HC70A WINTER 2005
PROFESSOR BOB GOLDBERG

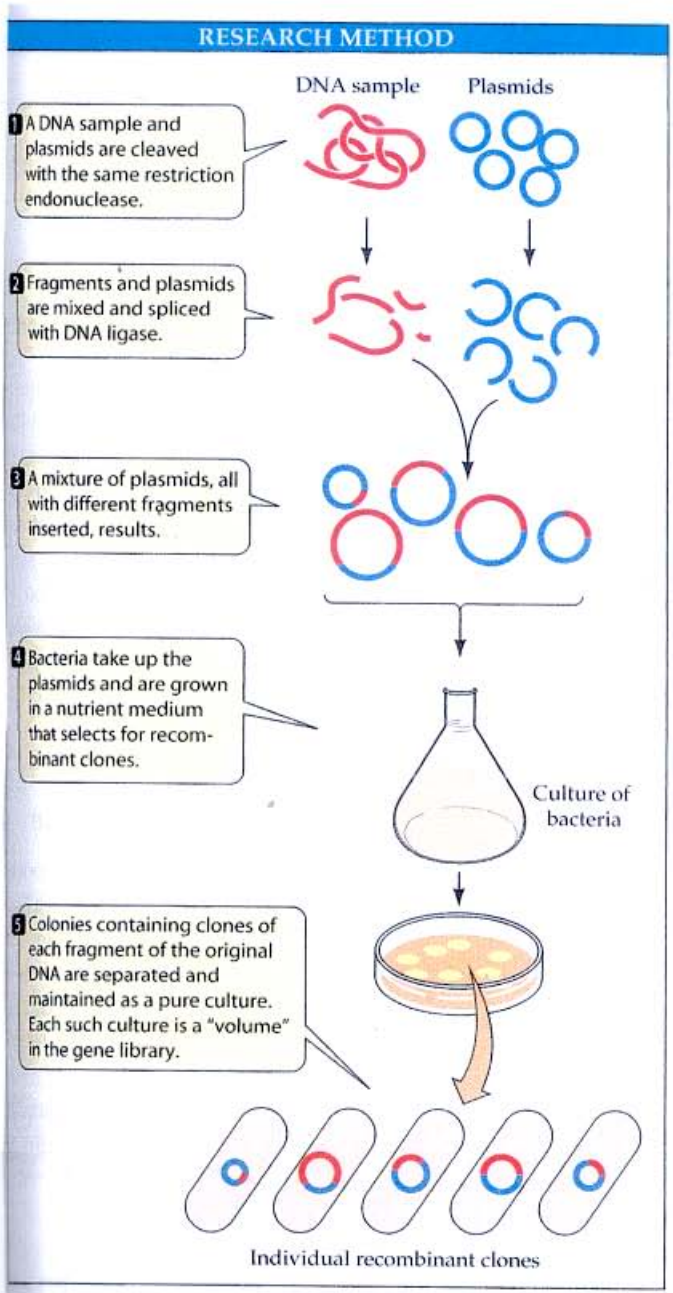
Lecture #3 1/18/05
HOW DO GENES WORK?

1/25/05

THEMES

1. Demonstrations – Bacterial "cloning" & Gel Electrophoresis 30' 1/18
2. Functions of Genes – Revisited
3. Replication of DNA & Genotype / ORI
4. PCR & How it Revolutionized Genetic Engineering 30' Stop 1/25 — 10' (FILM KERRY MULLIS & PCR)
5. Mutations & Genetic Diversity – CHANGING the Phenotype
6. Using Pedigrees to Follow Mutations
7. Genes to Proteins / An Overview
8. The Genetic Code – Implications For Genetic Engineering
9. RNA vs. DNA
10. Proteins – Unique Proteins → Unique Functions
11. Gene Expression in Bacteria & Eukaryotes
12. Introns & RNA Splicing
13. Yo – It's in the Sequences – "Material" For Genetic Engineering
14. How to Engineer a Plant to Make a Human Protein & How to Engineer a Goat to Make a DRUG!
15. Genetic Engineering – Any Biological Limits?
16. Thinking About Genetic Engineering in the context of How Genes Work & What they are! – Stop 1/28 120'

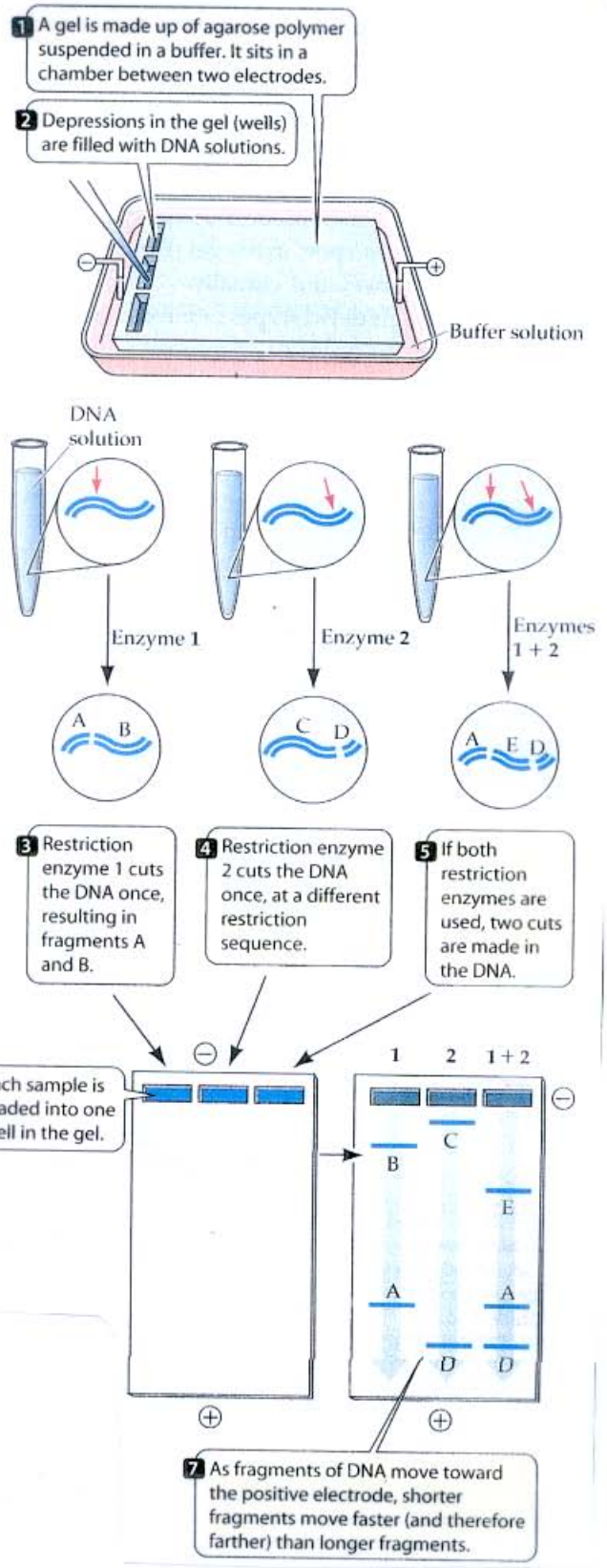
DEMONSTRATION - Bacteria "Cloning" EXPERIMENT



16.7 **Constructing a Gene Library** Human chromosomes are broken up into fragments of DNA using restriction enzymes. The fragments are inserted into vectors (plasmids are shown here) and taken up by host bacterial cells, each of which then harbors a single fragment of the human DNA. The information in the resulting bacterial cultures and sets of colonies constitutes a gene library.

DEMONSTRATION - SEPARATING DNA FRAGMENTS BY SIZE USING Gel Electrophoresis

RESEARCH METHOD

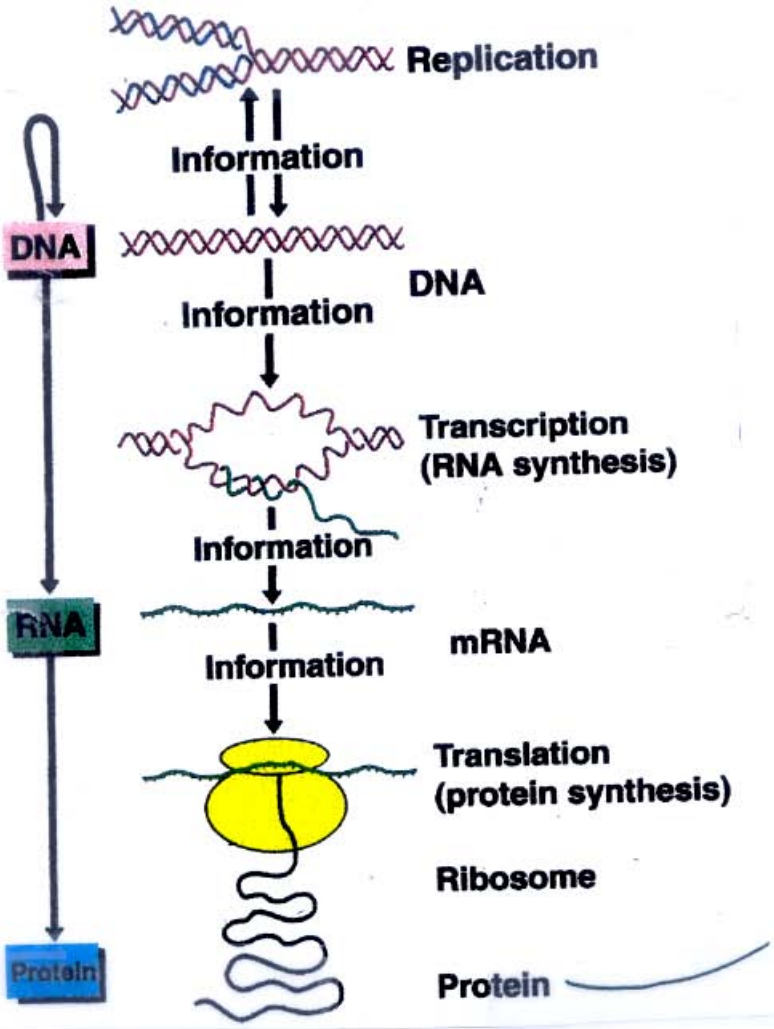


How Visualize the DNA?

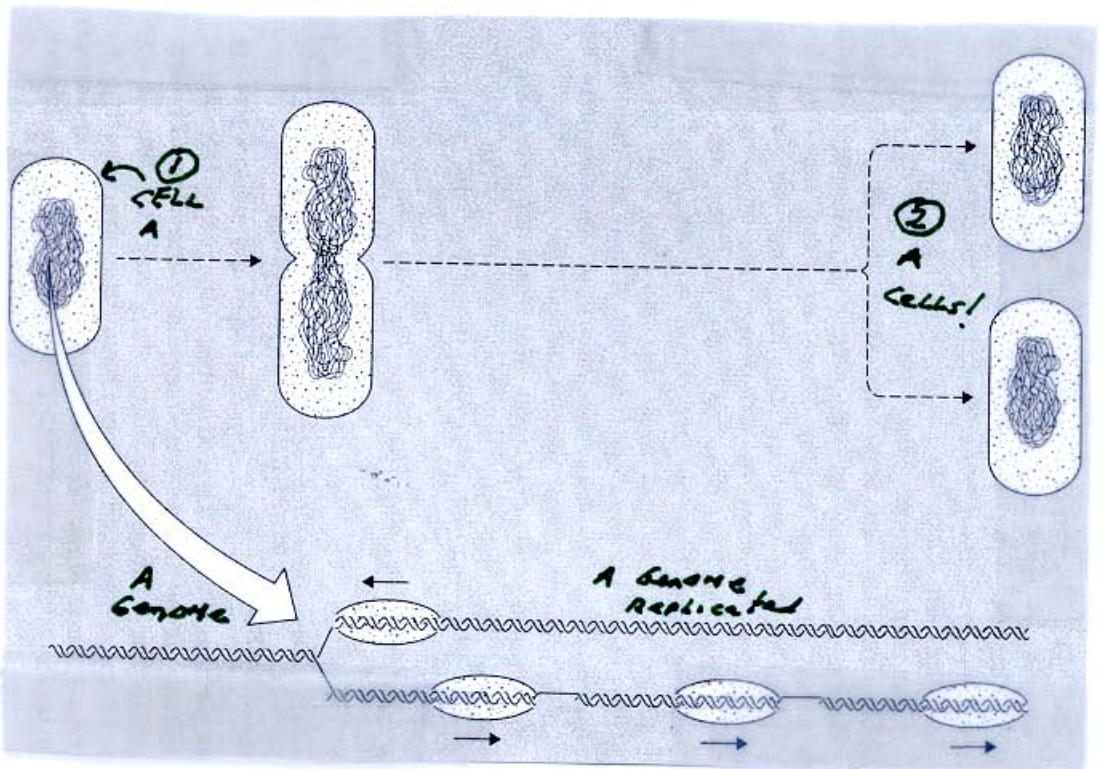
16.2 Separating Fragments of DNA by Gel Electrophoresis A mixture of DNA fragments is placed in a gel and an electric field is applied across the gel. The negatively charged DNA moves toward the positive end of the field, with smaller molecules moving faster than larger ones. When the electric power is shut off, the now separated fragments can be analyzed.

ii

HOW DO GENES WORK?



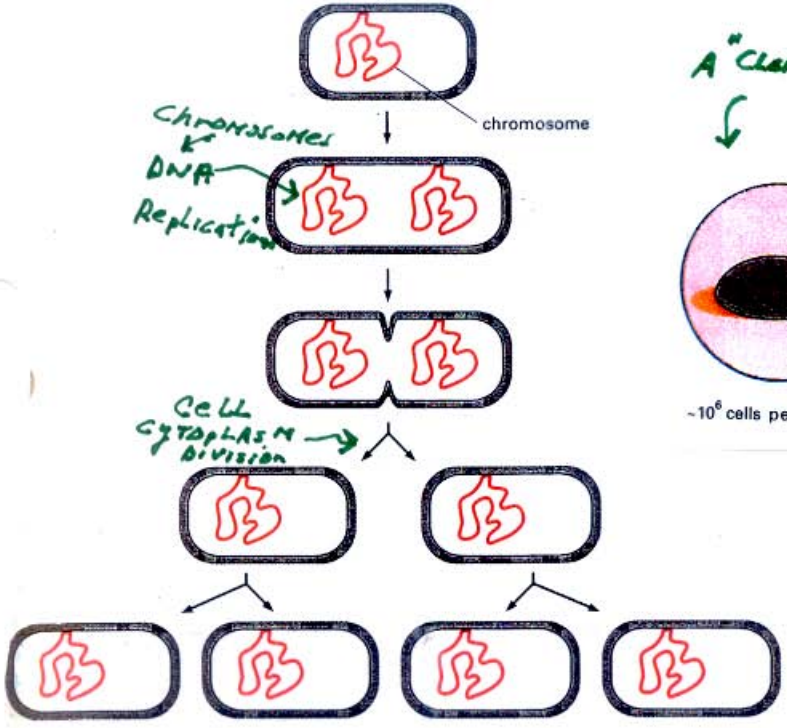
HOW ARE GENES REPLICATED EACH CELL GENERATION?



HOW IS THE DNA SEQUENCE COPIED / REPLICATED EACH CELL DIVISION?

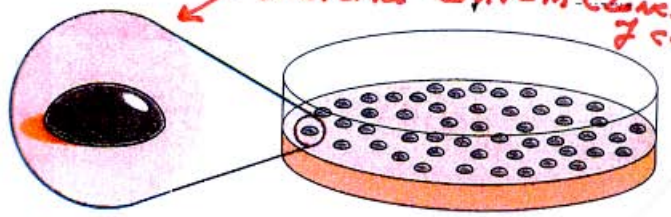
PASS ON GENES TO NEXT Generation Precisely?

GENES ARE REPLICATED DURING EACH CELL DIVISION



A "Clone"

Note - Each Clump of Bacteria Contain Clones of cells



A BACTERIAL COLONY CONTAINS MANY COPIES OF SAME CELL or CLONES WHICH ARE GENETICALLY IDENTICAL!

EACH DAUGHTER CELL CONTAINS THE SAME COLLECTION OF GENES

CLONES!

MAJOR PROPERTIES OF GENETIC MATERIAL

Replication & Stability

THE SEQUENCE OF EACH DNA STRAND MUST BE MAINTAINED DIVISION AFTER DIVISION

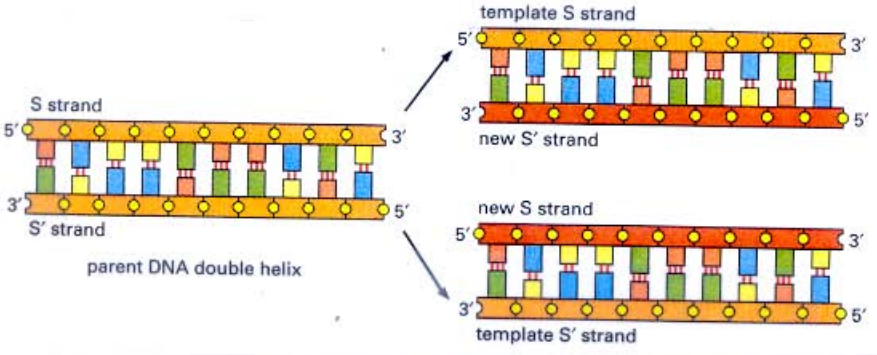


Figure 5–2 The DNA double helix acts as a template for its own duplication. Because the nucleotide A will successfully pair only with T, and G only with C, each strand of DNA can serve as a template to specify the sequence of nucleotides in its complementary strand by DNA base-pairing. In this way, a double-helical DNA molecule can be copied precisely.

HOW DOES THAT OCCUR?
PROPERTY OF THE DNA MOLECULE

DNA REPLICATION OCCURS SEMI-CONSERVATIVELY

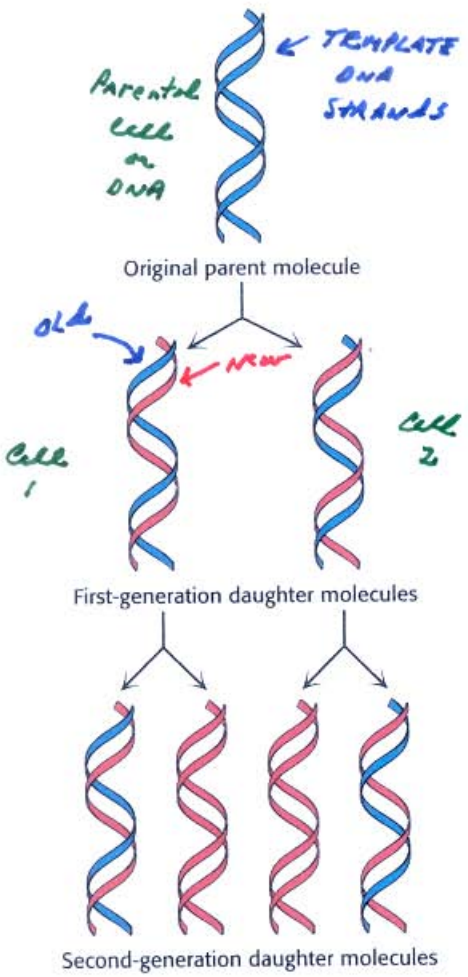


FIGURE 5.16 Diagram of semiconservative replication.

(1) DNA STRUCTURE ALLOWS DNA sequence to be Maintained

Complementary BASES

(2) EACH STRAND Serves as a Template for the Synthesis of a complementary Strand of DNA

(3) New Molecules of DNA are PRECISE COPIES OF PARENTAL DNA – ONE TEMPLATE STRAND + ONE NEWLY Synthesized Complementary STRAND!

Meselson & Stahl 1957

DNA SEQUENCE OF ONE STRAND IS A TEMPLATE FOR THE NEW STRAND

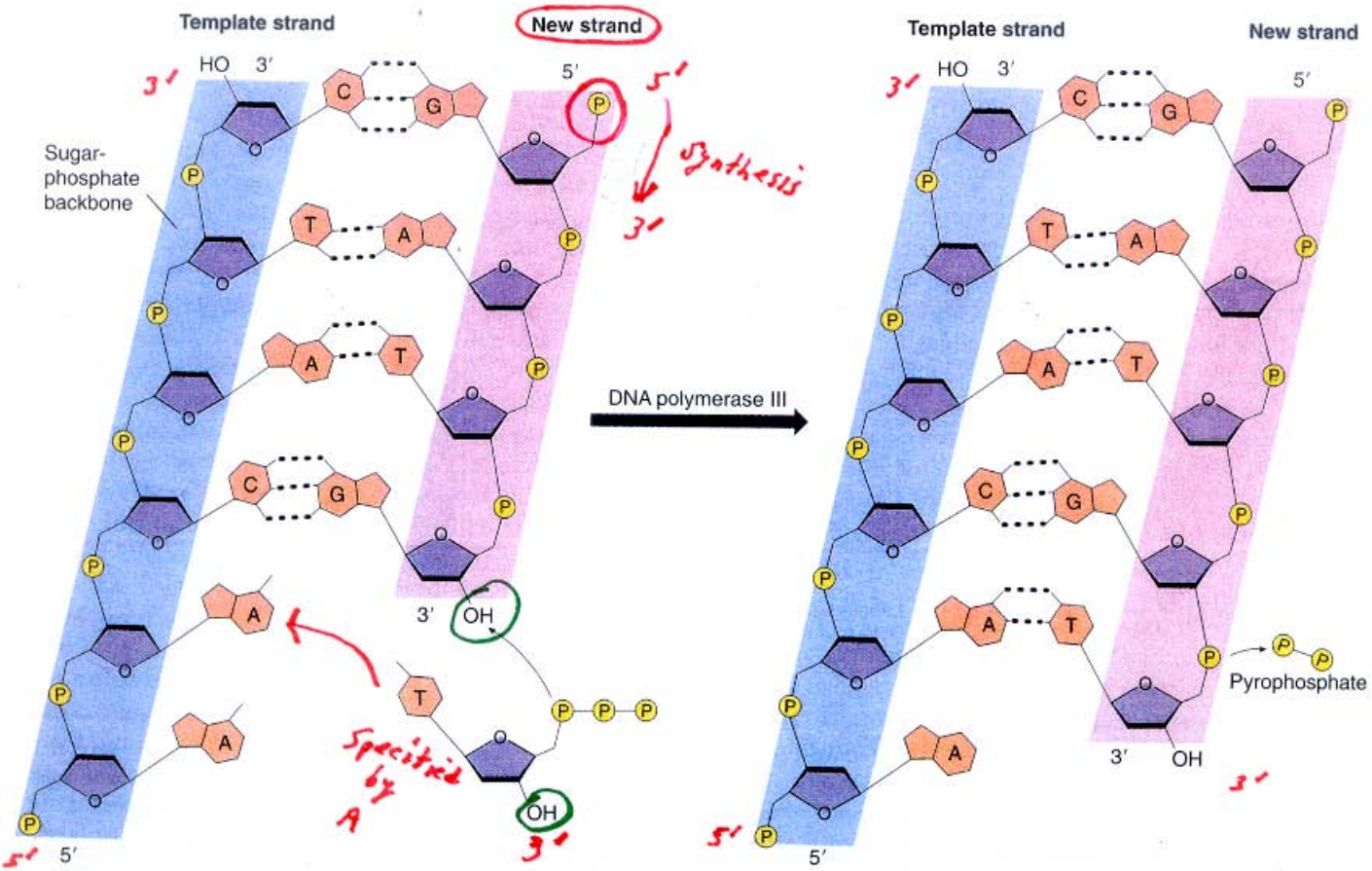
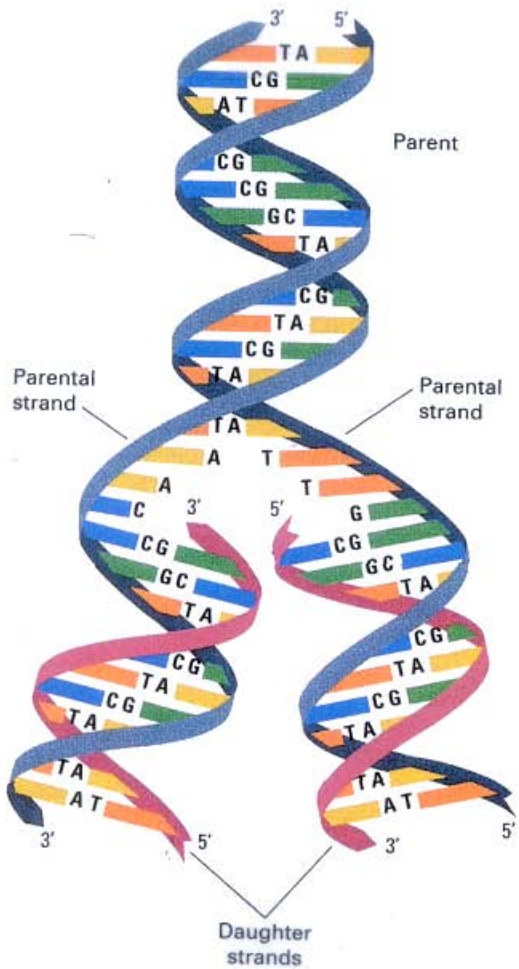


FIGURE 14.14
How nucleotides are added in DNA replication. DNA polymerase III, along with other enzymes, catalyzes the addition of nucleotides to the growing complementary strand of DNA. When a nucleotide is added, two of its phosphates are lost as pyrophosphate.

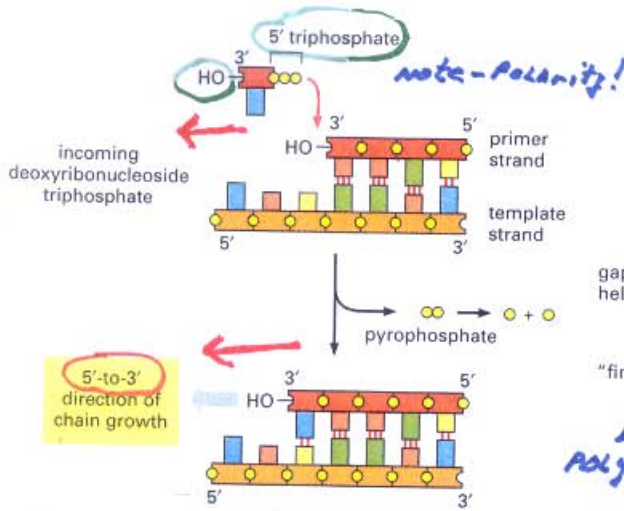
SEQUENCE IS SPECIFIED BY COMPLEMENTARY BASES

THE DNA SEQUENCE IS MAINTAINED GENERATION TO GENERATION

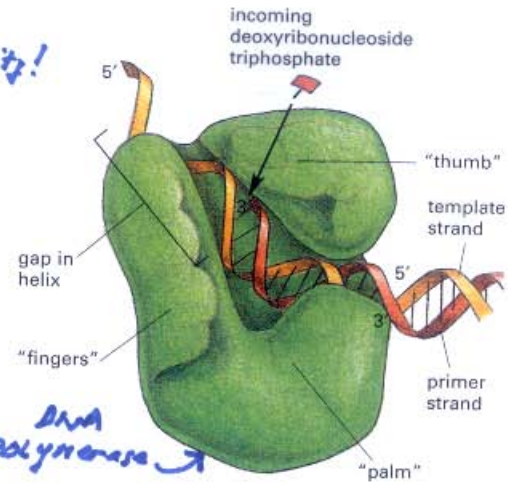


THE DNA SEQUENCE "LIVES" FOREVER!

DNA REPLICATION REQUIRES AN ENZYME - DNA POLYMERASE



(A)

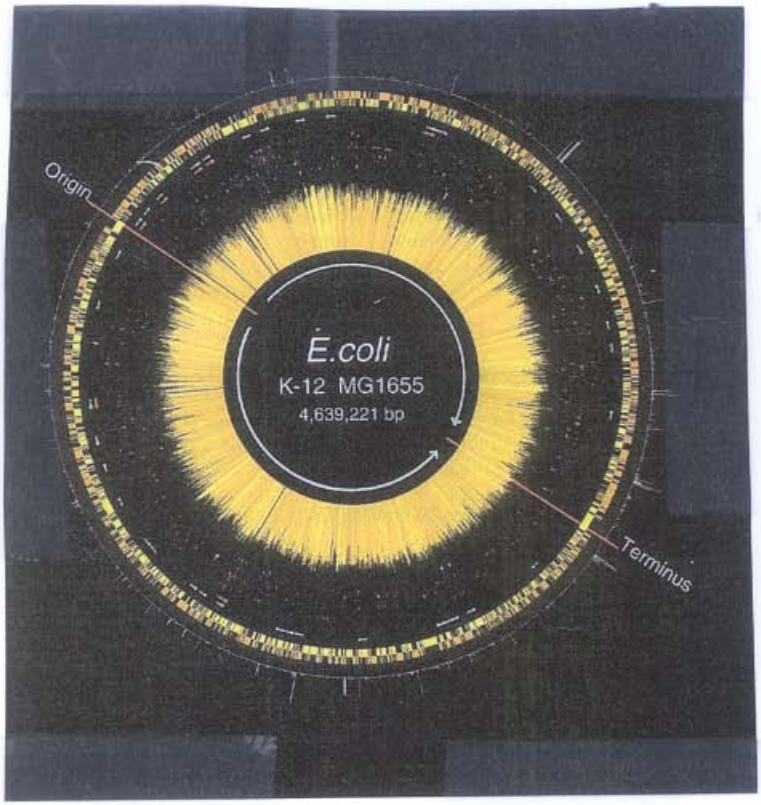


(B)

DNA POLYMERASE CATALYZES PHOSPHODIESTER BONDS AND "COPIES" THE TEMPLATE

NUCLEOTIDES ARE ALSO NEEDED

DNA Replication Starts at the ORIGIN OF REPLICATION



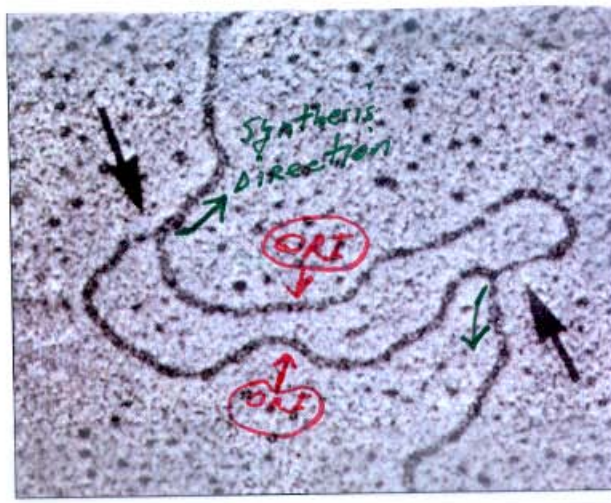
Key: — tRNA genes; — rRNA genes; — origin and terminus of replication

DNA Replication is bi'directional from the ORI!!

Hypothesis FOR TWO DIRECTION Synthesis?

DNA Polymerase BINDS to the origin of Replication (ORI) TO Begin DNA Synthesis

DNA in The Process of Being Replicated



Replication Moves Bidirectionally FROM ORIGIN

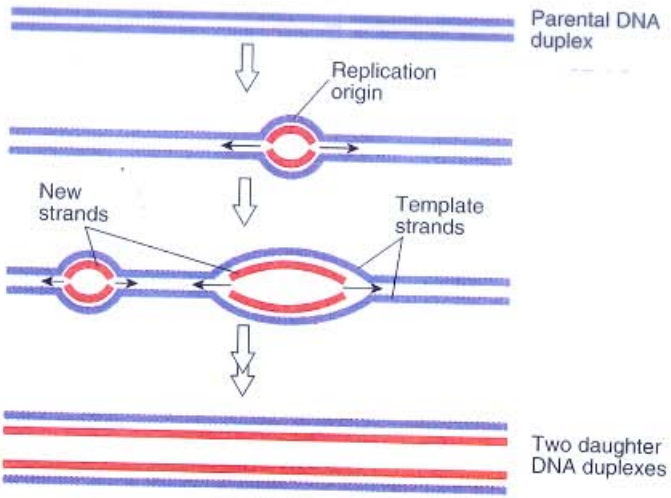


FIGURE 14.13
Origins of replication. At a site called the replication origin, the DNA duplex opens to create two separate strands, each of which can be used as a template for a new strand. Eukaryotic DNA has multiple origins of replication.

Foreign DNA segments use ori of chromosomes/DNA they are inserted into

e.g. bacteria insect^R gene
↳ use plant ori

The ORIGEN OF REPLICATION IS A SPECIFIC SEQUENCE

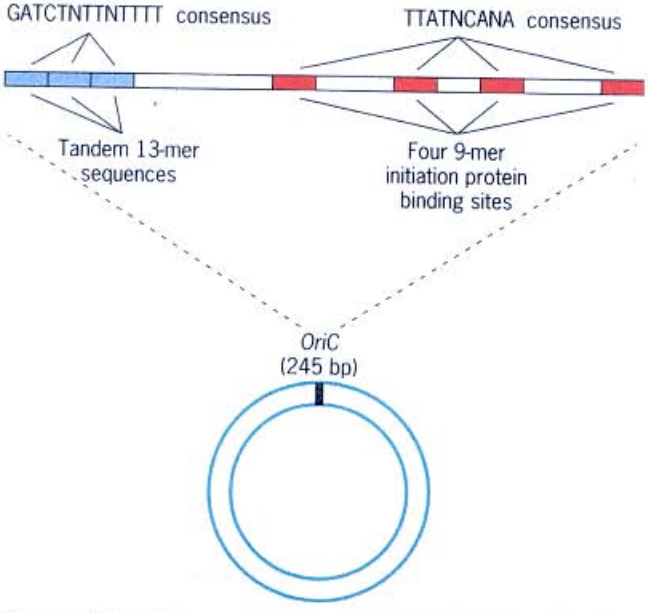


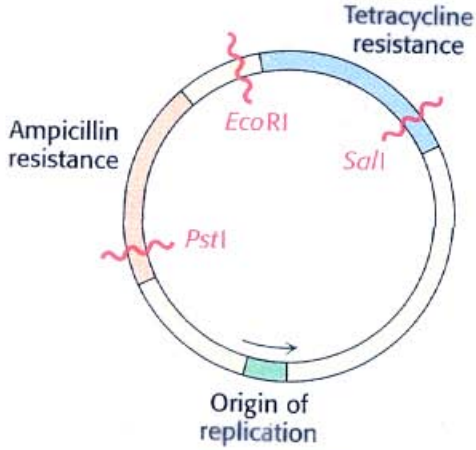
Figure 11.6 Structure of *OriC*, the single origin of replication in the *E. coli* chromosome.

HOW CLONE AN ORIGIN OF REPLICATION?

What is the Significance FOR Genetic Engineering?

CAN Replicating "CHROMOSOMES" Be Made?

VECTORS ARE NEEDED TO REPLICATE GENES IN SPECIFIC CELLS



Plasmid pBR322

(1) ORI is a specific sequence

(2) ORI is Genome & ORGANISM Specific

(3) DNA Polymerases ARE specific for EACH ORGANISM

∴ Need correct ORI to replicate gene in a specific ORGANISM!

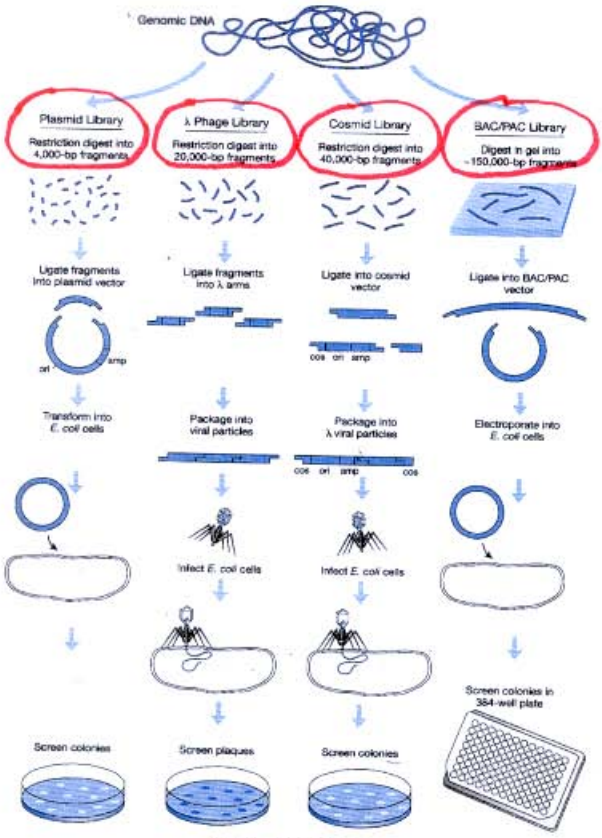
Note → Need BACTERIAL ORI to clone human gene in bacteria. Need human ori to replicate a bacterial gene in human cell.

Yo! It's in the sequence = Function

∴

Vectors CAN Be Engineered!
ORI's CAN BE CLONED/synthesized!

There ARE MANY Types of Vectors
ALL Require AN ORI



Making a Genomic Library

The Polymerase Chain Reaction or PCR is a Molecular Xerox Machine

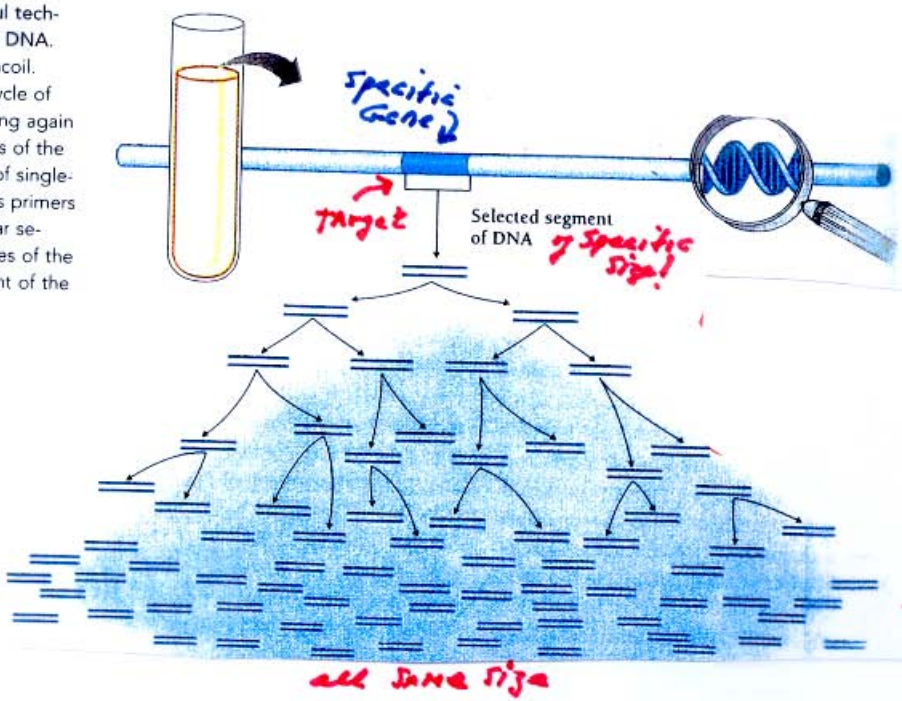


Figure 13-2 PCR is a simple, powerful technique for multiplying specific sequences of DNA. A. When DNA is heated, the two strands uncoil. They are then cooled and replicated. The cycle of heating, cooling, replicating, and then heating again is repeated until millions or billions of copies of the sequence 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.

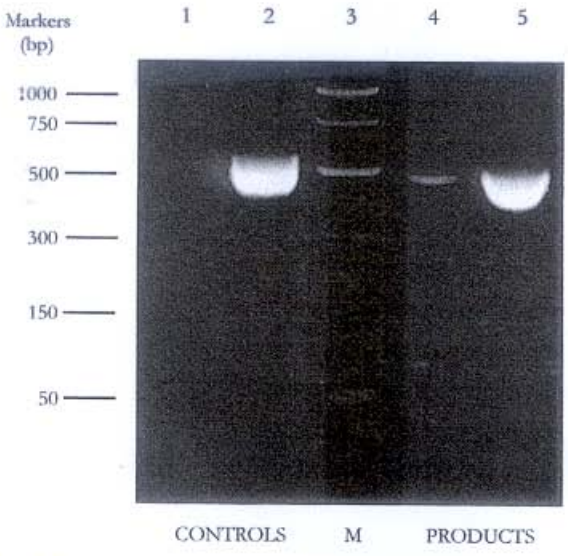
How many copies after 10 Replication Cycles?

PCR has revolutionized DNA analysis!
SPECIFIC DNA sequences/genes can be "copied" directly from "tiny" Amounts of DNA!

NO cloning needed!
but need sequence!
∴ have to clone "gene" first

DNA Polymerase

USING GEL ELECTROPHORESIS TO VISUALISE PCR PRODUCTS



TARGET specific BAND

Specific Diagnostic DNA Band Unique to DNA Sequence Being Amplified

Fig. 7.8. Visualisation of PCR products of ornithine decarboxylase on an agarose gel. Lane 1 – negative control (no DNA); lane 2 – positive control (cloned ornithine decarboxylase fragment, 460 bp); lane 3 – PCR size markers; lanes 4 and 5 – PCR product using rat liver genomic DNA and the ornithine decarboxylase primers used in lane 2. Lane 4 shown product after 15 cycles, lane 5 after 30 cycles of PCR. Photograph courtesy of Dr F. McKenzie.

PCR Has MANY Uses That have Changed MANY Fields

1. Amplify any DNA Sequence or Gene from "TINY" Amounts of DNA. No Need For Bacteria or Vector!
2. Study DNA FROM Limited Sources! a single hair, an Ancient insect/plant, a bone fragment, cheek cell
3. Used in: Forensics, DNA Fingerprinting, Law, Evolution, Disease Diagnosis, Identification, Pathogen Identification, Basic Molecular Biology, Evolution Studies, mRNA Detection

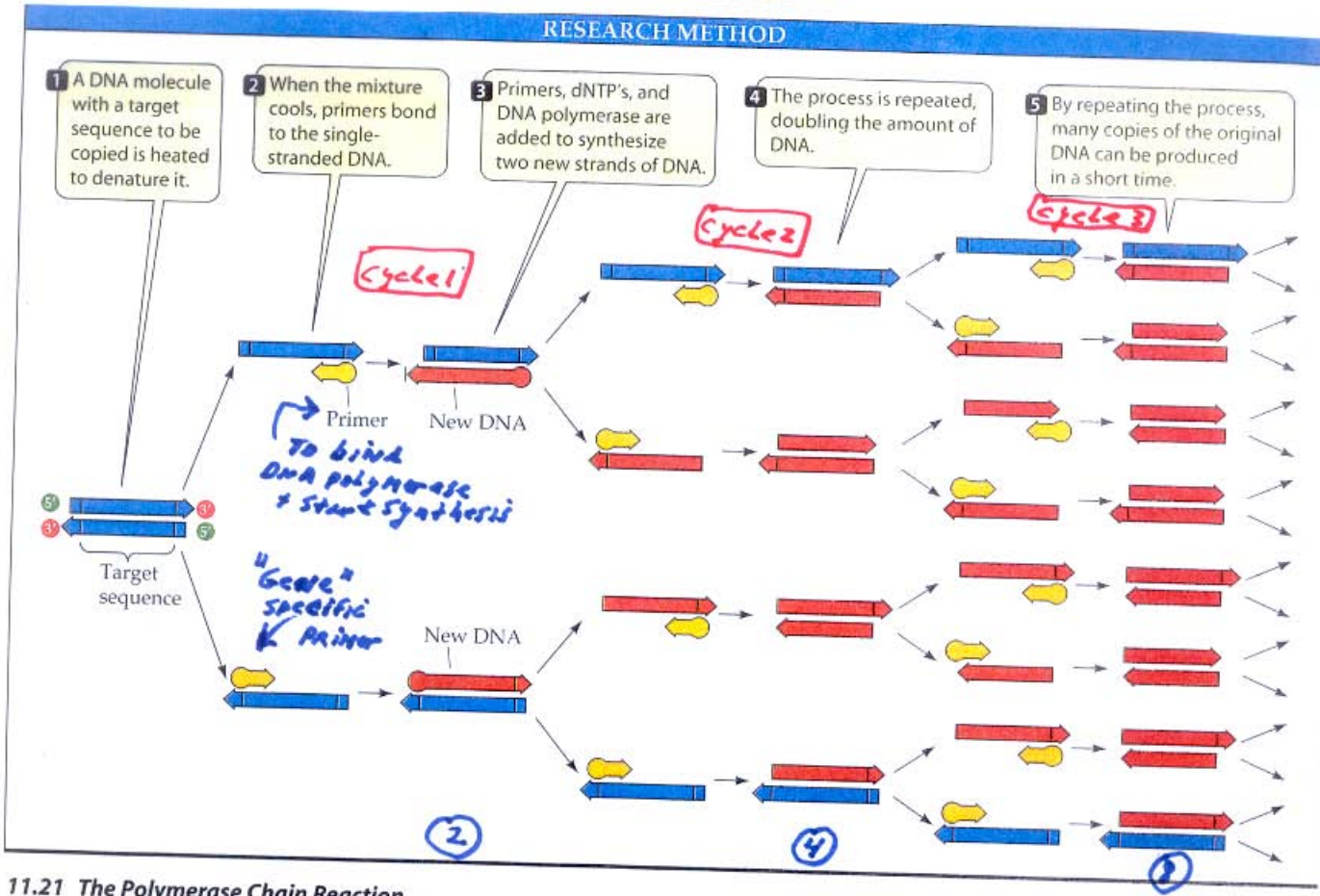
Need as little as ONE Molecule of DNA!

CAN Make an ∞ Amount of DNA!

But- Need Sequence of DNA Segment to be used for PCR! i.e. have to clone a Sequence FIRST!

REVOLUTIONIZED MANIPULATING DNA

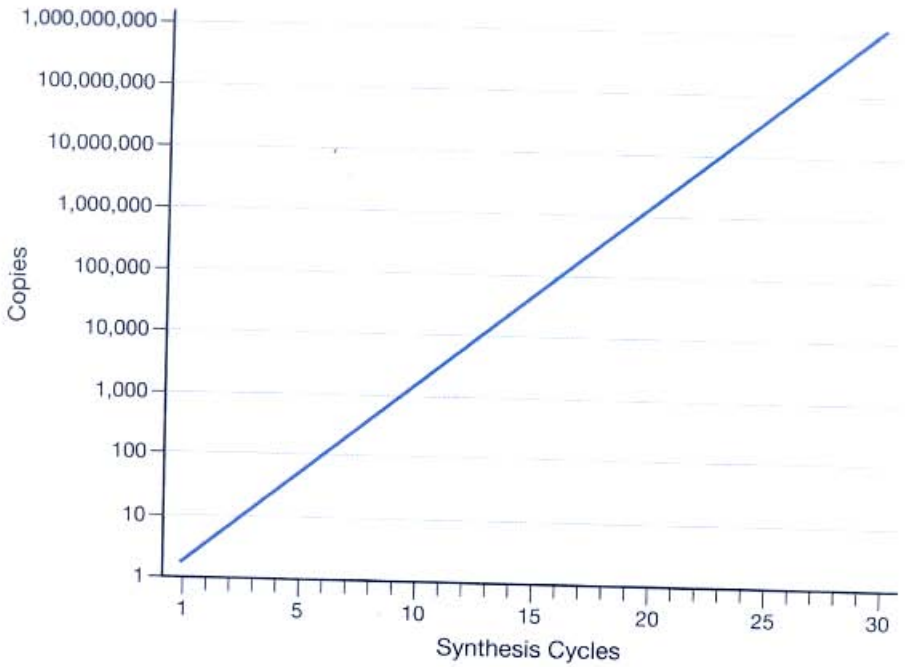
PCR IS A CYCLIC PROCESS OF DNA REPLICATION



11.21 The Polymerase Chain Reaction
The steps in this cyclic process are repeated many times to produce multiple copies of a DNA sequence.

$2^{\#}$ molecules of DNA where # = # cycles

MAKING LARGE AMOUNTS OF DNA USING PCR



Polymerase Chain Reaction Theoretical Amplification

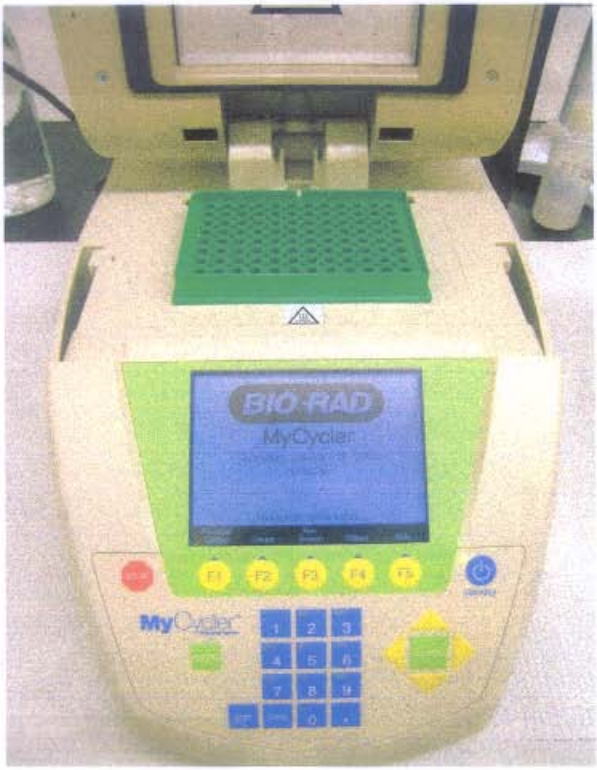
PCR Requirements

1. Knowledge of DNA Sequence :: Must clone DNA the "old fashioned way" first
2. DNA Polymerase - Heat Stable to ~100°C!! (where isolated?)
3. Thermo programmer / cycler to Heat + Cool DNA in cycles - Separate Strands & Allow new strands to form
4. Primers - Recognize specific DNA Sequences & Initiate DNA Synthesis & Binding of DNA Polymerase Note: are not needed in test tube!

It's ALL in the DNA Sequences
Know Sequence - can "make" an infinite Amount of DNA Sequence!

In 1 hour can do what took months before PCR!

A PCR Machine!



PCR APPLICATIONS

1. PGD
2. Sperm Genotyping
3. Ancient DNA
4. DNA Fingerprints

PCR CAN BE USED TO ANALYZE GENES IN A SINGLE HUMAN EMBRYO CELL OR SPERM!

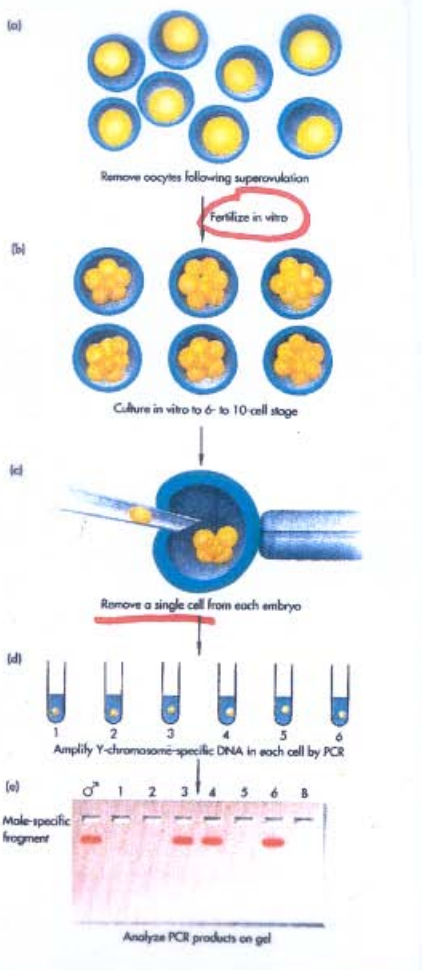
Determining Embryo Sex

PGD →

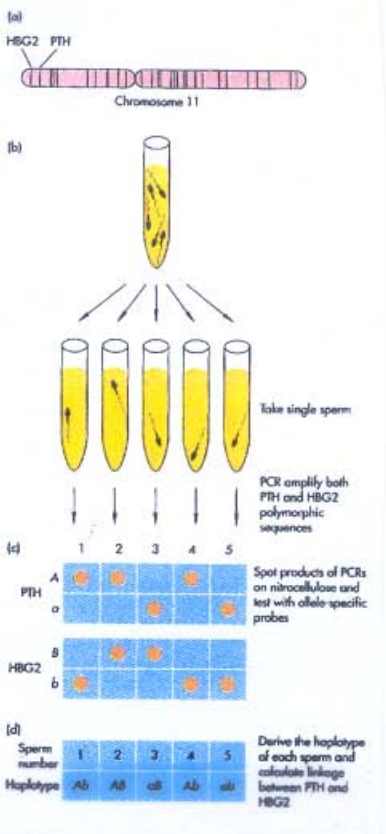
Pre-Implantation Genetic Diagnosis

TOTIPOTENCY STEMCELLS

SRY-gene



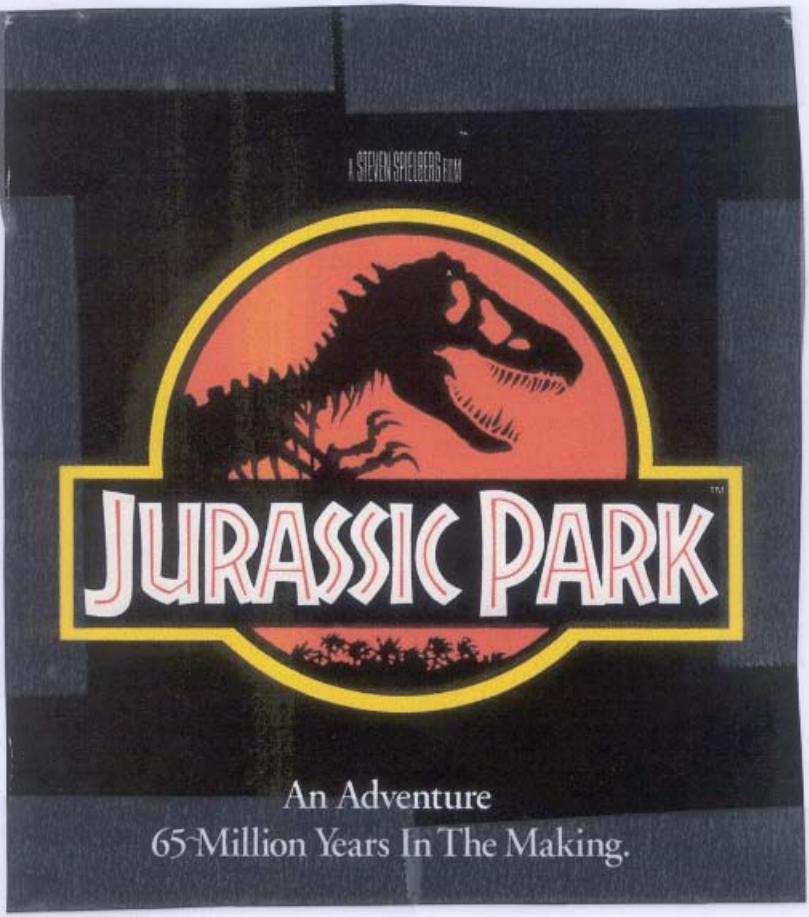
Determining Sperm Genotype



SEX determination in 8-cell EMBRYOS!

Genotypes of specific sperm!

What Are the IMplications of this procedure considering that Human Genome Has Been Sequenced?


A STEVEN SPIELBERG FILM
JURASSIC PARK
An Adventure
65 Million Years In The Making.

USING PCR TO DETECT GENES IN ANCIENT DNA

Ancient DNA Milestones

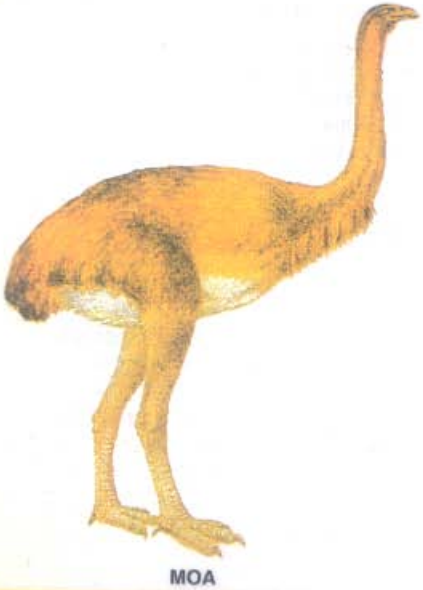
These extinct organisms have yielded meaningful genetic sequences.

AMBER INSECTS
40 MILLION YEARS OLD

FOSSIL LEAVES
17 MILLION

MAMMOTH
40,000

SMILODON
13,000



MOA
4,300

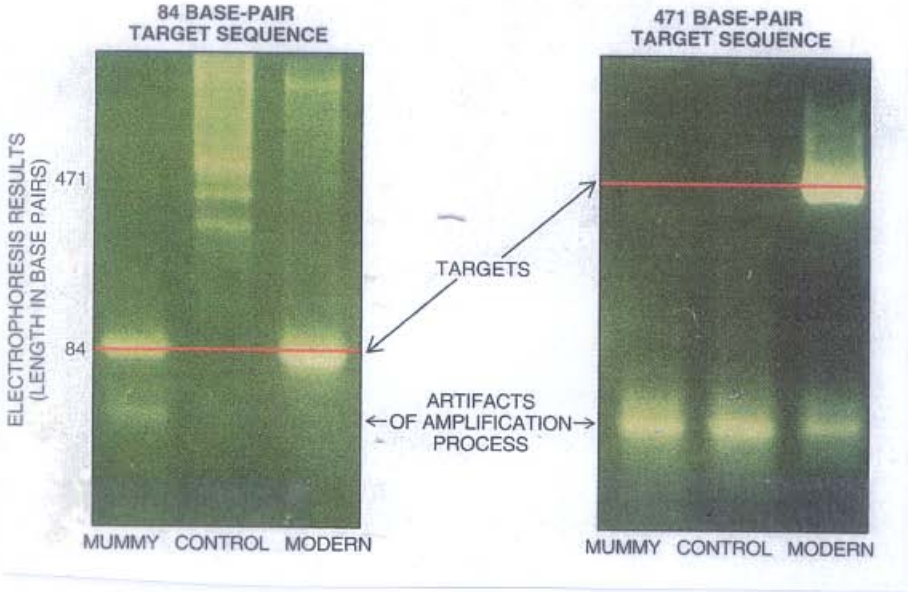
QUAGGA
140

THYLACINE
80

PRESENT

JUST NEED ONE MOLECULE OF DNA

Using PCR to detect genes in mummy DNA



Neanderthal DNA vs. Homo sapien (Human) DNA

SEQUENCE TO DETERMINE RELATIONSHIPS

USING PCR in CRIME SCENES

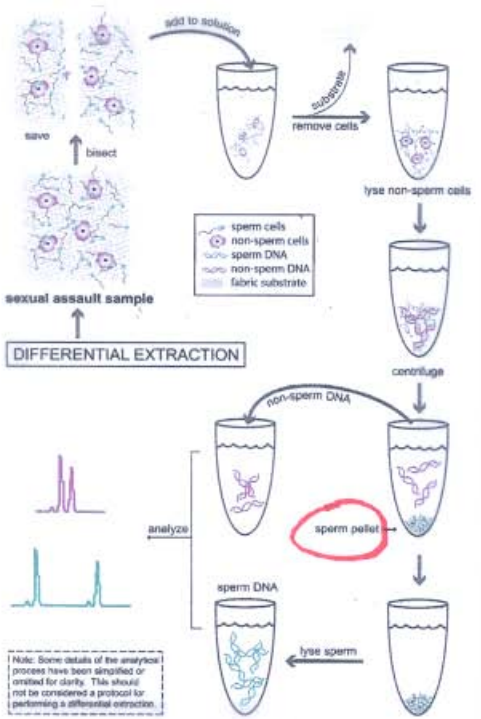
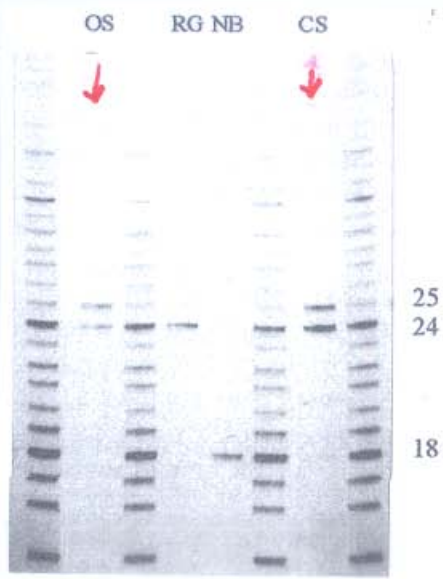


Plate 4 Differential extraction.

suspect victim crime scene



"Match" what is probability that this will occur by chance?

OS = suspect
CS = CRIME SCENE
RG and NB = VICTIMS

DNA DOESN'T "LIE"!!

FILM - KERRY MULLIS AND PCR

DNA REPLICATION IS PRECISE BUT MISTAKES OCCUR!

Gene A

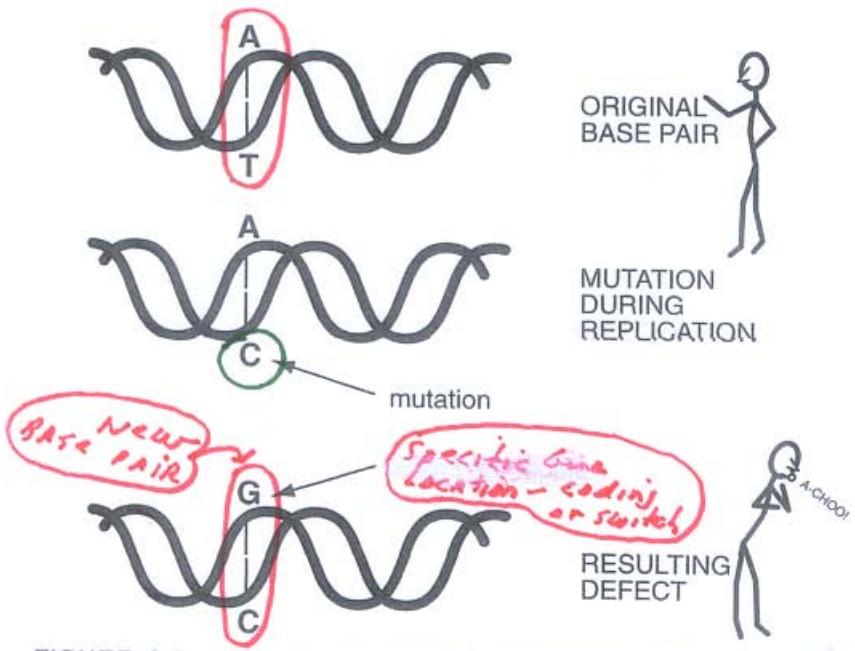


FIGURE 1.3 A mutated gene makes a defective protein that leads to an illness in an individual.

Gene A' VARIANT OF SAME Gene!

SEE MUTATION AS change in Phenotype

CHANGE IN DNA SEQUENCE
↳ CHANGE IN PROTEIN ∴ FUNCTION

CAN HAPPEN in PCR – BUT Minimize with Correct DNA Polymerase

MUTATIONS in GENES ARE RARE
BUT ARE INHERITED

① one gene per gamete

② 2 genes per somatic cell

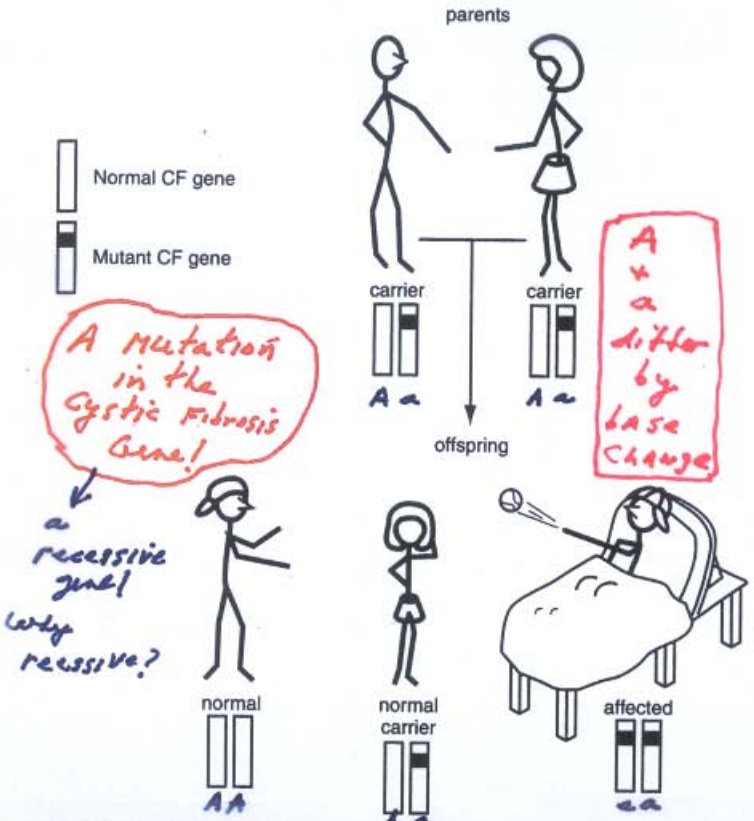


FIGURE 1.4 Two parents heterozygous for CF and producing normal or affected kids.

A v a differ by base change

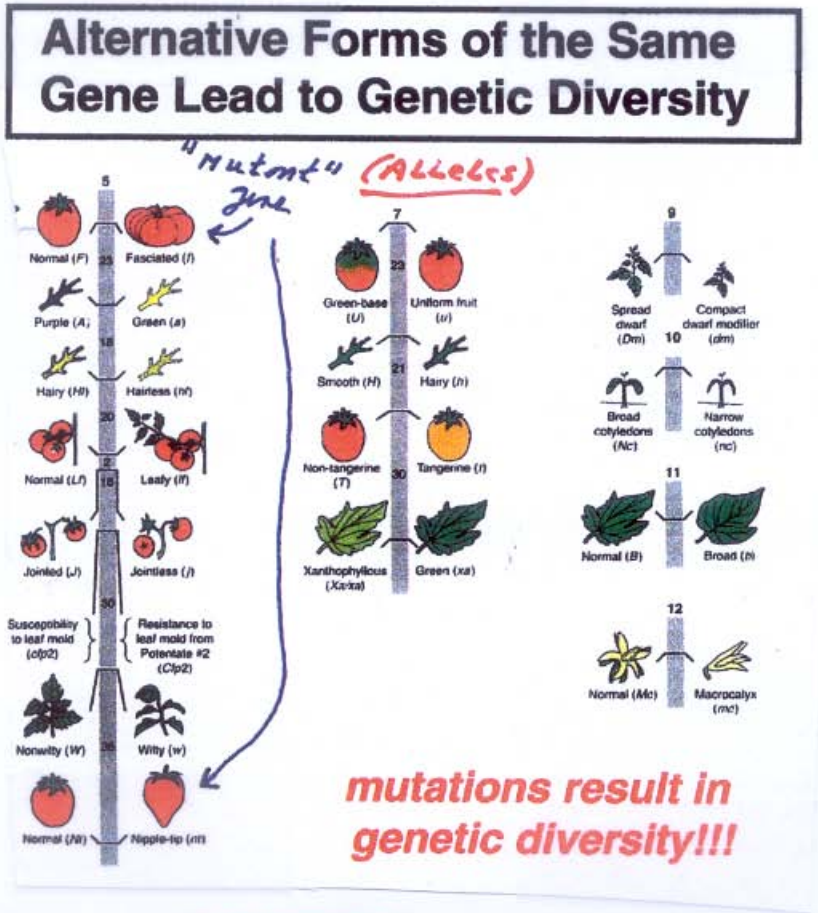
FOLLOWS Mendelian Rules

Eugenics

↳ How Remove "bad" Allele from Population? Can you do this?

How FOLLOW Inheritance?
What ALLOWS Disease to be Followed?

MUTATIONS GIVE RISE TO GENETIC DIVERSITY



Change in DNA Sequence!

How know Sequence changed?

Different forms of SAME Gene

SNP - Single Nucleotide Polymorphism!

VARIability Acted on & Used By "Nature" & By our ANCESTORS / EARLY Gene Engineers!

SAME PROCESS → DIVERSITY OF HUMAN GENES!

HOW KNOW MUTATION? ARE MUTATIONS GOOD, BAD, NEUTRAL?

MUTATIONS CAN OCCUR DIFFERENT WAYS

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-PAIR CHANGE

② ADD/DELETE BASE PAIRS

BASE SEQUENCE OF GENE CHANGES!

③ MOVE GENE OR PART OF GENE TO NEW LOCATION!

SWITCHES CHANGE!

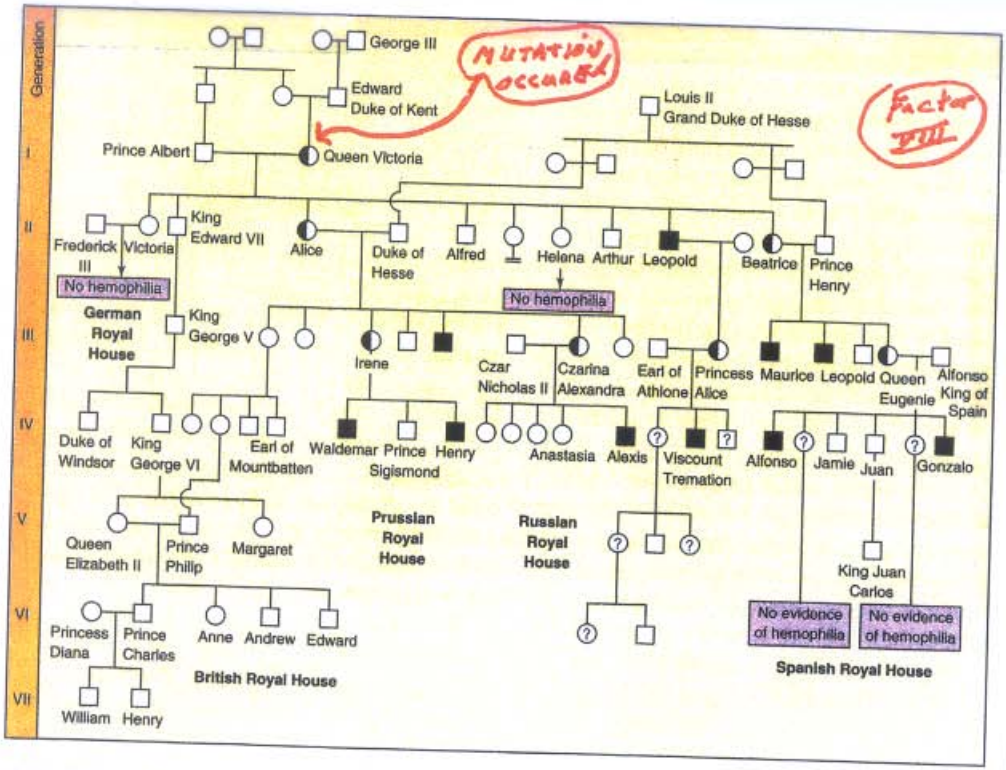
HUMAN GENETIC DISORDERS OCCUR AS A RESULT OF MUTATIONS

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

GARROD - INBORN ERRORS OF METABOLISM

PEDIGREES CAN BE USED TO FOLLOW DISEASE GENES IN HUMAN FAMILIES



Hemophilia

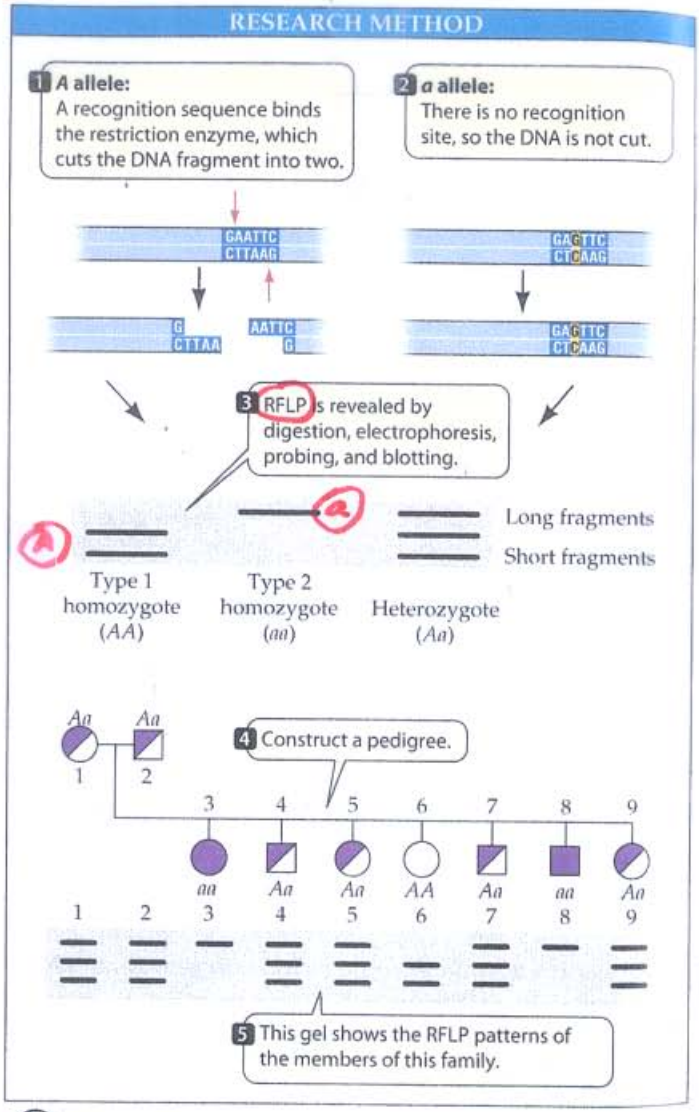
Followed by Bleeding Phenotype

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.

OR FOLLOW BY DNA TESTS USING MOLECULAR METHODS (e.g., PCR)

RESEARCH METHOD



17.7 RFLP Mapping Restriction fragment length polymorphisms are differences in DNA sequences that serve as genetic markers. Thousands of such markers have been described for the human genome.

A

a

use PCR &/or other method

← DNA Fingerprint

RFLP

← Follow in Family

Implications? Combined with sequence of Human Genome, Embryo testing (PGD), & PCR ?!!!

HOW DOES A GENE LEAD TO A PHENOTYPE?

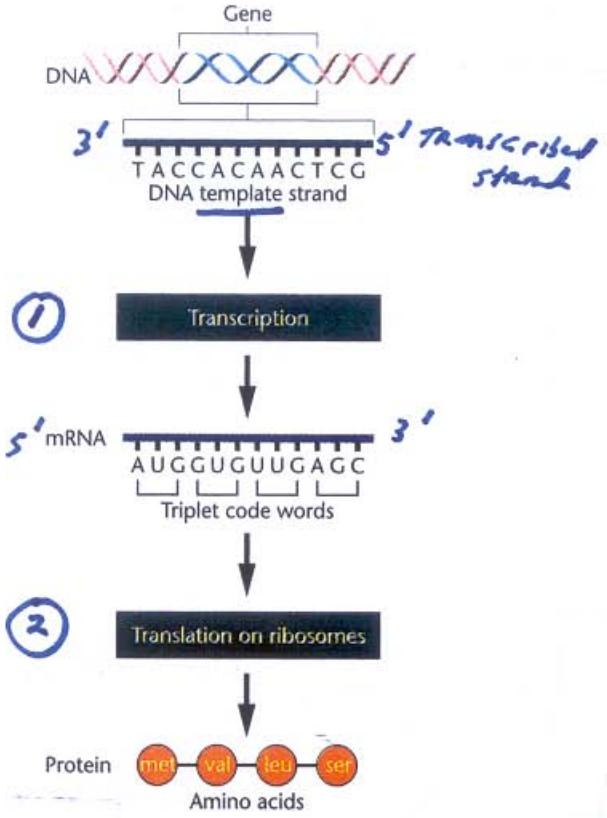


FIGURE 13.1 An overview of the concept of the flow of genetic information encoded in DNA to messenger RNA to protein.

(1) mRNA synthesized by TRANSCRIPTION

COMPLEMENTARY to TRANSCRIBED, NONSENSE STRAND

SAME SEQUENCE as SENSE STRAND

(2) mRNA TRANSLATED into PROTEIN by TRANSLATION of the Genetic Code

Genetic Code on mRNA TRANSLATED to Protein Sequence

∴ Sequence of Gene
↳ Sequence of mRNA
↳ Sequence of protein

KNOW SEQUENCE
KNOW PROTEIN
↳ ENGINEER NEW PROTEINS

MUTATIONS - CHANGE DNA SEQUENCE & CHANGE CORRESPONDING PROTEIN SEQUENCE !!

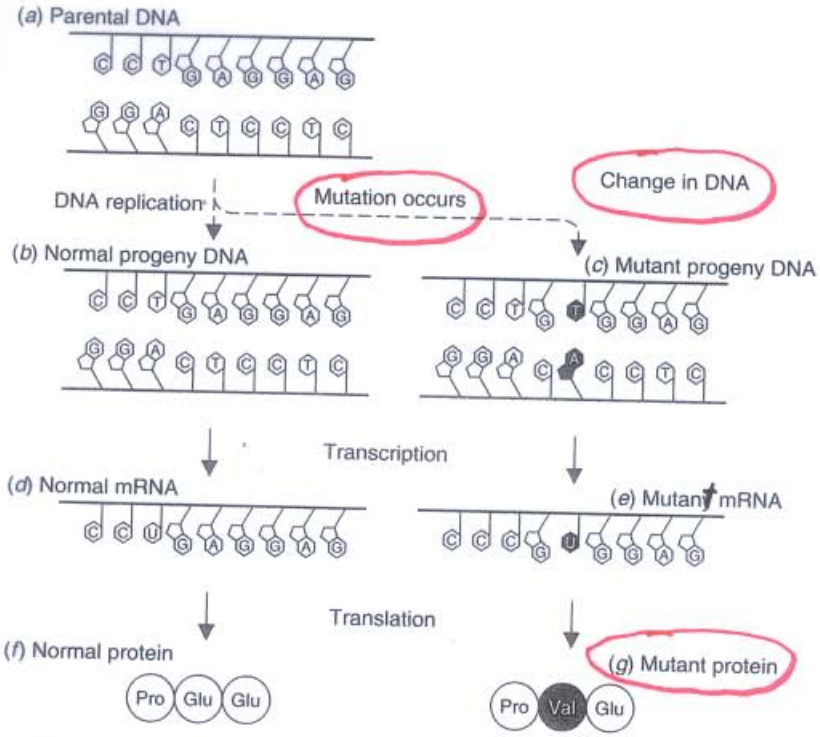
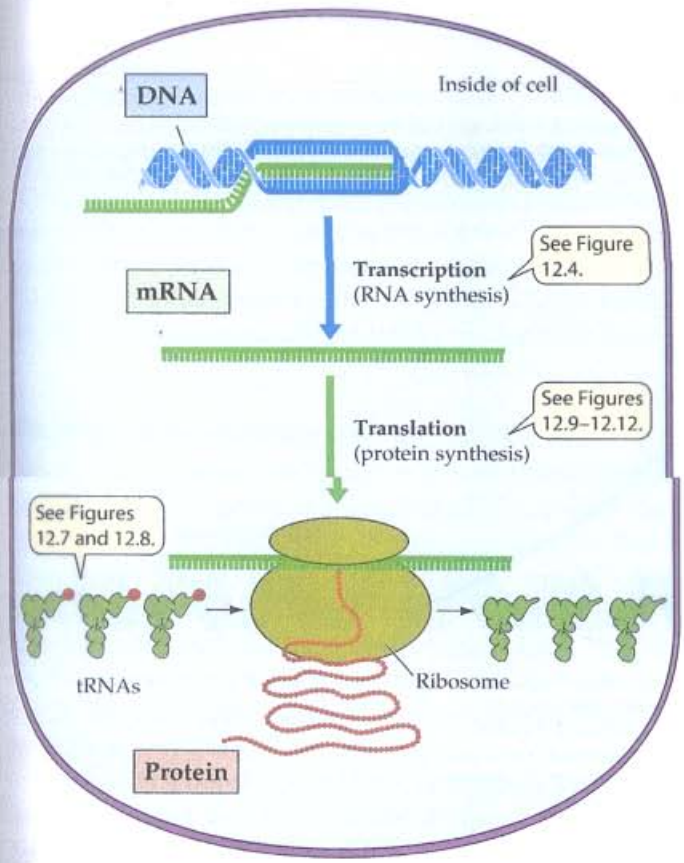


Figure 3-5. A Point Mutation Changes the Sequence of Amino Acids in a Protein. DNA replication is very accurate, so the nucleotide sequence in the progeny DNA **(b)** is usually identical to that of normal parental DNA **(a)**. Occasionally an error is made. In this example, a particular A · T base pair in parental DNA changes to a T · A pair in the mutant, progeny DNA **(c)**. During transcription, the information in DNA is converted into messenger RNA. The mutation in DNA results in a conversion of particular GAG codon in normal messenger RNA **(d)** into a GUG codon in mutant messenger RNA **(e)**. During translation of the information into protein, GAG codes for the amino acid glutamic acid (Glu) **(f)**, while GUG codes for valine (Val) **(g)** (see Figure 2-6). The two amino acids have very different chemical properties. Since the structure of the resulting protein is determined by the precise order of the amino acids, the mutant protein will differ significantly from the normal protein. The differences between the normal and mutant molecules shown are identical to those found between healthy people and patients suffering from sickle-cell disease.

AN ELABORATE CELLULAR MACHINARY REQUIRING THOUSANDS OF GENES IS REQUIRED TO PRODUCE PROTEINS ENCODED BY SPECIFIC GENES!



12.3 **From Gene to Protein** This diagram summarizes the processes of gene expression in prokaryotes. In eukaryotes, the processes are somewhat more complex.

IT TAKES GENES TO EXPRESS (and replicate) A GENE!!

LARGE NUMBERS OF GENES ARE NEEDED TO MAKE PROTEINS!

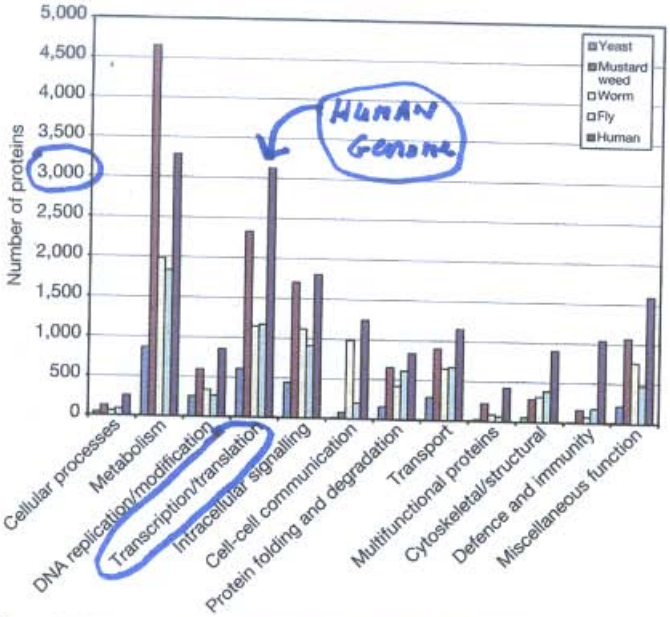


Figure 37 Functional categories in eukaryotic proteomes. The classification categories were derived from functional classification systems, including the top-level biological function category of the Gene Ontology project (GO; see http://www.geneontology.org).

~ 3,000 Genes needed for TRANSCRIPTION & TRANSLATION in Human Cells

GENETIC CODE ALLOWS THE SEQUENCE of NUCLEOTIDES in mRNA / Sense STRAND of Gene to be TRANSLATED into Sequence of Amino Acids in Proteins

mRNA
protein

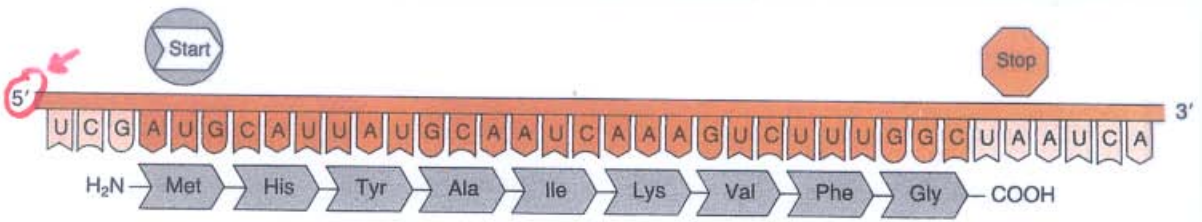


Figure 3.4 Decoding a messenger RNA sequence into a polypeptide.

NOTE: SEQUENCE in mRNA (= Sense Gene STRAND) IS TRANSLATED 5' → 3' (= beginning of sense STRAND to end) & protein made in N → C direction ∴ order nts in gene = order aa in protein!

The Genetic Code is Universal!

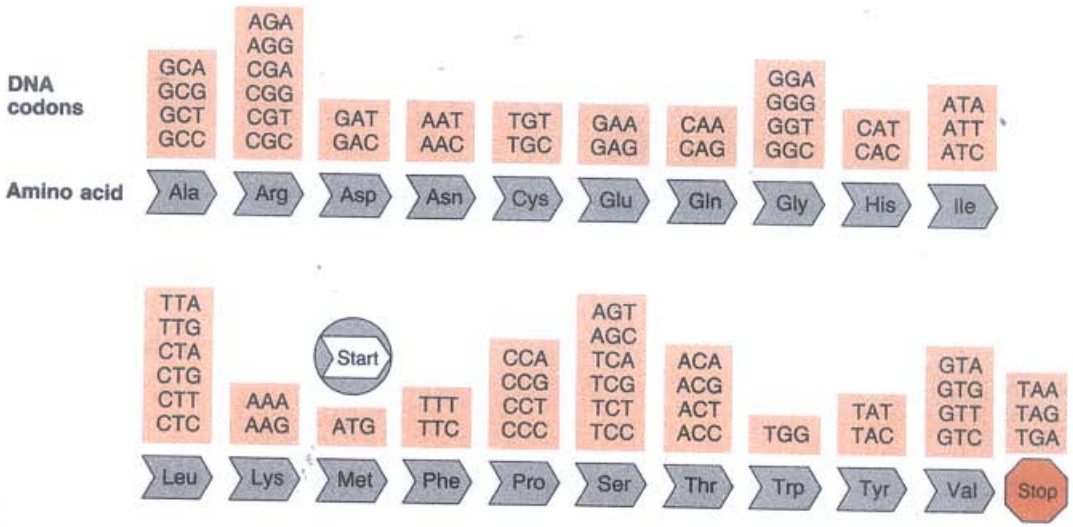


Figure 3.3 The genetic code. The codons shown for each amino acid are those for DNA. For RNA, the Ts are replaced by Us.

(1) Universal (2) Triplet (3) Punctuation
(4) Degenerate

KNOW SEQUENCE OF GENE - KNOW SEQUENCE OF PROTEIN USING GENETIC CODE

Big Implication For Genetic Engineering! CAN MAKE Genes, Genomes & Specify Proteins Wanted! CAN Express Genes FROM ONE Organism in Another!

→ DESIGN AN EXPERIMENT TO SHOW UNIVERSAL!

There is a colinearity between nucleotide sequence of a gene & amino acid sequence of protein

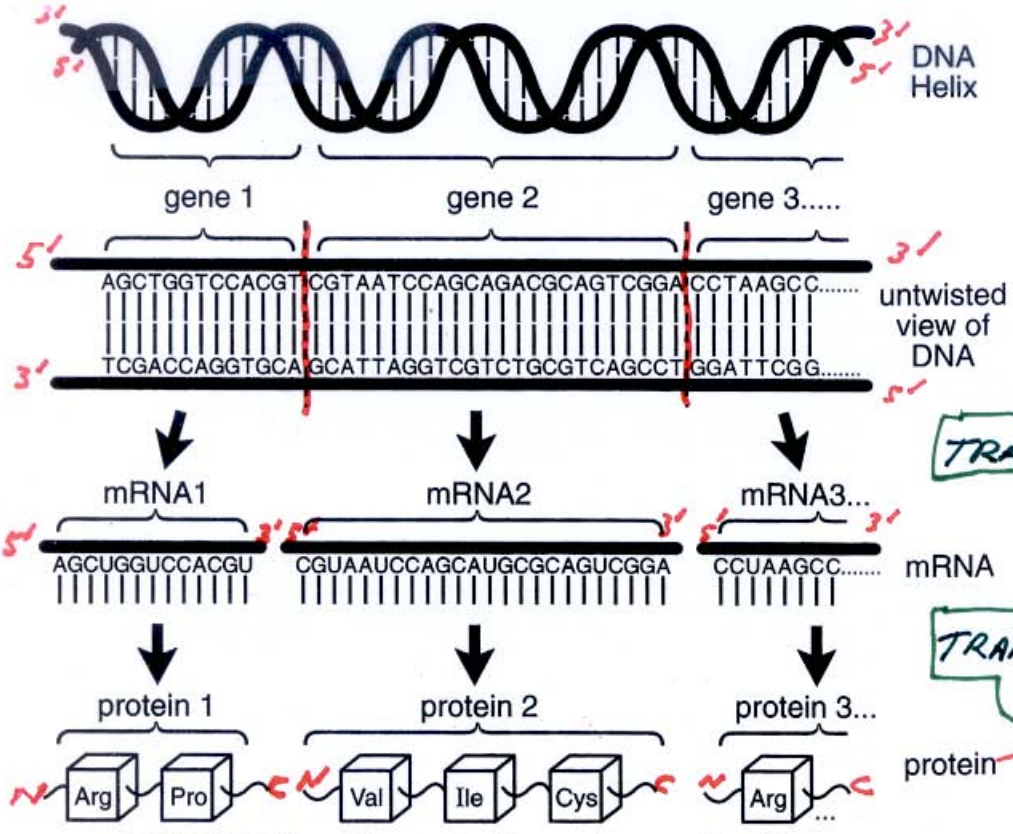


FIGURE 2.6 Adjacent sets of base pairs comprising different genes.

Specific TRAIT 1 → FUNCTION 1

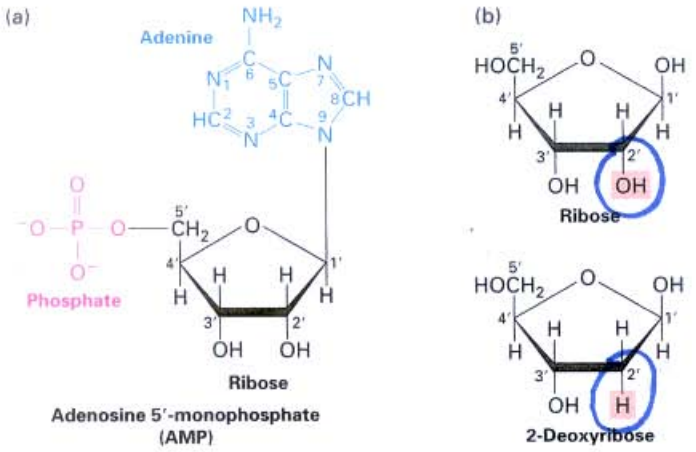
Specific Trait 2 → Function 2

Specific trait 3 → FUNCTION 3

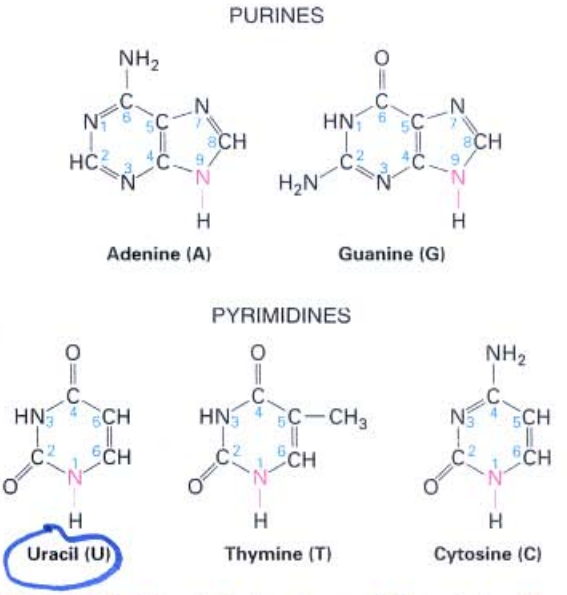
ONE GENE → ONE PROTEIN → ONE FUNCTION

MOST OF THE TIME!

RNA or RiboNucleic Acid contains Ribose Sugar and Uracil



▲ **FIGURE 2-14 Common structure of nucleotides.** (a) Adenosine 5′-monophosphate (AMP), a nucleotide present in RNA. By convention, the carbon atoms of the pentose sugar in nucleotides are numbered with primes. In natural nucleotides, the 1′ carbon is joined by a β linkage to the base (in this case adenine); both the base (blue) and the phosphate on the 5′ hydroxyl (red) extend above the plane of the furanose ring. (b) Ribose and deoxyribose, the pentoses in RNA and DNA, respectively.



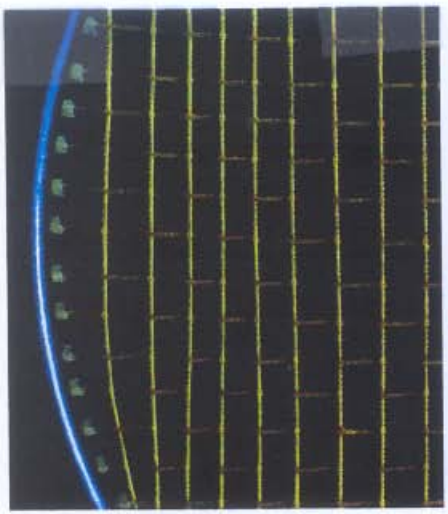
▲ **FIGURE 2-15 Chemical structures of the principal bases in nucleic acids.** In nucleic acids and nucleotides, nitrogen 9 of purines and nitrogen 1 of pyrimidines (red) are bonded to the 1′ carbon of ribose or deoxyribose. U is only in RNA, and T is only in DNA. Both RNA and DNA contain A, G, and C.

IN PLACE of Deoxyribose Sugar and Thymine!

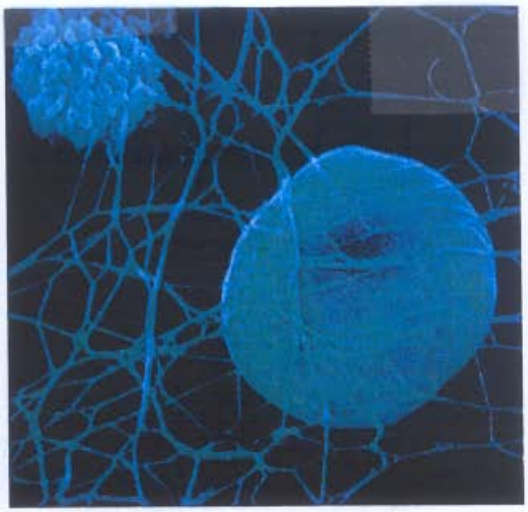
It's all in the Chemistry → Function!

UNIQUE PROTEINS CARRY OUT UNIQUE CELL FUNCTIONS

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.

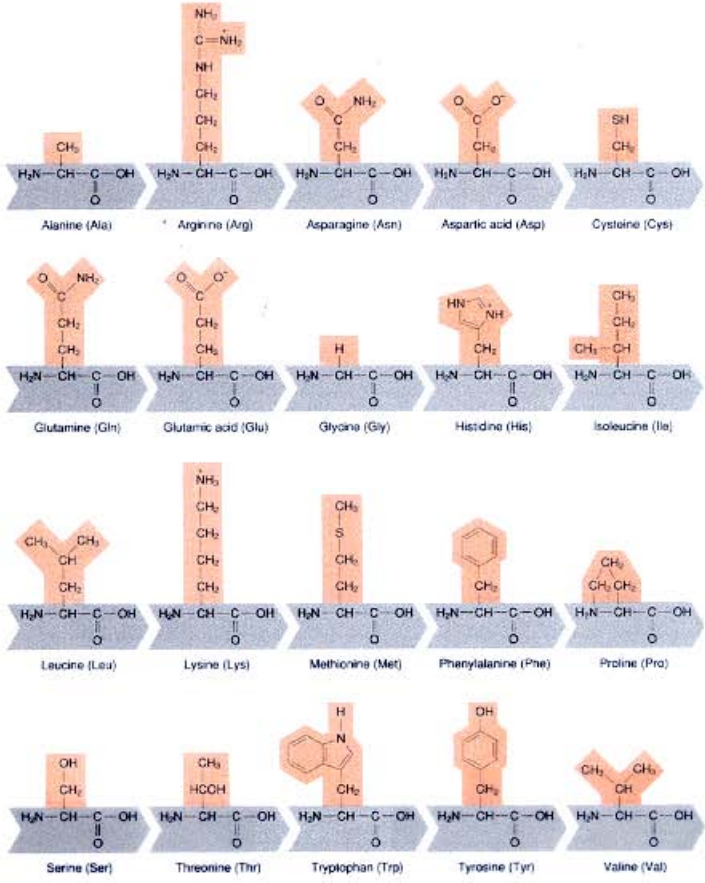
PROTEINS CARRY OUT DIVERSE CELL FUNCTIONS AND ARE UNIQUE BECAUSE OF SEQUENCE!

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 Tpase, 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

PROTEINS ARE MADE OF AMINO ACIDS



20 AMINO ACIDS DIFFER BY CHEMISTRY

Chemistry of Proteins → Biology

AMINO ACIDS ARE JOINED BY Peptide Bonds

corresponds to 5' → 3' end of mRNA / sense strand of gene!

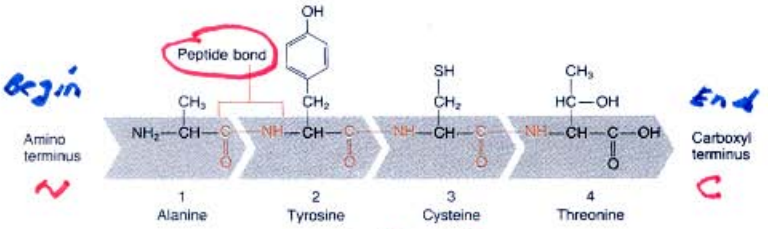
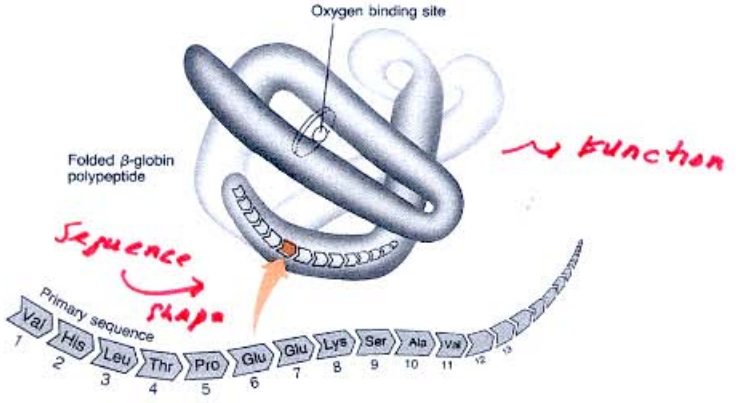


Figure 2.16 Peptide bonds between amino acids in a tetrapeptide (four amino acids).

Mutations!

Change Shape
Change Function
Change Phenotype



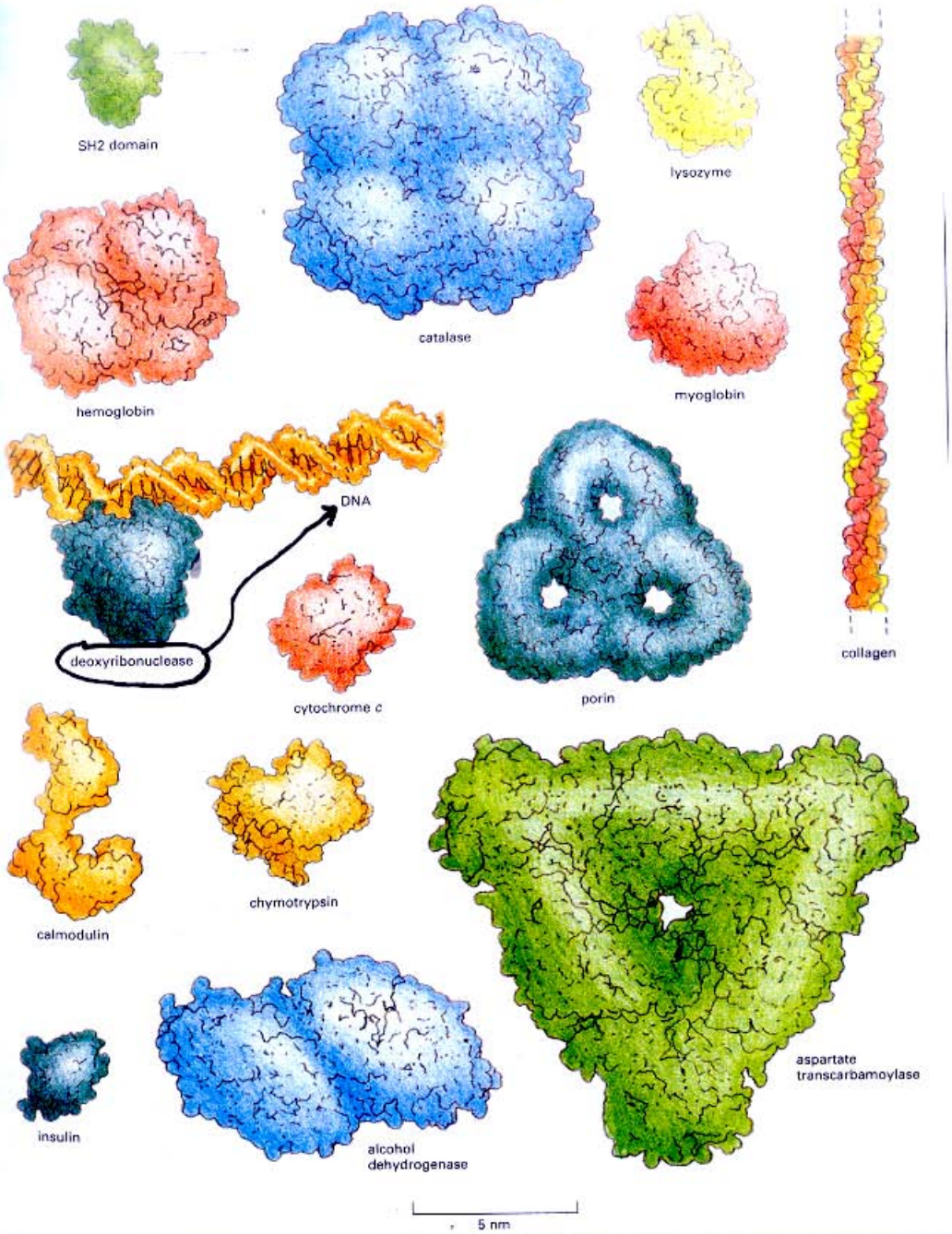
In sickle-cell hemoglobin, the Glu at position 6 is replaced by Val

Figure 2.17 A portion of the primary structure of the β-globin polypeptide and its location in the folded, complete polypeptide. Also shown is the amino acid that is altered in the β-globin polypeptide in sickle-cell disease.

ORDER OF AMINO ACIDS → Specific Protein Shape & Function

→ Phenotype

UNIQUE GENES IN A GENOME GIVE RISE TO UNIQUE PROTEINS



igure 3–24 A collection of protein molecules, shown at the same scale. For comparison, a DNA molecule bound to a protein is also ustrated. These space-filling models represent a range of sizes and shapes. Hemoglobin, catalase, porin, alcohol dehydrogenase, and aspartate anscarbamoylase are formed from multiple copies of subunits. The SH2 domain *(top left)* is presented in detail in Panel 3–2 (pp. 138–139). (After avid S. Goodsell, Our Molecular Nature. New York: Springer-Verlag, 1996.)

HOW DOES Gene # Relate to Protein #?

Note:

FORM → FUNCTION!

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.....

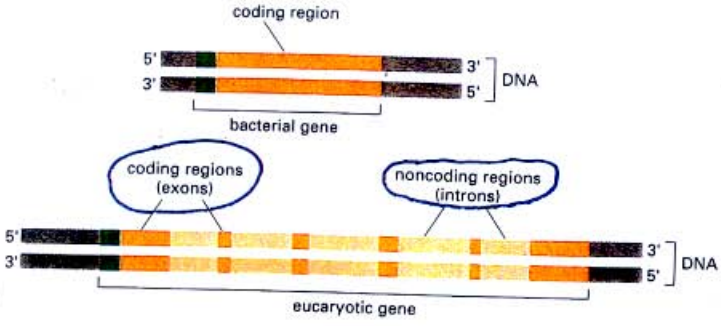


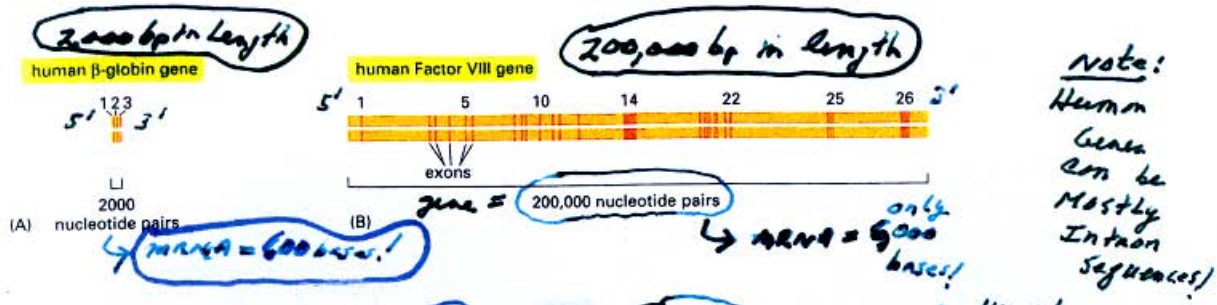
Figure 7–13 Comparison of a bacterial gene with a eucaryotic gene. The bacterial gene consists of a single stretch of uninterrupted nucleotide sequence that encodes the amino acid sequence of a protein. In contrast, the coding sequences of most eucaryotic genes (*exons*) are interrupted by noncoding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

38

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

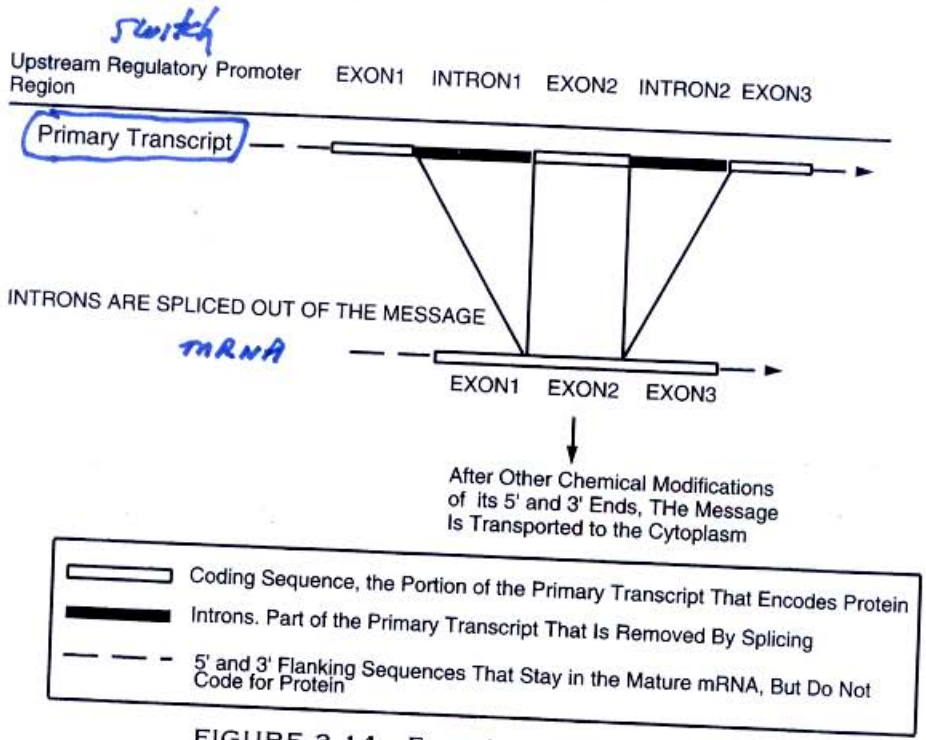
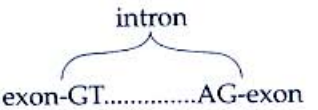


FIGURE 3.14 Exons, introns, and splicing.

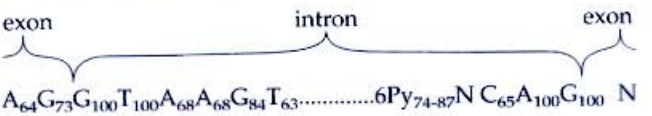
Implication for Engineering Eukaryotic genes in Bacteria?

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

Yo! It's in the Sequences!



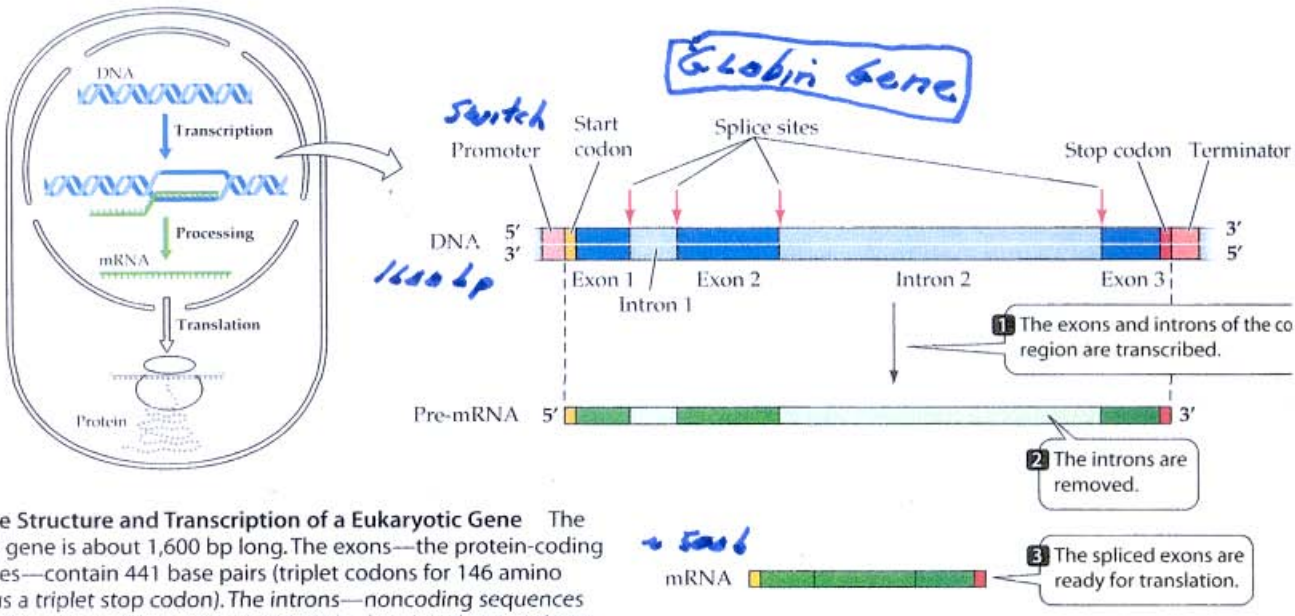
The sequences shown here are for the DNA nontemplate strand (equivalent to the RNA transcript, but with T rather than U). In addition, there are short consensus sequences at the exon–intron junctions. For nuclear genes, the consensus junctions are



Specific Sequences required FOR RNA Splicing!

What happens if these Sequences Are Mutated in a Gene?

RNA Splicing – Removing Non-Coding Sequences from Primary Transcripts to Generate Functional mRNAs

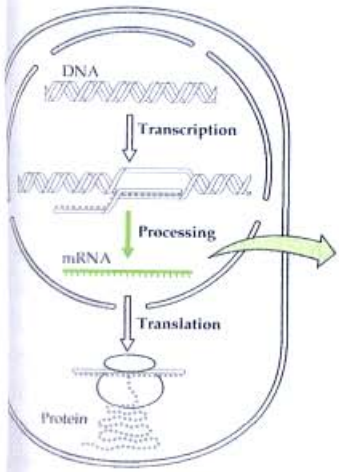


14.4 **The Structure and Transcription of a Eukaryotic Gene** The β-globin gene is about 1,600 bp long. The exons—the protein-coding sequences—contain 441 base pairs (triplet codons for 146 amino acids plus a triplet stop codon). The introns—noncoding sequences of DNA—between codons 30 and 31 (130 bp long) and 104 and 105 (850 bp long), are initially transcribed, but are spliced out of the initial mRNA transcript.

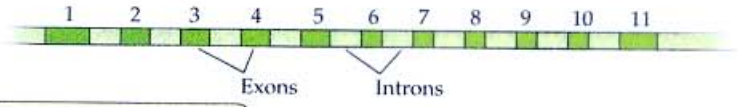
Mutations → Blood Disorders
Where can these occur?

Alternative Splicing – One Gene ↳ Several RNAs & Proteins!

Gene active in variety of cells
But ... !!!



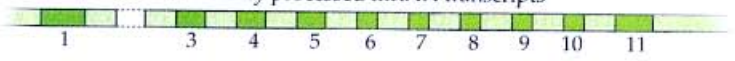
Primary RNA transcript for tropomyosin: 11 exons



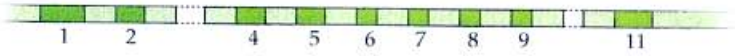
Different splicing patterns in different tissues result in a unique collection of exons in mRNA for each tissue.

Initially processed mRNA transcripts

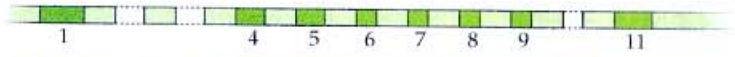
Skeletal muscle: missing exon 2



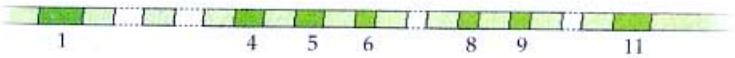
Smooth muscle: missing exons 3 and 10



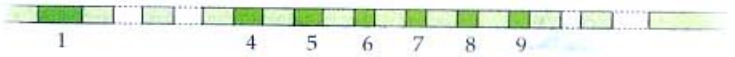
Fibroblast: missing exons 2, 3, and 10



Liver: missing exons 2, 3, 7, and 10



Brain: missing exons 2, 3, 10, and 11



5 different mRNAs!

4.20 Alternative Splicing Results in Different mRNAs and Proteins In mammals, the protein tropomyosin is encoded by a gene that has 11 exons. Tropomyosin pre-mRNA is spliced differently in different tissues, resulting in five different forms of the protein.

different mRNAs = different proteins = different functions!

IMPLICATION – HUMAN Genome Has only 30,000 Genes but Can Give Rise to Many More Proteins which are Responsible for producing the phenotype! ∴ human gene ∴ 3+ transcripts

∴ Reason why Human Genome do Contain Same # of genes as the fly & Yeast Genomes!

(40k) IMPLICATIONS for Genetic Engineering? Use Specific cDNA!

GENE ENGINEERING IMPLICATIONS!

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

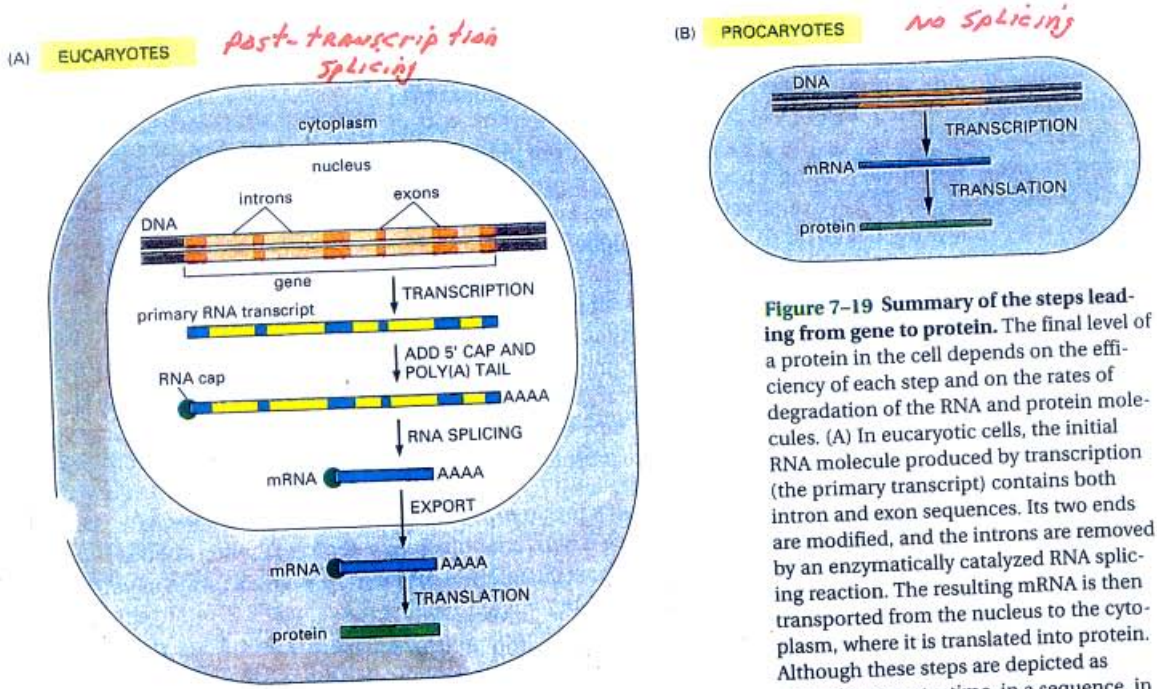


Figure 7–19 Summary of the steps leading from gene to protein. The final level of a protein in the cell depends on the efficiency of each step and on the rates of degradation of the RNA and protein molecules. (A) In eucaryotic cells, the initial RNA molecule produced by transcription (the primary transcript) 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!!

IMPLICATIONS FOR "DO-IT's in the DNA!!"

Modular organization of Sequences

1. DNA Replication
   - ORI
2. TRANSCRIPTION
   - Switch / Regulator
   - Terminator
3. Processing of RNA (Eukaryotes)
   - Splicing Sites
4. Translation
   - Start
   - Stop
   - Genetic Code / codons
5. Coding Sequence
   - Genetic Code

Modules → anything you want to do genetically!

Engineering Genes Requires:

1. The Gene & its Sequence
2. A Roadmap of where Coding Sequence & ALL switches located (What's the road map?)
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 → human protein in plant

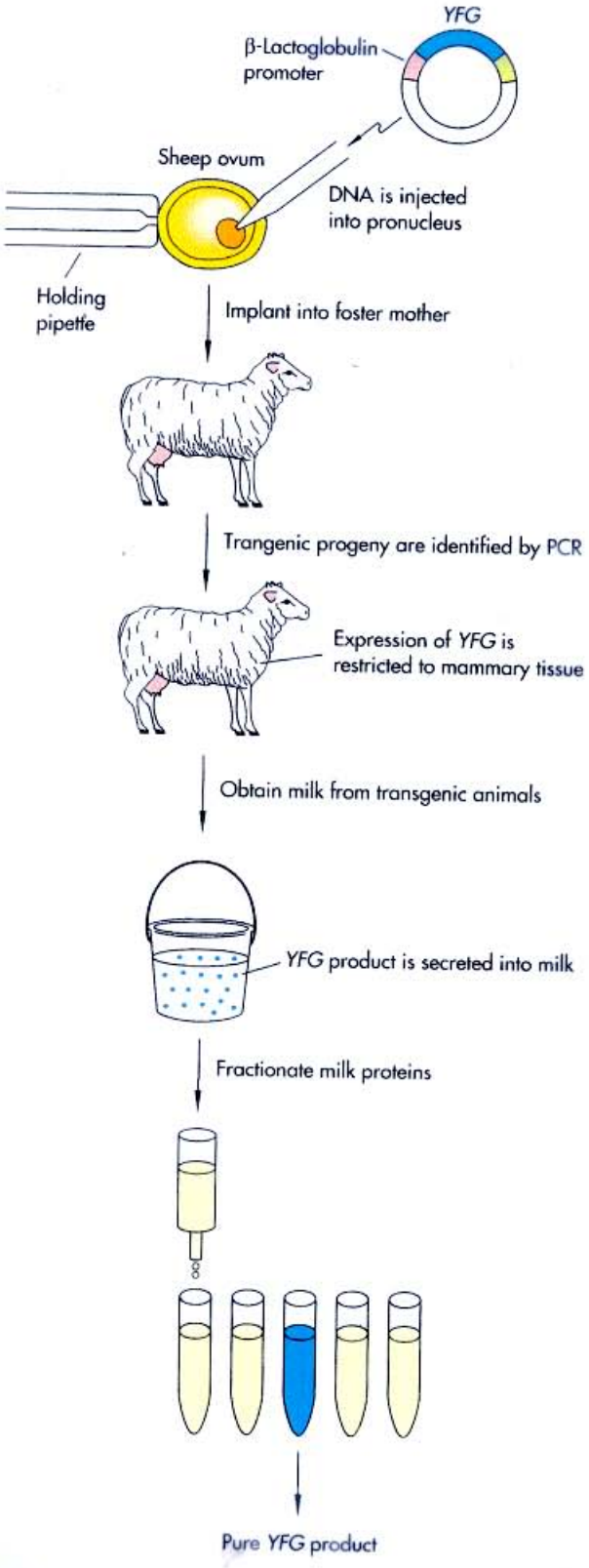
T) It's that easy Because living cells use SAME overall genetic processes!

P) There are NO LIMITS! The Limit?

HOW TO ENGINEER GOAT MILK TO CONTAIN A HUMAN PROTEIN!

(2) PROMOTER = SWITCH FOR MAMMARY GLAND/UTER

(1) YFG = your favorite gene



QUESTIONS?

a. How Identify YFG in Sheep Genome?

b. How Engineer YFG to be active only in Mammary cells?

c. How CAN YFG be expressed in Goats?

d. How does this experiment show that DNA is the genetic Material & the code universal?

(3) Pure Human YFG Protein (e.g., drug)

CAN ANIMAL GENES BE ENGINEERED TO WORK in PLANTS?

PLANT switch

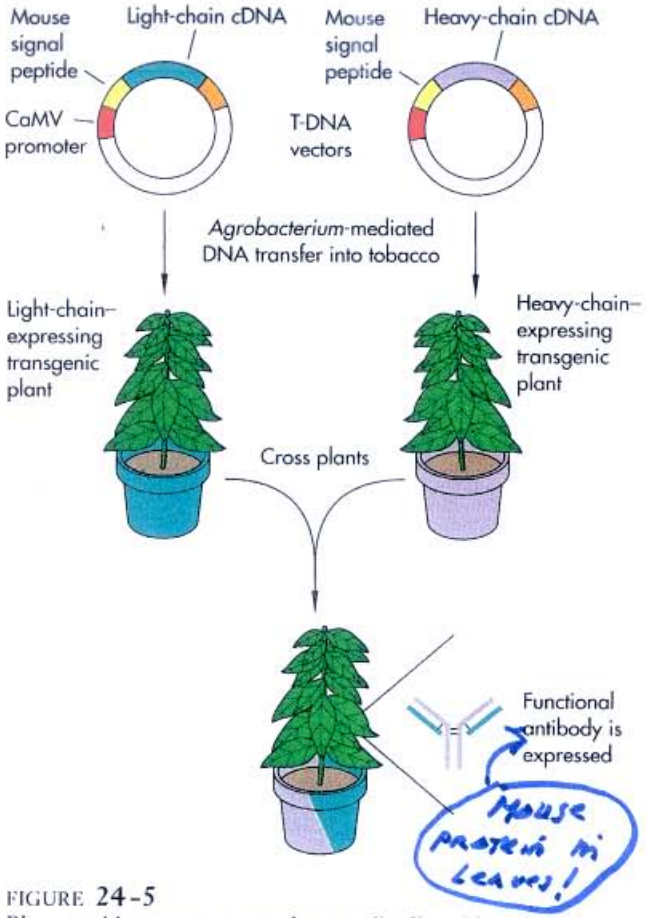


FIGURE 24-5
Plants as bioreactors to produce antibodies. Cloned cDNAs encoding the light and heavy chains from a mouse monoclonal antibody were ligated into separate T-DNA vectors and placed under control of a constitutive CaMV promoter. The plasmids were transferred separately into tobacco plants by *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.

QUESTIONS?

1. How identify Mouse Gene in Plant?
2. How Engineer Mouse Gene to be Active in Leaves?
3. What does this experiment tell us about genetic processes & genes = switches in plants and animals?

Remember the GloFish!

Yo! It's ALL in The Sequences!!

DNA, Gene, Switch, ori, mRNA, Protein!

NO HOCUS POCUS!

What does this IMPLY for BIOLOGY & Genetic Engineering!

ARE THERE ANY BIOLOGICAL LIMITS TO WHAT CAN BE GENETICALLY ENGINEERED?

HOW DO GENES WORK & WHAT ARE GENES IN CONTEXT OF....

↓

THINKING ABOUT THE CONSEQUENCES OF GMOS

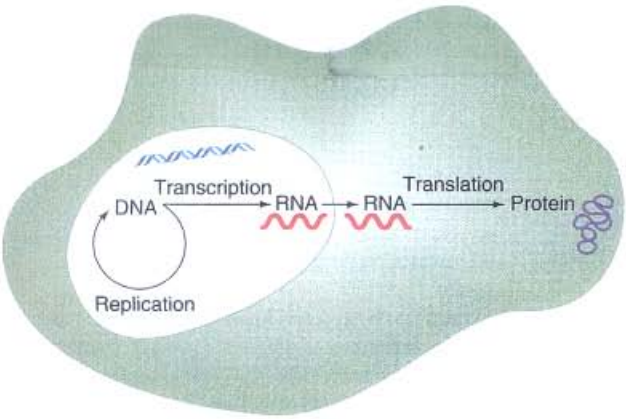


Figure 10-2 The three processes of information transfer: replication, transcription, and translation.

1. What is a Gene?
2. What is the Anatomy of a Gene?
3. How does the Gene Replicate?
4. How does the Gene direct Synthesis of a Protein?
5. Does the Gene work independently of other Genes?
6. What is the Sequence & Structure of the Protein?
7. How does it work in cell?
8. Does the Protein Structure imply any potential "Harm"
9. Does the Gene Change the organism? Fitness?

Need Science-based Questions & Science-based solutions

There's NO HOCUS POCUS all hypotheses are Testable!!