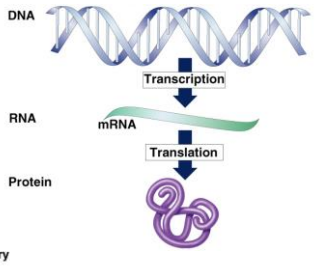
Chapter 15.1 – 15.3

Mutations



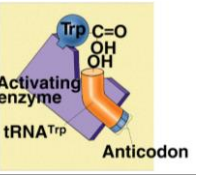
10 µm

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

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

Universal Code

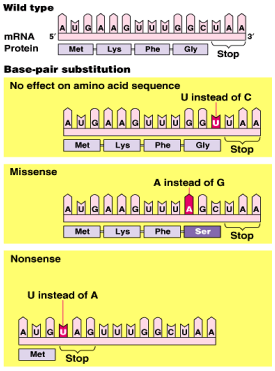
- Code is redundant
  - several codons for each amino acid
  - "wobble" in the tRNA
  - "wobble" in the aminoacyl-tRNA synthetase enzyme that loads the tRNA



First base (5′ end)	Second base: U	C	A	G	Third base (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

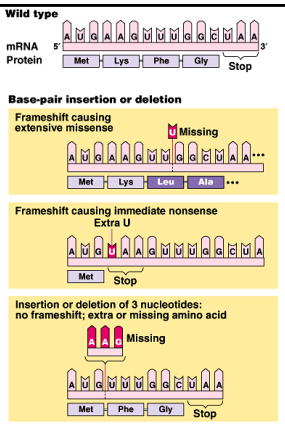
Mutations

- 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

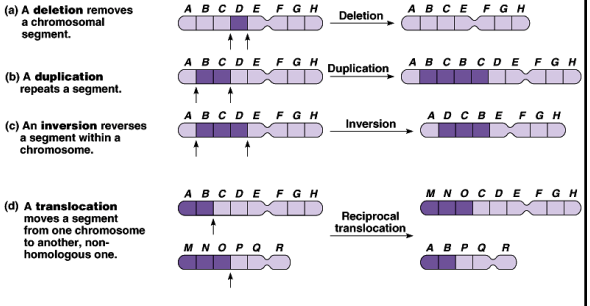


Mutations

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

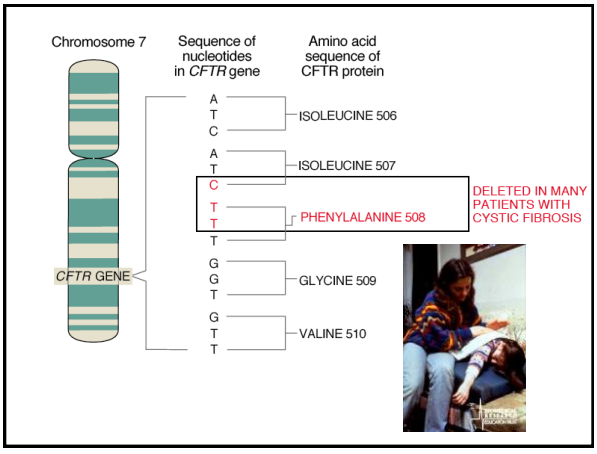


Changes in Chromosome Structure

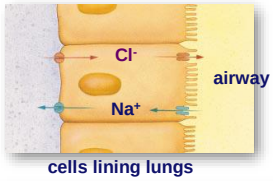


Cystic Fibrosis

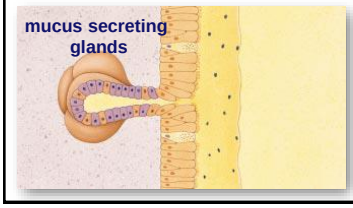
- Primarily Caucasians of European descent
  - strikes 1 in 2500 births
    - 1 in 25 'whites' is a carrier (Aa)
  - normal allele codes for a membrane protein that transports Cl^- across cell membrane
    - defective or absent channels fail to transport Cl^-
    - thicker & stickier mucus coats around 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



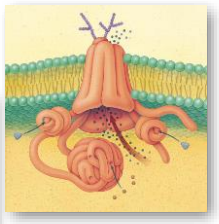
Normal Lungs



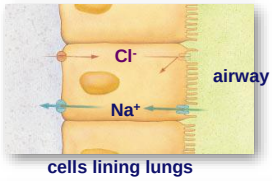
cells lining lungs



Chloride channel
Transports chloride through protein channel out of cell.
Osmotic effects: H_2O follows Cl^-



Lungs with Cystic Fibrosis



cells lining lungs

bacteria & mucus build up

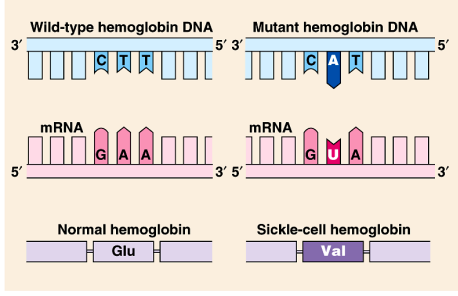
Tay-Sachs

- 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 gangliosides in brain cells
    - symptoms begin few months after birth
    - seizures, blindness & degeneration of motor & mental performance
    - child dies before 5yo

Sickle Cell Anemia

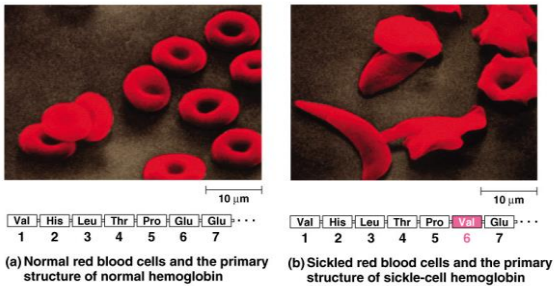
- Primarily Africans
  - strikes 1 out of 400 African Americans
  - (high frequency in sub-Saharan Africans)
  - caused by substitution of a single amino acid in hemoglobin
  - when oxygen levels are low, sickle-cell hemoglobin crystallizes into long rods
    - deforms red blood cells into sickle shape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

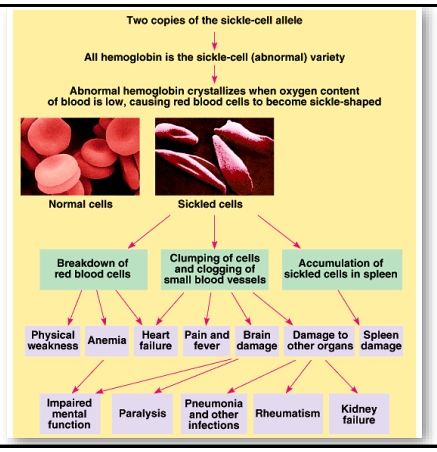
A Mutation Leads to Sickle Cell Anemia



Sickle Cell Anemia

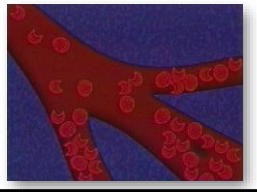
- Substitution of one amino acid for another in a polypeptide chain





Sickle Cell Trait…

- 1 out of 12 African Americans
- hybrids; 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…

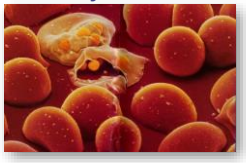


Heterozygote Advantage

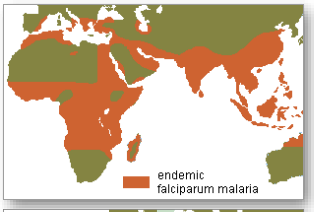
- Sickle cell frequency
  - high frequency of heterozygotes is unusual for allele with severe detrimental effects in homozygotes
- Suggests some selective advantage of being heterozygous
  - sickle cell: resistance to malaria?
  - cystic fibrosis: resistance to cholera?

Heterozygote Advantage

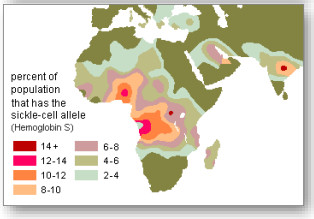
- Malaria
  - single-celled eukaryote parasite spends part of its life cycle in red blood cells
- In tropical Africa, where malaria is common:
  - homozygous normal individuals die of malaria
  - homozygous recessive individuals have shorter median life span
  - heterozygote carriers are relatively free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



Prevalence of Malaria



Prevalence of Sickle Cell Anemia



Genetics & Culture

- Why do MANY 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 do we have?

- 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

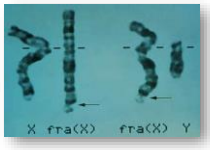
Repetitive DNA & other non-coding sequences account for most of eukaryotic DNA

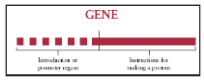
Table 19.1 Types of Repetitive DNA

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

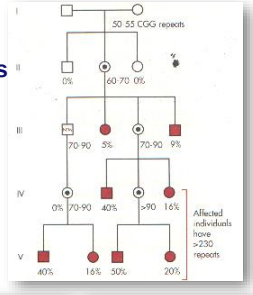
- Fragile X syndrome
  - most common form of inherited mental retardation
  - defect in X chromosome
    - mutation of *FMR1* gene causing many repeats of CGG triplet in promoter region
      - 200+ copies
      - normal = 6-40 CGG repeats
    - *FMR1* gene not expressed & protein (FMRP) not produced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

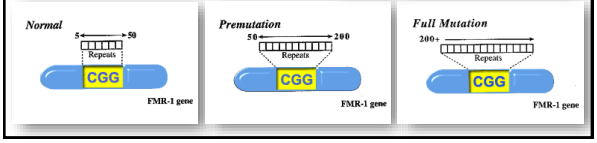




Fragile X Syndrome

- 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





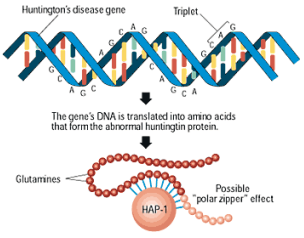
Huntington's Disease

- 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
    - normally 11-30 CAG repeats
    - mutation contains 40-100+ copies
    - CAG codes for glutamine amino acid

Huntington's Disease

- Abnormal protein (huntingtin) produced
  - chain of charged glutamines in protein
  - bonds tightly to brain protein, HAP1

Woody Guthrie



Interspersed Repetitive DNA

- Repetitive DNA is spread throughout genome
  - interspersed repetitive DNA (SINEs Short INterspersed Elements) 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 (Ch. 17/18)

1960s|1978

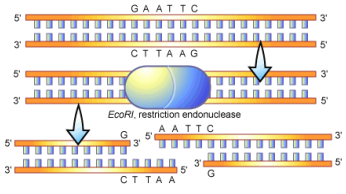
Discovery of Restriction Enzymes

Werner Arber

Daniel Nathans

Hamilton O. Smith

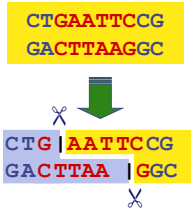
Restriction enzymes are named for the organism they come from:
EcoRI = 1st restriction enzyme found in E. coli



Restriction Enzymes

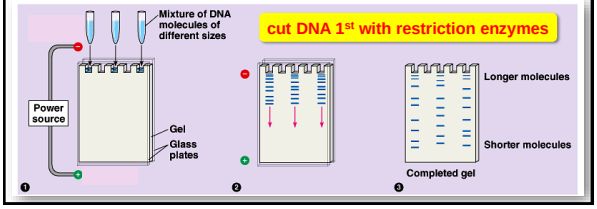
- Action of enzyme
  - cut DNA at specific sequences
    - restriction site
  - symmetrical "palindrome"
  - produces "ends"
    - sticky ends
    - blunt ends
- Many different enzymes
  - named after organism they are found in
    - EcoRI, HindIII, BamHI, SmaI

Madam I'm Adam

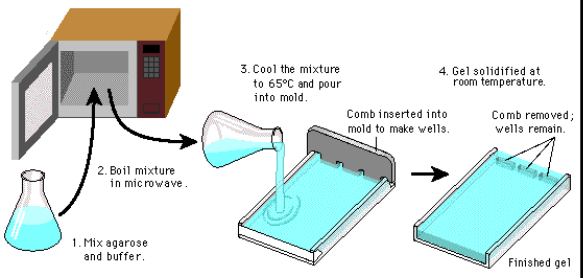


Gel Electrophoresis

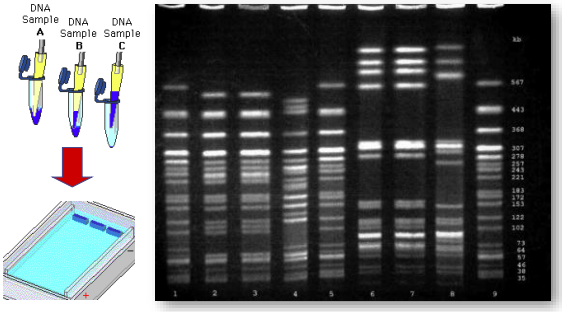
- Separation of DNA fragments by size
  - DNA is negatively charged
    - moves toward + charge in electrical field
  - agarose gel
    - "swimming through Jello"
    - smaller fragments move faster



Gel Electrophoresis

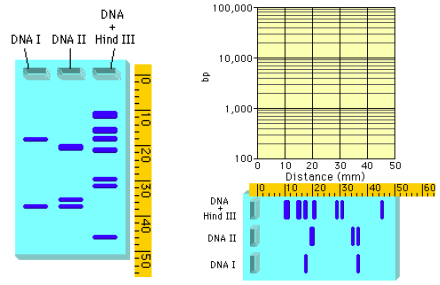


Gel Electrophoresis



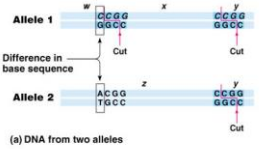
Measuring Fragment Size

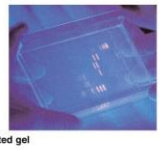
- compare bands to a known "standard"
  - usually lambda phage virus cut with HindIII
  - we use a 1kb ladder: 1000, 2000, 3000, etc…

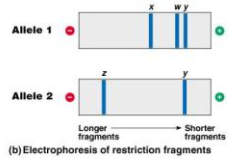


RFLP

- 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)