

Review:

- 1. What's virus?

Obligate intracellular parasites

- 2. Where does virus come from?

Bacteria  plasmid/transposon 
Co-evolution 

- 3. What's viroid?

Infectious RNA molecules

Preview:

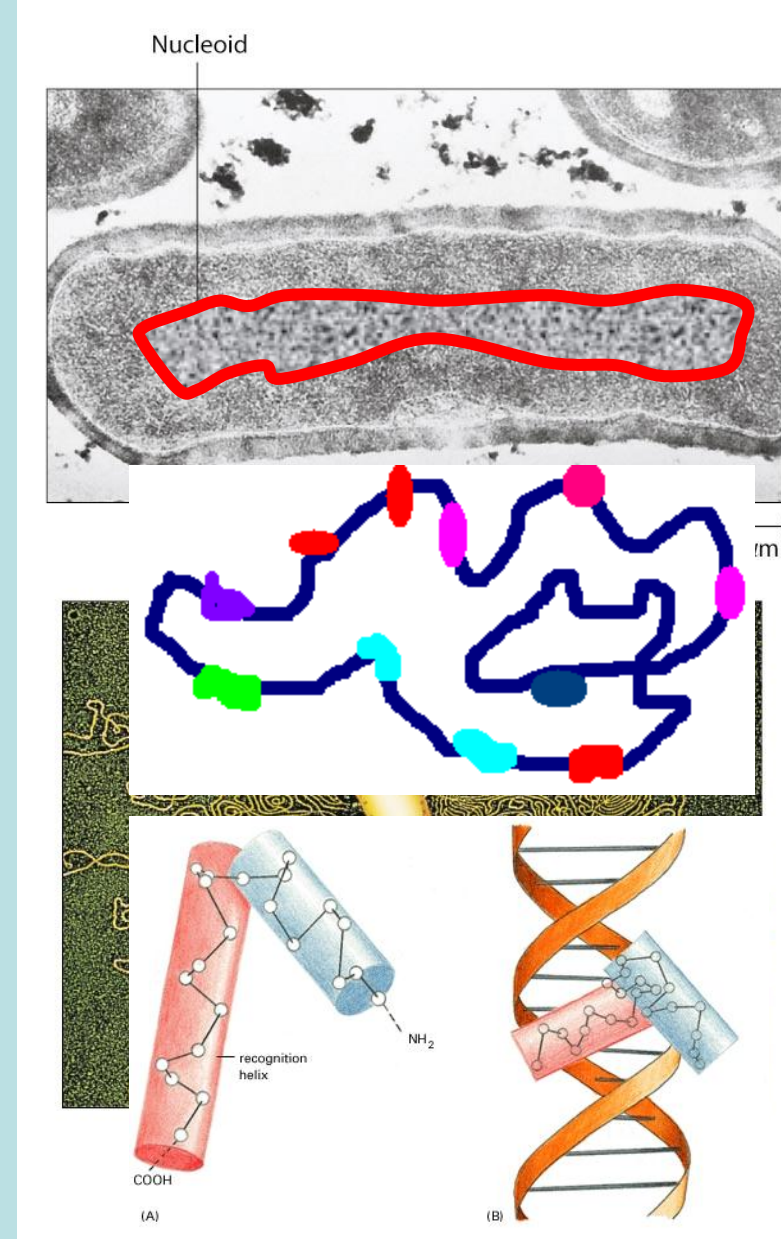
What's gene?

What's mutation?

What's resistance?

Microbial genetics

- Structure of bacteria genome:
- Circular single DNA molecule
- With proteins binding on DNA
- **Nucleoid**: the cellular region containing bacterial DNA
- **DNA**: polymer macromolecule that is composed of deoxyribonucleic acids storing genetic information



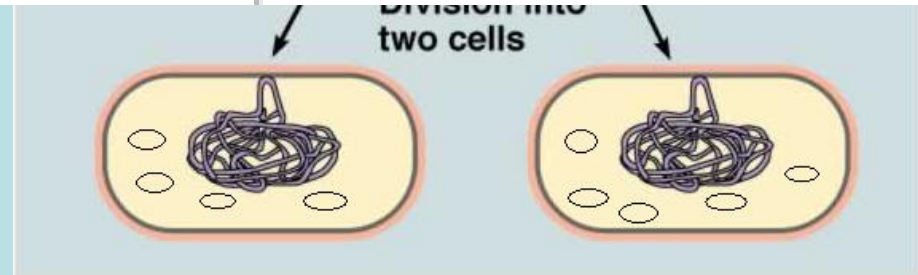
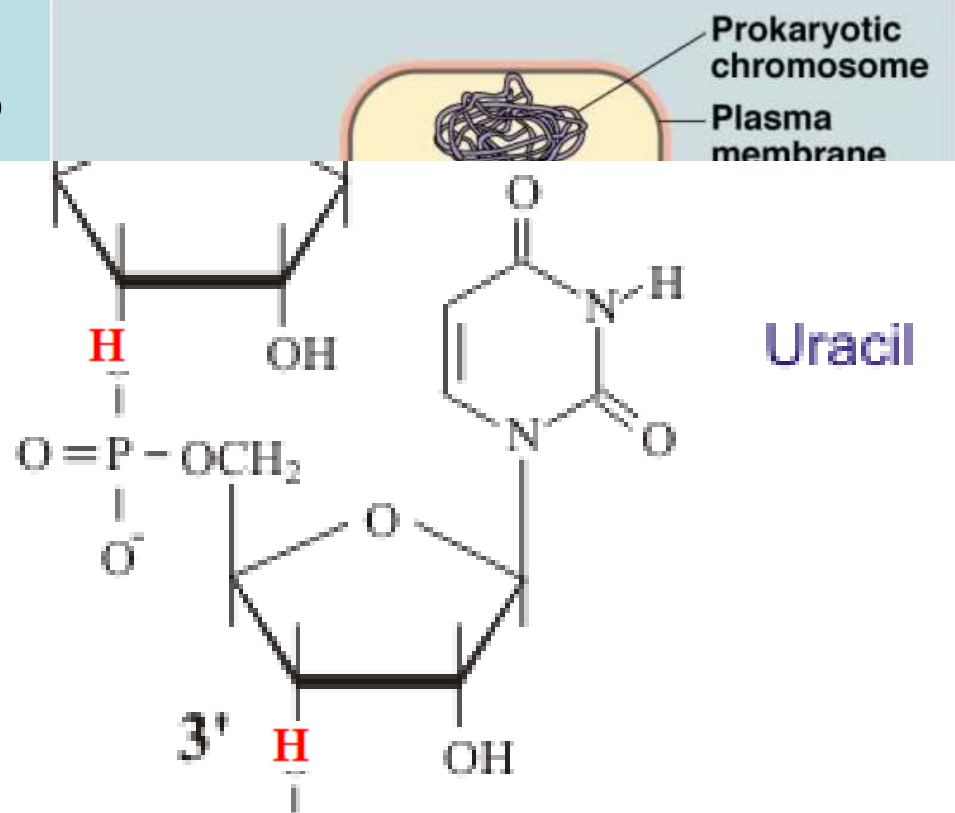
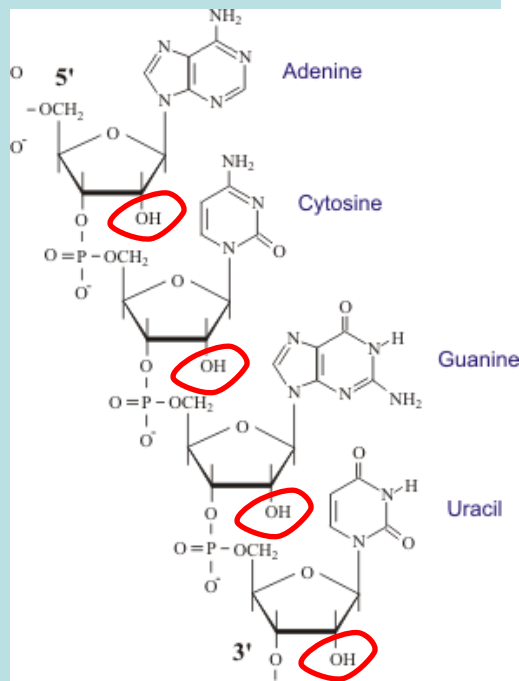
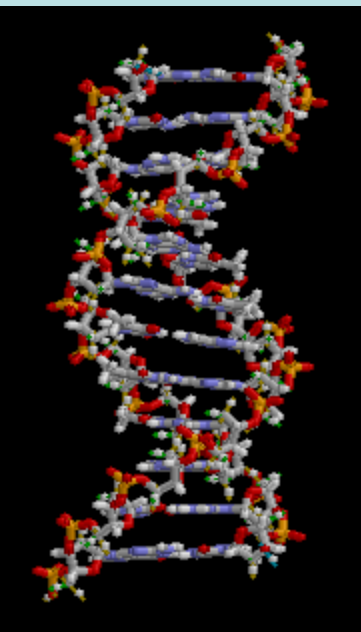
Microbial genetics

- **Gene**: a basic unit of heredity in an organism

Usually a fragment of DNA or RNA sequence

Plasmid

RNA: ribonucleic acid



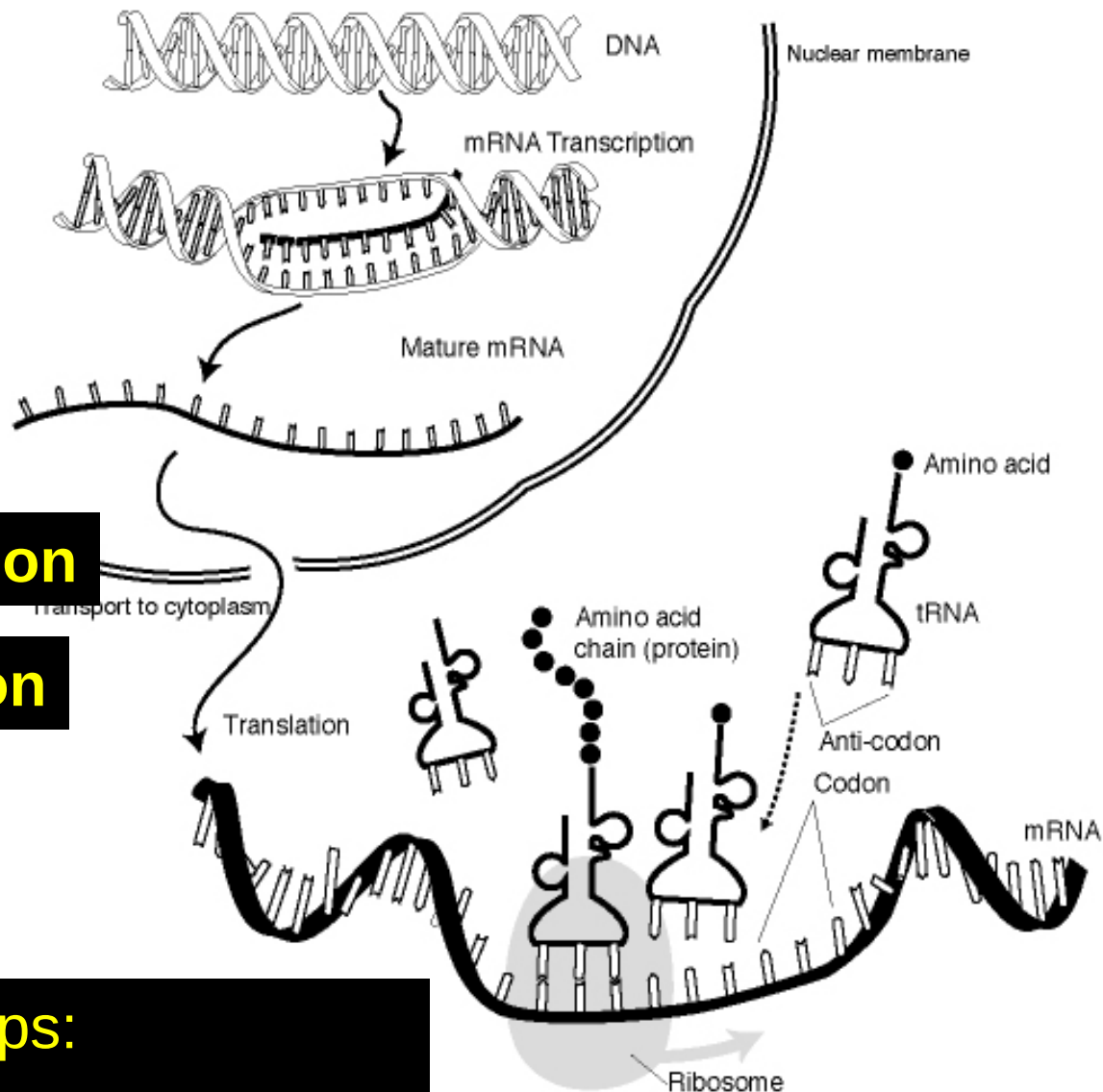
Cell division: DNA replication

<http://www.youtube.com/watch?v=teV62zrm2P0>

Gene expression: transcription, translation

Central dogma: the process that genetic information pass from DNA through RNA to proteins

DNA >> **transcription**
RNA >> **translation**
protein



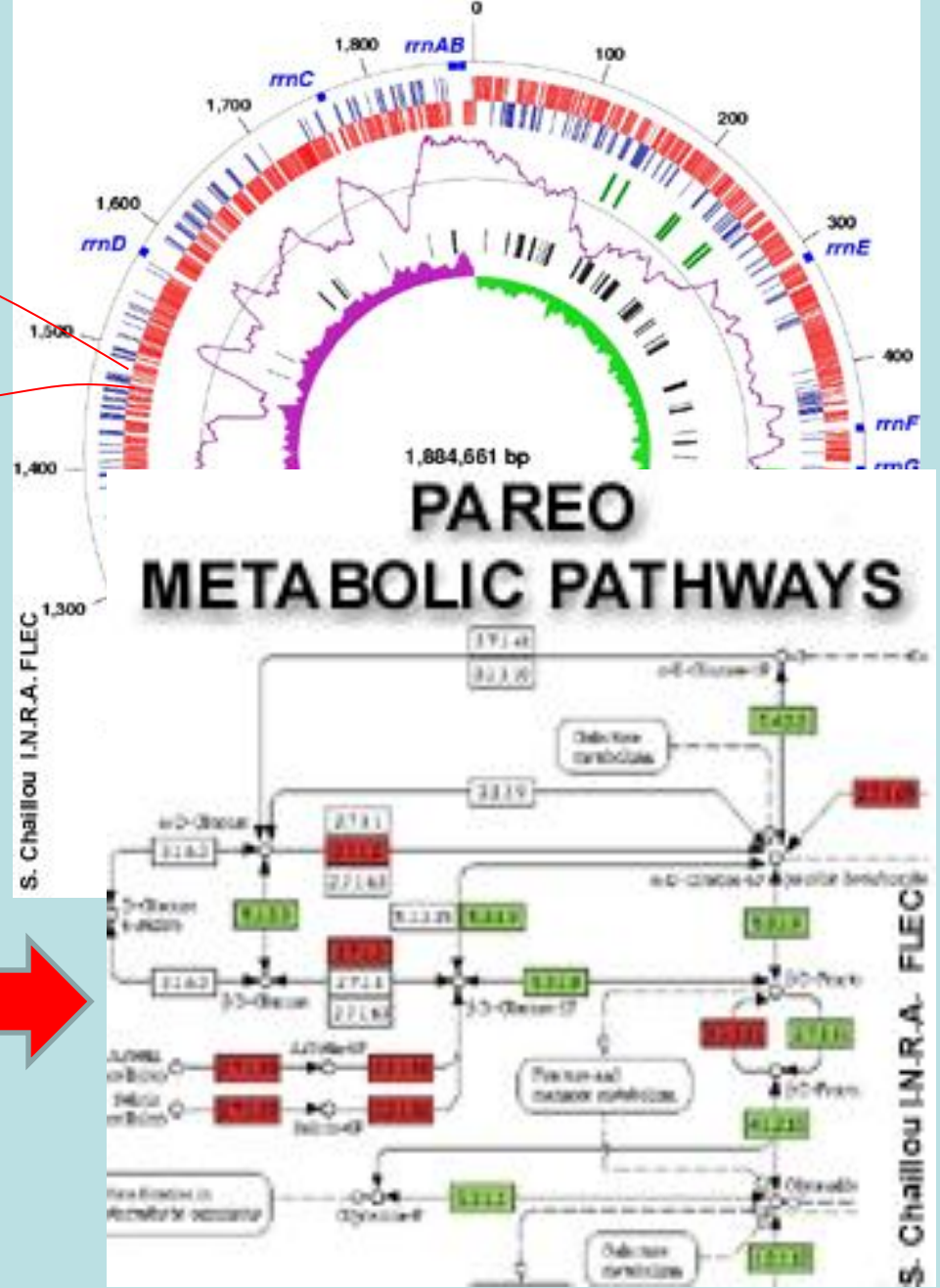
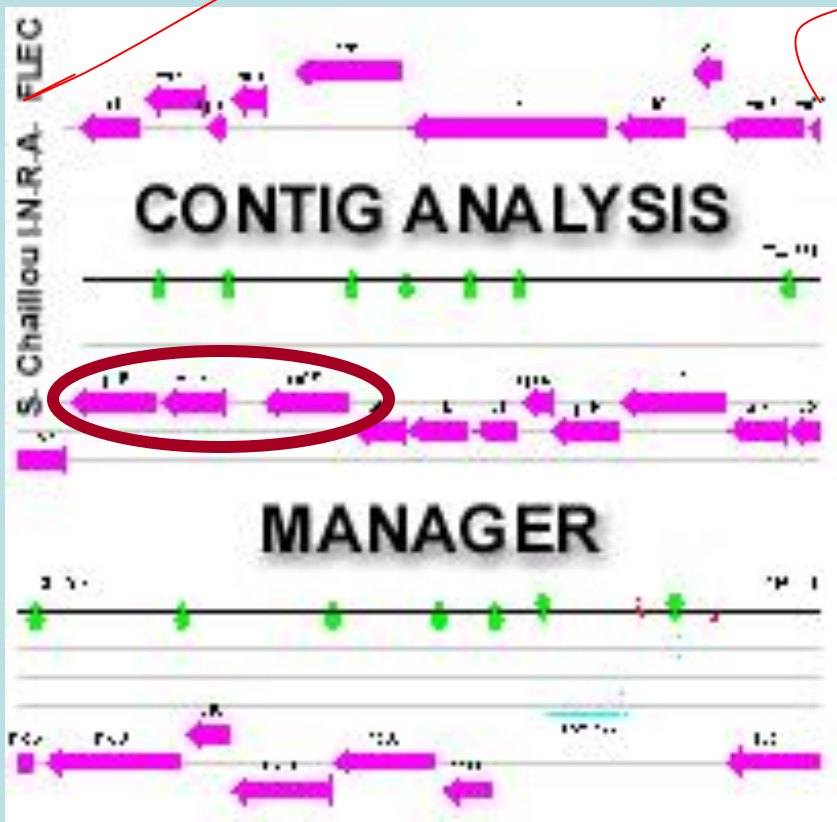
Gene expression, two steps:

transcription: <http://www.youtube.com/watch?v=WsofH466lqk&feature=related>

translation: <http://www.youtube.com/watch?v=5bLEDd-PSTQ&feature=related>

- Bacteria genome

Lactobacillus sakei 23K



Operon: a group of genes express together.

Spontaneous Mutation

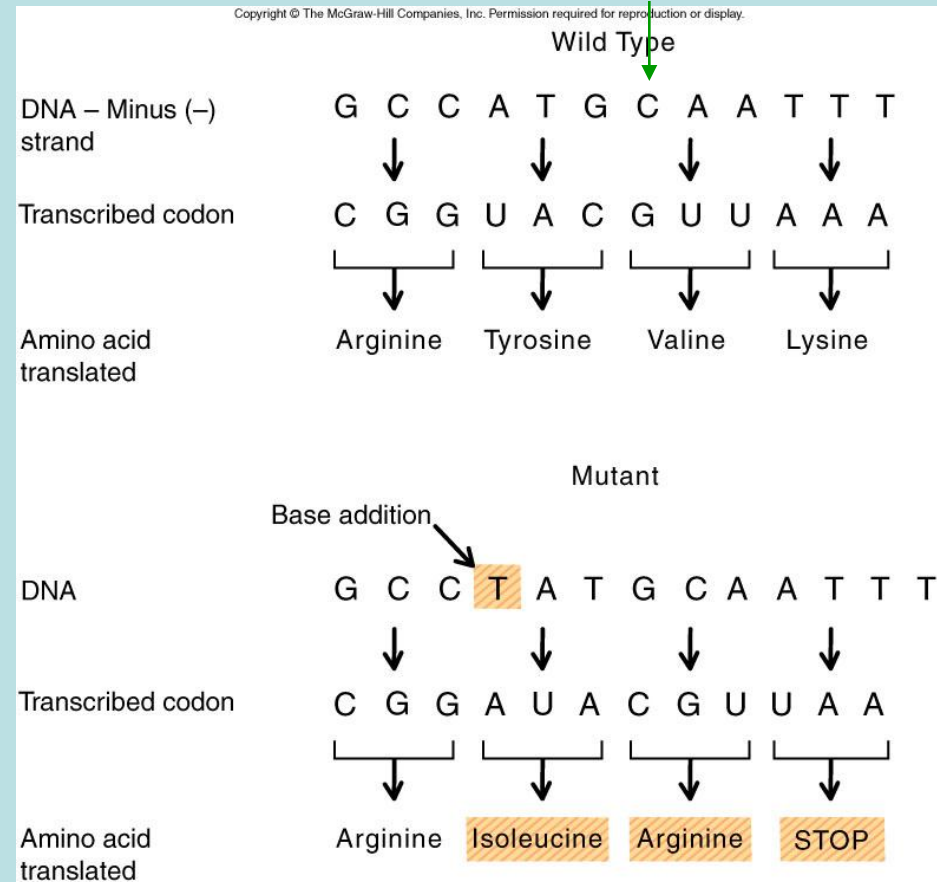
- **Spontaneous mutations** occur in natural environment
 - Occur infrequently and randomly
 - **Genetic mutation**: accidentally alteration of DNA sequence <http://www.youtube.com/watch?v=kp0esidDr-c&feature=related>
- **Rate of mutation**
 - Probability that a mutation will be observed in a given gene each time the cell divides
 - Rate is generally between 1 in 10,000 and 1 in a trillion
 - Low rate is due to cellular repair mechanisms

Spontaneous Mutation

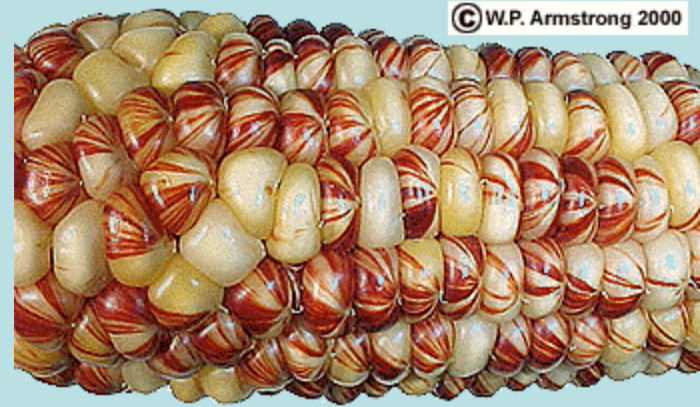
- Removal and addition of nucleotides
 - Shifts the translational reading frame
 - Shifts the codons
 - Mutation called frameshift mutation
 - Affects all amino acids downstream from addition or deletion
 - Mutations frequently result in premature stop codons

Base substitutions

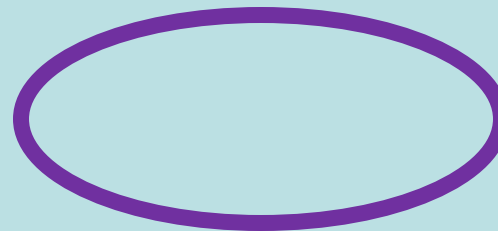
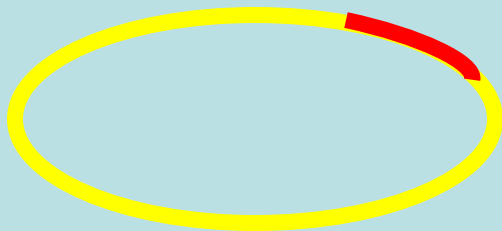
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Spontaneous Mutation

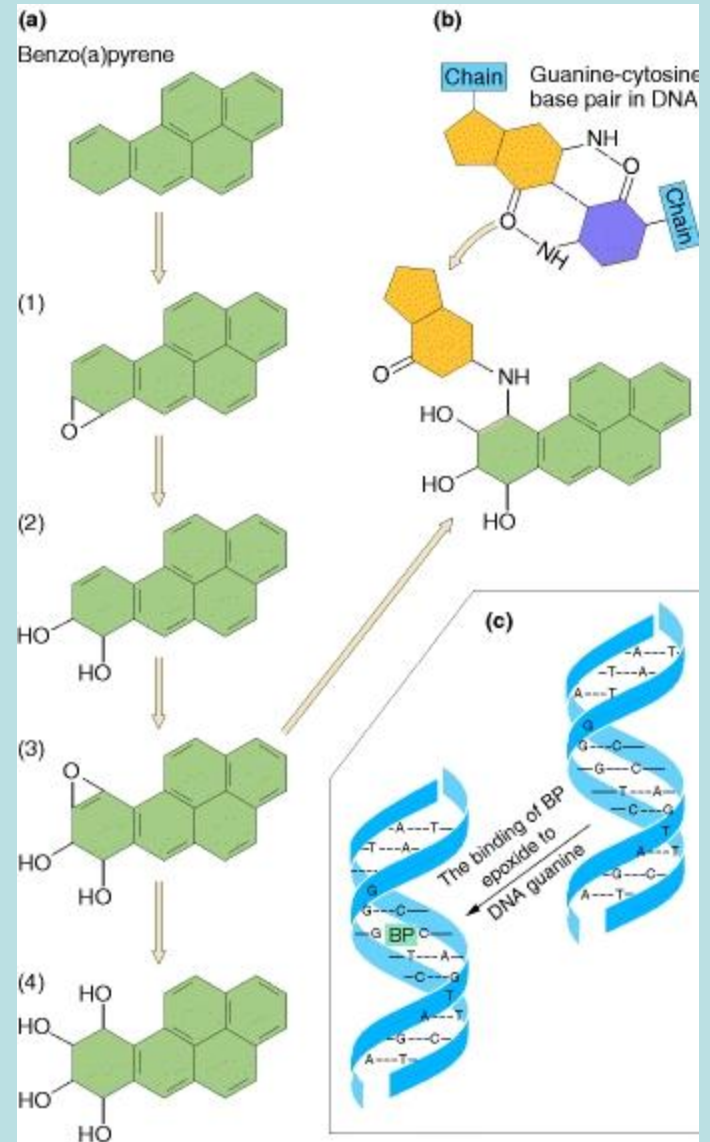


- Transposable elements
 - Special segments of DNA that move spontaneously from gene to gene
 - Elements called **transposons**
 - Transposons disrupt proper function of gene
 - Gene or gene product generally non-functional



Induced Mutations

- Mutations are essential for understanding genetics
 - Mutations can be intentionally produced to demonstrate function of particular gene or set of genes
 - These mutations are termed induced
- Mutations can be induced via
 - Chemical mutagens
 - Transposition
 - Radiation



Induced Mutations

- Chemical mutagens

- Nitrous acid

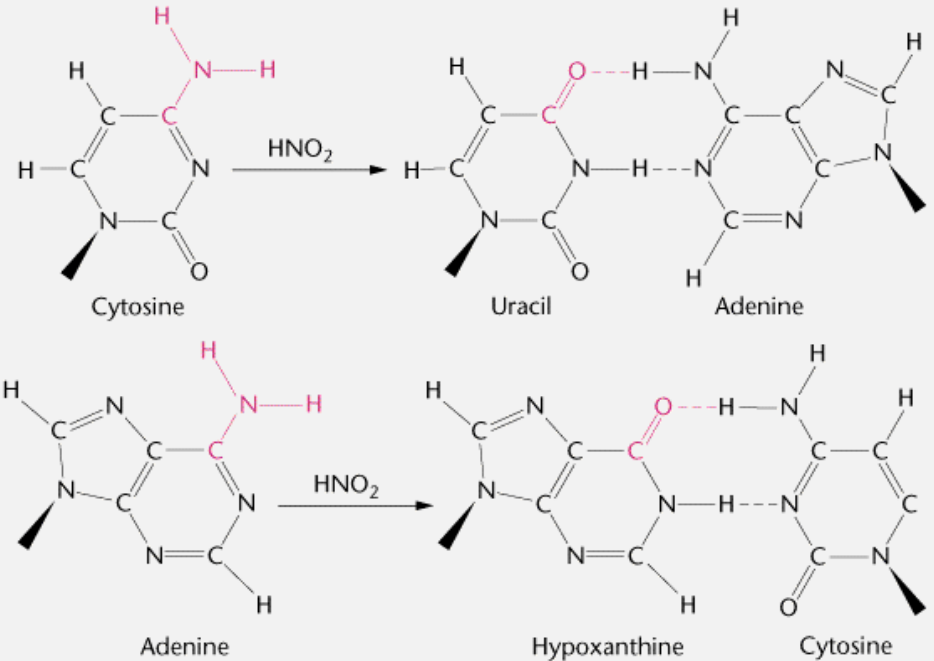
- Converts amino group to a keto group

- Changes cytosine to uracil (Uracil binds to adenine while cytosine binds to guanine)

- Alkylating agents

- Largest group of chemical mutagens
 - Alters hydrogen bonding of bases
 - Nitrosoguanine is common alkylating agent

Mutagenesis by Nitrous Acid (HNO_2)



(Klug & Cummings 1997)

Induced Mutations

- Chemical mutagens
 - Intercalating agents
 - Molecules that insert themselves between adjacent bases
 - Create space between bases
 - » Extra base is often added to fill space
 - Ethidium bromide is common intercalating agent
 - Potential carcinogen

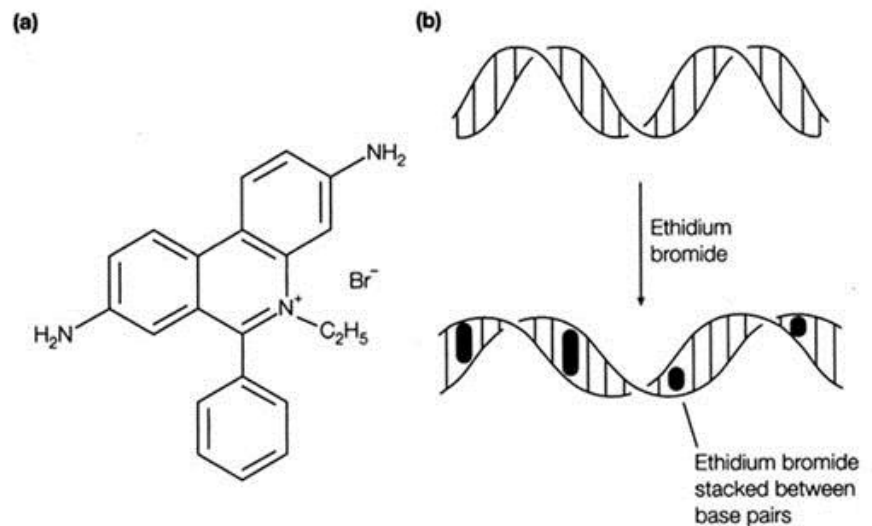
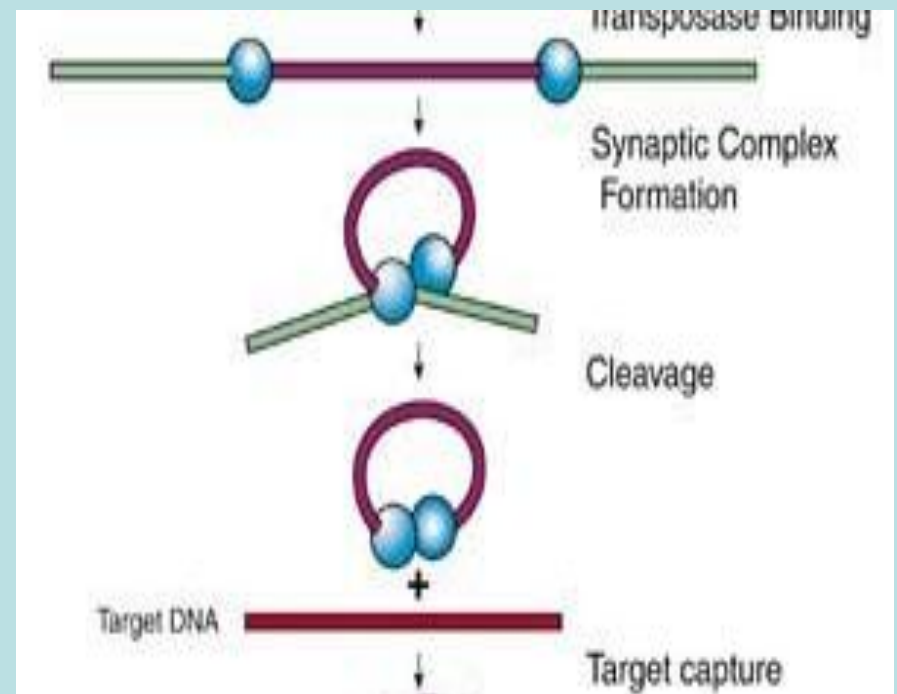


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

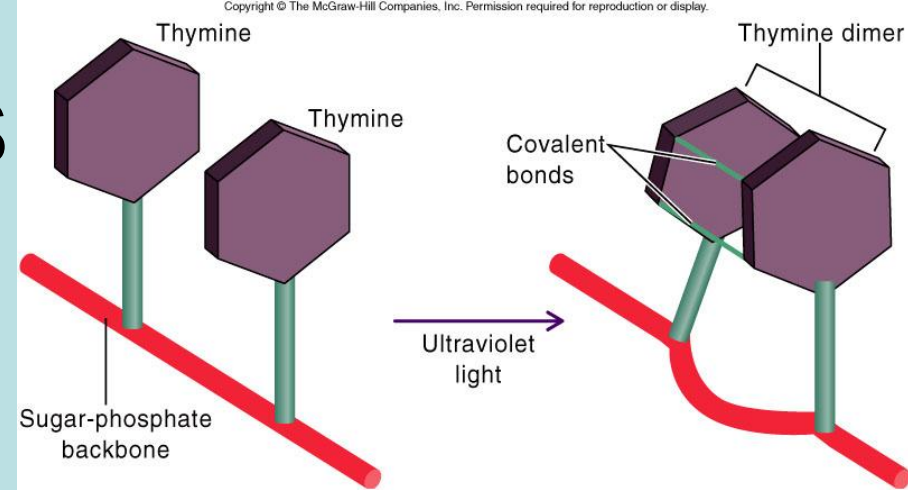
Induced Mutations

- Transposition
 - Common procedure used to induce mutation in laboratory
 - Gene that receives transposon will undergo a knockout mutation
 - Termed insertion mutation



Induced Mutations

- Radiation
 - Two types
 - Ultraviolet light
 - Causes covalent bonding between adjacent thymine bases
 - » Forms thymine dimers which distorts DNA
 - X rays
 - Cause breaks and alterations in DNA
 - » Breaks that occur on both strands are often lethal



Q: Both bacteria and virus can have mutation, but

A. **Virus has more mutation**

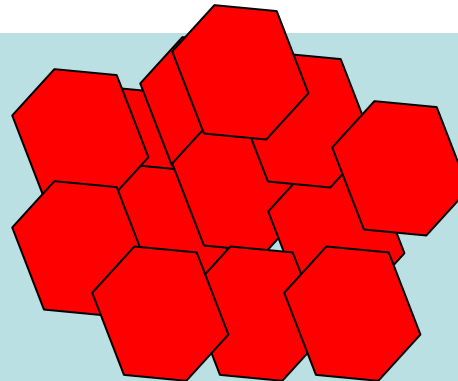
B. **Bacteria has quicker mutation**

C. **They have different mutation types**

D. **Virus has quicker mutation**

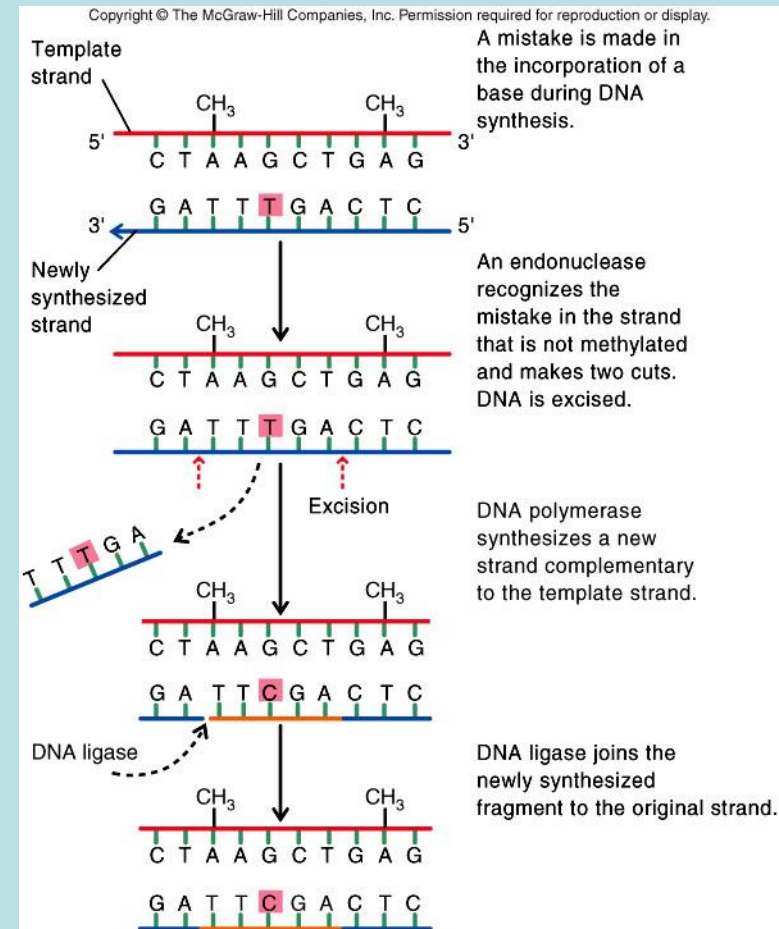
Virus mutation rate: 10^{-4}

Bacteria mutation rate: 10^{-8}



Repair of Damaged DNA

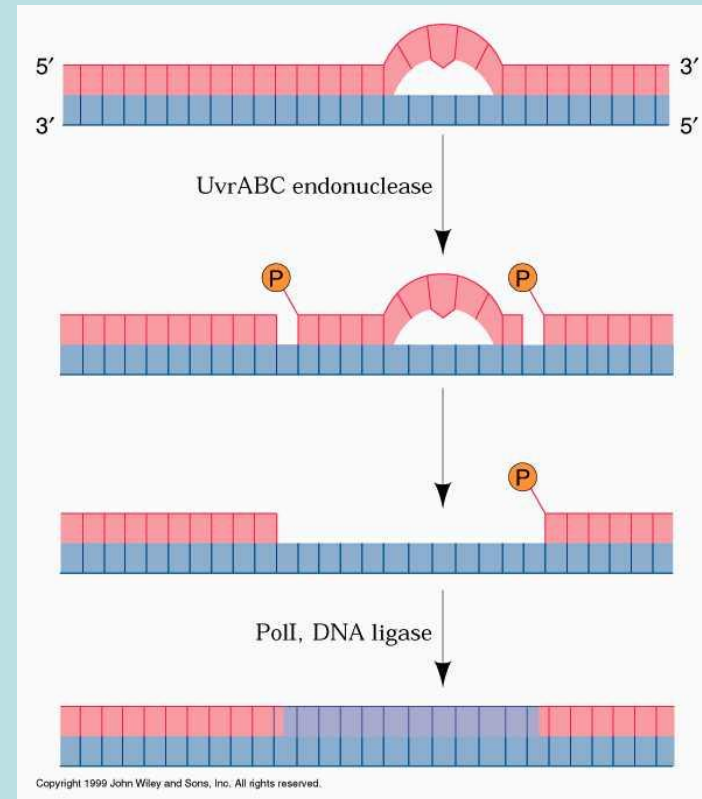
- Repair of base substitution
 - Cells develop two methods of repair
 - Proofreading
 - DNA polymerase has proofreading function
 - Able to excise incorrect base and replace with correct one
 - Mismatch repair
 - Endonuclease enzyme removes short stretch of nucleotide
 - DNA polymerase fills gap
 - DNA ligase joins ends



Mismatch repair

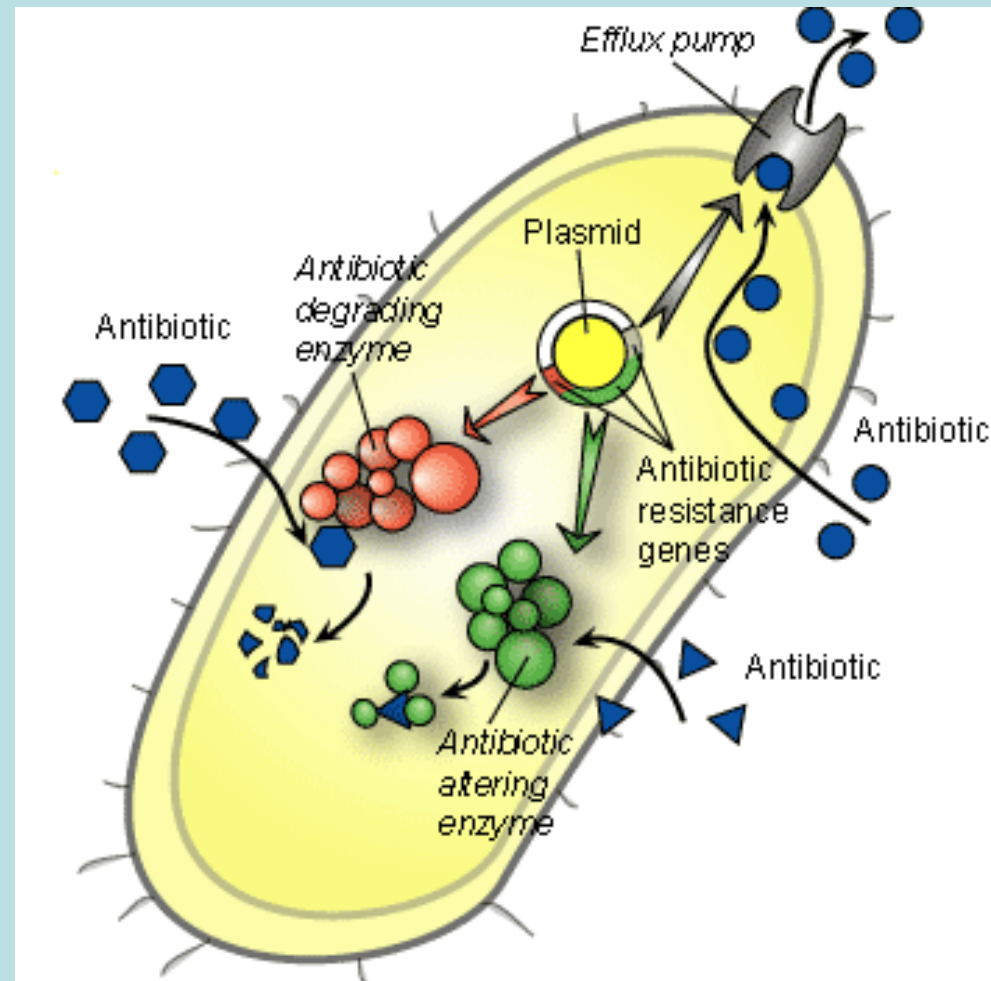
Repair of Damaged DNA

- Repair of modified bases
 - Enzyme cuts DNA backbone and removes base
 - DNA polymerase incorporates new base
- SOS repair
 - Last ditch effort to bypass damage
 - Damage induces SOS system
 - Produces new DNA polymerase
 - Highly error prone
 - » Mutations can arise from synthesis with new enzyme



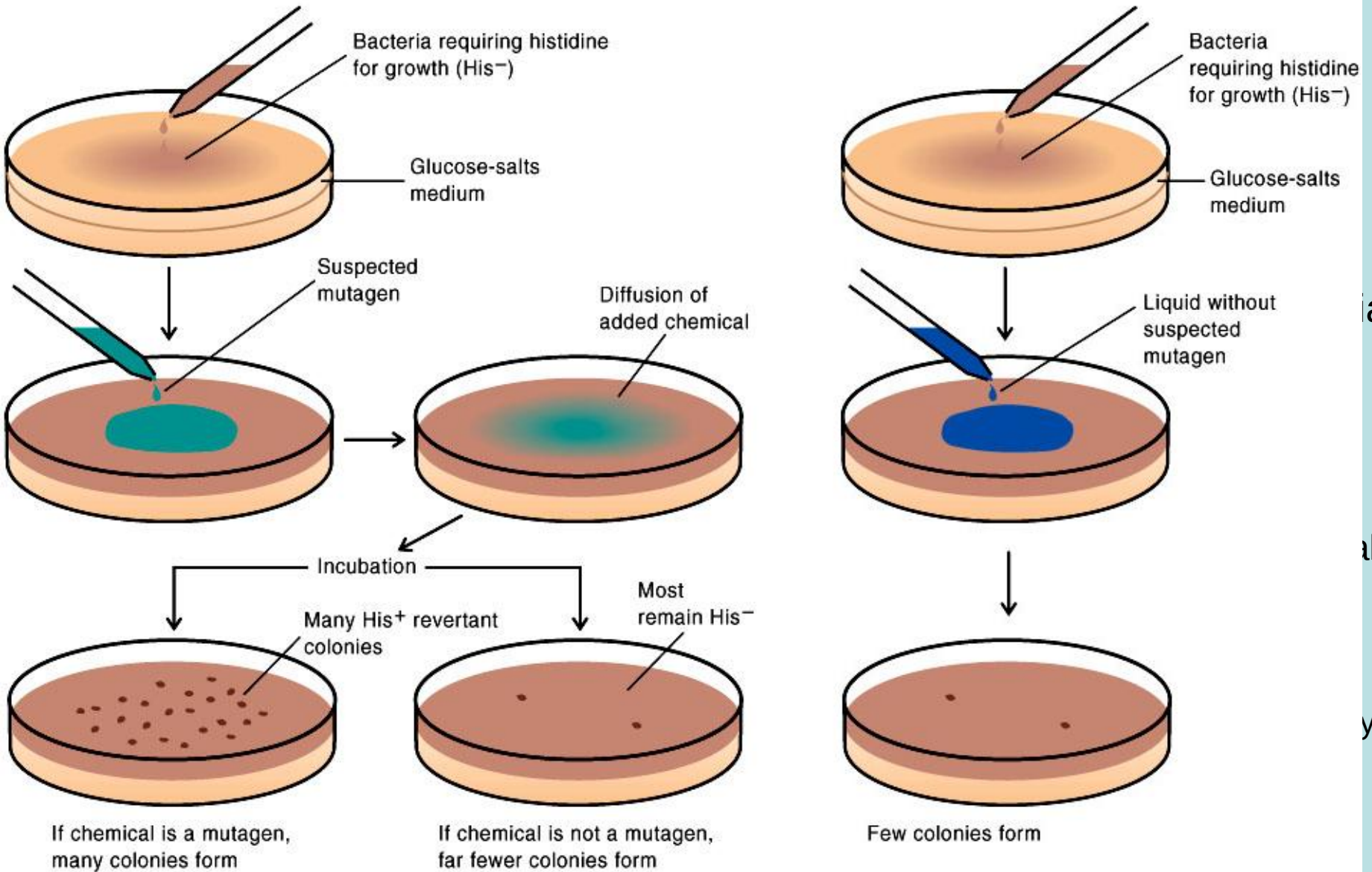
Mutations and Their Consequences

- Mutations provide organism way to respond to changing environments
- Environment selects for cells suited to survive
- Adaption and resistance



Mutation Selection

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(a) Test plates

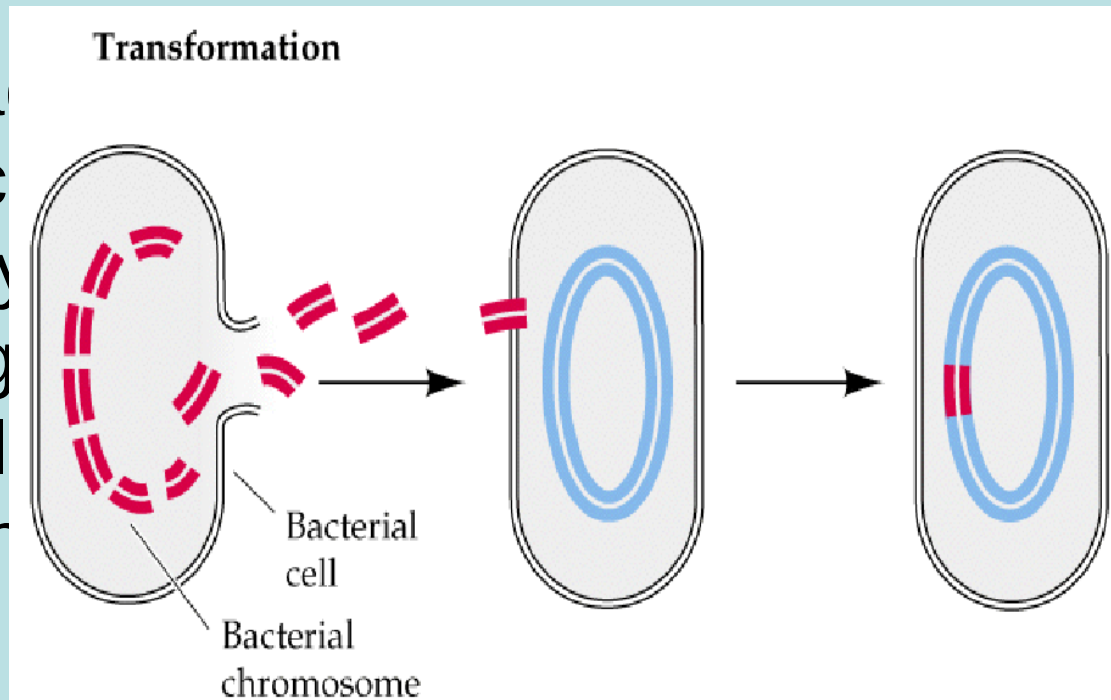
(b) Control plate

Gene transfer: Genes are naturally transferred between bacteria using three mechanisms

Transformation: DNA molecules directly delivered from one location (species) to another

Transduction: DNA fragments delivered from one location (species) to another by

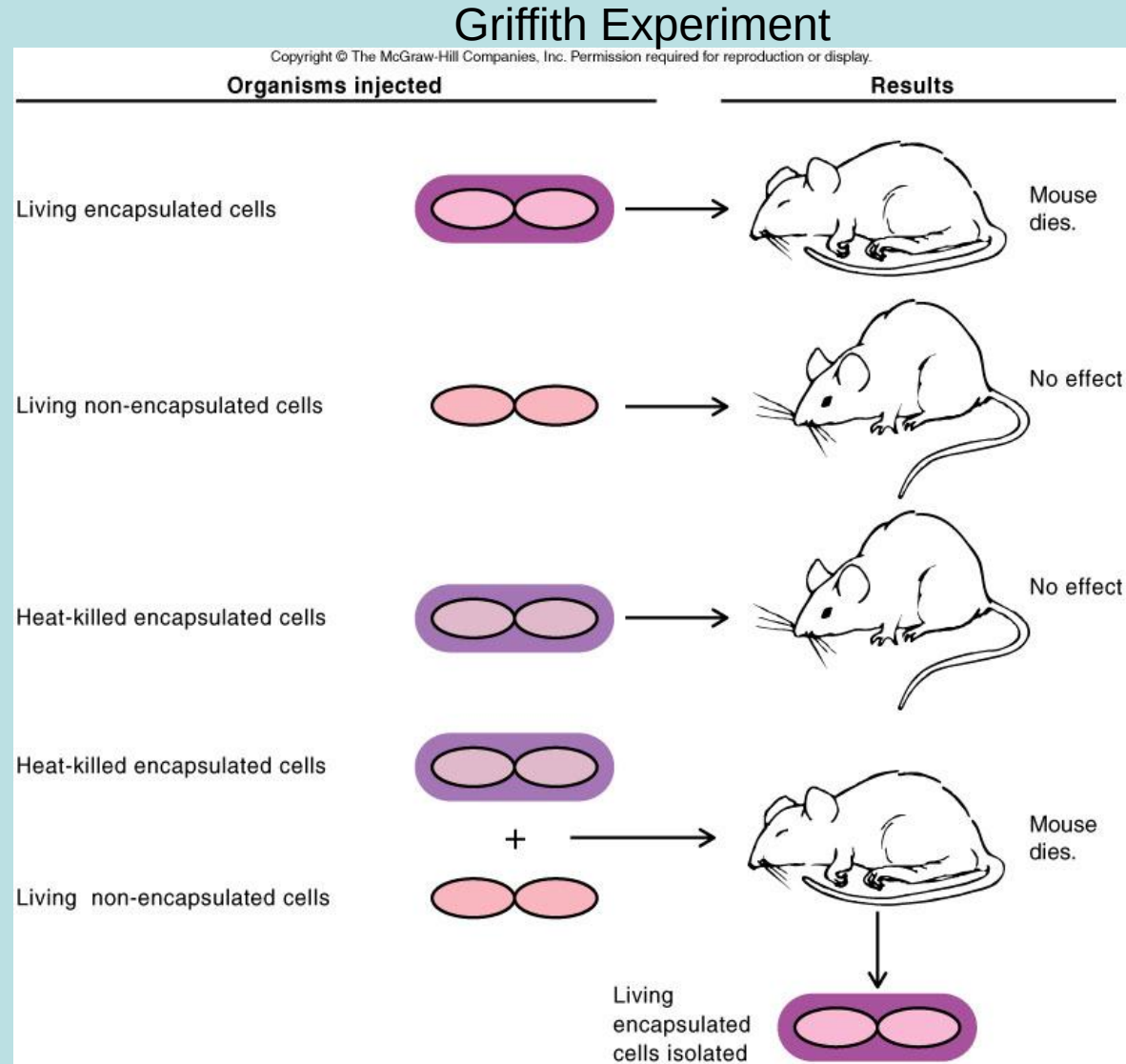
Conjugation: DNA fragments delivered from one cell by physical connection

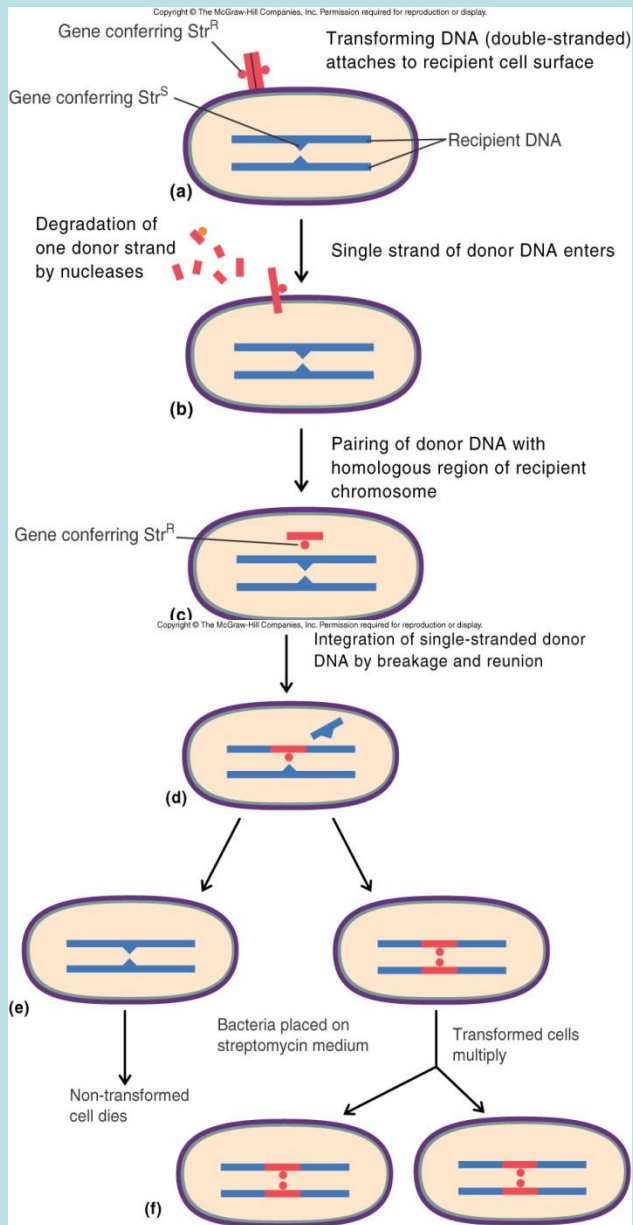


Mechanisms of Gene Transfer

- DNA-mediated transformation

- DNA fragment can be natural degrading products or artificial manufactured

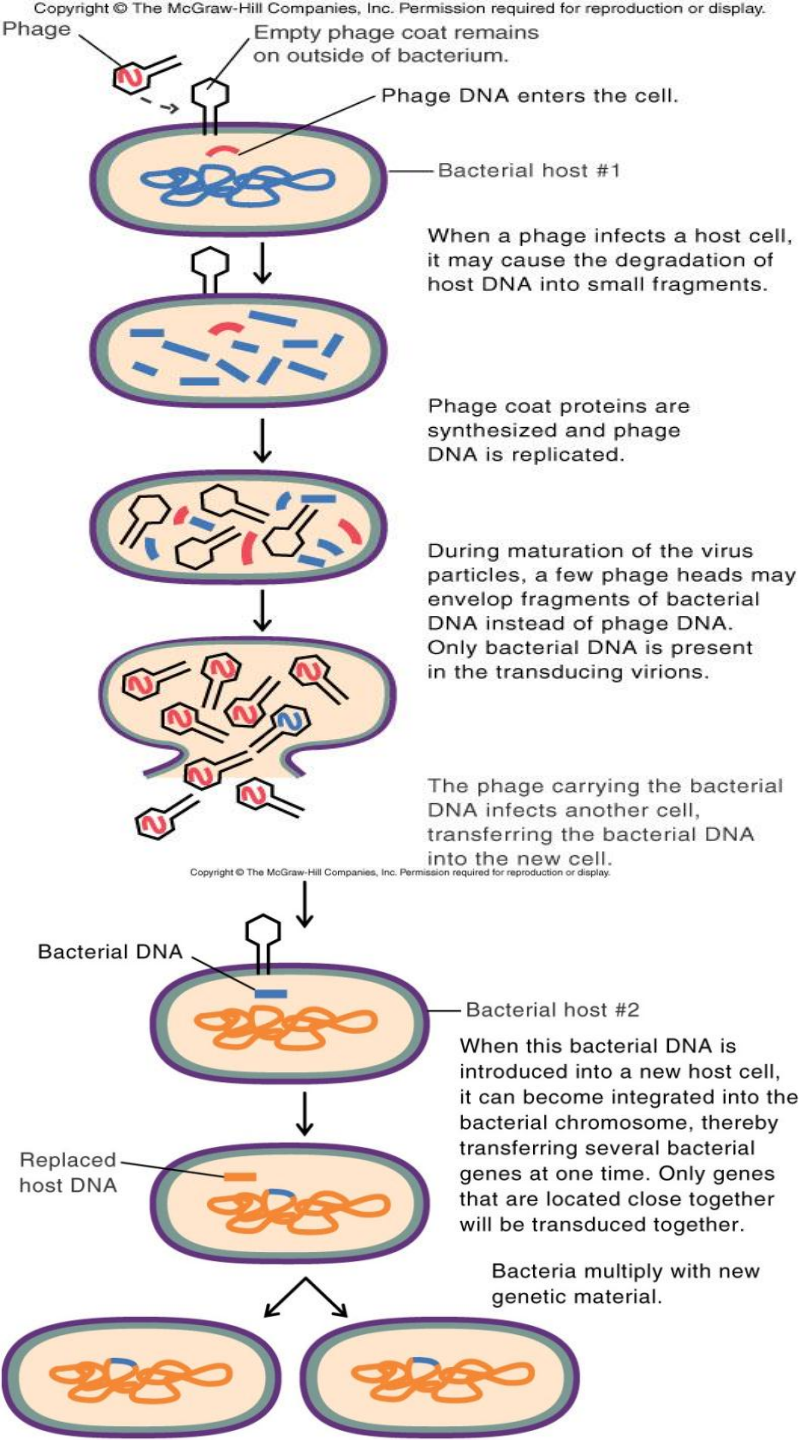




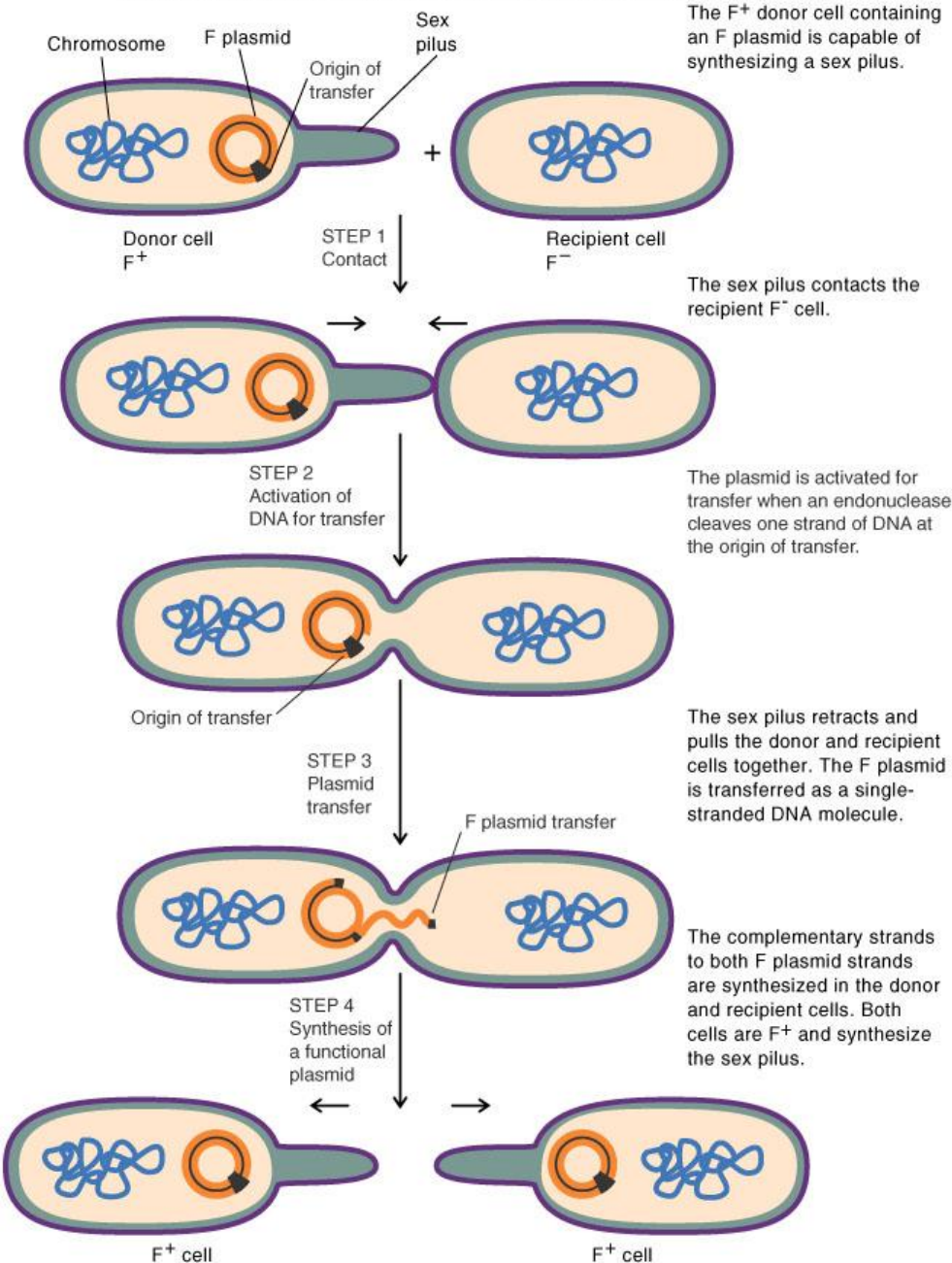
• Natural transformation

occurs in four stages

- Entry of the DNA
 - Only single strands enter, double strands are degraded
- Integration of the donor DNA
 - Donor DNA is integrated by hydrogen bonding
 - Enzymes cleave to recipient DNA
 - Donor DNA is put in place
- Mismatch repair
 - Repair mechanism removes either donor or recipient DNA that doesn't match
 - Repairs with correct nucleotides
- Cell multiplication
 - Transformed cells multiply under selective conditions in which non-transformed cells will not grow

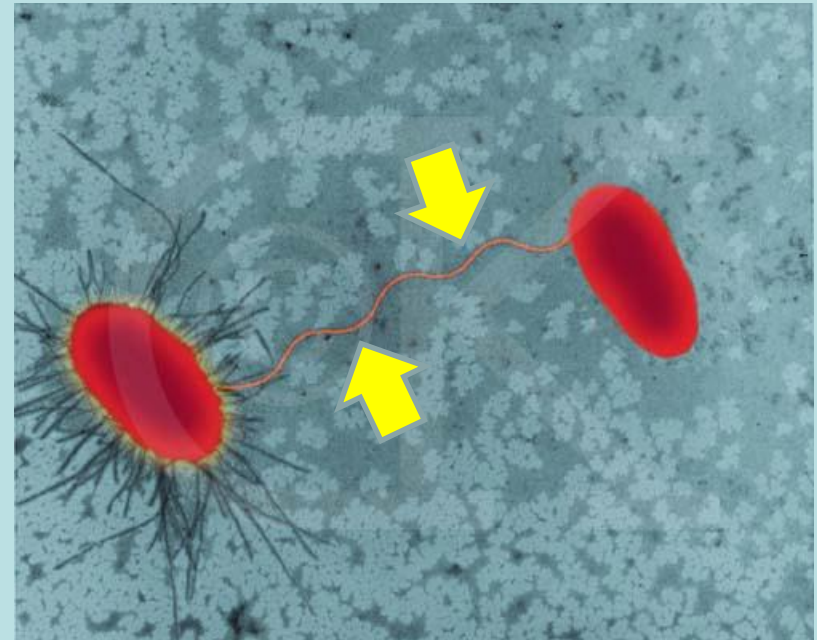


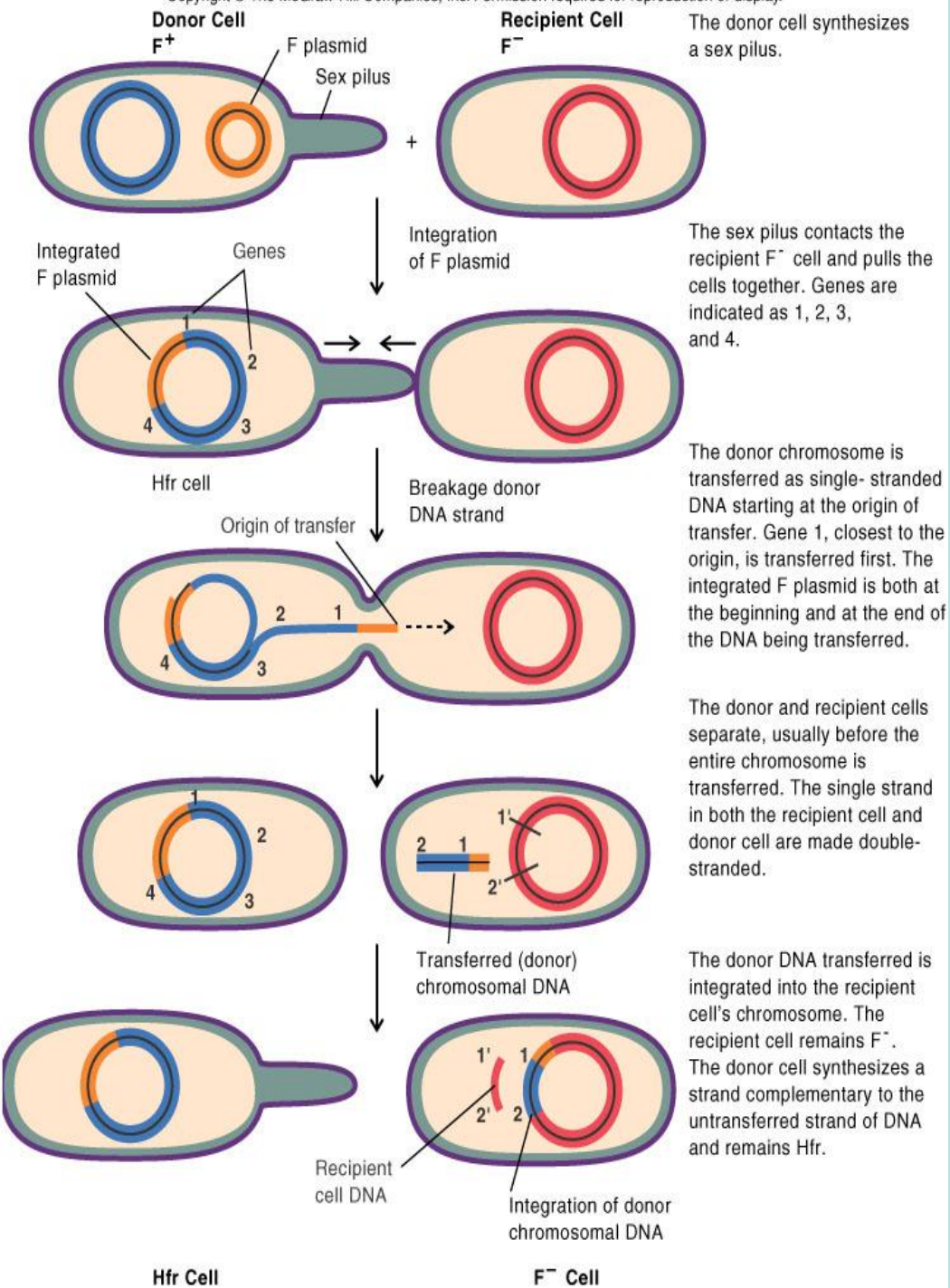
- **Transduction** is a mis-packaging of DNA during viral replication
 - The mis-packaged phage infects a new bacterial cell and inserts the donor DNA into the recipient cell
 - The donor DNA is integrated and mis-matched pairs are repaired



During **conjugation**, the plasmid is replicated in the donor cell and is transferred to the recipient

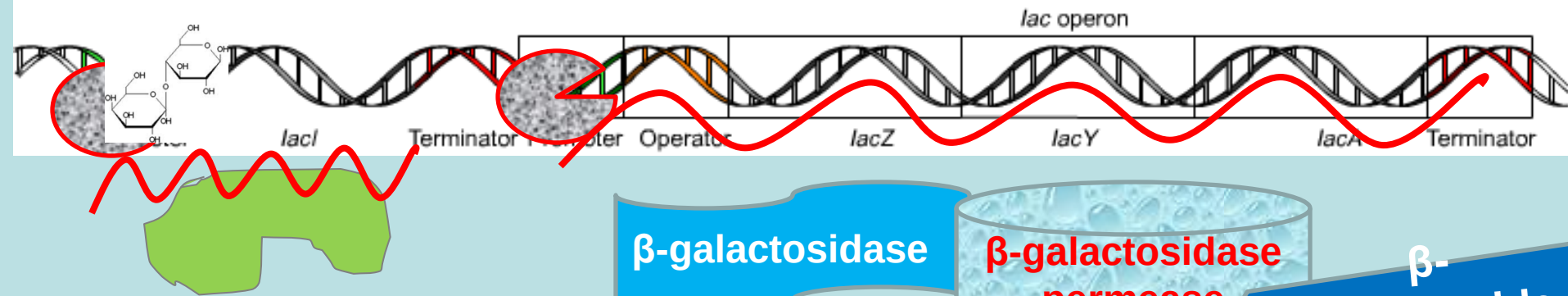
- After, plasmid is transferred, F^- cell becomes F^+





- In some F^+ cells, the F factor integrates onto the host chromosome
 - Converts F^+ to Hfr
 - Hfr= High frequency of recombination
 - Conjugation between Hfr and F^- cell results in only a portion of the F factor being replicated and transferred.
 - F^- remain F^-
 - F^- has new information but may not have the F factor gene

Bacteria gene organization: operon

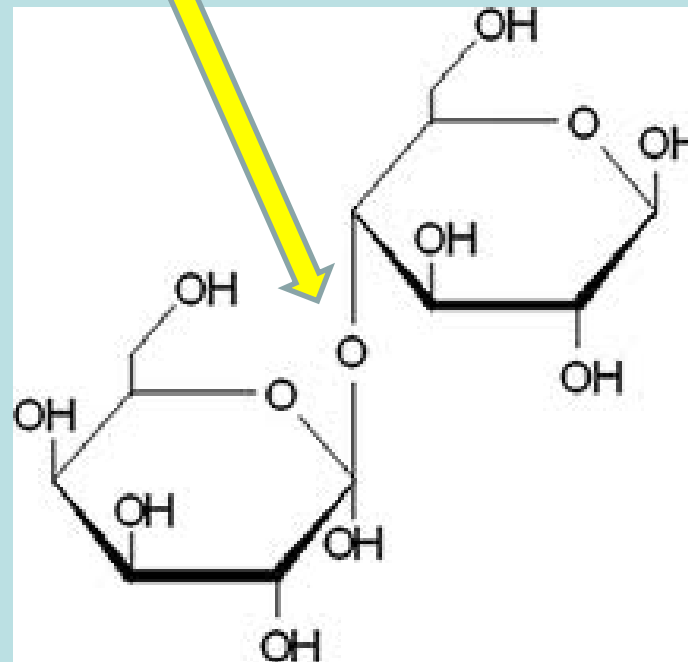


RNA polymerase

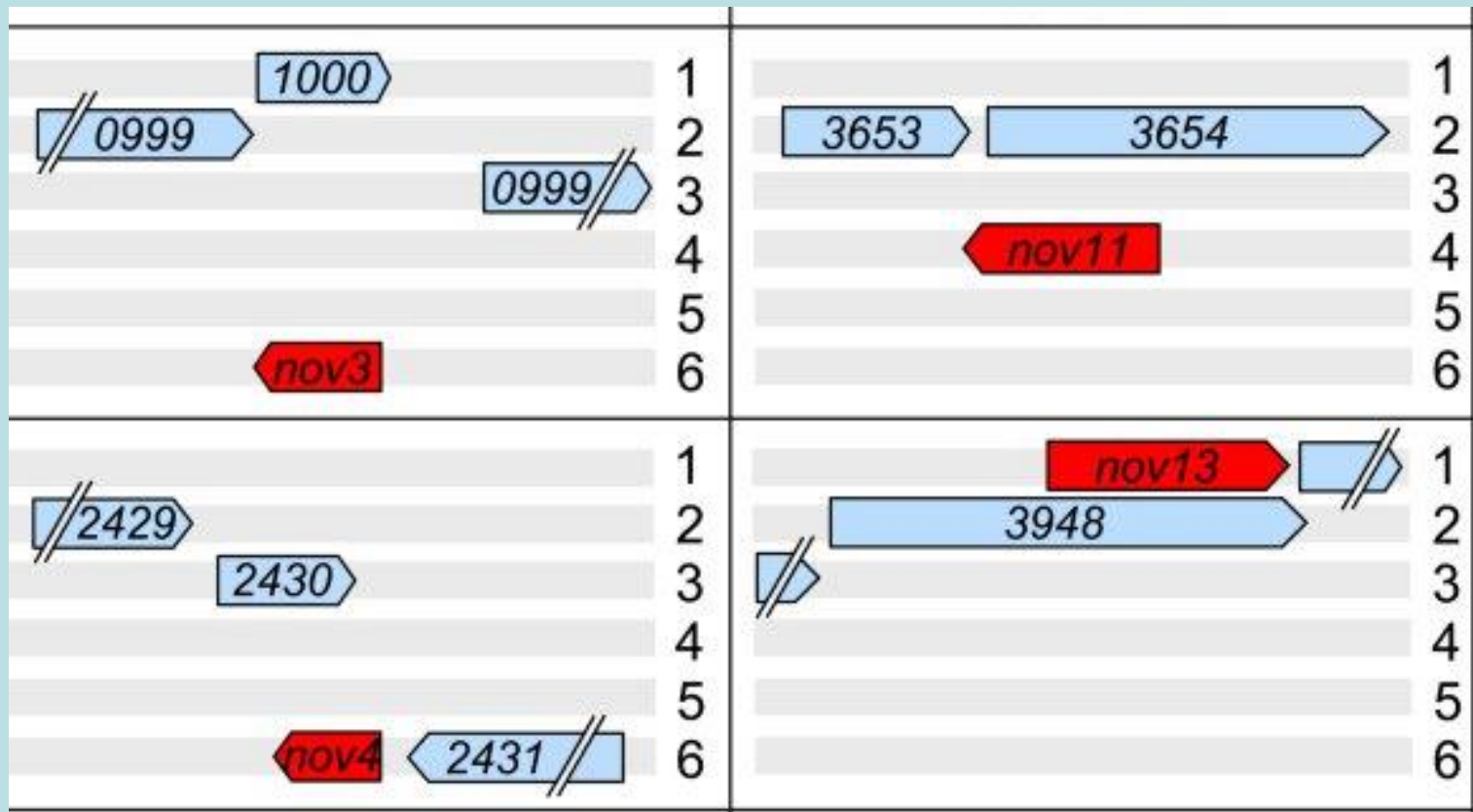
β -galactosidase

β -galactosidase
permease

β -
Galactoside
transacetylase

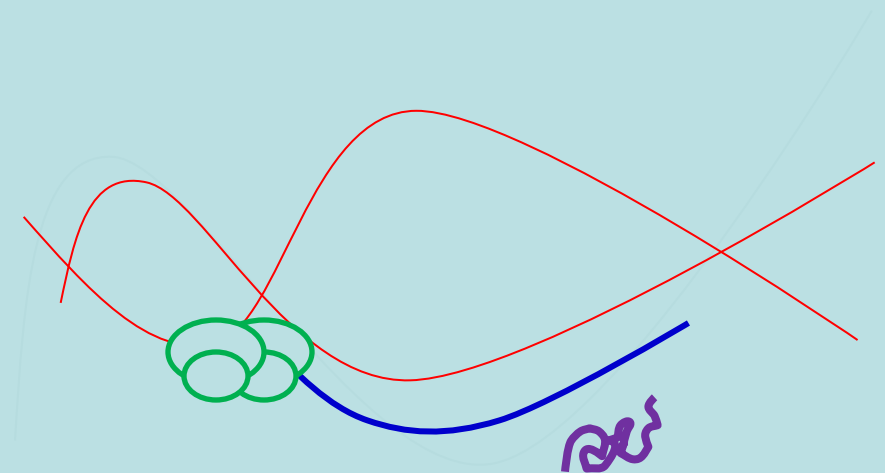
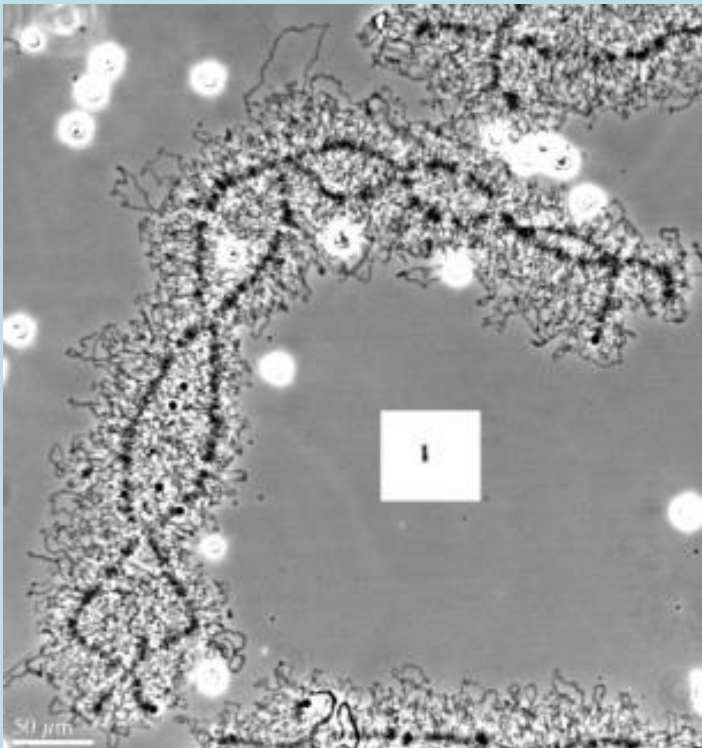


Bacteria gene organization: overlapped genes

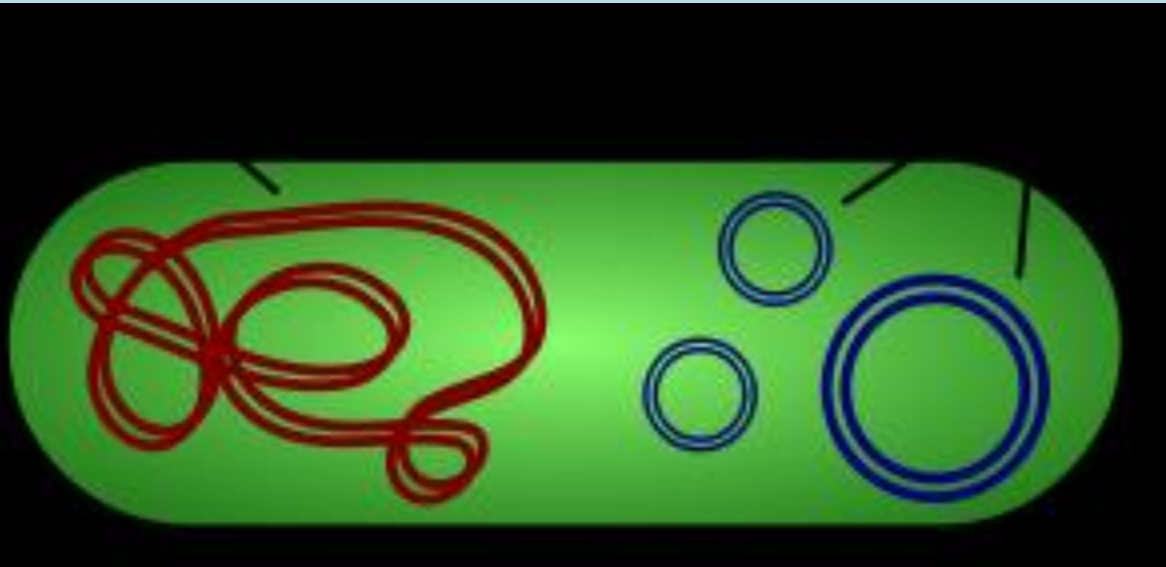


Organization of ten novel open reading frames (ORFs) overlapping predicted genes in *P. fluorescens* Pf0-1.

Bacteria gene expression: lamp-brush chromosome



Plasmid: size, replication and expression



The term *plasmid* was first introduced by the American molecular biologist Joshua Lederberg in 1952

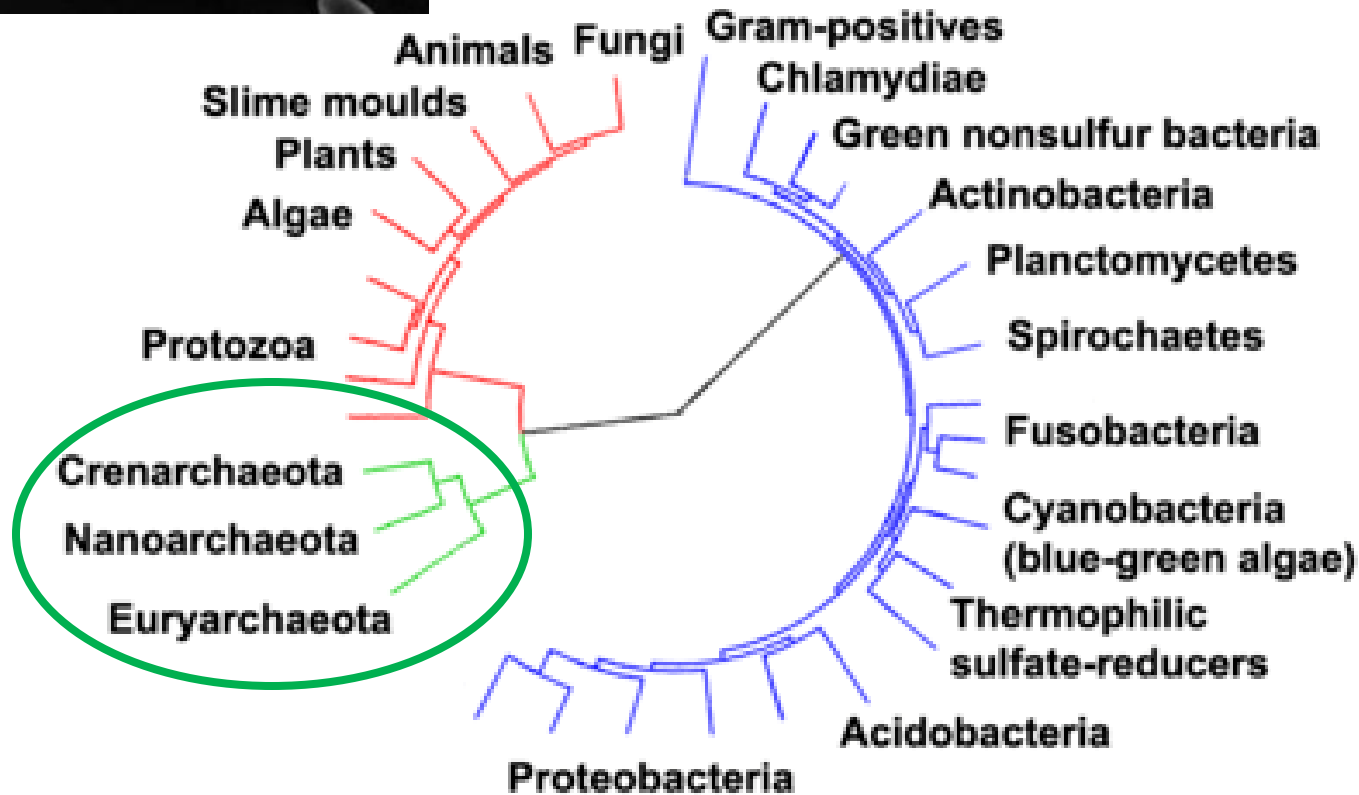
1-1000 Kb

1-400 copies

Independent promoter



Archaea genetics



1 circular
genome: 500
kb-1 MB

Have plasmid

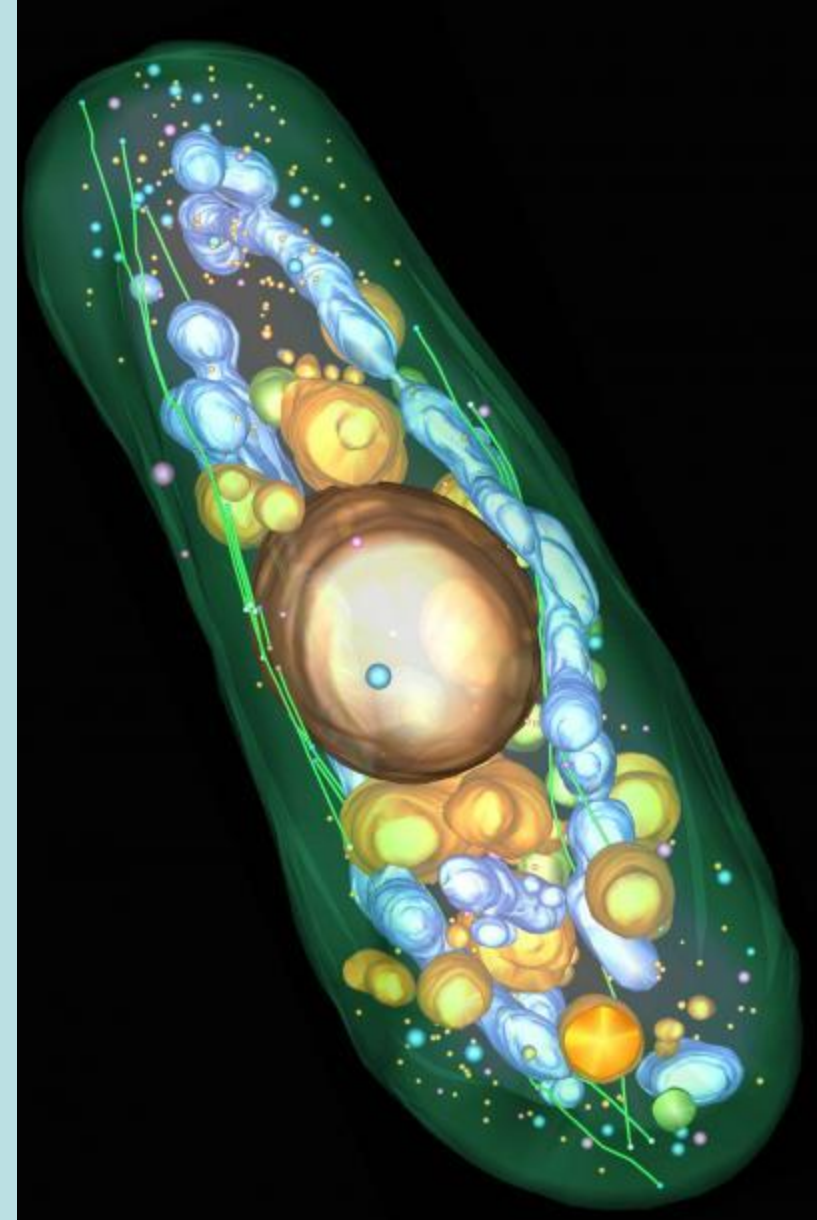
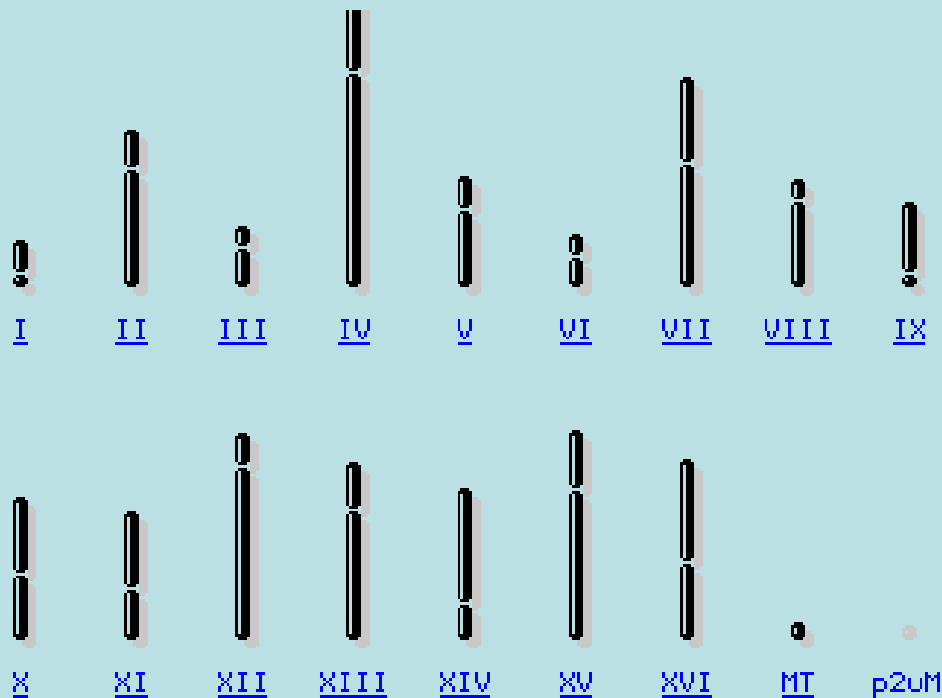
No spores
formed

Single layer
membrane

Yeast

*Eucaryotic cells: with nucleus and organelles

*Has larger genome, multiple chromosomes



Eucaryotic gene expression:

http://www.youtube.com/watch?v=OEWOZS_JTgk

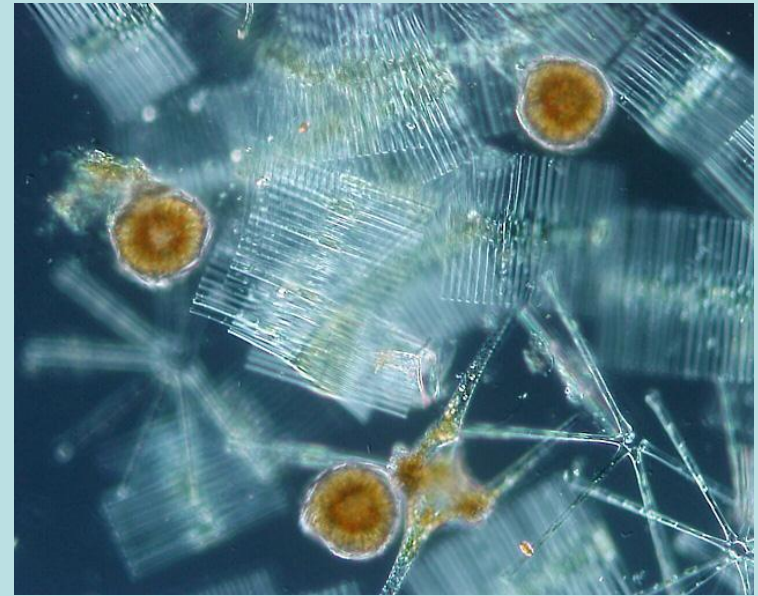
Algae genome



Red Algae



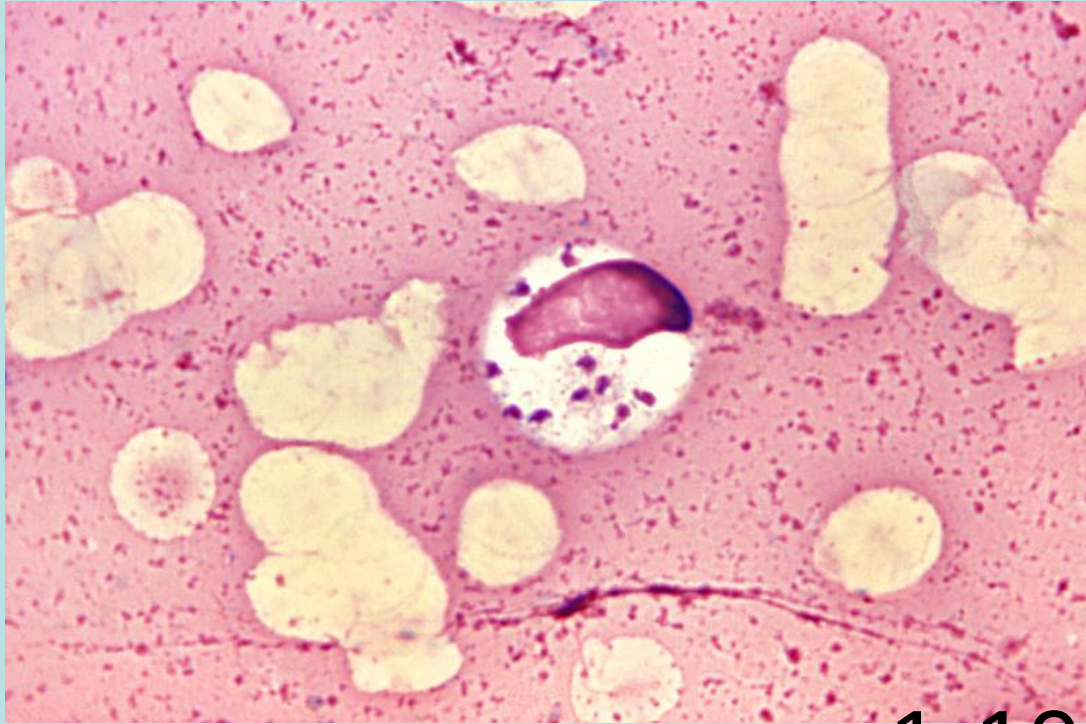
kelp



Phytoplankton

1-1000 Mb,
multiple chromosomes
Like plants

protozoa genome



Leishmania *donovani*, (a species of protozoa) in a bone marrow cell

1-100 Mb,
multiple chromosomes
Like animals

Review:

What's gene?

The basic unit of genetics, usually a fragment of DNA or RNA molecule with independent function

What's mutation?

The structural change of a gene

What's resistance?

The ability of a microorganism to withstand the effects of antibiotics or other compounds