

HC 70A Winter 2006

Professor Bob Goldberg

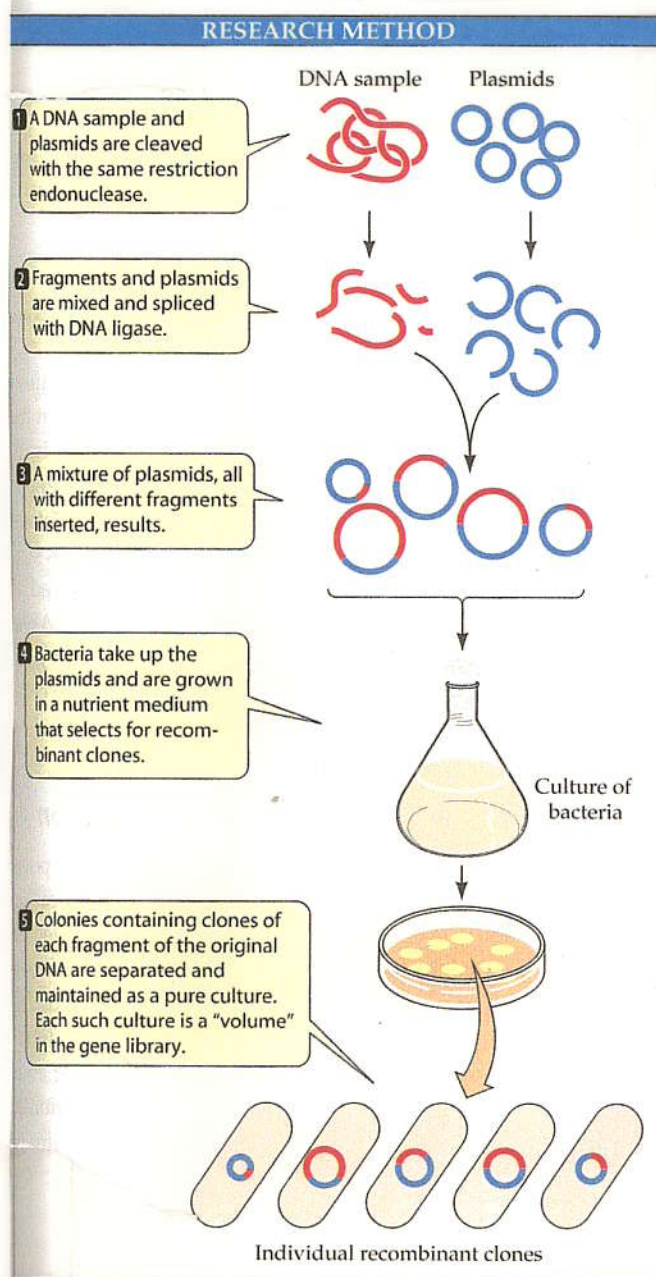
Lecture # 3

How Do Genes Work?

THEMES

- 1) Demonstrations - Bacterial Cloning "Call Student names" 1/24/06
- 2) Functions of Genes Revisited
- 3) Replication of DNA
- 4) PCR & How it Revolutionized Genetic Engineering
Stop 2/9/06
- 5) Kerry Mullis & PCR Film
- 6) Mutations & Genetic Diversity - Changing the Phenotype
- 7) Using Pedigrees to Follow Mutations
- 8) Genes → Proteins / An overview
- 9) The Genetic Code - Implications for Genetic Engineering
- 10) RNA vs. DNA
- 11) Proteins - Unique Structure → Unique Function
- 12) Gene Expression in Bacteria & Eukaryotes
- 13) Introns & RNA Splicing
- 14) Yes! - It's in the Sequences - Material for Genetic Engineering
- 15) Engineering Animals & Plants to Make Drugs - Yes!
- 16) Do there any Biological limit to Genetic Engineering?
- 17) Thinking about Genetic Engineering in Relation to How Genes work & what they are - Classical & Molecular!!
Stop 2/14/06

DEMONSTRATION - Bacteria "CLONING" EXPERIMENT

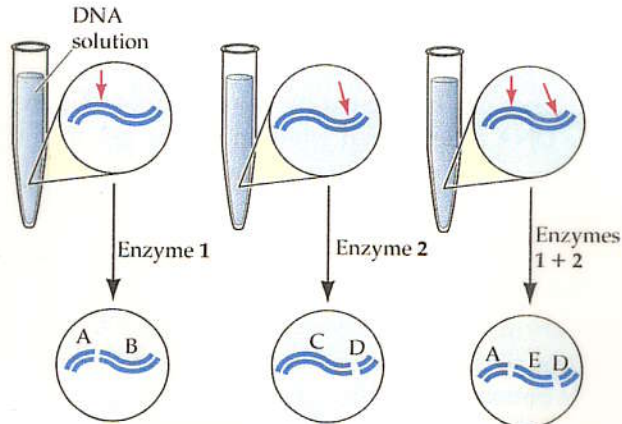
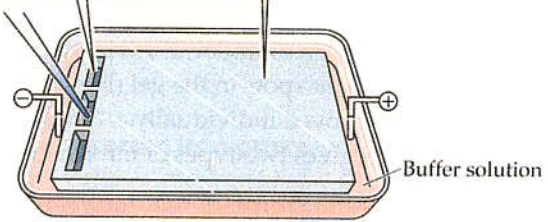


16.7 Constructing a Gene Library Human chromosomes are broken up into fragments of DNA using restriction enzymes. The fragments are inserted into vectors (plasmids are shown here) and taken up by host bacterial cells, each of which then harbors a single fragment of the human DNA. The information in the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

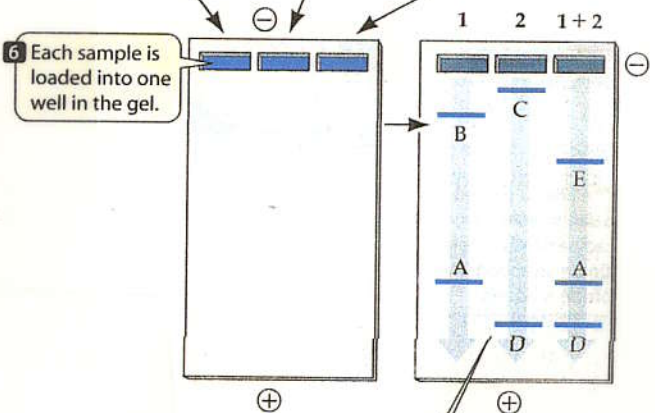
DEMONSTRATION - SEPARATING DNA FRAGMENTS By size using Gel Electrophoresis

RESEARCH METHOD

- 1** A gel is made up of agarose polymer suspended in a buffer. It sits in a chamber between two electrodes.
- 2** Depressions in the gel (wells) are filled with DNA solutions.



- 3** Restriction enzyme 1 cuts the DNA once, resulting in fragments A and B.
- 4** Restriction enzyme 2 cuts the DNA once, at a different restriction sequence.
- 5** If both restriction enzymes are used, two cuts are made in the DNA.



6 Each sample is loaded into one well in the gel.

7 As fragments of DNA move toward the positive electrode, shorter fragments move faster (and therefore farther) than longer fragments.

How visualize the DNA?

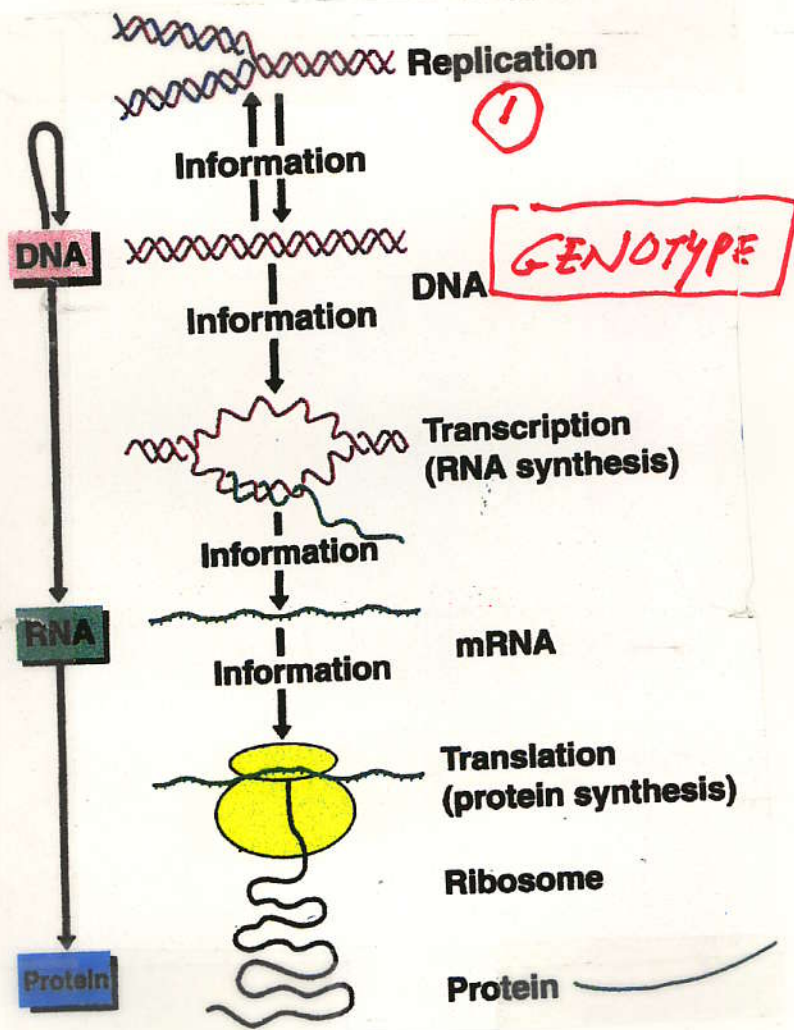


16.2 Separating Fragments of DNA by Gel Electrophoresis
A mixture of DNA fragments is placed in a gel and an electric field is applied across the gel. The negatively charged DNA moves toward the positive end of the field, with smaller molecules moving faster than larger ones. When the electric power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

FILM - KERRY MULLIS
AND PCR

HOW DO GENES WORK?

①
Genome Replication



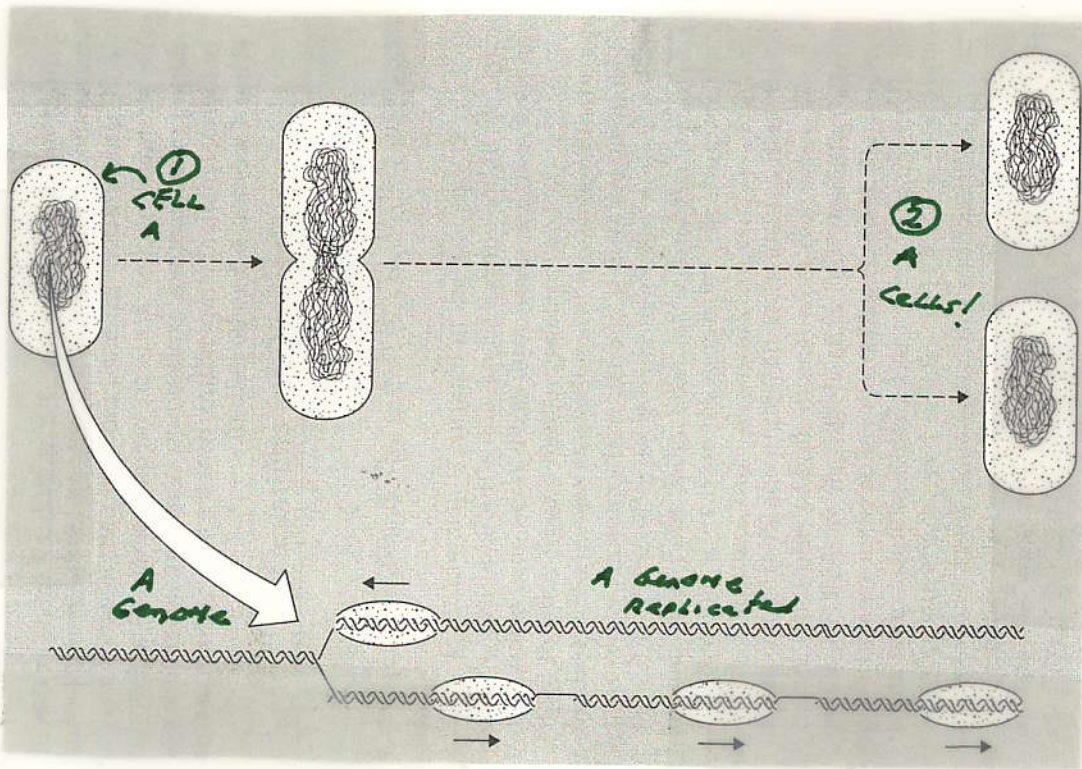
②
Gene Activity
↓
Protein
↓
Function
↓
Phenotype

A Gene is NOT Expressed unless A **FUNCTIONAL** Protein Produced!

→ **PHENOTYPE**

①

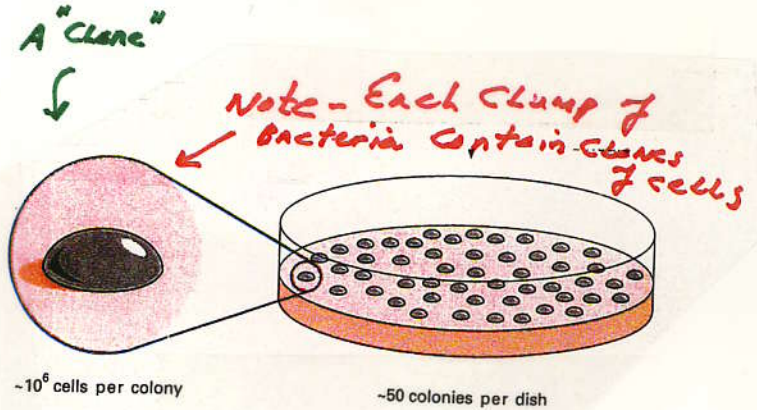
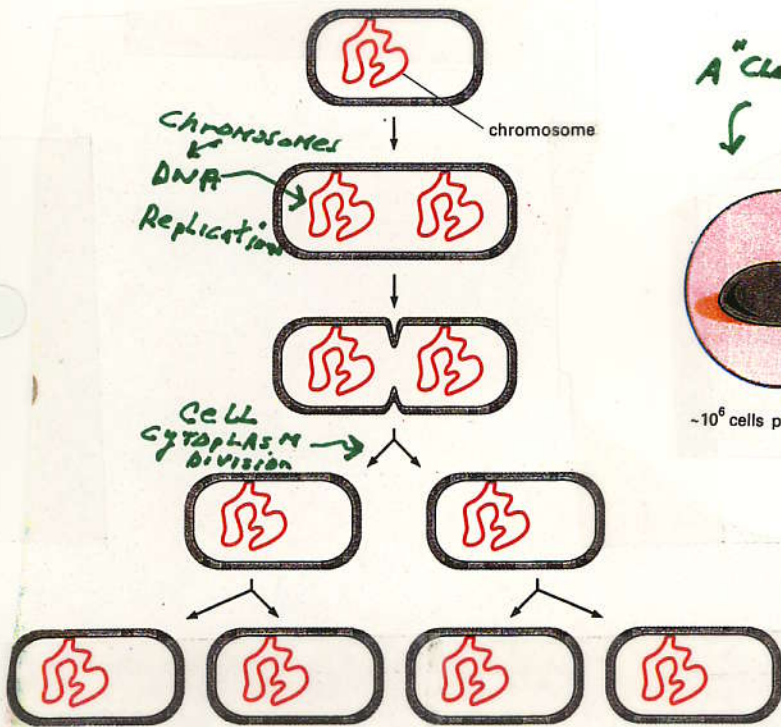
HOW ARE GENES REPLICATED EACH CELL GENERATION?



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 BACTERIAL colony contains many copies of SAME CELL or clones which are genetically identical!

EACH DAUGHTER CELL CONTAINS THE SAME COLLECTION OF GENES

MAJOR PROPERTIES OF GENETIC MATERIAL
Replication + stability

CLONES!

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

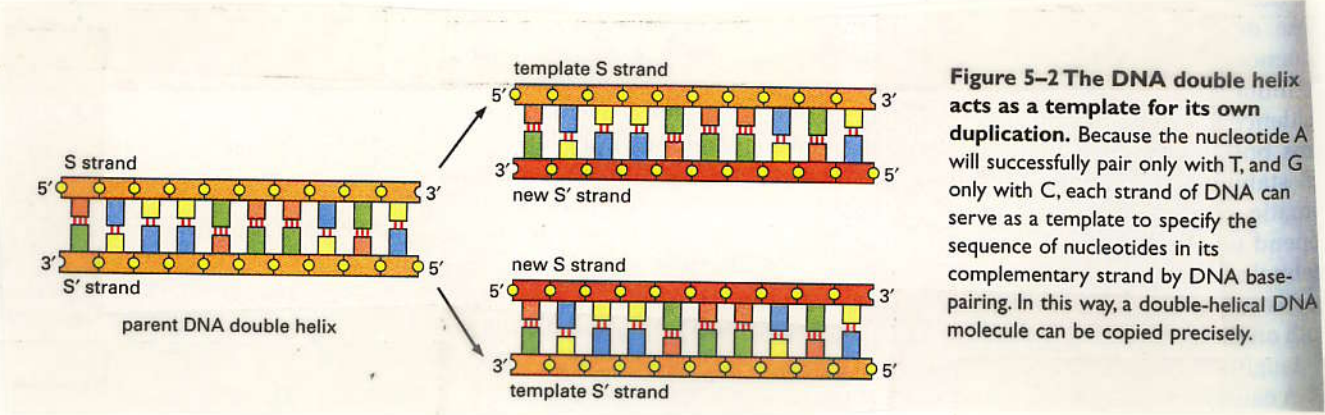


Figure 5-2 The DNA double helix acts as a template for its own duplication. Because the nucleotide A will successfully pair only with T, and G only with C, each strand of DNA can serve as a template to specify the sequence of nucleotides in its complementary strand by DNA base-pairing. In this way, a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

HOW DOES THAT OCCUR?
PROPERTY OF THE DNA MOLECULE

NOTE → SEQUENCE & POLARITY

DNA REPLICATION OCCURS SEMI-CONSERVATIVELY

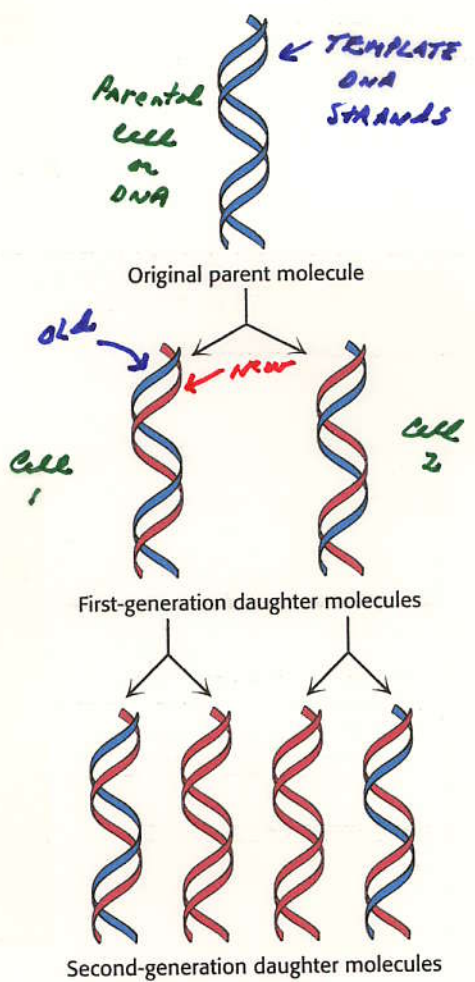


FIGURE 5.16 Diagram of semiconservative replication.

- 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

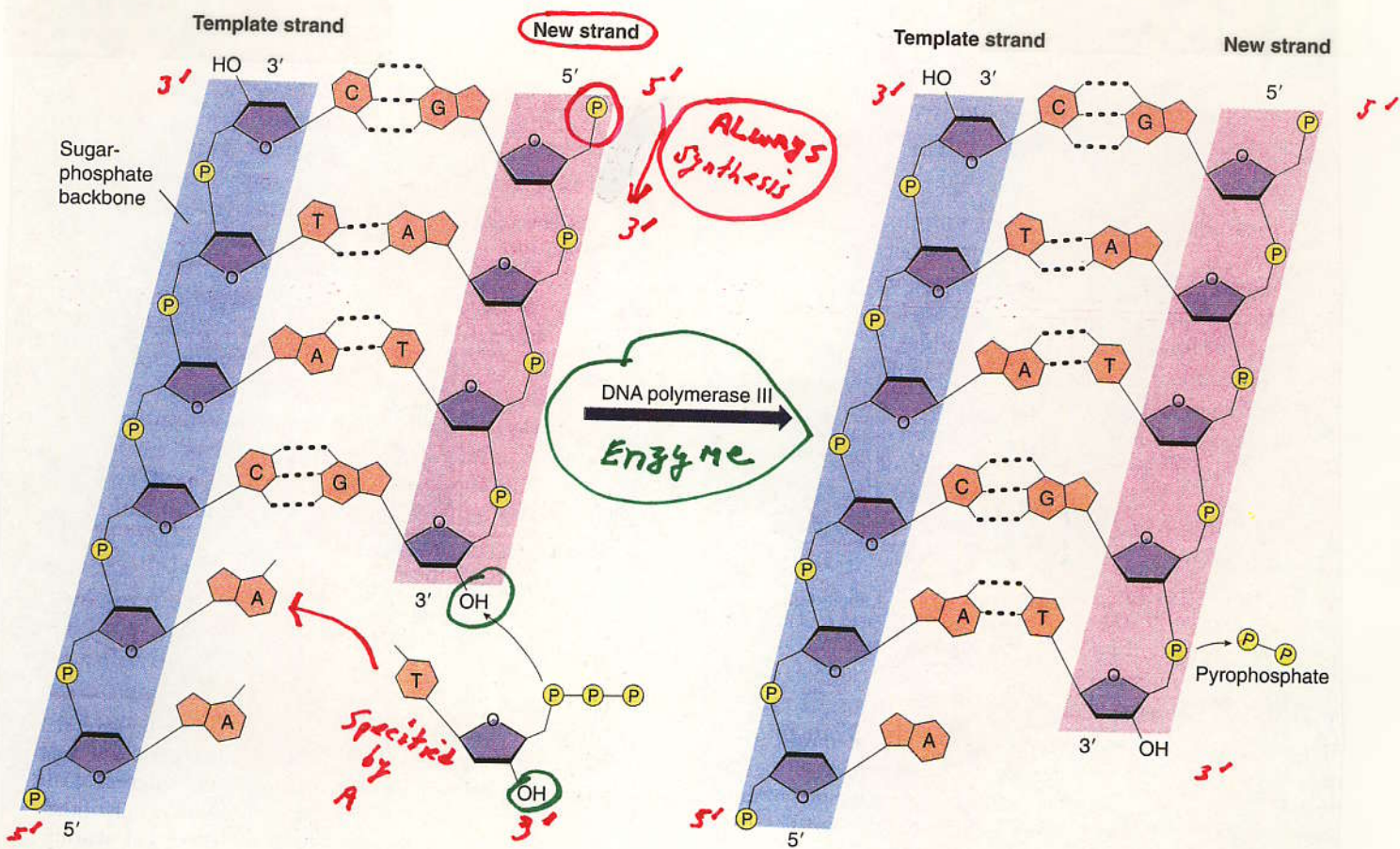


FIGURE 14.14 How nucleotides are added in DNA replication. DNA polymerase III, along with other enzymes, catalyzes the addition of nucleotides to the growing complementary strand of DNA. When a nucleotide is added, two of its phosphates are lost as pyrophosphate.

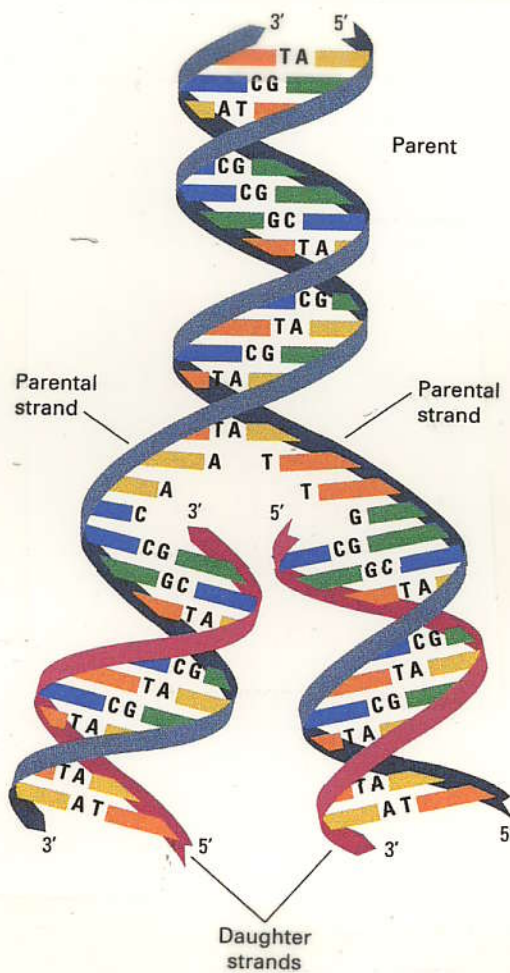
SEQUENCE IS SPECIFIED BY COMPLEMENTARY BASES

NOTE 5' (P) + 3' (OH)

(6)

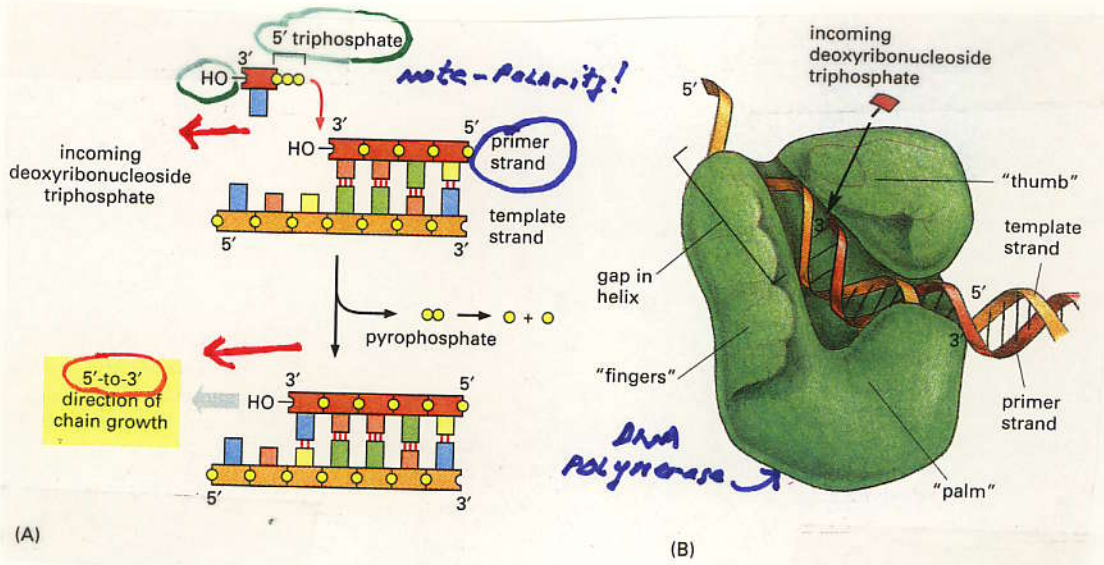
POLARITY II SEQUENCE

THE DNA SEQUENCE IS MAINTAINED
GENERATION TO GENERATION



THE DNA SEQUENCE "LIVES"
FOREVER!

DNA REPLICATION REQUIRES AN ENZYME - DNA POLYMERASE



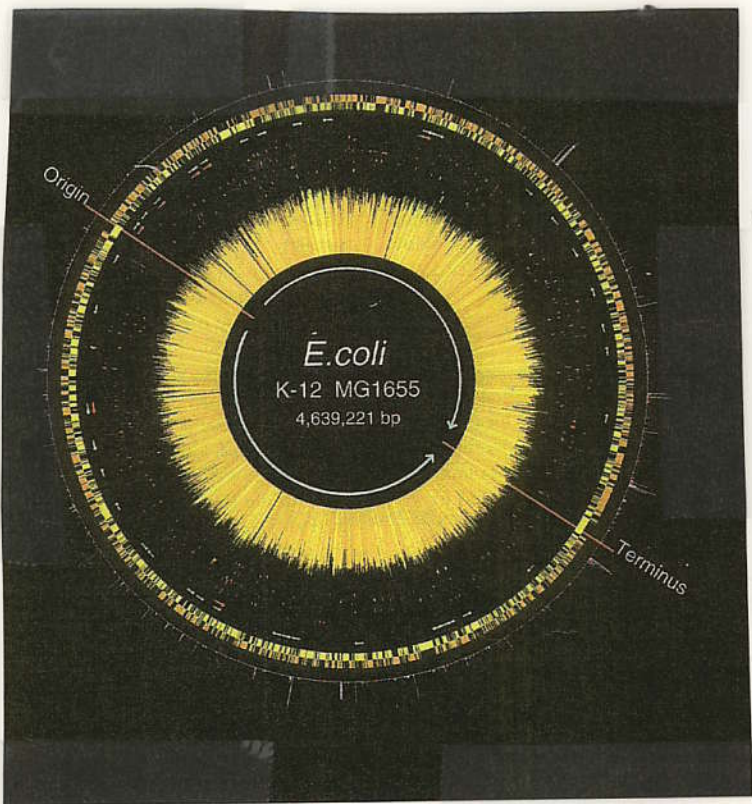
DNA POLYMERASE CATALYZES PHOSPHODIESTER BONDS AND "COPIES" THE TEMPLATE

NUCLEOTIDES ARE ALSO NEEDED

NEED A PRIMER + TEMPLATE + DNA POLYMERASE + NTS

ORI

DNA Replication Starts at the ORIGIN OF REPLICATION



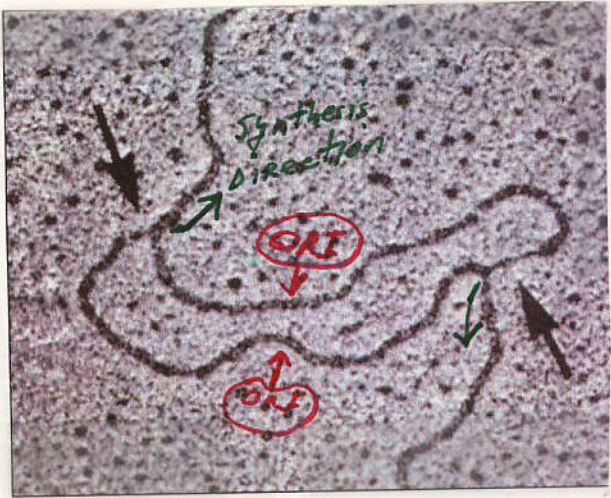
DNA Replication is bidirectional from the ori!!



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

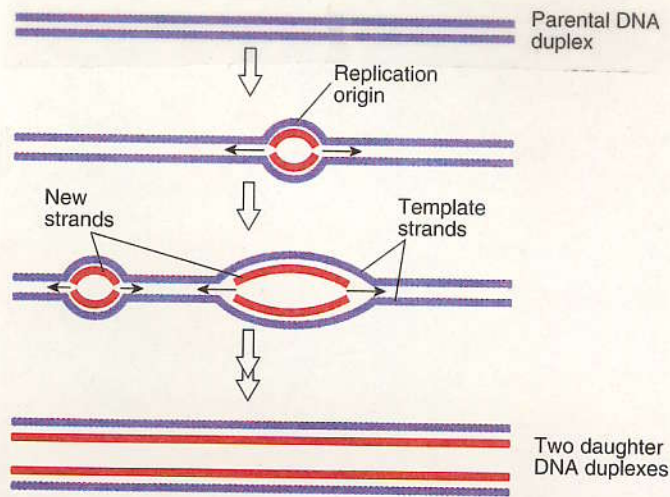


FIGURE 14.13
Origins of replication. At a site called the replication origin, the DNA duplex opens to create two separate strands, each of which can be used as a template for a new strand. Eukaryotic DNA has multiple origins of replication.

CONCEPT!



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

e.g., bacteria insect gene
 ↳ use plant ori

The ORIGIN OF REPLICATION IS
A SPECIFIC SEQUENCE

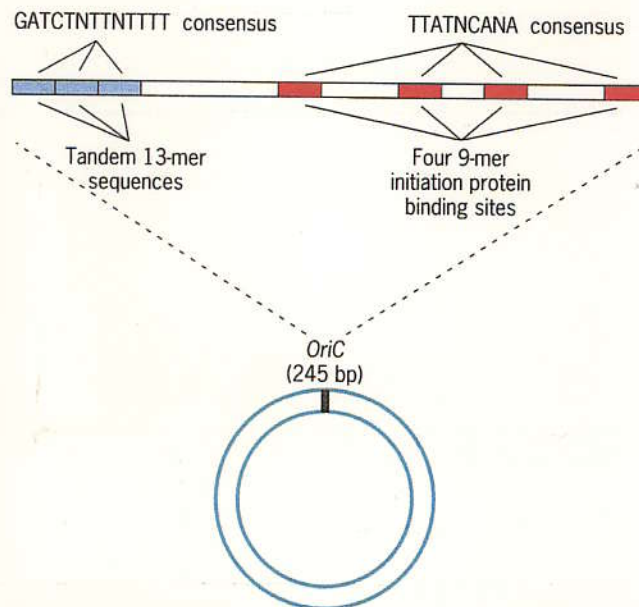


Figure 11.6 Structure of *OriC*, the single origin of replication in the *E. coli* chromosome.

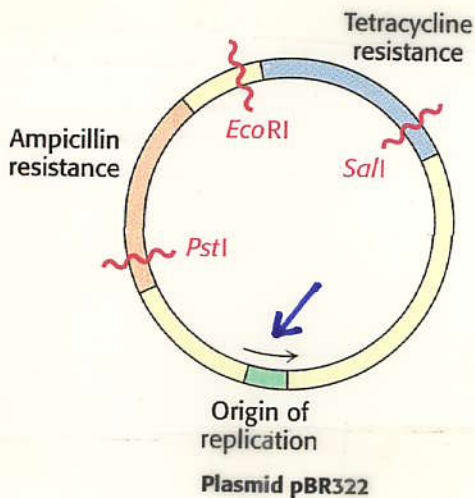
How CLONE
AN ORIGIN
OF
REPLICATION?

Specific
Sequence
What does this
mean for
Genetic
Engineering?

What is the significance for
Genetic Engineering?

CAN Replicating "Chromosomes"
BE MADE?

VECTORS ARE NEEDED TO REPLICATE GENES IN SPECIFIC CELLS



- 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.

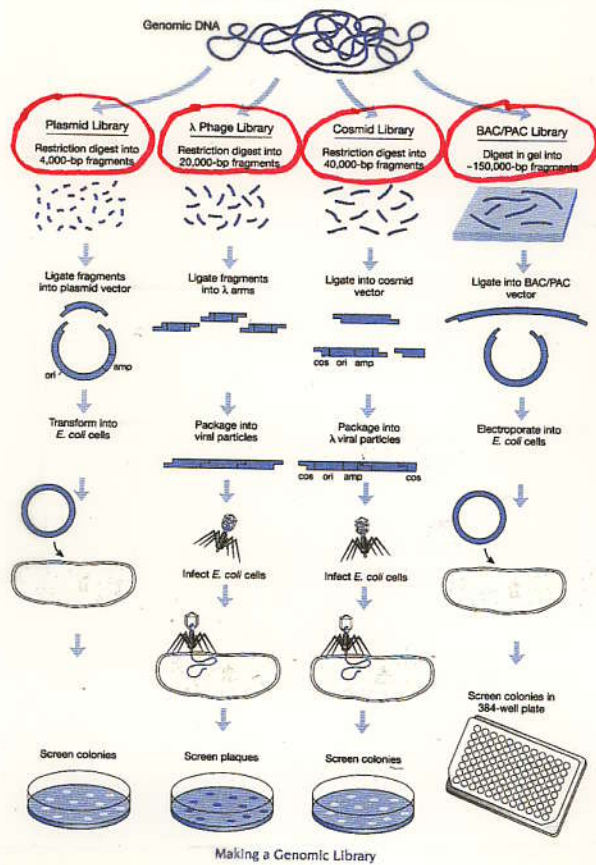
Yo! It's in the sequence = function

∴
Vectors can be engineered!
ORIs can be cloned/synthesized!

MODULAR

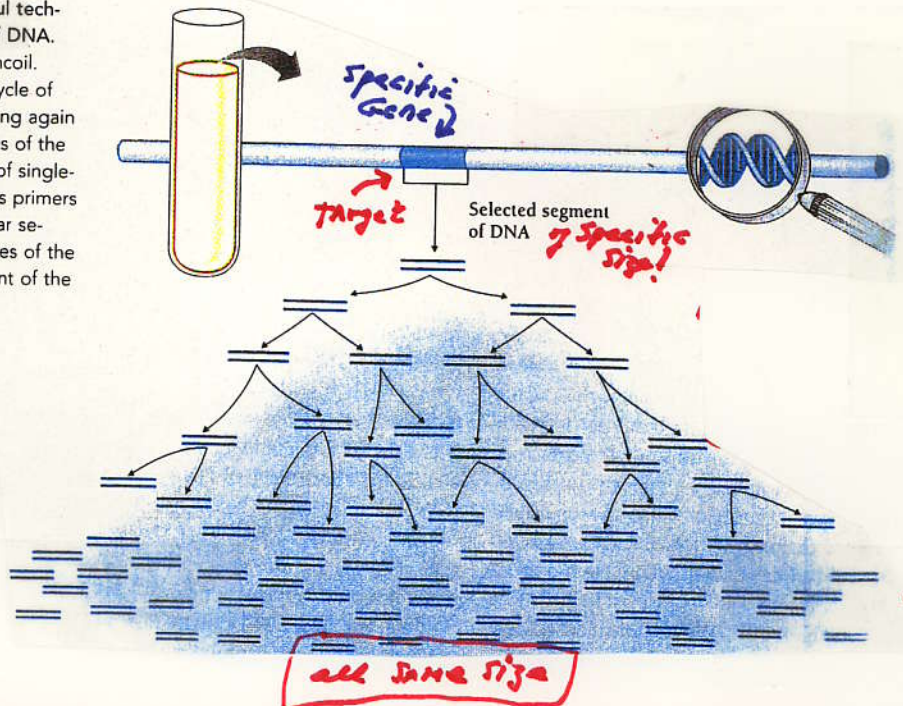
⑬

There ARE MANY Types of Vectors
ALL REQUIRE AN ORI



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!

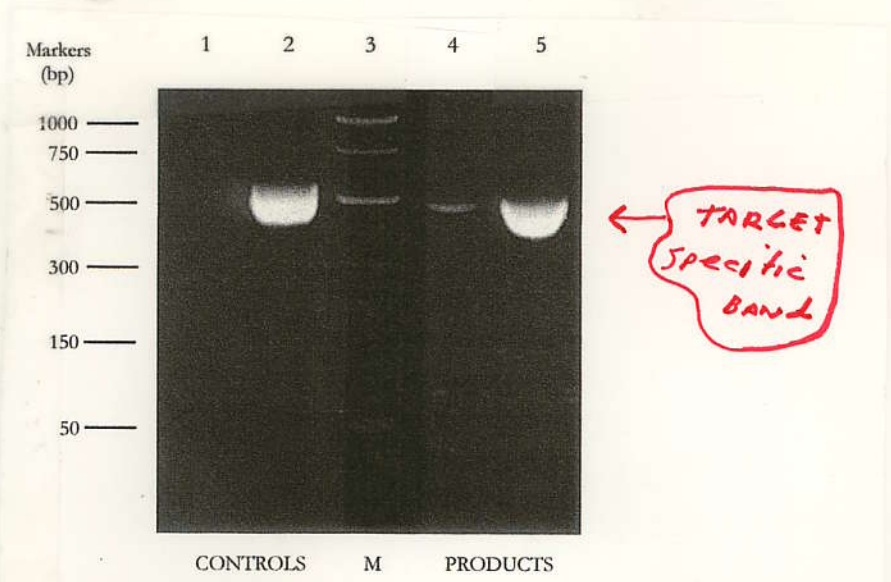
NO CLONING NEEDED!

but need sequence!

∴ have to clone "gene" first

DNA Polymerase
↑ ↑ ↑

USING GEL ELECTROPHORESIS TO VISUALIZE PCR PRODUCTS



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

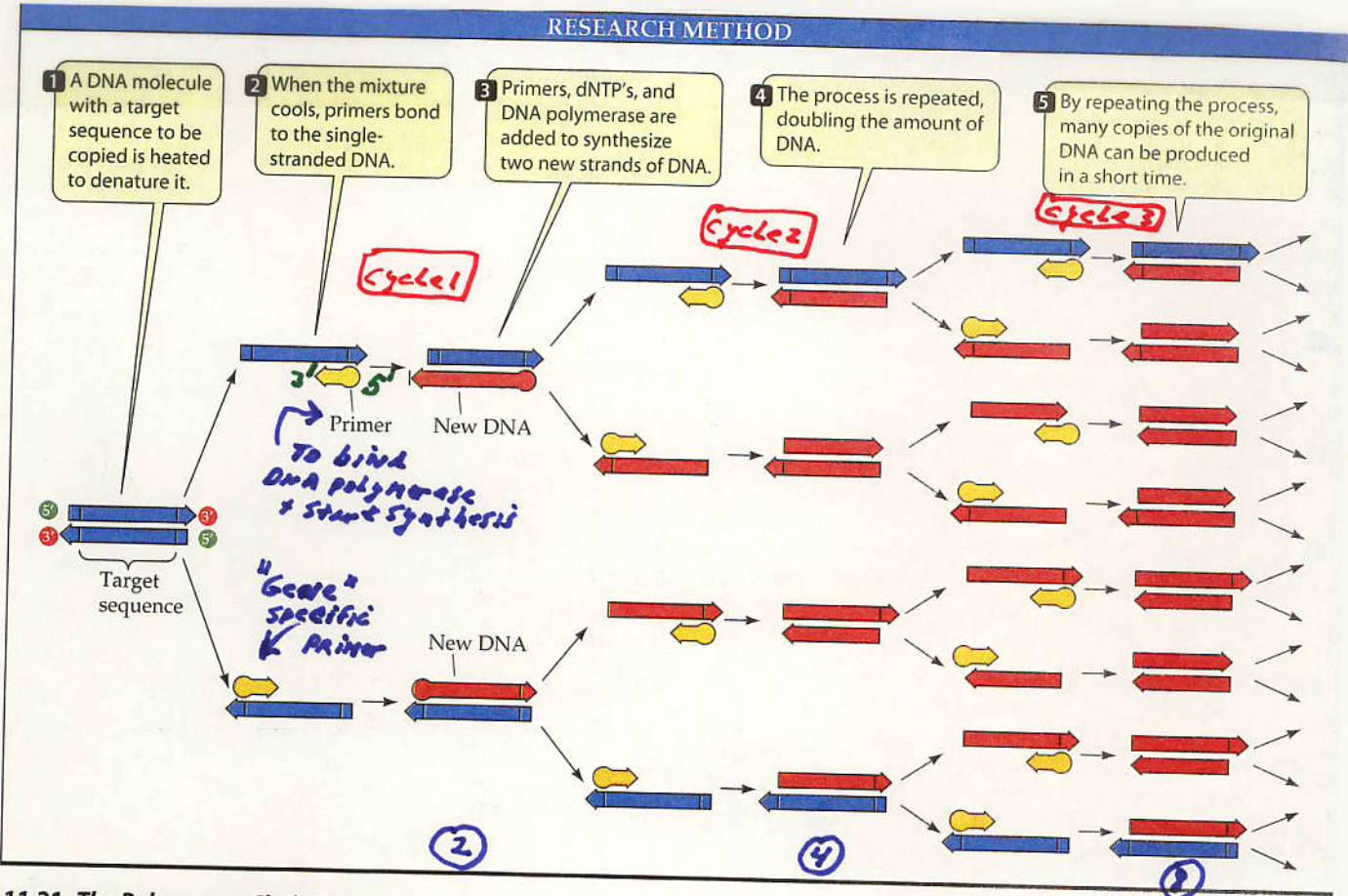
Need as little as ONE Molecule of DNA!

CAN Make an ∞ amount of DNA!

BUT - Need sequence of DNA segment to be used for PCR! *is have to clone + sequence first!*

REVOLUTIONIZED MANIPULATING DNA

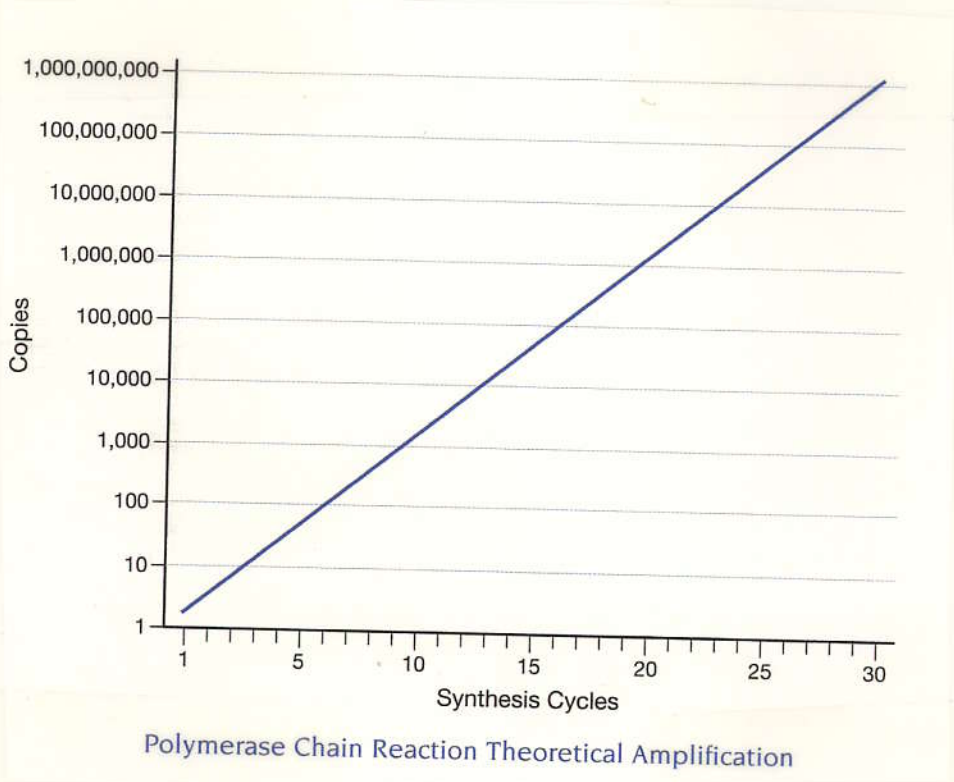
PCR IS A CYCLIC PROCESS OF DNA REPLICATION



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 $\infty = \# \text{ cycles}$

MAKING 2 AMOUNTS of DNA
USING PCR



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: 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 (Pre-Implantation Genetic Testing)
- ② Sperm Genotyping Diagnosis
- 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

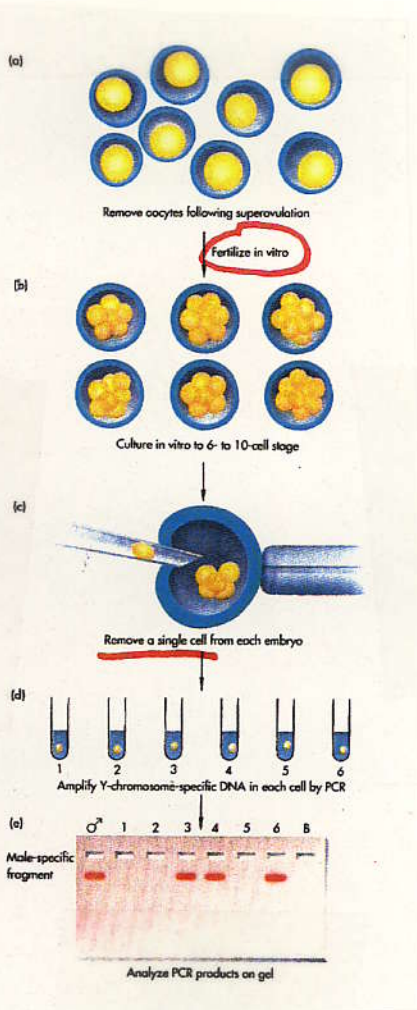
Pre-implantation

Genetic

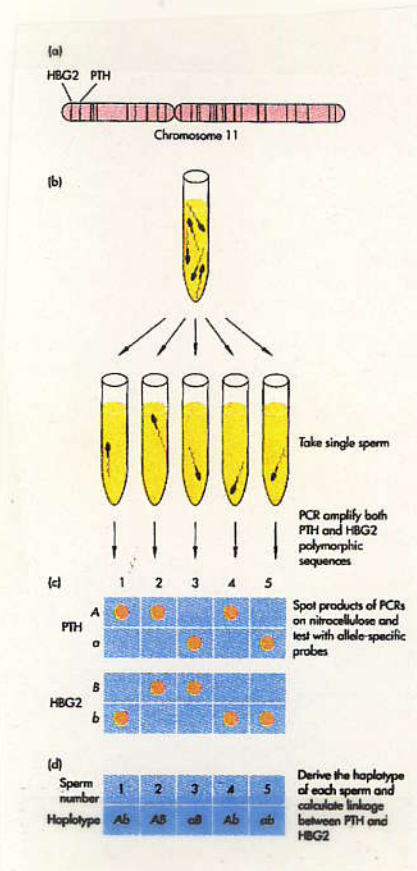
Diagnosis

TOTIPOTENCY Stem cells

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



An Adventure
65 Million Years In The Making.

19a

USING PCR TO DETECT GENES IN ANCIENT DNA

Ancient DNA Milestones

These extinct organisms have yielded meaningful genetic sequences.



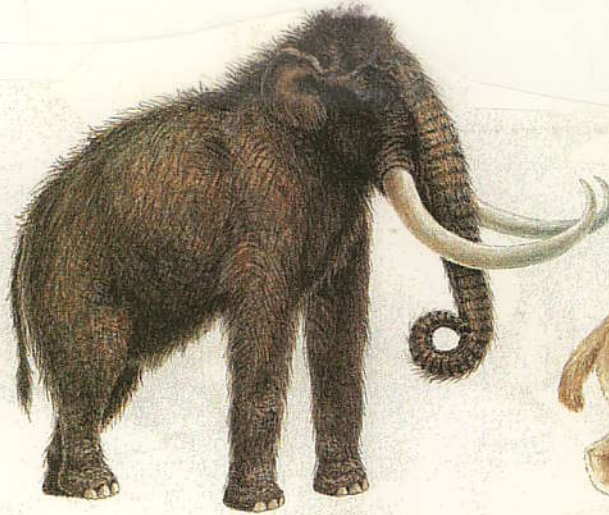
AMBER INSECTS

40 MILLION YEARS OLD



FOSSIL LEAVES

17 MILLION



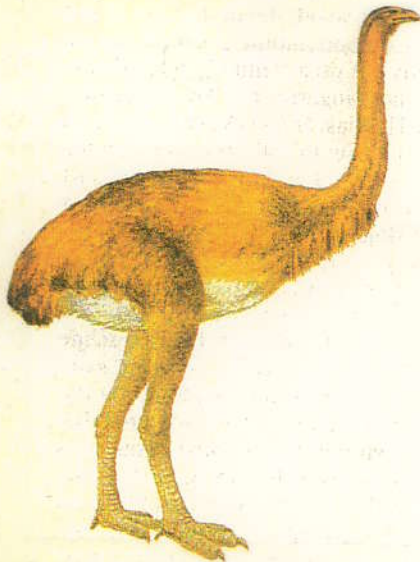
MAMMOTH

40,000



SMILODON

13,000



MOA

4,300



QUAGGA

140



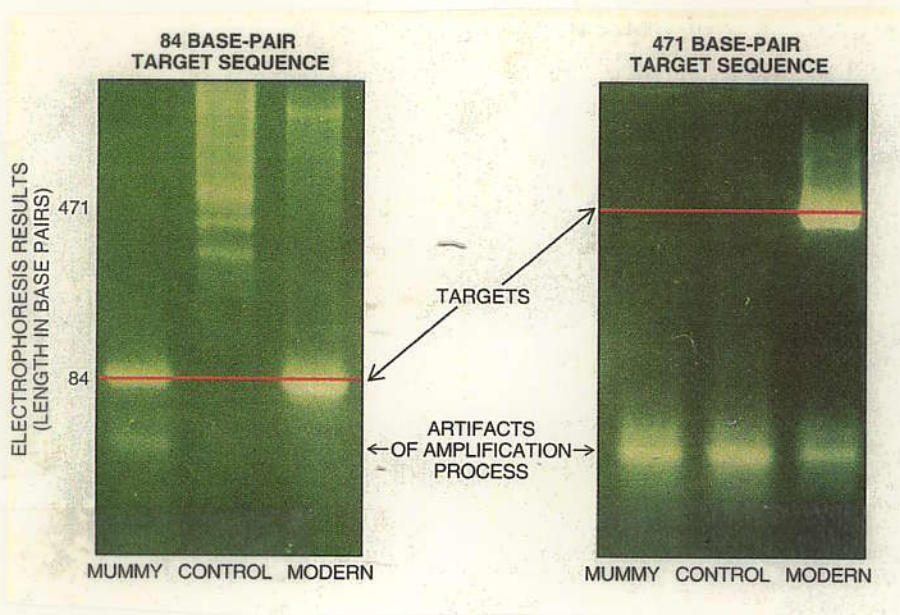
THYLACINE

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

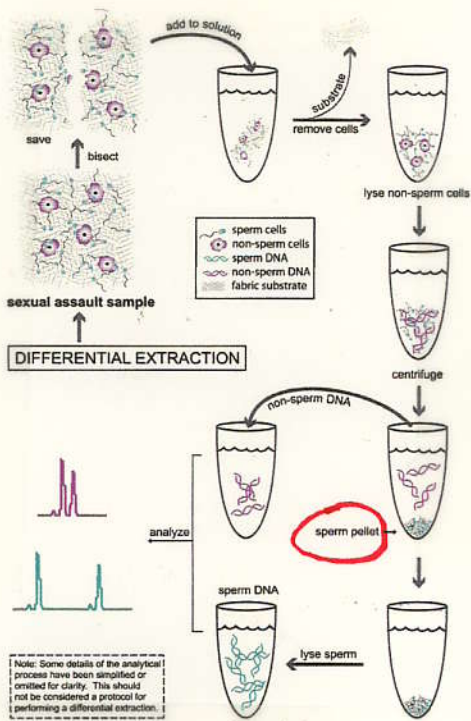
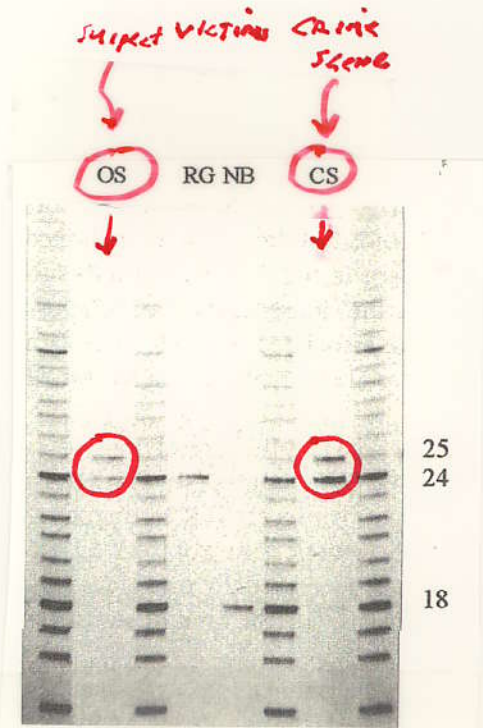


Plate 4 Differential extraction.



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

OS = SUSPECT
 CS = CRIME SCENE
 RG + NB = VICTIMS

DNA DOESN'T "LIE"!!

ABC NEWS
NIGHTLINE



ABC NEWS
NIGHTLINE

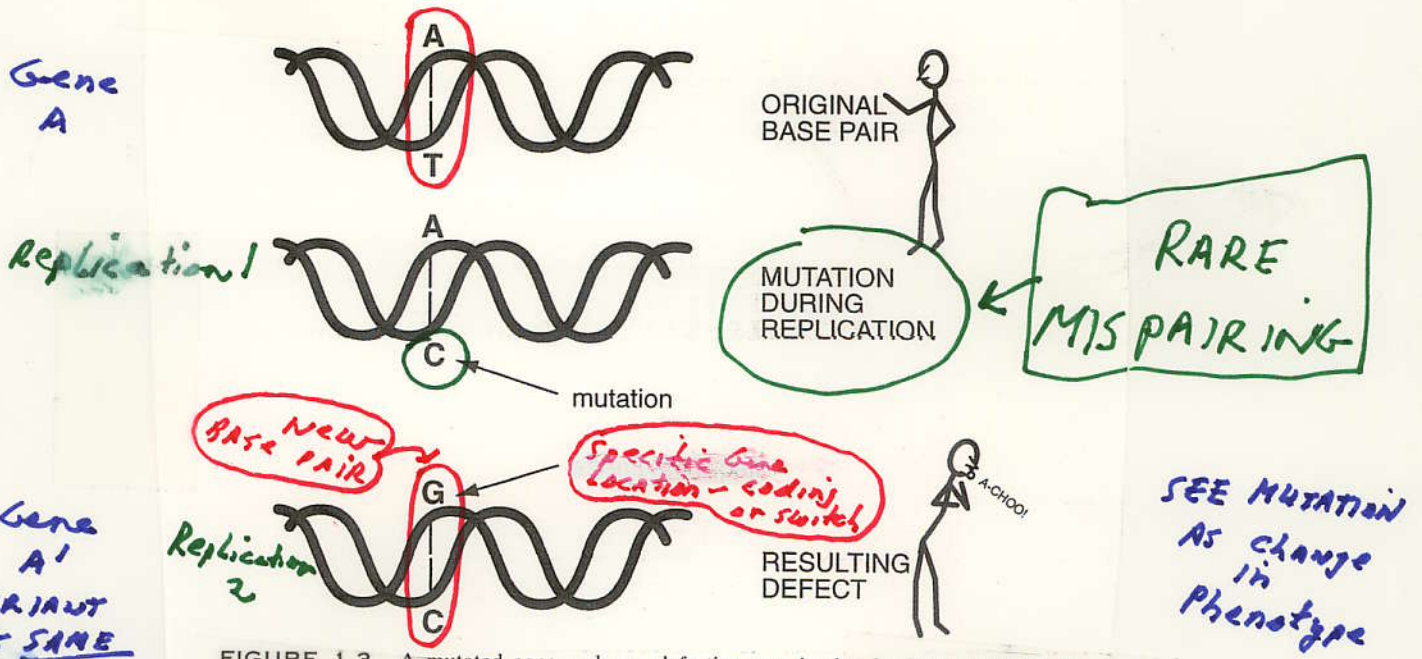
NOBEL
LAUREATE
BIOCHEMIST
KARY MULLIS

022104



An MPI Home Video Presentation
of an ABCNEWS Production.

DNA REPLICATION IS PRECISE BUT MISTAKES OCCUR!



Gene A
Variant of same Gene!

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

**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 zygote

② 2 genes per somatic cell

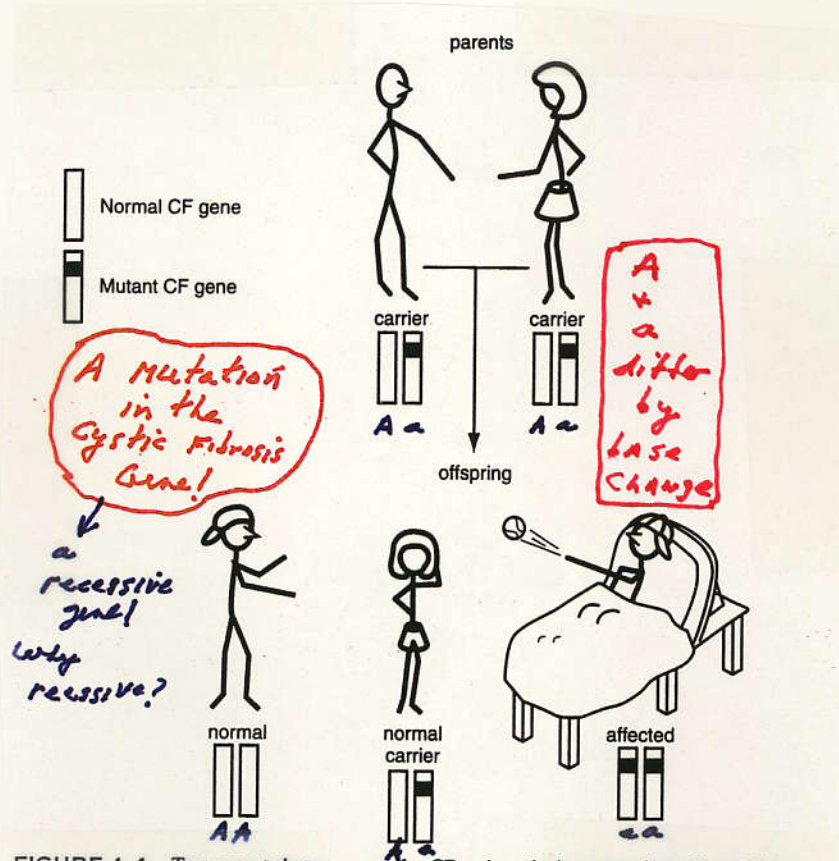


FIGURE 1.4 Two parents heterozygous for CF and producing normal or affected kids.

Follows Mendelian Rules

Eugenics

How Remove "aa" Allele from Population? Can you do this?

MARKERS

How FOLLOW INHERITANCE?
What Allows Disease to be Followed?

SELECTED FOR in populations - protection can be a founder mutation! 20

MUTATIONS in HUMAN Genes

- ① Different Mutations in Different Families in SAME Disease Gene

Hemophilia A

Need Markers for EACH Family

- ② SAME Mutation in Different Families

- "Hot Spot" - Dwarfism / FGFR3 or Achonroplasia
- Founder Mutation - Sickle Cell Anemia or β -globin gene

CAN Use SAME Marker for Most/ALL Families

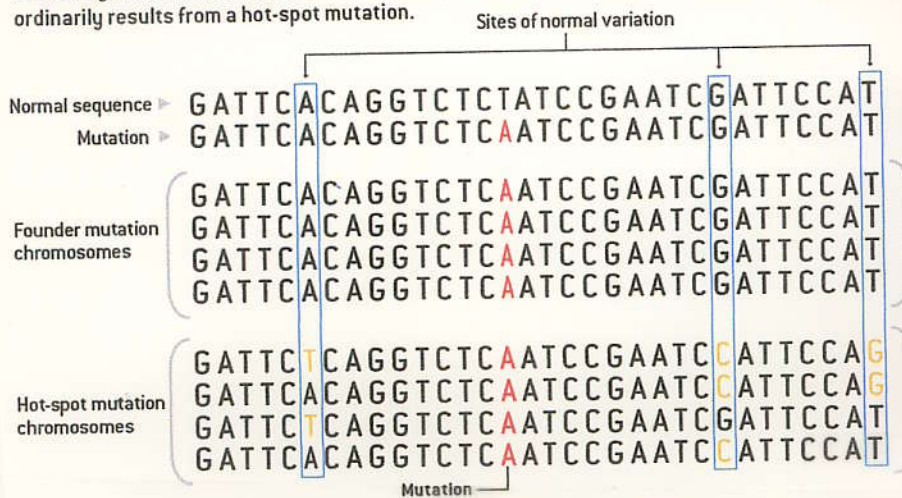
SELECTION in SPECIFIC POPULATION FOR founder Mutations & SIMILAR "New-comers"

AN OLD ORIGINAL VS. NUMEROUS NEWCOMERS

If a group of patients with the same disease all had the same mutation at a given spot in their DNA, how could physicians know whether they were looking at a hot spot or a founder mutation? They could tell by analyzing the surrounding DNA sequences.

Suppose that in all patients the code at one spot changed from a T to an A (red, below). If A were a founder mutation, the surrounding sequences in all patients would be identical—the patients would have inherited the full sequence from the same distant ancestor. But if A were a hot-spot mutation, having occurred spontaneously at a place where DNA is prone to error, the surrounding sequences would also show other differences (gold) at sites where DNA codes normally tend to vary without causing disease.

Sickle cell disease, marked by misshapen red blood cells (top photograph), is usually caused by a founder mutation. Achondroplasia, a form of human dwarfism (bottom photograph), ordinarily results from a hot-spot mutation.

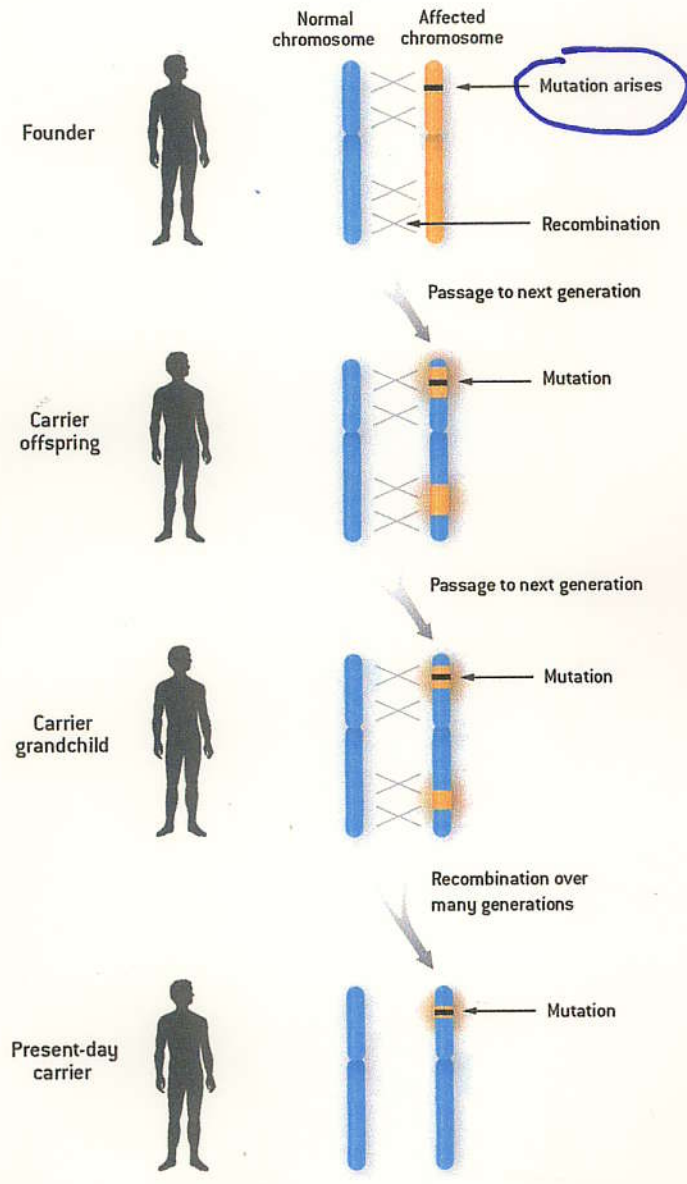


BALANCING SELECTION keeps a potentially deleterious gene circulating. In regions with malaria, spread by mosquitoes, having a single copy of a mutation in the hemoglobin gene is protective. Individuals with that mutation have higher survival rates. But those who inherit two copies of the mutation suffer from sickle cell disease and have lower survival rates. The competing forces lead to a stable level of the sickle cell mutation in the population.

Founder Mutations Are Maintained By Selection

GETTING SHORTER WITH AGE

The uniquely identifiable chromosome region—the haplotype—that surrounds a founder mutation gets shorter over generations as chromosomes mix in a process called recombination. In this example, the yellow chromosome in the founder holds the founder mutation, and the blue chromosome comes from a normal parent. When the founder produces sperm or eggs, the two chromosomes exchange sections. Carrier offspring inherit a newly mixed chromosome that includes the mutation and other parts of the founder haplotype (yellow region). Chromosomal mixing over generations inevitably leads to a shortened haplotype.



Selected Founder Mutation

MUST HAVE Selection Pressure - Adaptive Significance or Cost!

Founder Mutations & Human Disease

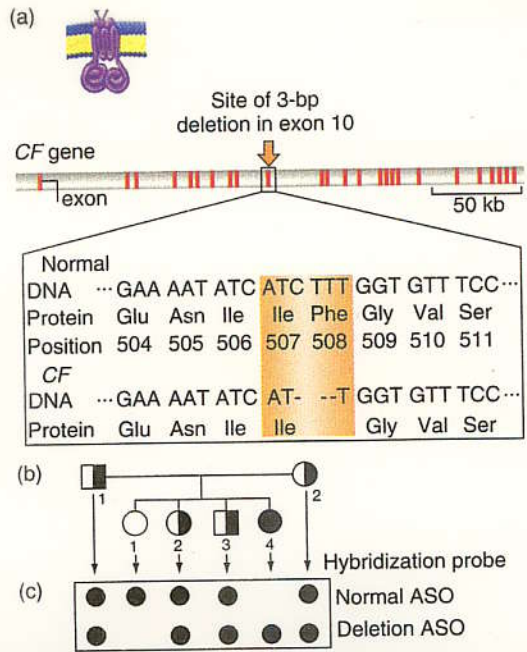
Noteworthy Founder Mutations

Affected gene	Condition	Mutation origin	Migration	Possible advantage of one copy
HFE	Iron overload	Far northwestern Europe	South and east across Europe	Protection from anemia
CFTR	Cystic fibrosis	Southeast Europe/Middle East	West and north across Europe	Protection from diarrhea
HbS	Sickle cell disease	Africa/Middle East	To New World	Protection from malaria
FVLeiden	Blood clots	Western Europe	Worldwide	Protection from sepsis
ALDH2	Alcohol toxicity	Far East Asia	North and west across Asia	Protection from alcoholism, possibly hepatitis B
LCT	Lactose tolerance	Asia	West and north across Eurasia	Allows consumption of milk from domesticated animals
GJB2	Deafness	Middle East	West and north across Europe	Unknown

CAN be use to TRACE Ancestry &
Found in Higher Frequency in Specific
Populations

Mutations CAN ALSO Arise in these genes in
different nucleotides spontaneously - May or
May not Cause SAME Disease - e.g., globin gene

70% of CF Alleles Have a 3 bp Deletion in Exon 3



Use this Marker
70% of cases

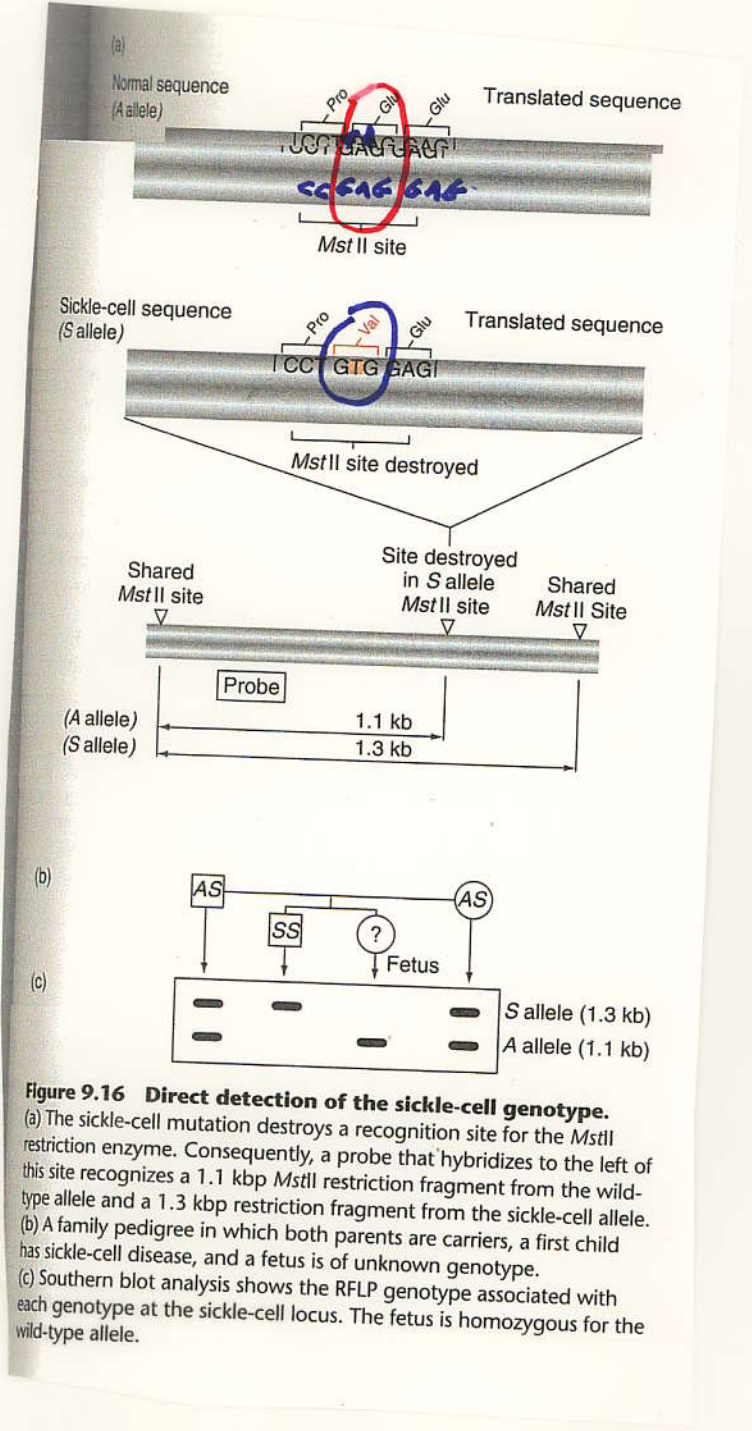
Figure 9.17 Direct detection of the most common cystic fibrosis mutation. The CF gene extends across 250,000 base pairs and is organized in 27 exons. It encodes a protein with 1480 amino acids. (a) The most common disease-causing mutation in the CF gene is a deletion of three bases in exon 10. It is possible to amplify the region containing the site of the most common mutation by PCR and divide the PCR products into two aliquots. You then blot the aliquots onto filter paper and probe with ASOs for the wild-type and mutant alleles. The ASO for the mutant allele differs by the absence of three bases from the ASO for the wild-type allele. (b) Pedigree of a family in which one daughter (child 4) has cystic fibrosis. (c) Analysis of the results of an ASO hybridization test provide direct information on the CF genotype of all family members.

30% of CF Alleles Due over 600 Different Mutations!

Use Marker Linked to Gene if Possible

21e

100% of sickle cell Alleles
Due to same Mutation



use this
marker -
Loss of
MstII site

Figure 9.16 Direct detection of the sickle-cell genotype.
 (a) The sickle-cell mutation destroys a recognition site for the *MstII* restriction enzyme. Consequently, a probe that hybridizes to the left of this site recognizes a 1.1 kbp *MstII* restriction fragment from the wild-type allele and a 1.3 kbp restriction fragment from the sickle-cell allele.
 (b) A family pedigree in which both parents are carriers, a first child has sickle-cell disease, and a fetus is of unknown genotype.
 (c) Southern blot analysis shows the RFLP genotype associated with each genotype at the sickle-cell locus. The fetus is homozygous for the wild-type allele.

21f

FUTURE - SEQUENCE ENTIRE
Genes Directly

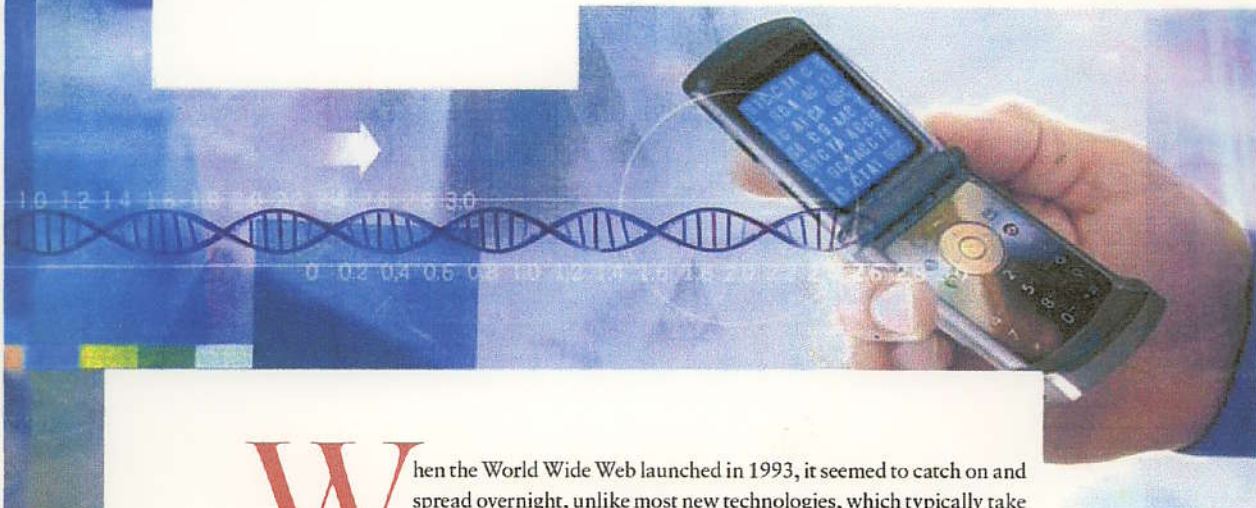
Personalized
Genome
article!

Genomes for ALL

Next-generation technologies that make reading DNA fast, cheap and widely accessible are coming in less than a decade.

Their potential to revolutionize research and bring about the era of truly personalized medicine means the time to start preparing is now

By George M. Church



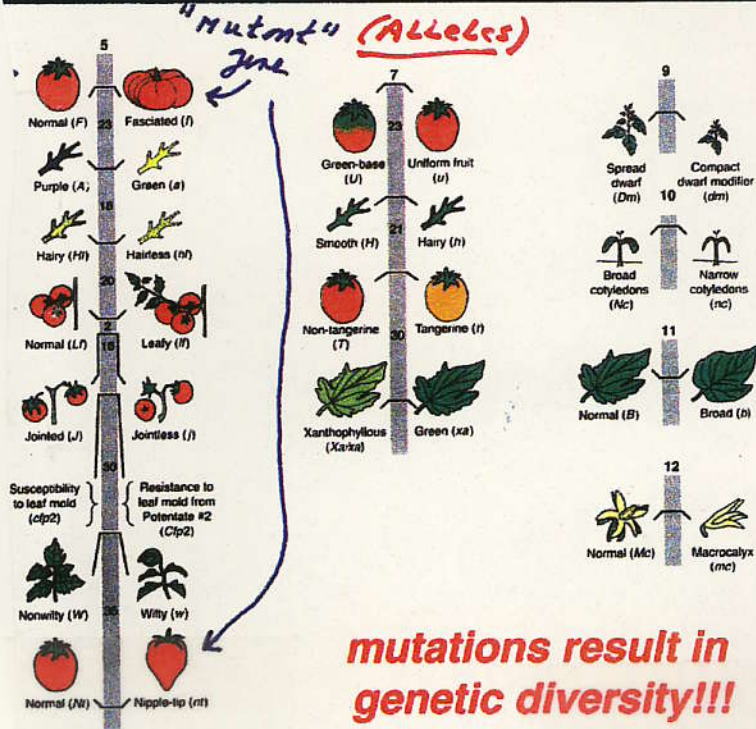
When the World Wide Web launched in 1993, it seemed to catch on and spread overnight, unlike most new technologies, which typically take at least a decade to move from first “proof of concept” to broad acceptance. But the Web did not really emerge in a single year. It built on infrastructure, including the construction of the Internet between 1965 and 1993, as well as a sudden recognition that resources, such as personal computers, had passed a critical threshold.

Vision and market forces also push the development and spread of new technologies. The space program, for example, started with a government vision, and only much later did military and civilian uses for satellites propel the industry to commercial viability. Looking forward to the next technological revolution, which may be in

219

MUTATIONS GIVE RISE TO GENETIC DIVERSITY

Alternative Forms of the Same Gene Lead to Genetic Diversity



mutations result in genetic diversity!!!

Change in DNA Sequence!

How know sequence changed?

Different forms of same gene



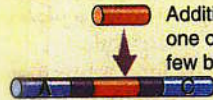


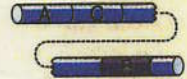
SNP - Single nucleotide polymorphism!

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 Substitution of one or a few bases 	B protein is inactive because changed amino acid disrupts function.
Insertion Addition of one or a few bases 	B protein is inactive because inserted material disrupts proper shape.
Deletion Loss of one or a few bases 	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 Change!

③ Move Gene or PART of Gene to New Location!

Switches Change!

LOSS OF FUNCTIONAL PROTEIN

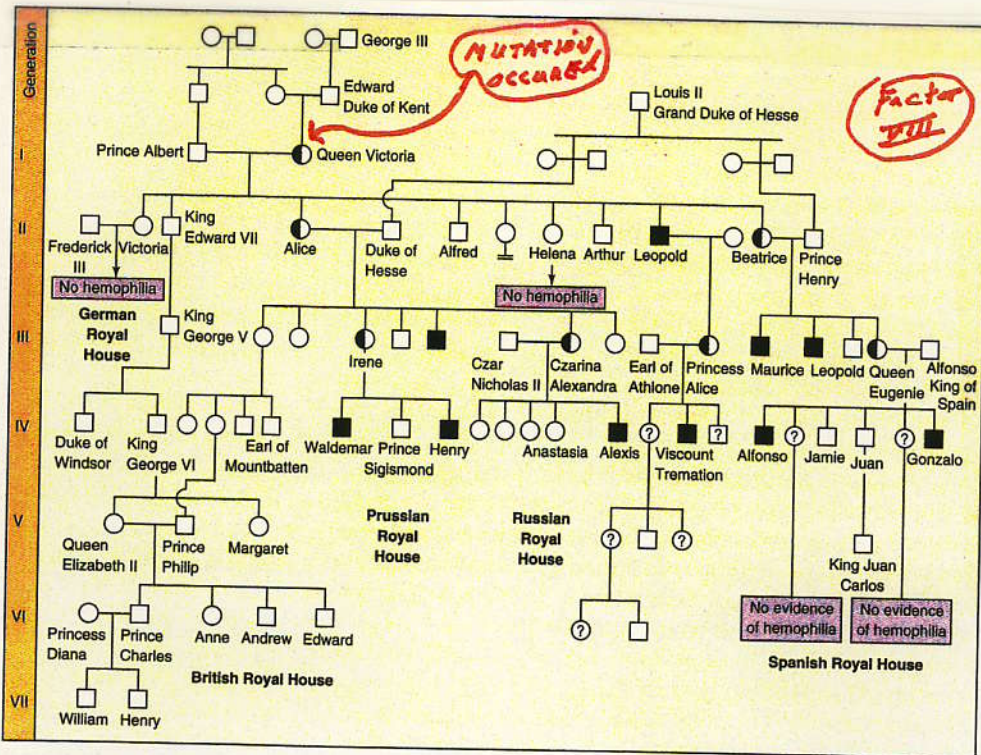
HUMAN GENETIC DISORDERS OCCUR AS
A RESULT OF MUTATIONS

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

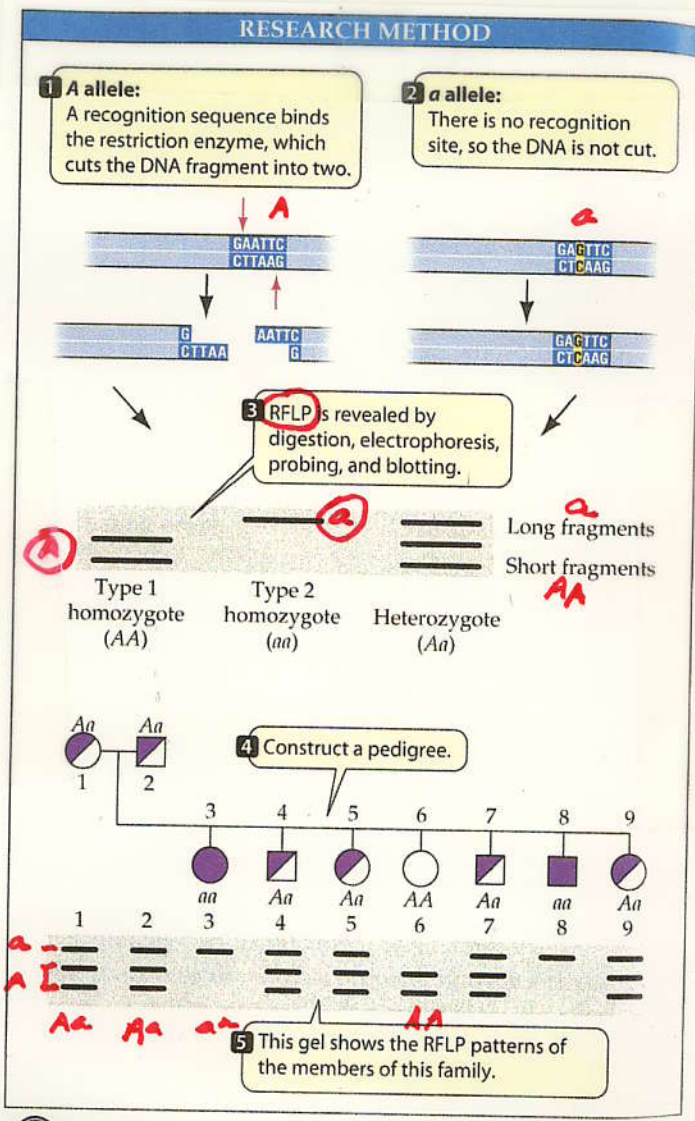


Hemophilia

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)



CUT - A

USE PCR + for OTHER Methods

a - NOT CUT

DNA Fingerprint
RFLP

Follow in Family



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.

Implications? Combined with Sequence of Human Genome, Embryo testing (PGD), + PCR ?!!!

HOW DOES A GENE LEAD TO A PHENOTYPE?

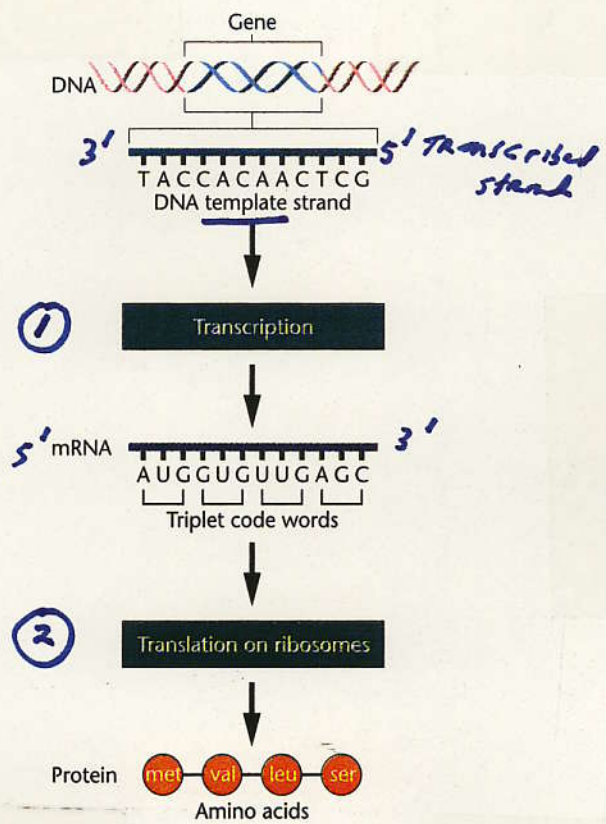


FIGURE 13.1 An overview of the concept of the flow of genetic information encoded in DNA to messenger RNA to protein.

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

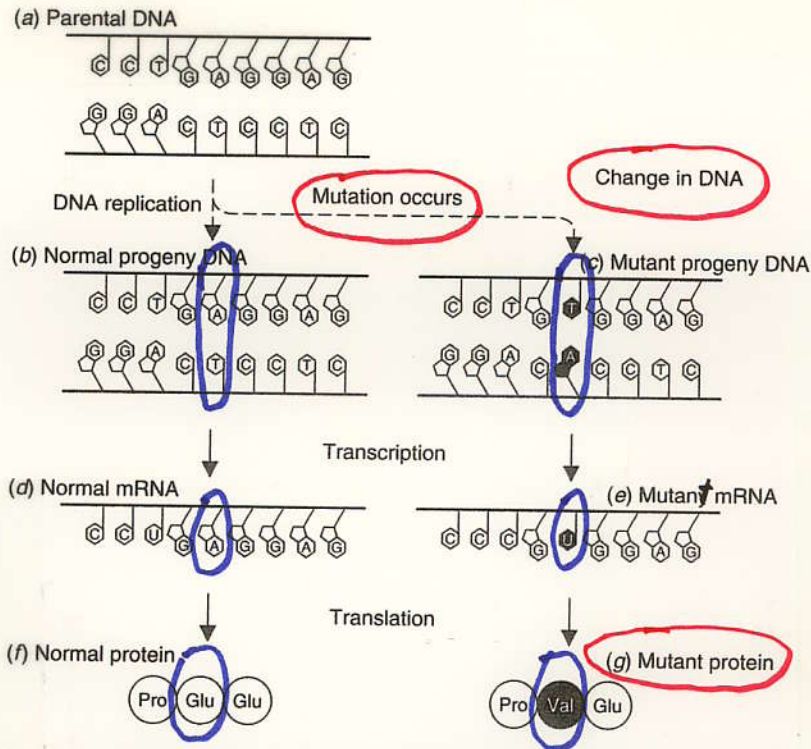
Genetic Code on mRNA TRANSLATED to protein sequence

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

KNOW SEQUENCE
 KNOW PROTEIN

↳ ENGINEER NEW PROTEINS

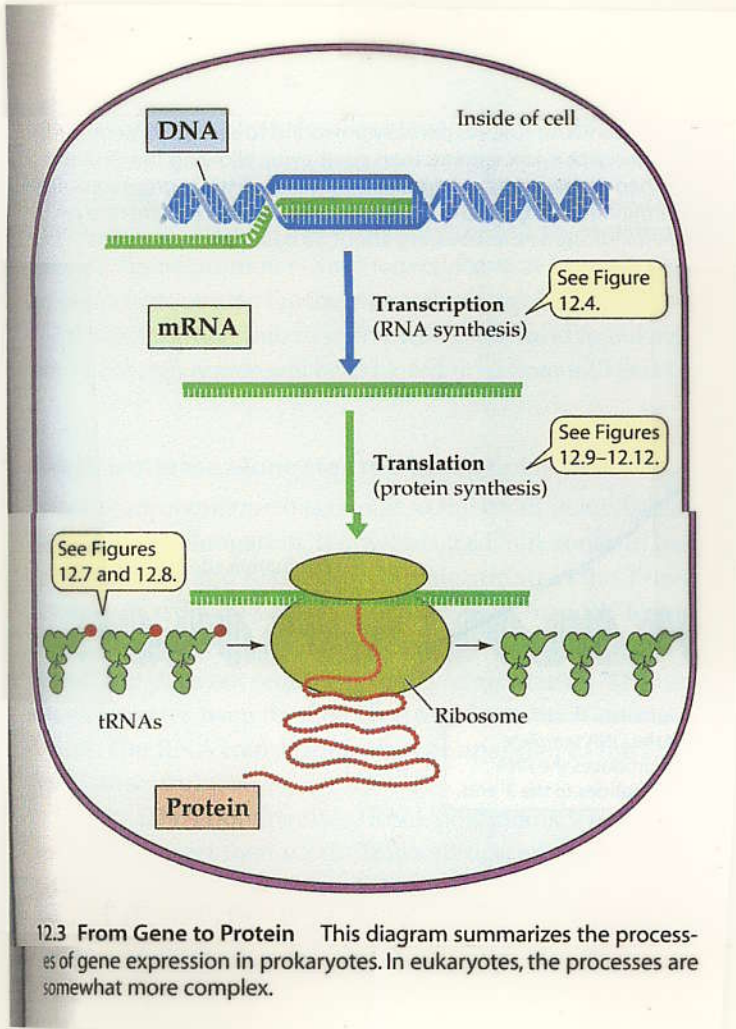
**MUTATIONS - CHANGE DNA SEQUENCE
 ↓ CHANGE CORRESPONDING PROTEIN
 SEQUENCE !!**



Change in DNA
 ↓
 Change in mRNA
 ↓
 Change in Protein
 ↓
 Change in Phenotype

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 MACHINERY
 REQUIRING THOUSANDS OF GENES
 IS REQUIRED TO PRODUCE
 PROTEINS ENCODED BY SPECIFIC
 GENES!



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

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

GENETIC CODE ALLOWS THE SEQUENCE OF NUCLEOTIDES in mRNA / sense strand of Gene to be TRANSLATED into Sequence of AMINO ACIDS in Proteins

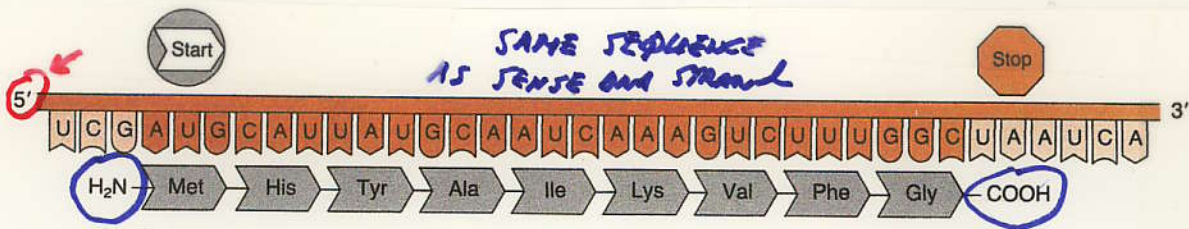


Figure 3.4 Decoding a messenger RNA sequence into a polypeptide.

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!

LARGE NUMBERS OF GENES ARE NEEDED TO MAKE PROTEINS!

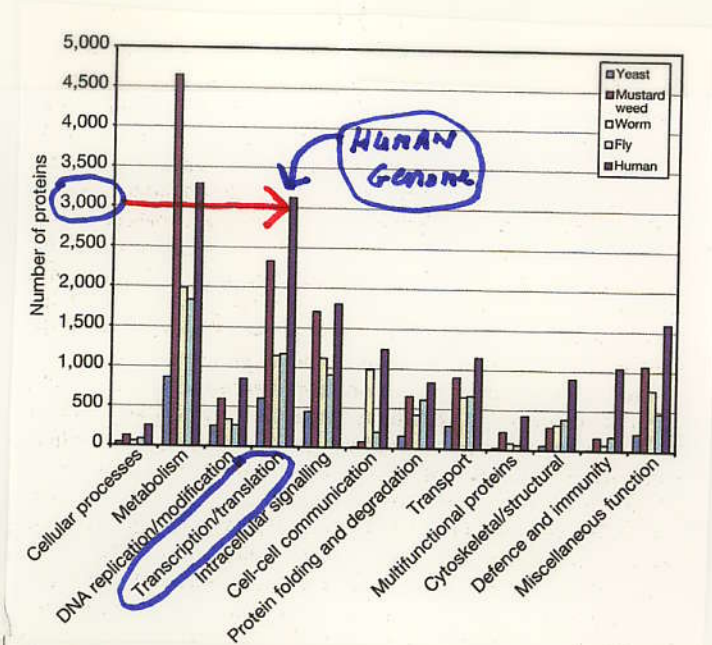


Figure 37 Functional categories in eukaryotic proteomes. The classification categories were derived from functional classification systems, including the top-level biological function category of the Gene Ontology project (GO; see <http://www.geneontology.org>).

~ 3,000 Genes needed for transcription & translation in human cells

The Genetic Code is Universal!

How Know?

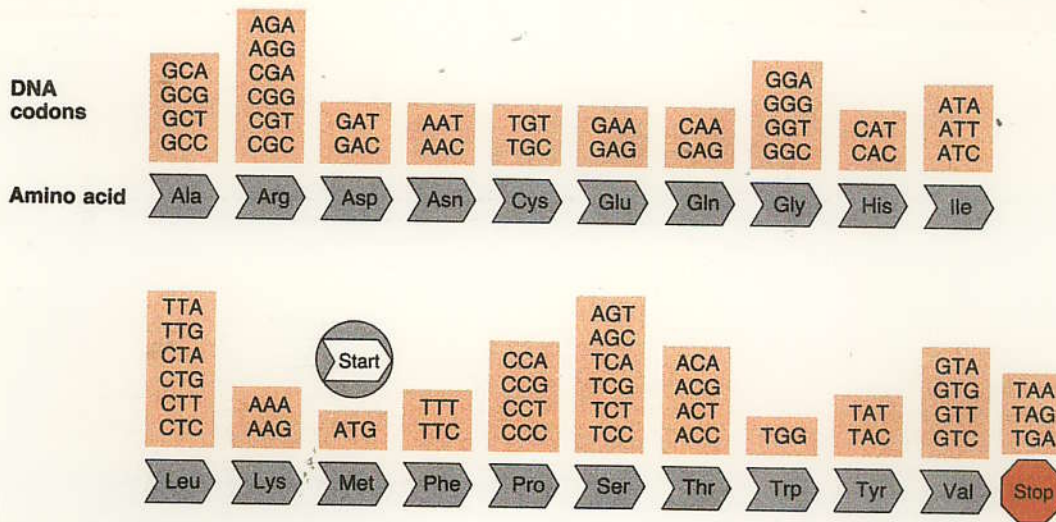


Figure 3.3 The genetic code. The codons shown for each amino acid are those for DNA. For RNA, the Ts are replaced by Us.

- 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, OR PROTEIN

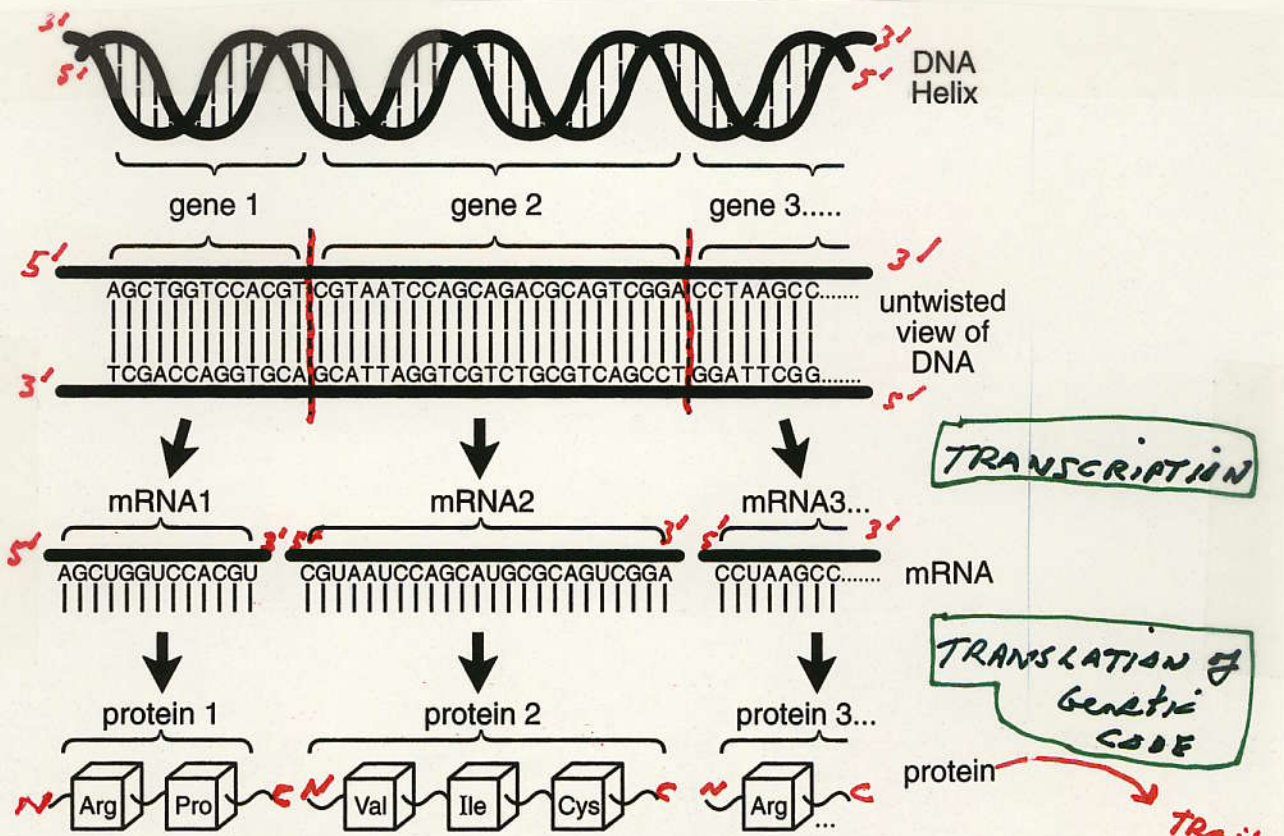


FIGURE 2.6 Adjacent sets of base pairs comprising different genes.

Specific TRAIT 1
FUNCTION 1

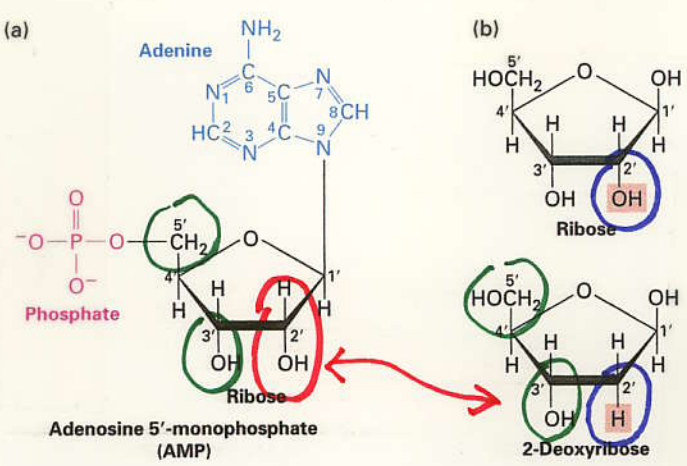
Specific TRAIT 2
FUNCTION 2

Specific TRAIT 3
FUNCTION 3

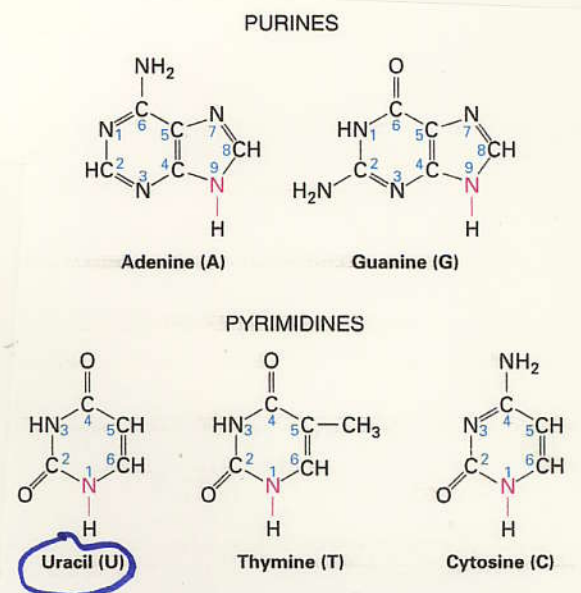
ONE GENE → ONE PROTEIN → ONE FUNCTION

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.



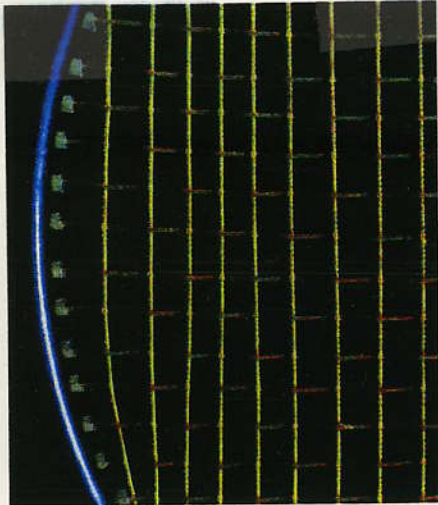
▲ FIGURE 2-15 Chemical structures of the principal bases in nucleic acids. In nucleic acids and nucleotides, nitrogen 9 of purines and nitrogen 1 of pyrimidines (red) are bonded to the 1' carbon of ribose or deoxyribose. U is only in RNA, and T is only in DNA. Both RNA and DNA contain A, G, and C.

IN PLACE of deoxyribose sugar and thymine!

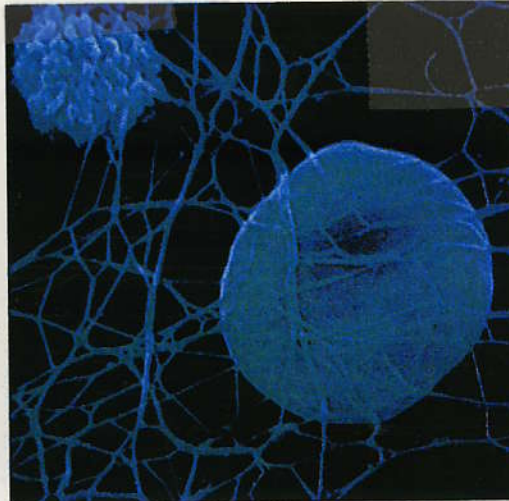
It's all in the Chemistry → Function!

UNIQUE PROTEINS CARRY OUT UNIQUE CELL FUNCTIONS

collagen

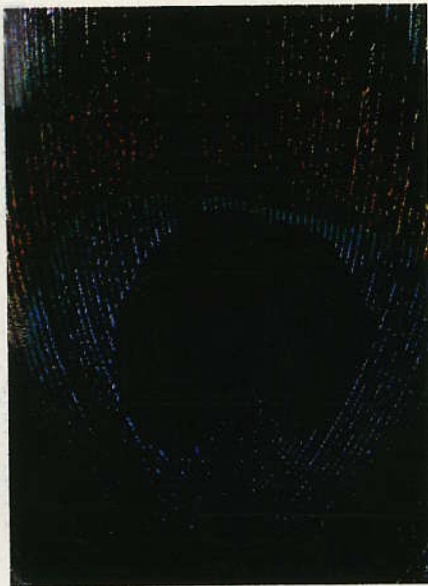


(a)



(b)

blood clot (protein C) 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 (3000x); (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
		Polymerases	Produce nucleic acids
		Kinases	Phosphorylate sugars and proteins
Defense	Immunoglobulins	Antibodies	Mark foreign proteins for elimination
Cell recognition	Toxins	Snake venom	Block nerve function
Transport throughout body	Globins	Hemoglobin	Carries O ₂ and CO ₂ in blood
		Myoglobin	Carries O ₂ and CO ₂ in muscle
Membrane transport	Transporters	Cytochromes	Electron transport
		Sodium-potassium pump	Excitable membranes
		Proton pump	Chemiosmosis
Structure/Support	Fibers	Anion channels	Transport Cl ⁻ ions
		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	Repressors	<i>lac</i> 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

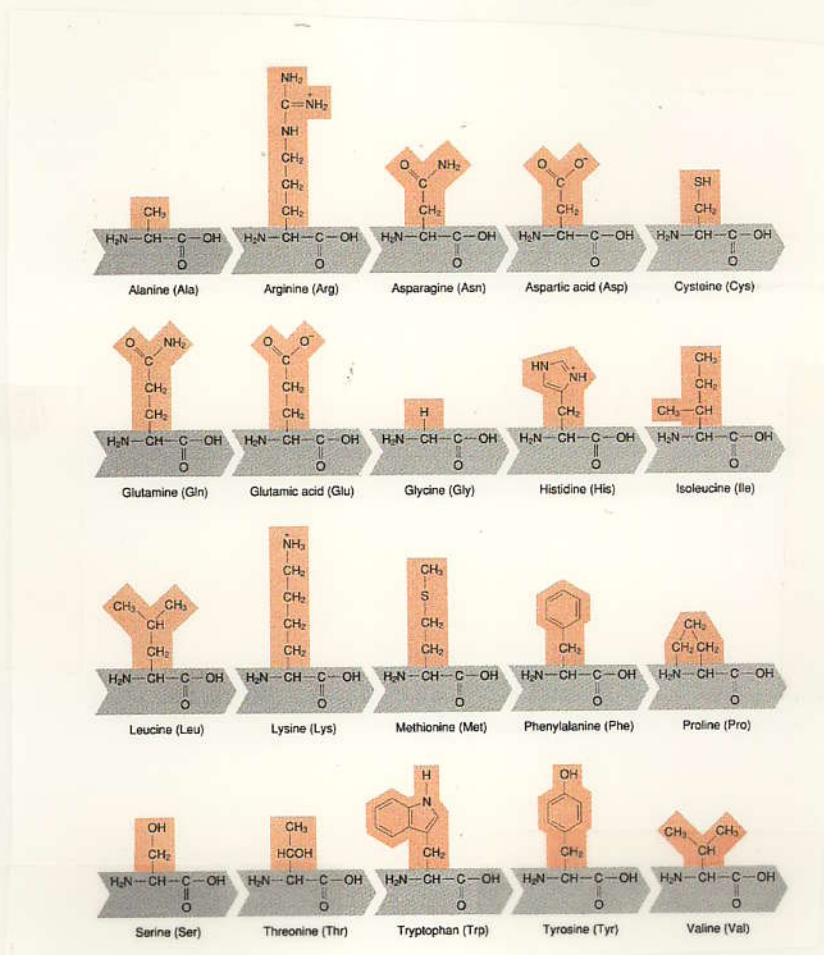
For Gene Engineer →

DNAPolymerase
Reverse Transc
Terminal Transferase
Restriction Enzymes

Regulate Switches!

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

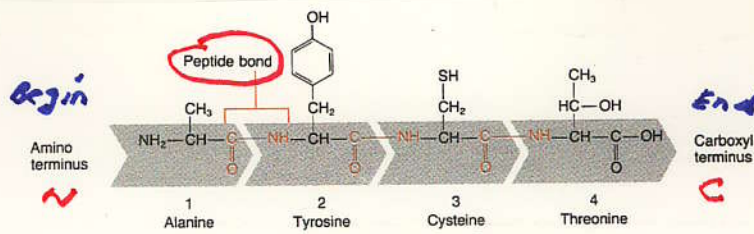
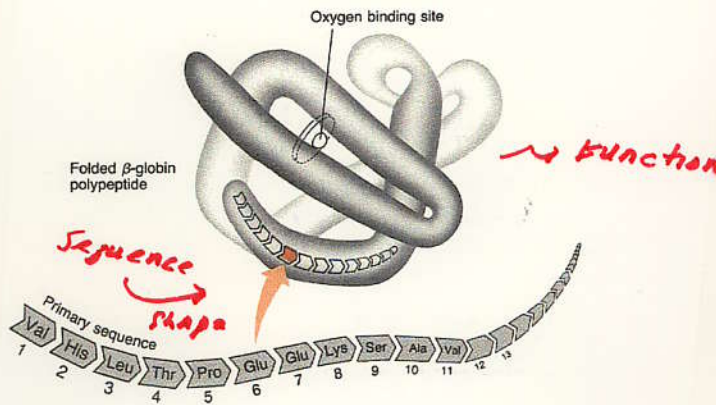


Figure 2.16 Peptide bonds between amino acids in a tetrapeptide (four amino acids).

Corresponds to 5' & 3' ends of mRNA / sense strand of gene!

Mutations!

Change Shape
Change Function
Change Phenotype



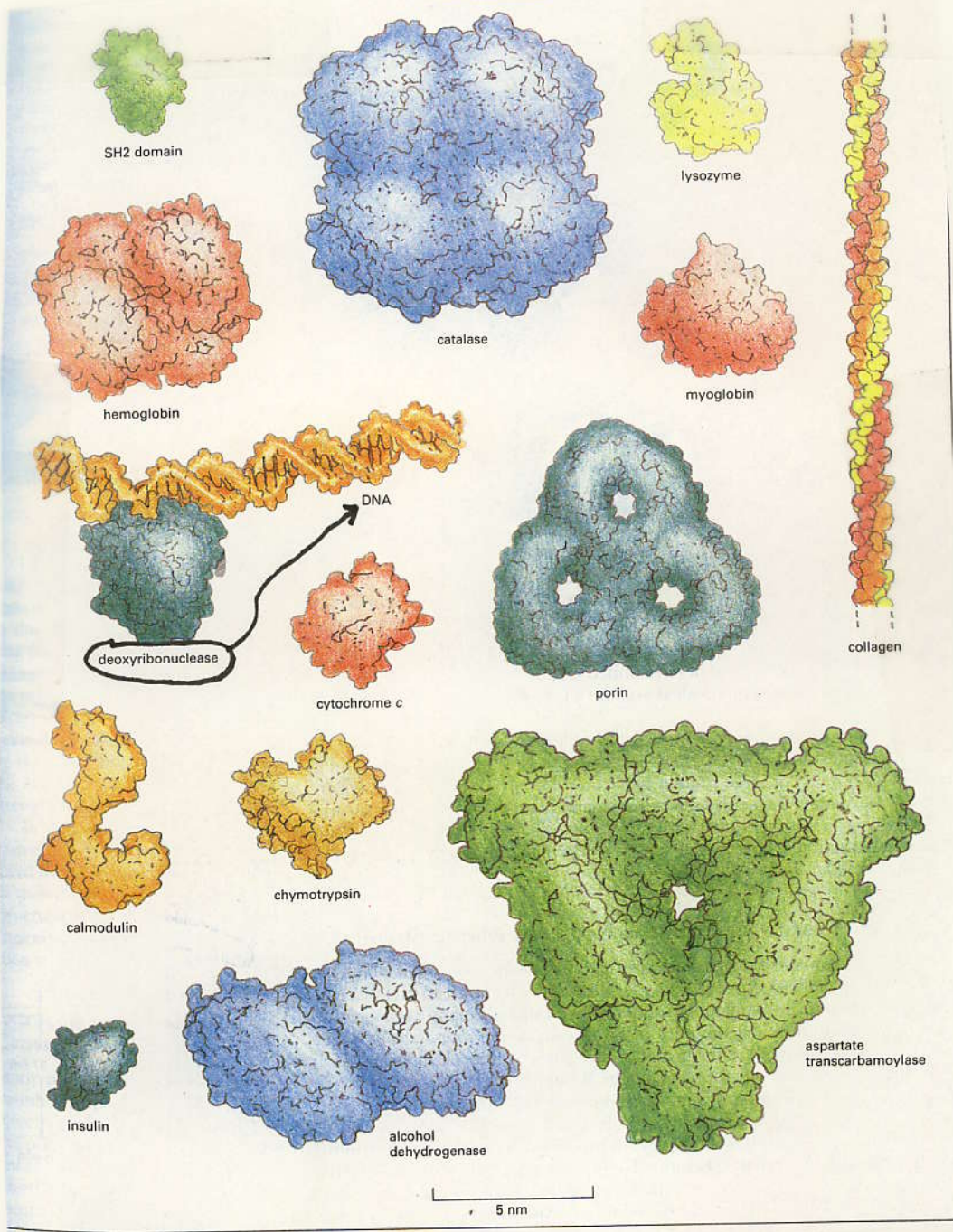
In sickle-cell hemoglobin, the Glu at position 6 is replaced by Val

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 \rightarrow Specific Protein Shape & Function

SHAPE \rightarrow FUNCTION \rightarrow Phenotype \rightarrow Specific

UNIQUE GENES IN A GENOME GIVE RISE TO UNIQUE PROTEINS



How does Gene # Relate to Protein #?

Note:
FORM
↳ FUNCTION!

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

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.....

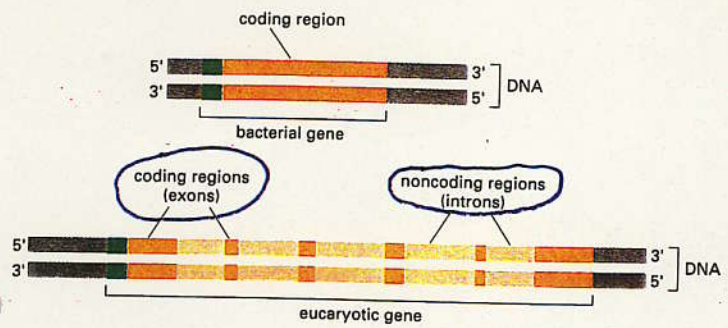


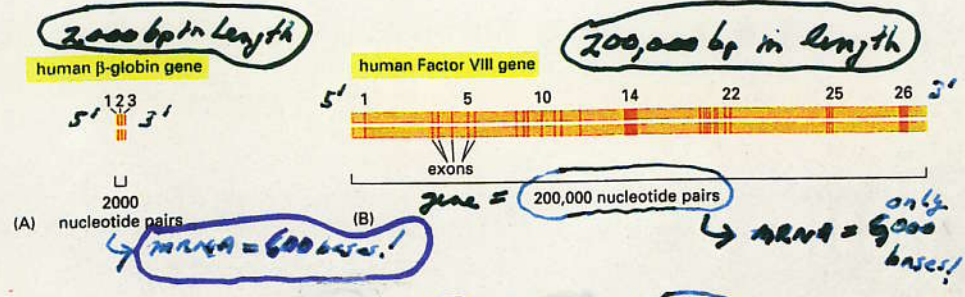
Figure 7-13 Comparison of a bacterial gene with a eucaryotic gene. The bacterial gene consists of a single stretch of uninterrupted nucleotide sequence that encodes the amino acid sequence of a protein. In contrast, the coding sequences of most eucaryotic genes (exons) are interrupted by noncoding sequences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

SWITCHES
UNIQUE
TO
BACTERIA
&
TO
PLANTS/ANIMALS

Eucaryotic Genes ^{CAN!} have non-coding regions "stuck" in coding regions

Prokaryotic Genes only have coding regions!

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



Note: Human Genes can be Mostly Intron Sequences!

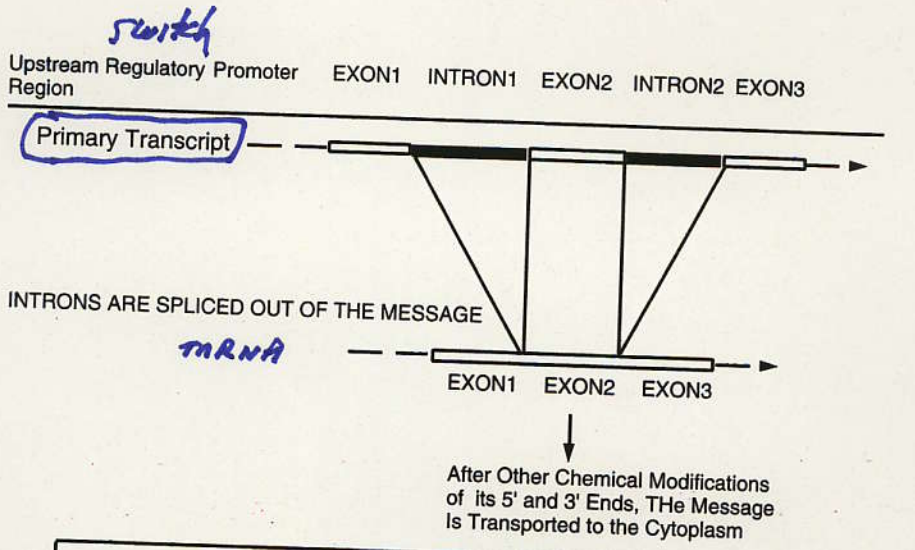
38

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



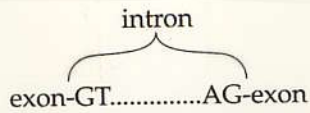
[Solid line] Coding Sequence, the Portion of the Primary Transcript That Encodes Protein
 [Thick solid line] Introns. Part of the Primary Transcript That Is Removed By Splicing
 [Dashed line] 5' and 3' Flanking Sequences That Stay in the Mature mRNA, But Do Not Code for Protein

FIGURE 3.14 Exons, introns, and splicing.

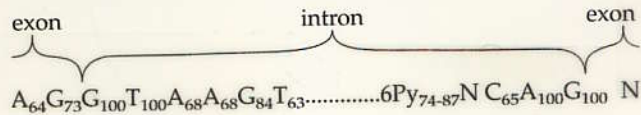
Implication for Engineering Eukaryotic genes in bacteria?

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

40! It's in the Sequences!



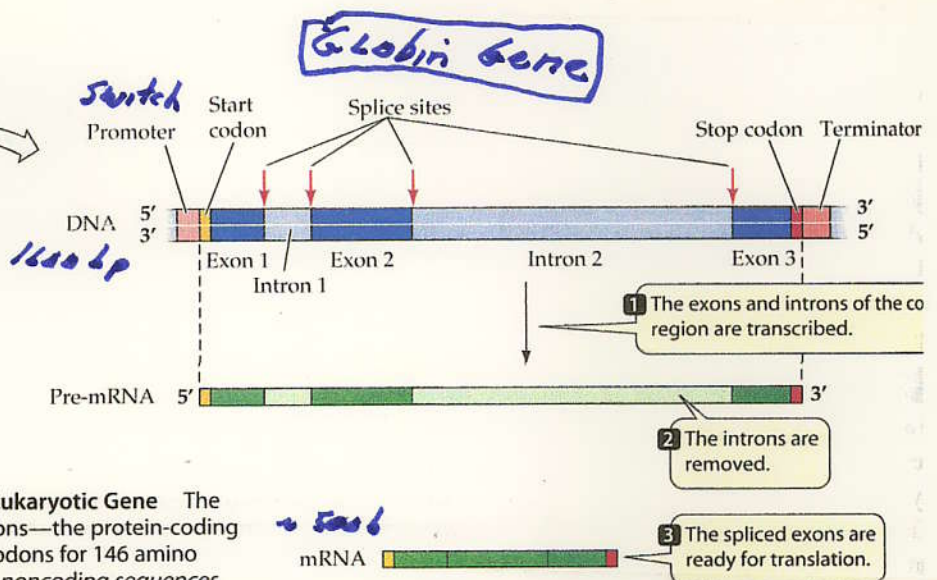
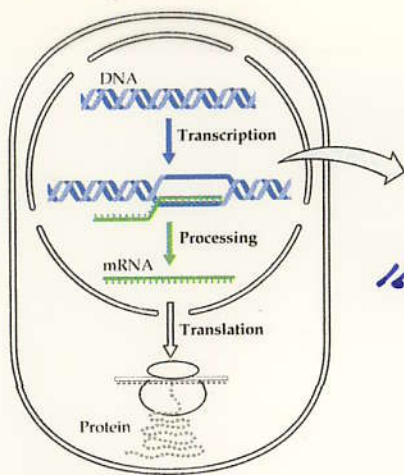
The sequences shown here are for the DNA nontemplate strand (equivalent to the RNA transcript, but with T rather than U). In addition, there are short consensus sequences at the exon-intron junctions. For nuclear genes, the consensus junctions are



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 & Generating Functional mRNAs

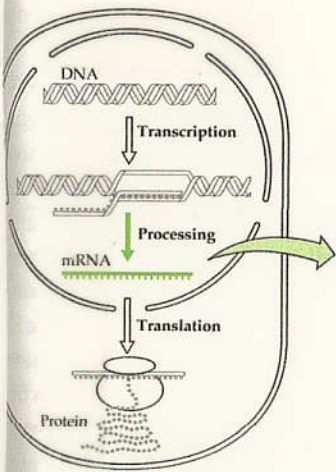


14.4 The Structure and Transcription of a Eukaryotic Gene The β -globin gene is about 1,600 bp long. The exons—the protein-coding sequences—contain 441 base pairs (triplet codons for 146 amino acids plus a triplet stop codon). The introns—noncoding sequences of DNA—between codons 30 and 31 (130 bp long)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

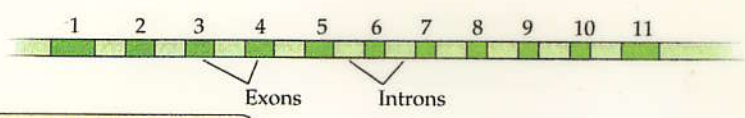
Mutations \rightarrow Blood Disorders
Where can these occur?

Alternative Splicing - One Gene ↳ Several RNAs & Proteins!

Gene Active in variety of Cells
But...!!!

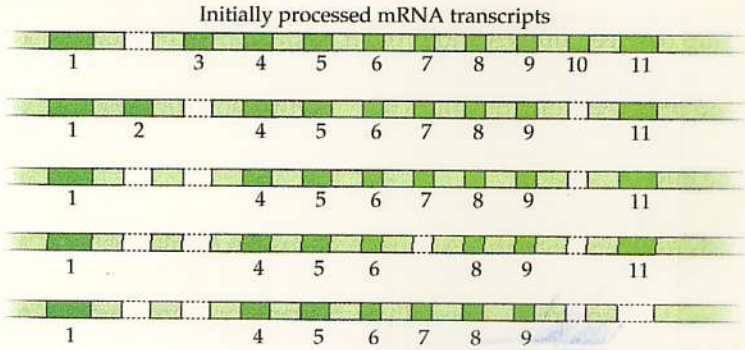


Primary RNA transcript for tropomyosin: 11 exons



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

- 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 contains same # of genes as the fly & mouse genomes!

406

Implications for Genetic Engineering? Use specific cDNA!

ONE GENE / MANY PROTEINS

The old axiom "one gene, one protein" no longer holds true. The more complex an organism, the more likely it became that way by extracting multiple protein meanings from individual genes

PROTEINS
↓
TRAITS

∴
Could have similar Genes but Different Proteins
e.g. Human vs. Mouse!

The Alternative Genome

BY GIL AST

GENES of mice and men are 88 percent alike. Many of the ways that humans differ from rodents arise from how we edit our genetic information.

Scientific American April 2005

406

Few Genes → Many Proteins → Many Functions & Phenotypes!

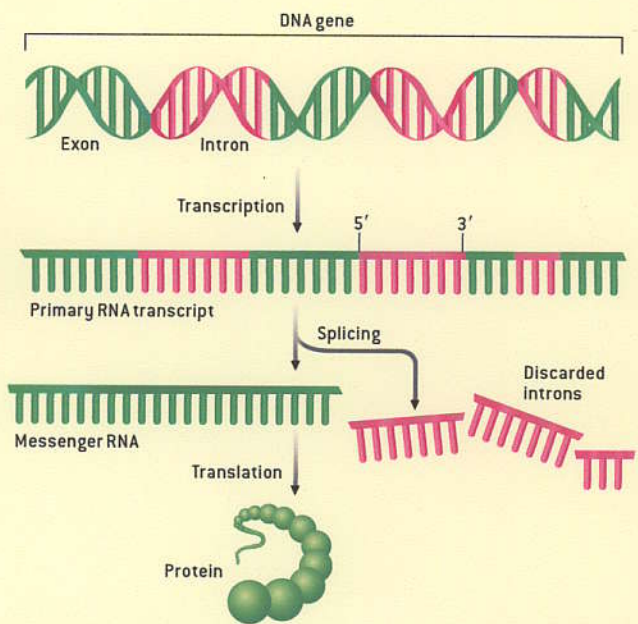
ONE GENE, MANY PROTEINS

The classical view of gene expression was simple: a DNA gene is first transcribed into RNA form, then cellular splicing machinery edits out "junk" stretches called introns and joins meaningful portions called exons into a final messenger RNA (mRNA) version, which is then translated into a protein. As it

turns out, these rules do not always apply. In complex organisms, the initial RNA transcript can be alternatively spliced—exons may be discarded and introns, or portions of them, retained—to produce a variety of mRNAs, and thus different proteins, from a single gene.

CLASSIC GENE EXPRESSION

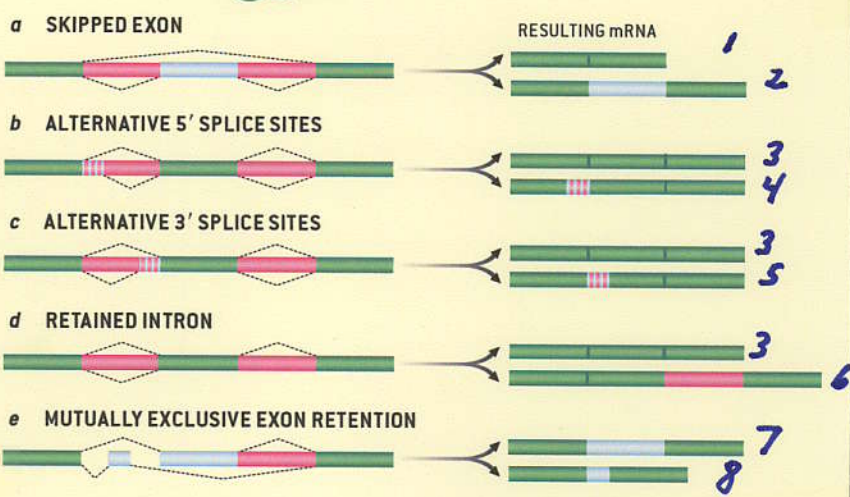
A DNA sequence is transcribed into a single-stranded copy made of RNA. Cellular machinery then "splices" this primary transcript: introns—each of which is defined by distinctive nucleotide sequences at its beginning and end, known, respectively, as the 5' (five-prime) and 3' (three-prime) splice sites—are removed and discarded while exons are joined into an mRNA version of the gene that will be translated into a protein by the cell.



ALTERNATIVE SPLICING

A gene's primary transcript can be edited in several different ways, shown at the right, where splicing activity is indicated by dashed lines. An exon may be left out (a). Splicing machinery may recognize alternative 5' splice sites for an intron (b) or alternative 3' splice sites (c). An intron may be retained in the final mRNA transcript (d). And exons may be retained on a mutually exclusive basis (e).

- Exon always spliced in
- Exon alternatively spliced
- Intron



1 Gene → 8 mRNAs → 8 Proteins!

~ 30,000 genes in human genome → 100,000s of proteins
(40%) NO PROTEIN - NO FUNCTION FUNCTION

Spliced Gene Determines Objects of Flies' Desire

The male fruit fly is a winged Casanova. He pursues lady flies with a repertoire of song, dance, and well-placed licks that many find impossible to resist. Now, by creating genetically engineered female flies that mimic the male courtship display, researchers have taken important steps toward understanding the biological basis of this complex, instinctive behavior.

In a pair of papers in the 3 June issue of *Cell*, Barry Dickson and colleagues at the Institute of Molecular Biotechnology in Vienna, Austria, report that a gene called *fruitless (fru)* sets up the fly brain to produce male courtship behavior in *Drosophila melanogaster*. Female flies altered to use the *fru* gene to make proteins normally made only by males woo other females much as males do. Additional experiments by Dickson's team identify a circuit of neurons in the fly brain that appears to mediate such courtship behavior and sexual orientation.

"I think it's quite remarkable," says Catherine Dulac, a neuroscientist at Harvard University. The work convincingly demonstrates that a single gene can regulate a complex sequence of behaviors, she notes. The team's "very elegant experiments" represent "a start toward understanding how an innate behavior is laid down in a nervous system," says Edward Kravitz, a Harvard neuroethologist.

In the 1960s, scientists discovered that male flies with a mutated *fru* gene become sexually indiscriminate—courting males as well as females. Then, in the mid-1990s, two teams reported that the *fru* gene operates differently in males and females; the cells of each sex read the gene in distinct ways, splicing together different mRNA transcripts. In males, these transcripts produce up to three distinct proteins, whereas the female mRNAs seem to lead to none. The DNA sequence of *fru* suggests that it encodes proteins that regulate the expression of other genes—but no

one knows what those genes might be.

Scientists have hypothesized that male *fru* proteins are necessary and sufficient for male courting behavior, but Dickson's paper is the first to show that directly, says Daisuke Yamamoto of Tohoku University in Sendai, Japan, who led one of the teams that discovered the splicing difference.

The key was making very minor modifications to the region of *fru* that is spliced differently in males and females.



Going courtin'. Spliced the right way, *fru* establishes a "courtship" circuit of neurons (green) in the male fly brain and makes females court other females (*inset*).

forcing female flies, for example, to splice the gene as males normally do. Although the sexual anatomy of these females appeared to be entirely normal, their behavior was dramatically altered. They courted other female flies, using all steps of the male courtship ritual, short of attempting copulation. Yet, male flies altered to splice *fru* as females do barely courted at all. Dickson hypothesizes that

"behavioral switch genes" like *fru* provide a way to hard-wire adaptive behaviors into the brain so that an animal can perform them instinctively. Still, he and others caution against extrapolating the results to sexual behavior in humans. "Clearly, we are vastly more complicated creatures than flies, and our common experience tells us that our sexual interests are not irreversibly set by our genes," Dickson says.

To investigate how *fru* programs the courtship routine into the fly brain, Dickson's team engineered additional fly strains. In one, a genetic marker identified all of the neurons in male flies that normally express the male-specific mRNAs of *fru*. Many of the labeled neurons appeared to form a circuit. Key elements of this circuit are olfactory neurons that may be specialized for pheromone detection. Inactivating these cells abolished courtship behavior in male flies, Dickson's team found. Somewhat puzzlingly, the researchers also found a similar circuit of neurons in female flies. This suggests to Dickson that courtship behavior depends not on anatomical differences between the male and female brain but rather on how this circuit functions.

Kravitz suspects that *fru* may also be involved in other instinctive behaviors that differ between the sexes—a possibility he will be investigating with a visiting postdoc from the Dickson lab. "We're pretty sure these same genes are involved in whether flies fight like males or females," he says. If so, *fru* may turn out to make male fruit flies fighters as well as lovers.

—GREG MILLER

CREDITS (TOP TO BOTTOM): E. DEMIR AND B. J. DICKSON, CELL 121, 5 (2005); PETRA STOCKINGER

fru → Male Courtship → ♀
 Alter *fru* splice variant in Females → Female Courtship → ♀
 Like Males
 40e

GENE ENGINEERING IMPLICATIONS!

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

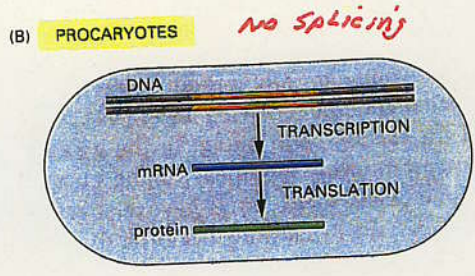
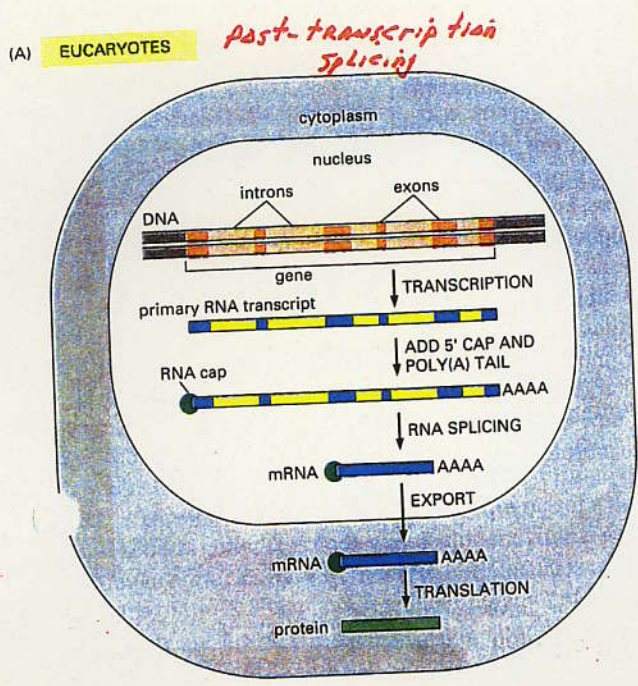


Figure 7-19 Summary of the steps leading from gene to protein. The final level of a protein in the cell depends on the efficiency of each step and on the rates of degradation of the RNA and protein molecules. (A) In eucaryotic cells, the initial RNA molecule produced by transcription (the primary transcript)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

Modular organization of sequences

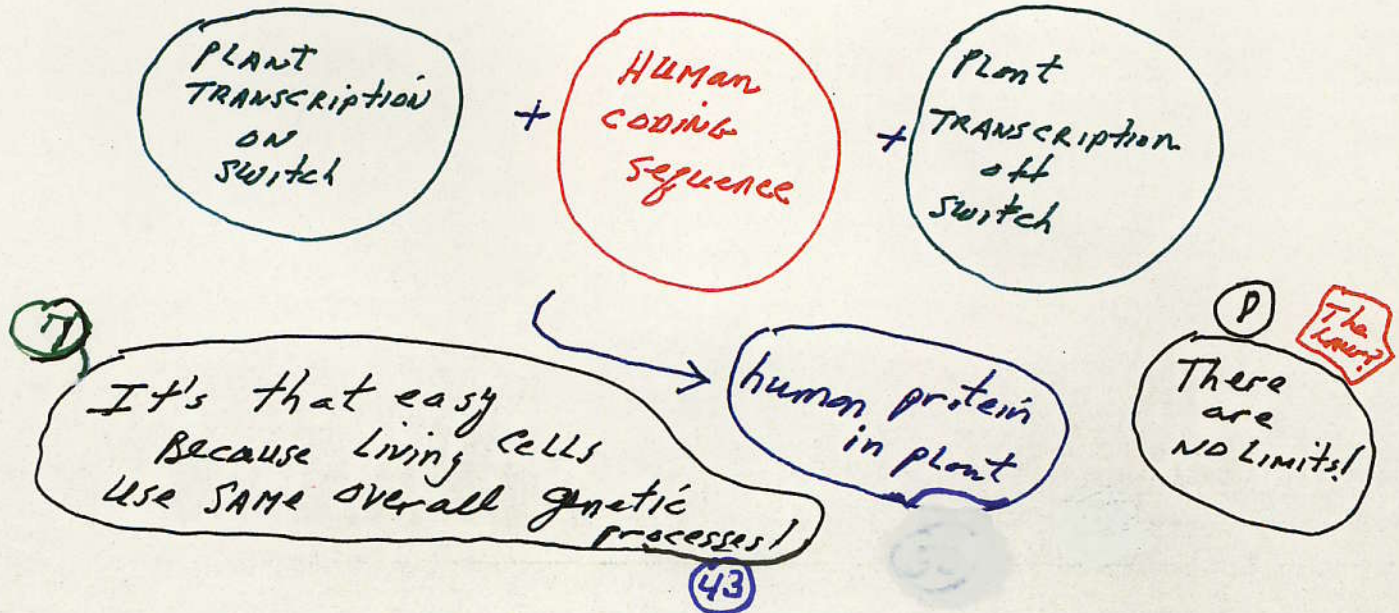
- ① DNA Replication
ORI
- ② TRANSCRIPTION
Switch/Regulator
Terminator
- ③ Processing of RNA (Eukaryotes)
Splicing Sites
- ④ Translation
Start
Stop
Genetic Code/Codons
- ⑤ Coding Sequence
Genetic Code

Modules → any thing you want to do genetically!

Engineering Genes Requires:

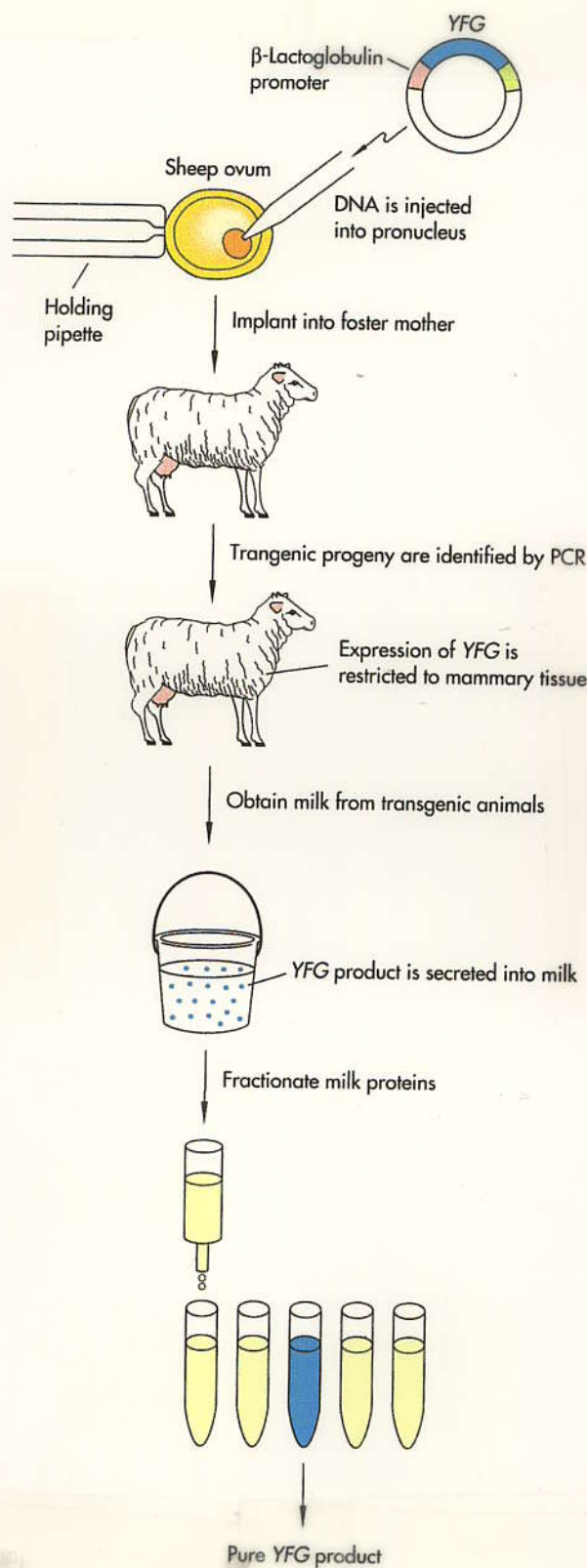
- ① The Gene & its Sequence
- ② A Roadmap of where Coding Sequence & ALL switches located (What's the road map?)
- ③ TRANSCRIPTION Start & Stop Switches
- ④ Coding Part of Gene / Genetic Code Part
- ⑤ TRANSLATION Start & Stop Switches
- ⑥ Kingdom - Specific Switches/Signals ←←

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!!



HOW TO ENGINEER GOAT MILK TO CONTAIN A HUMAN PROTEIN!

② PROMOTER = SWITCH FOR MAMMARY GLAND/UTTER



① YFG = your favorite gene

QUESTIONS?

- How identify YFG in Sheep Genome?
- How Engineer YFG to be active only in Mammary cells?
- How can YFG be expressed in Goats?
- 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

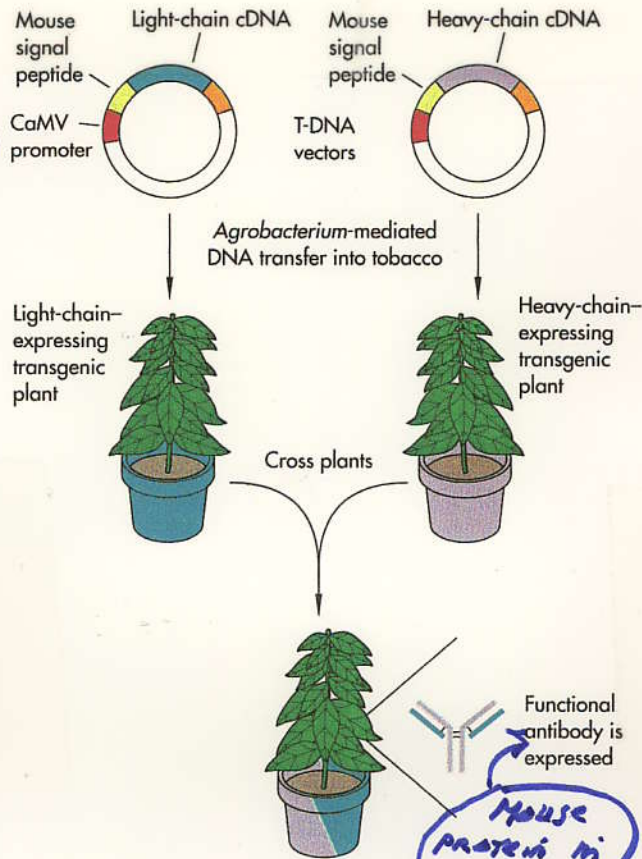


FIGURE 24-5
Plants as bioreactors to produce antibodies. Cloned cDNAs encoding the light and heavy chains from a mouse monoclonal antibody were ligated into separate T-DNA vectors and placed under control of a constitutive CaMV promoter. The plasmids were transferred separately into tobacco plants by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

Yo! It's ALL in The Sequences!!

DNA, Gene, Switch, Ori, mRNA, Protein!

NO HOCUS FOCUS!

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 GMS

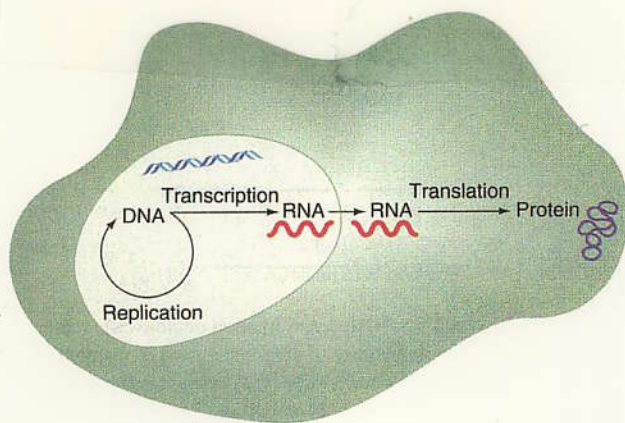


Figure 10-2 The three processes of information transfer: replication, transcription, and translation.

- 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 FOCUS
all hypotheses are
testable!!

"Behind" ALL TRAITS!

SAME PROCESSES!