

## Chapter 9

### Topics

- Genetics
- Flow of Genetics/Information

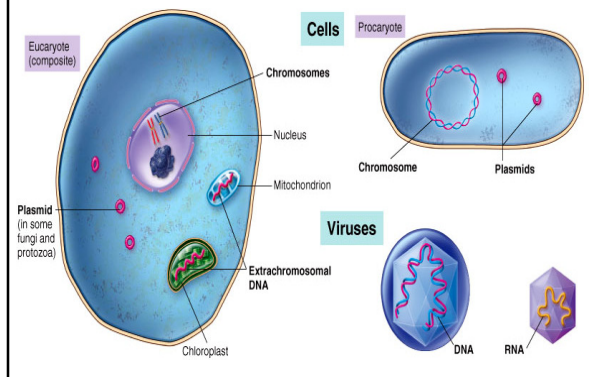
### Regulation

- **Mutation**
- **Recombination – gene transfer**

## Genetics

- **Genome** - the sum total of genetic information in an organism
- **Genotype** - the A's, T's, G's and C's
- **Phenotype** - the physical characteristics that are encoded within the genome

### Examples of Eukaryotic and Prokaryotic Genomes



## Chromosome

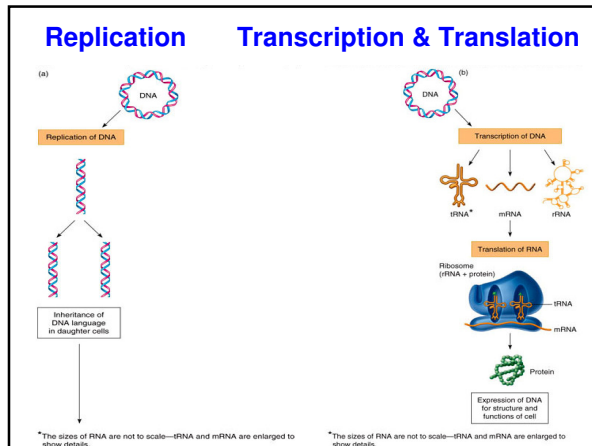
- Prokaryotic
  - 1 circular chromosome ± extrachromosomal DNA (**plasmids**)
- Eukaryotic
  - Many paired chromosomes ± extrachromosomal DNA (**Mitochondria or Chloroplast**)
- Subdivided into basic informational packets called **genes**

## Genes

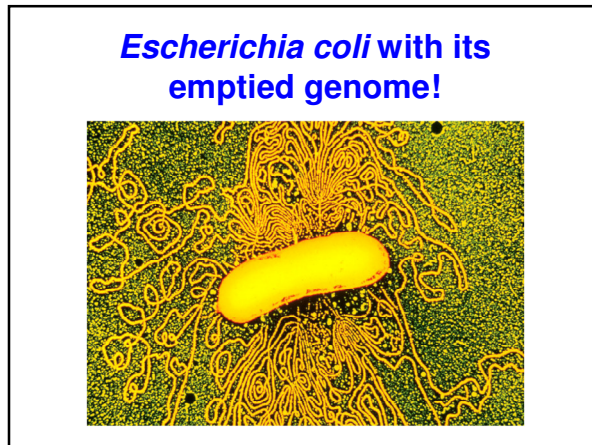
- Three categories
  - **Structural** - genes that code for proteins
  - **Regulatory** - genes that control gene expression
  - **Encode for RNA** - non-mRNA

### Flow of Genetics/Information The Central Dogma

- **DNA** → **RNA** → **Protein**
  - Replication - **copy DNA**
  - Transcription - **make mRNA**
  - Translation - **make protein**

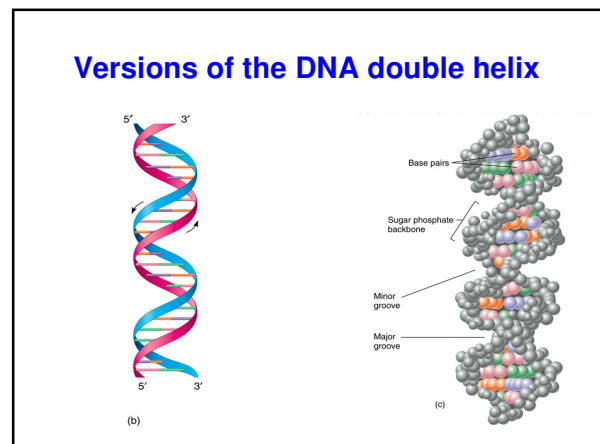


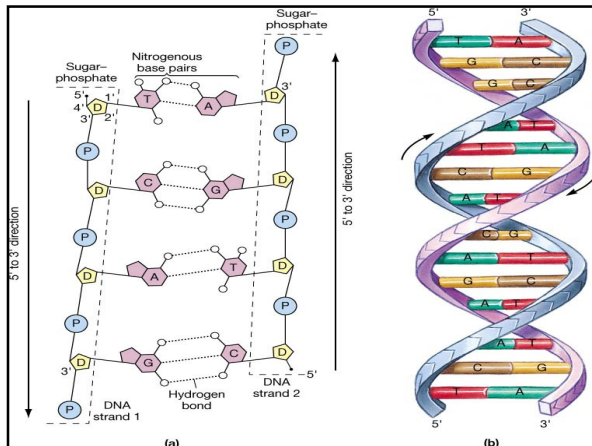
- ## DNA
- Structure
  - Replication
  - Universal Code & Codons



- ## Structure
- Nucleotide
    - Phosphate
    - Deoxyribose sugar
    - Nitrogenous base
  - Double stranded helix
    - Antiparallel arrangement

- ## Nitrogenous bases
- Purines
    - **A**denine
    - **G**uanine
  - Pyrimidines
    - **T**hymine
    - **C**ytosine





## Replication

- Semiconservative - starts at the **Origin of Replication**
- Enzymes
- Leading strand
- Lagging strand
  - Okazaki fragments

## Semiconservative

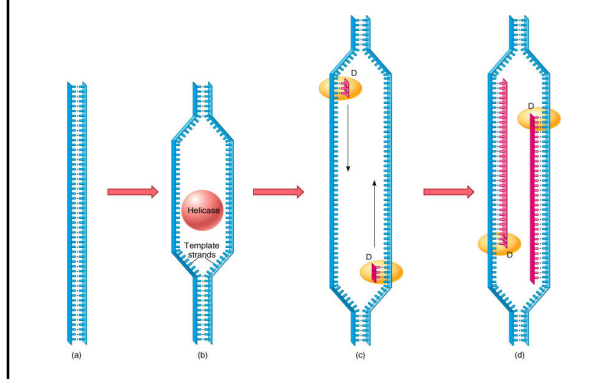
- New strands are synthesized in **5' to 3'** direction
- Mediated by DNA polymerase - only works in **5' to 3'** direction

## The function of important enzymes involved in DNA replication

**TABLE 9.1** Some Enzymes Involved in DNA Replication and Their Functions

Enzyme	Function
Helicase	Unzipping the DNA helix
Primase	Synthesizing an RNA primer
DNA polymerase III	Adding bases to the new DNA chain; proofreading the chain for mistakes
DNA polymerase I	Removing primer, closing gaps, repairing mismatches
Ligase	Final binding of nicks in DNA during synthesis and repair

## Semiconservative replication of DNA



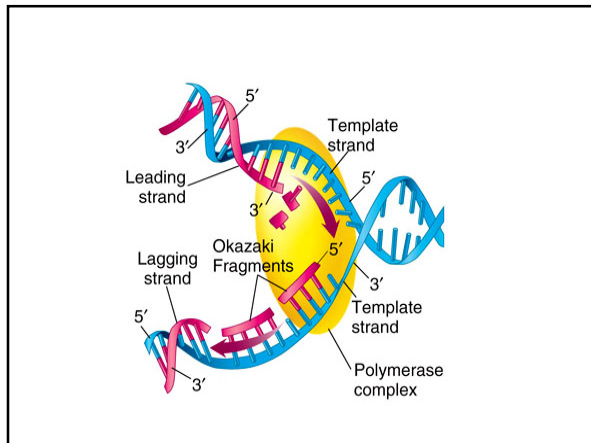
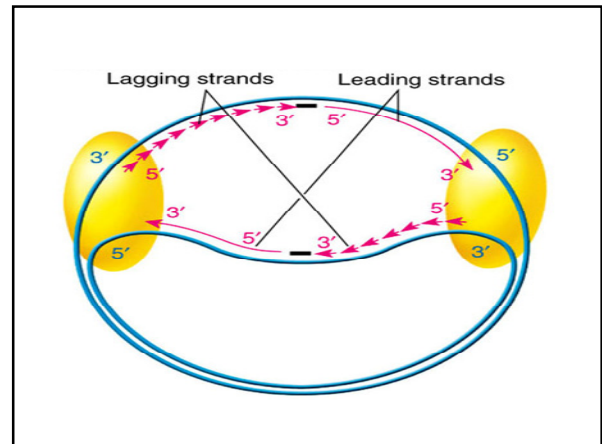
## Leading strand

- RNA primer initiates the **5' to 3'** synthesis of DNA in **continuous** manner

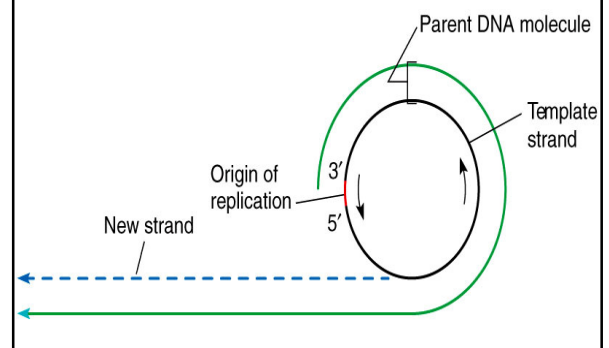
## Lagging strand

- Multiple **Okazaki fragments** are synthesized
- **Okazaki fragments** are ligated together to form one continuous strand

Look at the **DNA Fork Movie on McGraw Hill Website**



## Replication processes of plasmids and viruses involve a **rolling circle**



**Transcription** is the synthesis of RNA from a DNA template – second step in the central dogma -

RNA is transcribed from DNA

## RNA

- Transcription - 3 main types of RNA
  - **Message RNA** (mRNA)
  - **Transfer RNA** (tRNA)
  - **Ribosomal RNA** (rRNA)
- Codon - Remember that in RNA, there are no T's - **just U's**

**rRNA** combines with ribosomal proteins to form ribosomes which serve as sites for the assembly of amino acids into proteins

**tRNA** – select amino acids and transfer the amino acids to the growing chain of a protein

**mRNA** – carries the information for the proteins in the form of codons – one codon/one amino acid

## Codons

- Triplet code that specifies a given amino acid
- Multiple codes for one amino acid - **REDUNDANT**
- 20 amino acids
- Start codon
- Stop codons

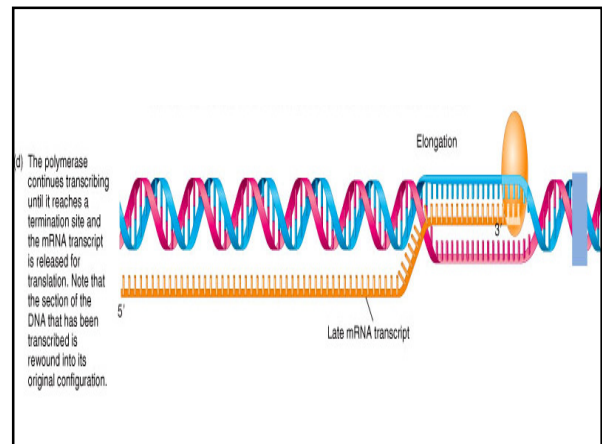
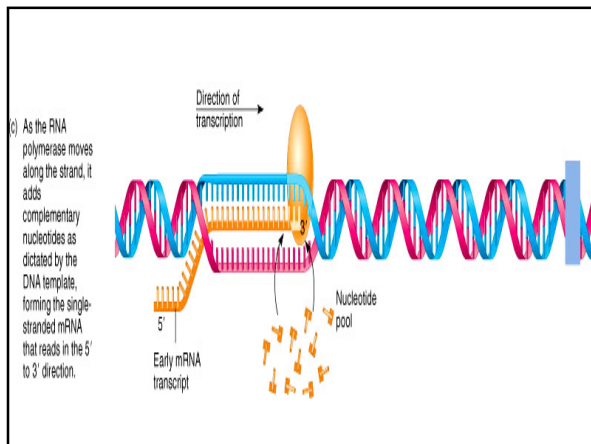
## The Genetic code - Wow!!!!

		Second Base Position				
		U	C	A	G	
U	UUU	Phenylalanine	UCU UCC	UAU UAC	UGU UGC	U
	UUC					C
	UUA	Leucine	UCA UCG	UAA UAG	UGA UGG	A
	UUG					G
C	CUU	Leucine	CCU CCC	CAU CAC	CGU CGC	U
	CUC					C
	CUA	Proline	CCA CCG	CAA CAG	CGA CGG	A
	CUG					G
A	AUU	Isoleucine	ACU ACC	AAU AAC	AGU AGC	U
	AUC					C
	AUA	Threonine	ACA ACG	AAA AAG	AGA AGG	A
	AUG <b>START</b> Methionine*					G
G	GUU	Valine	GCU GCC	GAU GAC	GGU GGC	U
	GUC					C
	GUA	Alanine	GCA GCG	GAA GAG	GGA GGG	A
	GUG					G

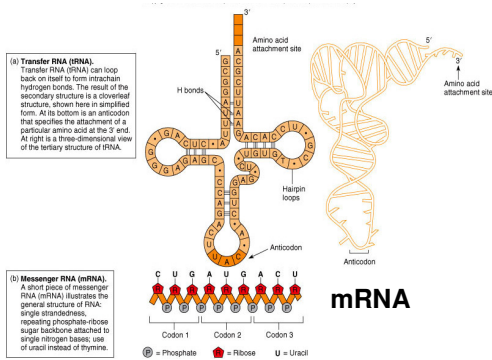
\*This codon initiates translation.  
For these codons, which give the orders to stop translation, there are no corresponding tRNAs and no amino acids.

## mRNA

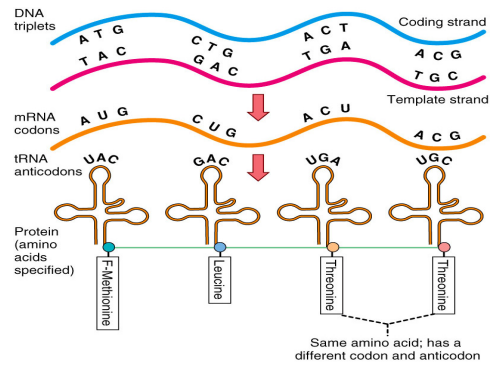
- Copy of a structural gene or genes of DNA
  - Can encode for multiple proteins on one message
- Thymidine is replaced by **URACIL**
- The message contains a codon (three bases)



## Relationship between tRNA and mRNA



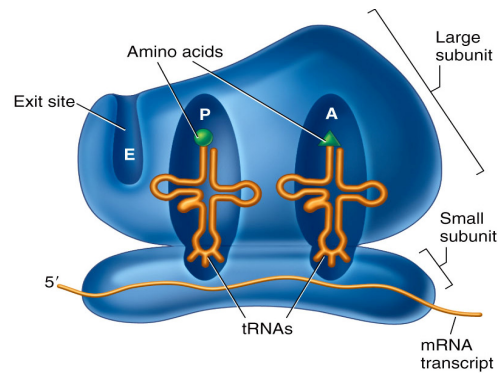
## Fig. 9.15 Interpreting the DNA code



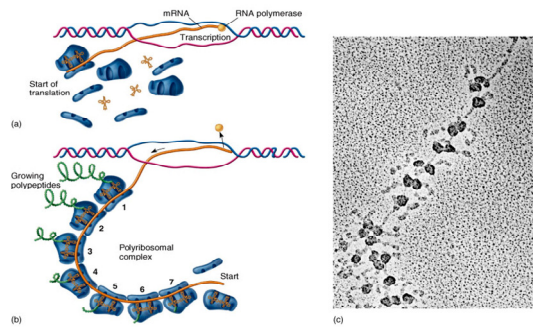
## Translation

- Translation
  - **Protein synthesis** have the following participants
    - mRNA
    - tRNA with attached amino acid - "loaded" tRNA
    - Ribosome

## The "players" in translation



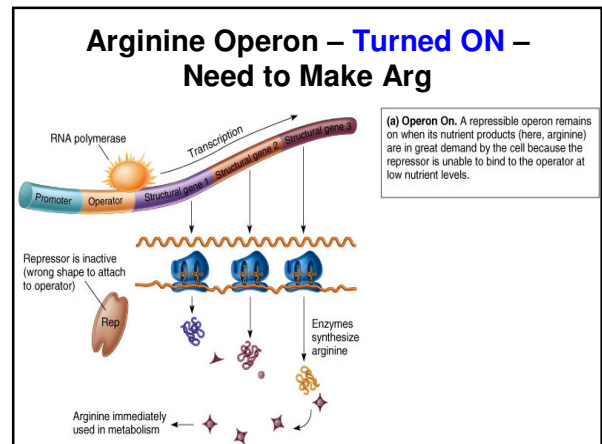
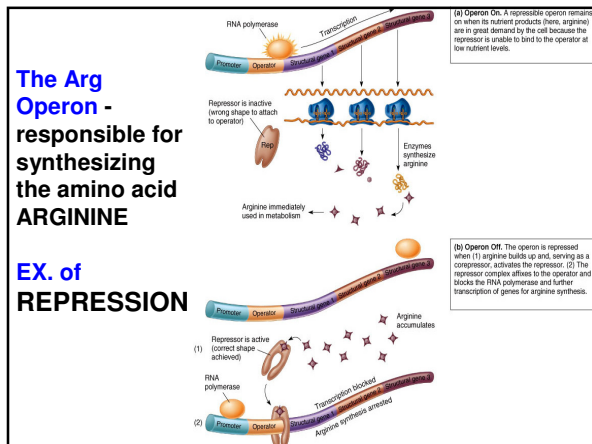
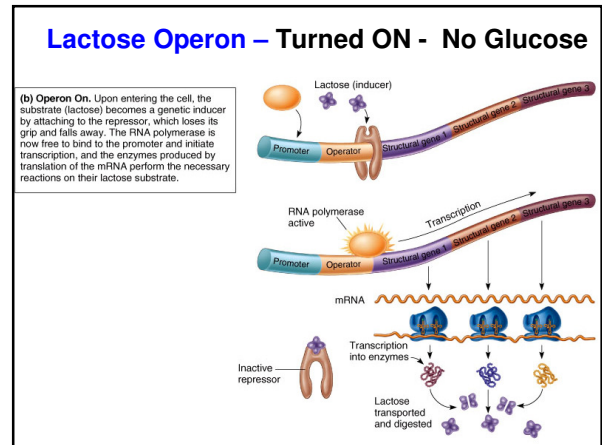
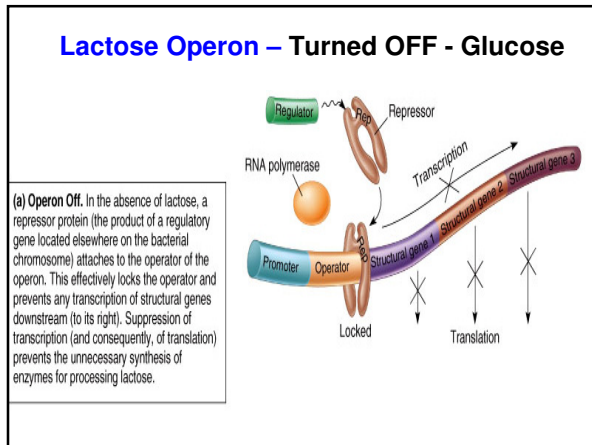
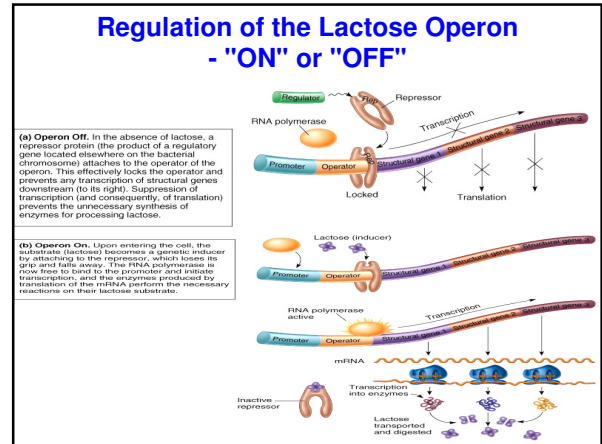
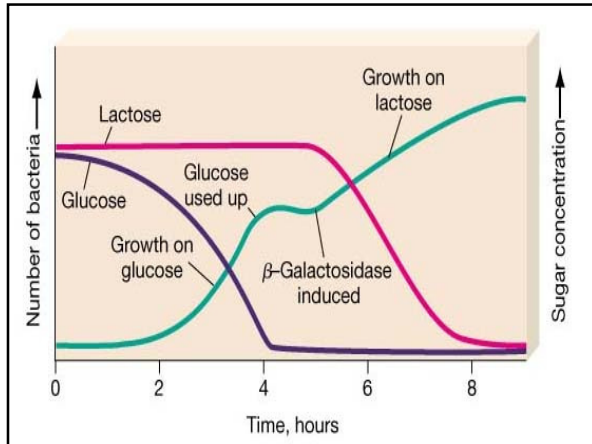
For prokaryotes, translation can occur at multiple sites on the mRNA while the message is still being transcribed



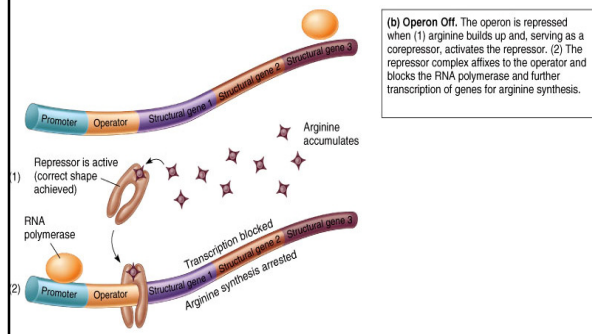
## Regulation

- **Lactose operon - genetic induction**
  - Utilize lactose as a food source
- **Repressible operon - genetic repression**
  - Amino acids, nucleotides





## Arginine Operon – Turned OFF – NO Need to Make Arg



## Comparison of Induction and Repression

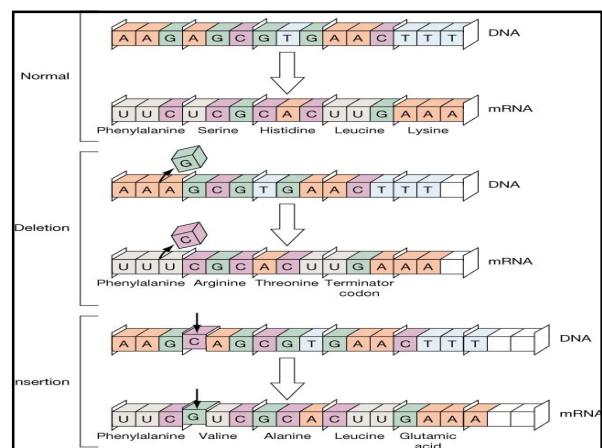
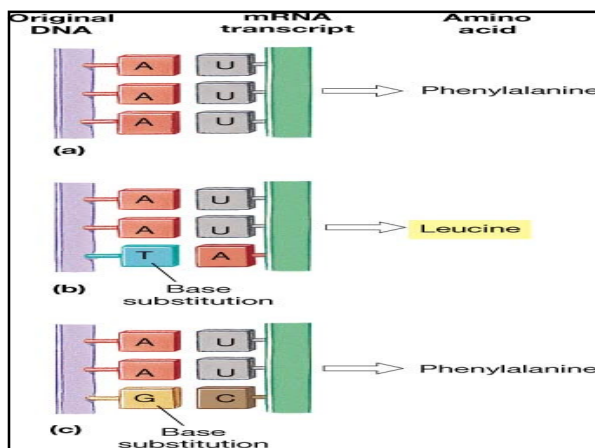
Regulatory Mechanism	Type of Pathway Regulated	Regulating Substance	Condition Leading to Gene Expression
Induction ( <i>lac</i> operon)	Catabolic Releases Energy	Nutrient (Lactose)	Presence of Nutrient
Repression ( <i>arg</i> operon)	Anabolic Uses Energy	End product (arginine)	Absence of End Product

## Mutations

- Changes made to the DNA - two main types
  - Spontaneous – random change
  - Induced – chemical, radiation
- Specific examples of mutations
  - Point – change a single base
  - Nonsense – change a normal codon into a stop codon
  - Frameshift – reading frame of the mRNA changes

**Point mutations** are a change in a single base – the **reading frame is not affected**, but the mutation may be either expressed or silent

**Frame-shift mutations** are the deletion or addition of one or more bases. These mutations change the reading frame of **all downstream codons**



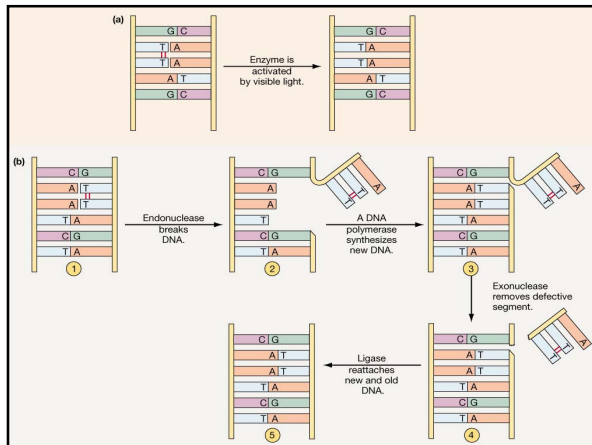


Spontaneous mutations are mutations that are caused by errors in the synthesis of DNA. Errors occur at the rate of 1 error every  $10^3$  or  $10^4$  nucleotides.

However, most organisms, both pro and eukaryotic, possess repair systems that lower the frequency of errors to one error in  $10^9$  to  $10^{11}$  nucleotides

Prokaryotes have repair systems that can repair damaged DNA. **Light repair** of DNA (photoreactivation) can repair thymine dimers induced by UV light.

**Dark repair** can identify and excise defective DNA and replace the defective DNA with the correct sequence based on the template strand.



### Eukaryotes have similar systems

Xeroderma pigmentosa is a genetic disease of humans that is due to an inherited defect in DNA repair

Exposure to sun (UV light) results in a dramatically increased rate of skin cancer due to UV induced mutation of DNA in the skin cells

### Xeroderma pigmentosa

Genetic disease where DNA repair process is damaged - patients lack DNA photolyase

Results in multiple skin cancers



The Ames test is used to screen environmental and dietary chemicals for mutagenicity and carcinogenicity without using animal studies.

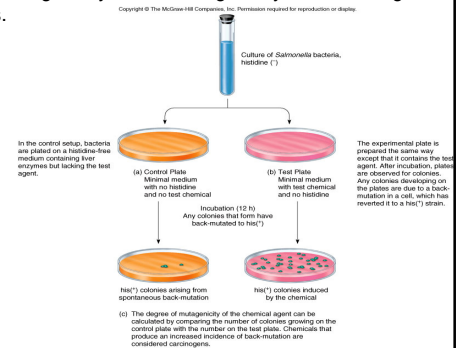


Fig. 9.23 The Ames test.

## Recombination

- Sharing or recombining parts of their genome
  - Conjugation
  - Transformation
  - Transduction

## Transformation – free DNA

- Nonspecific acceptance of free DNA by the cell (ex. DNA fragments, plasmids)
- DNA **can** be inserted into the chromosome
- **Competent cells** readily accept DNA

DNA released from a killed cell can be accepted by a live competent cell, expressing a new phenotype.

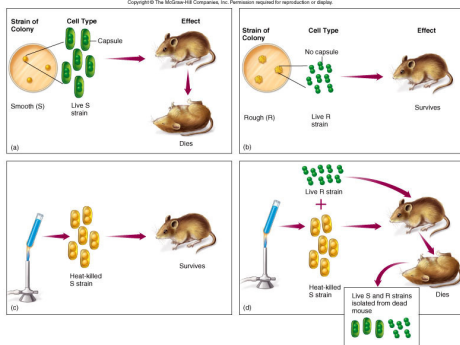


Fig. 9.25 Griffith's classic experiment in transformation

## Mechanism of Transformation

'Naked' DNA taken up by competent cell.

The DNA is free in the extracellular space. Cells are only competent to receive the DNA at certain periods of the life cycle.

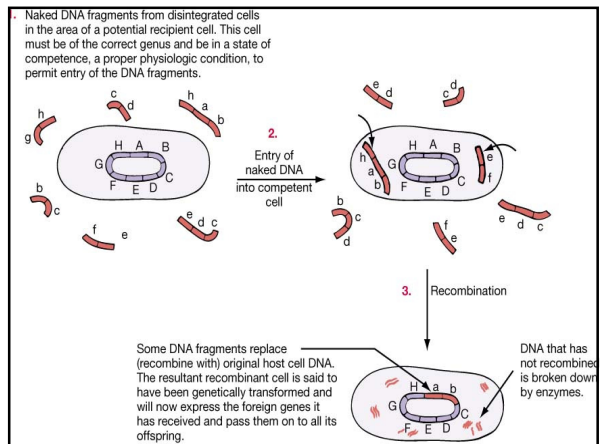
A competence factor is released by the cell and facilitates the entry of the DNA.

The amount of DNA that enters is small - less than 5% of the cell's genome.

To successfully transform cells, the DNA must be recombined into the recipient cell's genomic material.

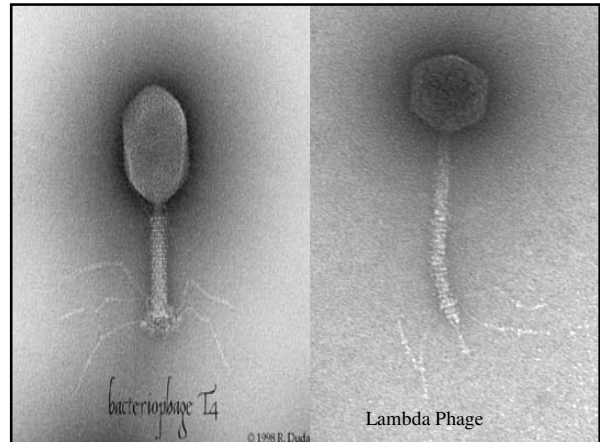
In recombinant DNA work, cells can be made "competent" to receive DNA. Then the recipient cells can be readily transformed.

Not all bacteria are subject to transformation - natural or induced.



## Transduction THINK BACTERIOPHAGE

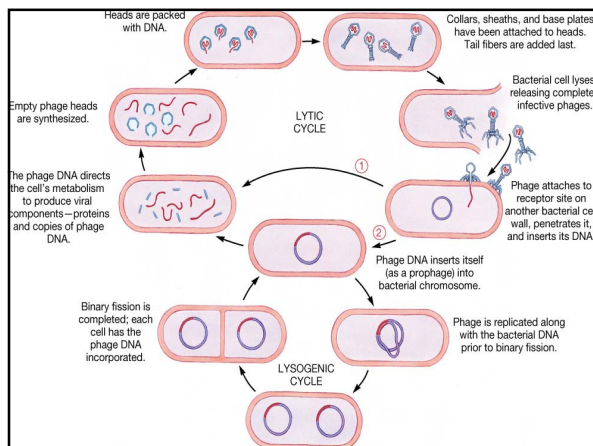
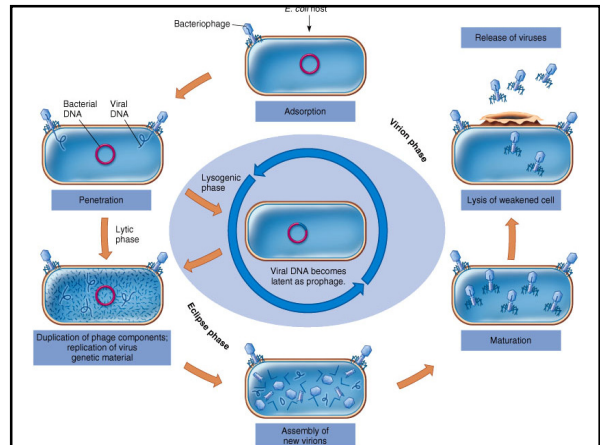
- Bacteriophage infect host cells
- Serve as the carrier of DNA from a donor cell to a recipient cell
  - Generalized
  - Specialized



A phage infects a bacterium and “chooses” a **lytic cycle** or **lysogenic cycle** - Figure 8.3.

If **lysogenic cycle** is chosen, the phage genome recombines into the bacterial genome and becomes a prophage.

Lysogenic phages are **specialized transducing phages** and can transduce only specific regions of the bacterial genome

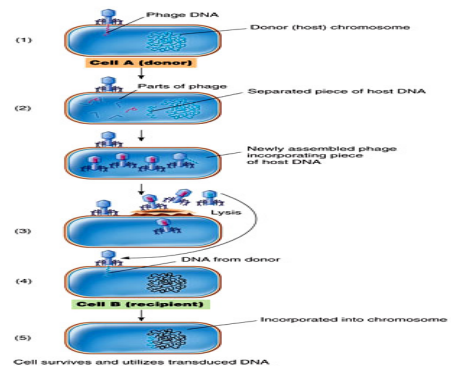


## Specialized and General Transduction

Generalized transducing phages undergo a lytic cycle and are capable of transducing any part of the donor's genetic information

**THIS IS A RANDOM EVENT**

### Genetic transfer based on generalized transduction



### Transduction is significant

The ability of a lysogenic phage to recombine into a bacterial genome suggests a parallel evolution of phage and bacteria since there must be sequence similarities at the site of integration.

Transduction is a mechanism to transfer genetic material from one cell to a second.

### Genetic transfer based on specialized transduction.

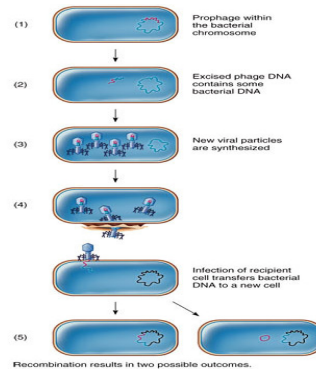
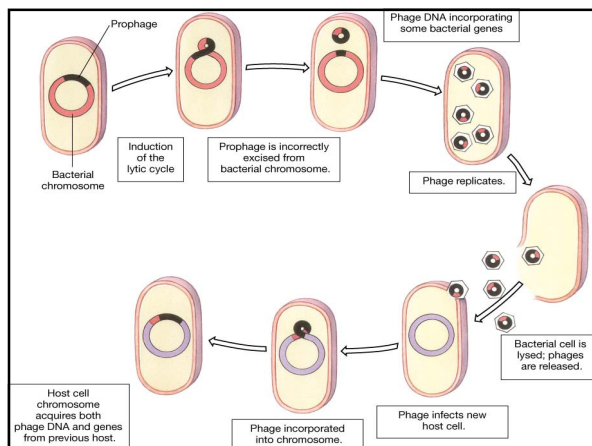


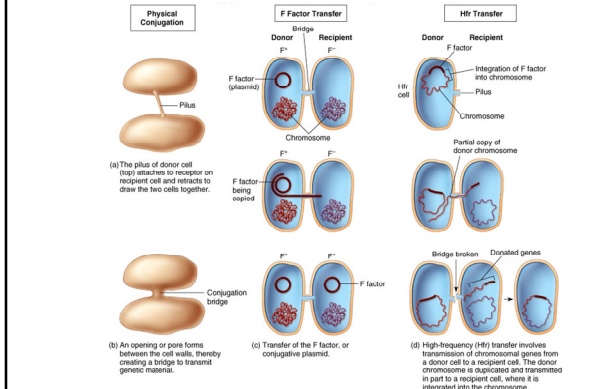
Fig. 9.27 Specialized transduction



### Conjugation

- Transfer of plasmid DNA from a  $F^+$  (F factor) cell to a  $F^-$  cell
- An  $F^+$  bacterium possesses a pilus
- Pilus attaches to the recipient cell and creates pore for the transfer DNA
- High frequency recombination (Hfr) donors contain the F factor in the chromosome

Conjugation is the genetic transmission through direct contact between cells.



**Conjugation**  
Lederberg discovered conjugation in 1946

**Mechanism of conjugation**

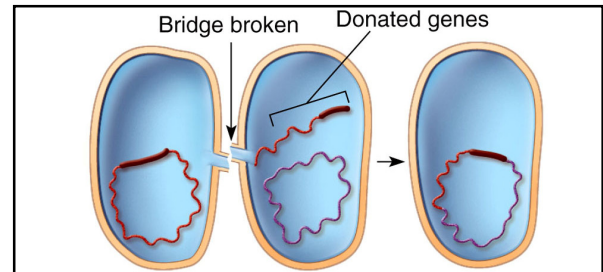
In one type of conjugation, the population of cells capable of conjugating contain two types of cells  $F^+$  and  $F^-$  - the former are the donor cells and the latter are the recipient cells. The donor cells have an F plasmid – sex pili and DNA Transfer.

Conjugation in this case is a transfer of the F plasmid from the donor to the recipient.

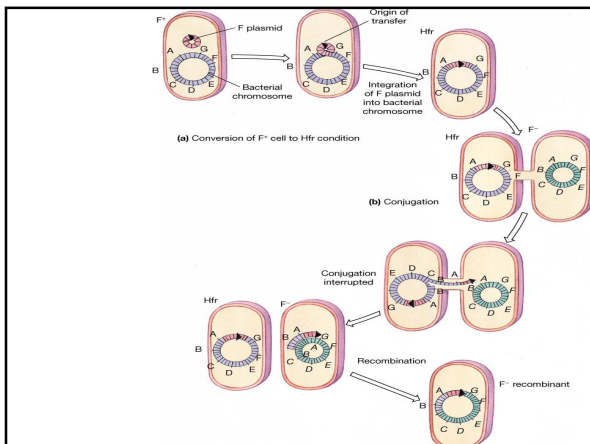
The F plasmid codes for the synthesis of pili which are instrumental in the formation of the conjugal bridge & DNA Transfer

A second type of conjugation is  $F^+$  to  $Hfr$  conversion.

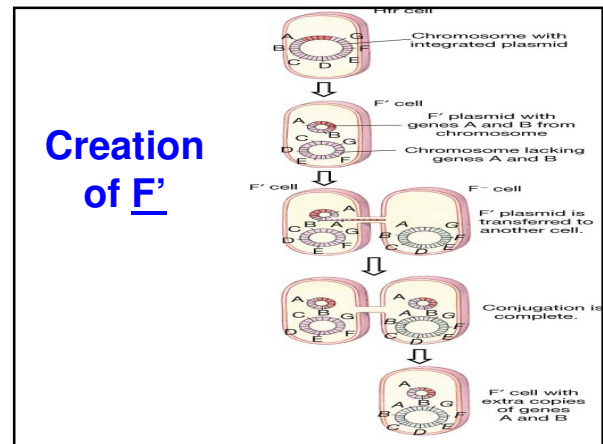
**$F'$  plasmids** are created when the  $Hfr$  plasmid recombines out of the bacterial genome imprecisely and carries with it a segment of the bacterial genome. That segment can be transferred to a recipient cell as in  $F^+$  conjugation.



(d) High-frequency ( $Hfr$ ) transfer involves transmission of chromosomal genes from a donor cell to a recipient cell. The donor chromosome is duplicated and transmitted in part to a recipient cell, where it is integrated into the chromosome.



**Creation of  $F'$**





### Significance of conjugation

In Hfr conjugation significant amounts of genetic material may be transferred.

Genetic information including determinants of pathogenicity or antimicrobial resistance may be transferred cell to cell.

Hfr conjugation is an excellent procedure to map genes of conjugable bacteria.

### The mechanisms of gene transfer are summarized –

> Table 8.2 Summary of the effects of various transfers of genetic information

Kind of transfer	Effects
<b>Transformation</b>	Transfers less than 1 percent of cell's DNA. Requires competence factor. Changes certain characteristics of an organism depending on which genes are transferred.
<b>Transduction</b>	Transfer is effected by a bacteriophage.
Specialized	Only genes near the prophage are transferred to another bacterium.
Generalized	Fragments of host bacterial DNA of variable length and number are packed into the head of a virus.
<b>Conjugation</b>	Transfer is effected by a plasmid.
F <sup>+</sup>	A single plasmid is transferred.
Hfr	An initiating segment of a plasmid and a linear sequence of bacterial DNA that follows the initiating segment are transferred.
F'	A plasmid and whatever bacterial genes adhere to it when it leaves a bacterium are transferred.