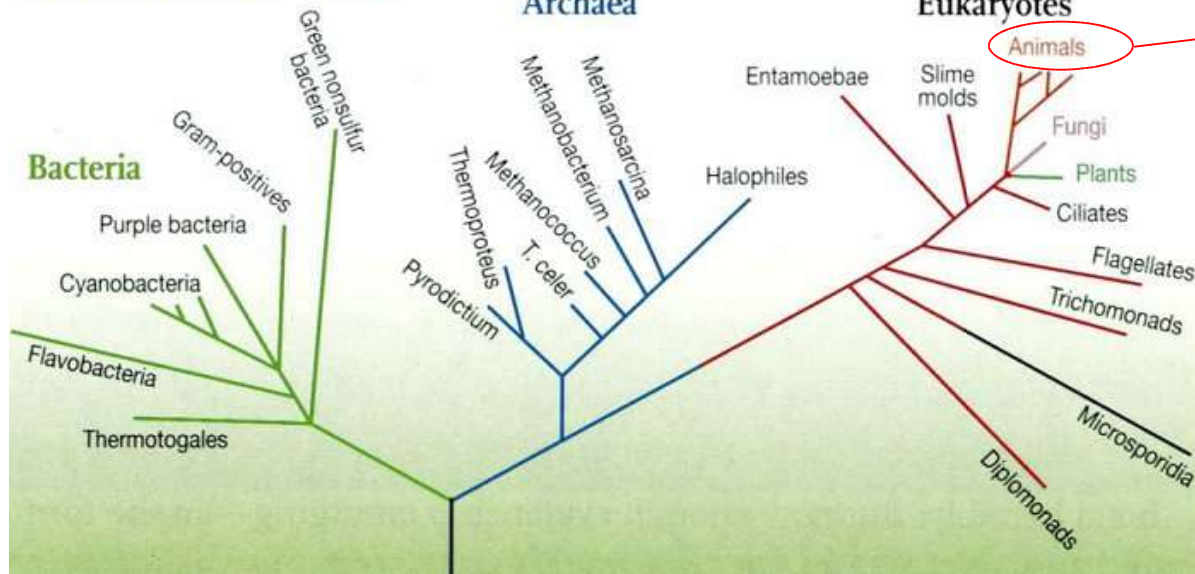


Some Overarching Organizational Rules:

- Biological parts self-assemble
- Biological parts are actively transported
- Biological parts interact specifically

Two Generic Cell Types:

THE TREE OF LIFE



Us

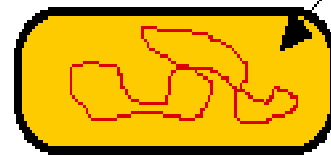
Higher Organisms



Eukaryote

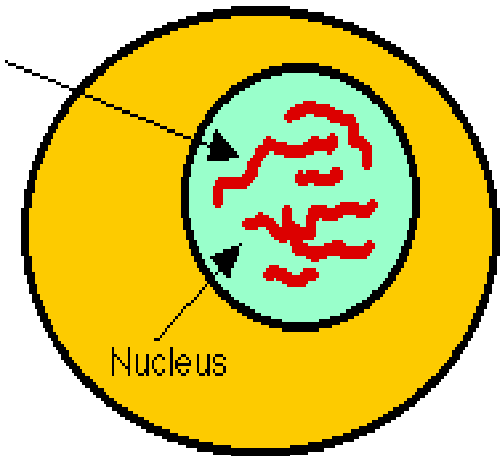
Bacteria

Prokaryote



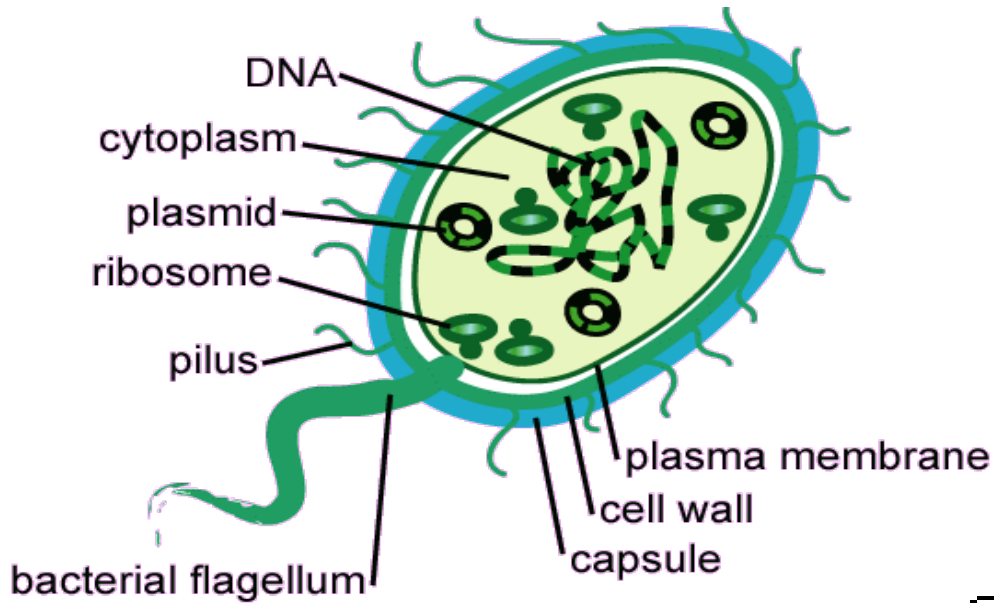
DNA organized in a single chromosome.
No nucleus. No mitosis.

DNA



DNA organized in multiple chromosomes inside a nucleus.
Mitotic division.

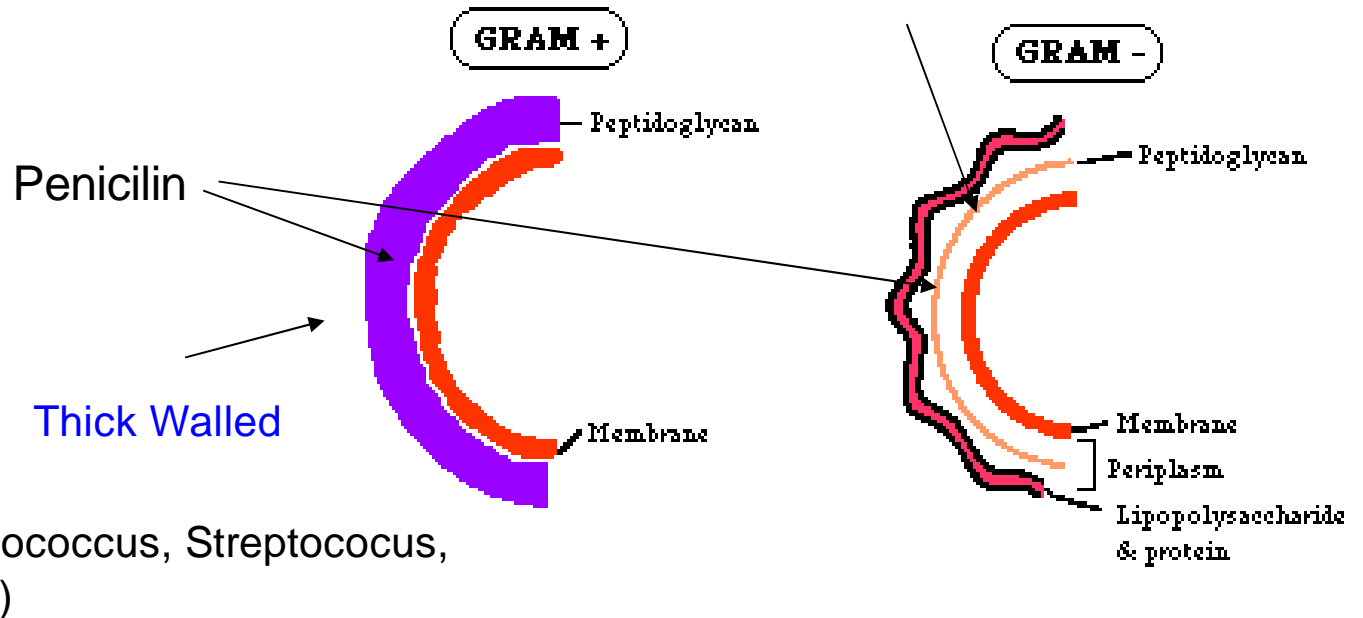
Prokaryotic Cells:



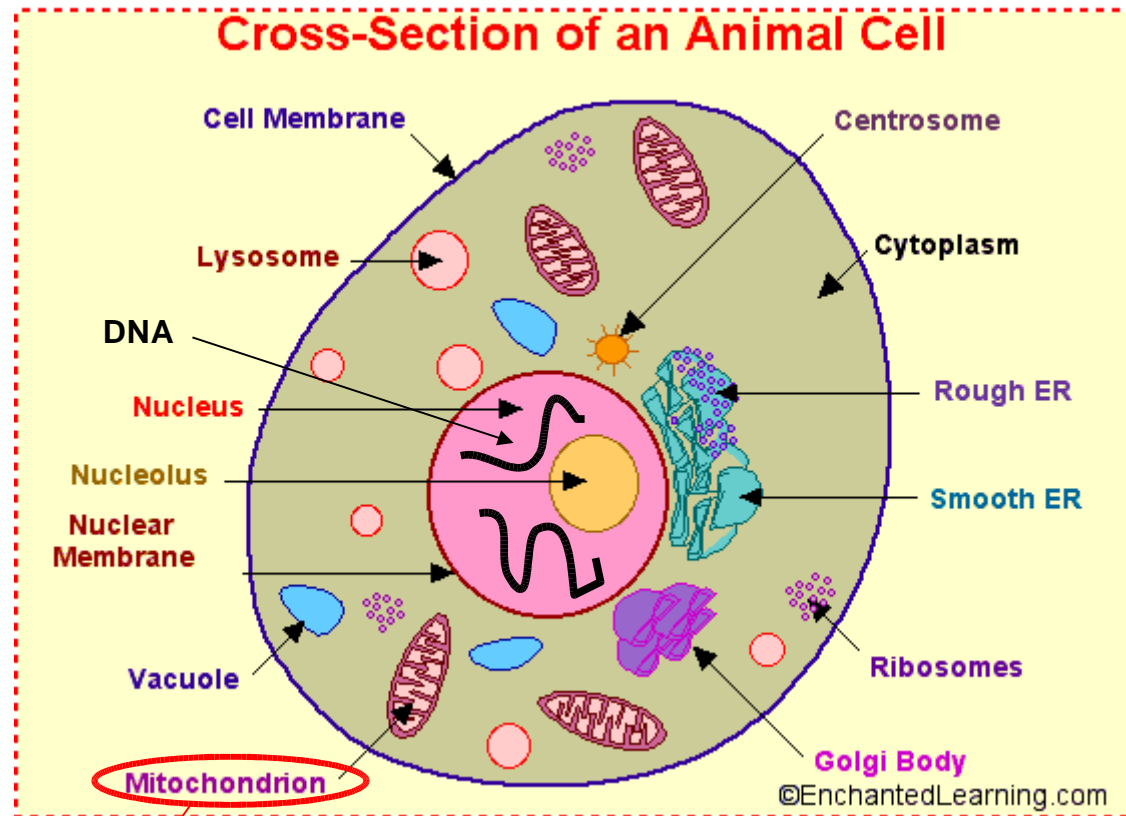
Bad pathogens

(the plague, salmonella, meningitis, E. coli, cholera)

Thin Walled

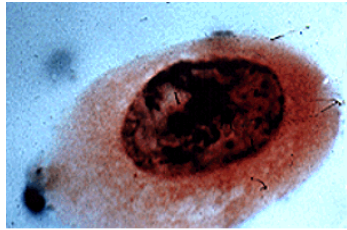


Eukaryotic Cells:

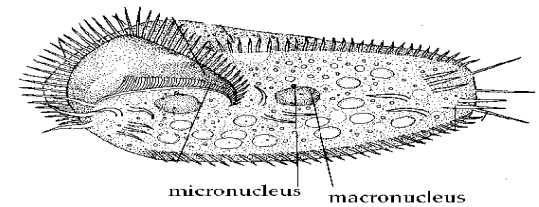
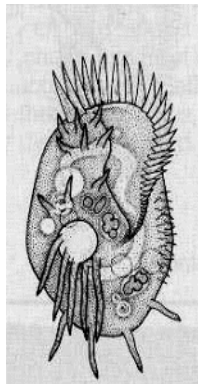
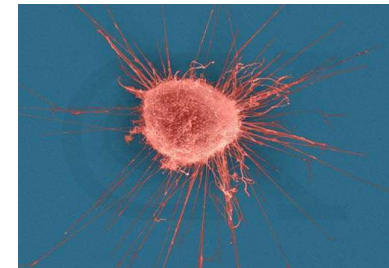
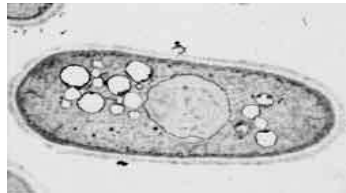
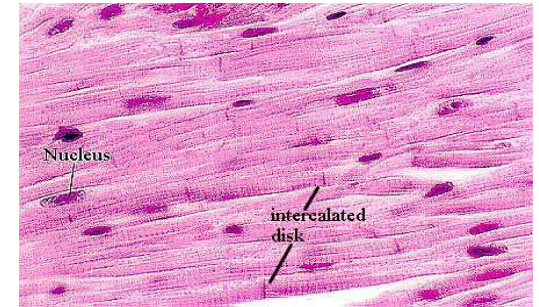
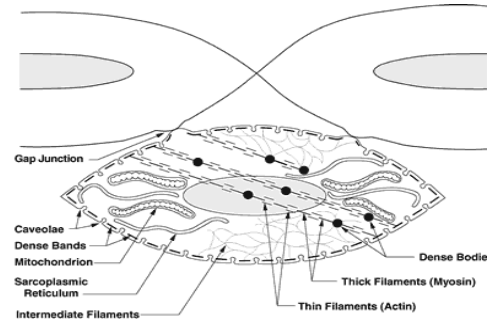
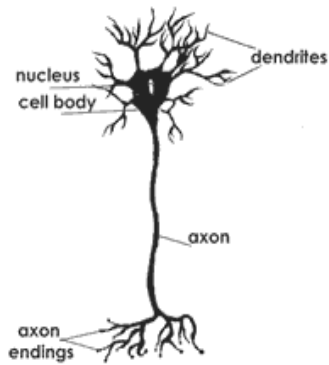


The mitochondria make energy – very important

Huge variety in cells:



©1995 Cornell University Medical College



The Sizes of Biological Parts

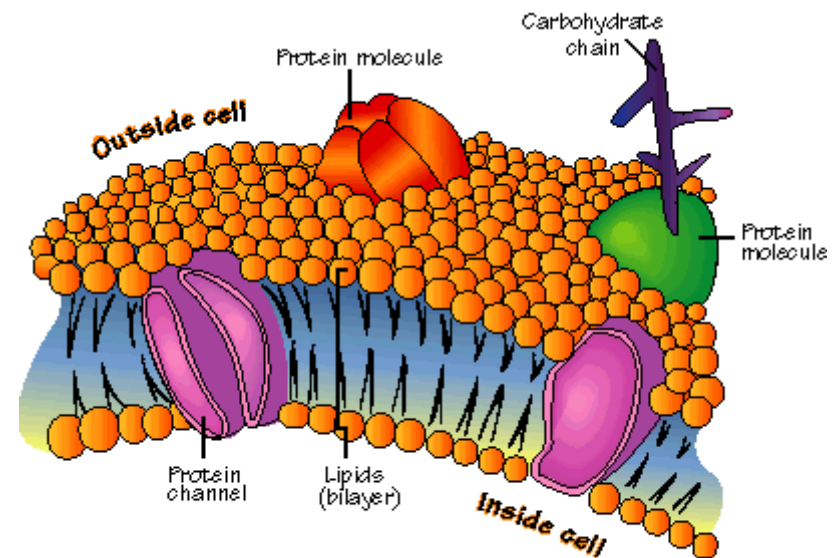
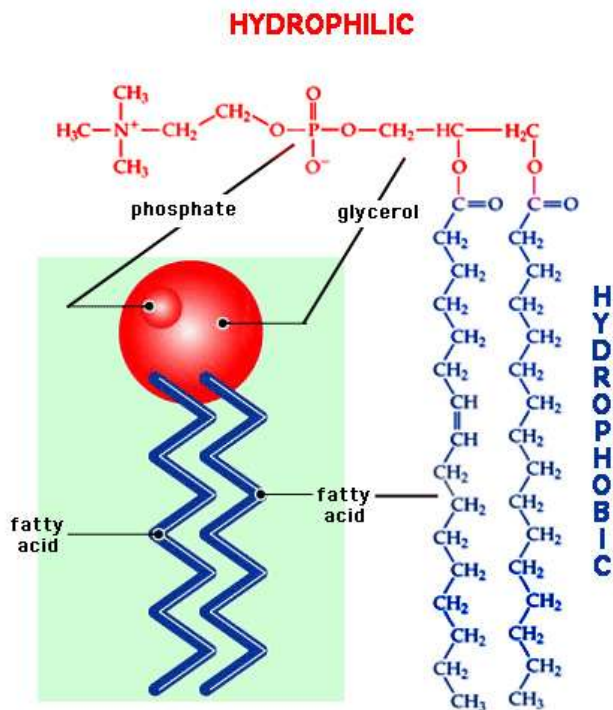
Atoms/small molecules	0.1 nm
Enzymes	10 nm
DNA	width ~ 2 nm length ~ mm to m
Microtubules	width ~ 25 nm length ~ μm
Viruses	0.1 μm
E.Coli	1 μm
White Blood Cell	0.01 mm
Nerve Cell	mm to cm to m

The stuff inside: small molecules

- water – we're 70% H₂O
- ions: H⁺, Na⁺, Ca²⁺, K⁺, etc. used to drive transport, pumps
- sugars – glucose, ribose, sucrose – substrates for many metabolic reactions
- nucleotides – form DNA & RNA
 - ATP (adenosine triphosphate – the big energy source of cells)
- amino acids – form proteins
- fatty acids – chain like molecules that are the basis of lipids that make the membrane

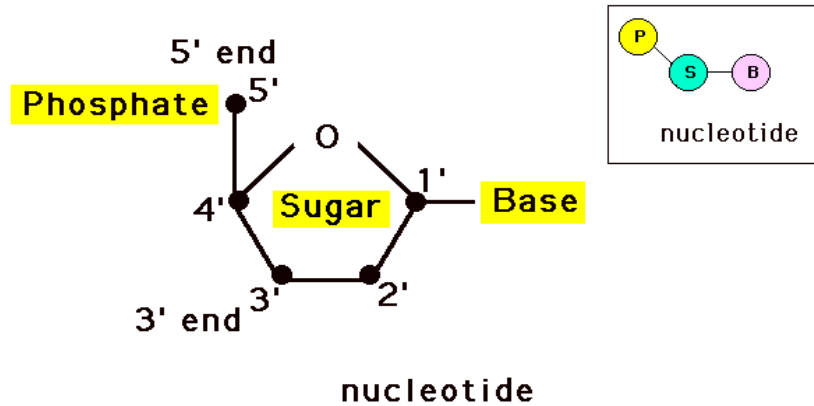
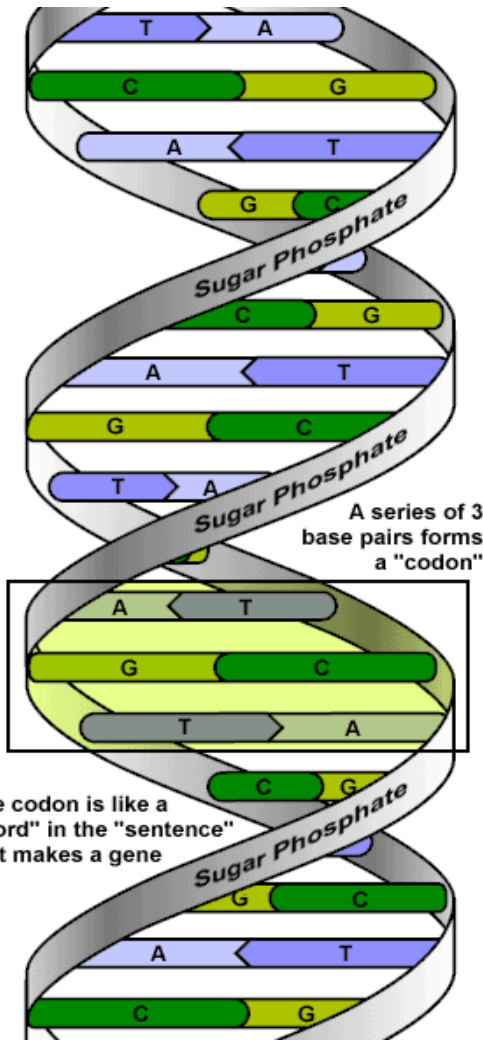
Lipids & Membranes: The packaging

- Lipids are molecules which form membrane bi-layers
- consist of two fatty acids joined by a 'head' molecule of glycerol
- head group likes water, tail does not like water
- Lipids spontaneously self-assemble into membranes

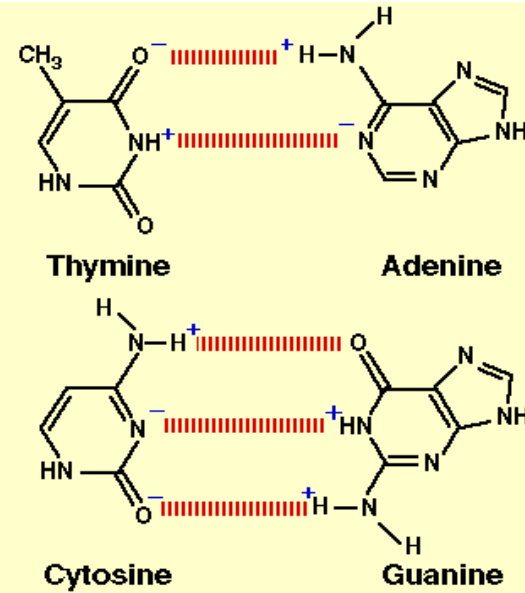


DNA:

Four different nucleotides



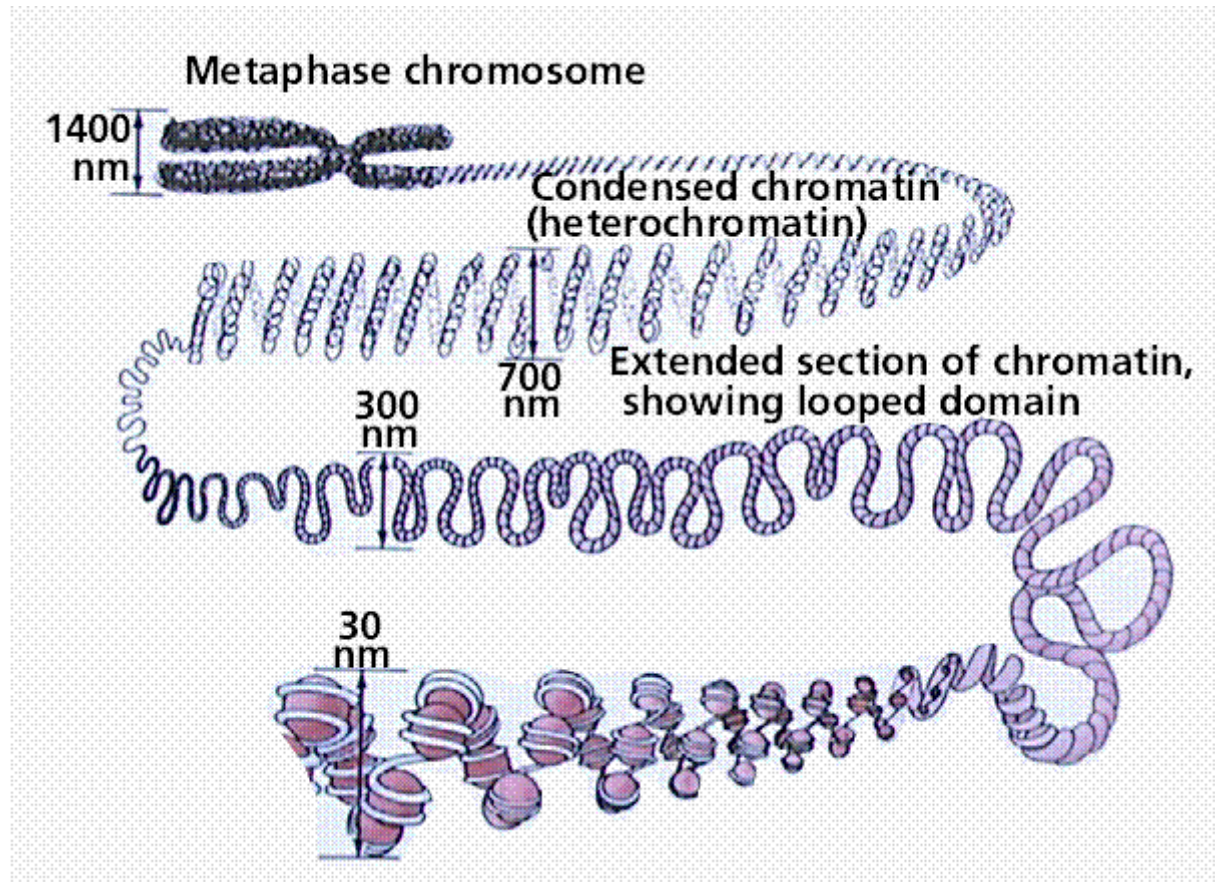
PYRIMIDINES PURINES



Watson-Crick Base Pairing

The Structure of DNA in Cells:

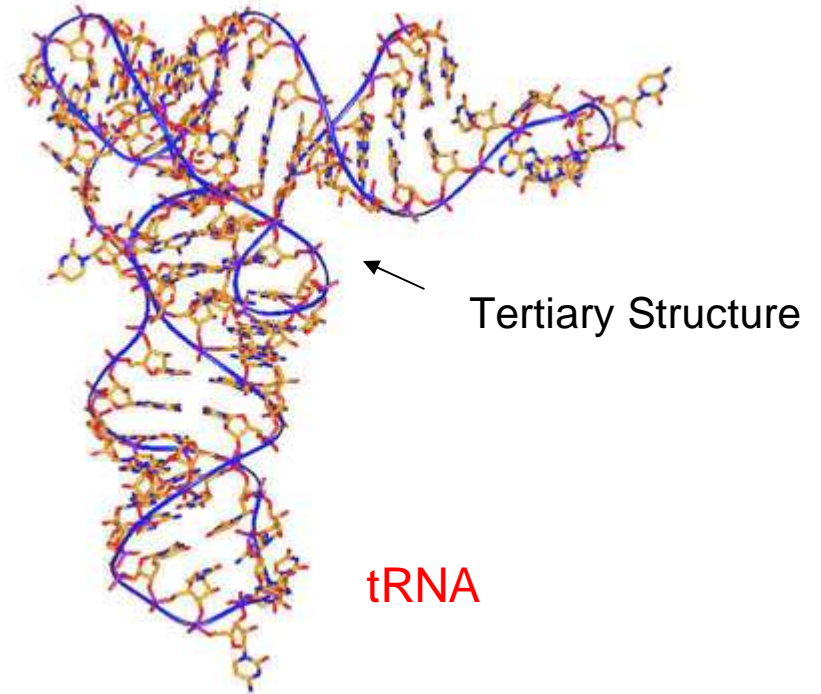
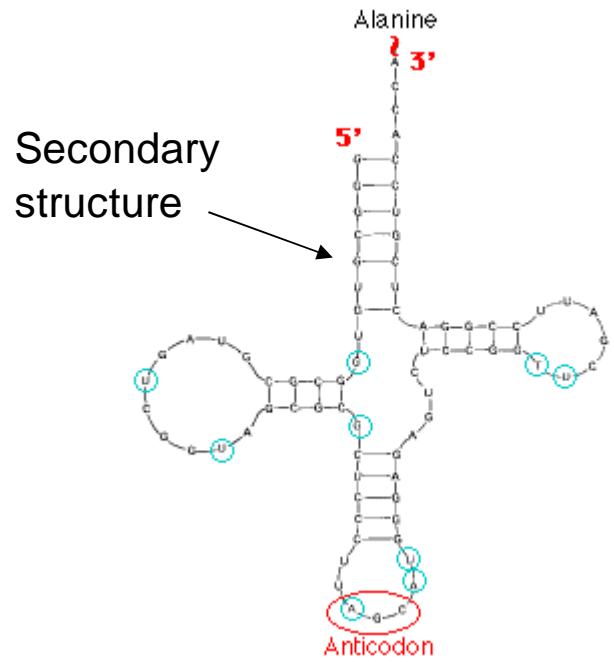
- How do you pack 1 m of DNA into a nucleus that is 2 μm in size?



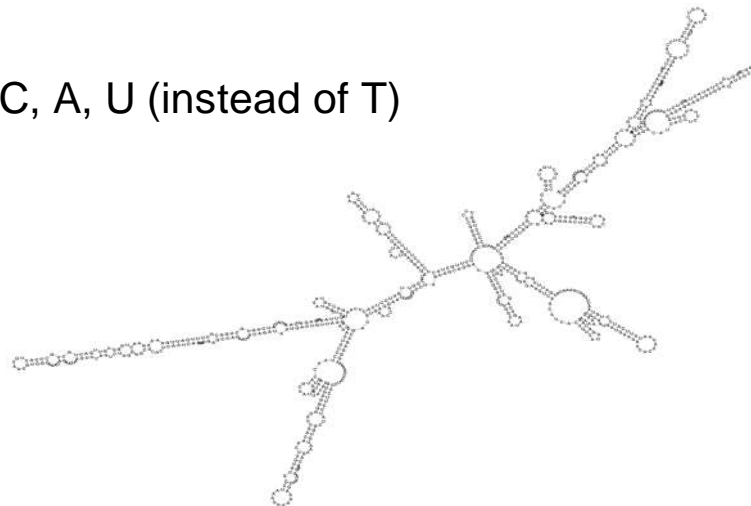
2nm DNA gets wrapped around histone complexes forming what are called nucleosomes (DNA -vely charged & histones are +vely charged)

These then wrap up and pack further and condense

RNA:



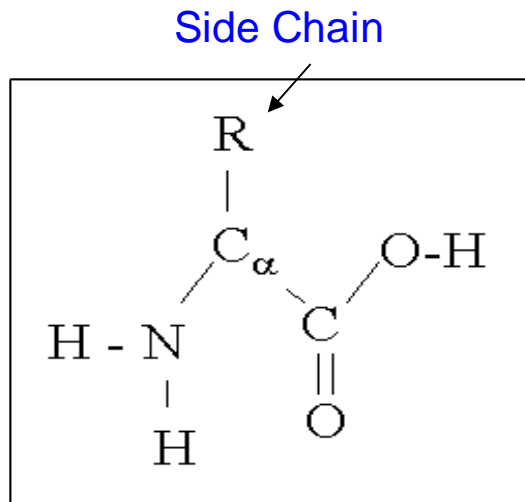
Alphabet = G, C, A, U (instead of T)



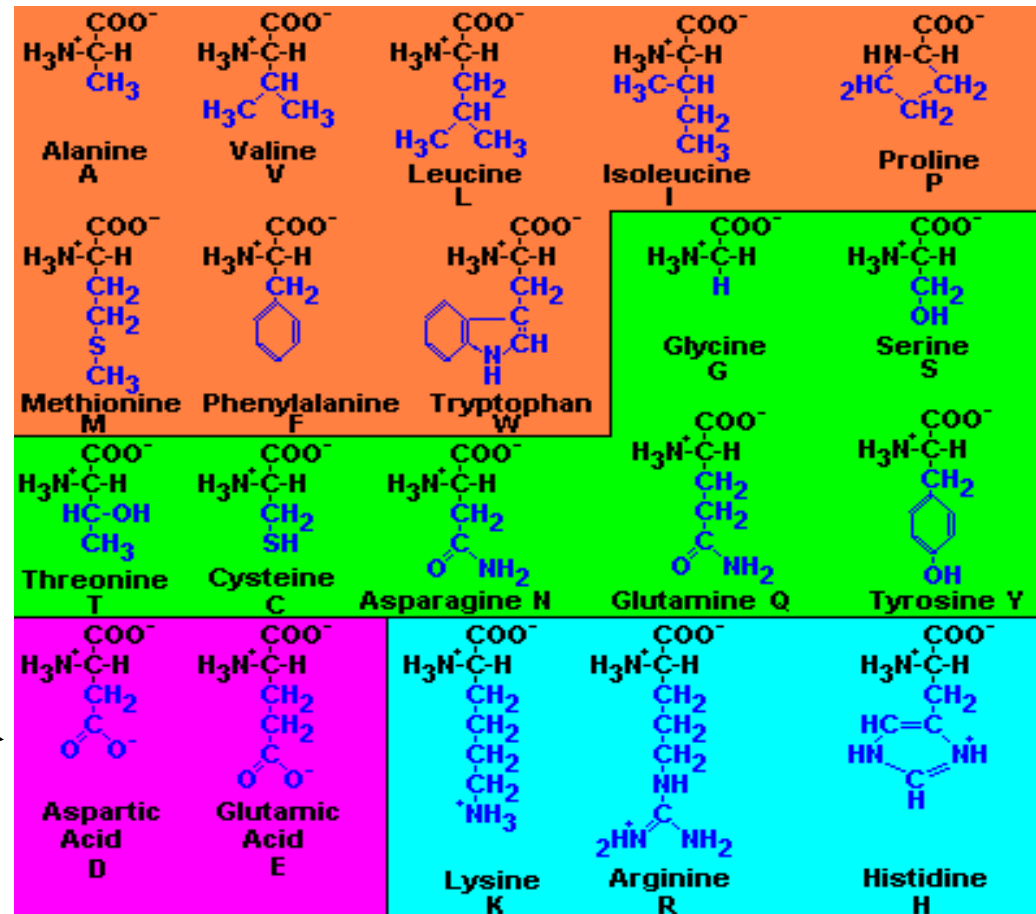
- carry the genetic information around as mRNA
- can carry out chemical functions

Proteins: Amino Acids

- Proteins are polymers built from 20 amino acids



Hydrophobic



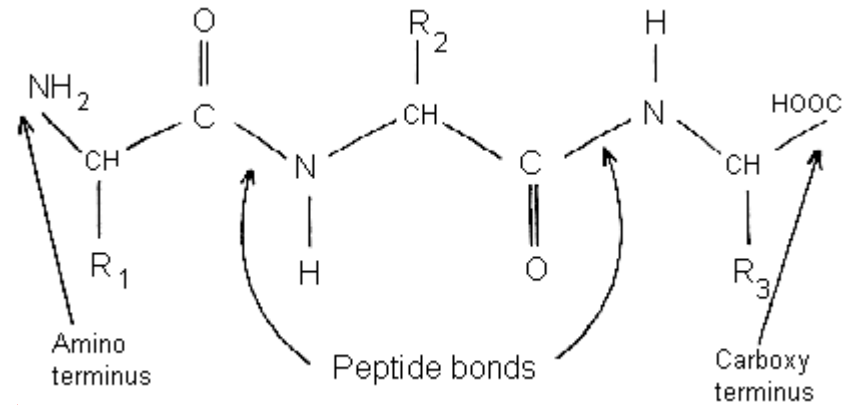
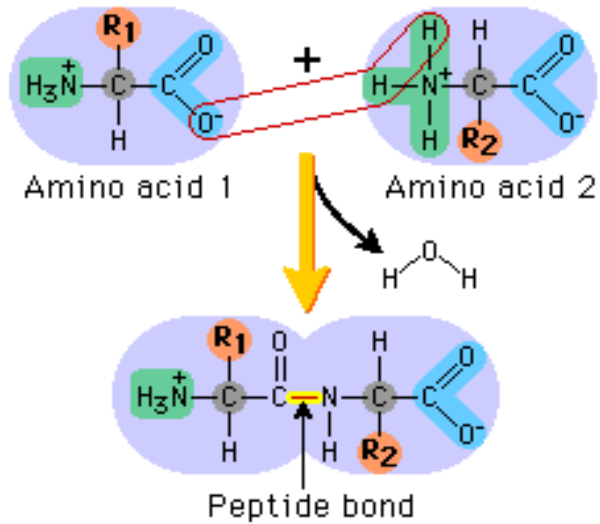
Polar



Charged

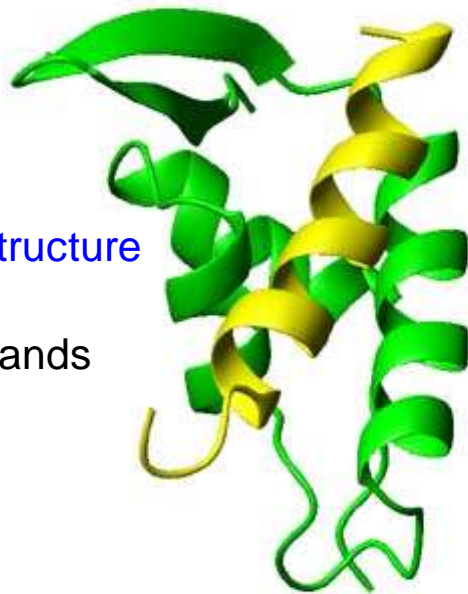


Proteins: Structure



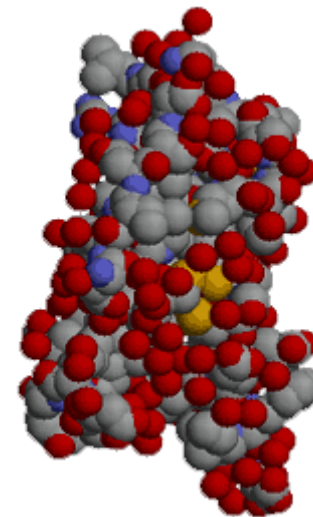
Secondary Structure

Helices & Strands



Tertiary Structure

Densely packed hydrophobic core



A final census:

Substance	% of total dry weight	Number of molecules
Macromolecule		
Protein	55.0	2.4×10^6
RNA	20.4	
23S RNA	10.6	19,000
16S RNA	5.5	19,000
5S RNA	0.4	19,000
Transfer RNA (4S)	2.9	200,000
Messenger RNA	0.8	1,400
Phospholipid	9.1	22×10^6
Lipopolysaccharide	3.4	1.2×10^6
DNA	3.1	2
Murein	2.5	1
Glycogen	2.5	4,360
Total macromolecules	96.1	
Small molecules		
Metabolites, building blocks, etc.	2.9	
Inorganic ions	1.0	
Total small molecules	3.9	

Table 2.1: Observed macromolecular census of an *E. coli* cell. Adapted from Neidhardt *et al.* and Schaechter *et al.*

Information Storage, Replication & Processing:

What does the cell use all these molecules/structure for?

- 1) Storing Chemical Information
- 2) Passing On/Replicating this information
- 3) Processing and calculating with this information

We'll look at each of these steps now

Information Storage:

- The information for running the cellular program is stored in the sequence of DNA
- DNA is like the hard drive of a computer
- It stores information, contains programs that get executed at specific times
- Each protein has a specific sequence in the DNA called a 'gene', and this DNA is called 'coding' DNA, as it codes for messages that make proteins
- The majority of DNA sequence does not code for 'genes' and is called non-coding DNA.
- It is the non-coding DNA which contains the 'programs' which determine what genes will get made at a given time or place
- different cells are running different programs and thus making different sets of genes

The Sizes of the Hard-drives

Bacteria (usually) have single circular chromosome – have many small plasmids

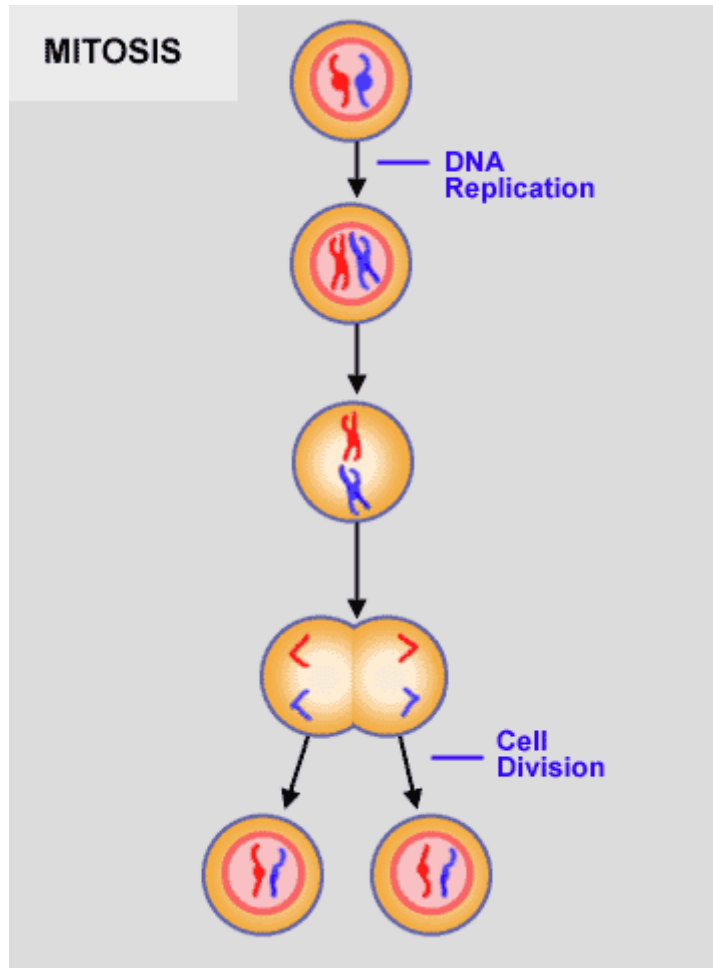
Eukaryotes have multiple linear chromosomes

- **haploid** = single copy of every chromosome
- **diploid** = two copies of every chromosome
- **polyploid** = multiple copies of every chromosome

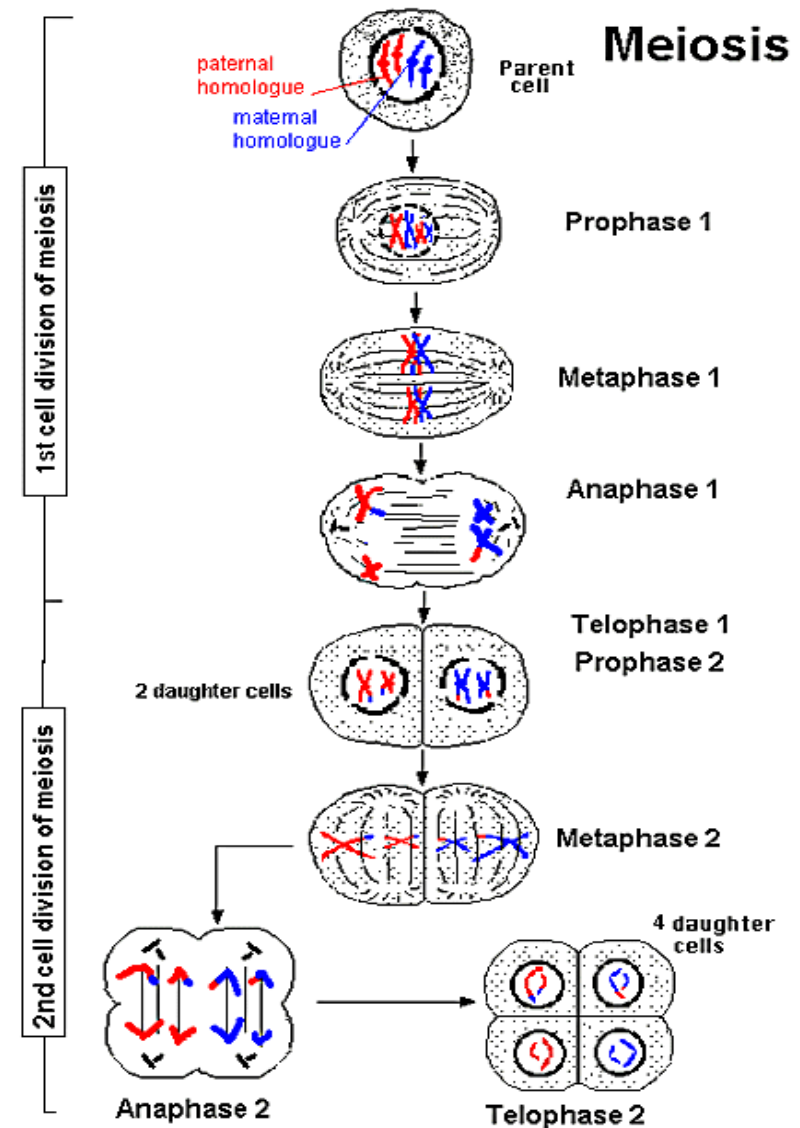
organism	#	T	size	genes
virus	1	H	5kb	10
E. coli	1	H	5Mb	4,377
S. Cervisiae	16	H	12Mb	5,570
C. elegans	16	D	100Mb	19,000
A. thaliana	5	D	115Mb	25,498
D. melanogaster	4	D	122Mb	14,000
H. sapiens	23	D	3Gb	25,000

Information Replication: Cell Division:

Asexual and normal cell division

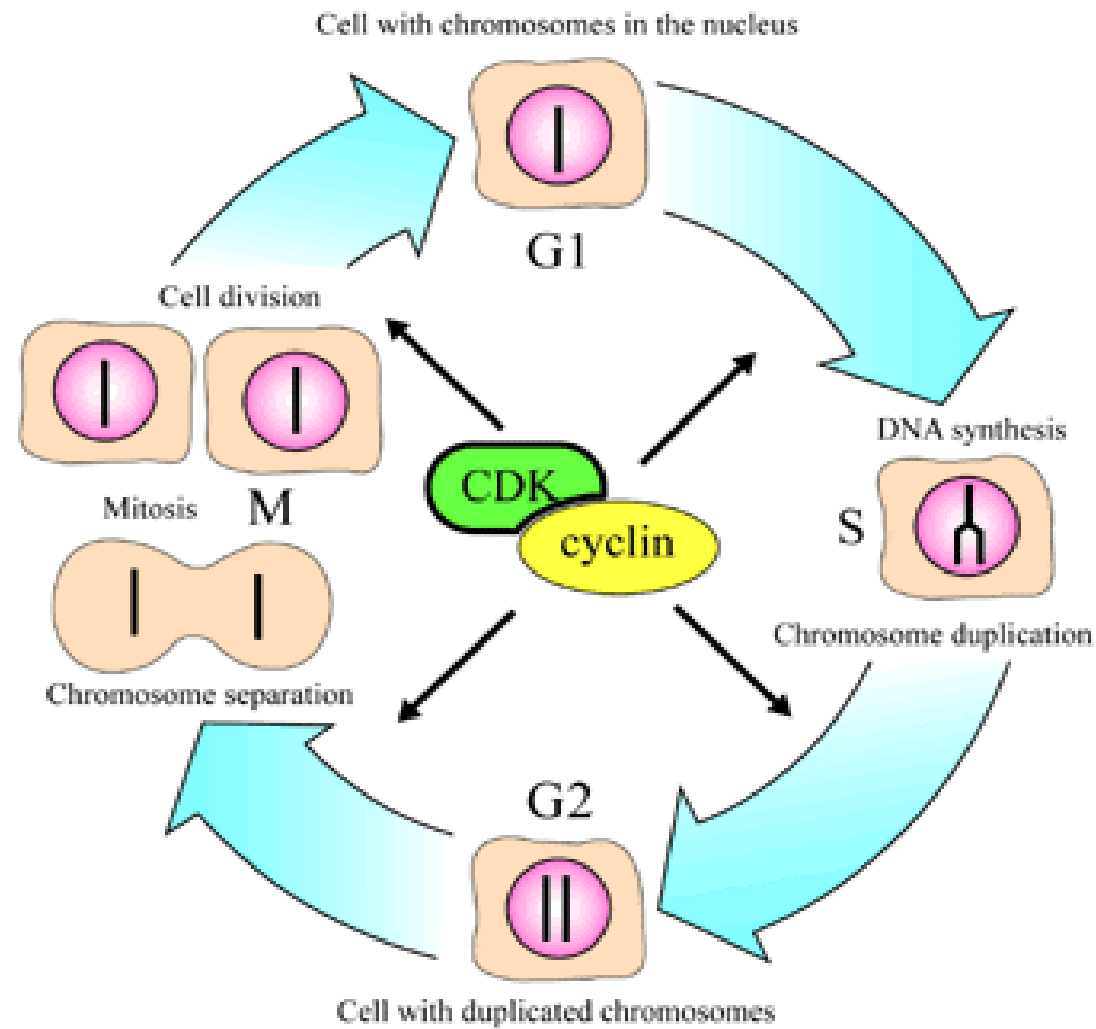


Sex cells = gametes = haploids

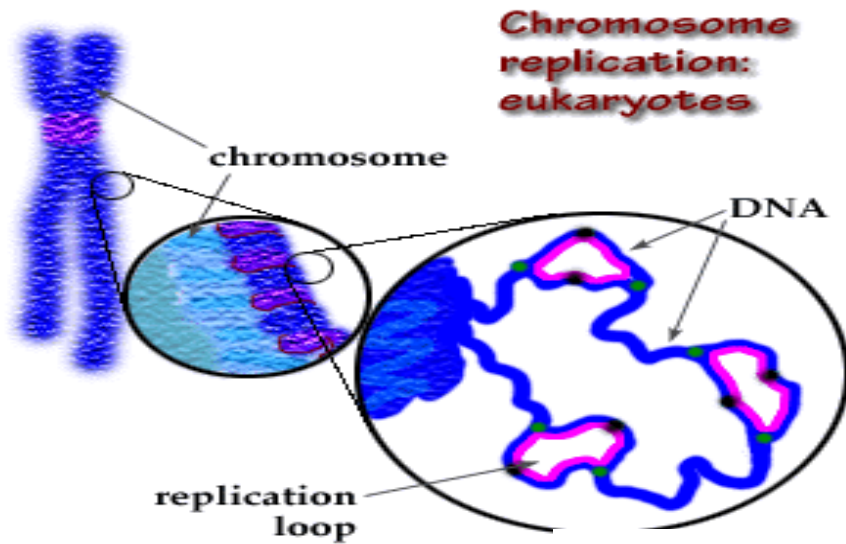


Cell Cycle:

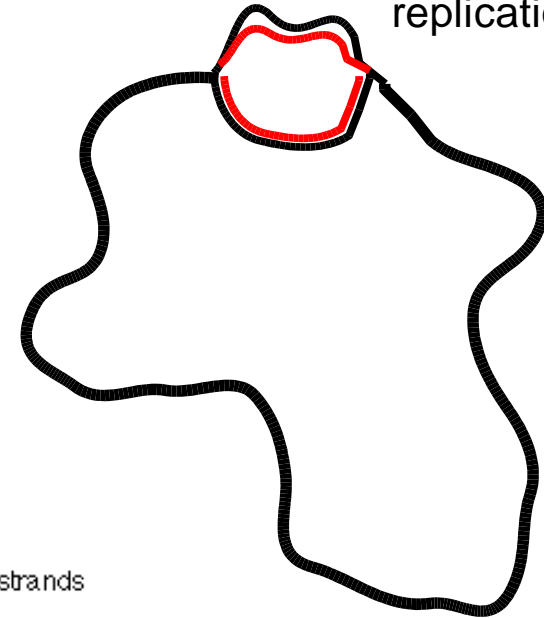
The Cell Cycle



DNA replication:



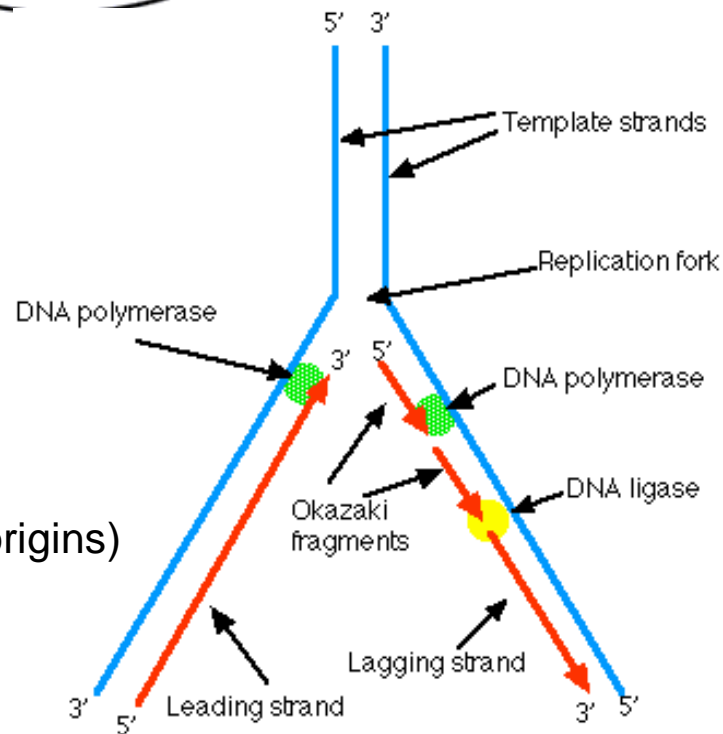
Bacteria have single origin of replication



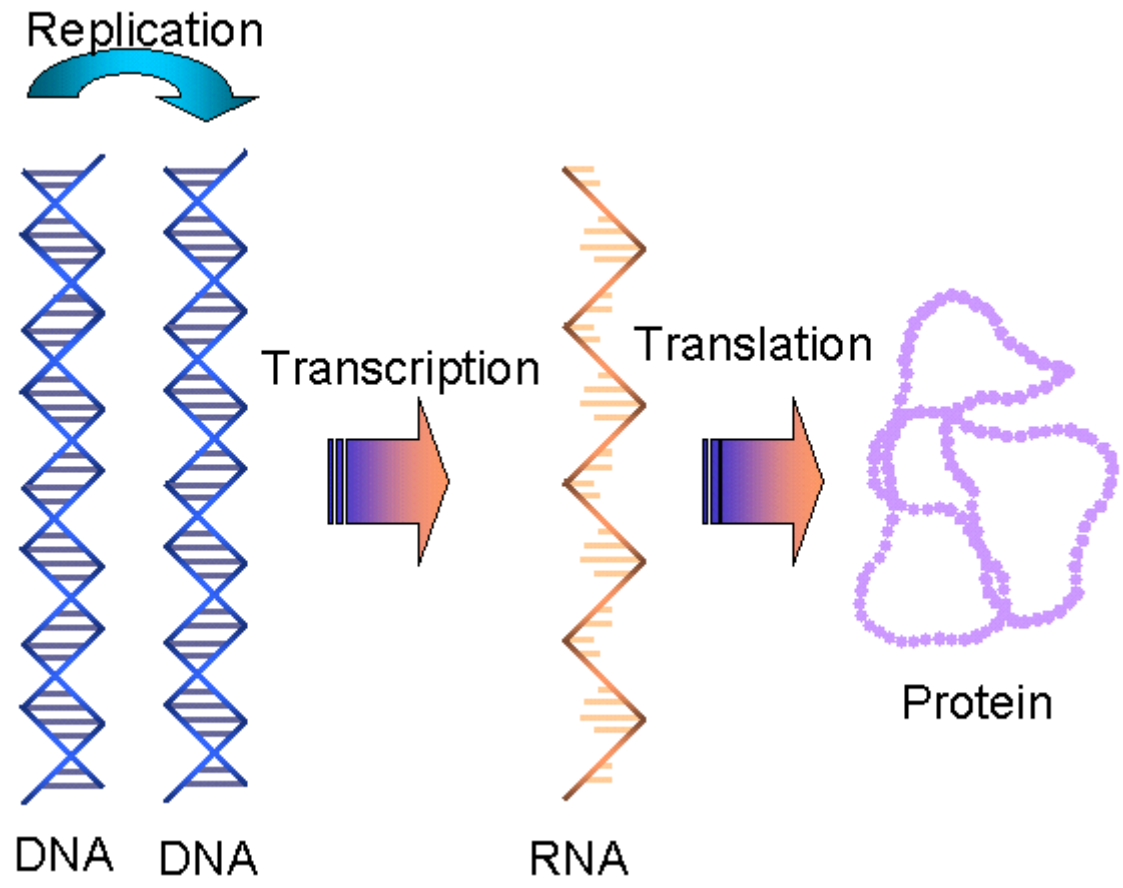
Replication Times:

bacteria ~ 40 mins (1 origin)
1000 nucleotides/s

humans ~ few hours (many origins)
100 nucleotides/s



Information Processing: Central Dogma:



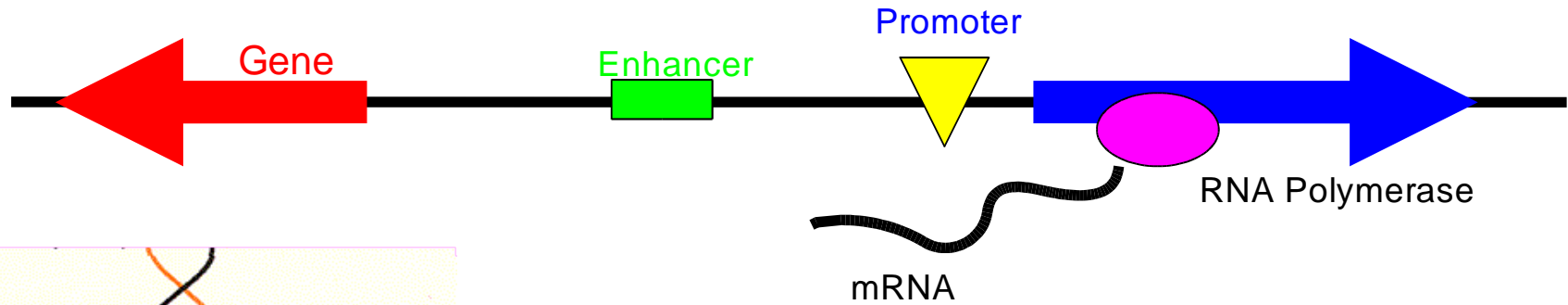
DNA to Proteins? Genetic Code



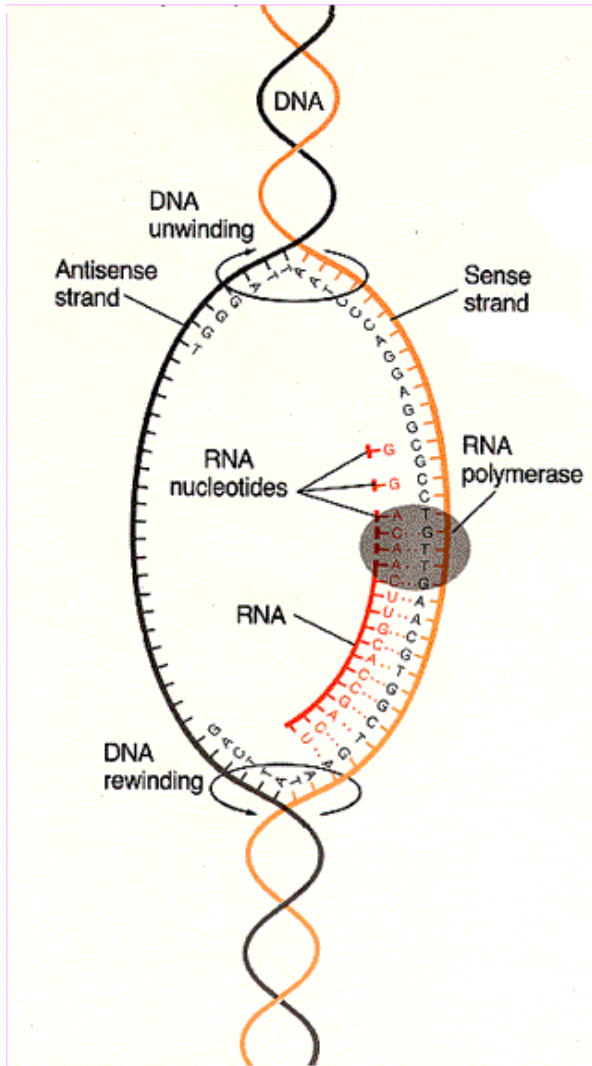
- 64 codons, with highly non-uniform mapping

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

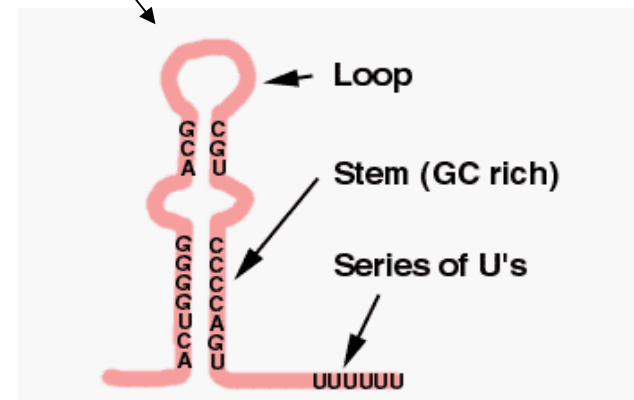
Gene Transcription:



- Bacteria = ON
- Eukaryotes = OFF, requires lots of other help



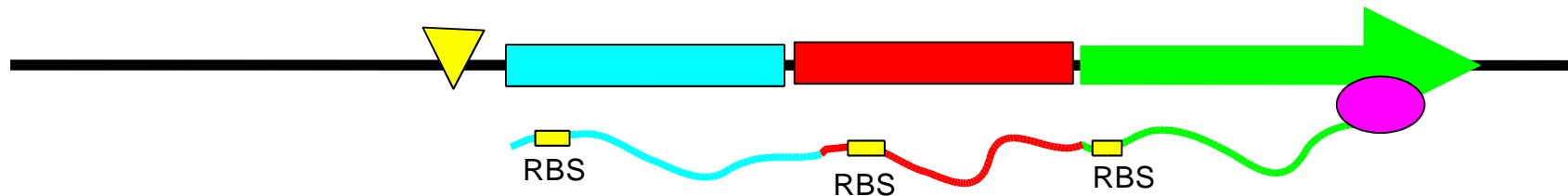
Pushes polymerase off of DNA



Messages are delivered differently:

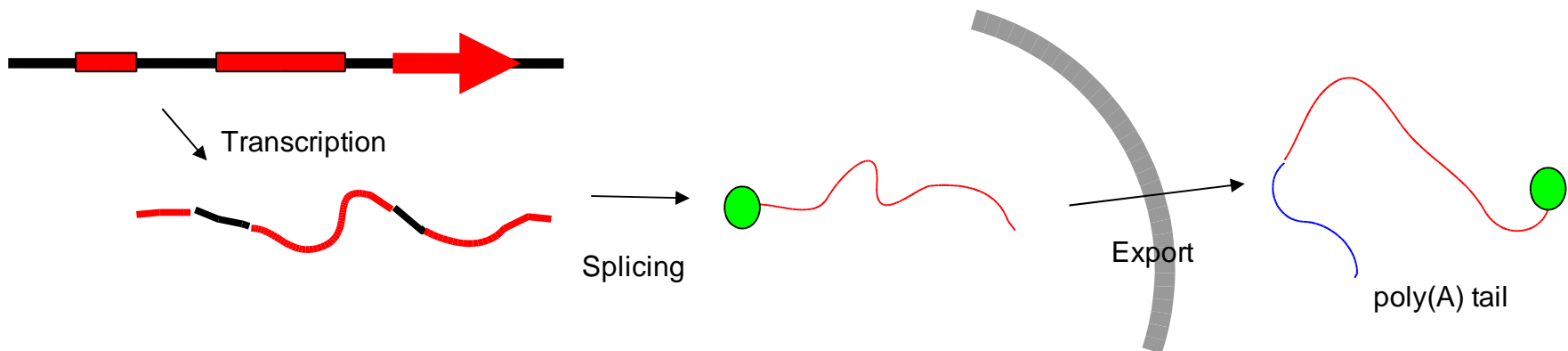
Prokaryotes (Simple):

- Genes in bacteria are organized in operons – one contiguous message RNA is generated

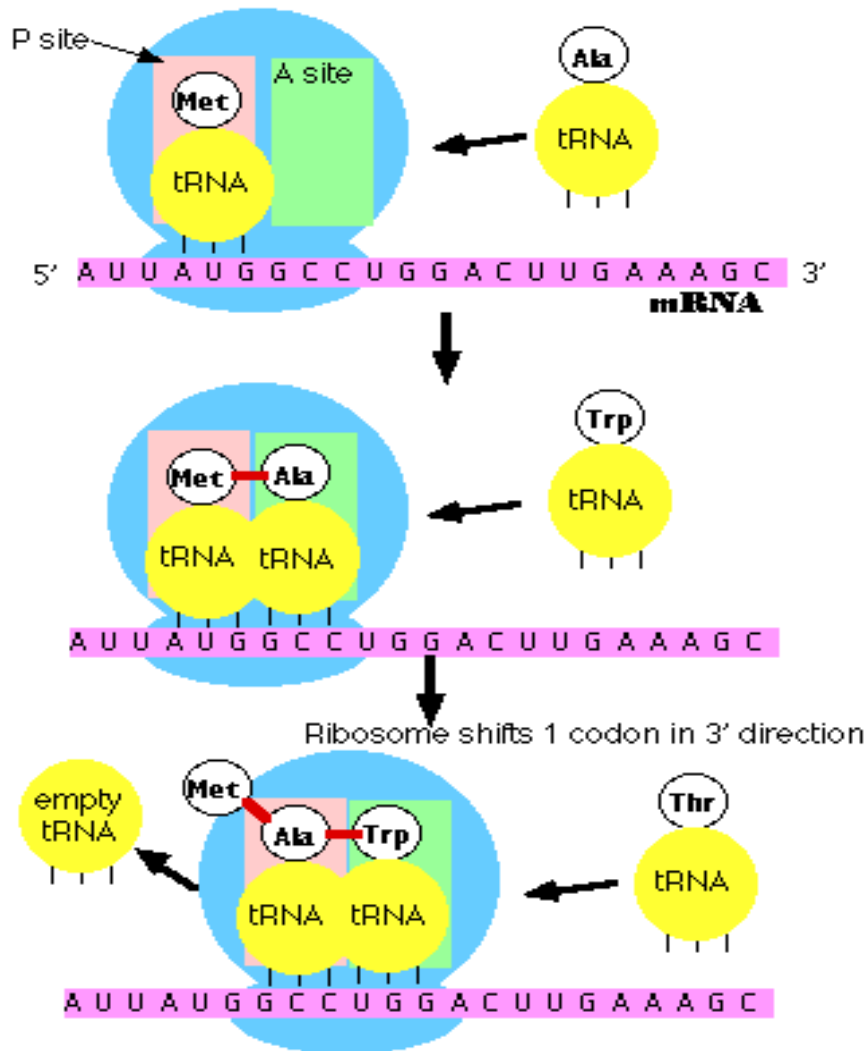


Eukaryotes (Complex):

- Genes contain coding (**exons**) and non-coding (**introns**) sequence
- Introns are spliced out and exons are assembled to make final mRNA
- final mRNA is exported out of nucleus and made into protein

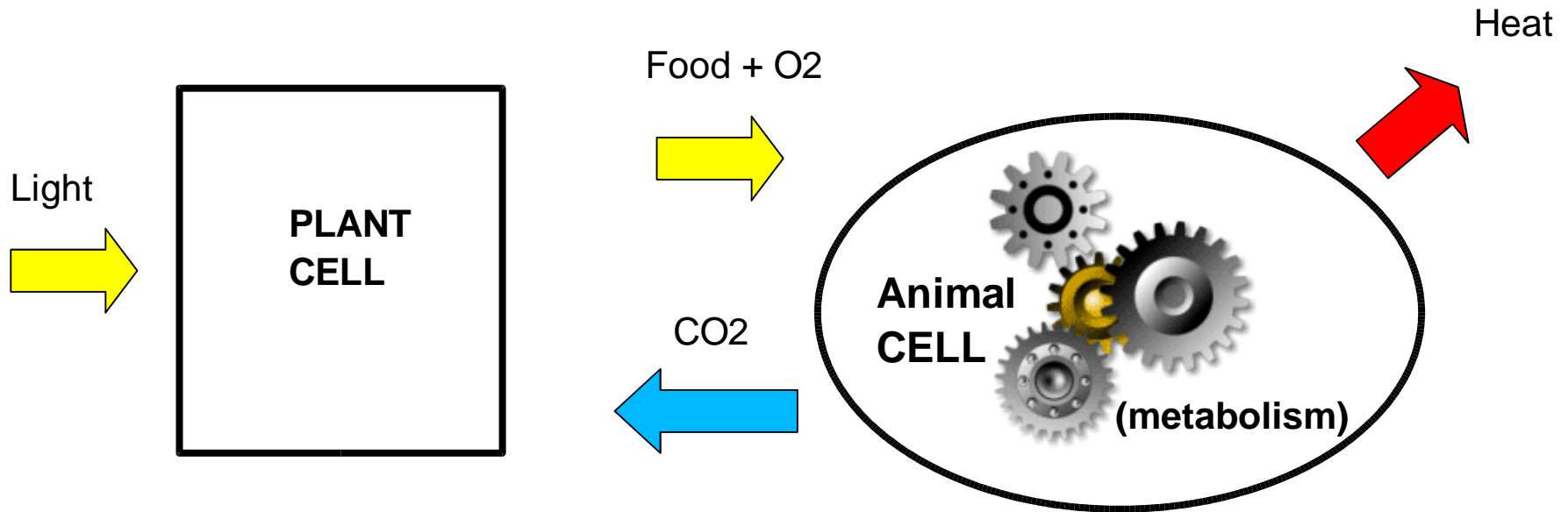


Translation:



- Ribosome is big machine that translates mRNA into protein
- In bacteria, Ribosome binds to specific site on mRNA
- In eukaryotes, Ribosome binds to 5' cap
- translation ends when 'STOP' codon is reached

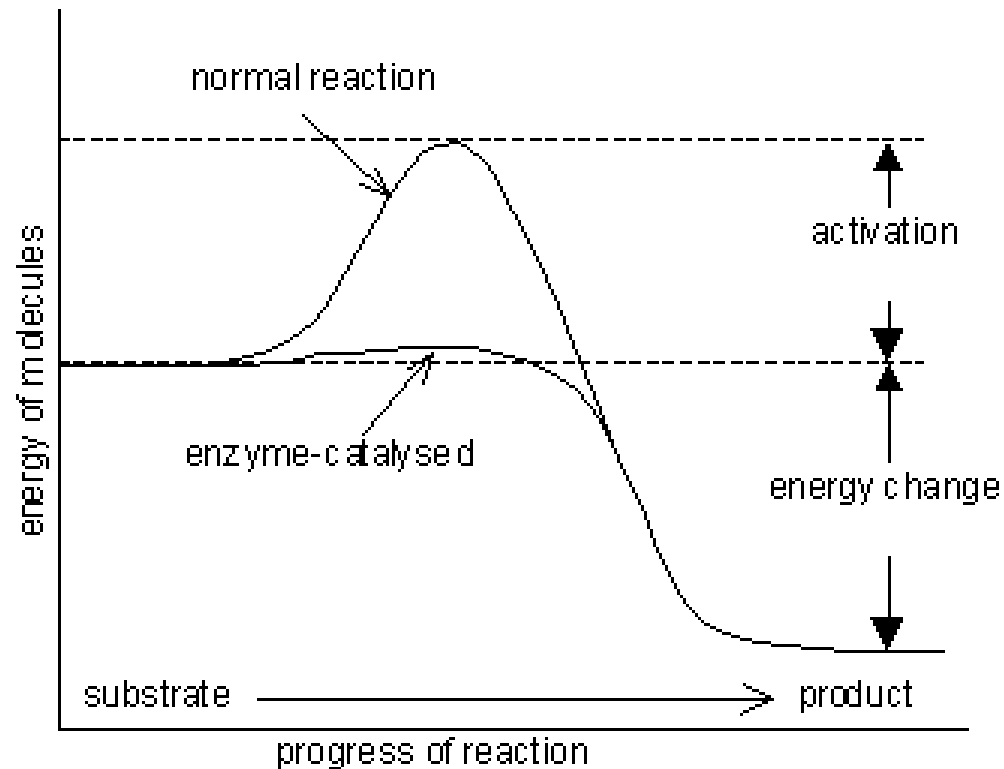
How cells get energy



- Oxidation of food CO_2 and H_2O and energy
- **Metabolism** = processes of converting/synthesizing molecules in cells
 - **Catabolism** = breaking down of molecules
 - **Anabolism** = synthesizing of molecules
- Two major energy reserves: **sugars**, **fatty acids**

Biochemical reactions

- Most chemical reactions in cells are mediated by proteins called **enzymes**
- Enzymes act as catalysts, by reducing the activation barrier between two states

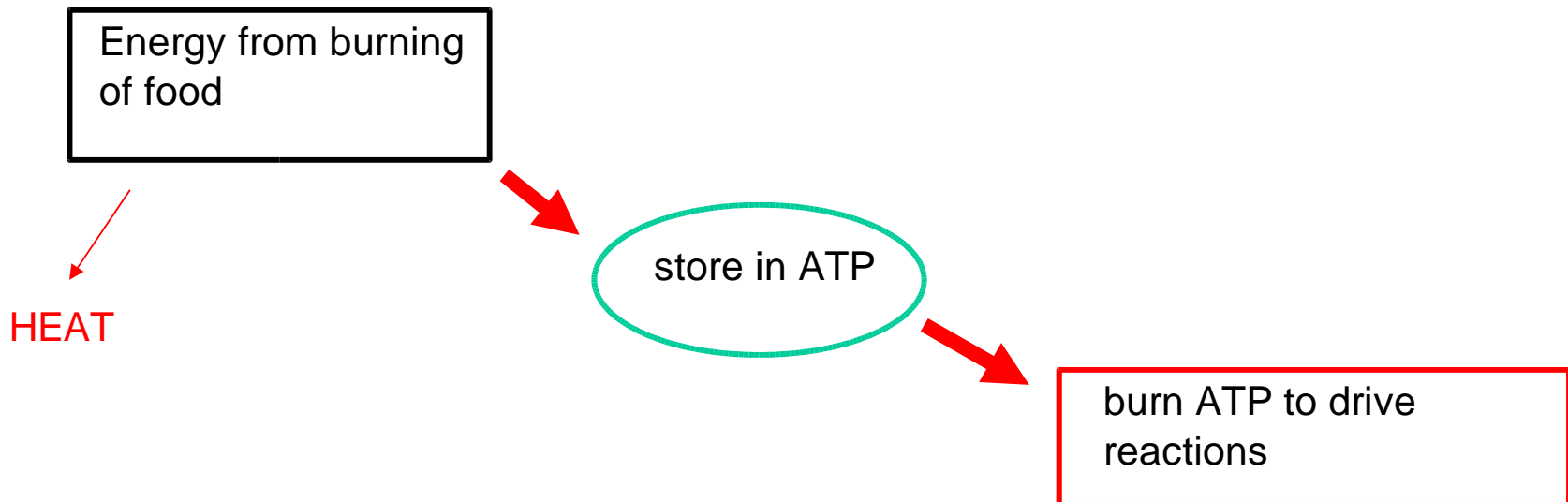


Energy Storage in Cells

- Some chemical reactions in cells are energetically unfavourable (e.g DNA & protein synthesis)
- Couple energetically bad pathway with favourable pathway using enzyme
- favourable pathway is driven by taking energy from **energy carriers**
- Most pervasive energy carrier is **ATP**



- DNA synthesis burns 2 ATP



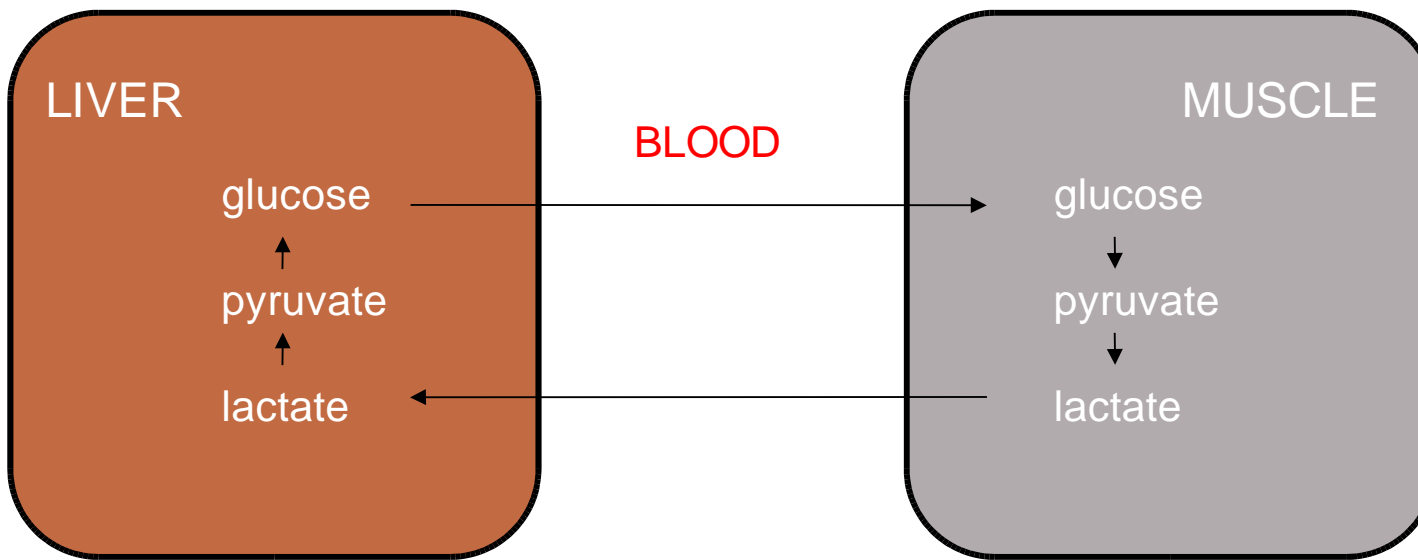
Converting Food to Useful Energy:

Glycolosis

- anaerobic
- converts glucose → 2 ATP

Citric Acid Cycle = Krebs Cycle:

- aerobic
- occurs in the mitochondria
- generates about 30 ATP from a single glucose!!!
- convert about 50% of possible energy (c.f. car ~ 20%)

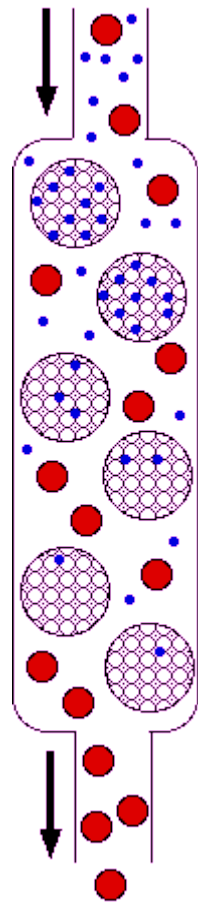


Molecular Biology Experimental Methods:

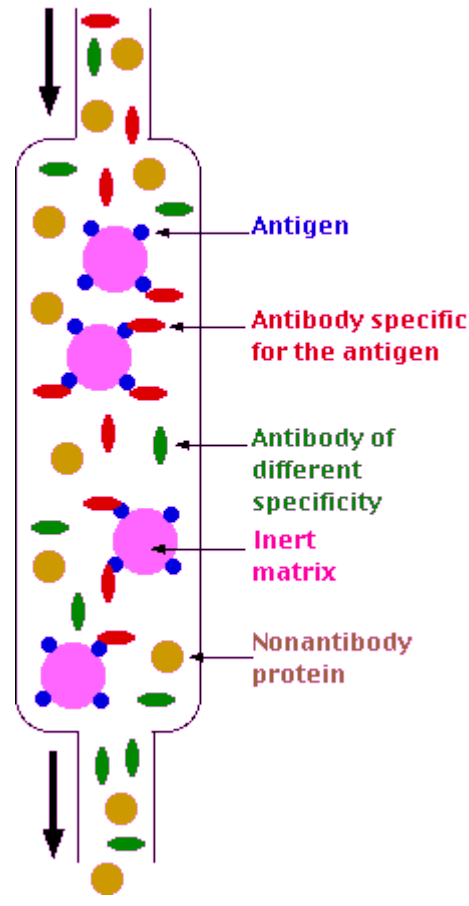
- Manipulating Proteins
- Manipulating DNA
- Sequencing
- Interactions
- Imaging

Chromatography:

- Chromatography is used to separate and purify molecules from a complex mixture



Gel-filtration



Affinity

Types:

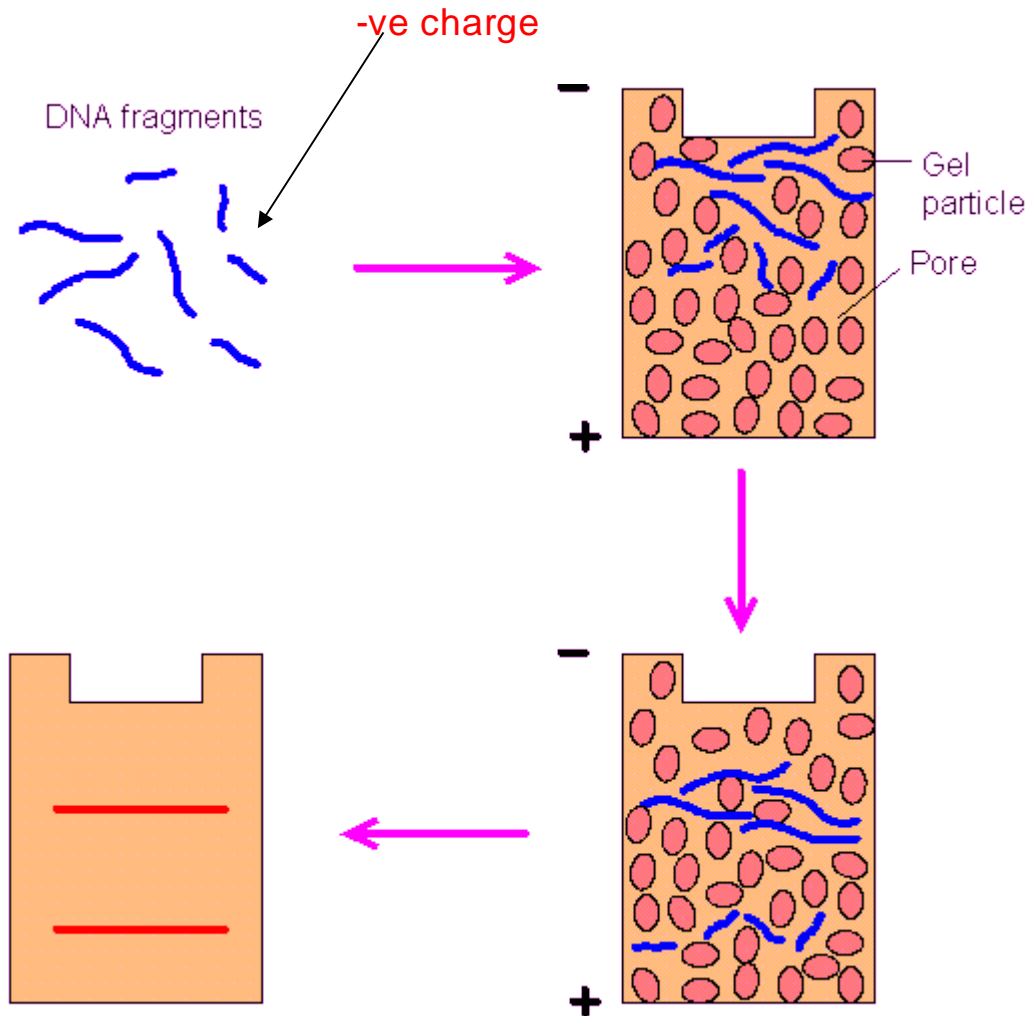
- **Charge** = ion-exchange chrom.
- **Hydrophobicity** = hydrophobic chrom.
- **Size** = gel-filtration chrom.
- **Binding** = affinity chrom.

Affinity chromatography can be used to elute very pure protein samples

- e.g. - DNA for DNA binding proteins
- antibodies for specific protein
- protein binding partners
- engineered tags (HIS, GST)
= Co-immune precipitation = CoIP

Gel Electrophoresis

- Used to determine size of biomolecules
- -vely charged molecules move in applied field through gel



- For proteins, SDS(detergent) is used to denature proteins and give them net negative charge
= **SDS-PAGE**

- 2D SDS-PAGE can be used to sort proteins by both size and native charge

-

DNA manipulation

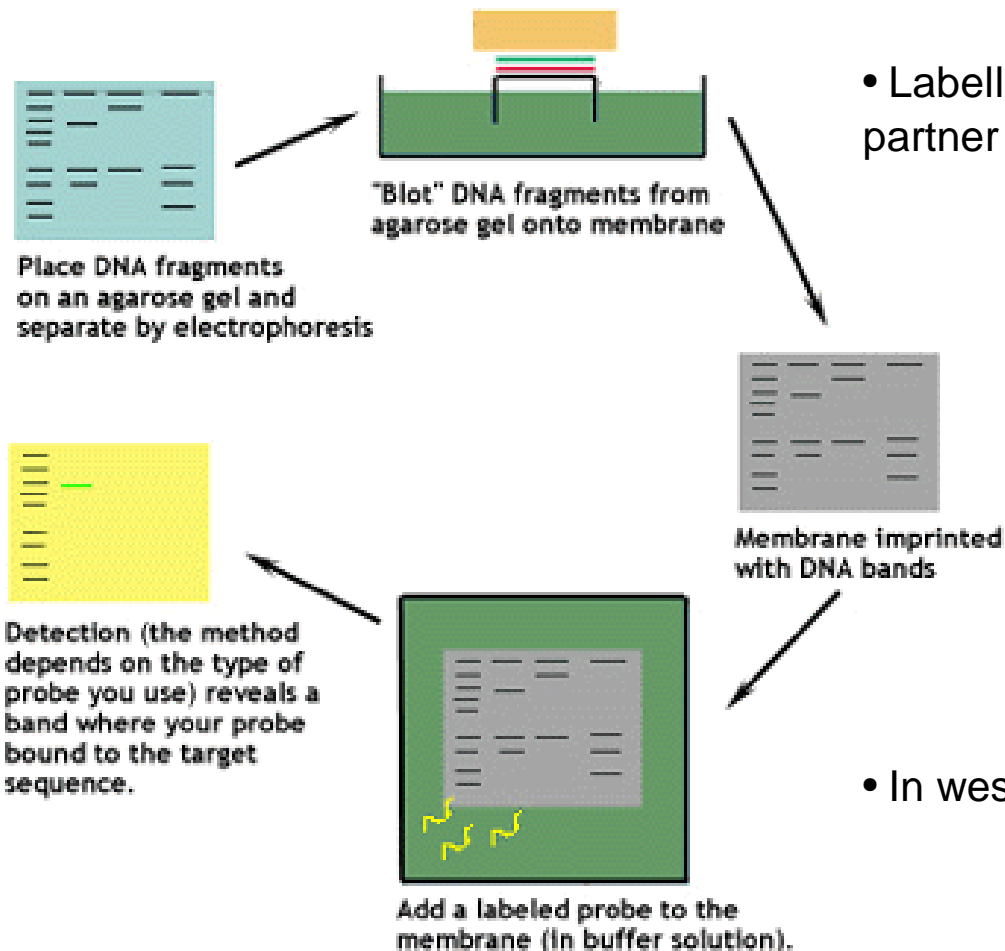
- Use **restriction enzymes** to cut specific nucleotide sequences (e.g. HpaI = GTTAAC; EcoRI = GAATTC)
- Some make blunt ends, some make dangling 'sticky' ends



- Used to chop up large DNA segments (i.e. Chromosome)
- Used to ligate (join) different DNA fragments -> genetic engineering

Northern, Southern and Westerns

- Used to detect quantitative amounts of specific **DNA (southern)**, **RNA (northern)** or **proteins (Western)** from a complex mixture (i.e. cellular extract).

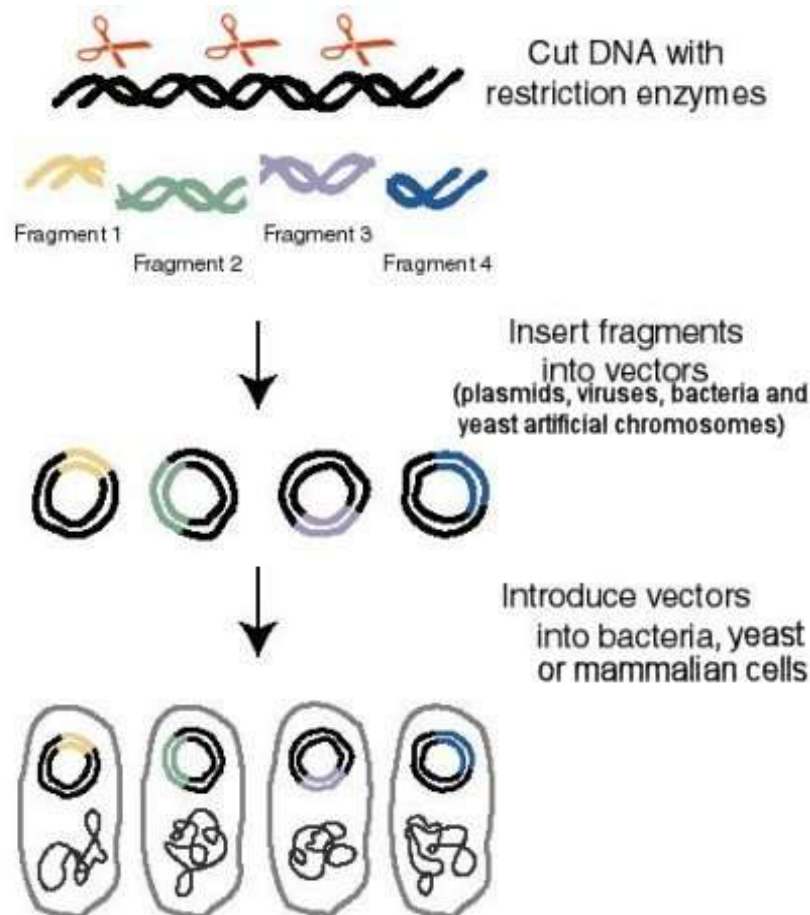


- Labelled DNA probe 'hybridizes' with matching partner in sample

- In westerns, a labelled antibody is used for probe

DNA Cloning

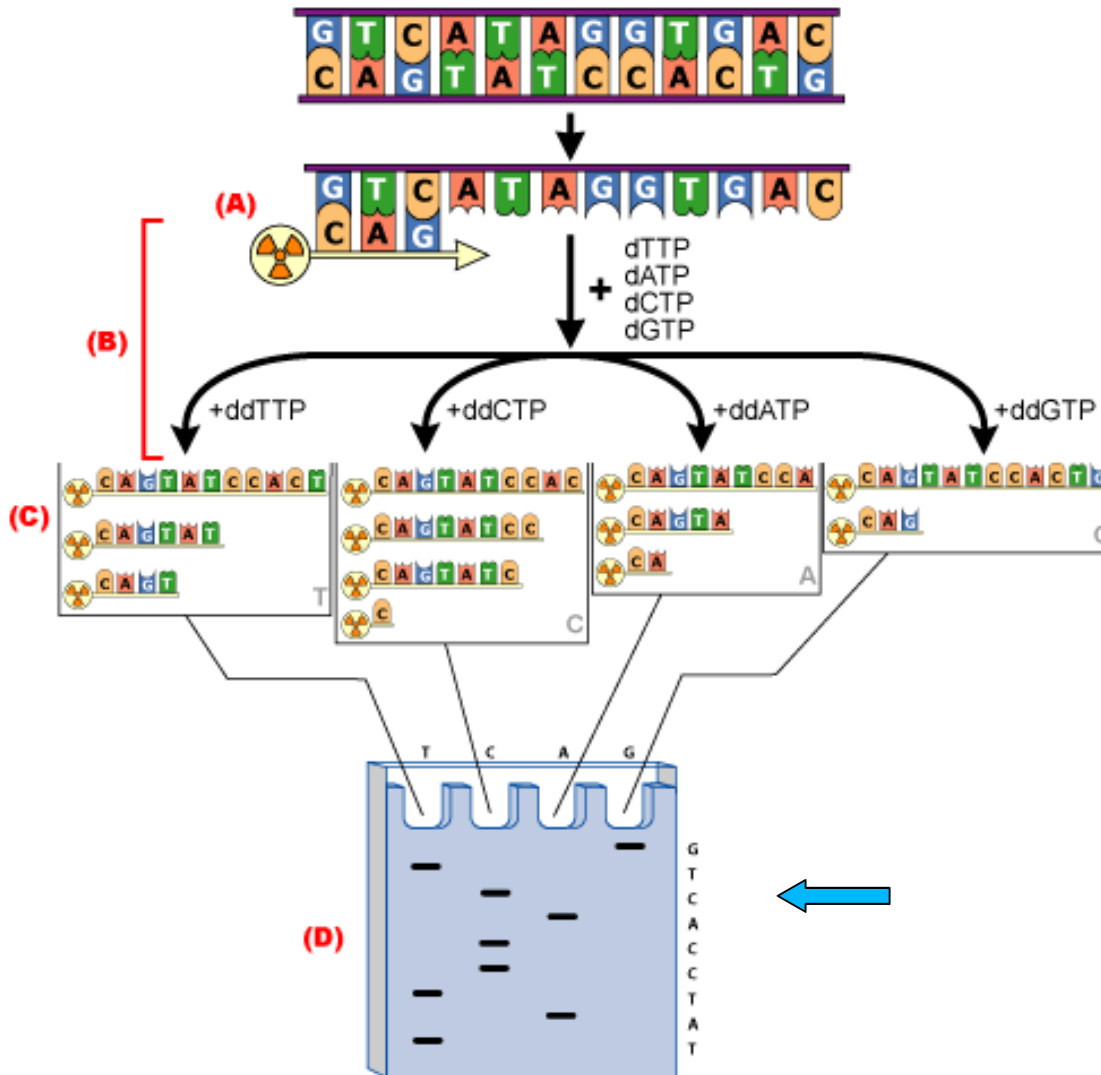
- Replicate specific DNA fragment in large quantities using bacteria
- Fragment is inserted into circular **plasmid** DNA and **transfected** into bacteria
- Large collection of bacteria colonies each with different fragment = **library**



- Types of libraries = genomic, cDNA
- cDNA library comes only from mRNA
- Use cDNA plasmid to express lots of specific protein = **expression vector**

DNA Sequencing

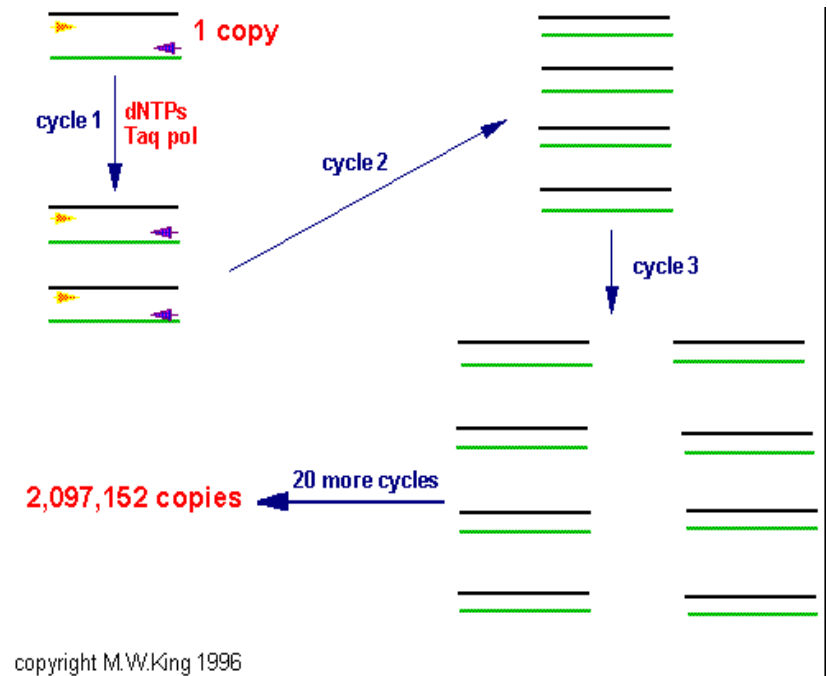
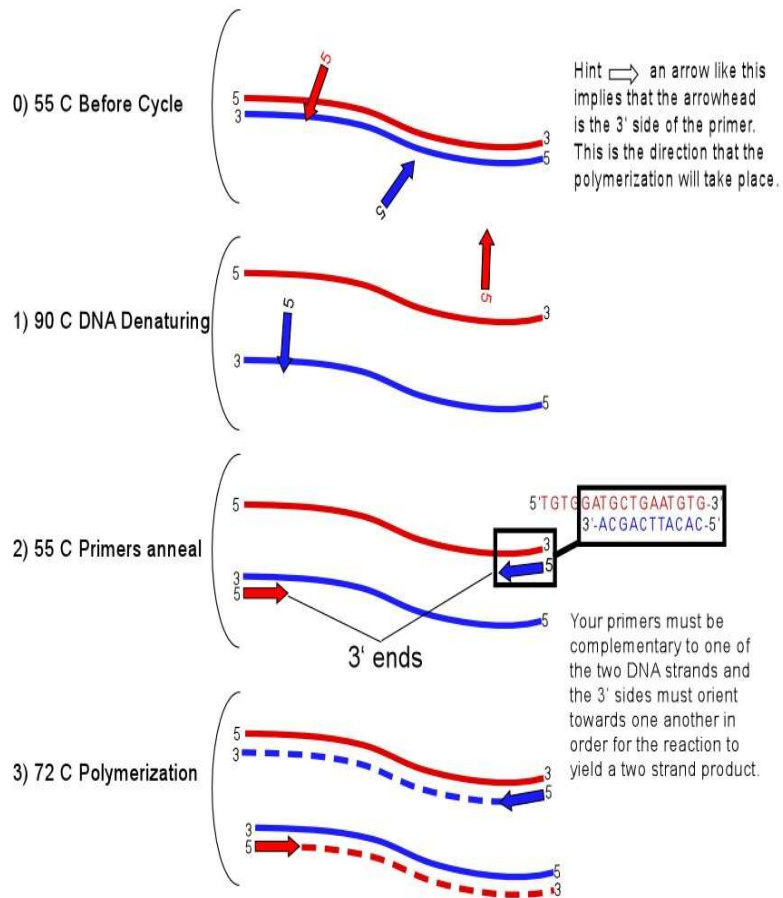
Dideoxy method: use special nucleotides that stop growth of DNA
--> produces DNA that stops at specific letter positions



Polymerase Chain Reaction (PCR):

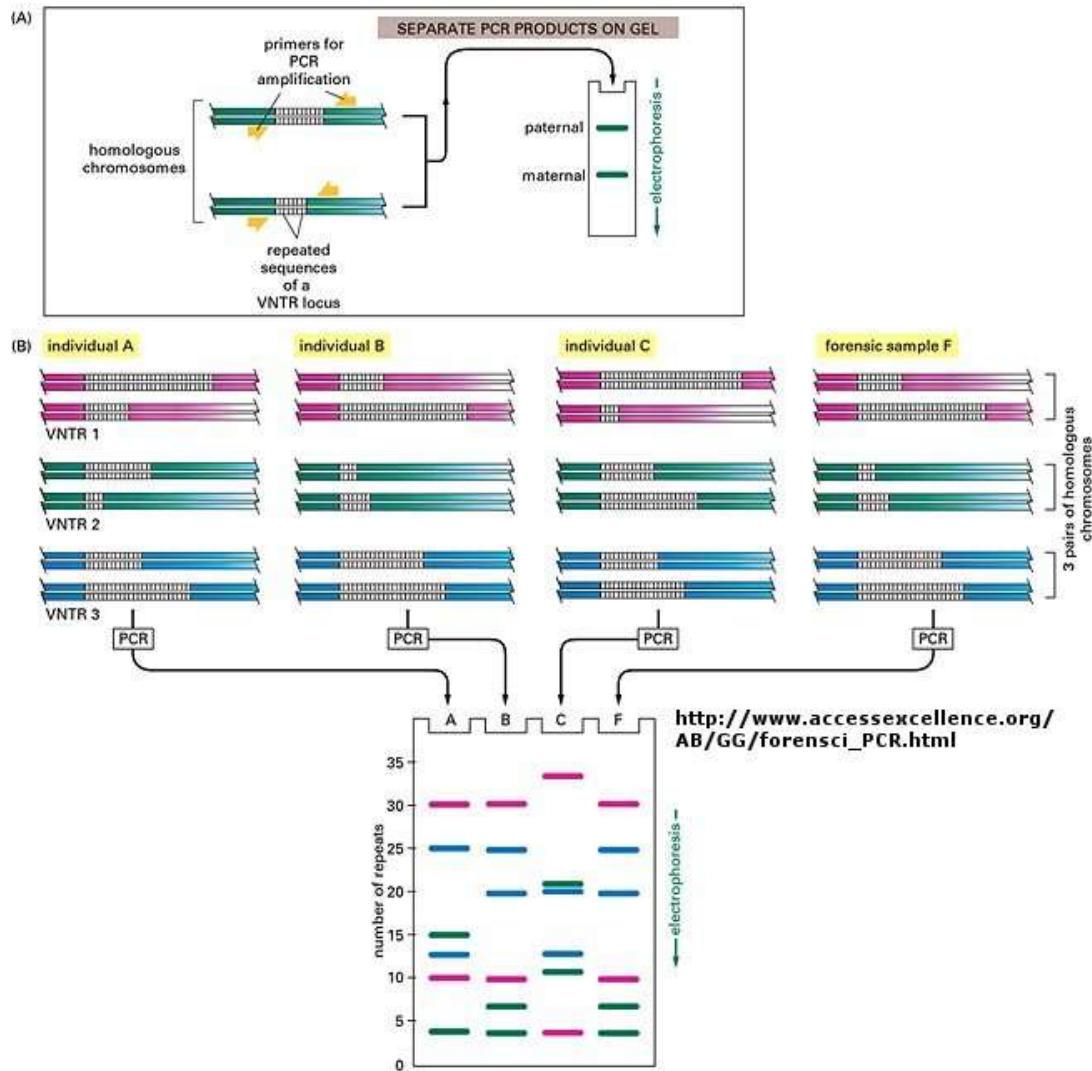
- Use 2 DNA **primers** to pull out and **amplify** specific region of DNA in a sample

Inside the PCR reaction tube...



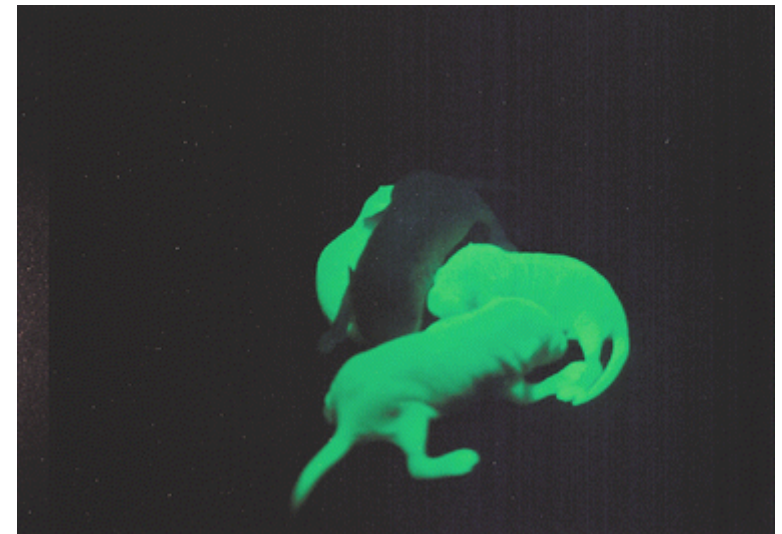
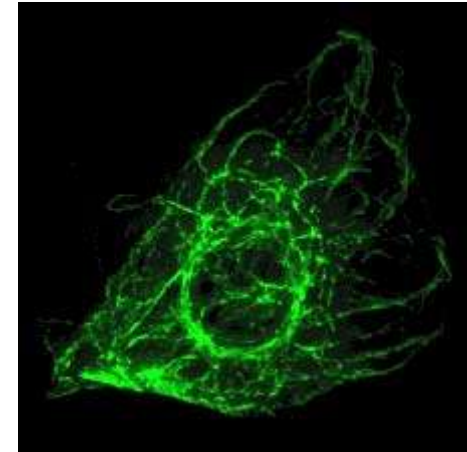
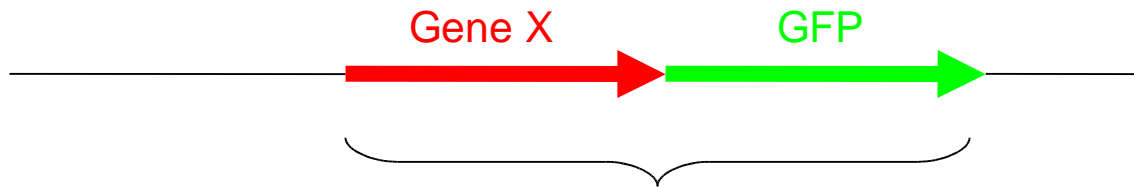
PCR and Forensics

- Our chromosomes contain regions of 'satellite' DNA that vary in length – each individual has specific length = fingerprint
- PCR amplify regions and then use gels to measure the lengths -> yields fingerprint



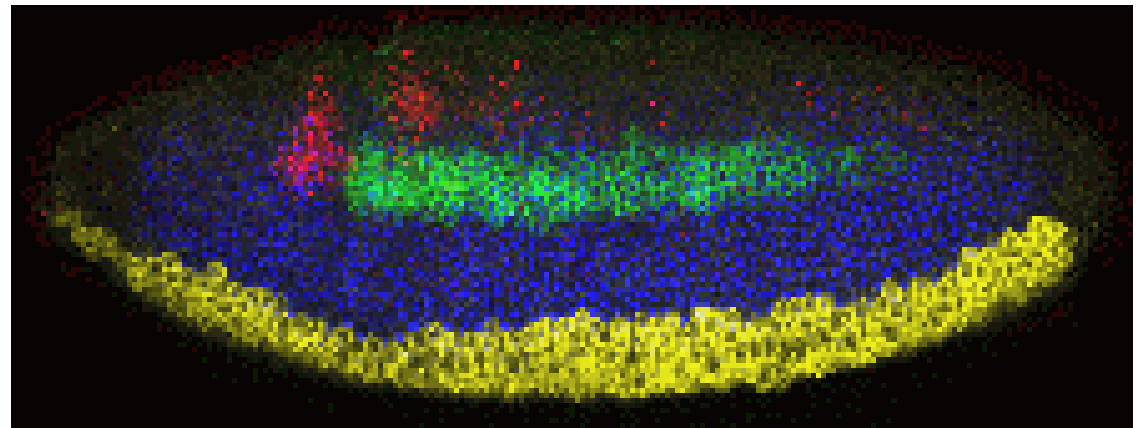
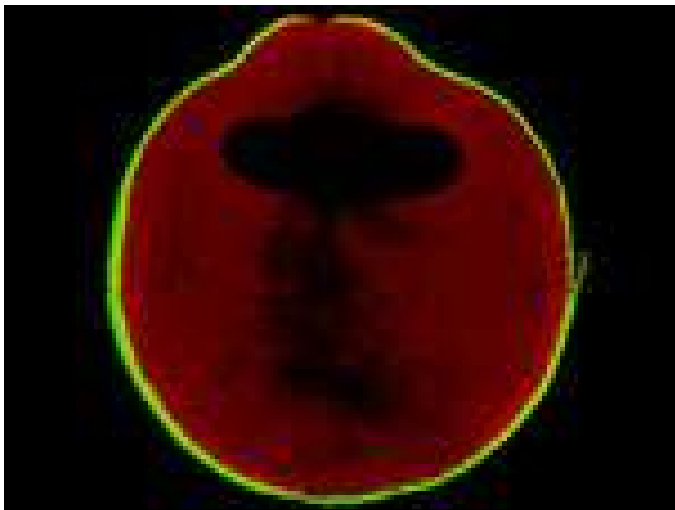
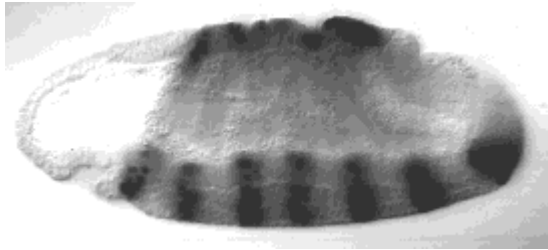
Imaging the action

- Reporter
- Green fluorescent protein GFP lacZ, labelled antibodies



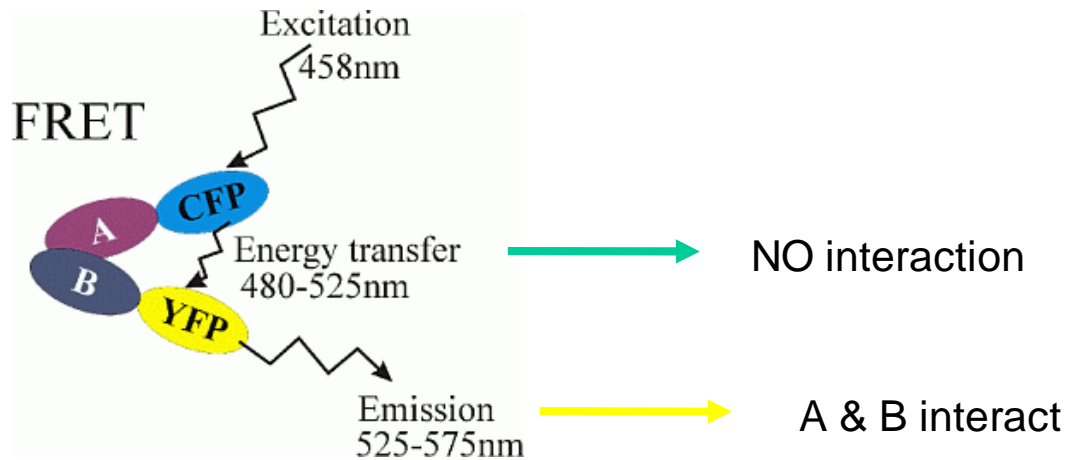
Imaging II

- **In-situ hybridization** measure the presence of mRNA of specific gene
- takes a snapshot of gene pattern – use fluorescently labelled RNA probe
- Use confocal microscope to image different 2D layers build 3D image

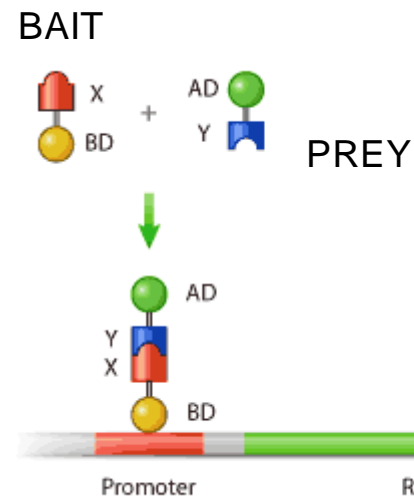
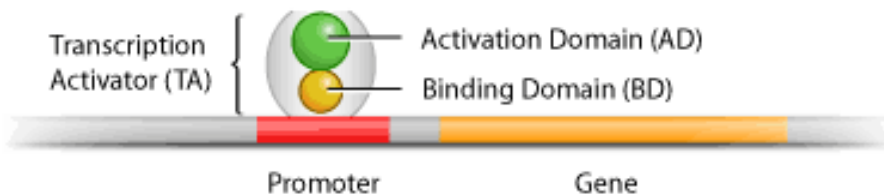


Detecting biomolecular interactions

FRET (Flourescence Resonance Energy Transfer)



Yeast Two Hybrid



Interactions II:

Phage Display:

- Phage = virus that infects bacteria. Has an external coat of protein
- Can fuse foreign proteins to coat proteins of phage
- Used to screen libraries of proteins for specific interaction drug design

