Chapter 9

Topics
- Genetics
- Flow of Genetics/Information

Regulation

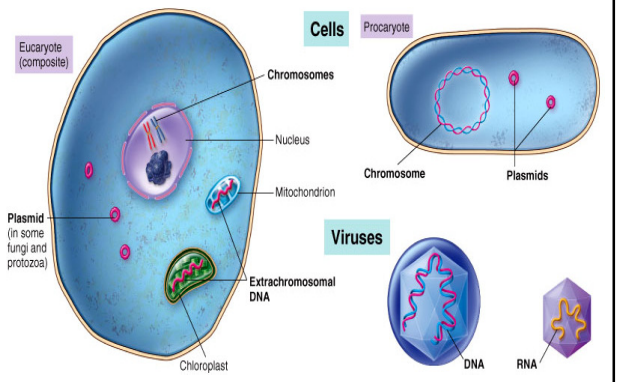
- Mutation

- Recombination – gene transfer

Genetics

- Genome - the sum total of genetic information in a 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 (*E. coli* ~ 4,288 genes)
  - 1 circular chromosome ± extrachromosomal DNA (plasmids)
- Eukaryotic (humans ~ 20 -25,000 genes)
  - Many paired chromosomes ± extrachromosomal DNA (Mitochondria or Chloroplast)
- Subdivided into basic informational packets called **genes**

Genes

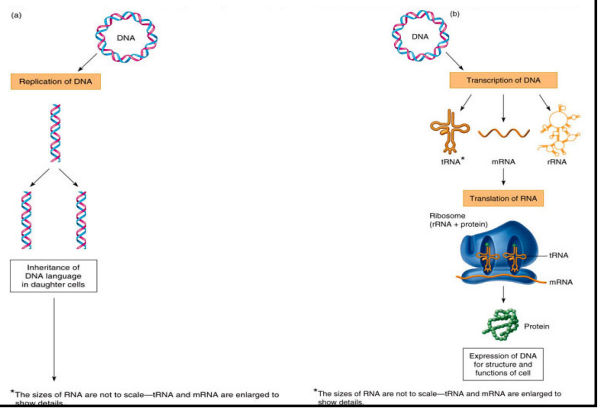
- **Three categories**
  - **Structural - genes that code for proteins**
  - **Regulatory - genes that control gene expression**
  - **Encode for RNA - non-mRNA**

Flow of Genetics/Information

The Central Dogma

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

Replication Transcription & Translation



DNA

- **Structure**
- **Replication**
- **Universal Code & Codons**

Escherichia coli with its emptied genome!

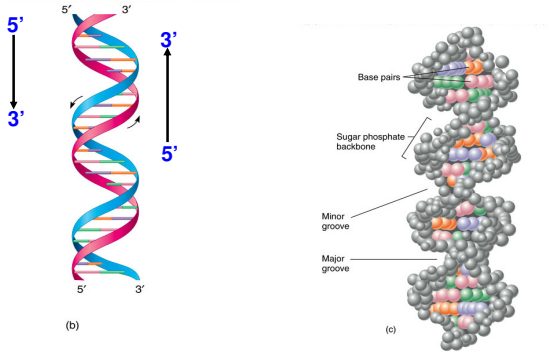
Structure

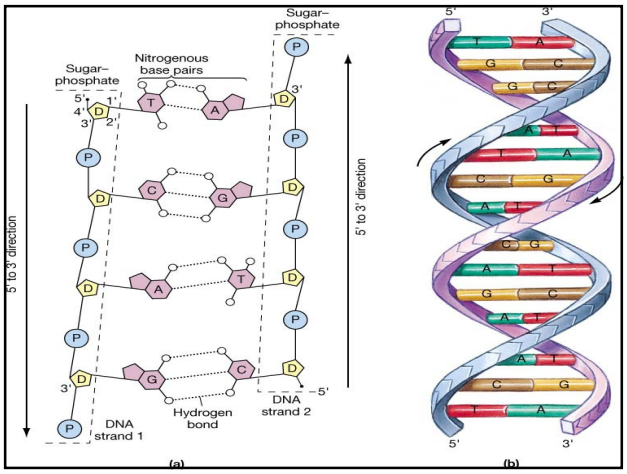
- **Nucleotide**
  - Phosphate
  - Deoxyribose sugar
  - Nitrogenous base
- Double stranded helix
  - Antiparallel arrangement

Nitrogenous bases

- Purines
  - **Adenine**
  - **Guanine**
- Pyrimidines
  - **Thymine**
  - **Cytosine**

Versions of the DNA double helix





Replication

- Semiconservative - starts at the Origin of Replication
- Enzymes
  - Helicase
  - Dna Pol III
  - DNA Pol I
  - Primase
  - Gyrase
  - Ligase
- Leading strand
- Lagging strand
  - Okazaki fragments

Semiconservative

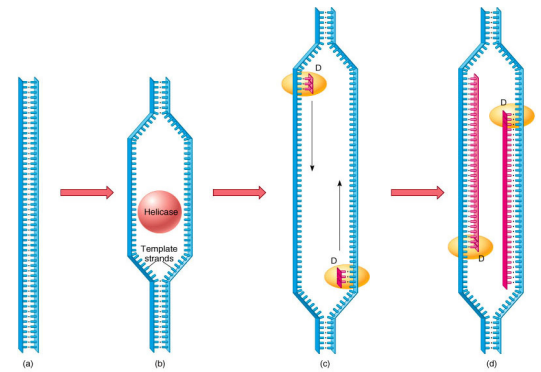
- **New strands are synthesized in 5' to 3' direction**
- **Mediated by DNA polymerase III- only works in 5' to 3' direction**

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



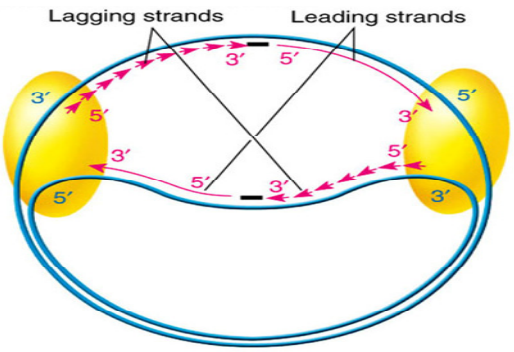
Leading strand

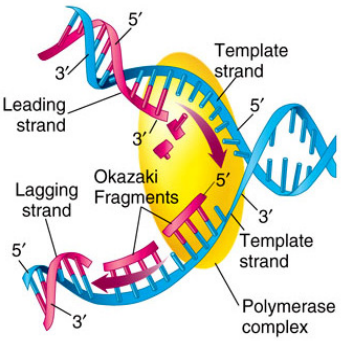
- **RNA primer initiates the 5' to 3' synthesis of DNA in continuous manner**

Lagging strand

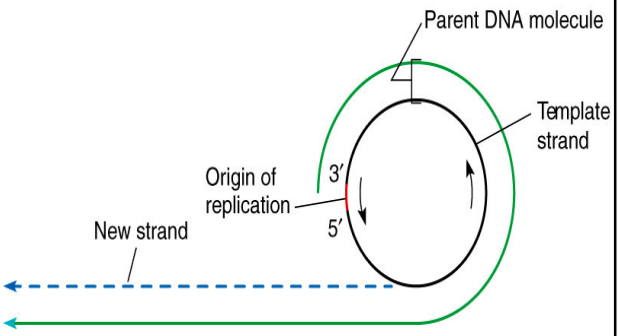
- Multiple **Okazaki fragments** are synthesized
- **Okazaki fragments** are ligated together to form one continuous strand

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 or DEGENERATE**
- 20 amino acids
- Start codon - AUG
- Stop codons – UAA, UAG, UGA

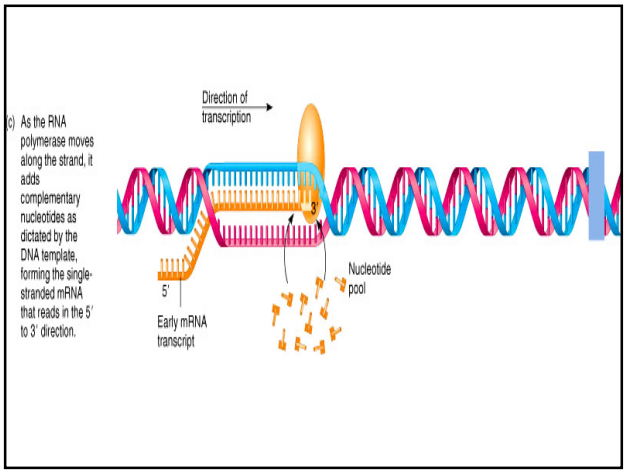
The Genetic code - Wow!!!!!

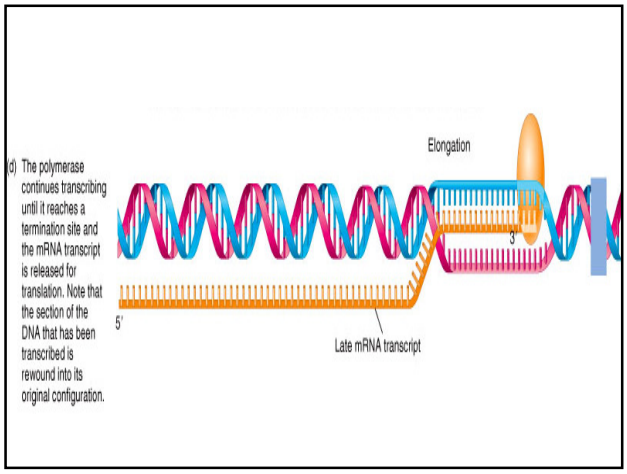
First Base Position	Second Base Position: U	Second Base Position: C	Second Base Position: A	Second Base Position: G	Third Base Position
U	UUU Phenylalanine	UCU Serine	UAU Tyrosine	UGU Cysteine	U
	UUC Phenylalanine	UCC Serine	UAC Tyrosine	UGC Cysteine	C
	UUA Leucine	UCA Serine	UAA STOP**	UGA STOP**	A
	UUG Leucine	UCG Serine	UAG STOP**	UGG Tryptophan	G
C	CUU Leucine	CCU Proline	CAU Histidine	CGU Arginine	U
	CUC Leucine	CCC Proline	CAC Histidine	CGC Arginine	C
	CUA Leucine	CCA Proline	CAA Glutamine	CGA Arginine	A
	CUG Leucine	CCG Proline	CAG Glutamine	CGG Arginine	G
A	AUU Isoleucine	ACU Threonine	AAU Asparagine	AGU Serine	U
	AUC Isoleucine	ACC Threonine	AAC Asparagine	AGC Serine	C
	AUA Isoleucine	ACA Threonine	AAA Lysine	AGA Arginine	A
	AUG START ;Methionine*	ACG Threonine	AAG Lysine	AGG Arginine	G
G	GUU Valine	GCU Alanine	GAU Aspartic acid	GGU Glycine	U
	GUC Valine	GCC Alanine	GAC Aspartic acid	GGC Glycine	C
	GUA Valine	GCA Alanine	GAA Glutamic acid	GGA Glycine	A
	GUG Valine	GCG Alanine	GAG Glutamic acid	GGG Glycine	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)

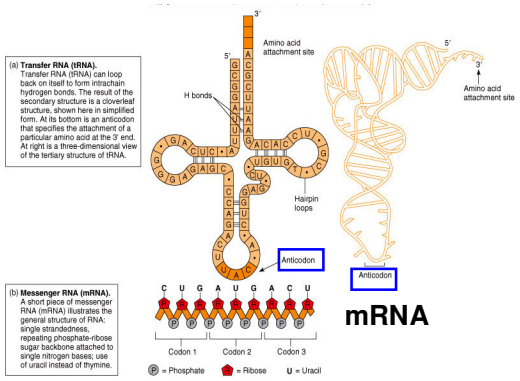




Trancription

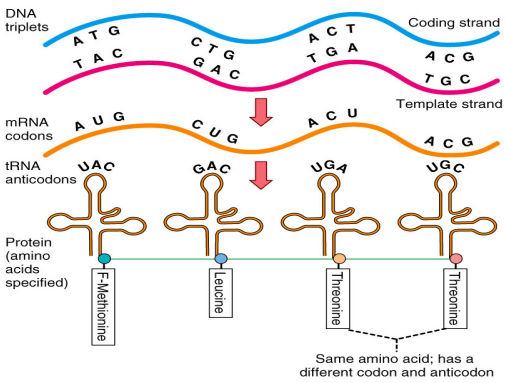
- -RNA Pol
- Template strand (3' → 5')
- Newly made mRNA (5' → 3')
- Promoter – binding site for RNA Pol
- Average size for mRNA – 1200 bases

Relationship between tRNA and mRNA



mRNA

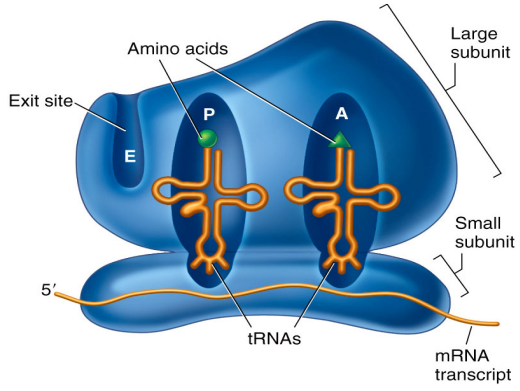
Fig. 9.15 Interpreting the DNA code



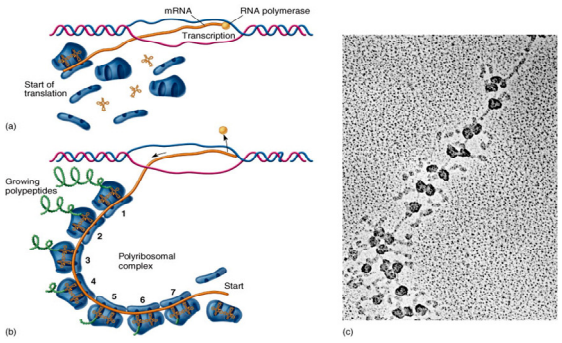
Translation

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

The "players" in translation



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

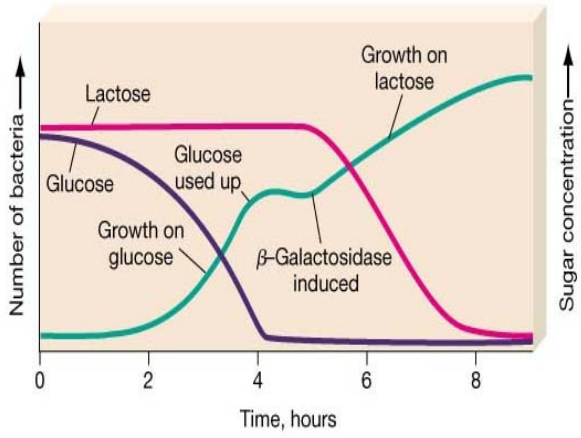


Transcription in Prokaryotes and Eukaryotes

- 1 mRNA = 1 protein
- 1 mRNA = several proteins (polycistronic)
- Different compartments for each event
- Presence of introns

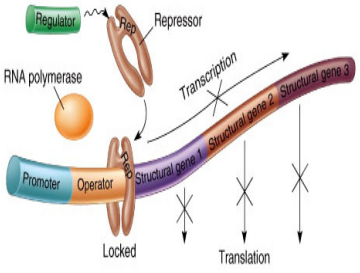
Regulation

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



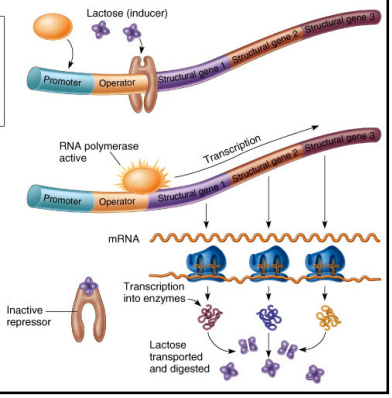
Lactose Operon – Turned OFF - Glucose

(a) Operon Off. In the absence of lactose, a repressor protein (the product of a regulatory gene located elsewhere on the bacterial chromosome) attaches to the operator of the operon. This effectively locks the operator and prevents any transcription of structural genes downstream (to its right). Suppression of transcription (and consequently, of translation) prevents the unnecessary synthesis of enzymes for processing lactose.



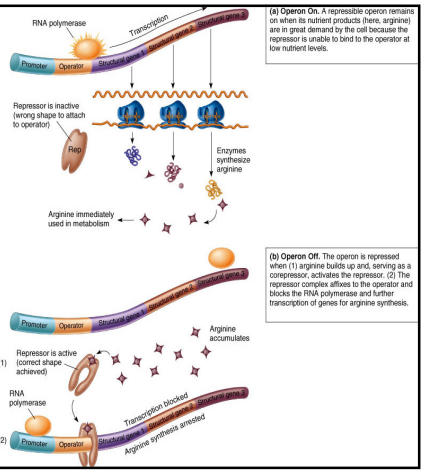
Lactose Operon – Turned ON - No Glucose

(b) Operon On. Upon entering the cell, the substrate (lactose) becomes a genetic inducer by attaching to the repressor, which loses its grip and falls away. The RNA polymerase is now free to bind to the promoter and initiate transcription, and the enzymes produced by translation of the mRNA perform the necessary reactions on their lactose substrate.

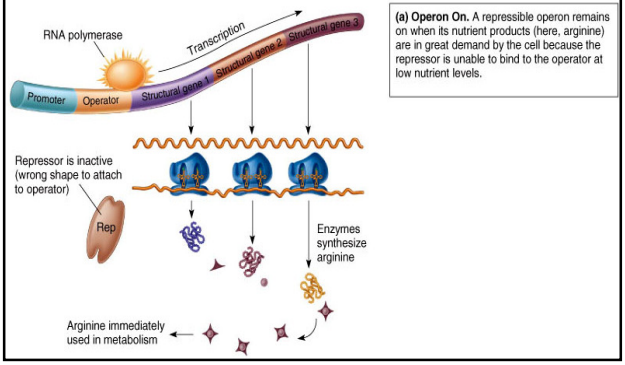


The Arg Operon - responsible for synthesizing the amino acid ARGININE

EX. of REPRESSION

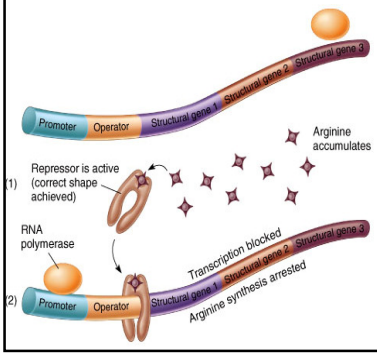


Arginine Operon – Turned ON – Need to Make Arg



(a) Operon On. A repressible operon remains on when its nutrient products (here, arginine) are in great demand by the cell because the repressor is unable to bind to the operator at low nutrient levels.

Arginine Operon – Turned OFF – NO Need to Make Arg



(b) Operon Off. The operon is repressed when (1) arginine builds up and, serving as a corepressor, activates the repressor. (2) The repressor complex affixes to the operator and blocks the RNA polymerase and further transcription of genes for arginine synthesis.

Comparison of Induction and Repression

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

Mutations

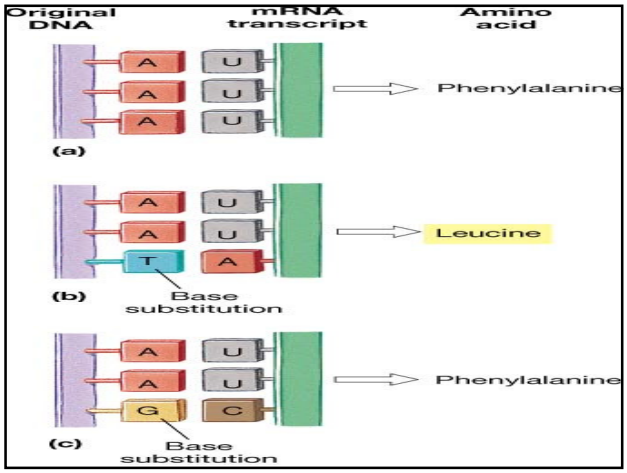
- **Changes made to the DNA - two main types**
  - **Spontaneous – random change**
  - **Induced – chemical, radiation**

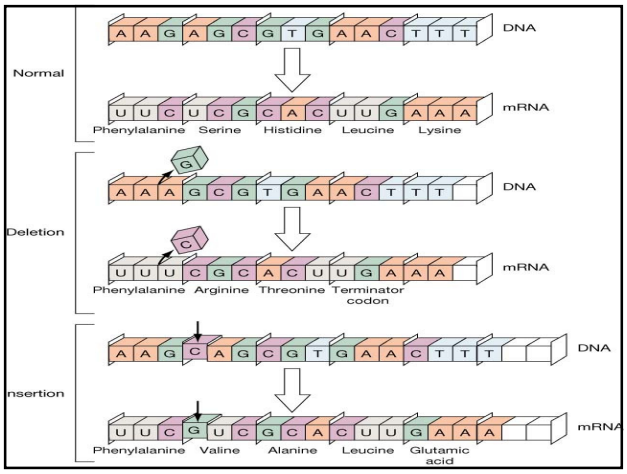
Specific examples of mutations

- **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



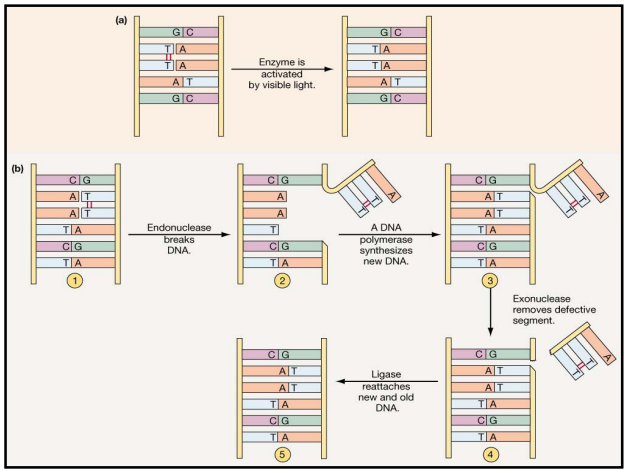


Spontaneous mutations are mutations that are caused by errors in the synthesis of DNA. Errors occur at the rate of 1 error every 10^3 or 10^4 nucleotides.

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

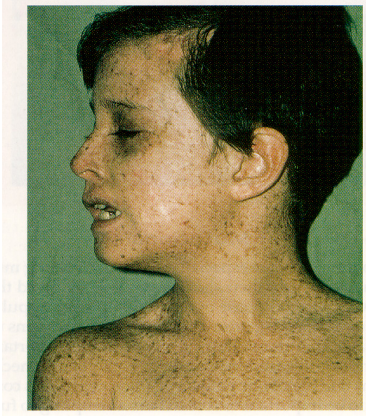
Xeroderma pigmentosa is a genetic disease of humans that is due to an inherited defect in DNA repair

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.

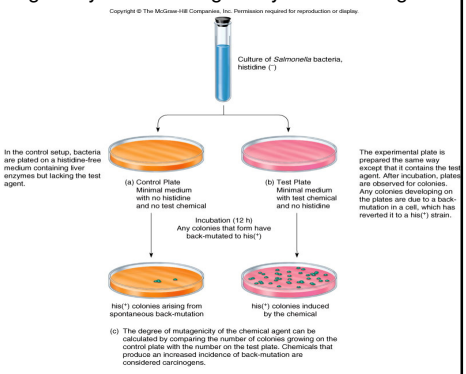


Fig. 9.22 The Ames test.

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.

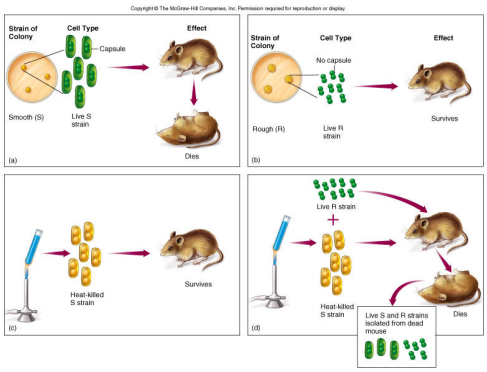


Fig. 9.24 Griffith's classic experiment in transformation

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.

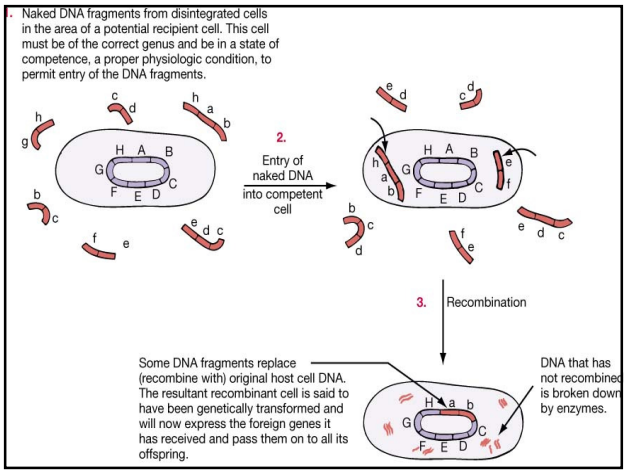
A competence factor is released by the cell and facilitates the entry of the DNA.

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.

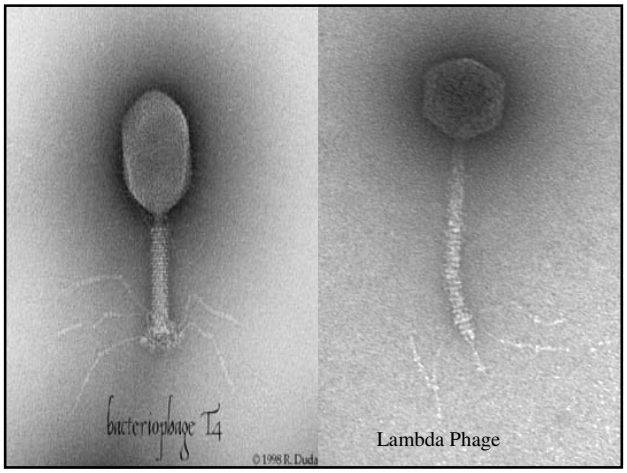
In recombinant DNA work, cells can be made "competent" to receive DNA. Then the recipient cells can be readily transformed.

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



Transduction
THINK BACTERIOPHAGE

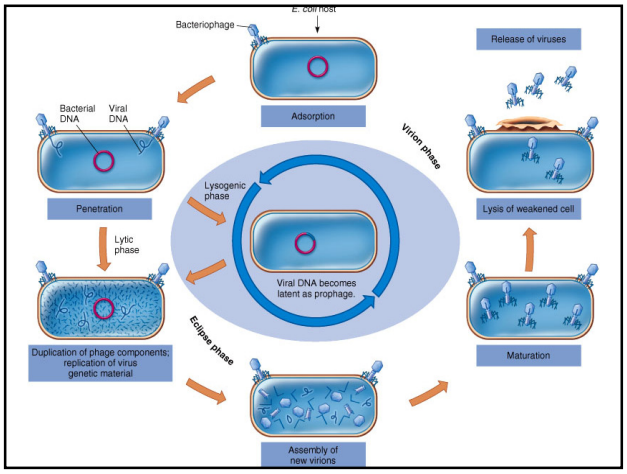
- Bacteriophage infect host cells
- Serve as the carrier of DNA from a donor cell to a recipient cell
  - –Generalized
  - –Specialized

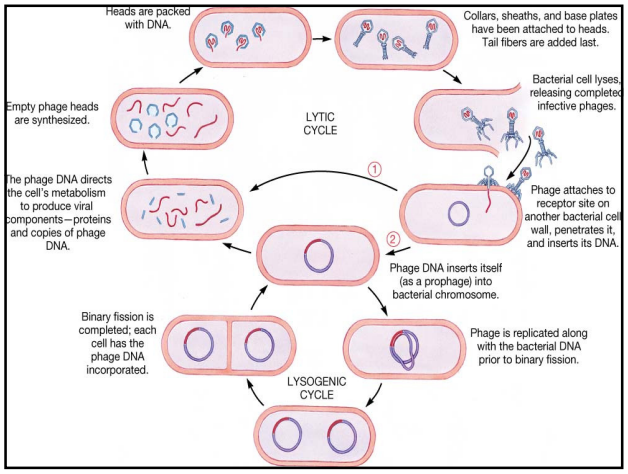


A phage infects a bacterium and "chooses" a lytic cycle or lysogenic cycle - Figure 8.3.

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



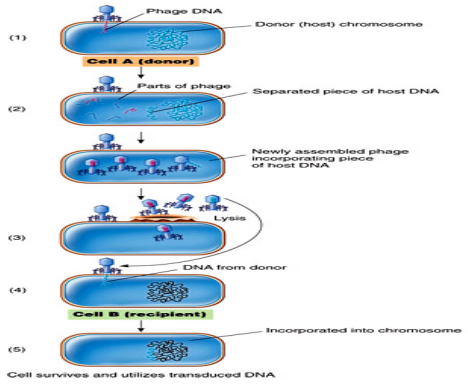


Specialized and General Transduction

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.

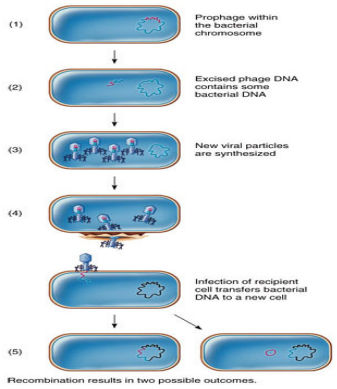
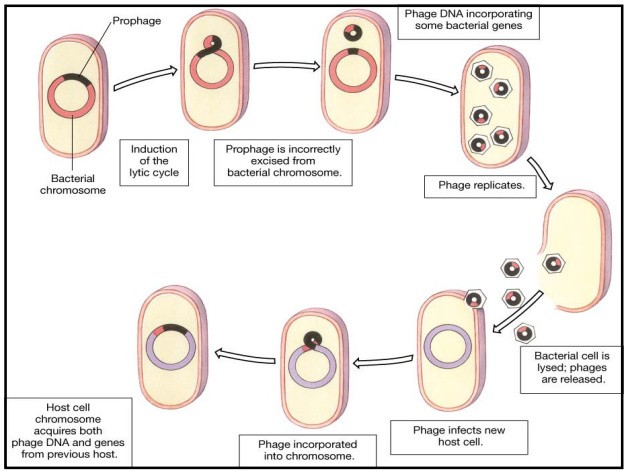


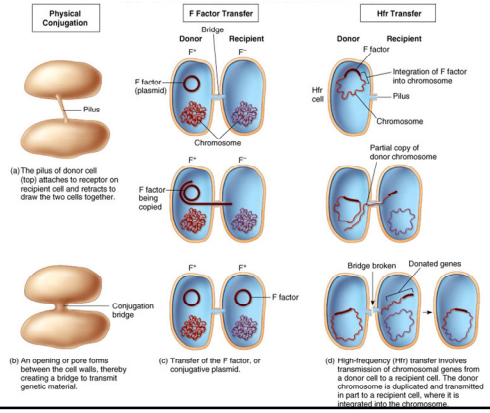
Fig. 9.27 Specialized transduction



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

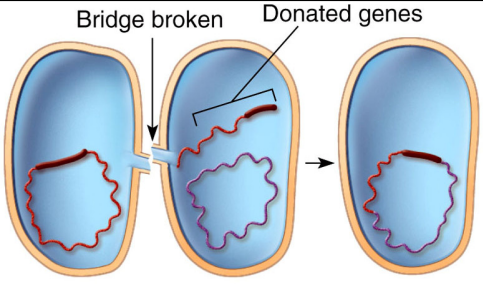
In one type of conjugation, the population of cells capable of conjugating contain two types of cells F^+ and F^- - the former are the donor cells and the latter are the recipient cells. The donor cells have an F plasmid – sex pili and DNA Transfer.

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

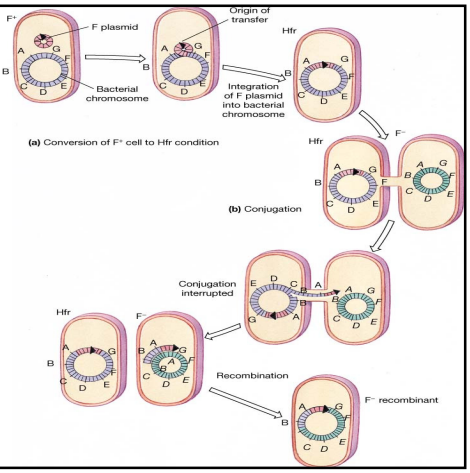
The F plasmid codes for the synthesis of pili which are instrumental in the formation of the conjugal bridge & DNA Transfer

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

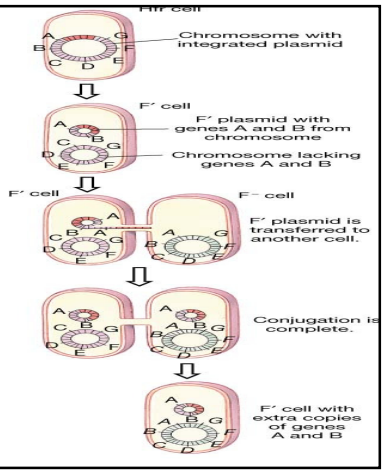
A third type of conjugation is F' plasmids are created when the Hfr plasmid recombines out of the bacterial genome imprecisely and carries with it a segment of the bacterial genome. That segment can the be transferred to a recipient cell as in F^+ conjugation.



(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 antimicrobic 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
Transformation	Transfers less than 1 percent of cell's DNA. Requires competence factor. Changes certain characteristics of an organism depending on which genes are transferred.
Transduction	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.
Conjugation	Transfer is effected by a plasmid.
F^+	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.