

Genetics

(see text pages 257-259, 267-298)

Remember what it is we want to address: How is it that prokaryotes gain new genetic ability?
The cells are haploid and reproduce by fission...so how does an genetic novelty arise?

By two mechanisms:

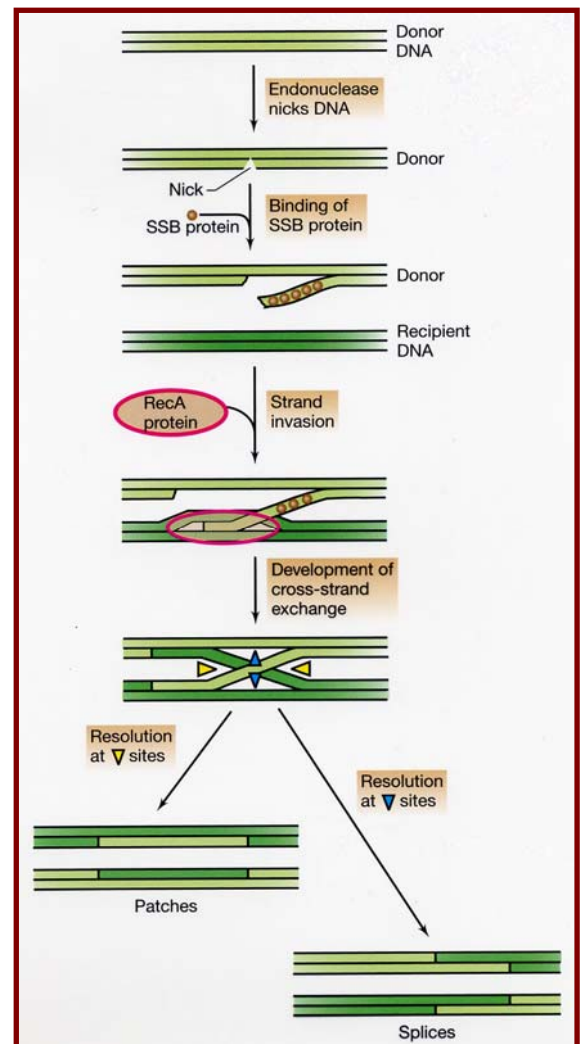
- Mutation
- Genetic Recombination

Mutation

- Mutation is a stable, heritable change in the nucleotide sequence of the DNA.
- Such change may occur spontaneously or in response to some mutagen
- There are three potential outcomes of a mutational event
 - Lethal: never revealed
 - Beneficial: for example increase enzyme activity
 - Neutral: No effect
 - Conditional: Not really a category but should be considered. Here a mutational event is only expressed under certain conditions...for example the ability to grow at an elevated temperature...or the ability to synthesize a certain compound only if a certain precursor is available.
- Rates of mutation are very low...very low (<1 in a million bases replicated) but because there are so many bacteria in a culture (> 10⁹ cells per mL) there may always be a mutant. (Where is Dr. Xavier!)

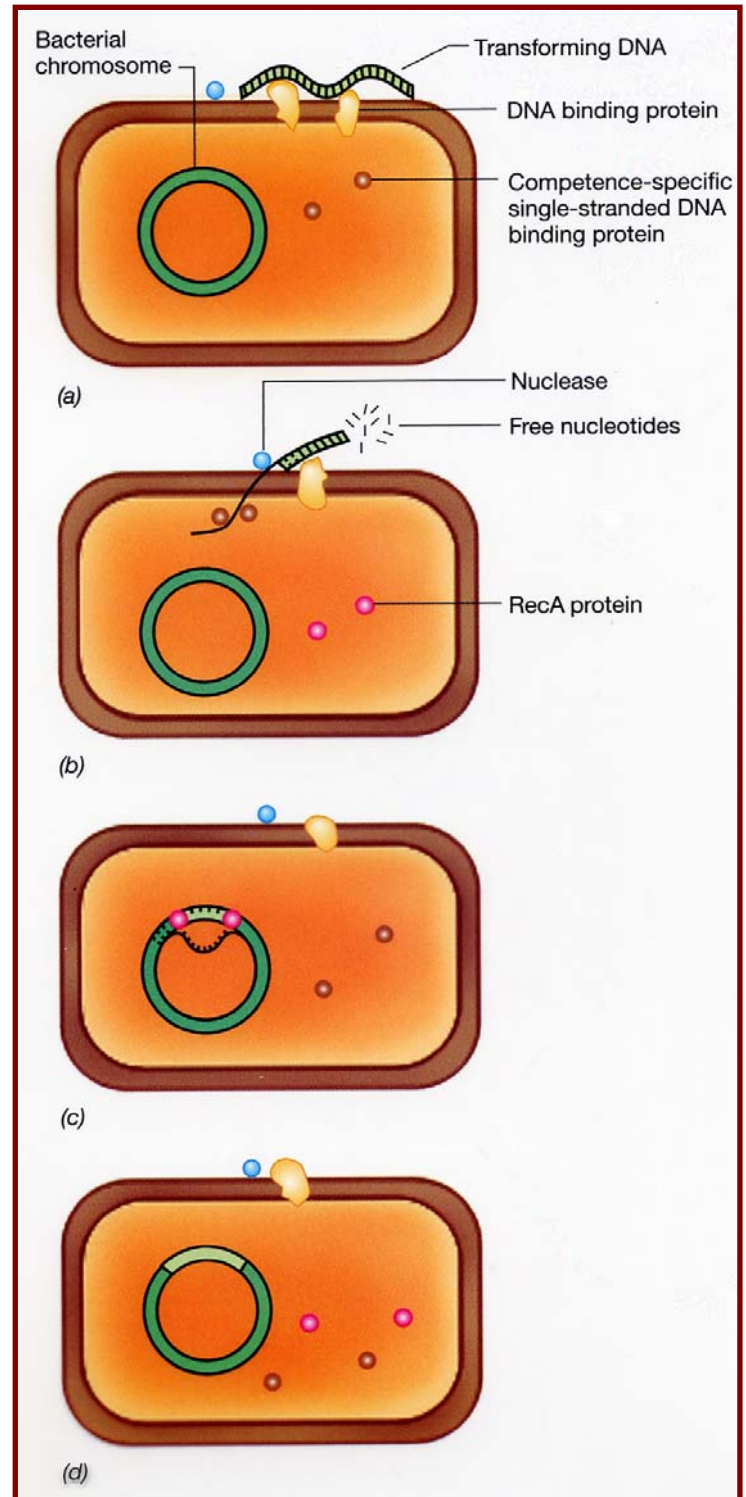
Genetic recombination

- Genetic recombination is the exchange of genetic material between two sources. The sources may be foreign to each other, or homologs within the same DNA molecule
- General or Homologous recombination
 - Not uncommon in the bacteria
 - Involves the participation of a specific protein (RecA) and DNA
- How
 - One strand of one of the DNA molecules (or homolog region) is nick by an endonuclease
 - The nicked strand is bound by SSBs with helicase activity
 - RecA binds to create a complex and permits invasion and annealing of free strand to the homolog region
 - Eventually the strands are cut and ligated to create crossover events. One section of DNA has been replaced with another.

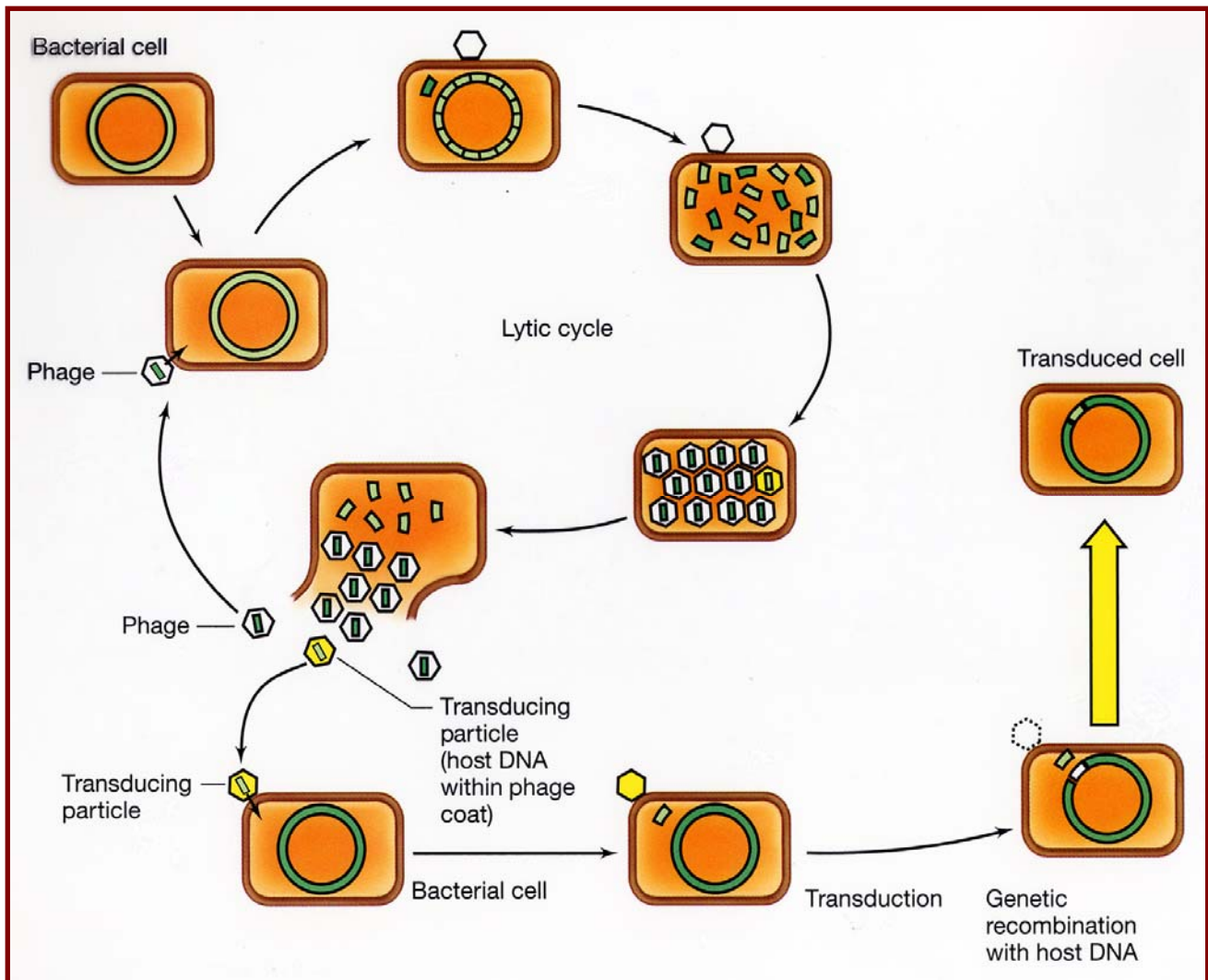


In what way does genetic recombination occur?

- Transformation
 - DNA existing in the environment is captured by 'competent' cells and incorporated into the genome
 - Lysis of a donor cell liberate DNA fragments
 - DNA fragments bound to the cell surface as double stranded
 - A single strand is transported into the cell
 - Quite a bit of DNA may be bound and transferred (~10 genes worth)
 - Once inside the cell, the fragment aligns with the homologous chromosomal DNA and parts may be recombined



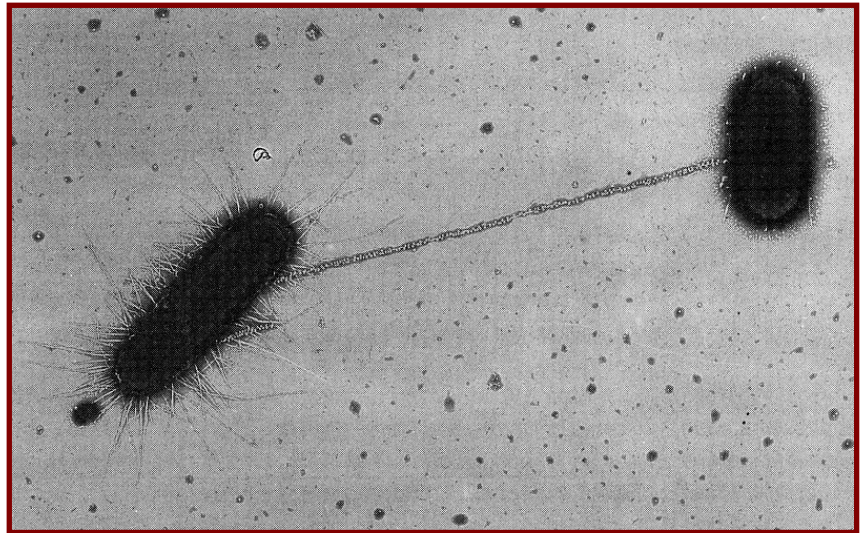
- Transduction
 - DNA is transferred from cell to cell by viruses
 - During packaging of the DNA by lytic or lysogenic phage an error is made and some host DNA is packaged.
 - Subsequent infection by the now TRANSDUCING particle results in the transfer of DNA as double stranded
 - The fragment may undergo genetic recombination

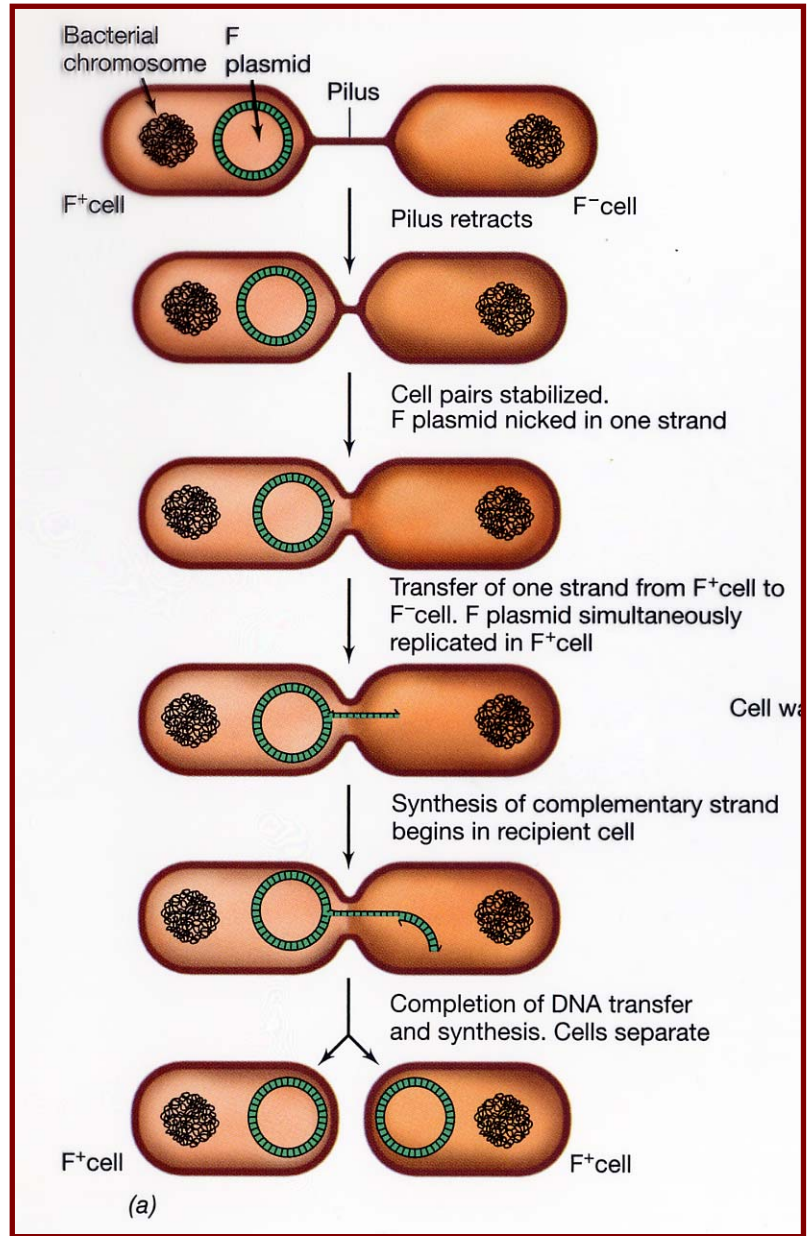
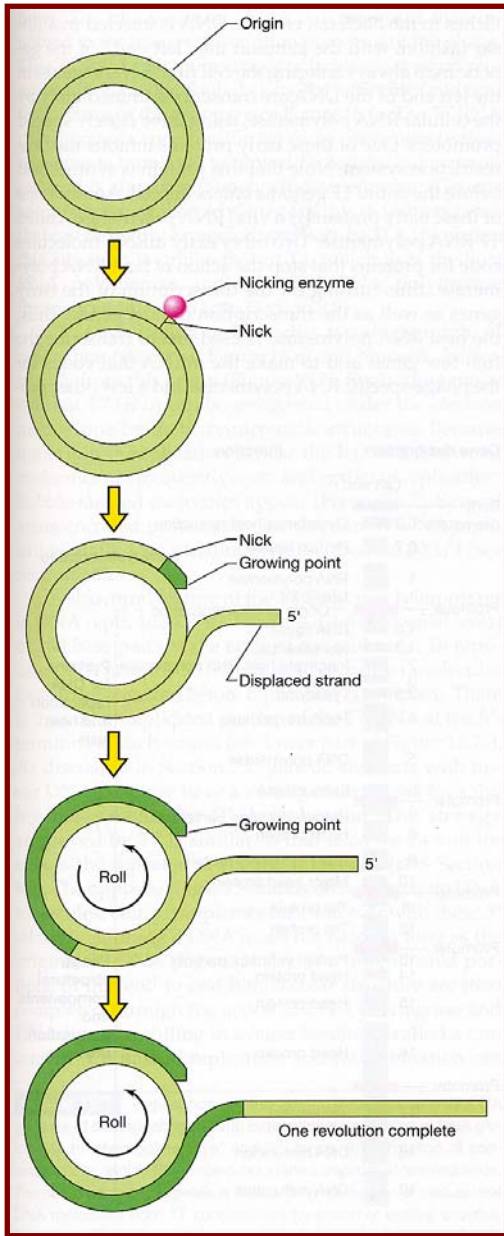


- Conjugation
 - Conjugation is the transfer of extra-chromosomal DNA known as plasmids
 - Plasmid are
 - Extra-chromosomal circular genetic elements that reproduce autonomously
 - Generally small, from 1 → 1000 kb
 - They may be 'cured' or removed from the cell carrying them
 - Some may be transferred to other cells in a process known as 'conjugation'
 - Some, may at times, be integrated into the host chromosome
 - They are double stranded DNA
 - The genes they carry impart new phenotypes onto the host
 - Examples of phenotypes
 - Antibiotic production
 - Ability to conjugate
 - Metabolic features (catabolic or anabolic)
 - Antibiotic resistance
 - Virulence factors

Conjugative Plasmids

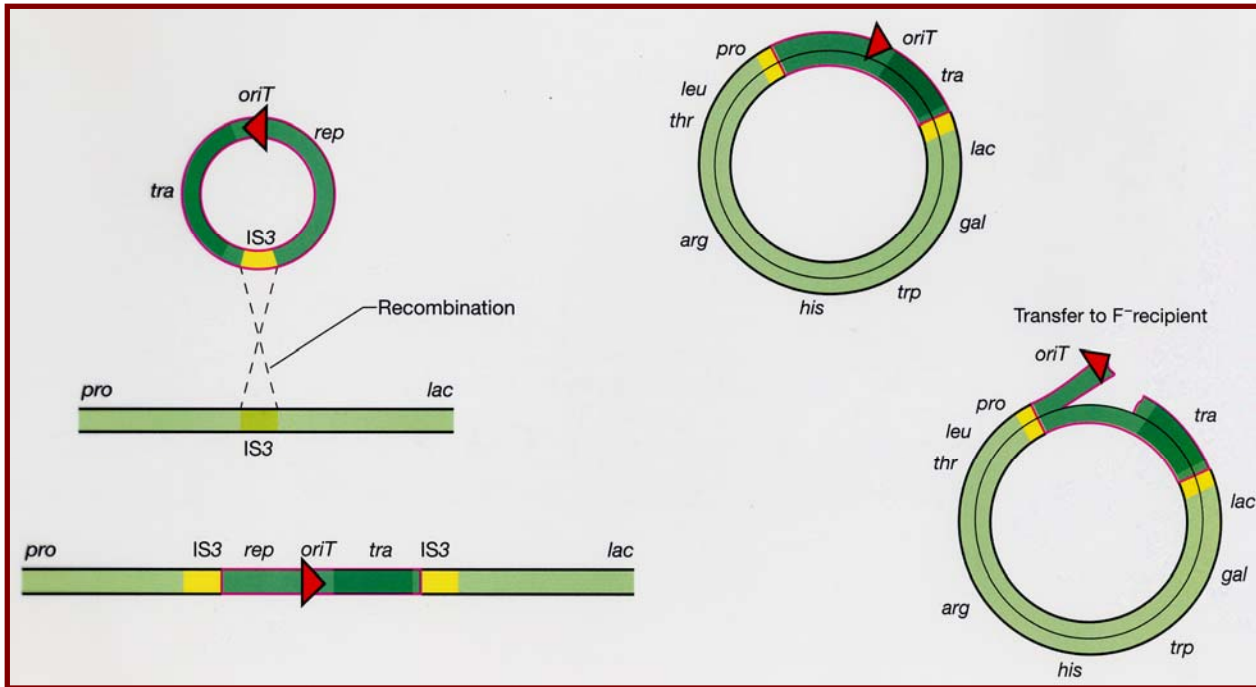
- Conjugation is a recombination event requiring cell-cell contact
- Plasmid carrying cell (F^+) carries the genetic information to produce a pilus (sex pilus) and the proteins necessary to transfer the plasmid
- Plasmid is replicated through a processes known as 'rolling circle replication'
- A nick is made in the plasmid and one strand is replicated
- The other strand is displaced and moved to the new host where it is also replicated.
- The whole process looks something like this.....





Integration of the plasmid into the host chromosome

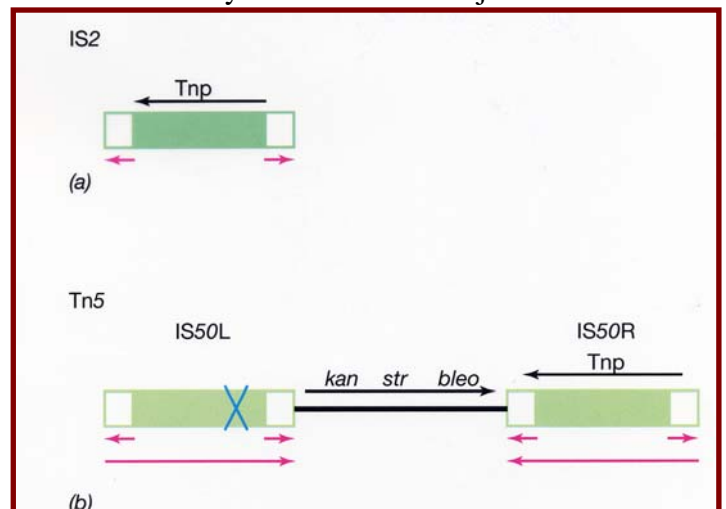
- Integration of the F^+ plasmid into the host chromosome may cause the chromosome to become mobilized. During conjugation, a large portion of the host chromosome may be transferred to the recipient cell.
- F plasmids integrated into the host chromosome are called Hfr strains.
- Integration take place at site known as Insertion sequences



- Due to manner in which insertion occurs, the origin of replication (*ori*) for the plasmid ends up in the middle of the inserted plasmid
- When replication (by rolling circle) begins only a section of the plasmid genome is transferred followed by segments of the host genetic information
- How much of the host genome is transferred depends on how long the cells are in contact.
- There are many insertion sites so many Hfr strains are possible.

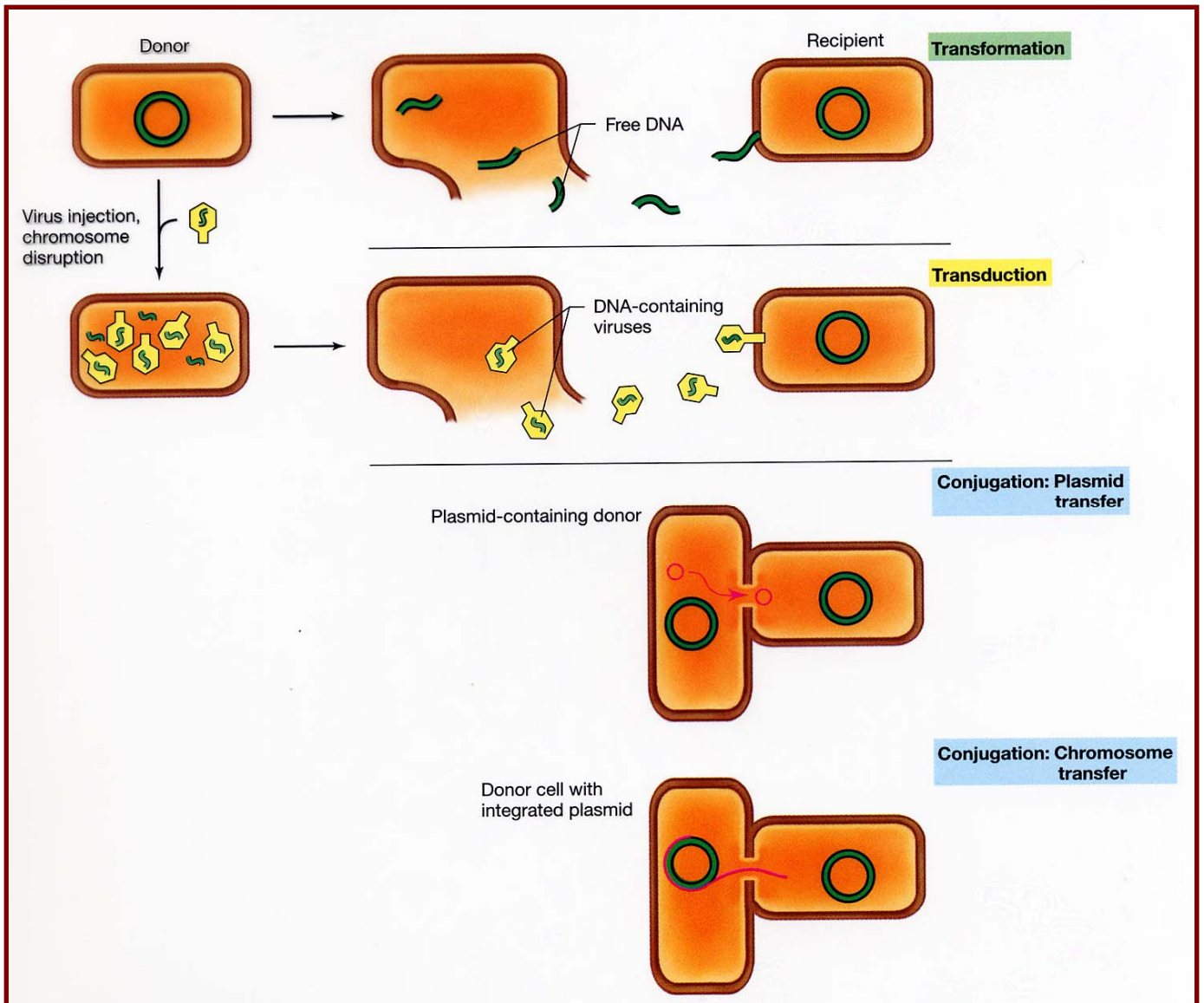
Insertion elements and Transposons

- These are regions of DNA that encode for the ability to move themselves to other regions of the DNA (hopping genes?). Both Insertion elements and Transposons encode for the enzyme Transposase which is required for them to move. Some of these elements may insert themselves only in certain regions of the DNA while others may insert themselves just about anywhere. Consequences.
- Insertion sequences carry no genetic information other than that necessary for the sequence to move to a new location.
- Transposons are larger than insertion sequences and carry other genes. In some case, Transposons may be 'composite transposons' which appear to be a blend of insertion sequences flanking additional genetic information.
- These genetic elements may move from one site to another on the same chromosome or to a plasmid or from plasmid to plasmid.



A quick summary of genetic recombination:

- Transformation
 - Transduction
 - Conjugation
-
- Insertion Sequences and Transposons are not really examples of recombination since 'foreign DNA' is not usually required.



Molecular Cloning (gene cloning)

(see pgs 307-311)

- The idea here is to take advantage of what we know about genetic system in the prokaryotes to manipulate the physiology of organisms and perhaps, have them do things that they normally would not do...the purpose would be
 - To gain better insights into how certain things function
 - To create bio-sensors of environmental conditions
 - For Biotechnology and the creation of useful products
- The approach is to move the desired gene from a large, complex genome to a small, simple one and then move it to the target organism
 - Basic system
 - Obtain source DNA
 - Move the DNA into a cloning vector
 - Cloning Vectors are independently replicating genetic elements ...plasmids or viruses
 - Introduce the cloned DNA into a host organism
 - Detect the DNA and reproduce it.

Cloning Vectors

- Plasmids
- Useful as vectors because they have
 - small size
 - their own origin of replication
 - multiple copy numbers (lots of signal)
 - selectable marker

Plasmid pBR322

- Identify the origin of replication
- Identify the markers
- Identify restriction sites (review restriction endonucleases)

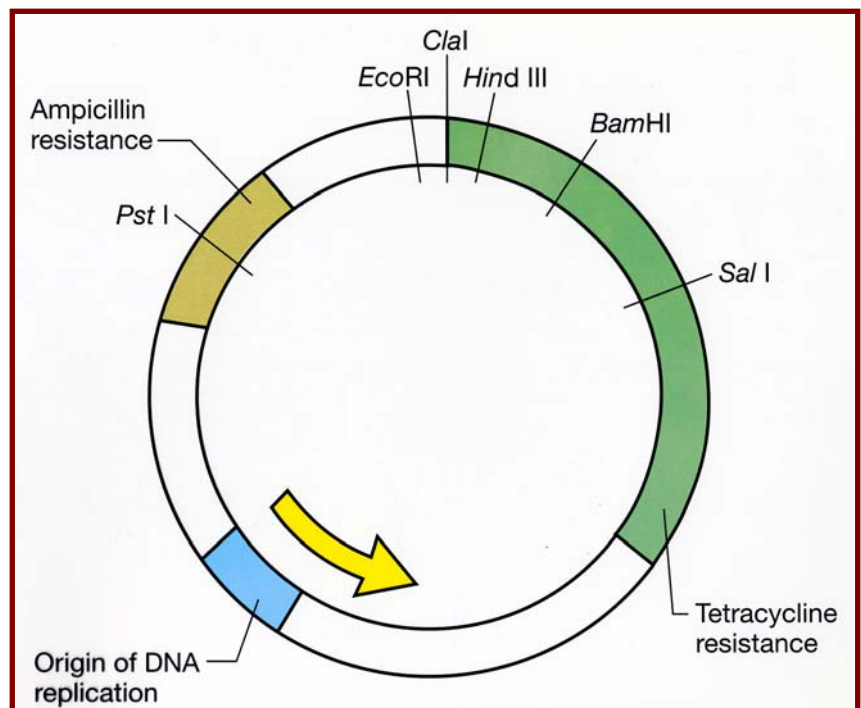
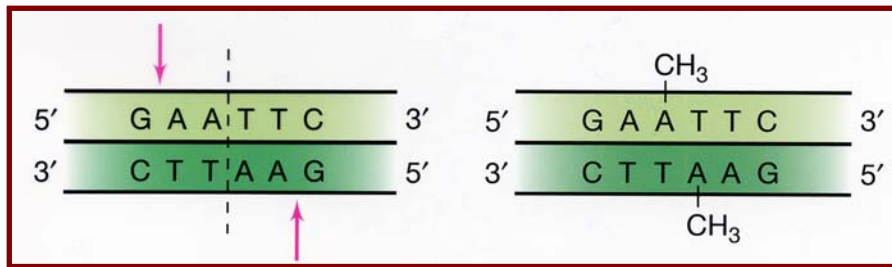


TABLE 10.4 Recognition sequences of a few restriction endonucleases

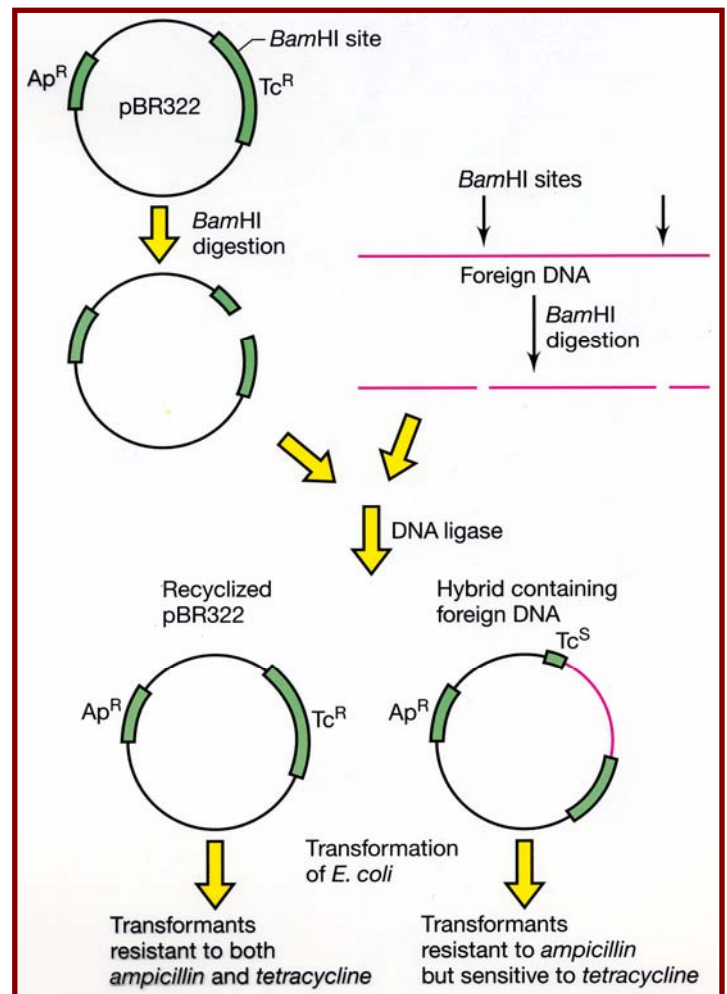
Organism	Enzyme designation	Recognition sequence ^a
<i>Bacillus subtilis</i>	BsuRI	GG↓*CC
<i>Brevibacterium albidum</i>	BalI	TGG↓*CCA
<i>Escherichia coli</i>	EcoRI	G↓AATTC
<i>Haemophilus haemolyticus</i>	HhaI	GCG↓C
<i>Haemophilus influenzae</i>	HindII	GTPy↓PuA*
<i>Haemophilus influenzae</i>	HindIII	A↓AGCTT
<i>Nocardia otitidis-caviarum</i>	NotI	GC↓GGCCGC
<i>Thermus aquaticus</i>	TaqI	T↓CGA*

^a Arrows indicate the sites of enzymatic attack. Asterisks indicate the site of methylation (modification). G, guanine; C, cytosine; A, adenine; T, thymine; Pu, any purine; Py, any pyrimidine. Only the 5' → 3' sequence is shown.



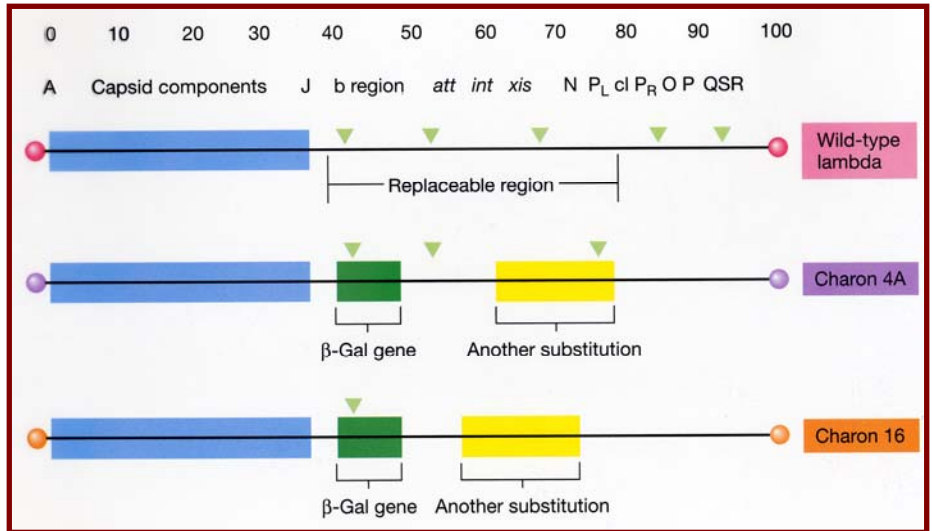
How might we use this plasmid

- Point out that insertion of the foreign DNA inactivates tetracycline resistance



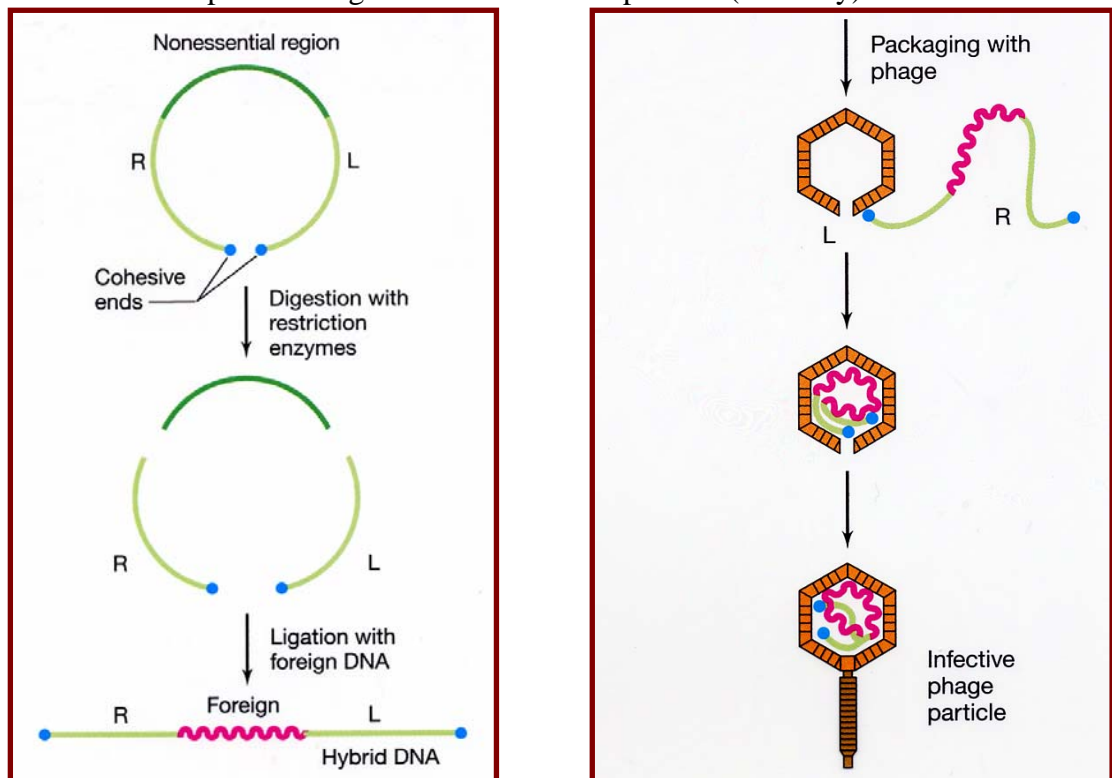
Viruses as cloning vectors

- Key here is a virus with a limited number of restriction sites for a given endonuclease...so you do not cut the viral DNA into lots of little pieces
- Point out how a piece of DNA may be *inserted* into the 'replaceable region of viral DNA' or how, with several restriction sites, DNA may be *replaced*



How is it done?

- Isolate vector DNA from virus
- Digest the DNA with a restriction endonuclease
- Add the foreign DNA and ligate the material
- Add cell extracts of head and tail proteins and all the formation of virus particles (spontaneous)
- Add to target organism and allow infection
- Check for the presence of recombination
 - Expression of the gene product
 - Develop something that attached to the product (antibody)



How is this useful? Practical applications of genetic engineering.
(see pages 981-992)

- Microbial fermentations
 - Increase yields, particularly useful for antibiotic production
- Vaccines
 - Virus vaccines may be an inactivated virus particle...since there is some room for error it may be better to isolate the gene coding for the virus capsid, express it another vector and then use the protein for a vaccine
- Mammalian proteins
 - Best example is the production of human insulin
- Transgenic plants and animals
 - Low fat cow
 - Plants that are resistant to certain herbicides (glyphosphate) or contain paracrystalline BT, flavr-savr tomatoe...the gene that causes pectin degradation is altered so the fruit may remain on the vine longer for a sweeter product, or harvested earlier because they will not spoil rapidly.
- Environmental biotechnology
 - Enzymes for detergents at high temperature
 - Reporter organisms
 - Physiological enhancement to destroy pollutants

TABLE 31.1 A few therapeutic products made by genetic engineering

Product	Function
Blood proteins	
Erythropoietin	Treats certain types of anemia
Factors VII, VIII, IX	Promote clotting
Tissue plasminogen activator	Dissolves clots
Urokinase	Blood clotting
Human hormones	
Epidermal growth factor	Wound healing
Follicle stimulating hormone	Treatment of reproductive disorders
Insulin	Treatment of diabetes
Nerve growth factor	Possible treatment of degenerative neurological disorders and stroke
Relaxin	Facilitates childbirth
Somatotropin (growth hormone)	Treatment of some types of growth failure and short stature
Immune modulators	
α -Interferon	Antiviral, antitumor, agent
β -Interferon	Treatment of multiple sclerosis
Colony stimulating factor	Treatment of infections and cancer
Interleukin-2	Treatment of certain cancers
Lysozyme	Anti-inflammatory
Tumor necrosis factor	Antitumor agent, potential treatment of arthritis
Replacement Enzymes	
β -glucocerebrosidase	Treatment of Gaucher disease, an inherited neurological disease
Vaccines	
Hepatitis B	Prevention of serum hepatitis
Lyme disease	Prevention of infection
Measles	Prevention of measles
Rabies	Prevention of rabies

All figures in this section are taken from Madigan et al. 2002

References:

Madigan, M. T., J.M. Martinko, and J. Parker. 2002. Brock Biology of Microorganisms 10th ed. Prentice Hall.