Today's Lecture (High-Points)

You can be identified by your own unique various regions

- Proteins + energy sources to replicate DNA.
  [DNA is a very stable structure.]
- RNA can be used as record to tell how DNA is active (making proteins). [Amazing useful in biotechnology.]
- Clinical use of Diagnosing DNA
- Human history via DNA Sequencing
- Evolution and DNA Sequencing: What was cavemen like?
- What was the original life form?

Proteins catalyze DNA Replication

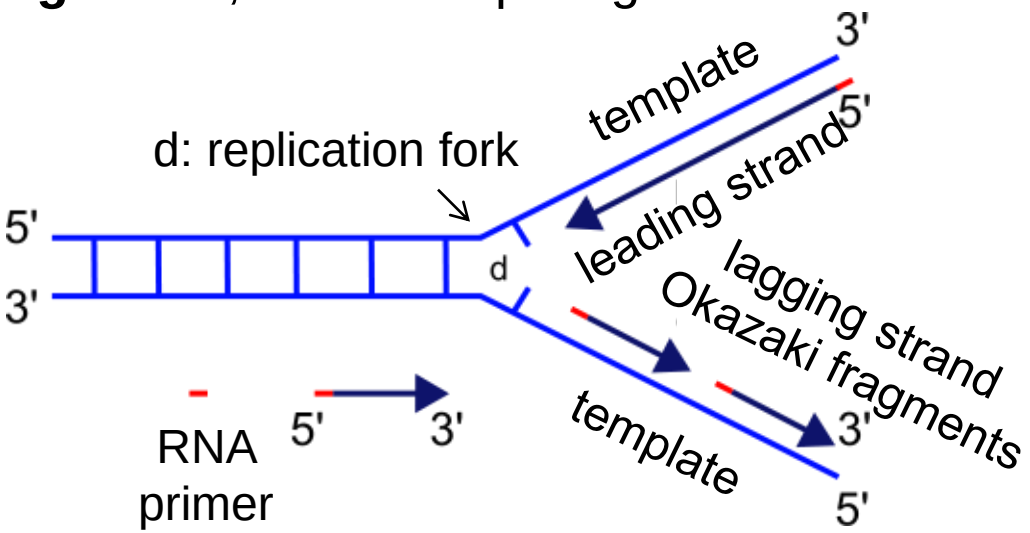
DNA is stable: need to catalyze rxn with Proteins & dNTPs

1. Helicase separates two strands.
2. SSB proteins stabilize ssDNA so stays open.
3. DNA polymerase adds new nucleotides

But:

One strand, (the leading strand) goes from 5'→ 3, DNA polymerase only goes from 5'→3'. No problem.

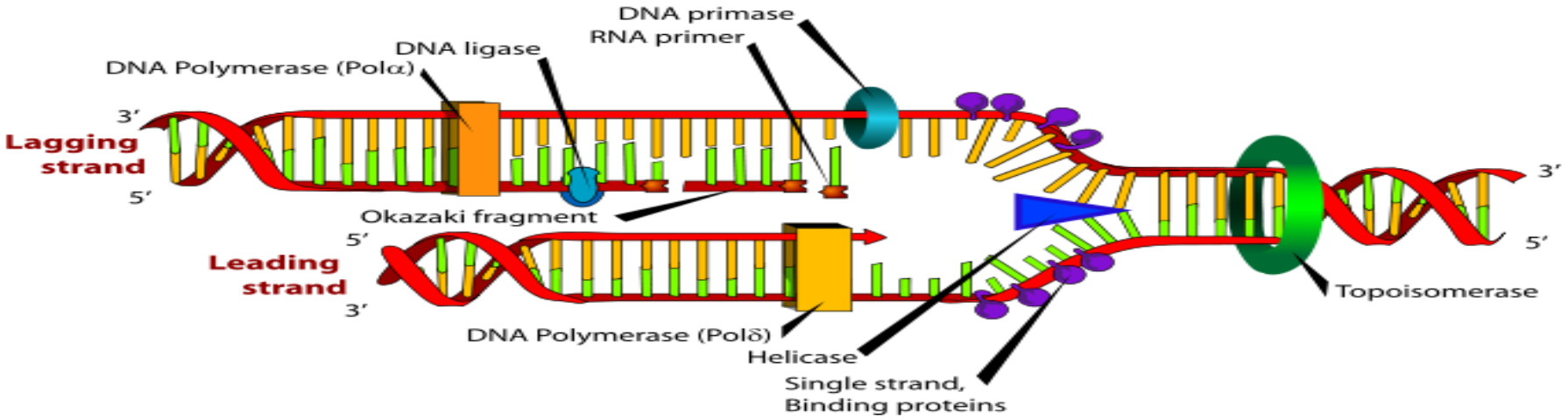
Other strand (the lagging strand) goes from 3' to 5'.It has to make **Okazaki fragments**, 100-200 bp long.



Proteins catalyze DNA Replication

Lots of proteins involved

Use ATP & dNTP as energy source



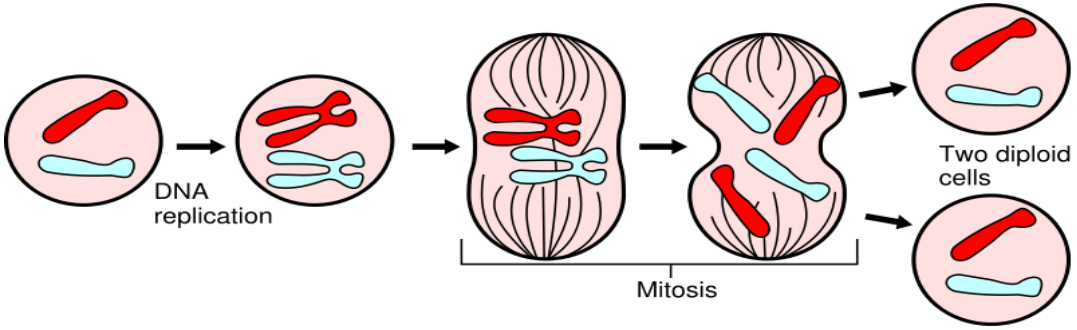
http://www.youtube.com/watch?feature=player_detailpage&v=hC_8y8fNkCw

Clinical Applications of DNA

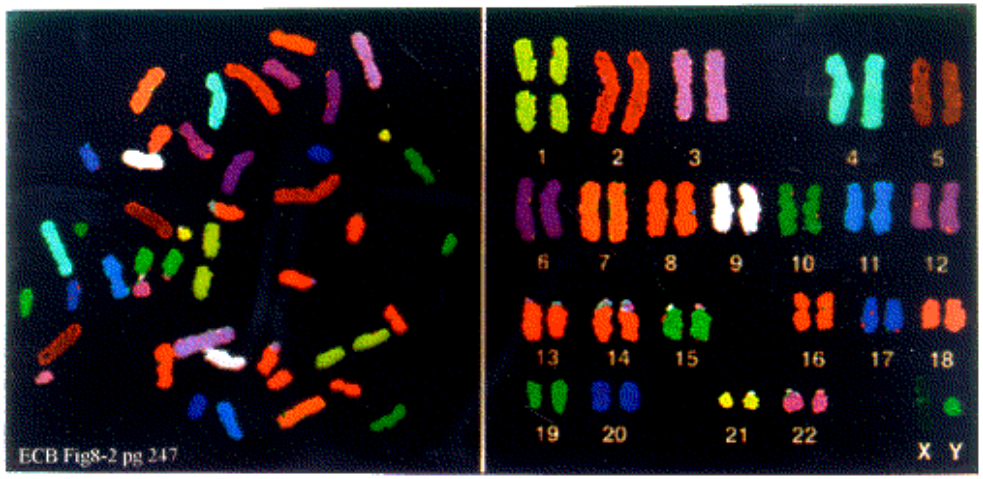
(Mitotic) Chromosomes can be identified by their unique DNA sequences

Each chromosome can be labeled uniquely

Mitosis

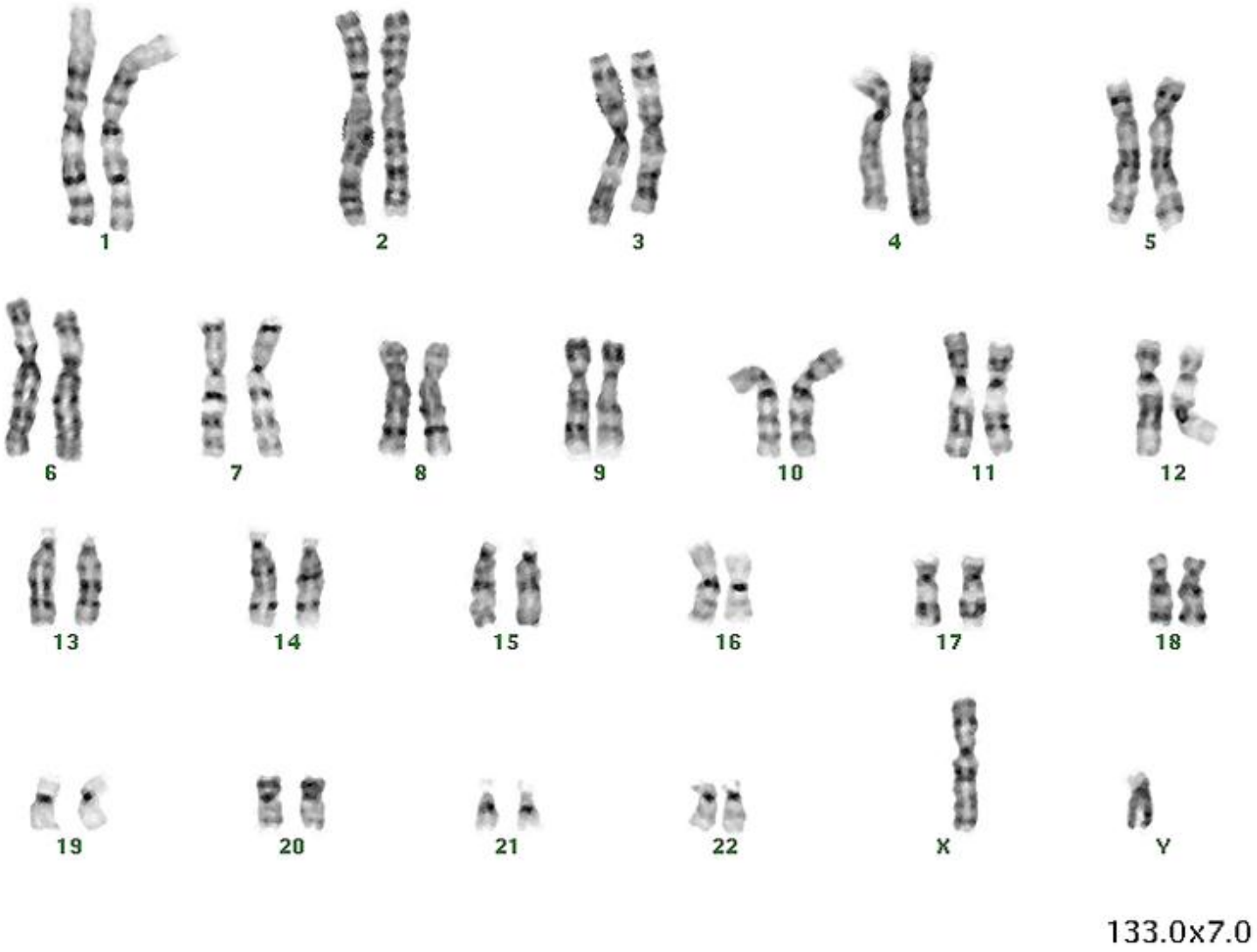


Fluorescently tagged DNA complementary to these unique sequences are used as markers.



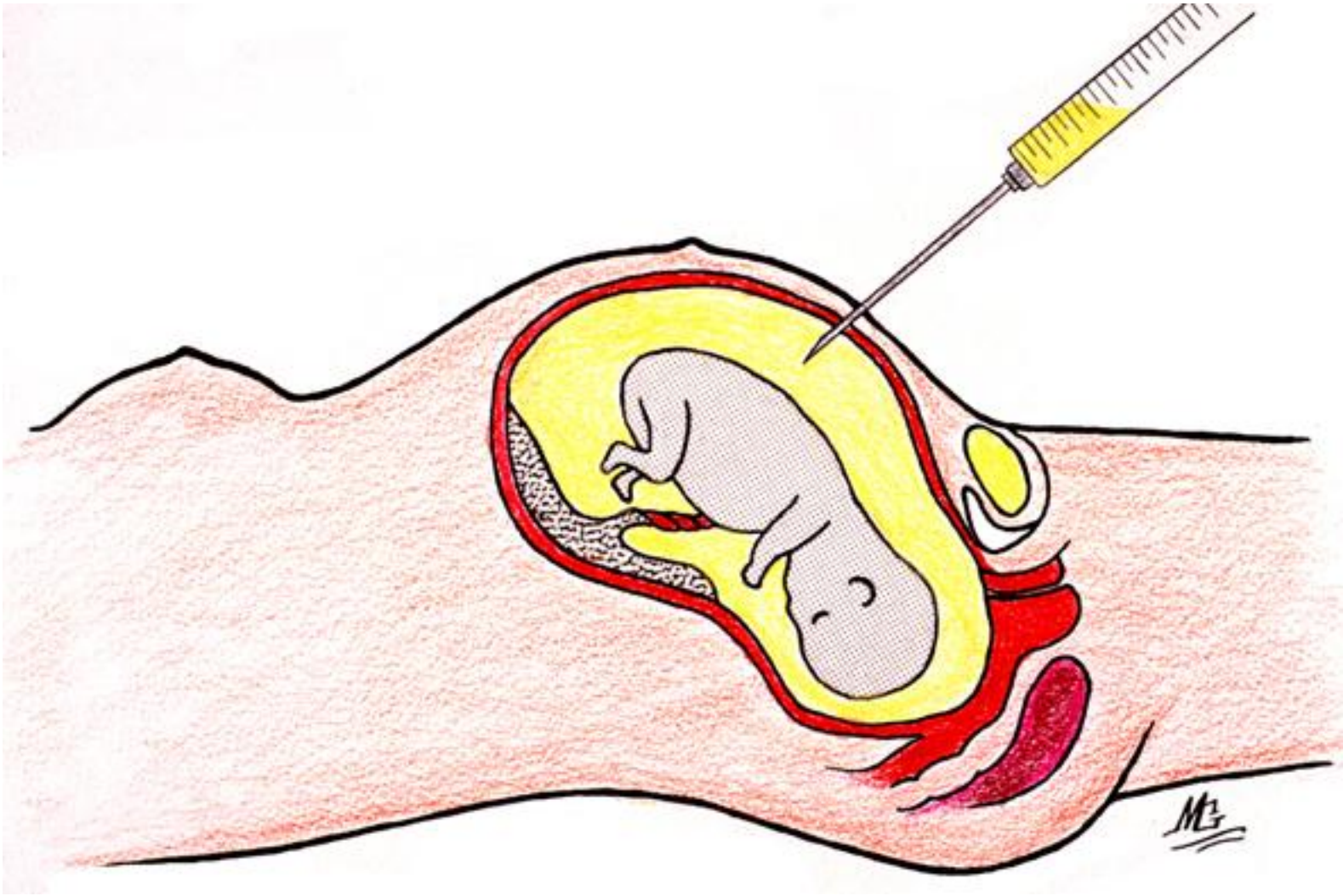
Cytogenetic Analysis

Cytogenetics – Karyotyping



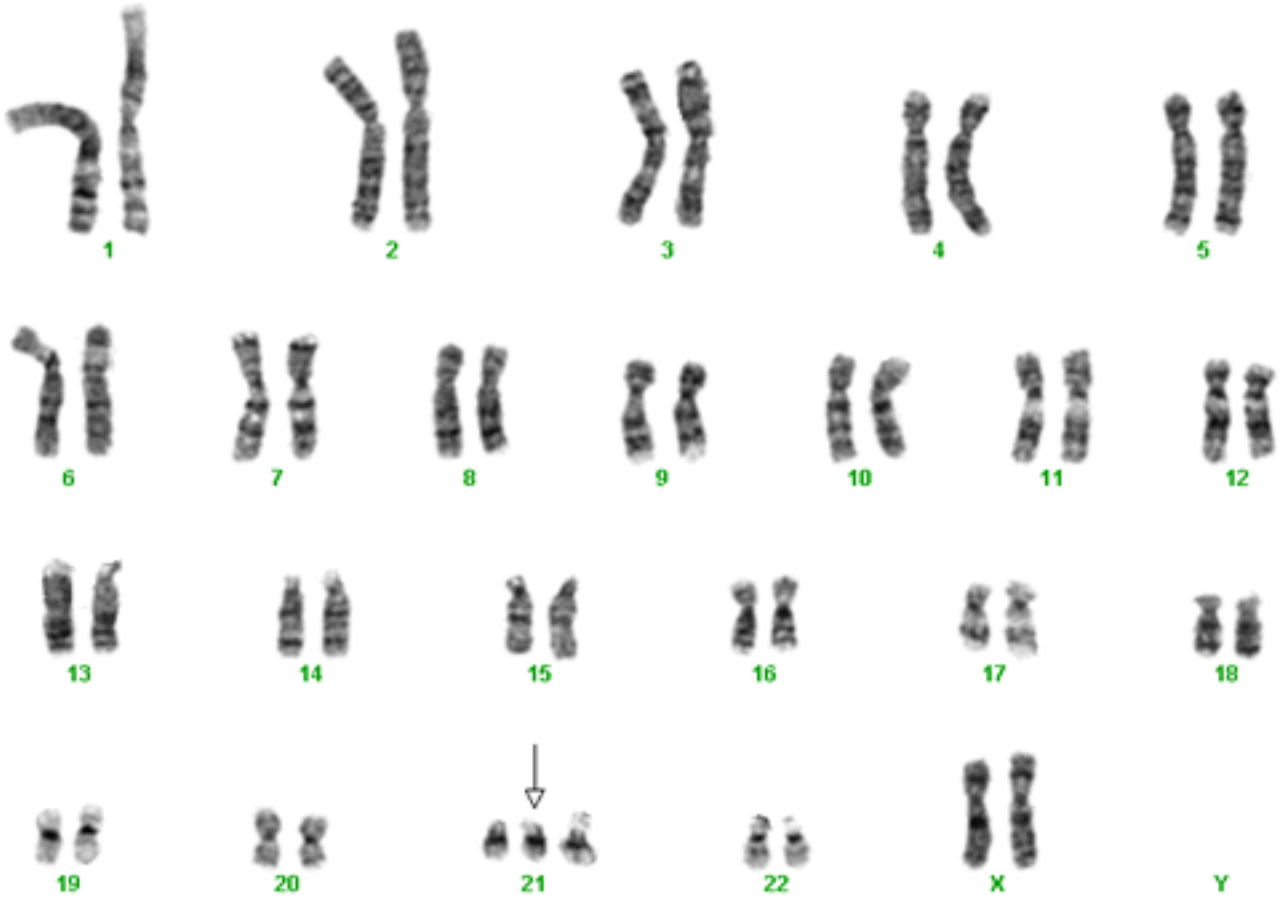
Can tell the genetics of sex (XX, XY)

Prenatal Diagnosis



http://atlasgeneticsoncology.org/Educ/Images/PrenatFig4.jpg

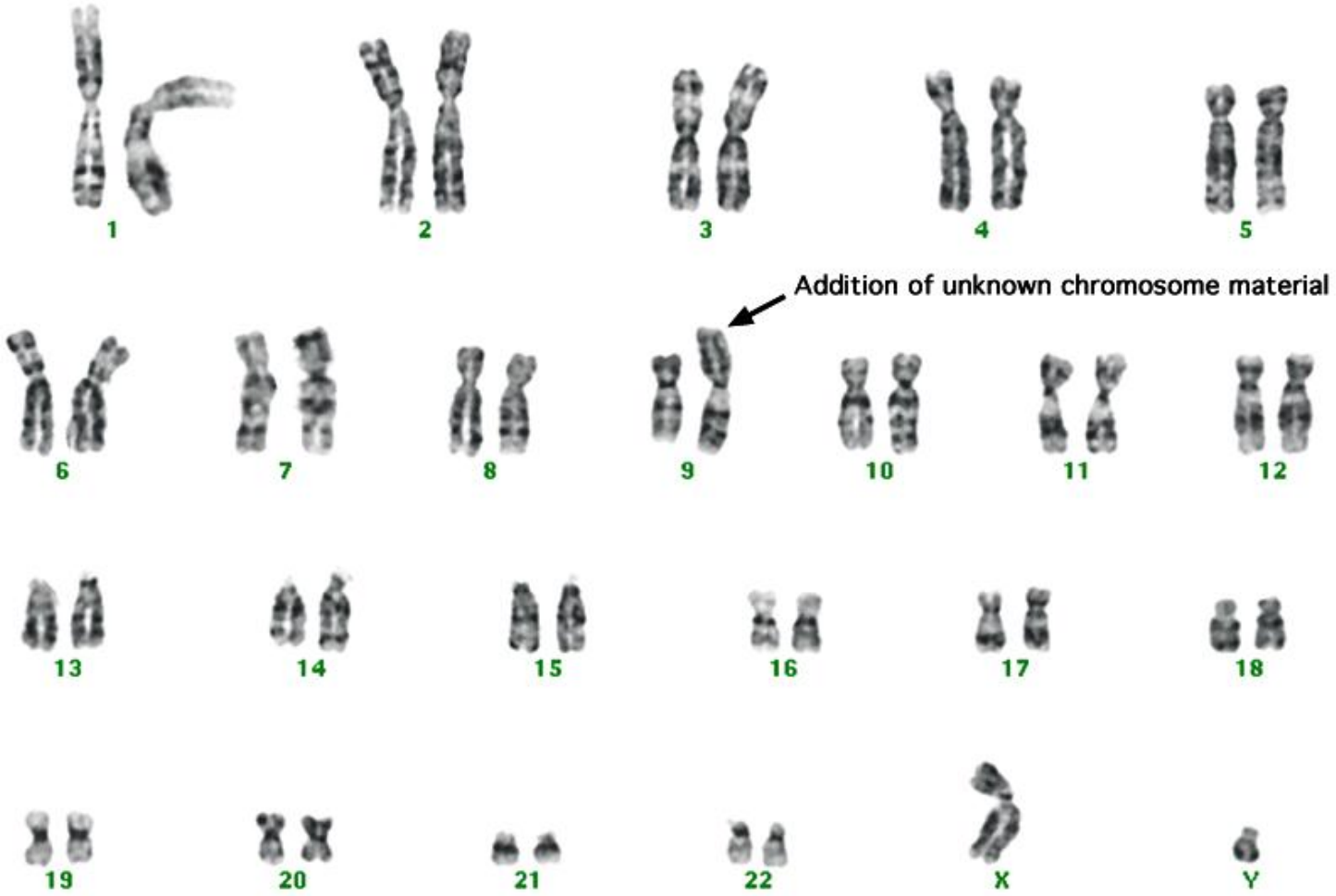
Cytogenetics – Prenatal Diagnosis



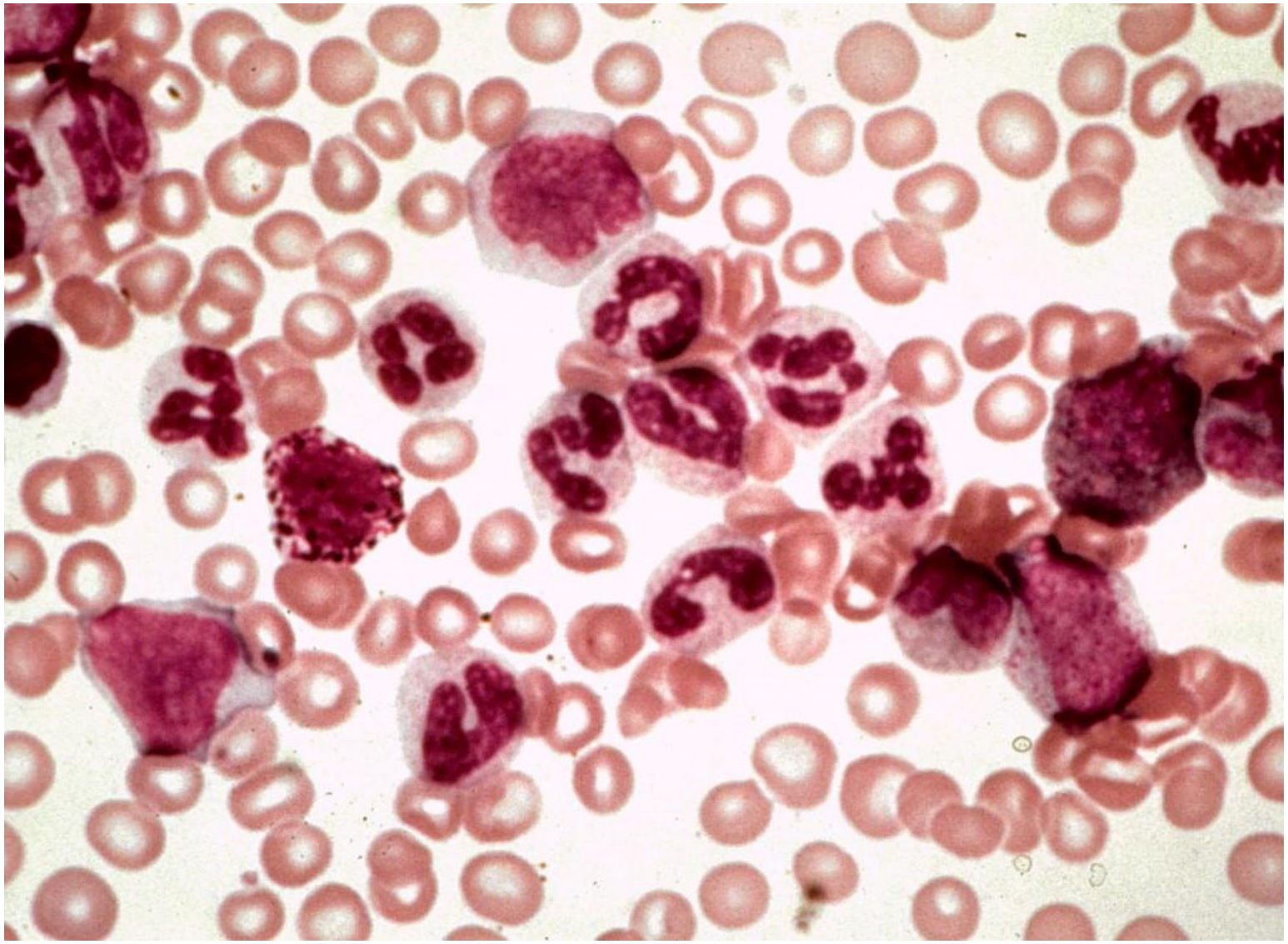
Trisomy 21: “Down’s Syndrome”

Postnatal Genetic Diagnostics

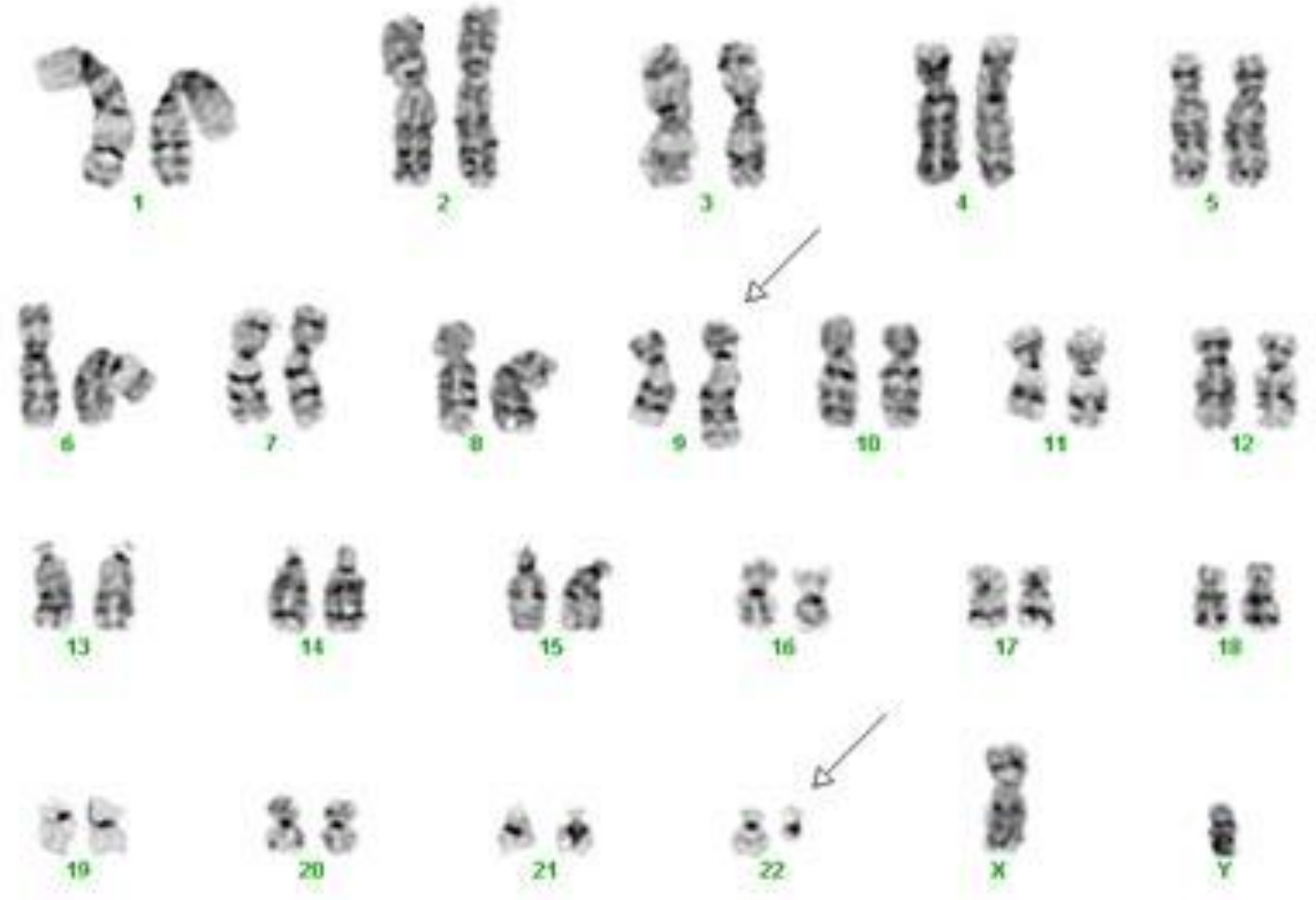
Postnatal Genetic Diagnosis



Cancer Genetic Diagnostics



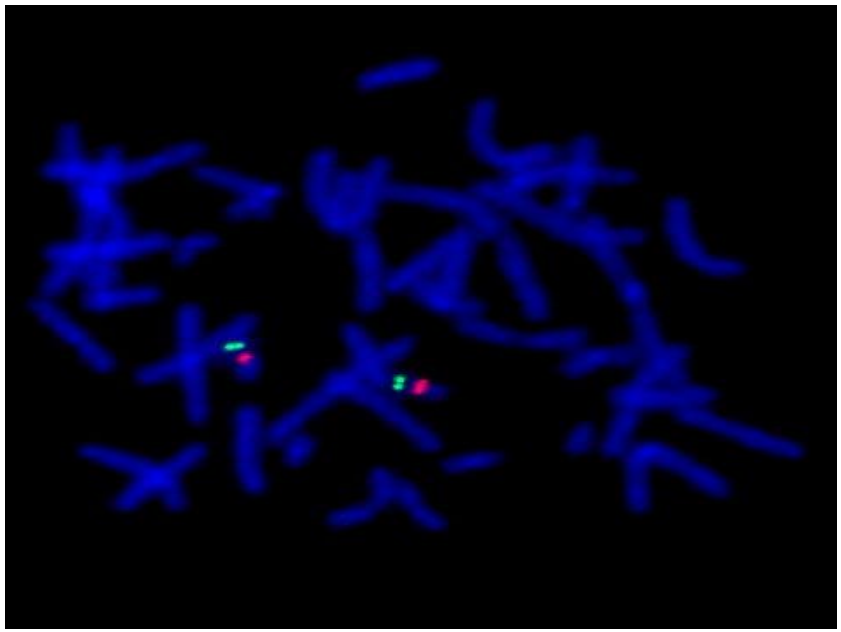
Cancer Genetic Diagnosis



Chromosomal Rearrangements can lead to Cancer

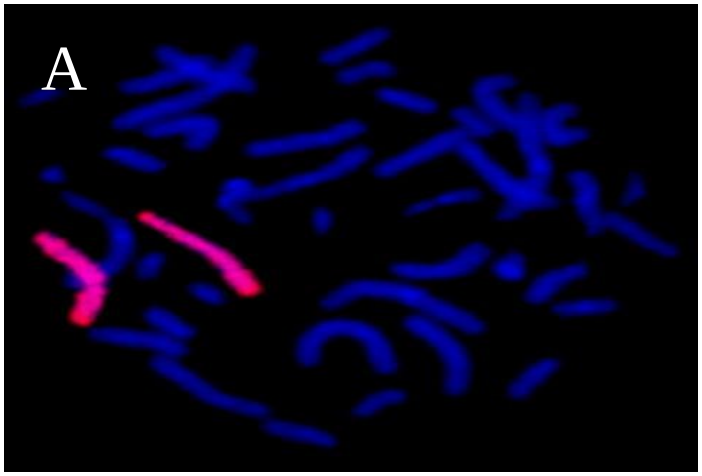
The location of genes on a chromosome can be determined

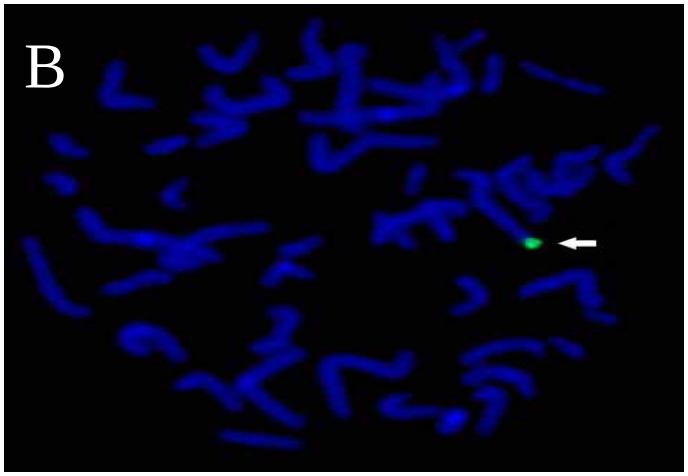
Fluorescence in Situ Hybridization (FISH)

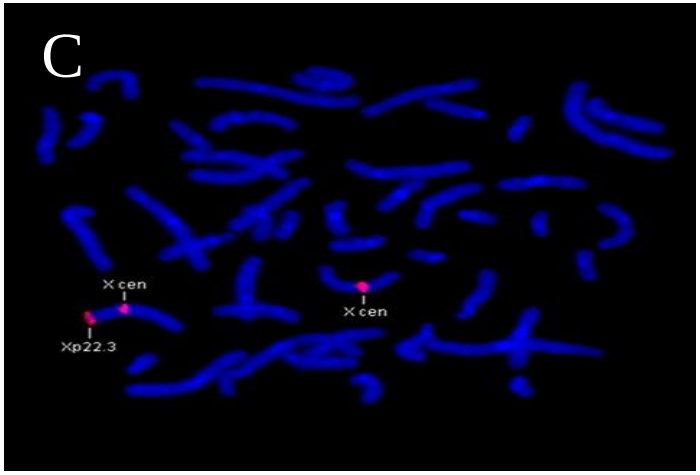


Green- # 22 marker- 22q13
Red- DiGeorge Syndrome region (if missing) at 22q11.2
(Person has 2→ normal)

FISH







A). Chromosone 4 "painted".

B) From same person in A, but hybridized with a probe for the terminal part of chromosome 4q. Only one green signal → one chromosome 4 is missing material from the terminal end of 4q.

C) X_{cen} → chromosome 22

Other: Steroid Sulfatase gene.

Two X chromosomes, 1 St.Su. gene → female carrier for Steroid Sulfatase Deficiency.

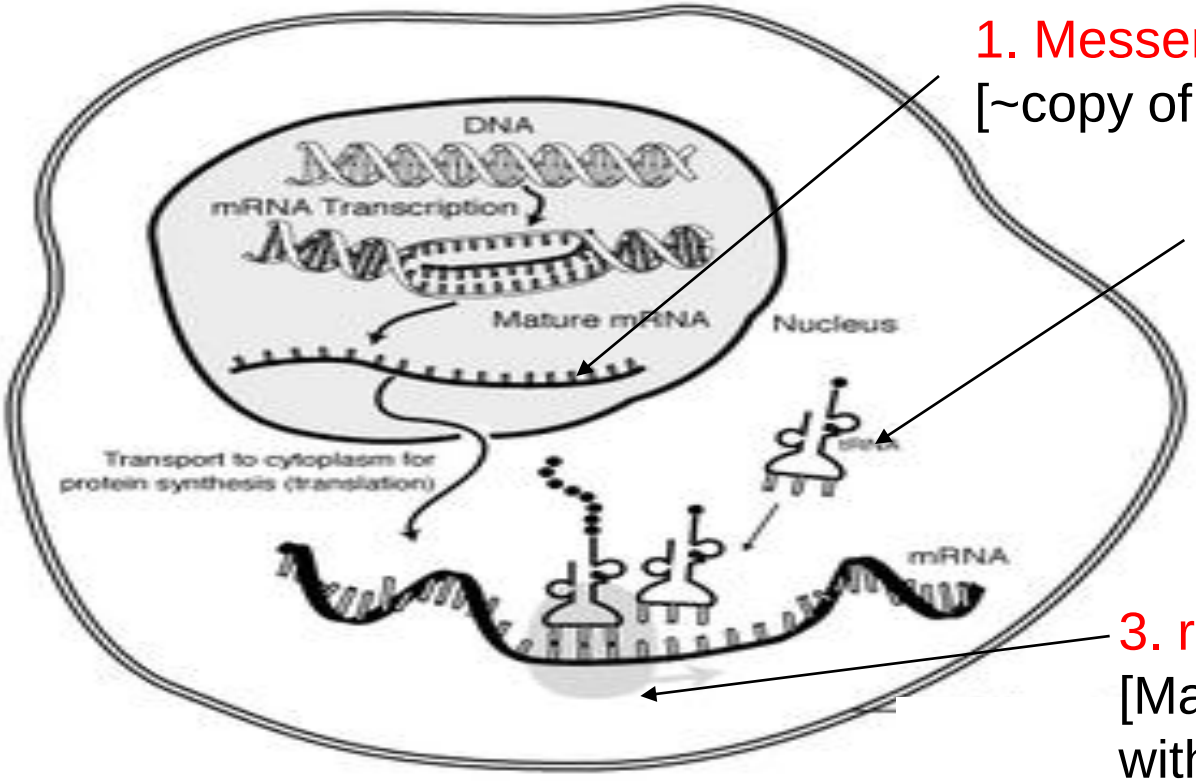
http://members.aol.com/chrominfo/metafish.htm

RNA

Sometimes want to look at Proteins to see what DNA is doing.
Hard to see proteins—use RNA

Details: 3 types of RNA
Messenger RNA (mRNA): "copy" of DNA
Transfer RNA– (tRNA) 3 bases of RNA→ amino acid
Ribosomal RNA—make protein using mRNA as copy

RNA has 3 different structures, names, and uses. mRNA, tRNA, rRNA



http://en.wikipedia.org/wiki/Messenger_RNA

1. Messenger RNA (mRNA)
[~copy of DNA]

2. transfer RNA (tRNA)
[binds to amino acid and codon for mRNA] 3 bases correspond to unique amino acid.

3. ribosomal RNA (rRNA)
[Makes up Ribosome, along with protein. Has catalytic activity– can form peptide bond.

We'll go into nitty-gritty details
about how amino acids are linked together

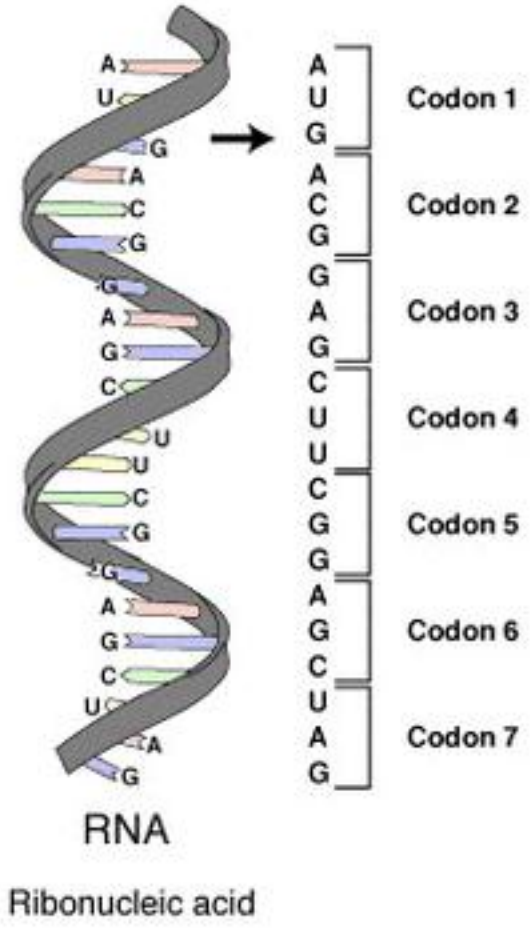
Why are there 3 nucleotides/codon (amino acid)?

Why not 2? 4?

There are 20 a.a.: need or x^3 (3^x ?) to code.

With x^3 , x=4, can code for $4^3 = 64$ amino acids.

Degeneracy…



RNA codon table

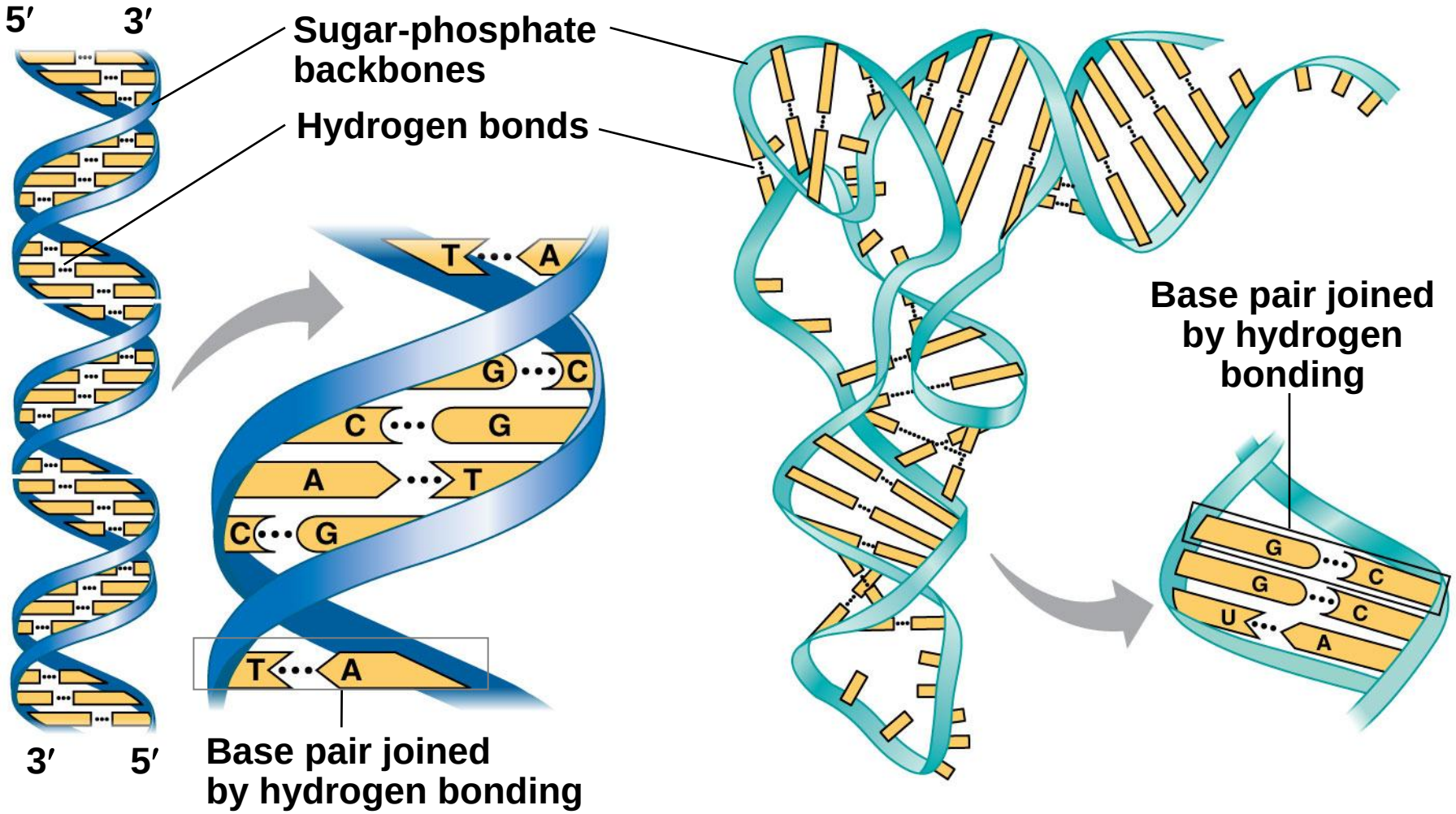
This table shows the 64 codons and the amino acid each codon codes for. The direction is 5' to 3'.

X = A,U,G,C

1st base	2nd base U	2nd base C	2nd base A	2nd base G
U	UUU (Phe/F)Phenylalanine UUC (Phe/F)Phenylalanine UUA (Leu/L)Leucine UUG (Leu/L)Leucine	UCX (Ser/S)Serine	UAU (Tyr/Y)Tyrosine UAC (Tyr/Y)Tyrosine **UAA Ochre (*Stop*)** **UAG Amber (*Stop*)**	UGU (Cys/C)Cysteine UGC (Cys/C)Cysteine **UGA Opal (*Stop*)** UGG (Trp/W)Tryptophan
C	CUX (Leu/L)Leucine	CCX (Pro/P)Proline	CAU (His/H)Histidine CAC (His/H)Histidine CAA (Gln/Q)Glutamine CAG (Gln/Q)Glutamine	CGX (Arg/R)Arginine
A	AUU (Ile/I)Isoleucine AUC (Ile/I)Isoleucine AUA (Ile/I)Isoleucine **AUG (Met/M)** **Methionine**, *Start*	ACX (Thr/T)Threonine	AAU (Asn/N)Asparagine AAC (Asn/N)Asparagine AAA (Lys/K)Lysine AAG (Lys/K)Lysine	AGU (Ser/S)Serine AGC (Ser/S)Serine AGA (Arg/R)Arginine AGG (Arg/R)Arginine
G	GUX (Val/V)Valine	GCX (Ala/A)Alanine	GAU (Asp/D)Aspartic acid GAC (Asp/D)Aspartic acid GAA (Glu/E)Glutamic acid GAG (Glu/E)Glutamic acid	GGX (Gly/G)Glycine

Notice/Recall that 3 bases cause more than 1 AA.

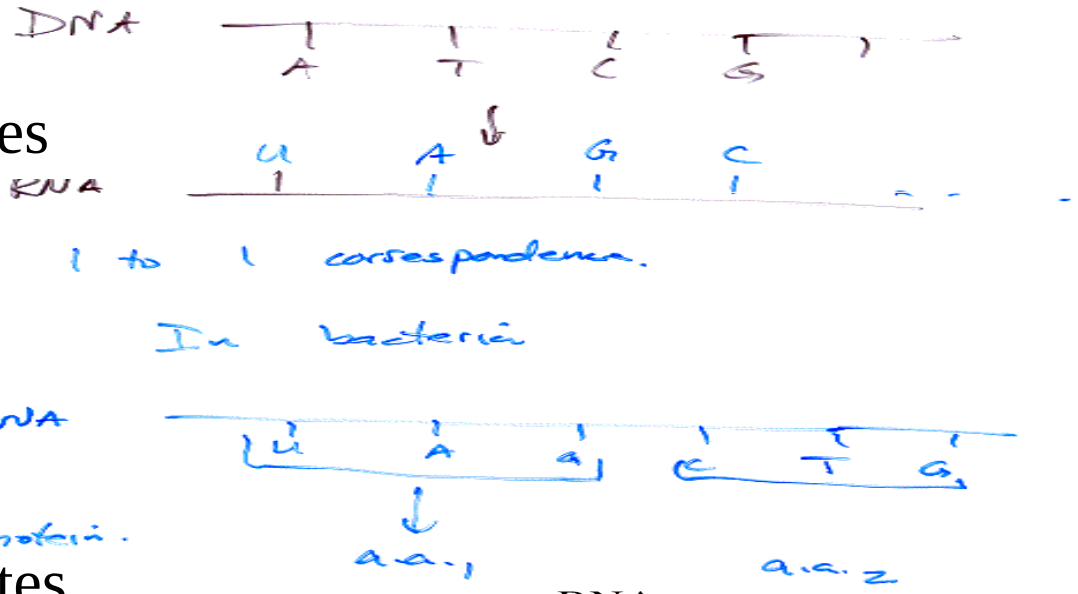
Figure 5.27



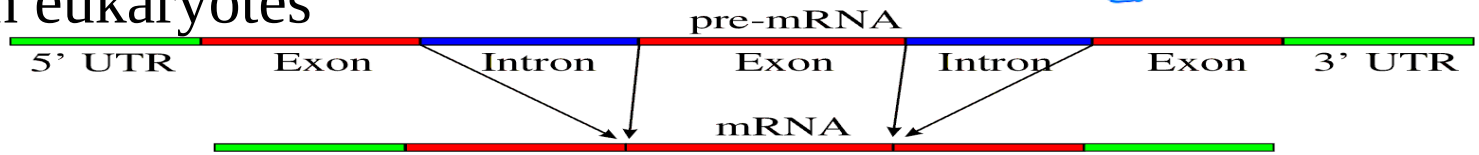
RNA is made from DNA

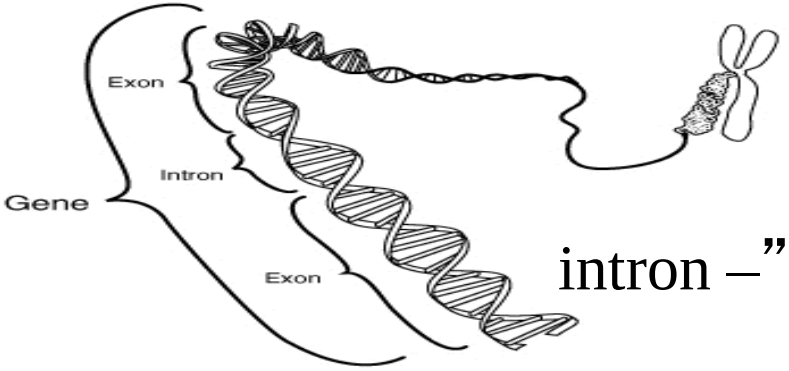
Introns and exons in Eukaryotes

In prokaryotes (messenger)



In eukaryotes





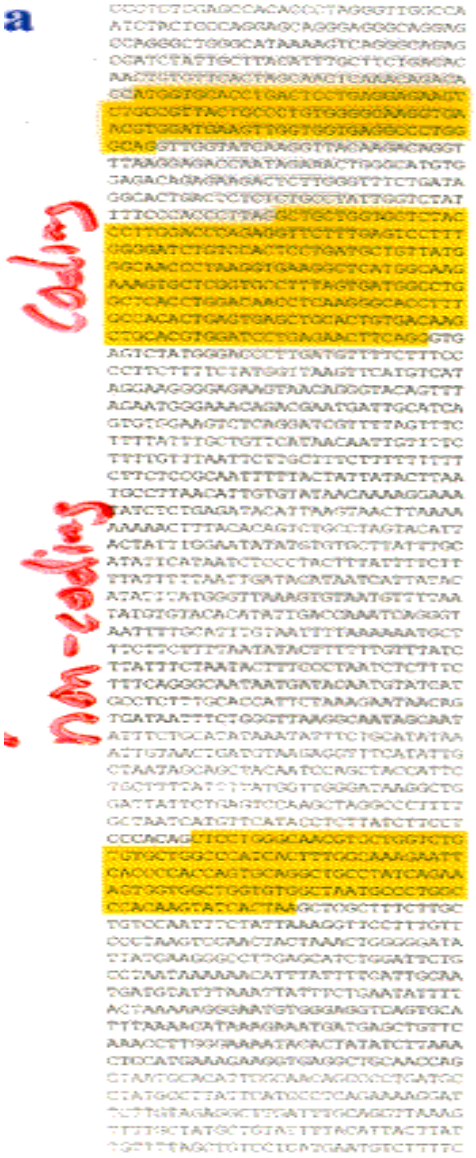
intron –"non-coding region" deleted

1993 Nobel Prize in Medicine to Phillip Allen and Richard J. Roberts

http://en.wikipedia.org/wiki/Intron

β-globin gene of Hemoglobin

example of introns/exons



Exploring the new world of the genome with DNA microarrays

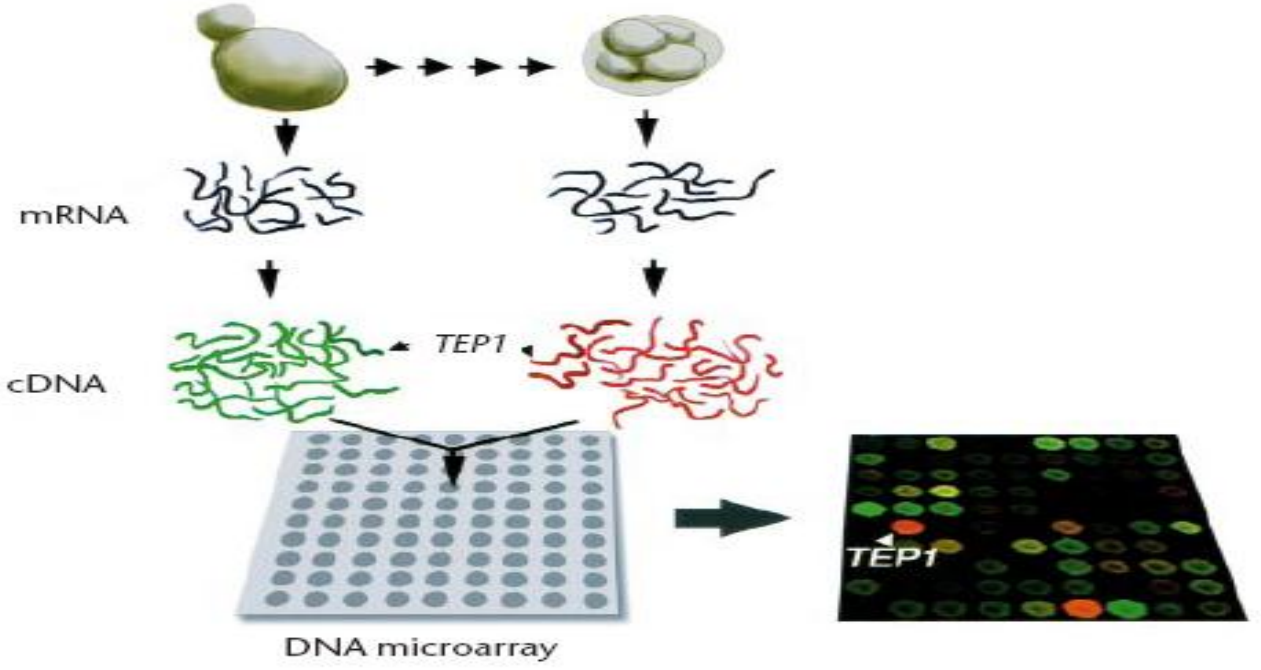
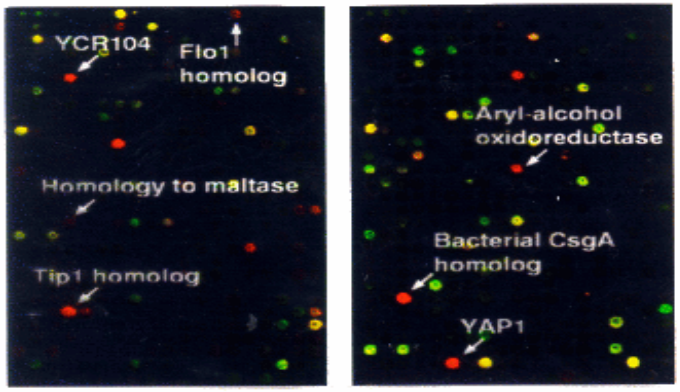


Fig. 1 Gene expression analysis using a DNA microarray.

Nature Genetics Supplement 21:33-37 (January 1999)

Gene Arrays ("Chips") can be made
Gene expression (i.e. RNA) can be detected on genome-wide scale : revolution!!

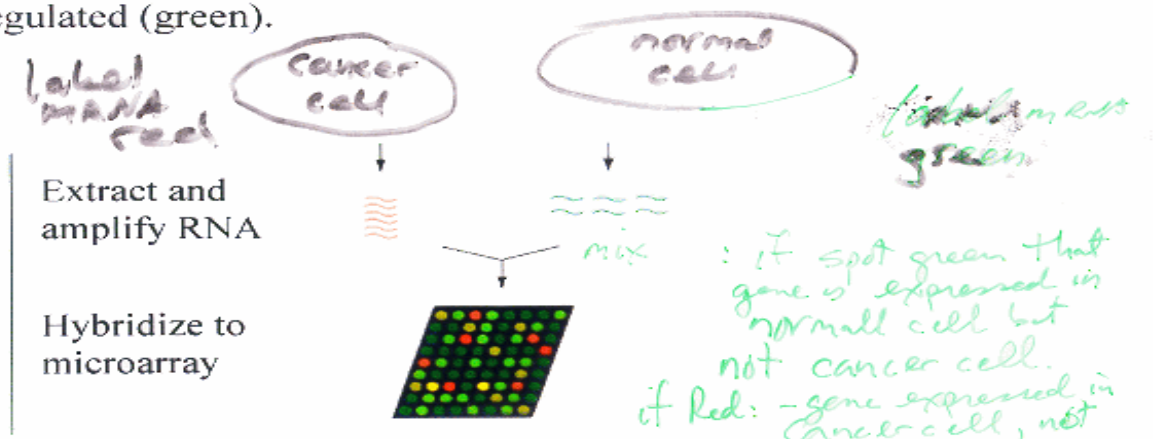
(Non-fluorescent) genes put on chip at defined position.



2 procedures

1. RNA from cell of interest extracted and fluorescently labeled e.g. with red dye. Added to chip, hybridized. Unbound washed away. Which position lights up tells which gene is active in cell!

2. By using red from "normal" cell, green from test cell, can tell which genes are up-regulated (red) and which are down-regulated (green).

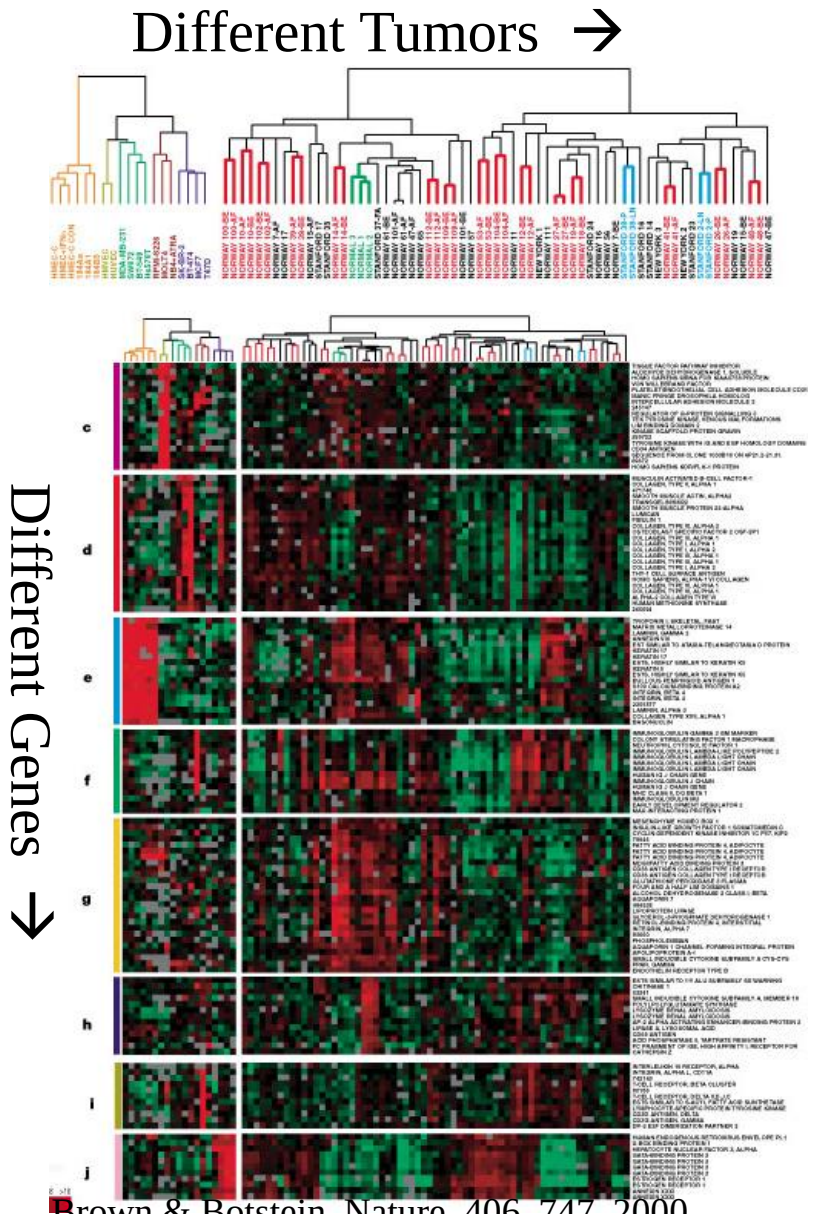


Molecular portraits of human breast tumours

Gene chips can be used to follow genetic changes during cancer and cancer treatment

FIG. 1. Variation in expression of 1,753 genes in 84 experimental samples.

Data are presented in a matrix format: each row represents a single gene, and each column an experimental sample. Green squares, transcript levels below the median; black squares, transcript levels equal to the median; red squares, transcript levels greater than the median; grey squares, technically inadequate or missing data.



Brown & Botstein, Nature, 406, 747, 2000

Affymetrix, 1992. Marriage of Silicon and Genes. Semiconductor manufacturing techniques could be united with advances in combinatorial chemistry to build vast amounts of biological data on a small glass chip.

Gene Chips can be used to follow genetic changes during development

Different spots light up, i.e. genes turned on,
at different times (developmental stages) in life cycle.

Molecular Evolution can be determined by DNA Sequences …or by protein sequences…or by protein structures

(Nice chapter in Berg, Tymoczko, Stryer, 5th ed.)

You and parents have same DNA by >>99.9%

You and me (unrelated humans) are 99.4% the same.

You and chimp: 99% the same.

We are related to a cauliflower! (about 50% DNA similarity)

Protein structures most closely related to function…best.

Can sometimes see similarities in structure even where a.a. or DNA sequences are very different …hard to tell.

http://en.wikipedia.org/wiki/Common_descent

www.famous-scientists.net/jane-goodall.html

Example: Myoglobin
Oxygen-carrying protein in muscle.

Amino acid sequence

Humans & Chimps myoglobin differ by 1 a.a. out of 153.

What's the probability that two peptide sequences are identical based on random chance?

$(1/20)^{153} \sim 0$

Just as you and parent look alike cause you came from parent, you and monkey…you and cauliflower have a common "parent".

We are all related

Cauliflower, Whales, Chimps, Humans...

Genes: Whale and Humans have similar DNA sequence for Maleness.

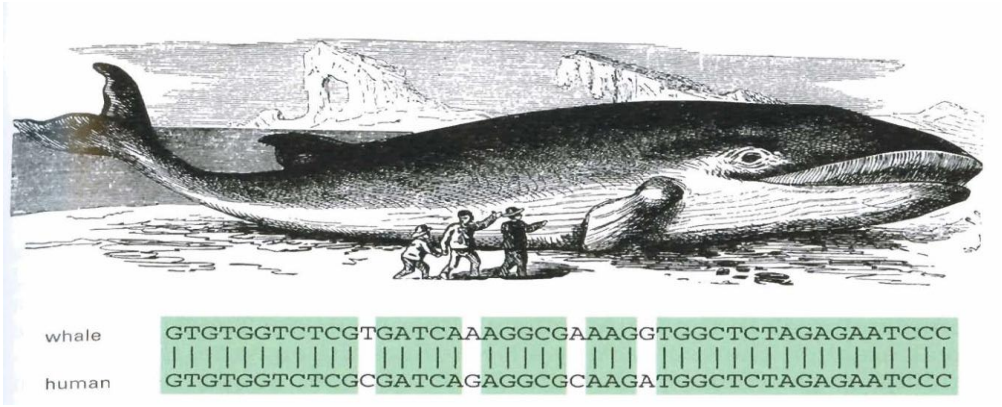


Figure 6-27 The sex-determination genes from humans and whales are unmistakably similar. Although their body plans are strikingly different, humans and whales are built from the same proteins. Despite the length of time since humans and whales diverged, the nucleotide sequences of many of their genes are still closely similar. The sequences of a part of the gene encoding the protein that determines maleness in humans and in whales are shown one above the other, and the positions where the two are identical are *shaded.*

Essential Cell Biology, p. 215

Charles Darwin

Big Ideas in Biology

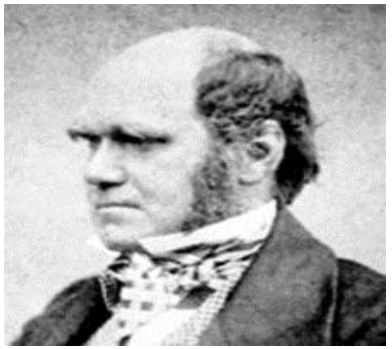
(Physics is about great laws…biology has one.)

"There is a grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one"-- Charles Darwin, Origin of Life, 1860.

Evolution

-- Life evolved from simpler forms

--One of the best tested scientific theories around



Evolution is a series of tricks/random events

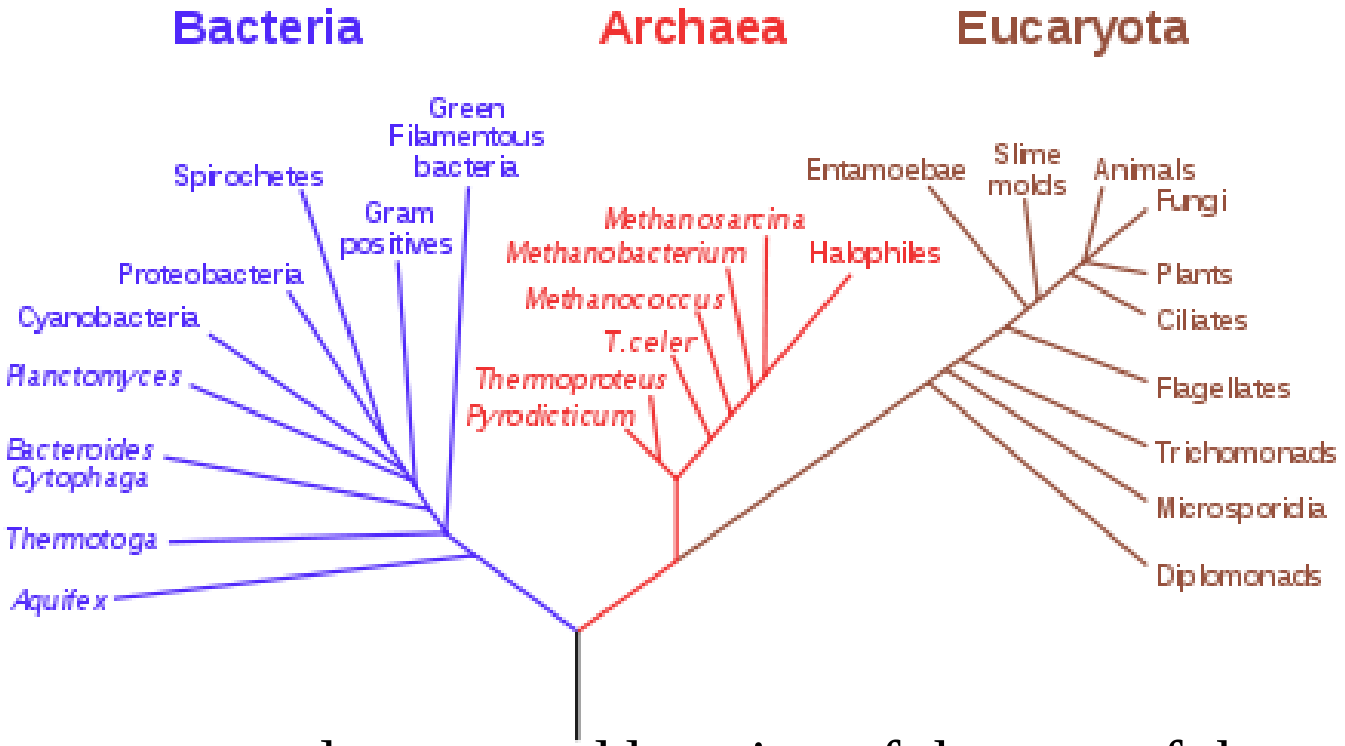
Build complex beings from simpler parts

Often many ways of doing this

Our life form is just one.

Scientists now believe that the most recent common ancestor of all currently living organisms appeared about 3.9 Billion years ago.

Phylogenetic Tree of Life



The most commonly accepted location of the root of the tree of life is between a monophyletic domain Bacteria and a clade formed by Archaea and Eukaryota of what is referred to as the "traditional tree of life" based on several molecular studies starting with Carl Woese.

http://en.wikipedia.org/wiki/LUCA

Artificial Selection

All dogs came from wolves but through artificial selection have bred certain dogs for certain traits.

http://en.wikipedia.org/wiki/Common_descent

Common vegetables such as cabbage, kale, broccoli are descendants of wild cabbage plant.

DNA Sequencing

Decoding 4,000 year old DNA (From Nature, 2009)

Less 10 years after first living person's DNA sequenced

"Inuk" died on an island off Greenland called Qeqertasussuk. He left bits of hair and bone that the permafrost preserved, including his complete genome.

Inuk's genes reveal he was a fairly young man, robustly built to exist in a frigid climate, with A-positive blood, dark skin, brown eyes, and thick, black hair on a scalp genetically susceptible to baldness. He was a palaeo-Eskimo, and by comparing his genome to other living people, they deduced that he was member of the Arctic Saqqaq, the first known culture to settle in Greenland whose ancestors had trekked from Siberia around the Arctic circle in pursuit of game.

Contamination a big problem: The best place to find it is entombed in ice, where it is preserved by the cold and protected from contamination. Hair doesn't as readily absorb contaminants, and its surface can be bleached clean. They also tagged the millions of fragments of extracted DNA with a barcode-like sequence to distinguish them from stray modern human DNA.

Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today's subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.