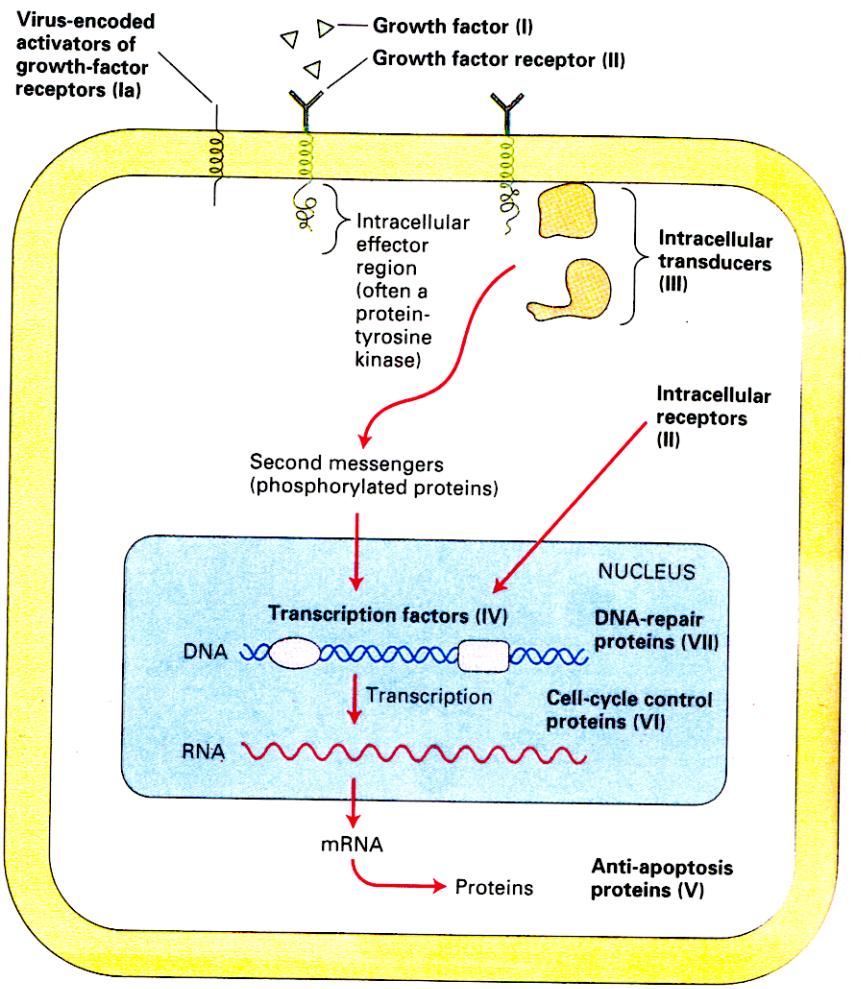


FIGURE 18.9
Portrait of a cancer. This ball of cells is a carcinoma (cancer tumor) developing from epithelial cells that line the interior surface of a human lung. As the mass of cells grows, it invades surrounding tissues, eventually penetrating lymphatic and blood vessels, both plentiful within the lung. These vessels carry metastatic cancer cells throughout the body, where they lodge and grow, forming new masses of cancerous tissue.

CANCER IS CAUSED BY MUTATIONS in Genes that Control Cell Division & DNA Replication

▶ **FIGURE 24-9 The seven types of proteins that participate in controlling cell growth.** Cancer can result from expression of mutant forms of these proteins: growth factors (I), growth-factor receptors (II), signal-transduction proteins (III), transcription factors (IV), pro- or anti-apoptotic proteins (V), cell-cycle control proteins (VI), and DNA-repair proteins (VII). Mutations changing the structure or expression of proteins in classes I–IV generally give rise to dominantly active oncogenes. The class VI proteins mainly act as tumor suppressors; mutations in the genes encoding these proteins act recessively to release cells from control and surveillance, greatly increasing the probability that the mutant cells will become tumor cells. Class VII mutations greatly increase the probability of mutations in the other classes. Virus-encoded proteins that activate growth-factor receptors (Ia) also can induce cancer.



CELL DIVISION CONTROL GENES!

These genes were identified by DNA Cloning Experiments — A major Benefit of Recombinant DNA Technology

GENES INVOLVED IN CANCER

CONTROL CELL GROWTH!!

MUTANT FORMS OF NORMAL GENE!

Table 18.4 Some Genes Implicated in Human Cancers

Gene	Product	Cancer
ONCOGENES	Cell division genes	
Genes Encoding Growth Factors or Their Receptors		
erb-B	Receptor for epidermal growth factor	Glioblastoma (a brain cancer); breast cancer
erb-B2	A growth factor receptor (gene also called *neu*)	Breast cancer; ovarian cancer; salivary gland cancer
PDGF	Platelet-derived growth factor	Glioma (a brain cancer)
RET	A growth factor receptor	Thyroid cancer
Genes Encoding Cytoplasmic Relays in Intracellular Signaling Pathways		
K-ras	Protein kinase	Lung cancer; colon cancer; ovarian cancer; pancreatic cancer
N-ras	Protein kinase	Leukemias
Genes Encoding Transcription Factors That Activate Transcription of Growth-Promoting Genes		
c-myc	Transcription factor	Lung cancer; breast cancer; stomach cancer; leukemias
L-myc	Transcription factor	Lung cancer
N-myc	Transcription factor	Neuroblastoma (a nerve cell cancer)
Genes Encoding Other Kinds of Proteins		
bcl-2	Protein that blocks cell suicide	Follicular B cell lymphoma
bcl-1	Cyclin D1, which stimulates the cell cycle clock (gene also called *PRAD1*)	Breast cancer; head and neck cancers
MDM2	Protein antagonist of p53 tumor-supressor protein	Wide variety of sarcomas (connective tissue cancers)
TUMOR-SUPRESSOR GENES	Regulators of cell division	
Genes Encoding Cytoplasmic Proteins	Control genes "Fail-Safe"	
APC	Step in a signaling pathway	Colon cancer; stomach cancer
DPC4	A relay in signaling pathway that inhibits cell division	Pancreatic cancer
NF-1	Inhibitor of ras, a protein that stimulates cell division	Neurofibroma; myeloid leukemia
NF-2	Inhibitor of ras	Meningioma (brain cancer); schwannoma (cancer of cells supporting peripheral nerves)
Genes Encoding Nuclear Proteins		
MTS1	p16 protein, which slows the cell cycle clock	A wide range of cancers
p53	p53 protein, which halts cell division at the G_1 checkpoint	A wide range of cancers
Rb	Rb protein, which acts as a master brake of the cell cycle	Retinoblastoma; breast cancer; bone cancer; bladder cancer
Genes Encoding Proteins of Unknown Cellular Locations		
BRCA1	?	Breast cancer; ovarian cancer
BRCA2	?	Breast cancer
VHL	?	Renal cell cancer

Regulate Switches

How detect if have cancer probes? Early Diagnosis! Genetic Predisposition?

NORMAL Gene "CANCER" MUTANT Gene

CANCER REQUIRES MUTATING SEVERAL GENES IN A single CELL

This TAKES TIME :: CANCER INCREASES with Age

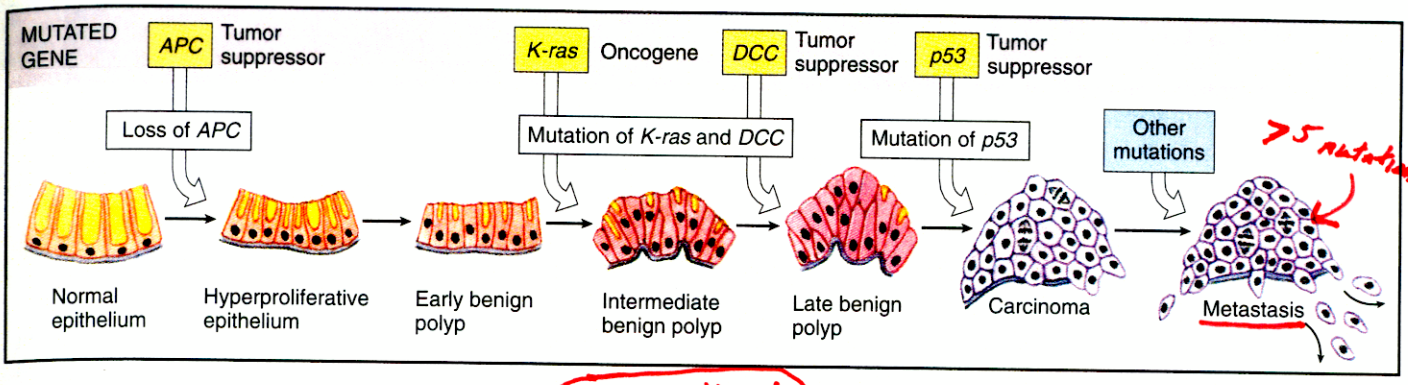


FIGURE 18.16
The progression of mutations that commonly lead to colorectal cancer. The fatal metastasis is the last of six serial changes that the epithelial cells lining the rectum undergo. One of these changes is brought about by mutation of a proto-oncogene, and three of them involve mutations that inactivate tumor-suppressor genes.

MULTI HIT!

BECAUSE MANY genes control cell division MUST MUTATE ALL in one cell → transform to cancer cell

Cancer-Causing Mutations Accumulate over Time

Cells control proliferation at several checkpoints, and all of these controls must be inactivated for cancer to be initiated. Therefore, the induction of most cancers involves the mutation of multiple genes; four is a typical number (figure 18.16). In many of the tissue culture cell lines used to study cancer, most of the controls are already inactivated, so that mutations in only one or a few genes transform the line into cancerous growth. The need to inactivate several regulatory genes almost certainly explains why most cancers occur in people over 40 years old (figure 18.17); in older persons, there has been more time for individual cells to accumulate multiple mutations. It is now clear that mutations, including those in potentially cancer-causing genes, do accumulate over time. Using the polymerase chain reaction (PCR), researchers in 1994 searched for a certain cancer-associated gene mutation in the blood cells of 63 cancer-free people. They found that the mutation occurred 13 times more often in people over 60 years old than in people under 20.

Cancer is a disease in which the controls that normally restrict cell proliferation do not operate. In some cases, cancerous growth is initiated by the inappropriate activation of proteins that regulate the cell cycle; in other cases, it is initiated by the inactivation of proteins that normally suppress cell division.

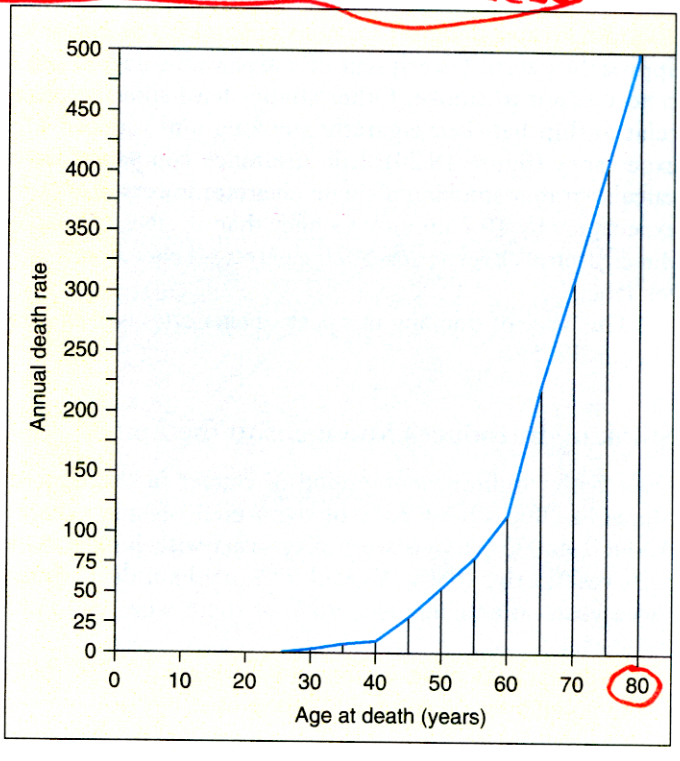


FIGURE 18.17
The annual death rate from cancer climbs with age. The rate of cancer deaths increases steeply after age 40 and even more steeply after age 60, suggesting that several independent mutations must accumulate to give rise to cancer.

CANCER IS A MAJOR KILLER!

Table 18.2 Incidence of Cancer in the United States in 2000

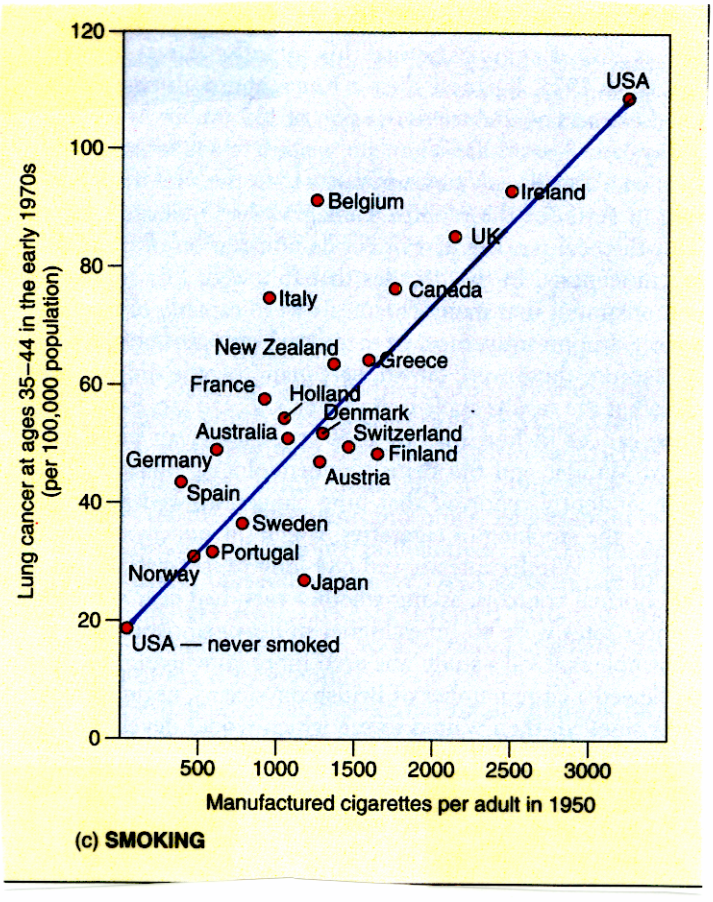
Type of Cancer	New Cases	Deaths	% of Cancer Deaths
Lung	164,100	156,900	28
Colon and rectum	130,200	56,300	10
Leukemia/lymphoma	93,100	49,200	9
Breast	184,200	41,200	8
Prostate	180,400	31,900	7
Pancreas	28,300	28,200	5
Ovary	23,100	14,000	3
Stomach	21,500	13,000	2
Liver	15,300	13,800	2
Nervous system/eye	18,700	13,200	2
Bladder	53,200	12,200	2
Oral cavity	30,200	7,800	2
Kidney	31,200	11,900	2
Cervix/uterus	48,900	11,100	2
Malignant melanoma	47,700	7,700	1
Sarcoma (connective tissue)	10,600	6,000	1
All other cancers	139,400	77,800	14

In the United States in 2000 there were 1,220,100 reported cases of new cancers and 552,200 cancer deaths, indicating that roughly half the people who develop cancer die from it.

Source: Data from the American Cancer Society, Inc., 2000.

Increases as people get older!

SMOKING CAUSES CANCER



(c) **SMOKING**

Can Control By Life Style & Eating Habits

SMOKE CONTAINS CARCINOGENS THAT MUTATE GENES IN LUNG CELLS

Reducing Life Expectancy by ~15 years!

GENETIC ENGINEERING AND GENE DISCOVERY PROJECTS OFFER HOPE FOR NEW CANCER MOLECULAR THERAPIES

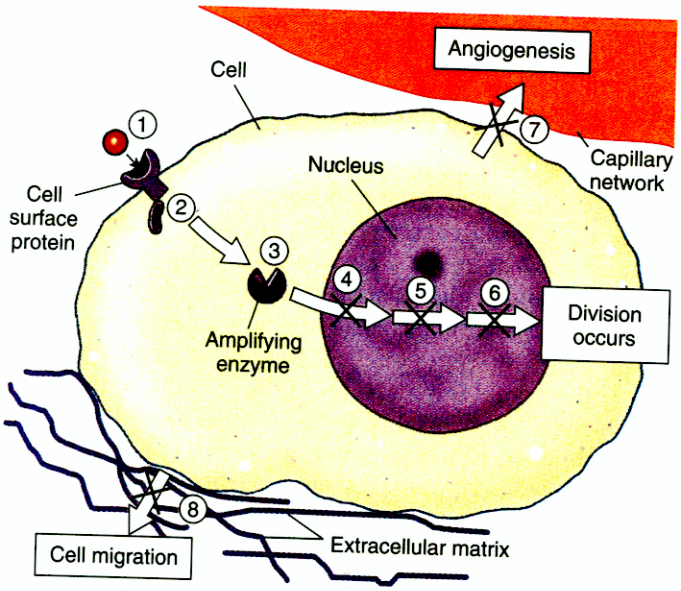


FIGURE 18.22
New molecular therapies for cancer target eight different stages in the cancer process. (*1*) On the cell surface, a growth factor signals the cell to divide. (*2*) Just inside the cell, a protein relay switch passes on the divide signal. (*3*) In the cytoplasm, enzymes amplify the signal. In the nucleus, (*4*) a "brake" preventing DNA replication is released, (*5*) proteins check that the replicated DNA is not damaged, and (*6*) other proteins rebuild chromosome tips so DNA can replicate. (*7*) The new tumor promotes angiogenesis, the formation of growth-promoting blood vessels. (*8*) Some cancer cells break away from the extracellular matrix and invade other parts of the body.

BLOCK MUTANT GENE ACTIVITY!

- DRUGS
- GENE THERAPY (HOW?)

(1) SWITCH "OFF" UNCONTROLLED GENE ACTIVITY OF CELL DIVISION CONTROL GENES !!

(2) PREVENT TUMOR GROWTH &/or METASTASIS !!