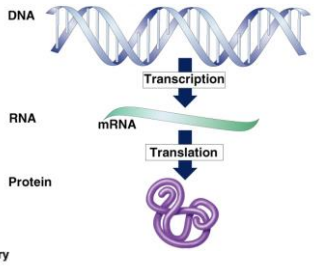
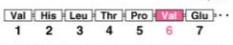
Chapter 15.1 – 15.3
Mutations

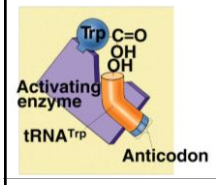


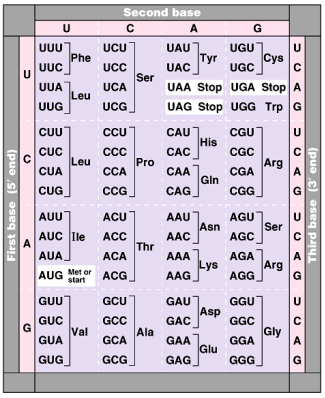


(b) Sickled red blood cells and the primary structure of sickle-cell hemoglobin

Universal Code

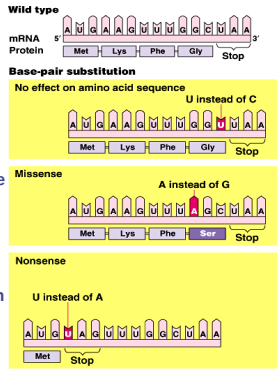
- Code is redundant
  - several codons for each amino acid
  - "wobble" in the tRNA
  - "wobble" in the aminoacyl-tRNA synthetase enzyme that loads the tRNA





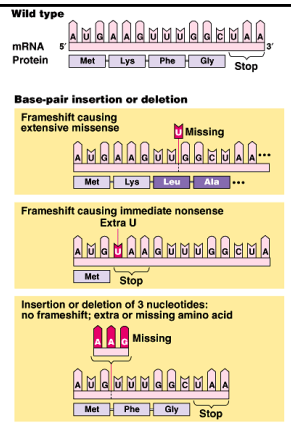
Mutations

- 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

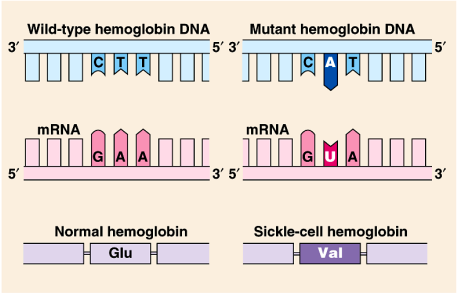


Mutations

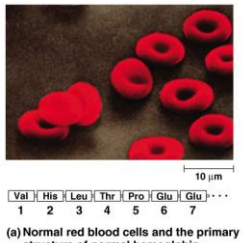
- Frameshift
  - shift in the reading frame
    - changes everything "downstream"
  - insertions
    - adding base(s)
  - deletions
    - losing base(s)



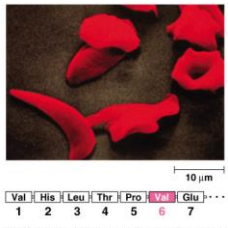
A Mutation Leads to Sickle Cell Anemia



Sickle Cell Anemia

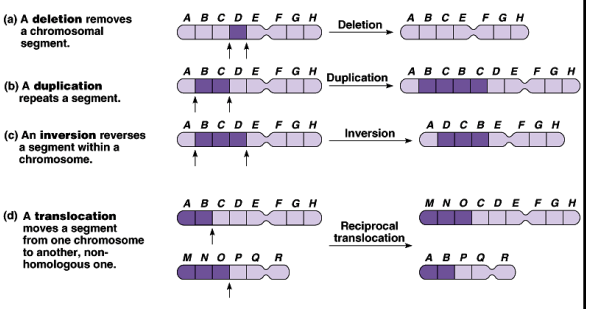


(a) Normal red blood cells and the primary structure of normal hemoglobin



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

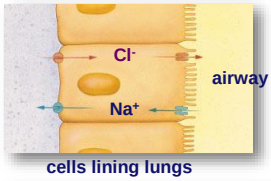
Changes in Chromosome Structure



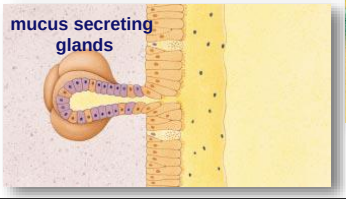
Cystic Fibrosis

- 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

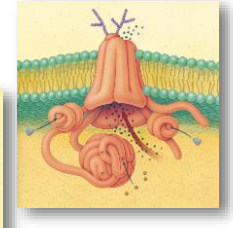
Normal Lungs



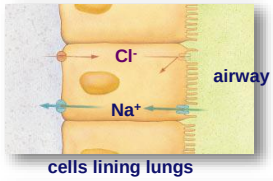
cells lining lungs



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

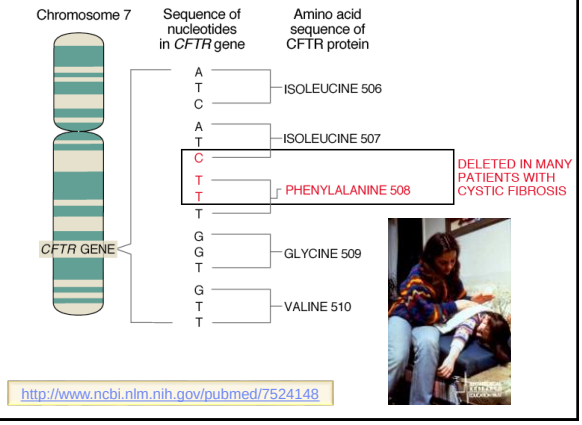


Lungs with Cystic Fibrosis



cells lining lungs

bacteria & mucus build up

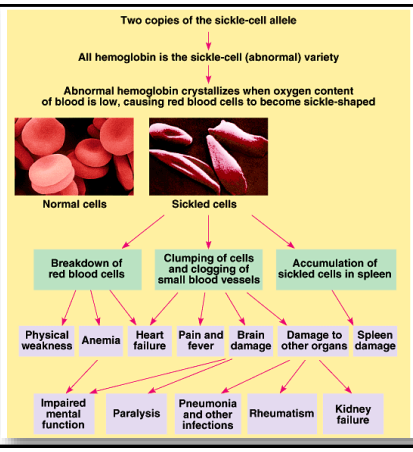


Tay-Sachs

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

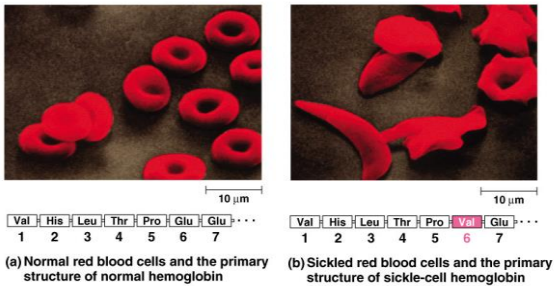
Sickle Cell Anemia

- 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



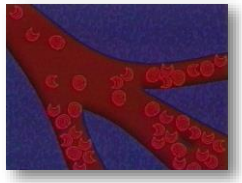
Sickle Cell Anemia

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



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…

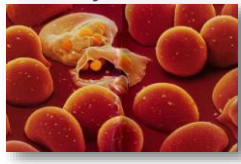


Heterozygote Advantage

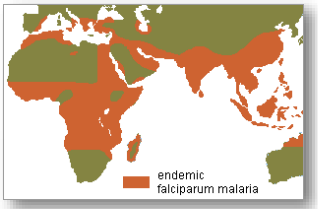
- Sickle cell frequency
  - high frequency of heterozygotes is unusual for allele with severe detrimental effects in homozygotes
    - 1 out of 400 African Americans
- Suggests some selective advantage of being heterozygous
  - sickle cell: resistance to malaria?
  - cystic fibrosis: resistance to cholera?

Heterozygote Advantage

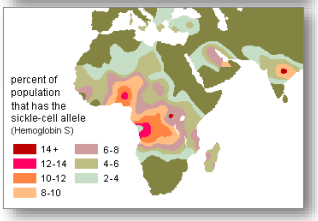
- Malaria
  - single-celled eukaryote parasite spends part of its life cycle in red blood cells
- In tropical Africa, where malaria is common:
  - homozygous normal individuals die of malaria
  - homozygous recessive individuals have shorter median life span
  - heterozygote carriers are relatively free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



Prevalence of Malaria



Prevalence of Sickle Cell Anemia



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

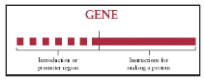
Repetitive DNA & other non-coding sequences account for most of eukaryotic DNA

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

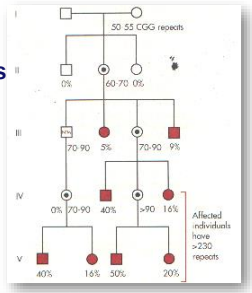
Genetic Disorders of Repeats

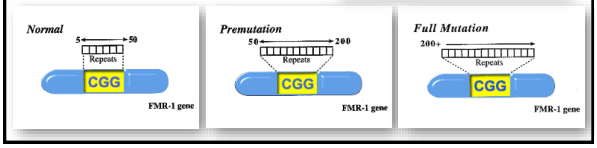
- Fragile X syndrome
  - most common form of inherited mental retardation
  - defect in X chromosome
    - mutation of FMR1 gene causing many repeats of CGG triplet in promoter region
      - 200+ copies
      - normal = 6-40 CGG repeats
    - FMR1 gene not expressed & protein (FMRP) not produced
  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





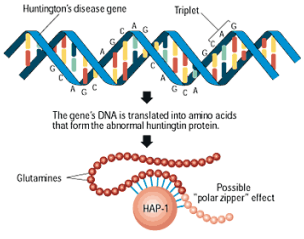
Huntington's Disease

- 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 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

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

CTGAATTCCG
GACTTAAGGC

CTG | AATTCCG
GACTTAA | GGC

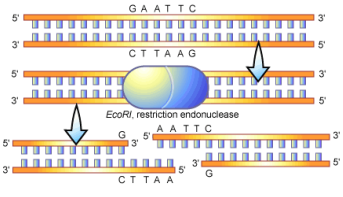
1960s|1978 Discovery of Restriction Enzymes

Werner Arber

Daniel Nathans

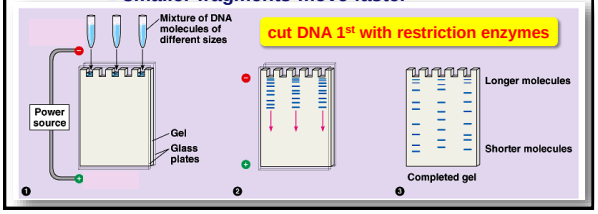
Hamilton O. Smith

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

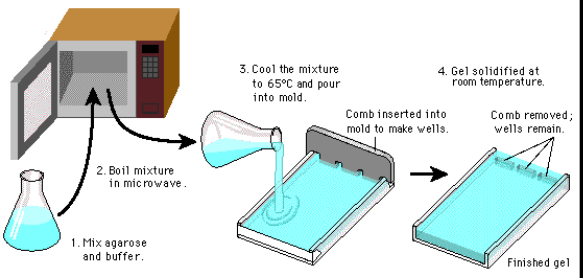


Gel Electrophoresis

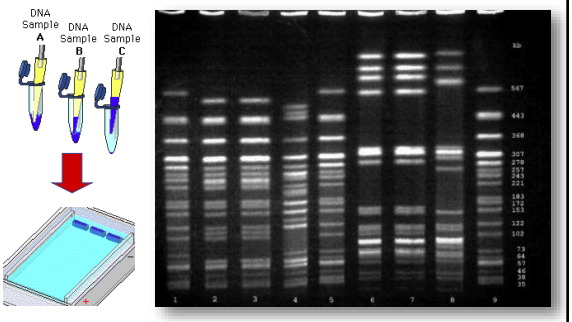
- Separation of DNA fragments by size
  - DNA is negatively charged
    - moves toward + charge in electrical field
  - agarose gel
    - "swimming through Jello"
    - smaller fragments move faster



Gel Electrophoresis

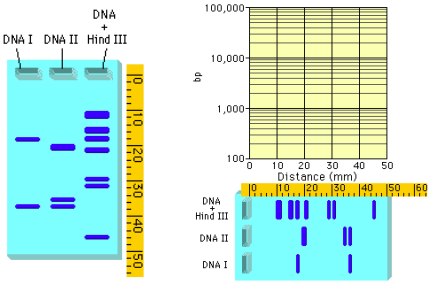


Gel Electrophoresis



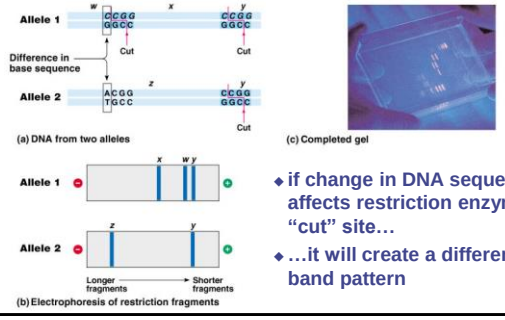
Measuring Fragment Size

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



RFLP

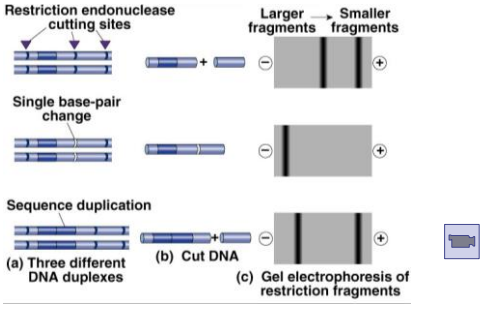
- Restriction Fragment Length Polymorphism
  - differences in DNA between individuals



- if change in DNA sequence affects restriction enzyme "cut" site…
- …it will create a different band pattern

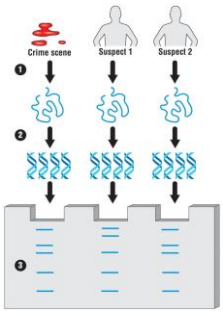
Polymorphisms in Populations

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



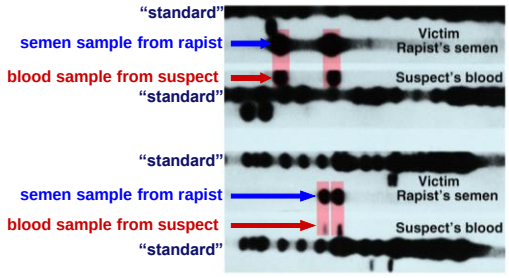
RFLP Use in Forensics

- DNA evidence



RFLP Use in Forensics

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



RFLP Use in Forensics

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