

Chapter 15.1 – 15.3

Mutations

DNA → Transcription → mRNA → Translation → Protein

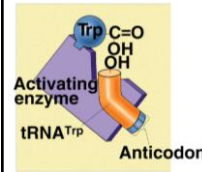
(b) Sickled red blood cells and the primary structure of sickle-cell hemoglobin

Val | His | Leu | Thr | Pro | Val | Glu | ...
1 2 3 4 5 6 7

Universal Code

Code is redundant

- several codons for each amino acid
- “wobble” in the tRNA
- “wobble” in the aminoacyl-tRNA synthetase enzyme that loads the tRNA



		Second base			
		U	C	A	G
U	U	UUU Phe	UCU	UAU Tyr	UGU Cys
	U	UUC	UCC	UAC	UGC
	U	UUA Leu	UCA	UAA Stop	UGA Stop
C	C	CUU	CCU	CAU His	CGU Arg
	C	CUC	CCC	CAC	CGC
	C	CUA Leu	CCA	CAA Gln	CGA Arg
A	A	AUU	ACU	AAU Asn	AGU Ser
	A	AUC	ACC	AAC	AGC
	A	AUA Leu	ACA	AAA Lys	AGA Arg
G	G	AUG Met or start	ACG	AAG	AGG
	G	GUU	GCU	GAU Asp	GGU Gly
	G	GUC	GCC	GAC	GGC
		GUA Val	GCA Ala	GAA Glu	GGA Gly
		GUG	GCG	GAG	GGG

Mutations

Point mutations

- single base change
- base-pair substitution
 - silent mutation
 - no amino acid change
 - redundancy in code
 - missense
 - change amino acid
 - nonsense
 - change to stop codon

Wild type

mRNA: A U G A A G U U U G C U A A
Protein: Met Lys Phe Gly Stop

Base-pair substitution

No effect on amino acid sequence
U instead of C
A U G A A G U U U G U U A A
Met Lys Phe Gly Stop

Missense
A instead of G
A U G A A G U U U A C U A A
Met Lys Phe Ser Stop

Nonsense
U instead of A
A U G A A G U U U G C U A A
Met Stop

Mutations

Frameshift

- shift in the reading frame
 - changes everything “downstream”
- insertions
 - adding base(s)
- deletions
 - losing base(s)

Wild type

mRNA: A U G A A G U U U G C U A A
Protein: Met Lys Phe Gly Stop

Base-pair insertion or deletion

Frameshift causing extensive missense
Missing U
A U G A A G U U U G G C U A A ...
Met Lys Leu Ala ...

Frameshift causing immediate nonsense
Extra U
A U G U A A G U U U G C U A A
Met Stop

Insertion or deletion of 3 nucleotides: no frameshift; extra or missing amino acid
Missing A A A
A U G A A G U U U G C U A A
Met Phe Gly Stop

A Mutation Leads to Sickle Cell Anemia

<p>Wild-type hemoglobin DNA</p> <p>3' C T T 5'</p> <p>mRNA: G A A</p> <p>Normal hemoglobin: Glu</p>	<p>Mutant hemoglobin DNA</p> <p>3' C A T 5'</p> <p>mRNA: G U A</p> <p>Sickle-cell hemoglobin: Val</p>
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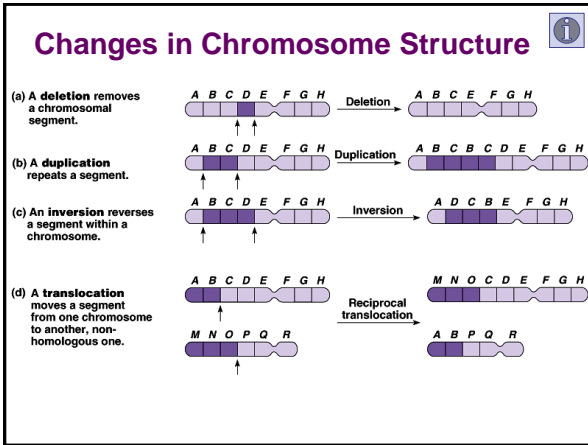
Sickle Cell Anemia

(a) Normal red blood cells and the primary structure of normal hemoglobin

(b) Sickled red blood cells and the primary structure of sickle-cell hemoglobin

Val | His | Leu | Thr | Pro | Glu | Glu | ...
1 2 3 4 5 6 7

Val | His | Leu | Thr | Pro | Val | Glu | ...
1 2 3 4 5 6 7

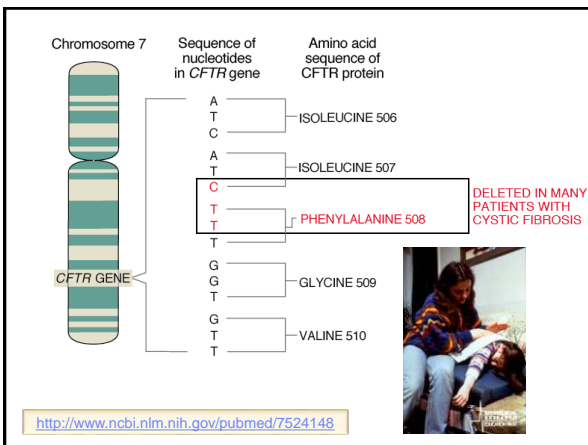
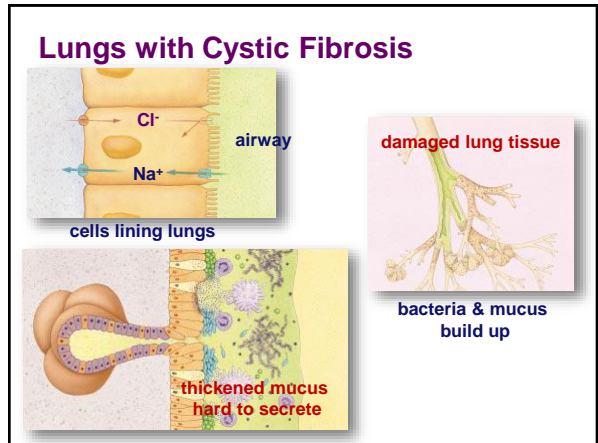
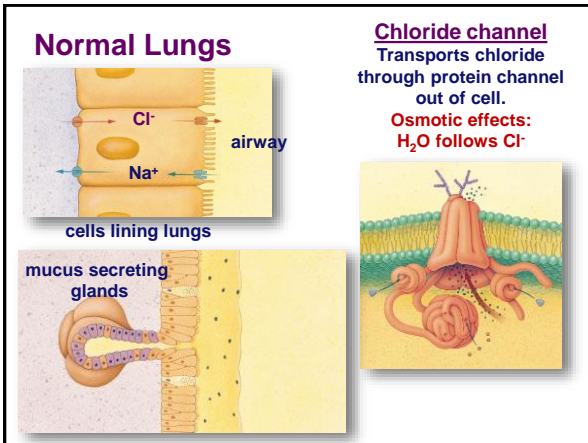


Cystic Fibrosis

■ Primarily Caucasians of European descent

- ◆ strikes 1 in **2500** births
 - 1 in 25 'whites' is a carrier (Aa)
- ◆ normal allele codes for a membrane protein that transports Cl⁻ across cell membrane
 - defective or absent channels fail to transport Cl⁻
 - thicker & stickier mucus coats around cells
 - mucus build-up in the pancreas, lungs, digestive tract & causes bacterial infections
- ◆ without treatment children die before 5; with treatment can live past their late 20s

normal lung tissue



Tay-Sachs

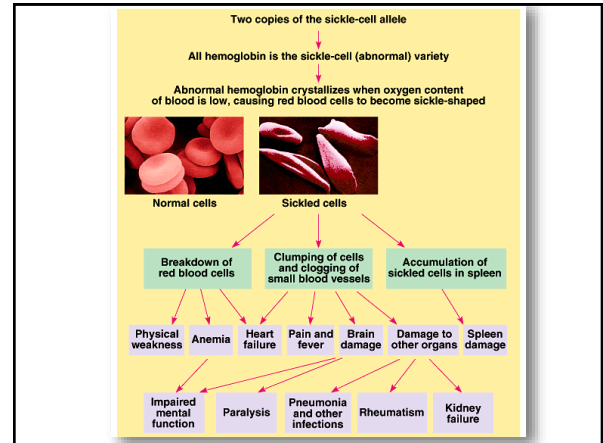
■ Primarily Jews of eastern European (Ashkenazi) descent & Cajuns

- ◆ strikes 1 in **3600** births
 - 100 times greater than incidence among non-Jews or Mediterranean (Sephardic) Jews
- ◆ non-functional enzyme fails to breakdown lipids in brain cells
 - symptoms begin few months after birth
 - seizures, blindness & degeneration of motor & mental performance
 - child dies before 5yo

Sickle Cell Anemia

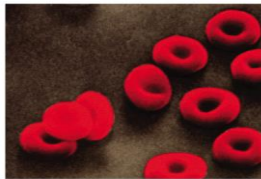
■ **Primarily Africans**

- ◆ strikes 1 out of **400** African Americans
- ◆ (high frequency in sub-Saharan Africans)
- ◆ caused by substitution of a single amino acid in hemoglobin
- ◆ when oxygen levels are low, sickle-cell hemoglobin crystallizes into long rods
 - deforms red blood cells into sickle shape
 - sickling creates **pleiotropic** effects = cascade of other symptoms



Sickle Cell Anemia

- Substitution of one amino acid for another in a polypeptide chain



Val His Leu Thr Pro Glu Glu ...

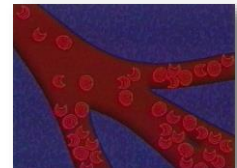
Val His Leu Thr Pro **Val** Glu ...

(a) Normal red blood cells and the primary structure of normal hemoglobin

(b) Sickled red blood cells and the primary structure of sickle-cell hemoglobin

Sickle Cell Phenotype

- Often a heterozygous individual
- The 2 alleles are both represented...
 - ◆ both normal & abnormal hemoglobins are synthesized in heterozygote (Aa)
 - ◆ carriers usually healthy, although some suffer some symptoms of sickle-cell disease under blood oxygen stress
 - exercise
 - high altitude...



Heterozygote Advantage

■ Sickle cell frequency

- ◆ high frequency of heterozygotes is unusual for allele with severe detrimental effects in homozygotes
 - 1 out of **400** African Americans
- Suggests some selective advantage of being heterozygous
 - ◆ sickle cell: resistance to malaria?
 - ◆ cystic fibrosis: resistance to cholera?

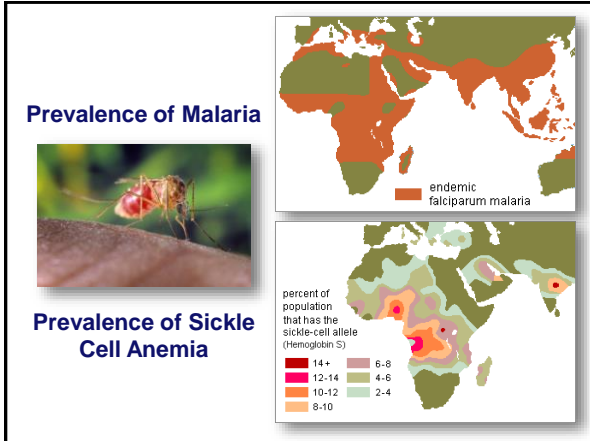


Heterozygote Advantage

■ Malaria

- ◆ single-celled eukaryote parasite spends part of its life cycle in red blood cells
- In tropical Africa, where malaria is common:
 - ◆ **homozygous normal** individuals die of malaria
 - ◆ **homozygous recessive** individuals have shorter median life span
 - ◆ **heterozygote carriers** are relatively free of both
- High frequency of sickle cell allele in African Americans is vestige of African roots





Genetics & Culture

- Why do all cultures have a taboo against incest?
 - ◆ laws or taboos forbidding marriages between close relatives are fairly universal
- Fairly unlikely that 2 carriers of same rare harmful recessive allele will meet & mate
 - ◆ but matings between close relatives increase risk
 - consanguineous matings
 - ◆ individuals who share a recent common ancestor are more likely to carry same recessive alleles

How many genes?

- Genes
 - ◆ only ~3% of human genome
 - ◆ protein-coding sequences
 - 1% of human genome
 - ◆ non-protein coding genes
 - 2% of human genome
 - ◆ tRNA
 - ◆ ribosomal RNAs
 - ◆ siRNAs
 - ◆ 'junk' DNA as part of the other 97%

What about the rest of the DNA?

- Non-coding DNA sequences
 - ◆ regulatory sequences
 - promoters, enhancers
 - terminators
 - ◆ "junk" DNA
 - introns
 - repetitive DNA
 - ◆ centromeres
 - ◆ telomeres
 - ◆ tandem & interspersed repeats
 - transposons & retrotransposons

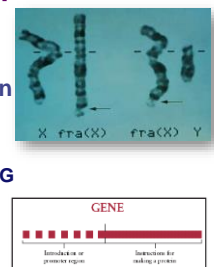
Repetitive DNA

Repetitive DNA & other non-coding sequences account for most of eukaryotic DNA

Table 19.1 Types of Repetitive DNA	
Tandemly Repetitive DNA (Satellite DNA)	
Repeated units at a site are usually identical	
Proportion of mammalian DNA:	10–15%
Length of each repeated unit:	1–10 base pairs
Total length of repetitive DNA per site, in base pairs:	
Regular satellite DNA	100,000–10 million
Minisatellite DNA	100–100,000
Microsatellite DNA	10–100
Interspersed Repetitive DNA	
"Copies" are very similar but not identical	
Proportion of mammalian DNA:	25–40%
Length of each repeated unit:	100–10,000 base pairs
Number of repetitions per genome:	10–1 million

Genetic Disorders of Repeats

- Fragile X syndrome
 - ◆ most common form of inherited mental retardation
 - ◆ defect in X chromosome
 - mutation of FMR1 gene causing many repeats of CGG triplet in promoter region
 - ◆ 200+ copies
 - ◆ normal = 6-40 CGG repeats
 - FMR1 gene not expressed & protein (FMRP) not produced
 - ◆ function of FMR1 protein unknown
 - ◆ binds RNA



Fragile X Syndrome

- The more triplet repeats there are on the X chromosome, the more severely affected the individual will be
 - ◆ mutation causes increased number of repeats (expansion) with each generation

Normal

FMR-1 gene

Premutation

FMR-1 gene

Full Mutation

FMR-1 gene

Huntington's Disease

- Rare autosomal dominant degenerative neurological disease
 - ◆ 1st described in 1872 by Dr. Huntington
 - ◆ most common in white Europeans
 - ◆ 1st symptoms at age 30-50
 - death comes ~12 years after onset
- Mutation on chromosome 4
 - ◆ CAG repeats
 - 40-100+ copies
 - normal = 11-30 CAG repeats
 - CAG codes for glutamine amino acid

Huntington's Disease

- Abnormal (*huntingtin*) protein produced
 - ◆ chain of charged glutamines in protein
 - ◆ bonds tightly to brain protein, HAP-1

Woody Guthrie

Interspersed Repetitive DNA

- Repetitive DNA is spread throughout genome
 - ◆ interspersed repetitive DNA (SINES Short **I**nterspersed **E**lements) make up 25-40% of mammalian genome
 - ◆ in humans, at least 5% of genome is made of a family of similar sequences called, *Alu* elements (PV92 anyone?!)
 - 300 bases long
 - *Alu* is an example of a "jumping gene" called a transposon; a DNA sequence that "reproduces" by copying itself & inserting into new chromosome locations

Restriction Enzymes

- Action of enzyme
 - ◆ cut DNA at specific sequences
 - restriction site
 - ◆ symmetrical "palindrome"
 - ◆ produces "ends"
 - sticky ends
 - blunt ends
- Many different enzymes
 - ◆ named after organism they are found in
 - EcoRI, HindIII, BamHI, SmaI

Madam I'm Adam

CTGAATCCG
GACTTAAGGC

CTG | AATCCG
GACTTAA | GGC

Discovery of Restriction Enzymes

1960s | 1978

Werner Arber Daniel Nathans Hamilton O. Smith

Restriction enzymes are named for the organism they come from:

EcoRI = 1st restriction enzyme found in *E. coli*

Gel Electrophoresis

- Separation of DNA fragments by size
 - ◆ DNA is negatively charged
 - moves toward + charge in electrical field
 - ◆ agarose gel
 - “swimming through Jello”
 - smaller fragments move faster

cut DNA 1st with restriction enzymes

Gel Electrophoresis

Gel Electrophoresis

Measuring Fragment Size

- compare bands to a known “standard”
 - ◆ usually lambda phage virus cut with HindIII
 - nice range of sizes with a distinct pattern

RFLP

- Restriction Fragment Length Polymorphism
 - ◆ differences in DNA between individuals

◆ if change in DNA sequence affects restriction enzyme “cut” site...
 ◆ ...it will create a different band pattern

Polymorphisms in Populations

- Differences between individuals at the DNA level (SNPs vs. STRs)

RFLP Use in Forensics

- DNA evidence

The diagram illustrates the RFLP process in three steps: 1. DNA extraction from a crime scene and two suspects. 2. Digestion of the DNA with restriction enzymes, creating fragments of varying lengths. 3. Gel electrophoresis, which separates the fragments based on size, resulting in a unique banding pattern for each sample.

RFLP Use in Forensics

- 1st case successfully using DNA evidence
 - ◆ 1987 rape case convicting Tommie Lee Andrews

The top gel shows a 'standard' DNA ladder, a semen sample from a rapist (indicated by a blue arrow), and a blood sample from a suspect (indicated by a red arrow). The bottom gel shows a 'standard' DNA ladder, a victim's semen sample (indicated by a blue arrow), and a blood sample from a suspect (indicated by a red arrow). The bands in the suspect's blood samples match the bands in the rapist's semen and victim's semen samples.

RFLP Use in Forensics

- Evidence from murder trial
 - ◆ Do you think suspect is guilty?

The gel image shows RFLP results for a murder trial. On the left, labels identify: blood sample 1 from crime scene, blood sample 2 from crime scene, blood sample 3 from crime scene, blood sample from suspect, blood sample from victim 1, and blood sample from victim 2. A 'standard' is also shown. Red circles highlight the bands in the suspect's blood sample, which match the bands in the victim 1 and victim 2 samples. Blue circles highlight the bands in the crime scene samples, which do not match the suspect's bands.