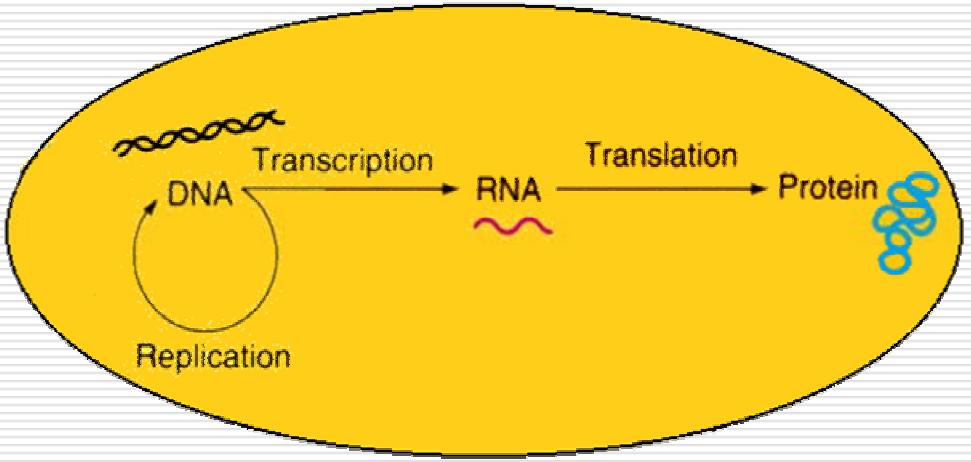
# Microbial Genetics

and biotechnology

# Define Terms

- Genetics
- Genome / Genomics
- Chromosomes
- Gene
- Genotype
- Phenotype
- Recombination

The "Central Dogma" in prokaryotic cells



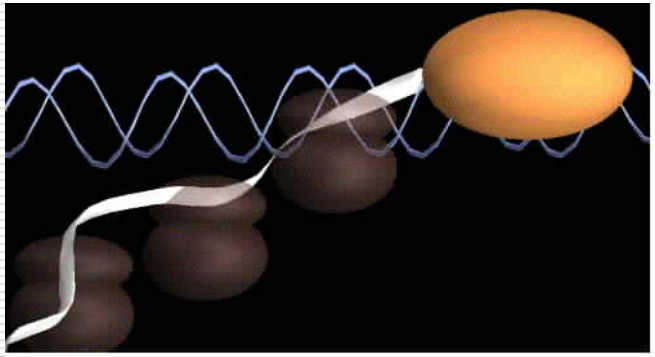

(after Griffiths et al. 1996)

# DNA Structure

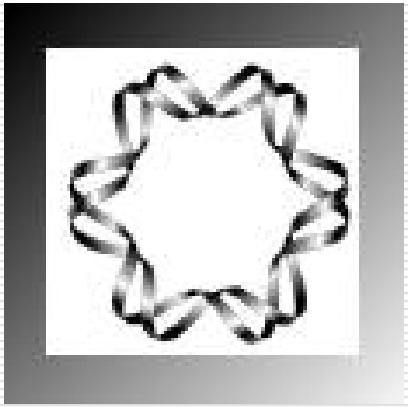
- Double stranded
- Nucleotide
  - Nitrogen Bases
  - Sugar
  - Phosphate
- Base Pairs
  - Hydrogen Bonds
    - A-T
    - C-G
  - Alpha helix
- 5’ – phosphate group
- 3’ – hydroxyl group

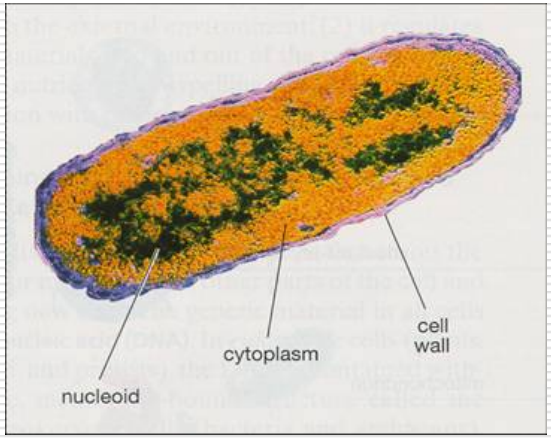
# RNA Structure

- Single strand
- Nucleotide
  - Nitrogen base
  - Sugar
  - Phosphate
- Base Pairs
  - A-U
  - C-G
- Three types
  - mRNA
  - rRNA
  - tRNA



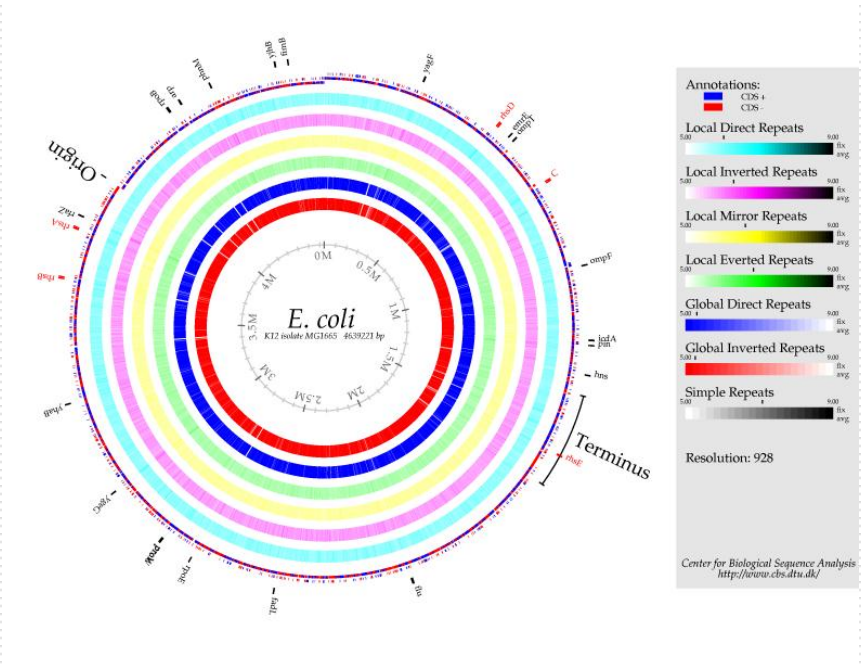
# Prokaryotic Chromosomes

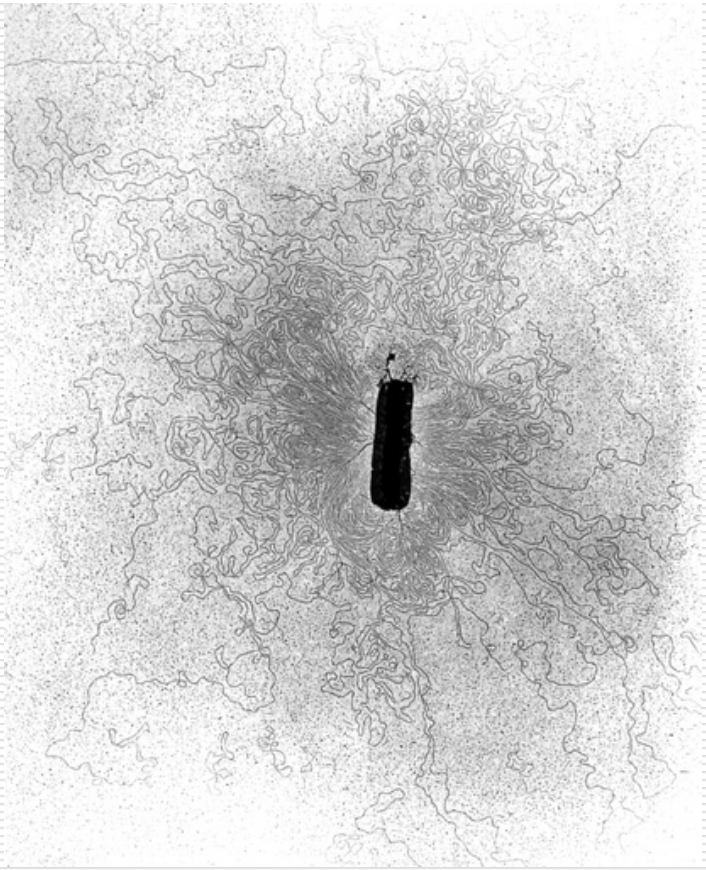





- Location
  - Nucleoid region
  - No membrane
- Number
  - Most have 1
  - Some species have 2, the second linear
- Appearance
  - Circular
  - Ds
  - Loops and coils

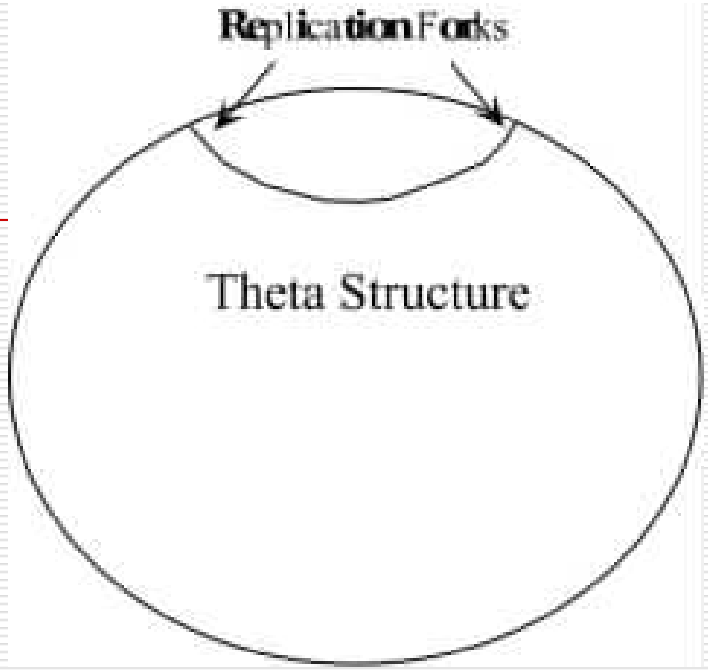
# E. coli genome / chromosome

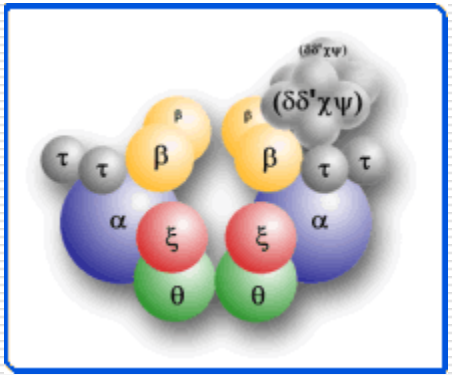


# DNA Replication

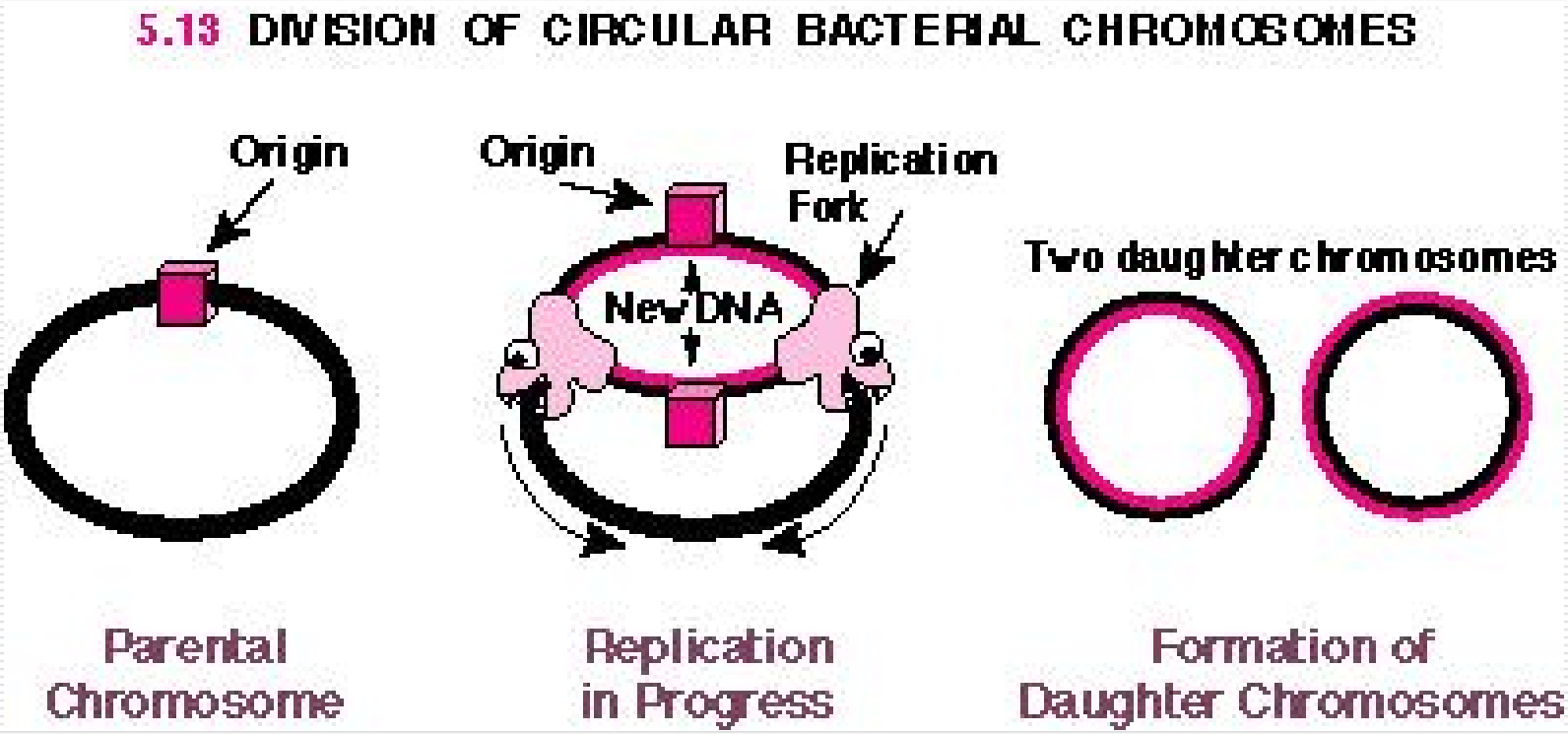
- Semiconservative
  - Replication fork
    - Single origin
    - Bidirectional
      - 2 Leading strands
      - 2 Lagging strands
  - Enzymes
    - Helicases
    - DNA polymerases 5’ to 3’
      - I for leading strand
      - II digest RNA primer
      - III for lagging strand
    - DNA ligase
    - DNA gyrase
  - Hydrogen bonds broken and reformed
  - Methylation of adenine bases
    - Initiation sites
    - Turn on or Turn off
    - Protect against viral infections
    - DNA repair



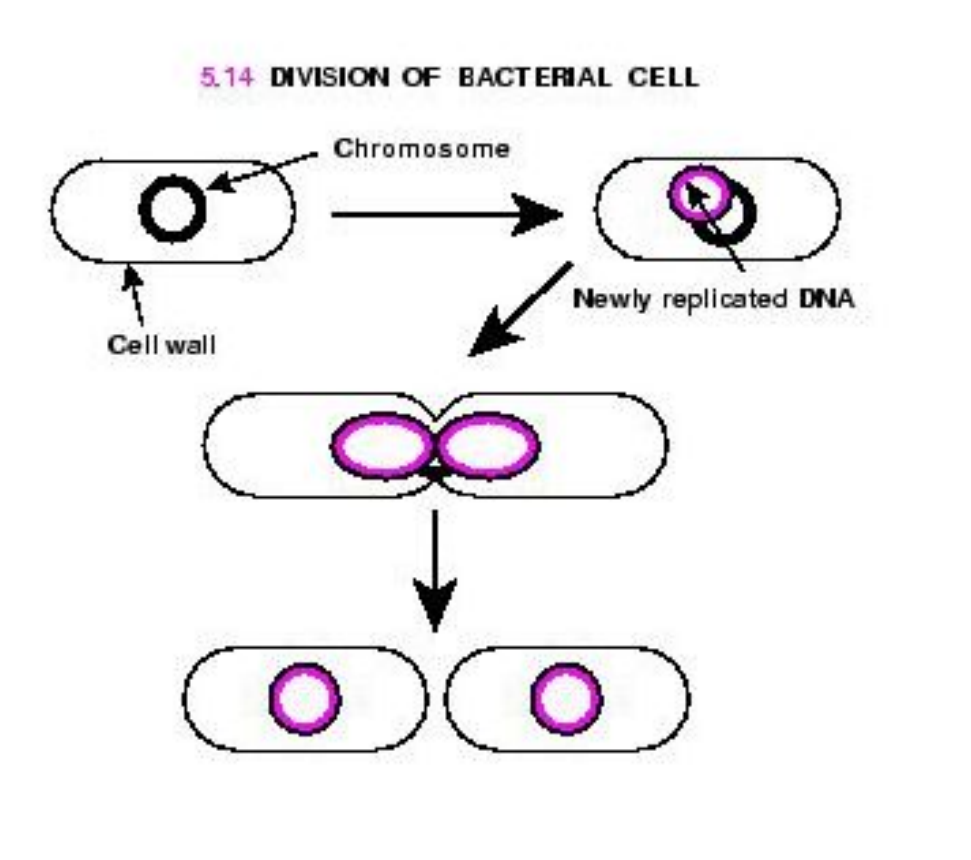




Polymerase I & II

# DNA Replication Overview



# Binary Fission

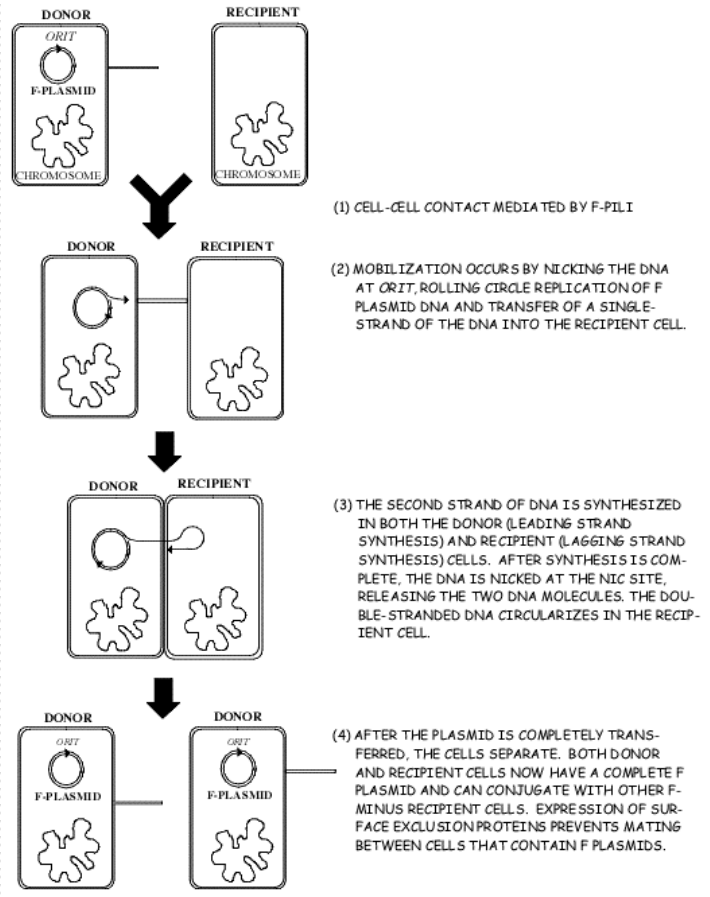


# Plasmids

- 2% of genetic information
- Ds, circular extra chromosomal DNA
- Independent replication
- Cellular Traits
  - F-Fertility
  - R-Resistance : inactivate AB, toxins, heavy metals
  - Dissimilation: catabolism of unusual substances
  - Bacteriocins
  - Virulence : enzymes, toxins, attachment

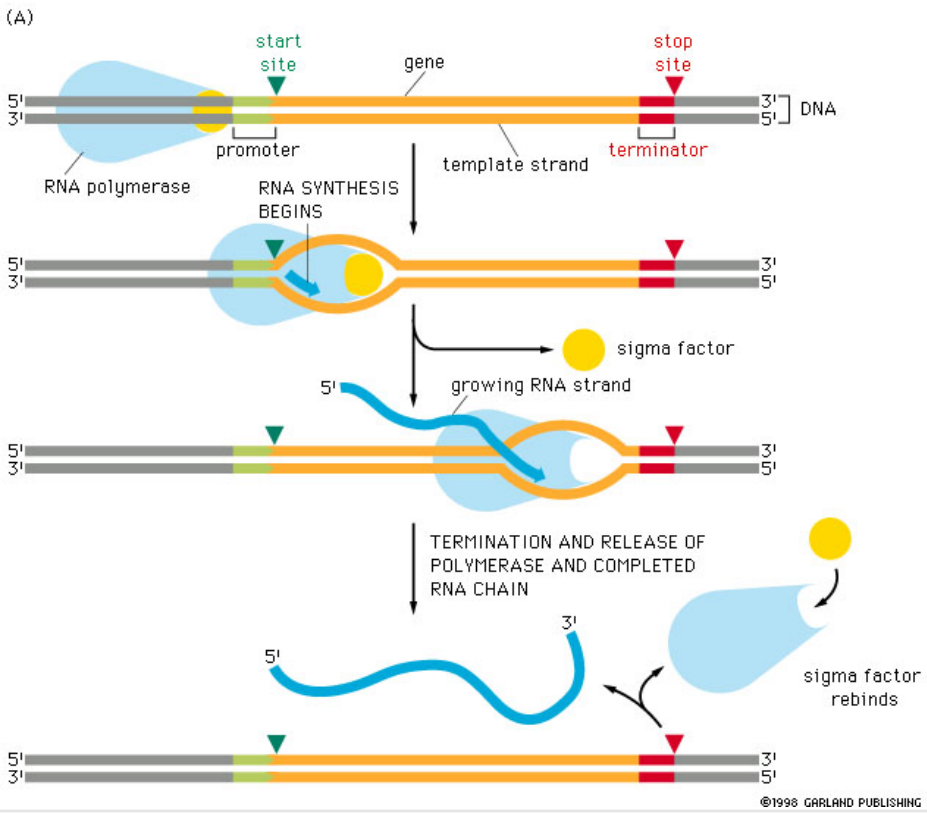
# Rolling Method for DNA replication and F-Plasmid

- Rolling Method
  - One strand remains in loop
  - Second strand breaks away and rolls of loop
  - Both strands serve as templates for daughter strand
  - Occurs during conjugation



# Transcription

- DNA → RNA
  - mRNA
  - rRNA
  - tRNA
- Initiation
  - Sigma factor on RNA polymerase
    - binds to promoter sequence on DNA
    - Will be release after 10 nucleotides
  - RNA polymerase
    - unzips, unwinds DNA
    - Lacks proof reading ability
- Elongation
  - 5' to 3', slower
    - Ribonucleotide sequences
      - Base pairs :
        - A-U [instead of Thymine]
        - C-G
- Termination
  - Self
    - Terminator sequence
    - G-C rich area
  - Protein-dependant
    - Terminator protein
    - Separates DNA and RNA polymerase



# Sigma Factors for RNA polymerase

## Sigma factors

**5th component of RNA Polymerase**

**70**
**rpoD**
**cellular growth**

**32**
**rpoH**
**heat shock**

**24**
**rpoE**
**extreme heat shock (>50 C)**

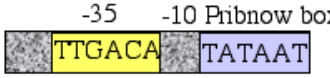
**54**
**rpoN**
**nitrogen metabolism**

**28**
**fliA**
**flagellaformation**

**38**
**rpoS**
**stationary phase**

**19**
**fecI**
**iron citrate transport**

**RNA Polymerase loosely binds DNA and scans 5' to 3'. A helix turn helix region within**

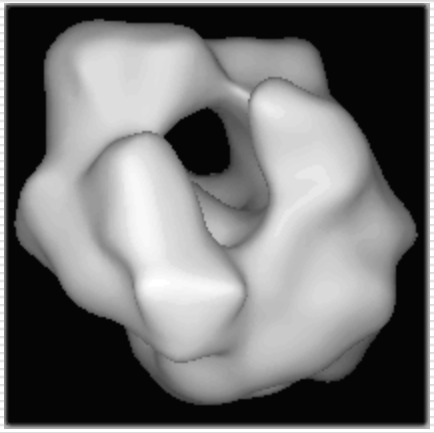



**Prokaryotic**

**Sigma factors recognizes promoter elements more Tightly. Alpha helices within the protein fit into The major groove of DNA. Different sigma Factors regulate genes for different conditions.**

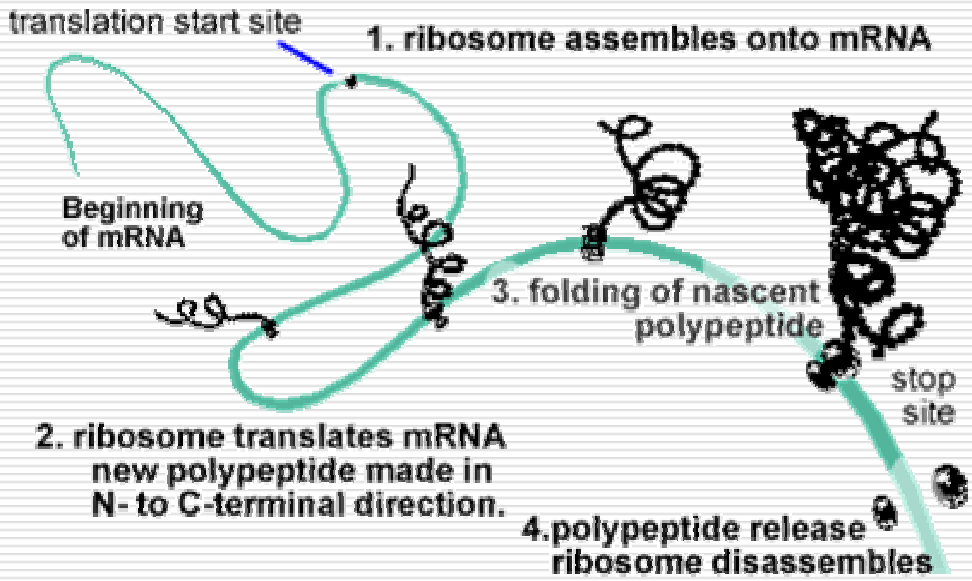
# Prokaryotic RNA

- Transcription = RNA → Polypeptides
- RNA
  - mRNA
    - Code for several polypeptides along strand
    - Each code has codons: Start and Stop
  - tRNA
    - Acceptor stem
    - Anticodon
    - Wobble
  - rRNA
    - 70S Ribosomes
      - 50S: 23S + 5S rRNA and 33 proteins
      - 30S: 16S rRNA and 21 proteins
    - Binding Sites on Ribosomes
      - A: **a**ccepts tRNA with AA
      - P: holds tRNA for base pairing anticodon to mRNA codon for **p**olypeptide
      - E: release [**e**xit] for tRNA

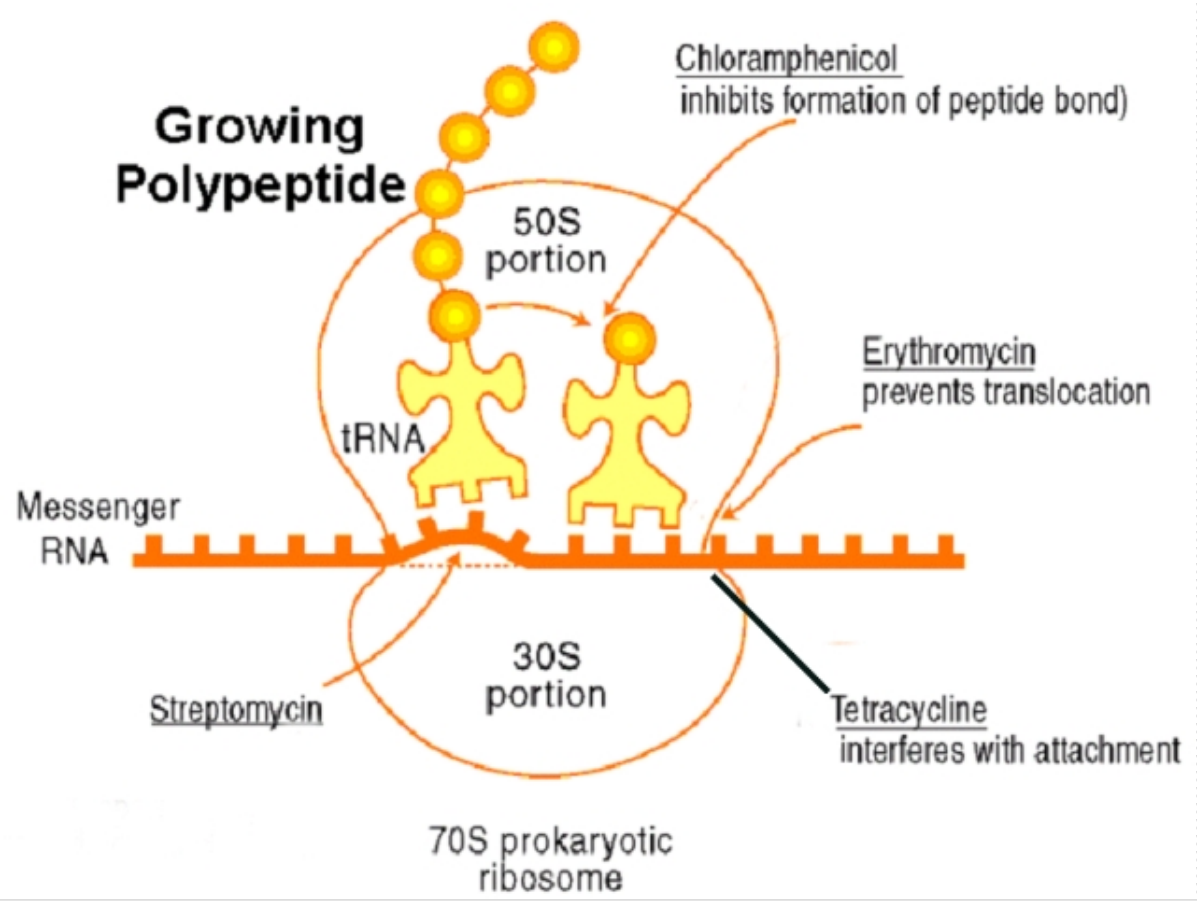


# Translation Steps

- Initiation
  - 30S
  - tRNA @ P site
  - 50S
  - GTP used
- Elongation
  - New tRNA @ A site
  - Ribozyme in 50S forms peptide bond
  - GTP used
- Termination
  - Release factor proteins
  - Stop codon on mRNA



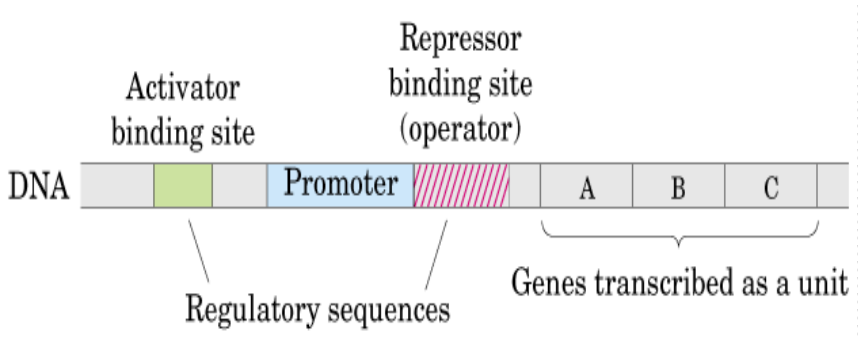
# Importance of rRNA structures



# Regulation of Gene Expression

- Constitutive
  - Not regulated
  - Always “on” at fixed rate
    - Transcription
    - Translation
  - 60-80%
  - Polypeptides need in large amounts
- Regulated
  - Only when needed
  - Control synthesis of enzyme : genetic control
    - Induction
    - Repression
  - Control enzyme activity: feedback
    - Noncompetitive inhibition
    - Competitive inhibition

# Genetic Control of Enzyme Synthesis and formation




- Operon Model
  - Operator (O)
  - Promoter (P)
  - Structural genes
- Regulatory genes
  - Makes repressor
    - Active binds to O
    - Inactive unable to bind to O
- Types
  - Inducible Operons
    - Repressor Active
    - Operon Off
    - Inducer needed
  - Repressible Operons
    - Repressor inactive
    - Operon On
    - Corepressor needed

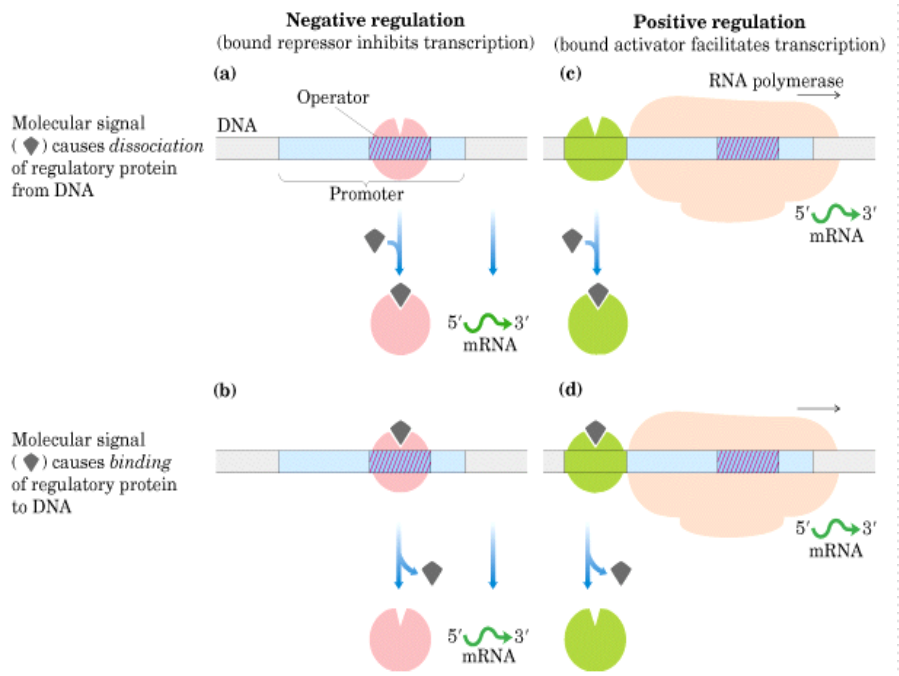
# Operator and Regulation

Operator

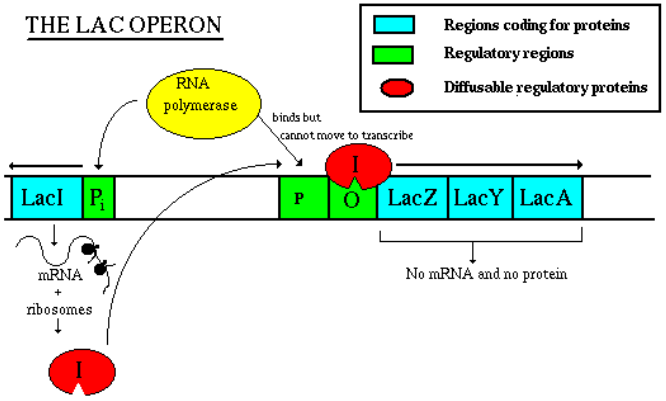
GAATTGTGAGCGGATAACAATT
CTTAACACTCGCCTATTGTTAA

-Systematic mutation defined contact points
-DNaseI footprinting showed the region of binding

Two fold symmetry of the operator sequence

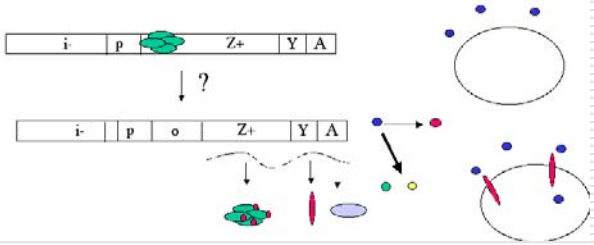


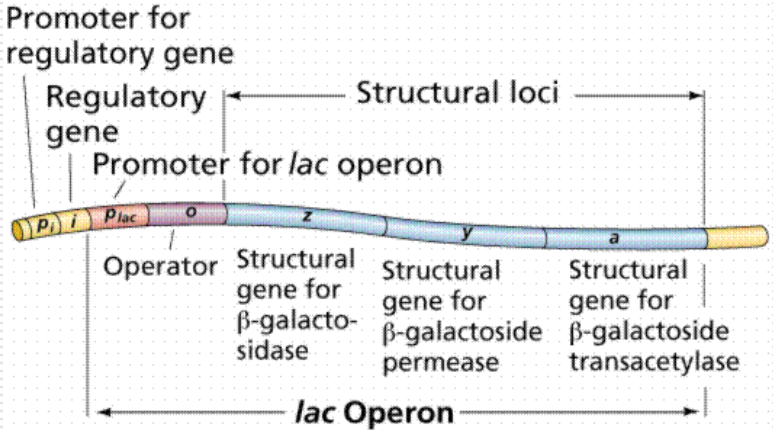
# Lac Operon: Inducible



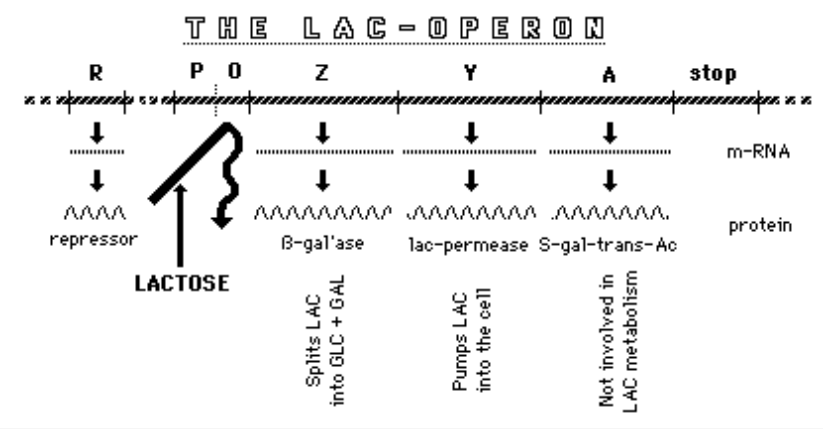

1. When lactose is absent--repressor binds The operon and the lac operon is shut down

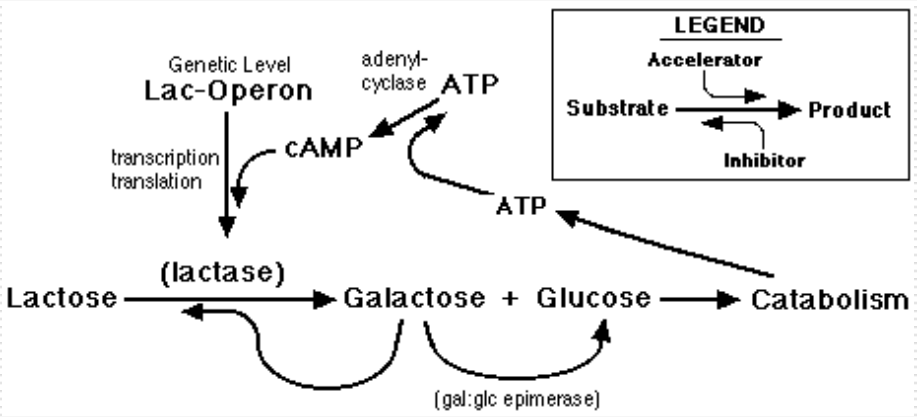
2. How then is permease (lacY) made to let the Lactose in, to turn the operon on?



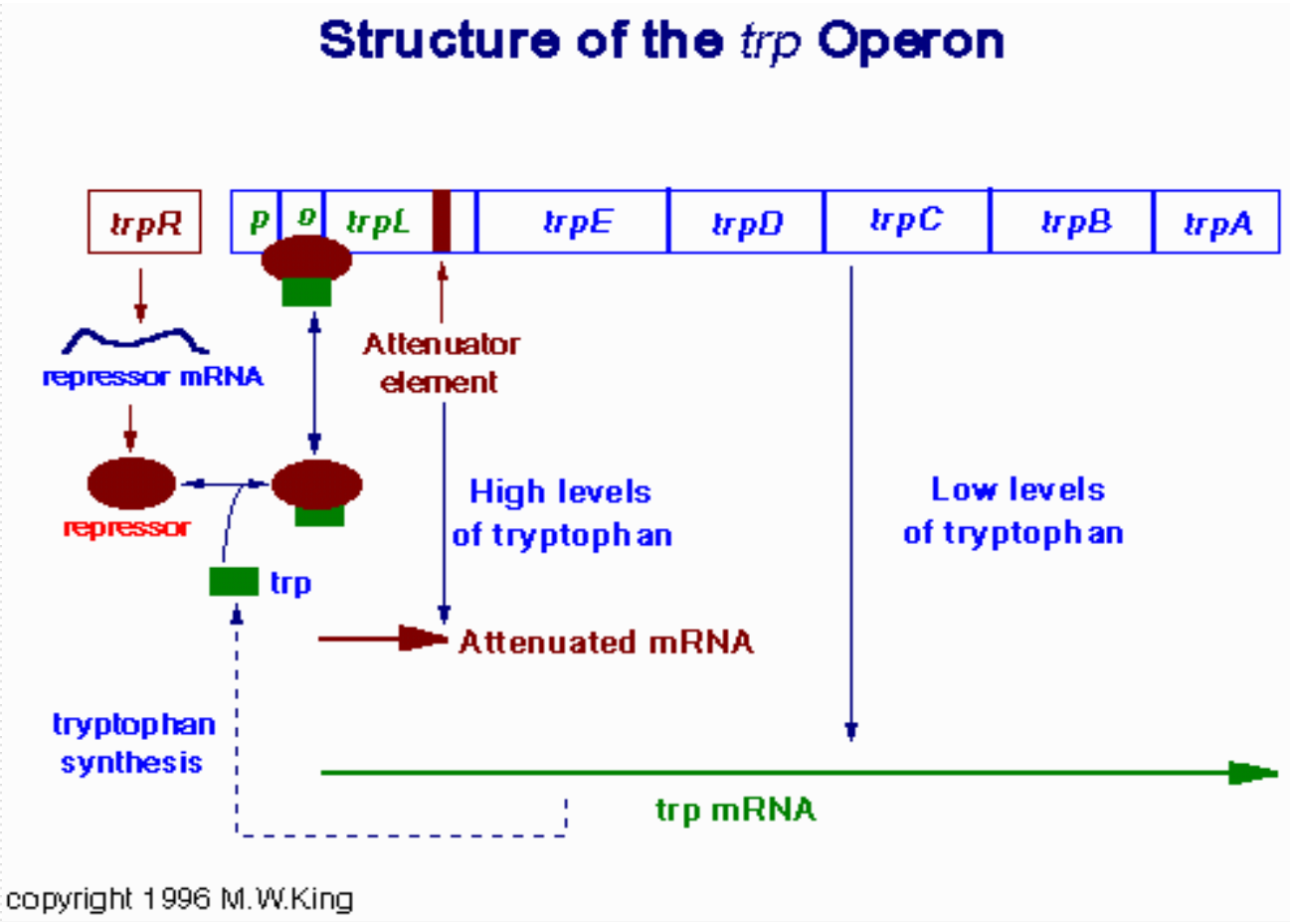



# Use of Lactose



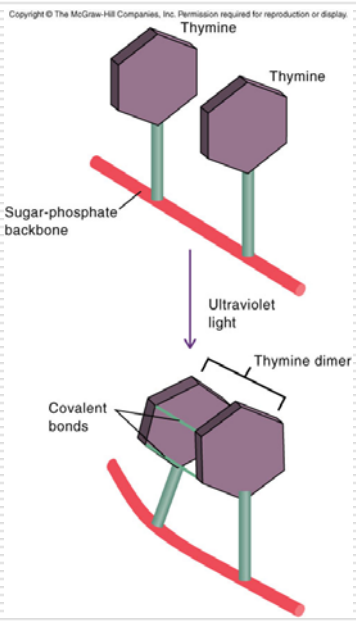



# TRP Operon: Repressible

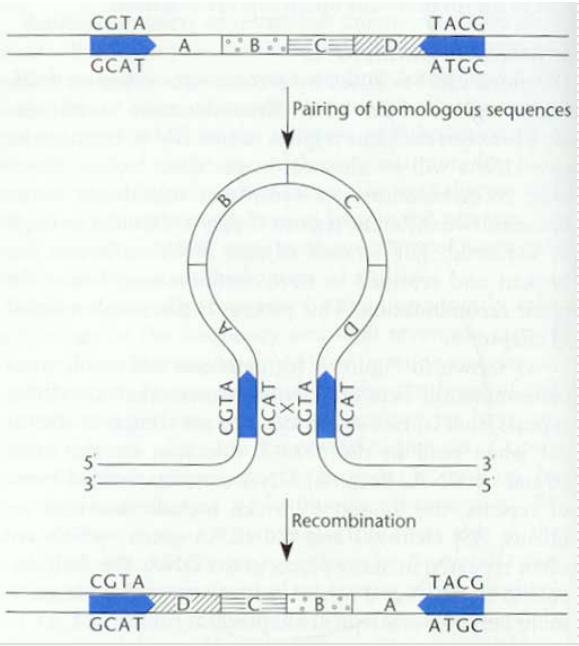


# Mutations

- Define
- Types
  - Silent
  - Point
    - Mis-sense
    - Non-sense
    - Sense [aka silent]
  - Substitution
    - Transition: purine for purine
    - Transversion: purine for pyrimadine
  - Frameshift
    - Insertions
    - Deletions
- Causes
  - Spontaneous
  - Induced
    - Chemical
    - Physical
  - Conditional
  - Adaptive
  - Transposons

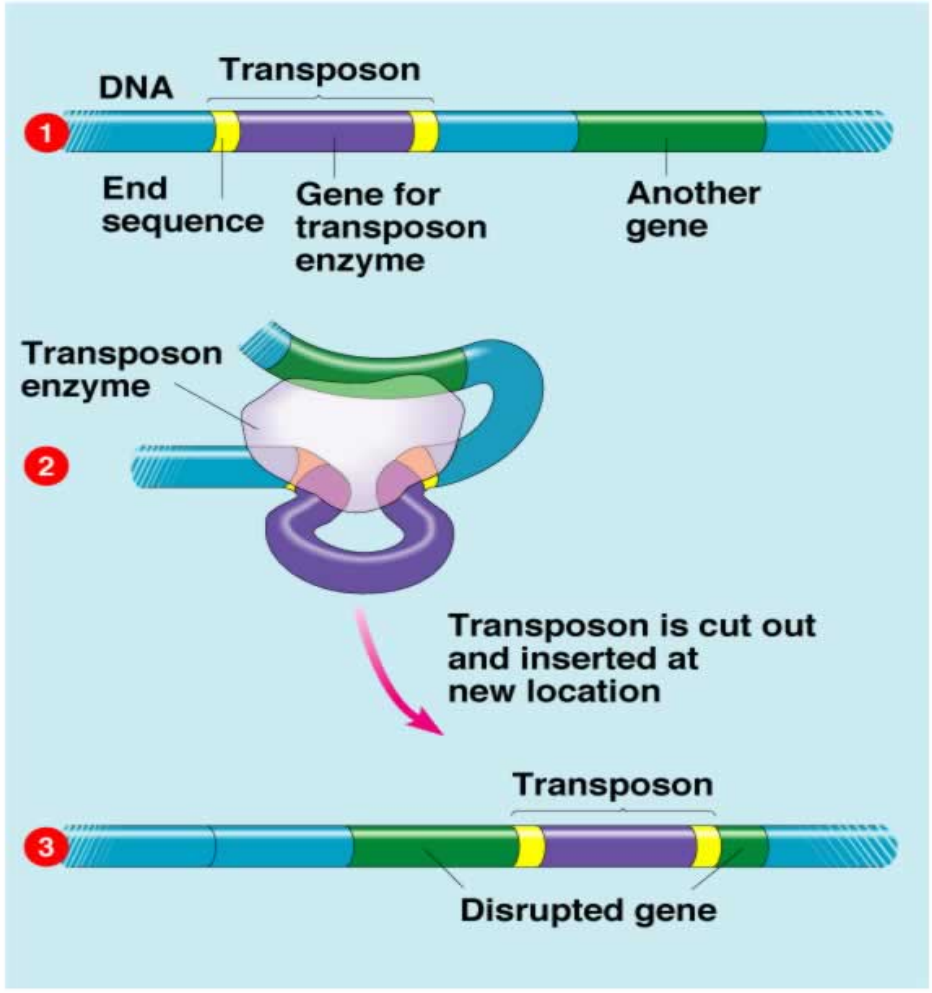



Thymine dimer

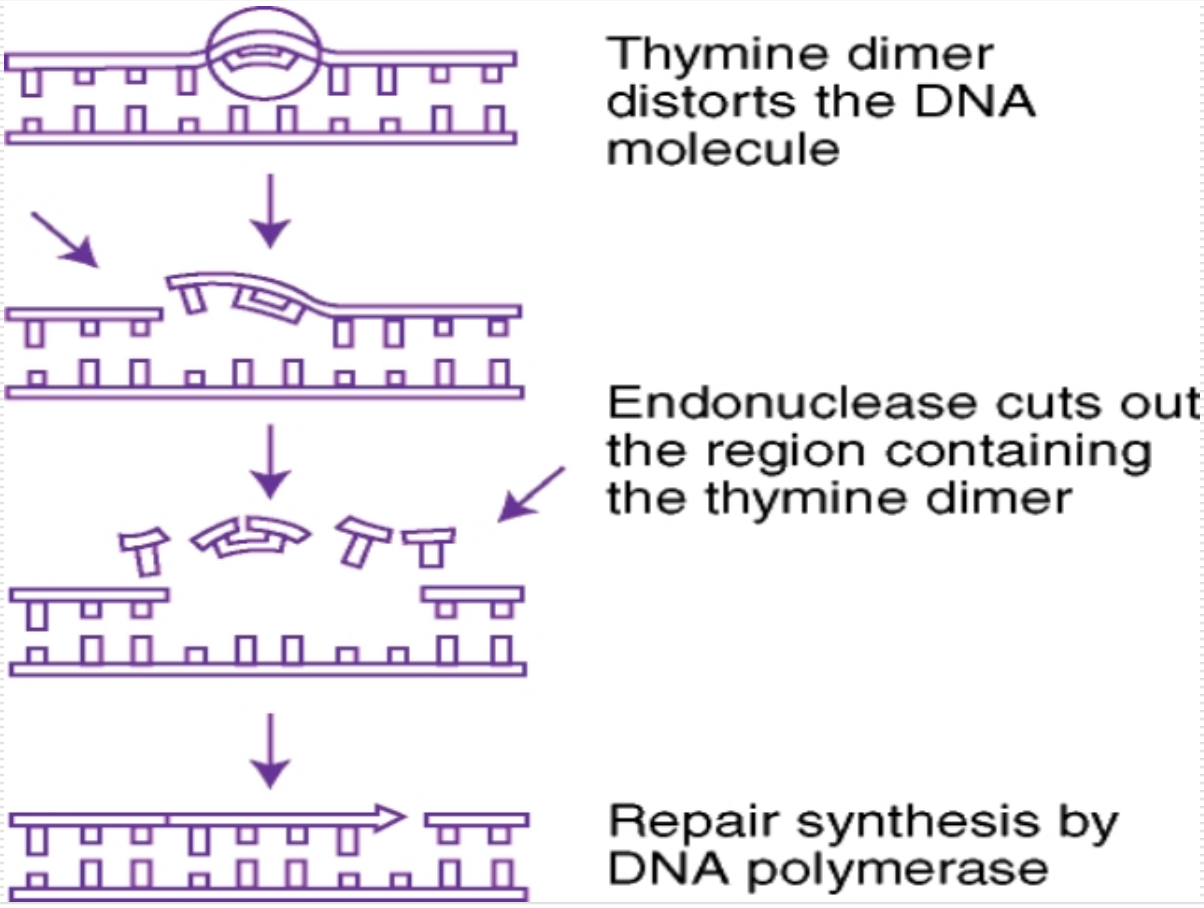



inversion

# Transposons



# Repair of Mutations

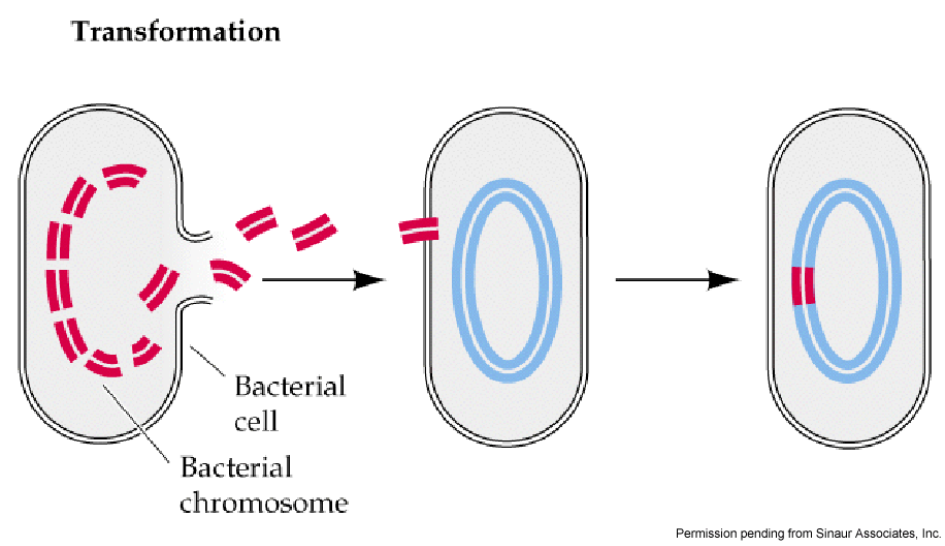


# Genetic Transfer

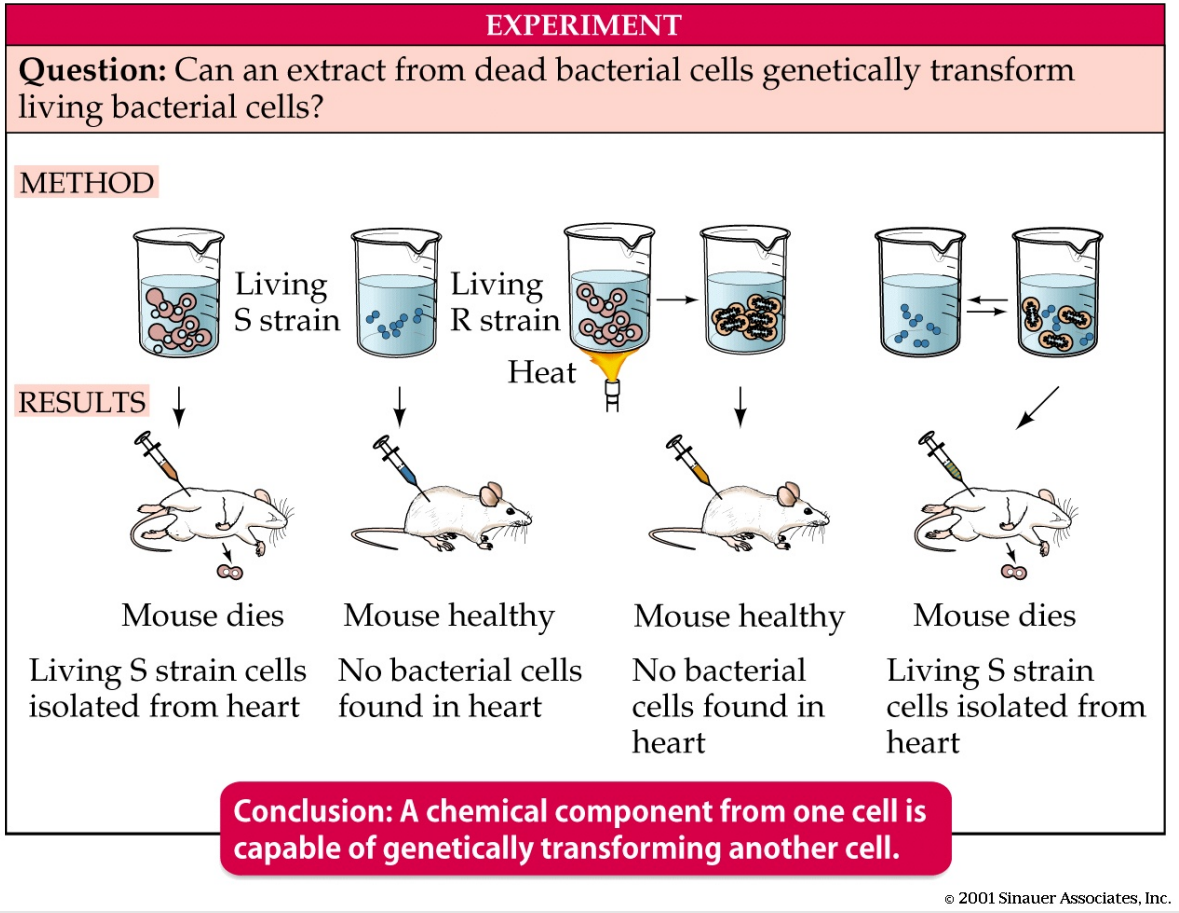
- Vertical
  - Parent to offspring
- Horizontal
  - Lateral transfer to same generation
  - Donor to recipient
  - DNA transfer
  - Plasmid transfer
  - Types
    - Transformation
    - Transduction
    - Conjugation

# Transformation

- Occurance
  - 1%
  - Random
  - Naturally in certain species
    - *Haemophilus*
    - *Neisseria*
    - *Pseudomonas*
    - *Streptococcus*
    - *Staphylococcus*
- Griffith experiment
- Genetic transfer
  - Environment
  - Competent cells
    - Cell wall
    - Plasma membrane
  - Bacterial lysis
    - DNA
    - Plasmids



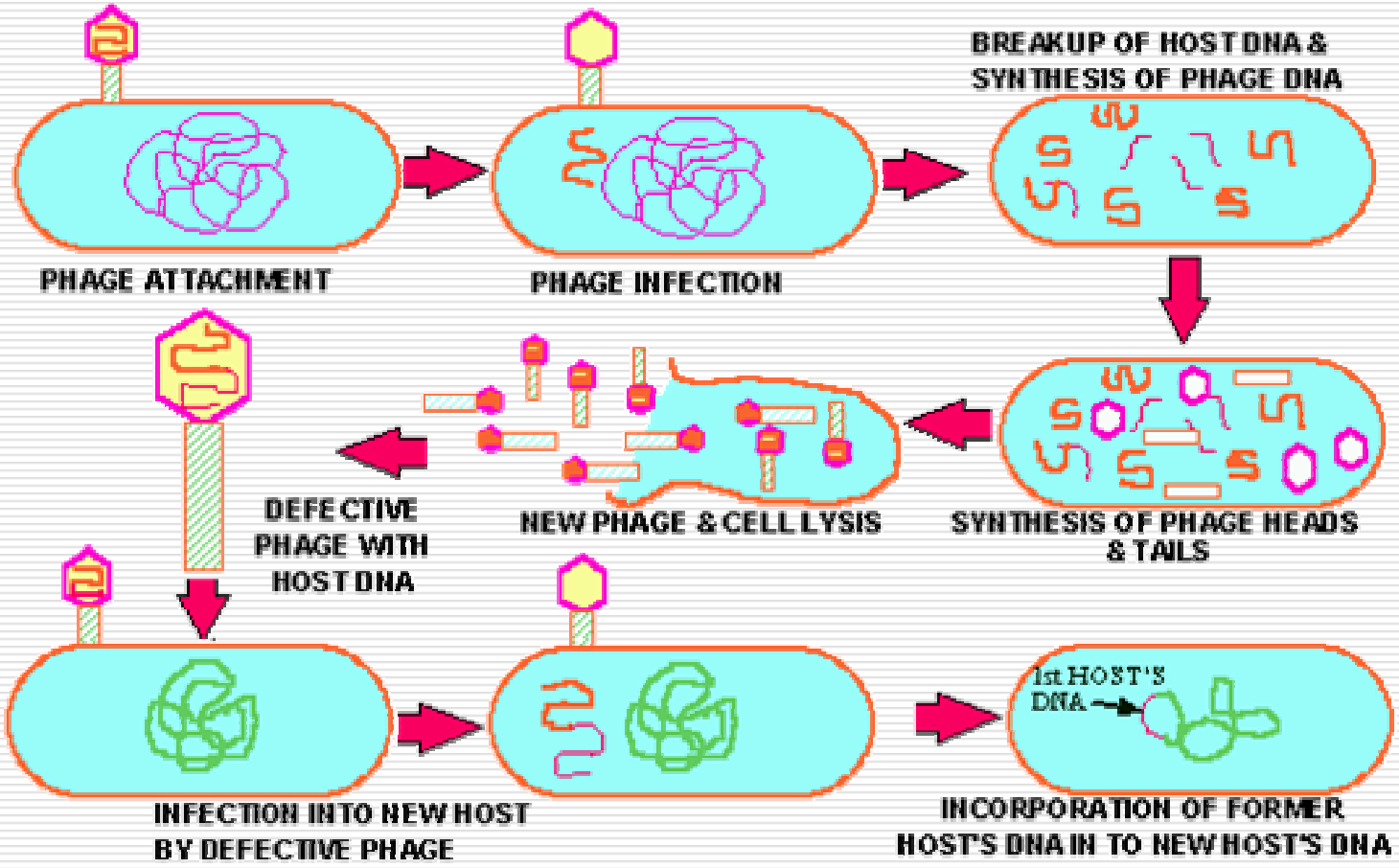
# Griffith Experiments



# Transduction

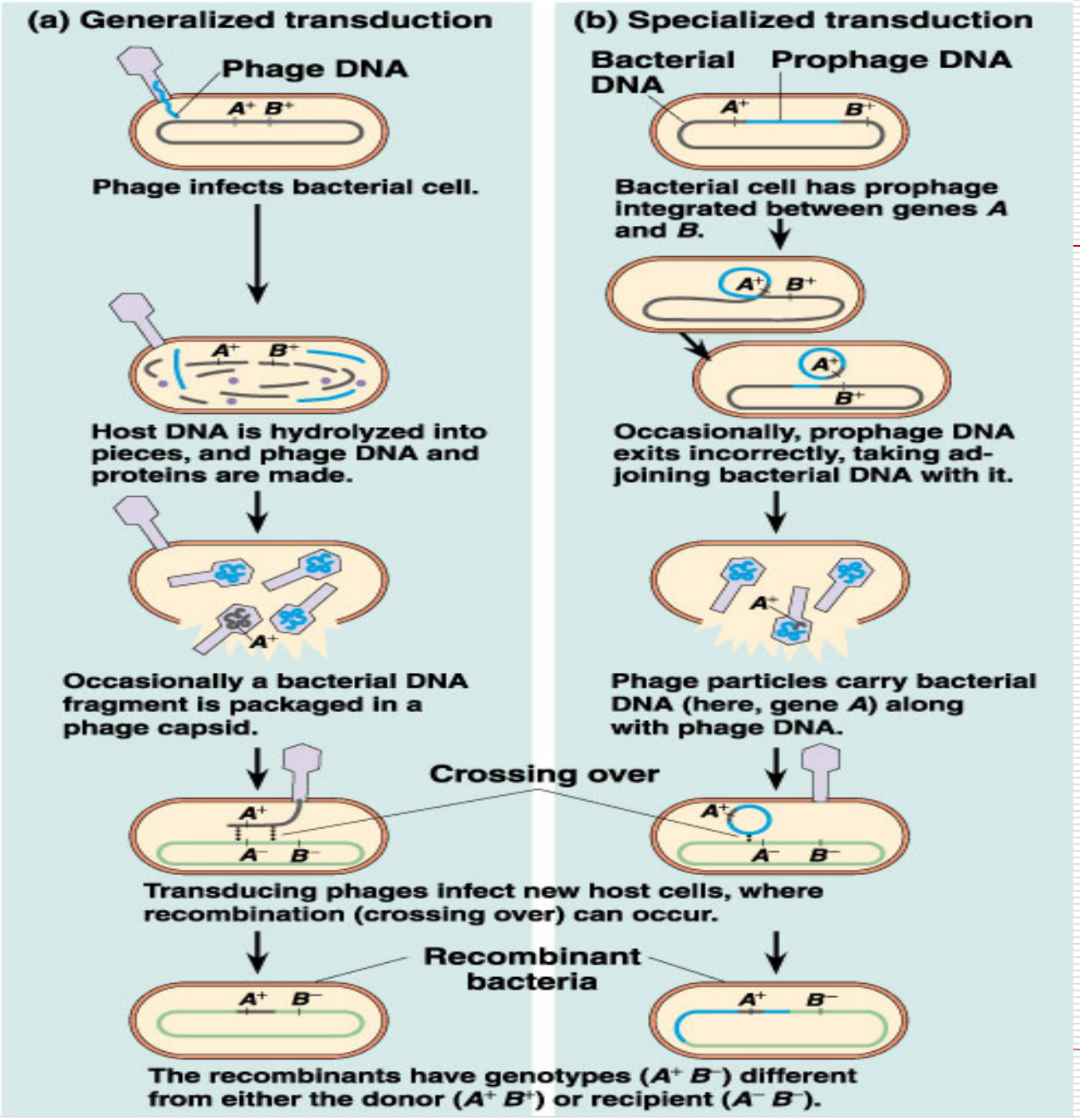
- Transfer of bacterial genes via viruses
  - Donor to recipient
  - Virus: Bacteriophages
- Types
  - Generalized
  - Specialized
- Replication Cycle
  - Lytic
  - Lysogenic

# Generalized Lytic Cycle

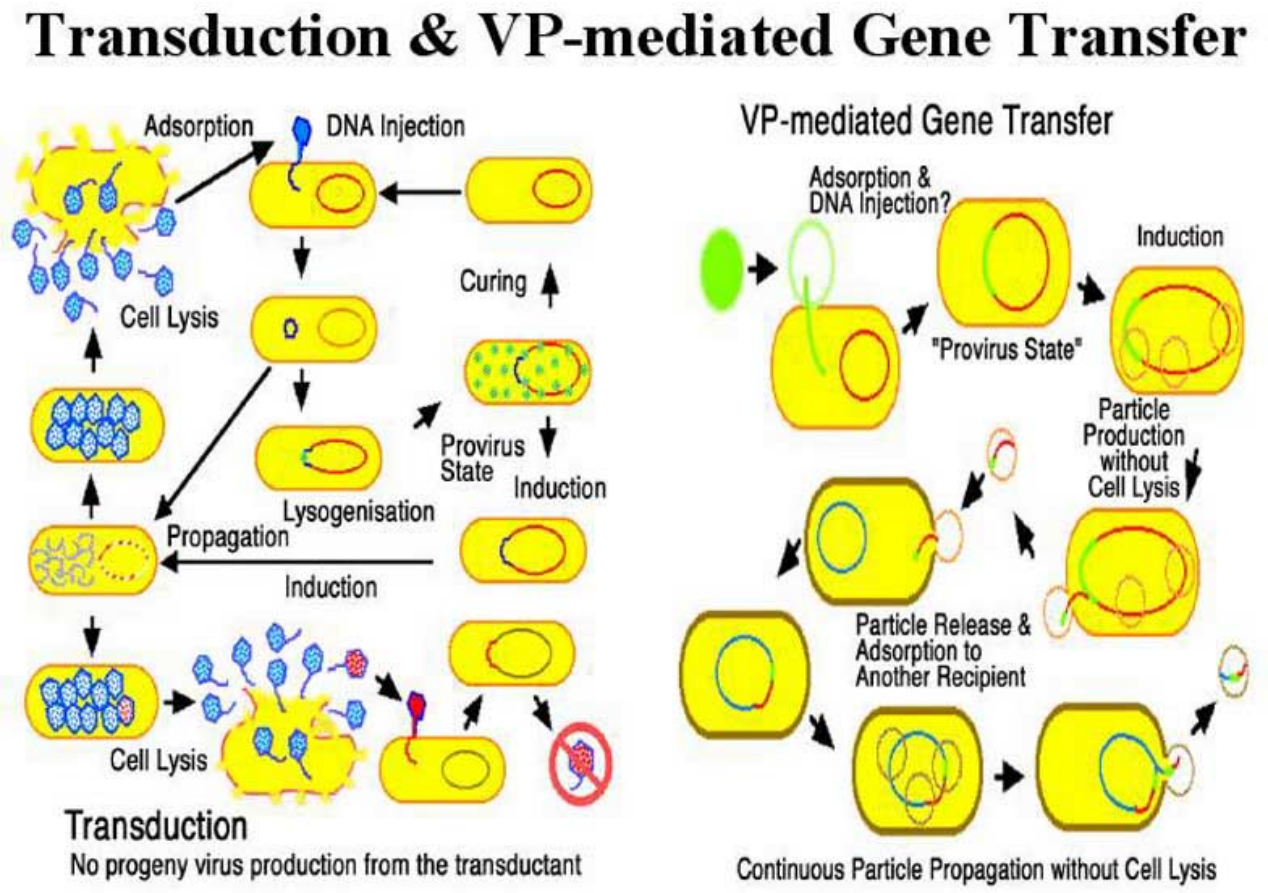


# Specialized Lysogenic Cycle

- Only certain bacterial genes are transferred
- Example: Toxins
  - *Corynebacterium*
    - Diphtheria toxin
  - *Streptococcus pyogenes*
    - Erythrogenic toxin
  - *E. coli*
    - Shiga-like toxin

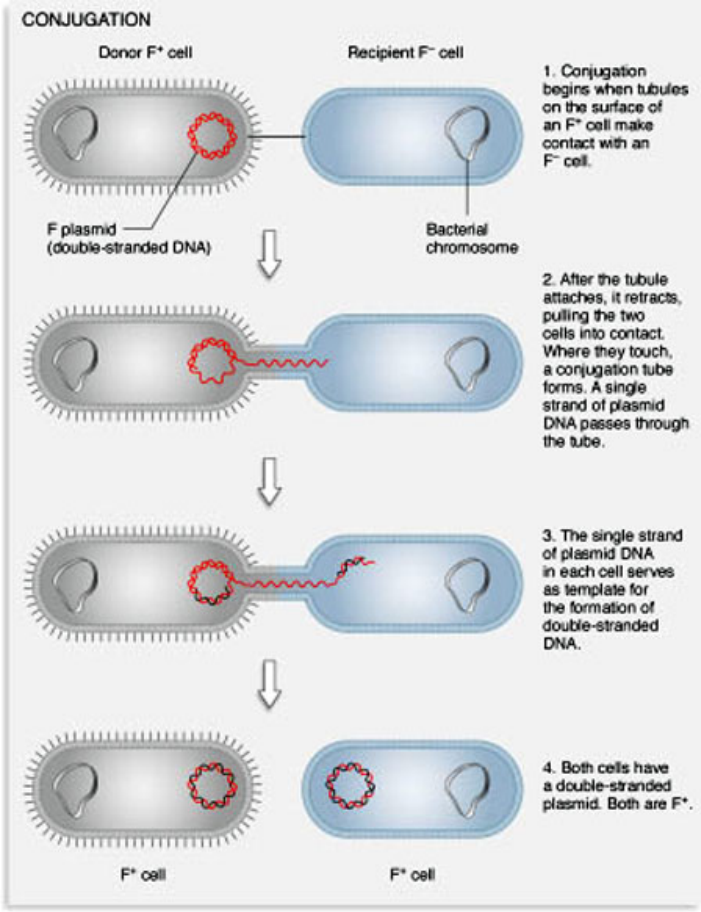

(a) Generalized transduction
Phage DNA
A+ B+
Phage infects bacterial cell.
Host DNA is hydrolyzed into pieces, and phage DNA and proteins are made.
Occasionally a bacterial DNA fragment is packaged in a phage capsid.
(b) Specialized transduction
Bacterial DNA
Prophage DNA
Bacterial cell has prophage integrated between genes A and B.
Occasionally, prophage DNA exits incorrectly, taking adjoining bacterial DNA with it.
Phage particles carry bacterial DNA (here, gene A) along with phage DNA.
Crossing over
A− B−
Transducing phages infect new host cells, where recombination (crossing over) can occur.
Recombinant bacteria
The recombinants have genotypes (A+ B−) different from either the donor (A+ B+) or recipient (A− B−).

# Transduction

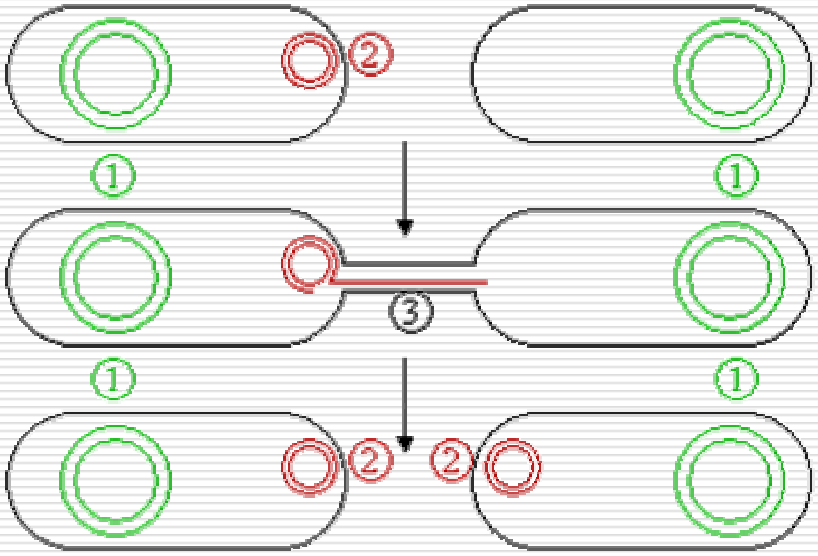


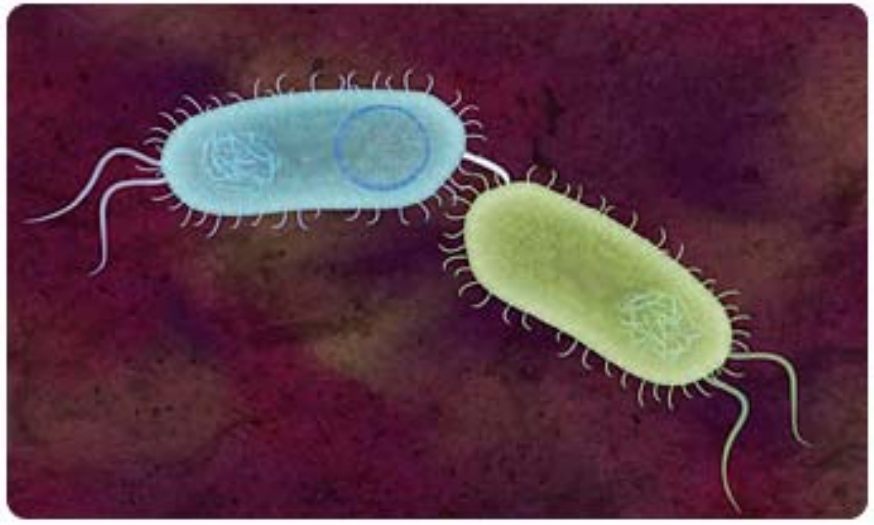
# Conjugation

- Define
- Bacteria
  - Gram Neg : F.pilus
  - Gram Pos: sticky surface molecules
- Types
  - F+ [plasmid]
  - R [plasmid]
  - Hfr [DNA]

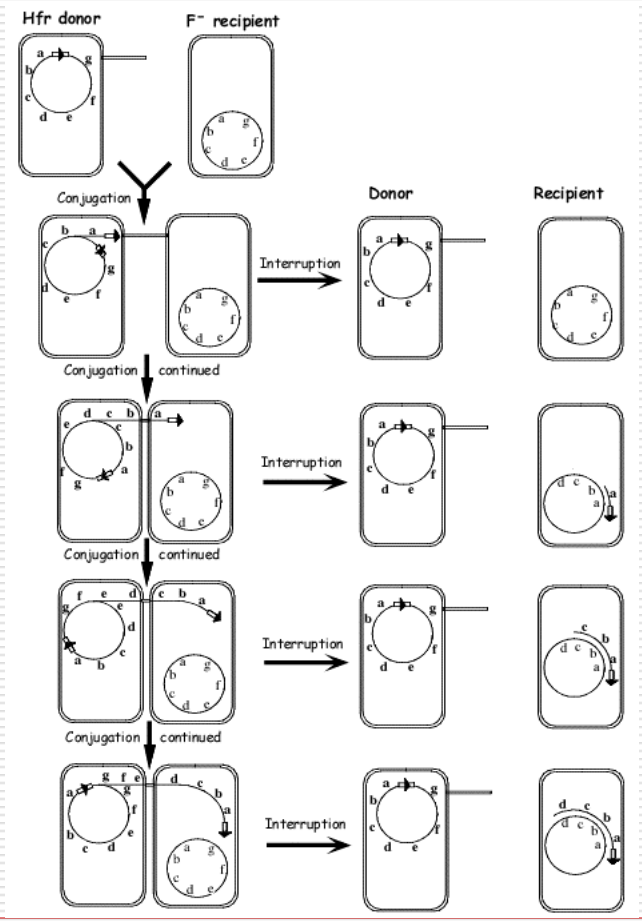


# Conjugative Plasmid



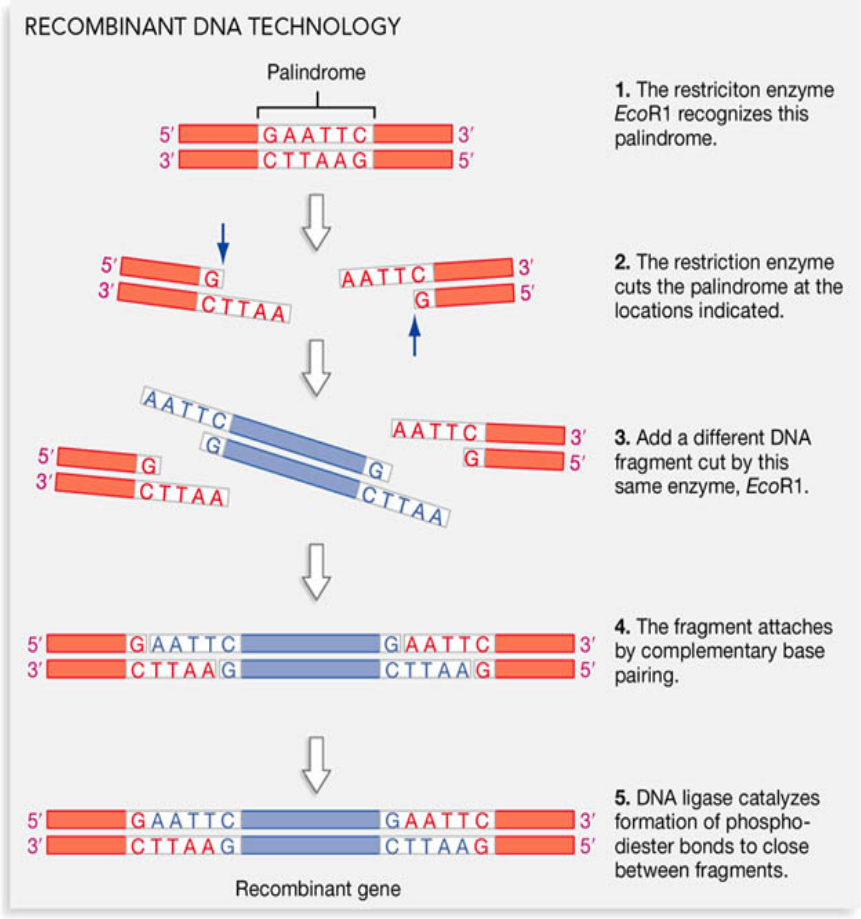
# Hfr Interrupted Stages

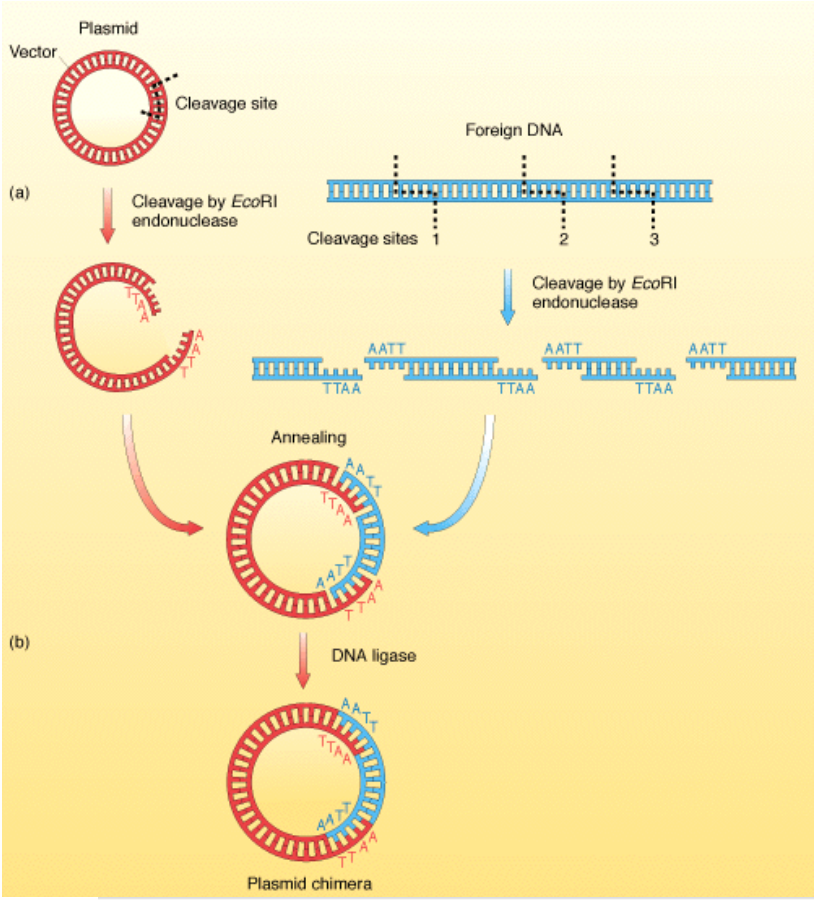


# Genetic Recombination

- General
  - Homologous chromosomes
  - Any location
  - DNA breakage and repair
- Site Specific
  - Nonhomologous
  - Viral genomes in bacterial chromosomes
- Replicative

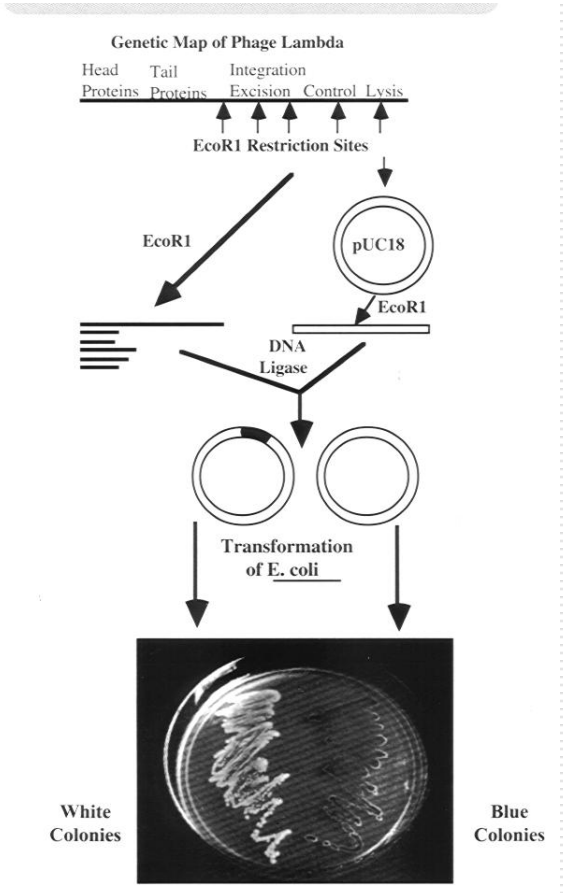
# Recombinant DNA



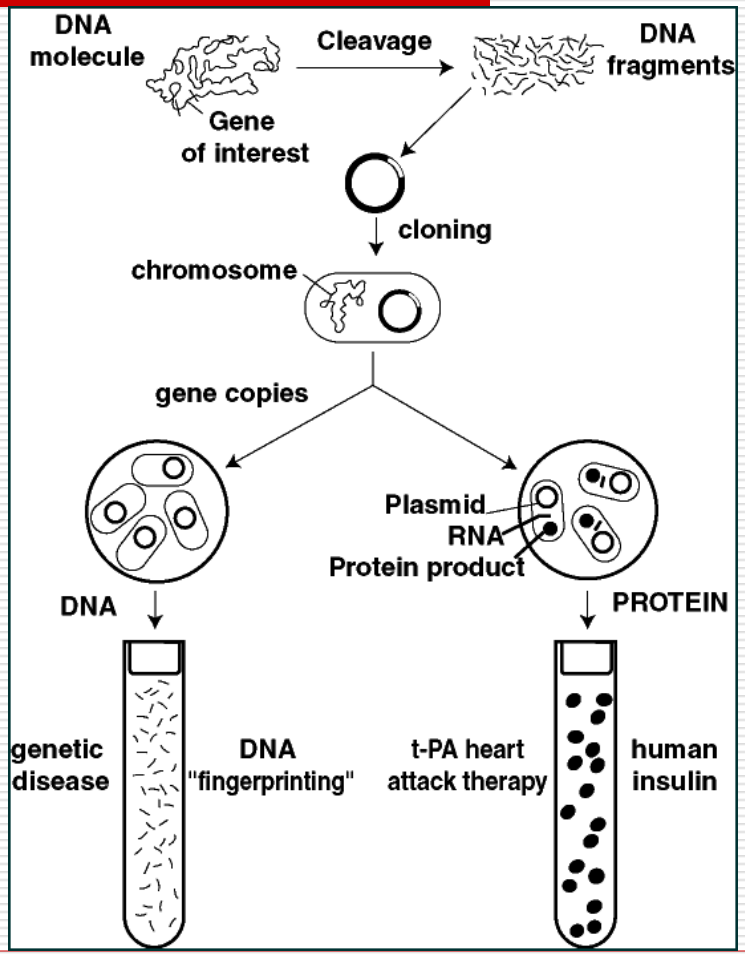



# Genetic Engineering

- Use
  - Plasmids
  - Recombinant DNA
- Applications
  - Therapeutic
    - Hormones
    - Enzymes
    - Vaccines
    - Gene therapy
  - Agricultural
  - Scientific
    - Southern Blot
    - ELISA tests



# Biotechnology



# Questions?