Recombination

Homologous recombination - requires extensive DNA homology, mediated by RecA protein. Will also need a lot of other proteins. Use w/ very similar DNA - if identical, no difference. 100's - 1000's of base pairs. Enz can revert process, or cut DNA at hybridization & leave crossovers.

Hybridization = crossing over (i.e., meiosis)

Straight DNA
Wiggly DNA

Straight w/ "wiggly" mutation → may be an entire gene
More DNA within chromosome,
Recomb. w/ new, foreign DNA
May get new DNA from plasmids

Site-specific recombination - recombination between specific short segments of DNA with only a short region of homology. Mediated by specific enzymes. Used by many bacteriophages for integration of their genome into the host chromosome.

Small seq. of DNA (10 base pairs) by specific enz for cleaving, inserting, etc.

Non-homologous ("illegitimate") recombination - no homology, unknown mechanism

Theory → DNA might come in close proximity w/ mutated, UNREPAIRED DNA.
Genetic regulation through DNA recombination:

* Good Advantage for att.

* Good Advantage for att.

E. coli: Synthesis of fimbriae (fibrils extended from the outer membrane, involved in attachment to eukaryotic cells).

Borrelia hermsii (causative agent of relapsing fever): Synthesis of different VMPs (variable membrane proteins) to escape recognition by antibodies.

* Many separate pieces of DNA; chromosomes are plasmid-like.

* Silent VMP: can encode VMP, but don't have promoter.

* We don't know how.

* Similar to Ig switching (IgM → IgA).

Try to make antibiotic for dormant phase → but ble ankle blocks rep, doesn't work.
Transformation

* Competence: the ability to take up naked DNA from the environment

![Diagram of transformation process]

Some species are naturally competent to take up extracellular DNA by transformation, including important pathogens such as *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*.

*S. pneumoniae* can take up any segment of DNA → not specific

**More specific** → highly efficient at taking up DNA

*Neisseria* and *Haemophilus* only take up DNA segments containing a specific sequence (DNA-uptake sequence or DUS). DUS are found frequently throughout the bacteria's genome, but rarely in other organisms. Both bacteria have specific receptors on their external surfaces that recognize DNA with the appropriate DUS.

Some bacteria (e.g. *S. pneumoniae*) develop competence in response to activating signals (quorum sensing). So, a little bit fussy in nature.

*Most DNA is stride. DNA ble large # of stride around*

Bacteria such as *E. coli* are not naturally competent to take up DNA, but can be induced to a state of artificial competence by chemical treatment.

*Not very efficient at taking up DNA.*

*Drawback to competence is that for unknown reason, many bacteri cells spontaneously lyst.*
What are the implications of natural competence in bacteria?

Spread of antibiotic resistance → significant problem
- Swap resistant DNA.

Increased genetic variation
- Broad pool of variants → difficult to kill all of them.

Increased virulence
- Better toxin, or prod. more toxin, or more resistant to MP.

Acquisition of beneficial genes
- Can live on new nutrient source → ie, new niche.

Increase in host range
- Was for horse → A & adapt to human.

* Neisseria meningitidis: m1, in throats of people (commensal) → diff. spp.
- DNA exA blw commensal bact. & N. meningitidis. → ie, virulence factor
* Can have shift blw Haemophilus to Neisseria → exA blw genera

Maintenance of integrity - DNA restriction systems
- ie: restriction endonucleases → make sure DNA they take up is DNA they want.

DNA methylase
- An enzyme which recognizes a specific DNA sequence,
  then modifies a base of that sequence by adding a methyl group.

Restriction endonuclease
- An enzyme which recognizes the same specific DNA sequence. → tag & says "self"

Does not cut the DNA if sequence is methylated → not digested.
(recognized as "self" DNA)

Cut the DNA if sequence is not methylated
(recognized as "non-self" DNA)

* It does make mistakes (ie, non-Mendelian recom.)
  - rare event
  - there are a lot of bact., so one lost is no big deal.
Plasmids, Bacteriophages and Transposons:

**Plasmids** (extrachromosomal DNAs, episomes)

- May be circular or linear
- *not req'd for host survival*

Generally _a metabolic drain on bacteria_.

- *making prod etc*.
- *if no benefit, will get lost (out-competition).*

**May confer selective advantage to bacteria by encoding:**

**Essential proteins**
- *bact need this prod*

**Antibiotic resistance** ("R-factors")

- Allows survival where other bacteria are killed

**Production of antibiotic**

- Kills off competing bacteria

**Specialized catabolic enzymes**

- Allows use of nutrients that cannot be digested by other bacteria

**Virulence determinants**

- Allows survival in hosts

* infectious w/ plasmid
Plasmid Host range

Determined by mechanism of plasmid replication

**Limited host range:**
Can replicate in only a single bacterial species or group of closely related bacteria. Use host DNA replication machinery.

**Broad host range:**
Can replicate in a wide variety of bacterial species. Generally encode own replication machinery

*Problem w/ spread of antibiotic resistance*

Some plasmids may integrate into the chromosome. Integrated plasmids may later be excised from the chromosome.

![Diagram of integration of F plasmid into bacterial chromosome](image)

*Figure 4-5. Integration of the F plasmid into a bacterial chromosome to form a high-frequency recombination (Hfr) chromosome, followed either by exact excision to re-form the F plasmid or by inexact excision to form an F' plasmid containing some bacterial chromosome genes.*
Conjugation: bact. "mating"

Plasmid transferred from donor ("male") cell into recipient ("female")

F+ cell

F- cell

F plasmid

Cell chromosome

Strand of F being introduced

Will repl & re-circularize

Complementary strand being synthesized

Conjugation bridge
Chromosomal DNA transfer

Conjugation of plasmid that contains an integrated chromosomal sequence (e.g. F')

Conjugation of integrated plasmid, carries along adjacent chromosomal sequences (e.g. Hfr)

*ble chromosome is involved, unlikely to lose plasmid.*
Bacteriophages (phages)

Bacterial viruses

Bacteriophages recognize appropriate hosts by interacting with a specific bacterial surface component (just as do human viruses).

**Lytic phage:** Produces multiple copies of phage particle, then lyses bacterial cell

**Lysogenic phage (temperate phage):** Replicates DNA along with host cell, does not kill cell

Integrand into chromosome

Episome (plasmid-like) repl outside of chromos.

The first step in the multiplication of a virus is its attachment to a host cell; more than one virus particle can simultaneously adsorb to a single cell.

Release of the viral chromosome. This is generally rare, may occur only once in 10,000 divisions of a lysogenic bacterium.

Lytic cycle (usually takes 15–60 min at 37°C)

Entrance of the viral chromosome into the host cell

Release of new virus particles by lysis of the host cell wall

The viral chromosomes are surrounded by newly synthesized protective coats.

Protection coat Viral chromosome

Multiplication of the viral chromosome

Lysogenic bacteria usually divide at the same rate as normal bacteria.

Prophage (inserted plasmid, etc.) may have potential to become active bac. phage.

Lytic cells

A inserts into e.coli

* bact turn on SOS sys. in response to DNA damage. A senses SOS, jump out, repl, lyse cell, SURVIVE.

* can’t get rid of it b/c w/in chromos.
Virulence properties are often carried by bacteriophages, including the genes encoding:

**Cholera toxin of Vibrio cholerae** if Vibrio is not infected, it can't infect us. Phage is specific for Vibrio (rec. & toxin)

**Shiga-like toxins of E. coli** (cause hemorrhagic colitis, infant diarrhea)

**Erythrogenic toxin of Group A Streptococci (GAS)**

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Transduction - exchange of DNA between bacteria through use of bacteriophage particles

Some bacteria utilize phage-like particles to exchange DNA, e.g. Serpulina hydysenteriae (agent of swine dysentery) and VSH-1. The bacteriophage co-opts bybaculovirus to become stable part of bacterial phage capsid. VSH-1 packages random fragments of chromosomal DNA into phage-like particles that are released into the environment.

VSH-1 particles inject their DNA into other S. hyodysenteriae bacteria. DNA recombines into chromosome by homologous recombination.

Many other bacteria possess similar gene transfer mechanisms.
**Transposable elements**: moves from one place to another on its own. Encodes its own enzyme, may or may not repl.

Important determinants in gene transfer and the spread of antibiotic resistance among and between species. Many transposons confer resistance & more indep. of host.

- Can go from an E. coli to a mycoplasma
  \* VERY FREQ.!!

Defined DNA segments that catalyze their own transfer from one site to another in the DNA of an organism. Not dependent upon homologous or site-specific recombination.

Features of bacterial transposable elements include:

1) **Defined ends with inverted terminal repeats**
   - discrete piece of DNA

2) **Encode an element-specific transposase** (enzyme that mediates transposition)

3) **Can cause mutations and mediate genetic rearrangements**
   - jumps around, integrate, etc.

4) **Generate a direct repeat in target DNA**
   - creates a mutation
Mechanism of transposition

i) replicates, both strands DNA, w/ blunt cut
ii) transposase cuts both strands DNA in staggered fashion
iii) transposon, blunt end, inserts in w/ transposase
iv) filled in w/ ligase \( \rightarrow \) get repeats
x created mutation blq repeats \( \rightarrow \) a reading frame, etc \( \Rightarrow \) gene is disrupted even if transposon comes out.

Transposase makes staggered cut

---ATGCA

---TACGT---

TE joins to the ends
Gaps filled by host enzyme

Staggered cut is reason why transposable elements are generally found flanked by directly repeated sequences
**Insertion elements**

Contain inverted terminal repeats and transposase gene(s)

Simplest transposable element

\[ \text{Recognize seq. as being } \square \text{ or } \square \text{ end of seq.} \]

---

**Transposons**

*Insertion seq.* + *other genes (enz, resistance)*

Contain inverted terminal repeats (may or may not be insertion sequences), transposase gene(s), and additional DNA sequences (may include genes encoding antibiotic resistance, etc.)
Bacteriophage Mu (both a phage and a transposon)

Mu forms infected phage particles.

Infects *E. coli*, injects DNA like any other phage

Can be lytic (replicate and lyse host cell)

Can be lysogenic

Lysogen integrates via transposition mechanism at random sites (not site-specific)
Conjugative transposons

Like "normal" transposons in that: sits still w/in one bacterium (moves w/in 1 bacterium) unless it gets help

- Have inverted repeats at both ends
- Encode enzymes needed for transposition $\rightarrow$ transposase
- Can carry antibiotic resistance genes $\rightarrow$ huge evolutionary niche
  - Even 2, 8, even 10

Different from "normal" transposons in that:

- Do not generate direct repeat upon integration $\rightarrow$ different form of integration $=$ straight cut
- Can transfer themselves between cells $\rightarrow$ conjugate themselves
- Utilize a different mechanism of transposition

Conjugative transposons are often sensitive to conditions within the bacterial cell

- Want variability, but not enough to kill host. $\times$ sensitive to bacteriophage
- Stresses to bacteria promote conjugation
  (bacterium is in trouble, I'm getting out of here!)
- **If** treat bacterium with antibiotic, promote spread of antibiotic resistance w/conjugative transposons.
  - SOS response $\rightarrow$ will try to go to as many bacteria as possible (up transposing).