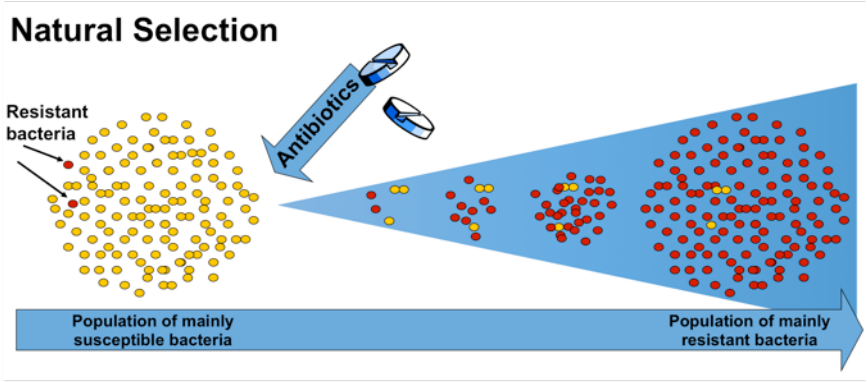
BACTERIAL GENETICS **Antibiotic resistance develops through natural selection:**



Resistant bacteria appear due to
mutation of existing gene(s)
OR
acquisition of genes
} **Bacterial Genetics**

Antibiotic targets:

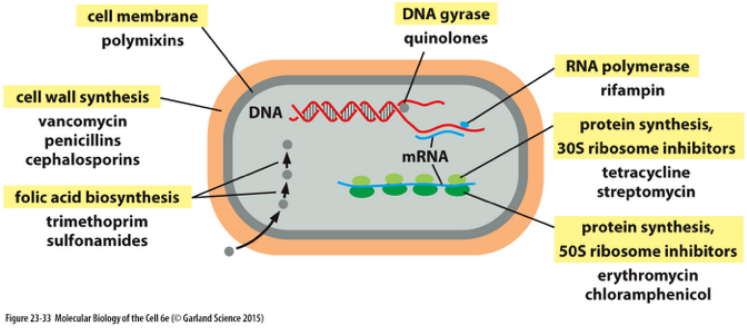


Figure 23-33 Molecular Biology of the Cell 6e (© Garland Science 2015)

Mechanism of resistance:

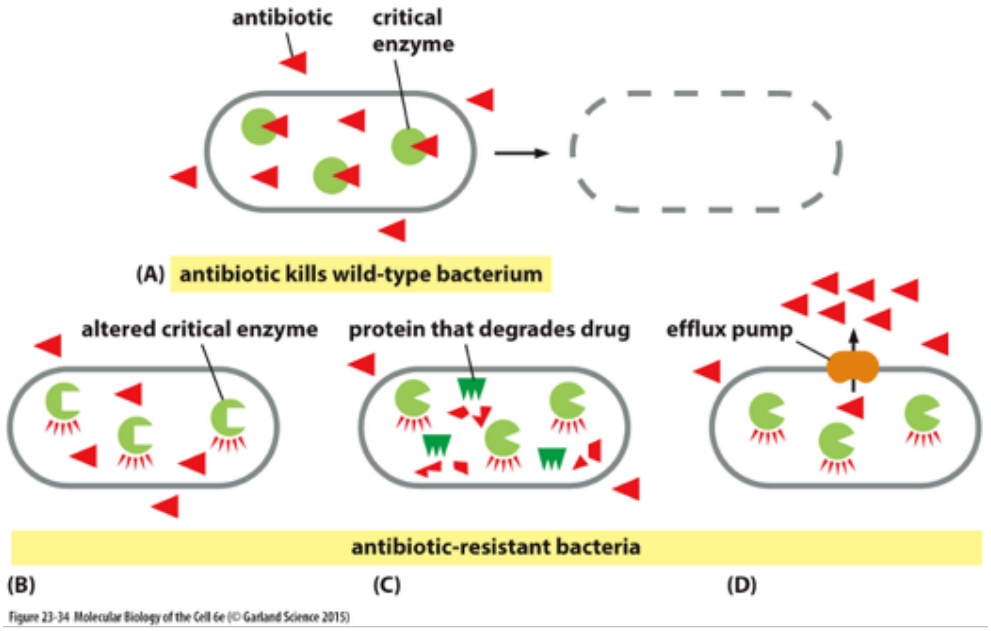
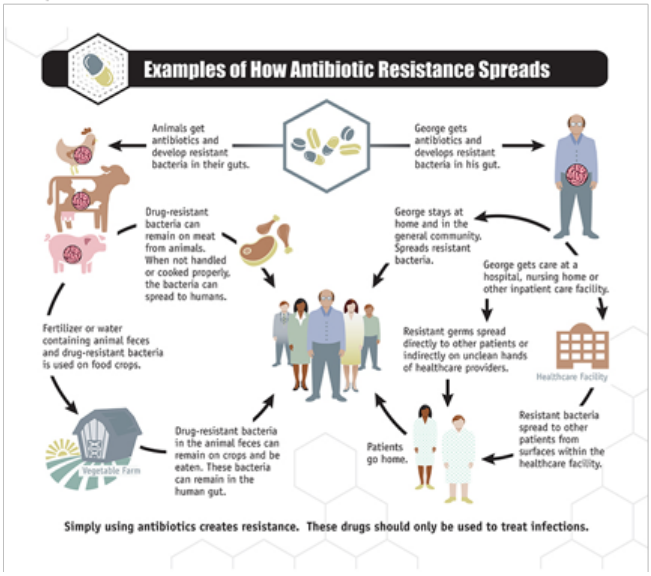


Figure 23-34 Molecular Biology of the Cell 6e (© Garland Science 2015)

Each mechanism of resistance is **genetically encoded**

1. Spread of **antibiotic resistant bacteria** through communities



Food for thought

How to prevent development of resistance?

How to prevent this type of spread?

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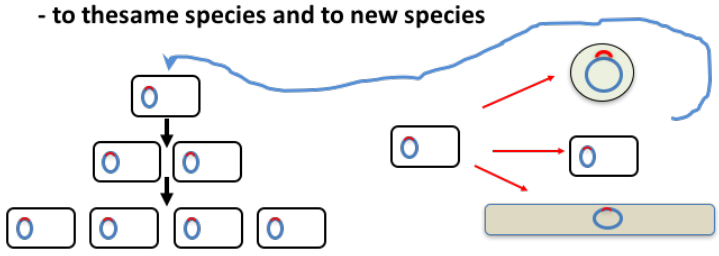
2. Spread of **antibiotic resistant genes** to other bacteria
- to thesame species and to new species



vertical gene transfer

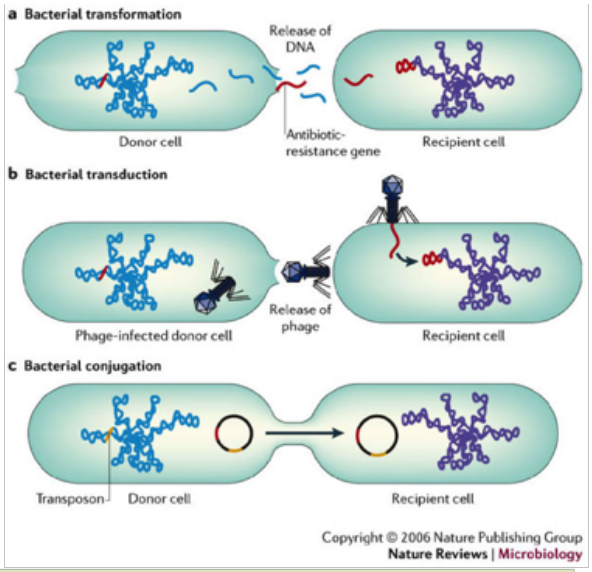
(parent to daughter cells)

horizontal gene transfer

(to a new strain of same species; or to a different species)
e.g. *Escherichia coli to Klebsiella pneumoniae*

11

Horizontal Gene Transfer



Transformation

Transduction

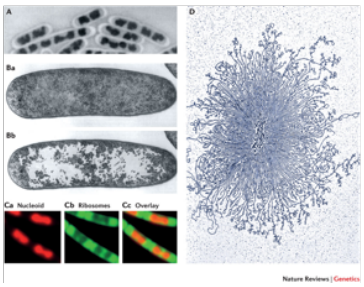
Conjugation

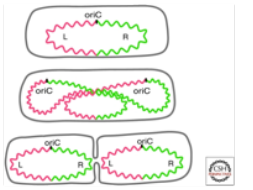
Important! - Allows bacteria to acquire new traits, rather than simply passing on existing traits

Horizontal gene transfer also termed lateral gene transfer

Bacteria have chromosomes

- (Usually) a single dsDNA circular chromosome, plus accessory plasmids which replicate autonomously
- Chromosome is highly organised and segregated
  - 1.3 mm of DNA packaged into 2μm cell
  - Genes always located in same place in daughter cells





E. coli chromosome segregation

- In bacteria, observational approaches were not possible (eye-colour, wing shape, shape of a pea, etc)
- **However: One gene - One enzyme Hypothesis** (Beadle and Tatum, 1941) provided link between bacterial physiology and genetics

- **Irradiated *Neurospora crassa (a fungus)***
  - *Isolated* ***mutants*** unable to synthesise particular nutrient or vitamin (so defective in a synthetic step)
    - **Biosynthetic Pathway of Arginine:**

precursor $\xrightarrow{\text{enzyme X}}$ ornithine $\xrightarrow{\text{enzyme Y}}$ citrulline $\xrightarrow{\text{enzyme Z}}$ arginine

  - **Such mutants were termed 'Auxotrophs'**
  - **Prototrophs** are wild-type for that characteristic

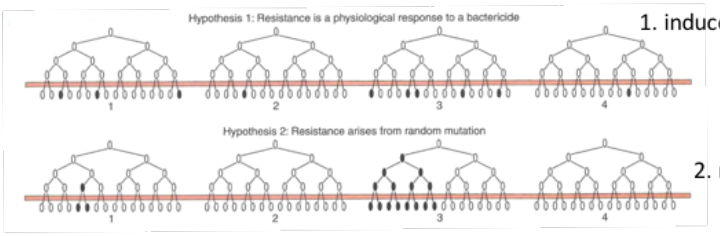
Conclusion:

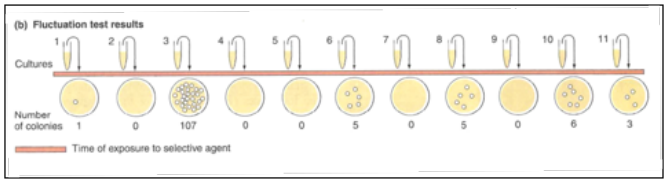
- genes produce enzymes
- produced linear progression for biochemical pathways
- pathway conservation was good phenotype to monitor
- but segregation must be discrete
  - (i.e. must follow **one gene = one enzyme hypothesis**)

- **How is genetic variability introduced and inherited in bacteria?**
- **Most organisms** followed 'Darwinian' Rules:
  - Genome changes occur randomly/spontaneously
  - Changes not driven by the environment
  - If changes are beneficial for survival in an environment, then more likely to be passed on to progeny
- **Bacteria** were thought to follow "directed" change / "adaptive-mutation" hypothesis - "Lamarckism"
  - Mutations induced by selection
  - Adaptation of organism to environment

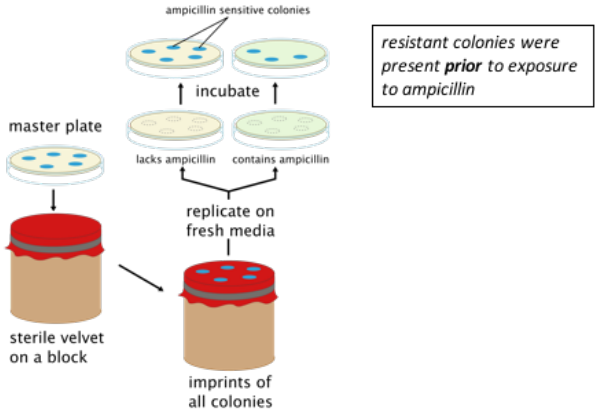
- **Salvador Luria and Max Delbruck (1943)**
  ***E. coli* resistance to bacteriophage T1**
  Bacteria can be grown on an agar plate to form a **lawn**
  If phage are present they will kill bacteria resulting in a pl**aque**
  Observation: shortly after the majority of bacteria are killed by phage (seen as a plaque), phage-resistant colonies will appear.
  Question:
  Are these mutations induced by the phage?
  Or are they already present in the population?
  A fundamental question in genetics

- Grew a liquid culture of *E. coli*
- Aliquoted into a series of tubes and grew again creating identical independent sub-cultures to be monitored **separately**
- Each sub-culture was plated on agar with phage and resistance to phage counted
  Now:
  If mutation was the result of exposure to the phage,
  (induced mutation; **A**) frequency of resistance in each culture should be similar
  If mutation was spontaneous, **B**, the frequency of mutation should be very variable.





Luria and Delbruck proposed that their results could be explained by the occurrence of a **constant rate of random mutations** in each generation of bacteria growing in the initial culture tubes.
Resistance arises from random mutation.



*resistant colonies were present **prior** to exposure to ampicillin*

- **Joshua and Esther Lederberg (1952)**
  - More direct proof of Darwinian Inheritance
  - Conclusively showed random variation occurred and not induction due to selective pressure

BACTERIAL TRANSFORMATION

Transformation - *isolated* DNA molecules are taken up from external surroundings and incorporated into the genome

- Process is solely encoded by the recipient bacteria (unlike transduction and conjugation)
- All required proteins encoded within recipient core genome
- Most transformable bacteria do not permanently express the proteins required for transformation
- Bacteria are described as **competent** when they are able to undergo transformation
- Only a fraction of the bacteria in a population become competent

First species to be discovered as transformable*:
Streptococcus pneumoniae Gram +ve (Griffiths)

- approx. 80 species now known to be transformable *
- both Gram +ve and Gram –ve species

Some Naturally transformable species

- *Bacillus subtilis* (Gram +ve)
- *Streptococcus pyogenes (Gm +ve)*
- *Neiserria gonorrheoa (Gm –ve)*
- *Haemophilus influenza (Gm–ve)*

WHY BE TRANSFORMABLE ?

- **Nutrition:**
  - Nucleotide source?
  - Some bacteria become competent at stationary phase
  - Inefficient, more efficient to catalyse complete DNA breakdown in extracellular environment
  - Use normal import channels to recover the nutrients
- **Genome maintenance/repair:**
  - Uptake of potentially homologous DNA allows damage repair?
  - Consistent with "self" DNA uptake requirement
  - Some evidence that competence systems are induced by DNA damage
- **Genome diversification:**
  - DNA uptake increases genetic diversity
  - Increasing diversity during stress (e.g. starvation) maximises likelihood of survivors
- **Disadvantages of DNA uptake?**
  - energy cost
  - new genes could be potentially harmful

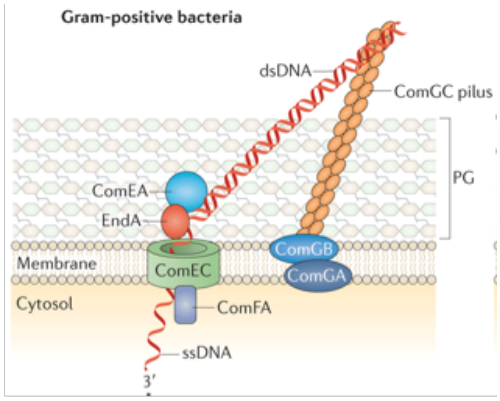
MECHANISMS OF TRANSFORMATION:

1. Bacteria develop "competence"
2. Cells bind double stranded DNA in the environment
3. Movement of DNA over cell membrane / cell wall
4. Conversion to single stranded DNA
5. Intracellular fate:
   - maintained in the genome as a plasmid **or**
   - homologous recombination into genome **or**
   - degradation

1. Bacteria develop competence naturally

- usually as a culture encounters **stress**
  e.g. nutrient limitation
- cells coordinately express a new set of genes,
  i.e. *a **regulon** is activated*
- cells synthesise new proteins
- construct a protein structure on the cell wall,
  the "**Com pilus**", and other surface proteins
- **Com pilus** is related to another structure found on bacteria - **type 4 pili (T4P)**

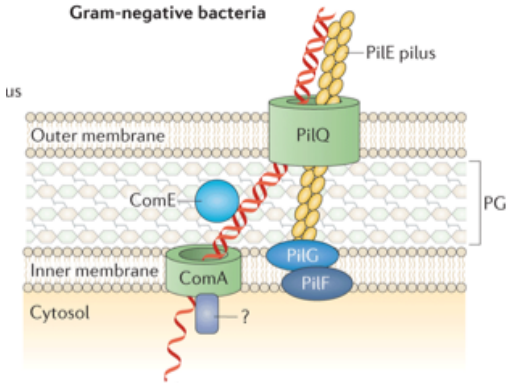
- **DNA binding and uptake: Gm +ve species**
  Capture of exogenous DNA by the transformation pilus* (ComGC pilus).
  Binding of dsDNA by the DNA binding protein ComEA.
  Recognition of the dsDNA by the nuclease EndA and **Conversion to ss DNA**
  Transport of the **ss DNA** strand by ComEC, driven by the ATP-dependent translocase ComFA

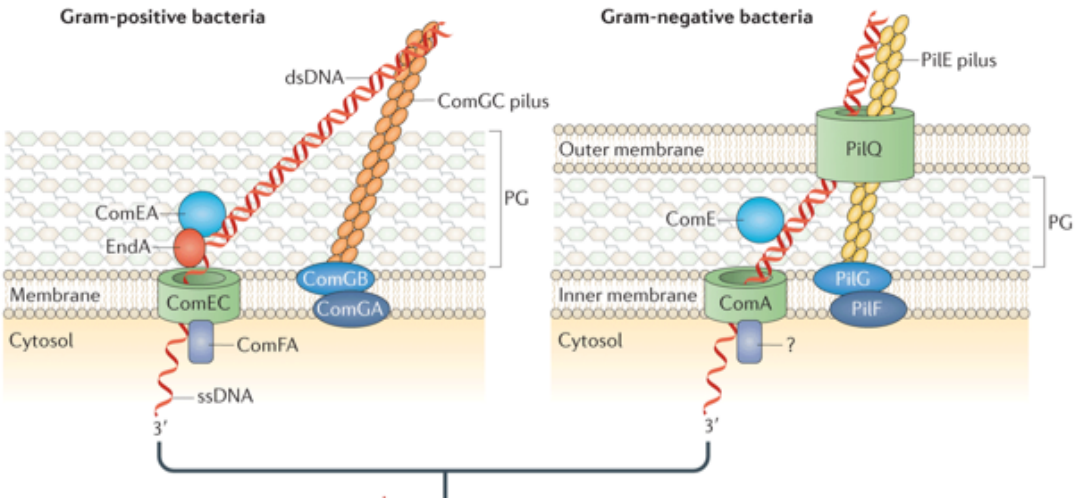


- this pilus has several names;
  - it is also known as a **type 4 pilus (Tfp, T4P)** or type 4 pseudo pilus

- **DNA binding and uptake: Gm -ve species**

PilQ secretome channel enables pilus to cross outer membrane, bind dsDNA (specific sequences) and transport it into periplasm.
Binding of dsDNA by the DNA binding protein (ComE).
Transport of the DNA strand across inner membrane by ComA (ComFA?)
Endonuclease probably exists but not found yet

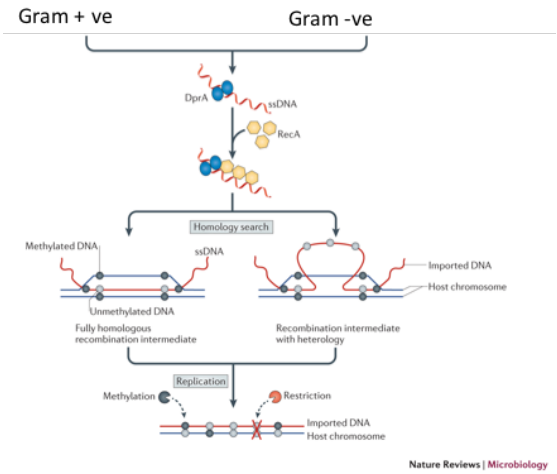




HOMOLOGOUS RECOMBINATION

1. Internalised ssDNA recruits ***RecA protein**
2. **RecA** polymerises on ssDNA
3. **RecA** promotes a homology search along chromosomal DNA

- **RecA protein** is required for recombination and DNA repair in bacteria

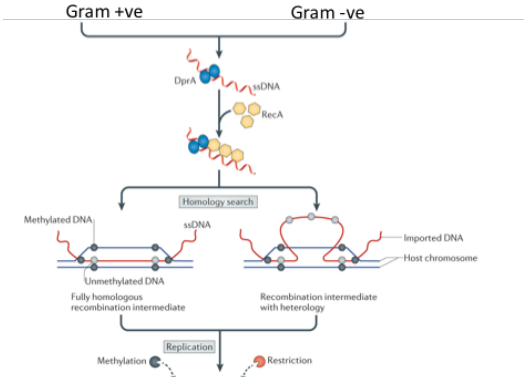


4. Strand exchange

a. Homologous

b. Heterologous

5. Replication (restriction/modification system competes for access of unmethylated DNA)



NATURAL TRANSFORMATION - SUMMARY

- Complex process that requires dedicated DNA uptake and integration systems
- Ancient process; but only a fraction of bacteria now appear capable of natural transformation
- Involved in **creating genetic diversity**, repairing DNA and potentially a nutrient source.
  - genetic diversity includes **addition of new genes** (e.g. AMR), **deletion of genes** and **replacement of genetic alleles.**
- A highly regulated process
  - Mechanism can differ between bacterial species
  - Normally system is "off"
  - Environmental and cellular cues required for induction

ARTIFICIAL TRANSFORMATION

- Many bacterial species can be transformed artificially, e.g. *E. coli, Salmonella, Staphylococcus*
- Uses **circular double-stranded DNA**
  (natural transformation prefers ds linear DNA)
- **Artificial transformation:**

- is relatively inefficient

- is a key tool in gene cloning (molecular biology, synthetic biology)

Electroporation:
- electric field (10-20 kV/cm) changes membrane permeability properties
  dsDNA then enters cell
  bacterial membrane repair restores natural permeability

Chemical transformation:
- $CaCl_2$ treatment of cells (usually *E. coli*) at 4°C
- other divalent cations used include Mg^{2+}, Mn^{2+}, Rb^{2+}
- causes membrane permeability change - allow plasmid entry

1. **Make bacteria competent,** e.g. with $CaCl_2$
   Add DNA and incubate on ice for 30 min
   - DNA can bind to the bacterial surface
2. **Heat shock the bacteria/DNA mixture** (42^oC ≈30 sec)
   - changes membrane permeability
   - allows uptake of DNA
3. **Put on ice** for 30 mins
   - restores normal bacterial membrane properties
4. **Add rich broth to cells and incubate** at 37^oC ≈1 hour
   - allows expression of selective marker e.g. Amp
5. **Plate on selection/screening medium**
   - To visualise transformants

ELECTROPORATION - replaces Steps 1, 2 3 with High voltage

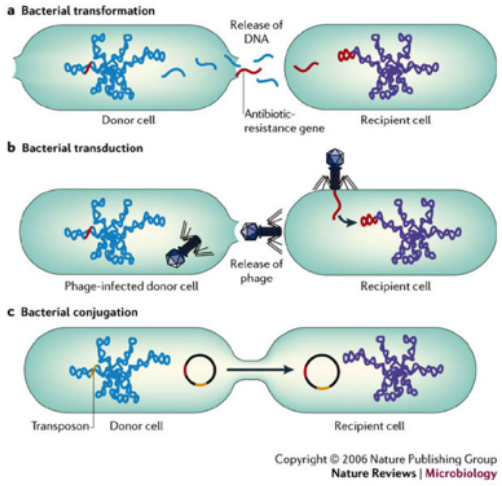
- **Genetic background of transformed bacteria**
  - Restriction system – why?
- **Preparation of artificially competent bacteria**
  - Manipulations that alter membrane permeability
- **Quality of the transforming DNA**
  - Ion chelators, salt contaminants, concentration, damaged DNA
- **Size of the transforming DNA**
  - Large plasmids are taken up less efficiently
- **Artificial transformation is still fairly inefficient.**
  - e.g. 10^8 transformants per mg DNA;
  - most bacteria in a sample are NOT transformed, e.g. 1 in 1000 are transformed

  Selection\screen is required to **identify transformants** from **non-transformants**

- **SELECTION**
  Most bacteria will not be transformed, so you must **select** for tranformants
  - usually an antibiotic resistant gene is encode in a plasmid vector.
- **SCREENING**
  Transformants will contain either **vector alone** OR **vector plus insert**
  - how will you tell the difference?

CONJUGATION

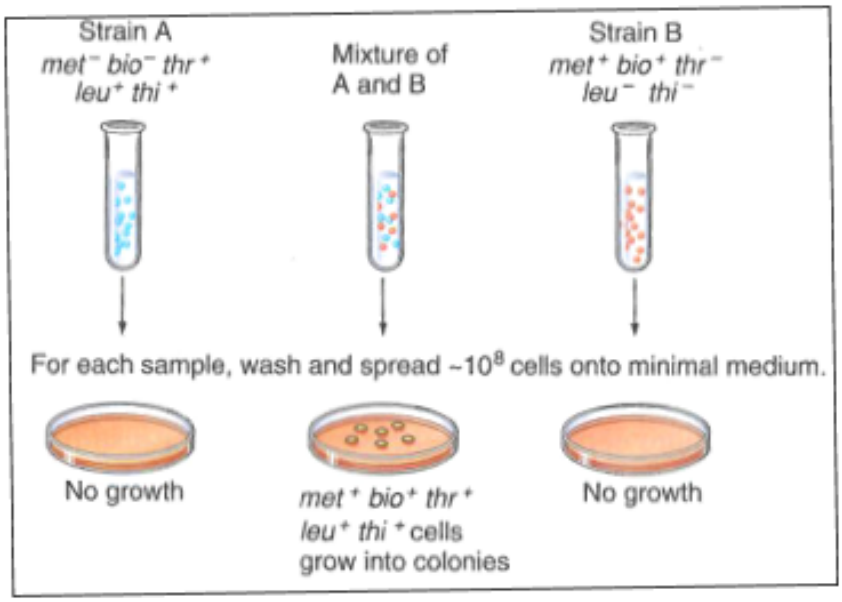
- Contrasts with vertical gene transfer (.i.e. inherited)
- Allows bacteria to gain genetic diversity (entirely new traits rather than altering existing ones)
- Three mechanisms:
  - (Natural) Transformation
  - Transduction
  - Conjugation



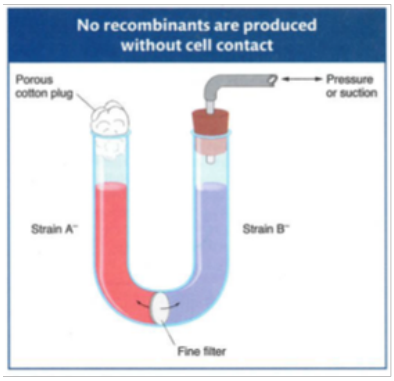
- DEMONSTRATION OF BACTERIAL MATING

Used 2 strains, each containing auxotrophic mutants
Q: can mutant assortment occur by co-incubation of these strains?

Basic mating strategy: A^+B^- x A^-B^+ ® A^+B^+,
(A and B = different amino acid requirements)



- Therefore either (a) exchange of genetic material (mating)
  or (b) cross-feeding was occurring



Bernard Davis:
conjugation required
cell-to-cell contact

Filter allows media to mix but prevents contact between donor and recipient cells

Filter absent: mating
Filter present: no mating

cell to cell contact is required

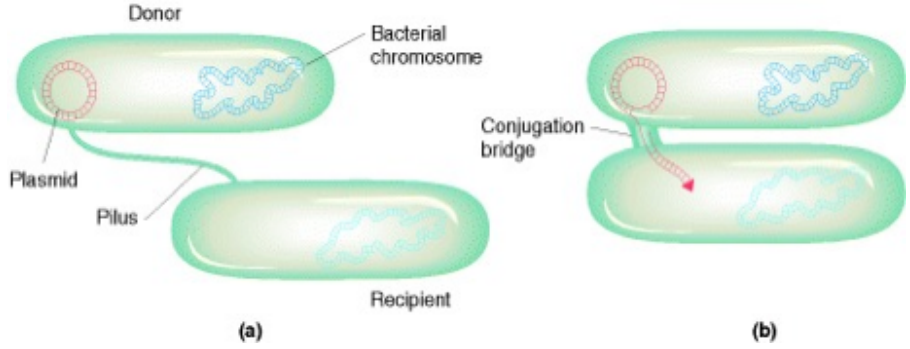
- GENERAL CHARACTERISTICS OF CONJUGATION

DNA transfer from one cell to another by means of cell-to-cell contact
Occurs mostly in Gram –ve bacteria
Some Gram +ve species: (Streptomyces, Streptococcus, Clostridia)
Prevalence suggests advantageous to the species
May be more prevalent than was thought previously

Also :
Usually transfers plasmids, rather than chromosome
DNA transfer is unidirectional: donor to recipient (William Hayes, 1953)

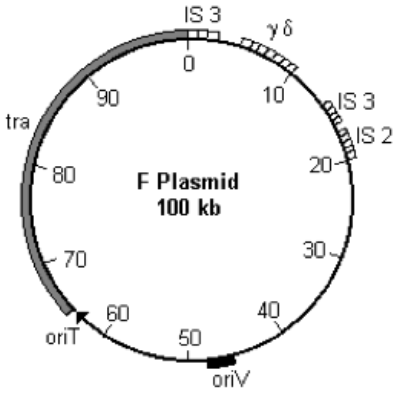
THE F FACTOR

- **Strains that were found to transfer genes by conjugation were designated as possessing a 'Fertility' factor (F)**
- **F factor:**
  - Conferred ability to donate DNA
  - Can be lost and regained easily
  - Strains carrying F are **donors** and designated **F** $^{+}$
  - Strains lacking F are **recipients** and designated **F** $^{-}$
  - F is a **conjugative plasmid** which encodes the machinery for conjugation



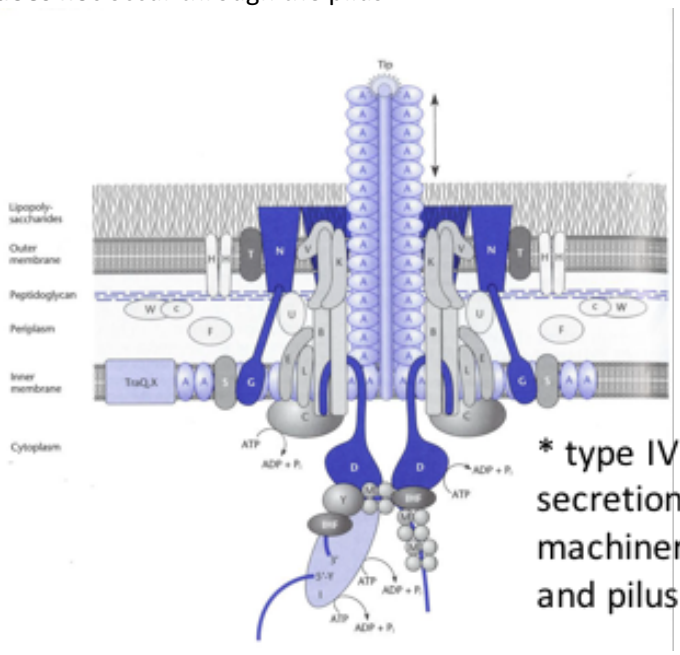
- F Plasmid contains genes that encode for:

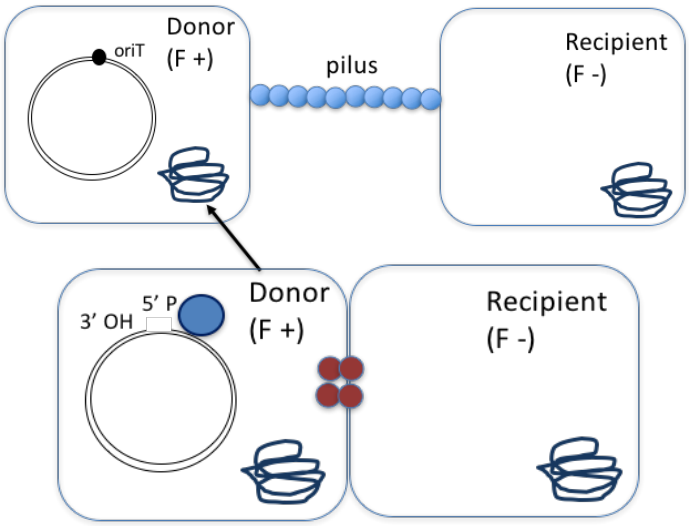
  a type 4 secretion system, a nanomachine, that synthesises the pilus (F pilus)
  "surface exclusion"
  stabilisation of mating pairs
  DNA transfer
  regulation



IS 3 & IS 2 = insertion sequences
γδ = transposon Tn1000
oriV = origin of replication
oriT = origin of conjugal transfer
tra = tra functions

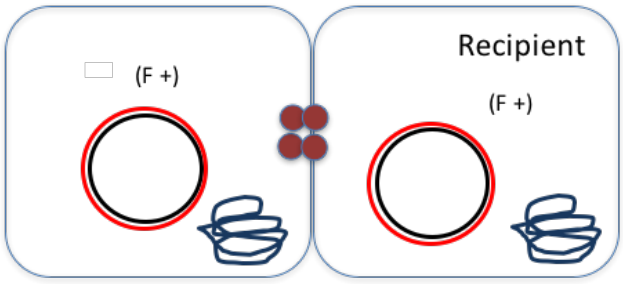
F pilus is synthesised by the donor cell
by the type IV secretion machinery
Once attached to a recipient, the cells come together.
DNA transfer then occurs.
DNA transfer **does not** occur through the pilus



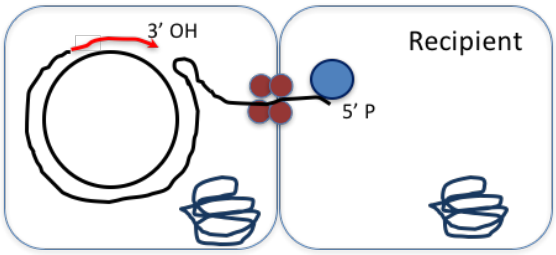


- pilus retraction, cells are brought together
- Tra proteins form a pore complex - "mating bridge" - between two cells
- DNA relaxase nicks one strand of F plasmid at oriT site and attaches to the 5'P

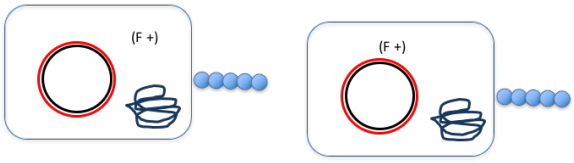
= DNA relaxase = Tra pore complex



- re-synthesis of F plasmid is completed in donor cell
- DNA transfer completed and ssDNA converted to ds circular DNA in recipient cell



- relaxase/ ssDNA migrates to Tra pore complex and transfers to recipient cell
- donor strand is resynthesised by DNA polymerase



- Cells separate
- Both cells now have F plasmid and can make F pilus
- Both can act as donors to new recipient cells
- Neither cell can be a recipient because of **surface exclusion**

- WHICH CELLS CAN BE RECIPIENT ?

The host range is defined by several factors:

- [1] Recognition of recipient by donor
- [2] Successful completion of DNA transfer
- [3] Replication of transferred plasmid

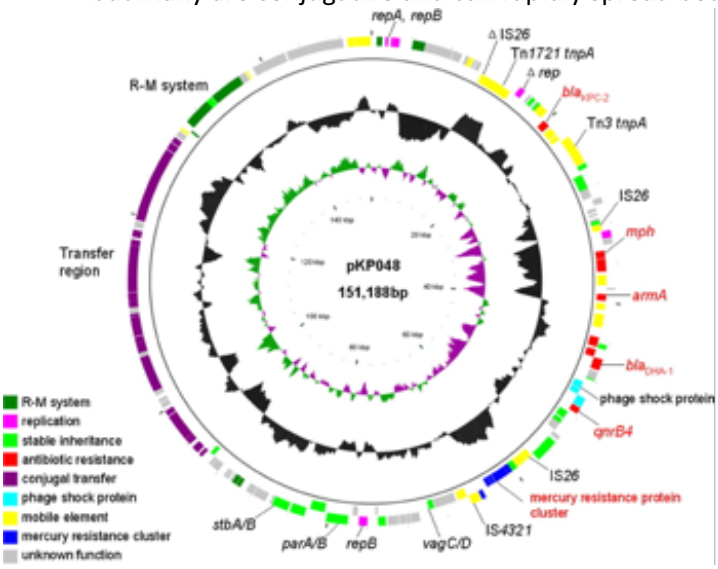
Plasmid	Conjugation host range [1]	Transfer host range [2]	Replication host range [3]
F	Narrow (Gram - ve enterics	broad	narrow
RP4	Broad (Gram -ve and Gram +ve)	broad	broad

- **Host range of some conjugal plasmids is very broad:**
  - Includes transfer to:
    - bacteria, yeast, plant cells, and mammalian cells
  - However, plasmid replication is often limited

R FACTORS:

Resistance (R) factor:

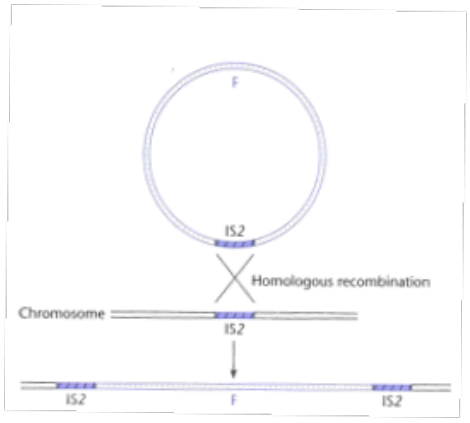
- Plasmids that **encode multiple antibiotic resistances**
- originally described in Shigella in the 1950s
- not necessarily conjugative plasmids
- but many are conjugative and can rapidly spread between diverse bacterial species



This plasmid (pKP048; 151 kb) specifies resistance to:
- carbapenems
- cephalosporins
- fluoroquinolones
- aminoglycosides

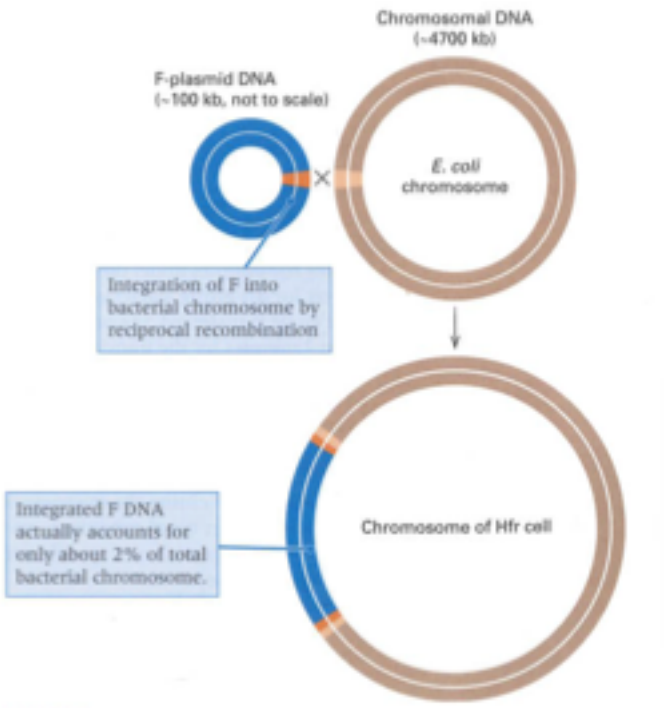
HFR STRAINS

- In some *E. coli* strains, the **F plasmid can be integrated into the chromosome**
- Occurs due to **homologous recombination** between insertional sequences (IS) (see L37) on plasmid and chromosome
- Multiple IS sites present throughout bacterial genomes



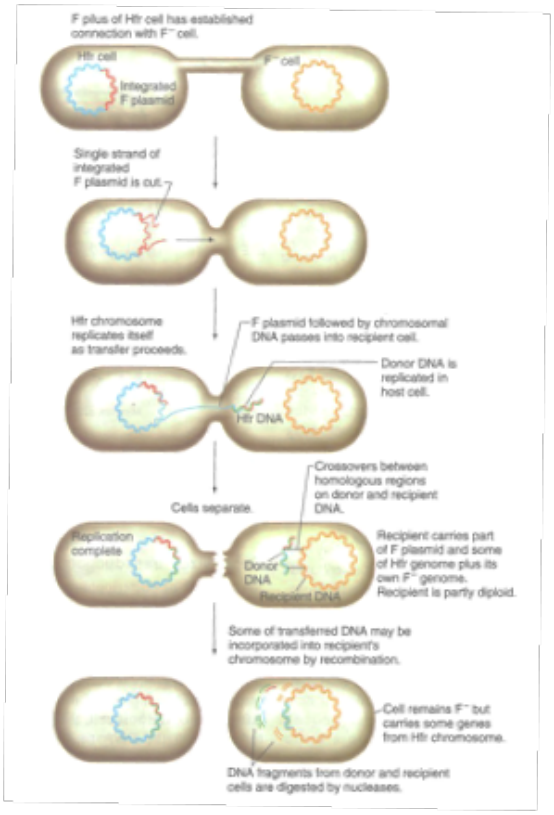
- **F-plasmid integration:**
  - can still initiate conjugation
  - can result in bacterial chromosome mobilisation

  (transfer of chromosomal genes to the recipient cell)
  - initiated at oriT site
  - rolling circle replication
- **Transfer machinery:**
  - still operates; does not distinguish between F plasmid alone or F plasmid integrated within bacterial chromosome
- **Integrated F-plasmid and chromosomal genes are transferred**

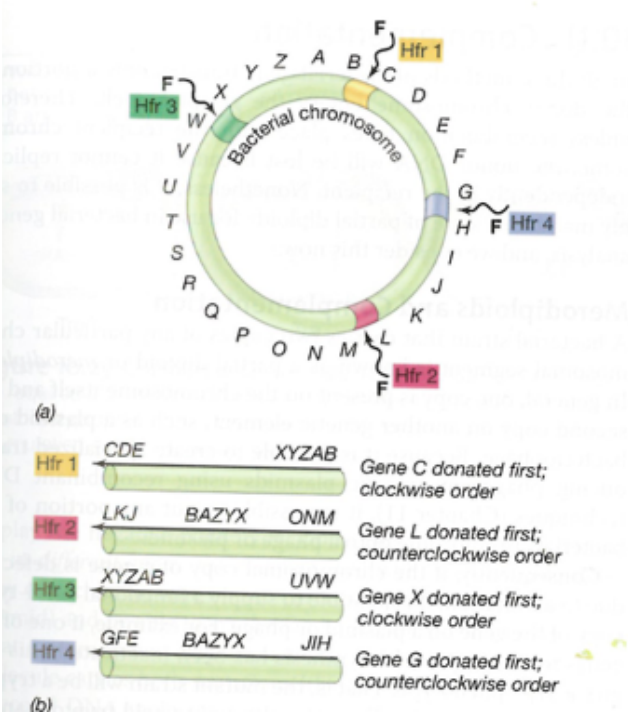


HFR CONJUGATION

- **Conjugation and Gene transfer between Hfr donor and F- recipients**
- just like described earlier with F plasmid
- but with bacterial chromosome section included
- recombination between chromosomal genes may occur if homology present
- the DNA strand transferred is very long and often breaks; the F plasmid would be the last piece transferred, therefore recipients do not become F+

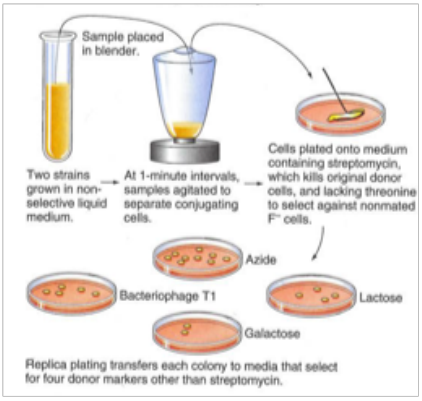


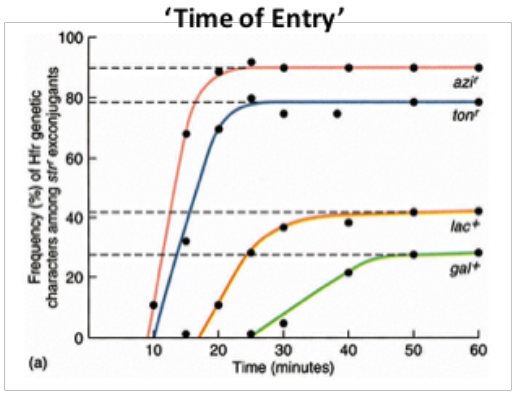
- **Linear transport of Hfr DNA**
  - directional
  - can use this to map gene order
  - based on transfer frequency to recipient
  - 'Time of Entry Mapping' by 'Interrupted Mating' experiments
- **Genes proximal and downstream of 'origin of transfer'**
  - more likely to be transferred.
  - transfer of proximal locus: ("a") occurs more frequently relative to distal loci ("b,c,d,…").



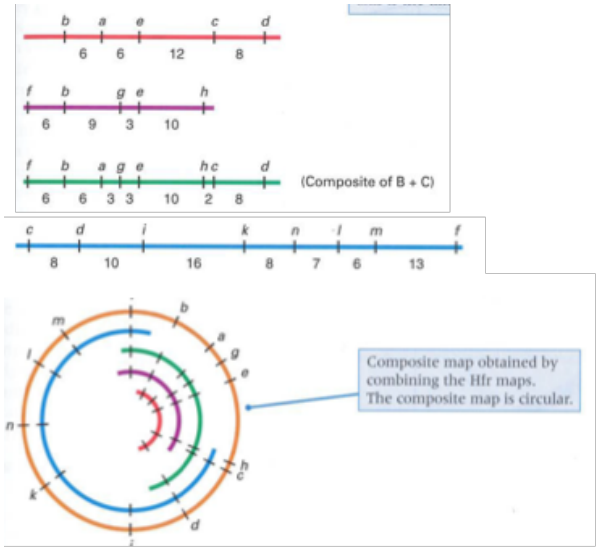
- **INTERRUPTED MATING EXPERIMENTS (SEX IN A BLENDER)**

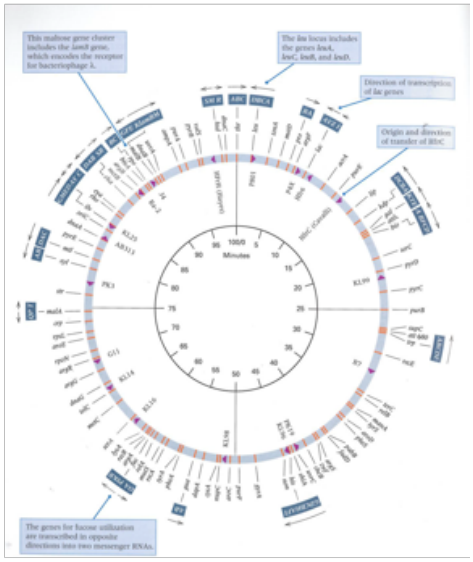
  - Hfr (ton^r gal$^+$ azi^r lac$^+$ str^S) and F- (ton^S gal$^-$ azi^S lac$^-$ str^R)





WHAT IS THE ORDER OF THE ABOVE GENES ON THE CHROMOSOME?





This was "state of the art" until 1980s
In 2000s, we sequence the entire genome

ISOLATION OF F'

- Remember : F plasmid can both integrate and excise from the genome **(a) (b)**
- Improper excision of integrated F can occur, resulting in F' (F prime)
- F' usually contains chromosomal genes
  (via **illegitimate recombination**) **(c) (d)**

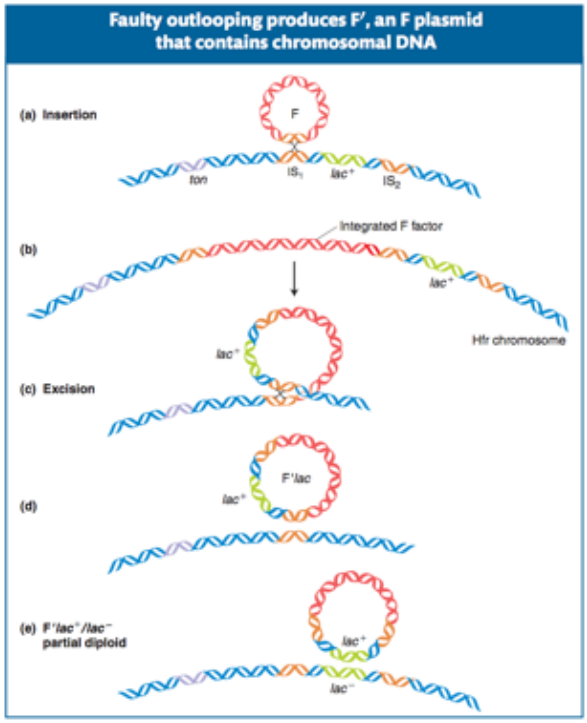
These F' plasmids:

- behave like F-plasmid (i.e. autonomously replicating, conjugation-competent)
- can incorporate host genomic fragments (replacing some plasmid sequences)
- are stable plasmid, with integration fairly rare because of the altered IS sites.

In F' x F$^-$ cross, F' remains F' and F$^-$ becomes F'

- recipient bacterium now known as a merodiploid or **partial diploid (e)**
- because of presence of 2 copies of a particular gene (chromosomal and F')

- **Hfr conjugation**
  - Hfr strain formed via F+ integration within bacterial chromosome
  - can transfer host chromosome
  - Transfer originates at *oriT* of inserted F plasmid
  - Transfer of entire chromosome is very rare (requires 100 minutes)
  - The acceptor strain remains F-
  - Was very useful in establishing a genetic map of *E. coli* genome (interrupted mating)

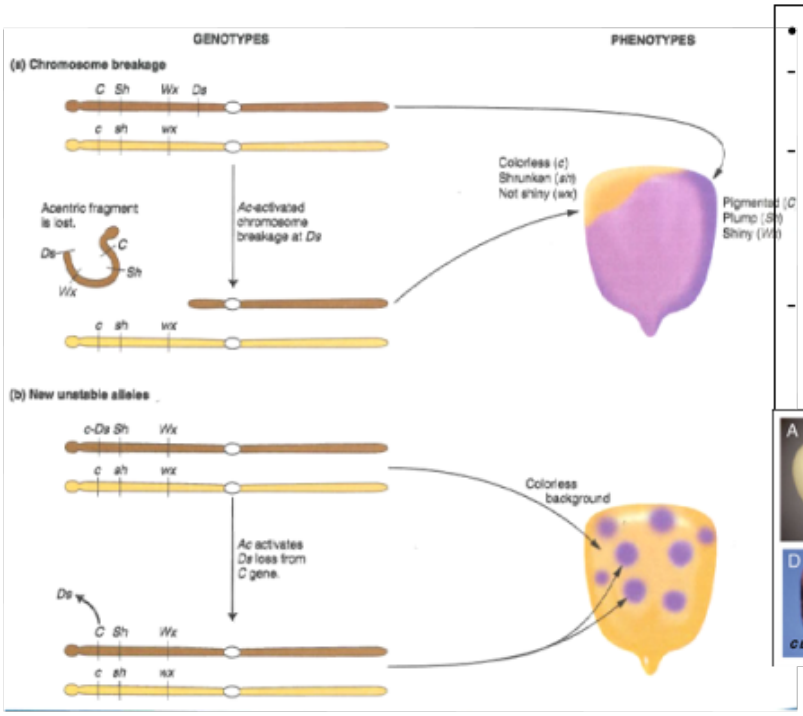


- **F' strains**
  - arise, via illegitimate recombination, from excision of integrated F plasmid
  - flanking host sequences are included in the resulting F' plasmid
  - can replicate and transfer DNA just like F+
  - Transferred DNA is a plasmid in recipient, but carries a portion of the donor genome
  - **F' x F - = F' and F'**

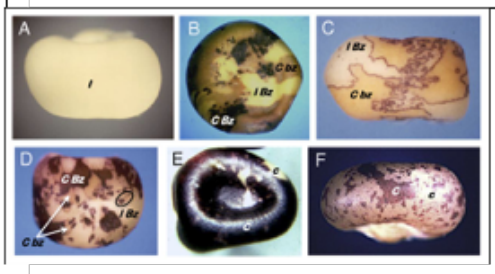
TRANSPOSITION

- **Barbara McClintock:**
  - colour variation in maize (Zea mays) 1940s
  - dogma was that genes were stable
  - she used staining and microscopy to track chromosomes
  - 9th chromosome regularly suffered breakages at the same position

- **Genetic basis**
  - '**Activator** (Ac)' and '**Dissociator** (Ds)'
  - **Ds** was unstable region on chromosome 9
  - **Ds could change position**
  - Ds breakage required a second element Ac (also mobile)



- **Pigmentation:**
- partially controlled by genes in chromosome 9
- chromosome 9 breakage at **Ds** resulted in altered pigmentation due to loss of these alleles
- **Ds** can also insert (unstably) at multiple locations resulting in pigment variation



Phenotypes of kernels that led to McClintock's discovery of chromosome breakage at *Ds*. *I*: dominant inhibitory allele of the *C* gene; *C*: full anthocyanin pigmentation when together with the wild-type *Bz* allele of the *Bronze* gene; *bz*: recessive allele of the *Bronze* gene, bronze pigmentation with *C*. The chromosome constitution of the kernels shown in *A–D* is *I Bz/C bz* (neglecting endosperm triploidy). (*A*) Colorless *I Bz/C bz* phenotype. (*B*) Random breakage of chromosome 9 results in loss of the *I* allele to reveal fully pigmented *C Bz* sectors, followed by loss of the *Bz* allele to reveal the bronze-colored *C bz* phenotype. (*C*) Chromosome breakage at a *Ds* transposon located proximal to both the *C* and *Bz* loci results in simultaneous loss of both the *I* and *Bz* alleles, giving only the colorless and bronze phenotypes. The colored rims result from complementation between the wild-type *C* allele in *C bz* tissue and the wild-type *Bz* allele in tissue containing the inhibitory *I* allele of the *C* gene. (*D*) Altered phenotype produced by chromosome breakage at *Ds* after transposition to a new site just proximal to the *C* gene at the distal end of chromosome 9. The initial chromosome break at *Ds* eliminates the *I* allele, revealing pigmented *C Bz* sectors. The circle highlights a twin sector arising from a dicentric chromatid

formed at the cleavage site and subsequent random breakage of the dicentric during cell division, giving rise to adjacent patches of *C Bz* and *C bz* tissue. (*E*) Phenotype resulting from chromosome breakage at the *Ds* just proximal to the *C* gene in a kernel having the genetic constitution *C Ds/c*, where *C* is the dominant allele (pigmented aleurone) and *c* is the recessive allele (colorless aleurone). The *C* → *c* variegation results from chromosome breakage at *Ds* and subsequent loss of the *C* allele. (*F*) Phenotype of an unstable mutation arising by transposition of *Ds* into the *C* gene, causing a mutation to the colorless *c* allele. The *c* → *C* variegation is caused by somatic transposition of *Ds* out of the gene, restoring the colored phenotype of the *C* allele.

Bacterial transposons are, like their eukaryotic counterparts, pieces of DNA that can move from one location to another

- **Background:**

  - discovered in *E. coli* late 1960s (Shapiro)
  - caused inactivation of galactose metabolism genes
  - mutants occurred at relatively high frequency (1 in 10^5)
  - mutations were **polar**
    - downstream gene expression also affected
  - mutant phenotypes were stable
  - foreign DNA was inserted into the mutant genes

Actually used λ phage carrying *gal* genes: λ*gal*

TRANSPOSABLE ELEMENTS AND TRANSPOSONS

Two types of elements can be inserted into bacterial DNA:

- **Insertion sequences (IS sequences)**
  - simplest transposable element
- **Transposons**
  - simple or composite

Mechanism of transposition

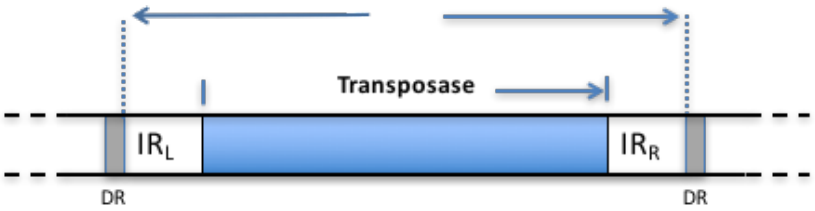
- replicative or non-replicative

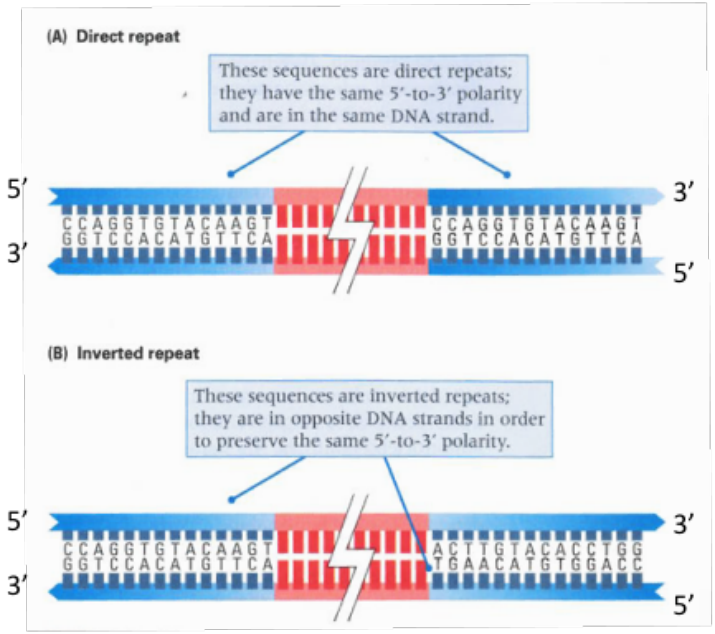
Insertion of IS sequences and transposons can alter gene expression:
- activate nearby genes in the genome;
- cause mutations;
- add new genes to the genome

INSERTION SEQUENCES (IS)

- simplest Mobile Genetic Element; also termed IS elements;
- short in length: ~ 750 bp – 2,000 bp
- only encode elements necessary for transposition
  - **inverted repeats** and **transposase**
- **transposase**
  - enzyme required for mobilisation and insertion
- **inverted repeats (IR)**
  - 10 – 50 bp
  - recognised by transposase

basic organisation of IS :





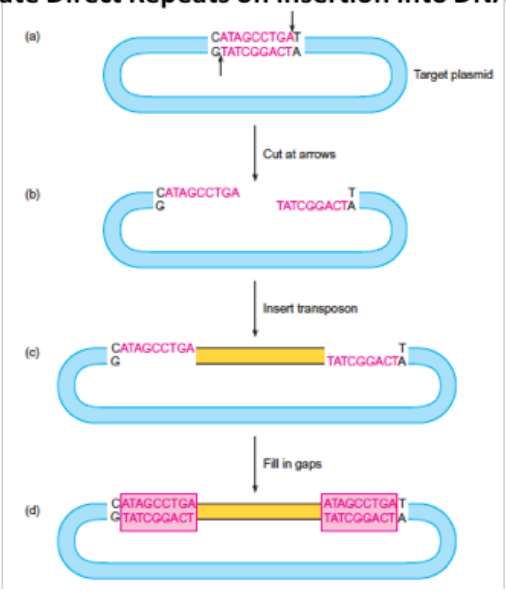
Most transposases generate Direct Repeats on insertion into DNA

(a) Transposase cuts target DNA at different points on each strand

(b) Single strand overhangs generated

(c) Transposon inserted

(d) Gaps are filled in generating **direct repeats** flanking transposon

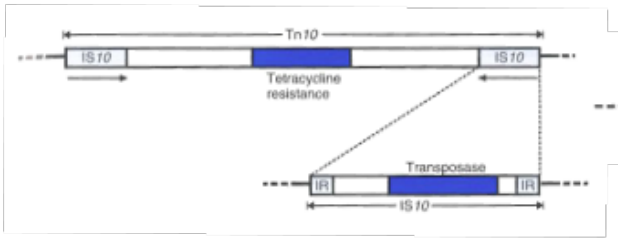


TRANSPOSONS

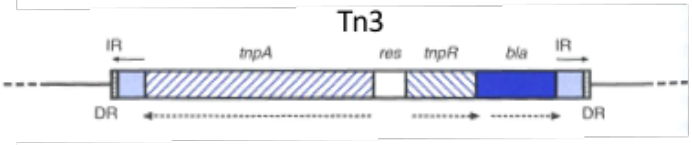
- Larger than IS elements (several kb)
- More complex – they carry 'passenger' DNA

2 classes of transposon:

- **'Composite' (Class I)**
- **'Simple' (Class II)**



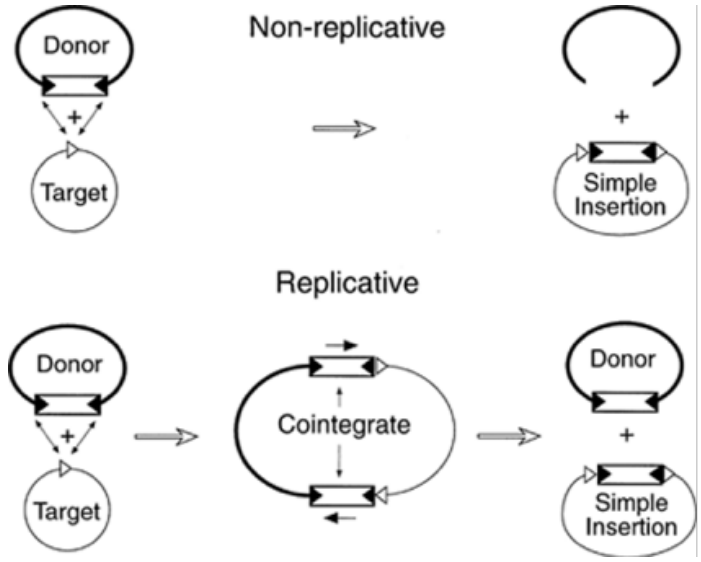
Two insertion sequences present – one at each end of the element; Passenger genes present e.g. *tetR*

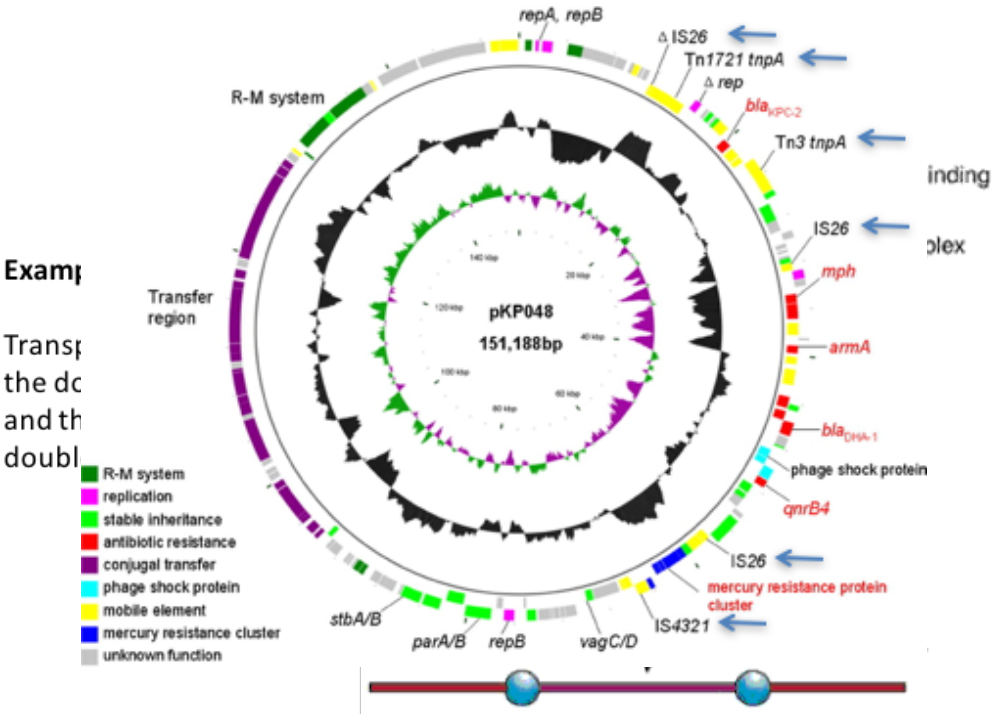


Essentially an enlarged IS element; passenger genes carried, e.g. *bla;* transposase and resolvase

Mechanism of transposition:

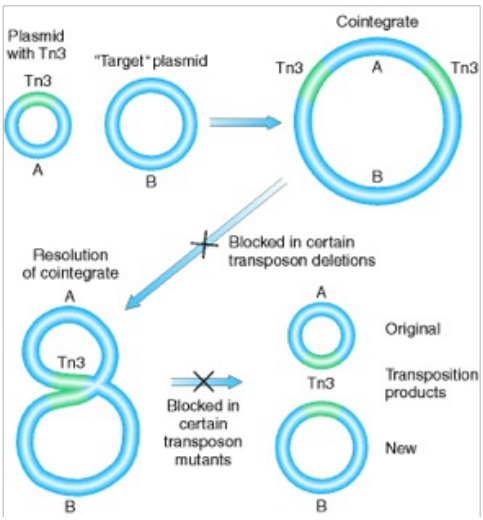
- **Transposition**- the movement of a transposable element
- Two stages
  - **Excision**
  - **Insertion**
- Two different methods
  - **Non-replicative**/conservative (transposon removed from old site, e.g. Tn10)
  - **Replicative** (new copy of transposable element generated, e.g. Tn3) "copy and paste" *(although that's not a term widely used)*





NON REPLICATIVE TRANPOSITION

Some transposons jump form one location to another, leaving behind a copy of the transposon – **replicative transposition.** The **source** and **targets** can be chromosomal DNA but are often bacterial **plasmids**.

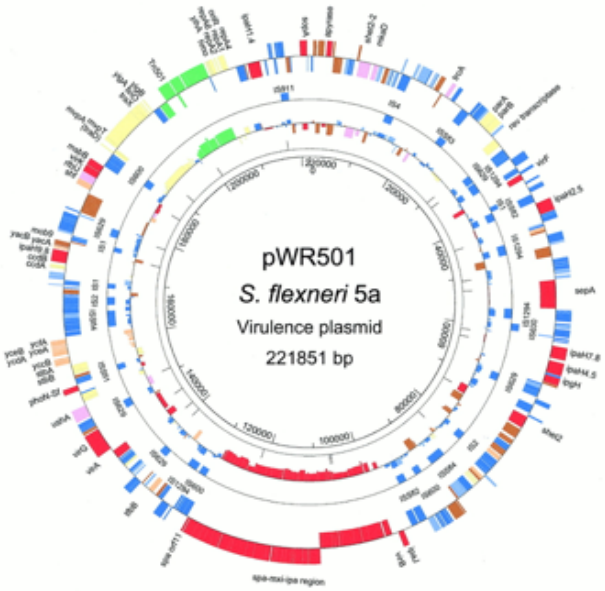


The scheme on the left was worked out using mutants that were blocked in various stages of transposition

REPLICATIVE TRANSPOSITION:

- Mobile genetic elements are everywhere, they carry important genes such as antibiotic resistance; often found on plasmids but also on chromosomes.

- *Shigella* is highly related to, and evolved from, *E. coli*
- Acquired a large 221 kb virulence plasmid
- Large virulence plasmid
  IS material represents **46%**
  of the plasmid
  26 full length IS elements;
  and extensive scars from ancestral rearrangements

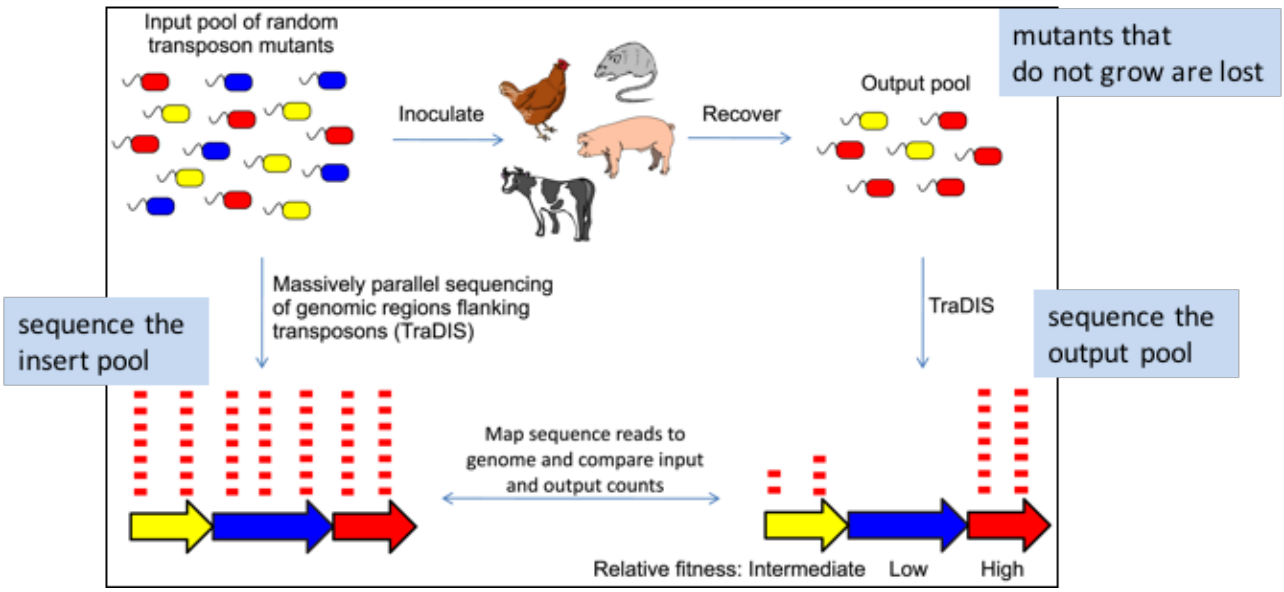


- Ability to integrate randomly in a large genome makes them good genetic tools
- An important use - **insertional inactivation** - disrupt gene function

Question: a bacterial genome has 3000 genes.
Which genes are essential for a specific process,
e.g. formation of biofilm, growth in an animal host?
Obviously many genes are essential for growth in "normal" lab conditions, but are there specific genes that are required for growth *in vivo*?

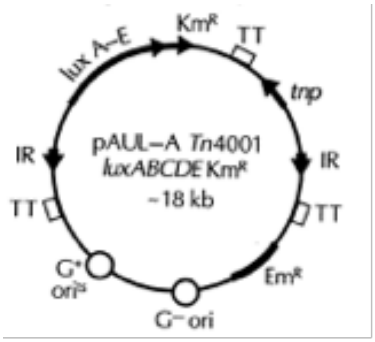
Construct a pool of transposon mutants in the bacterium, such that every bacteria has an insertion, but at a different location.
3,000 genes; maybe 10,000 insertions, >1 per gene.
Can make pools of >100,000 mutants

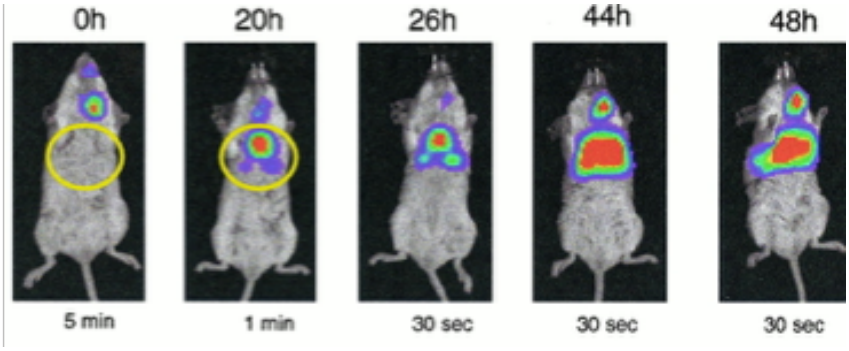
TRaDIS – transposon directed insertion site sequencing



Results will indicate which genes are **not** present in output pool
– these are important for survival in the host.
Advantages – only a few animals are needed for such an experiment. Think how many you would need of each gene had to be mutated and the mutant tested in an animal!

- Ability to integrate randomly makes them good genetic tools
- **Another important use – can be engineered to carry new genes**

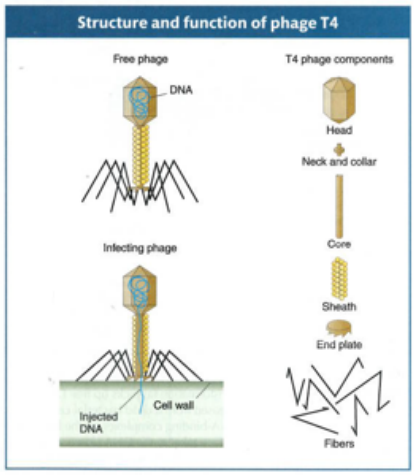




PHAGE TRANSDUCTION

BACTERIOPHAGE:

- **Most abundant lifeform* in biosphere**
  - estimated** that 10 x more phage exist than bacteria; 10^{30} bacteria; 10^{31} phage
- **Viruses of bacteria**
  - most bacteria are susceptible to phage
- **Nomenclature:** T4, l, P22 etc
- **Structure:**
  - Nucleic Acid (DNA/RNA)
  - Proteins – capsid head, core, sheath etc



PHAGE-MEDIATED TRANSFER OF GENETIC MATERIAL

- **2 forms:**
  - generalised
  - specialised
- **Discovery (1951):**
  Salmonella typhimurium
  - mixed 2 auxotrophic mutants together
  - obtained prototrophic strain !

  Repeated with a U-tube with filter:
  - No prototrophic formation
  - Not conjugation
  - Altered pore size of filter: small pores **did** stop prototroph formation

  Concluded genetic transfer was mediated by bacteriophage

VIRULENCE PHAGE LYTIC CYCLE

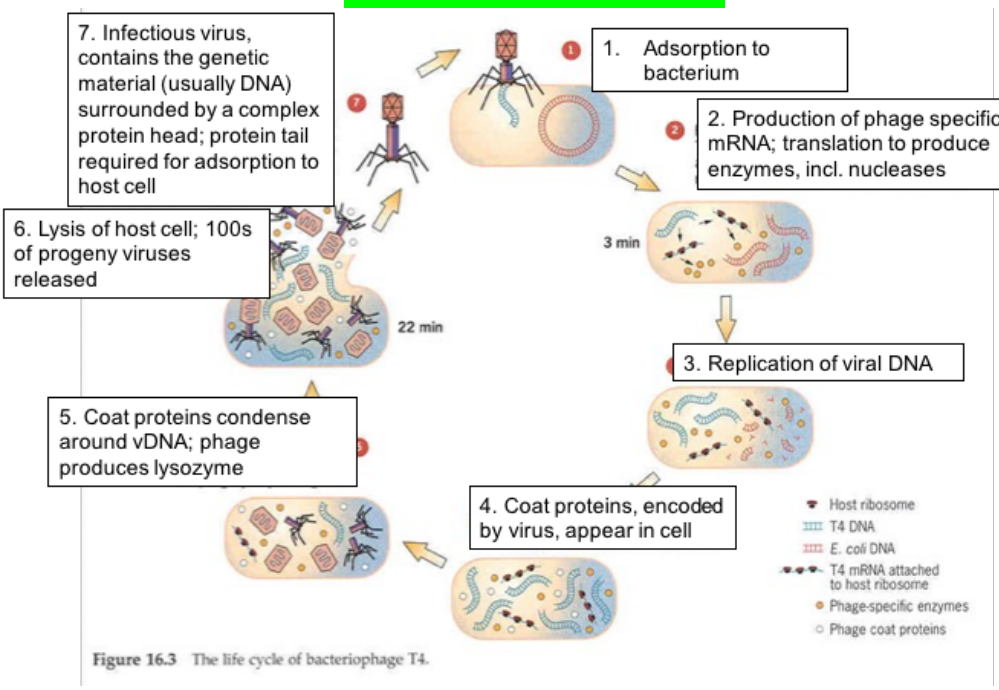
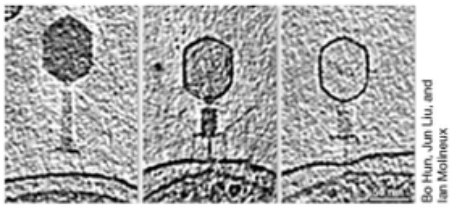
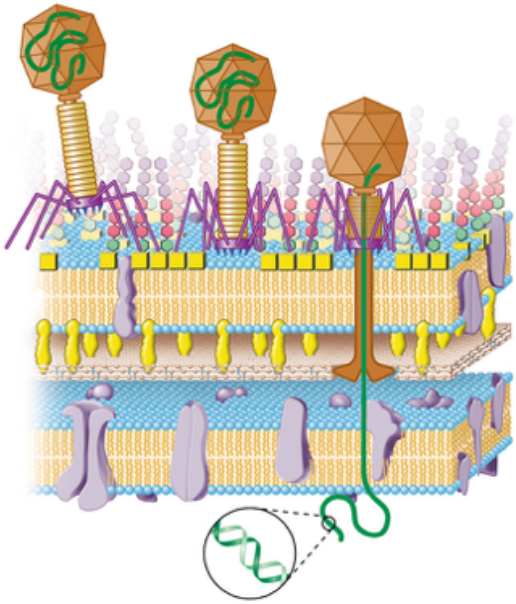


Figure 16.3 The life cycle of bacteriophage T4.

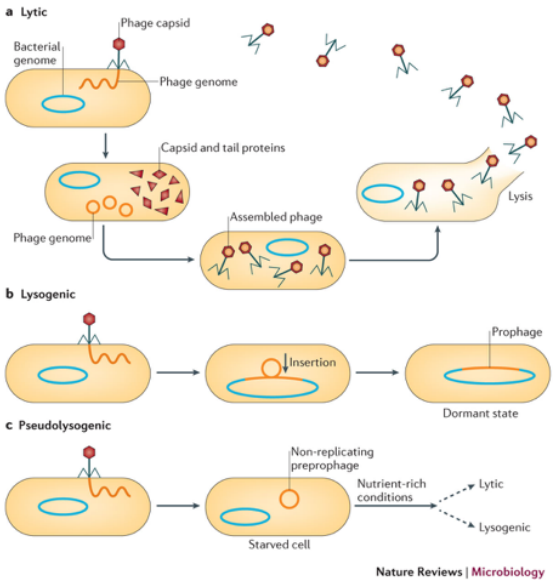




Contraction and delivery is acturally been used by bacteria to produce a system to inject toxins from one another. Type VI secretion system--> secrete across members, to exchange toxins. It resembles the structure of a phage (the sheath).

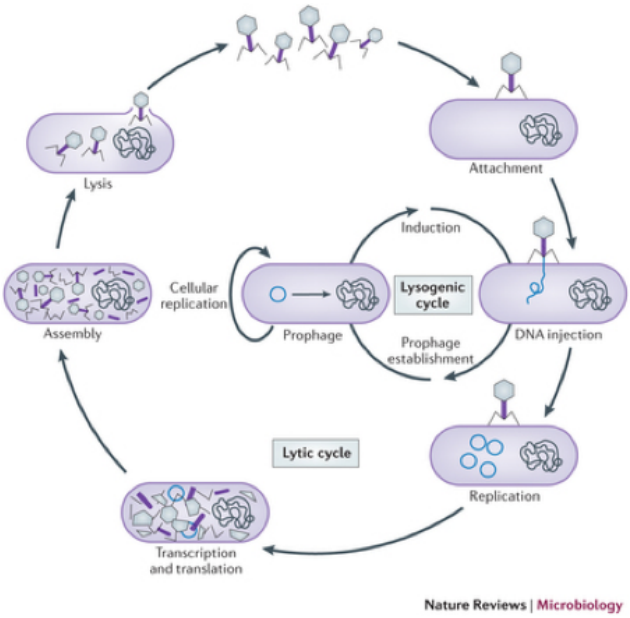
BACTERIOPHAGE LYFE CYCLE

- > 100 phage particles released from a bacterium
- a phage "lysate" is produced in culture
- on agar plates, a visible plaque is seen



Two phage types:

- **Virulent phages;**
  only undergo lytic cycle e.g., T4 (previous slides)
  always kill host cell
- **Temperate Phages;**
  Can undergo **lysogenic** and **lytic** cycles
  e.g., λ
  Can exist in host for prolonged periods as prophage
  Can introduce genetic information to host



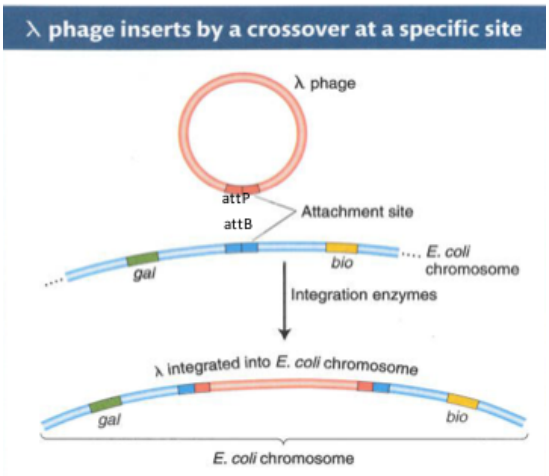
Lyogenic can be induced into lytic

- **A lysogen** is a bacterium that contains a phage integrated into its chromosome
- **A prophage** is phage integrated into its chromosome

In a lysogen:

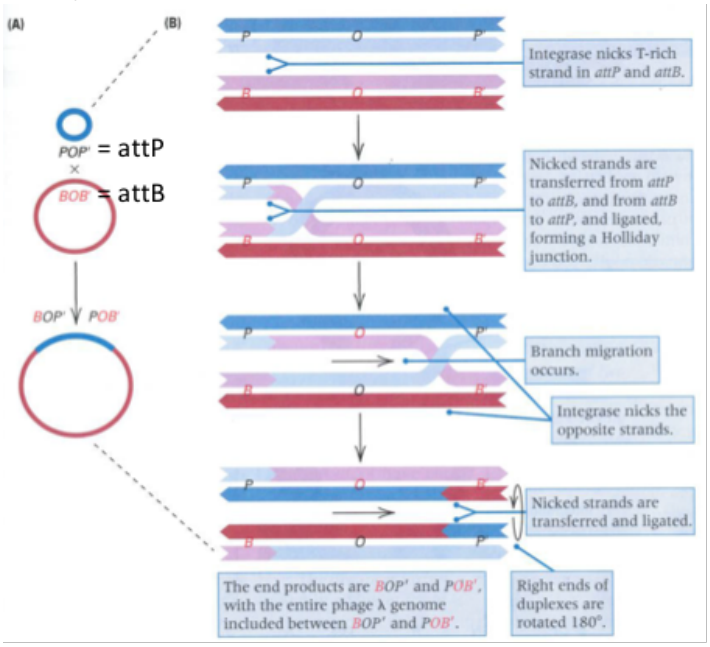
- Bacterium grows happily
- Most phage genes repressed (exception is the "repressor")
- No phage particles constructed
- Phage nucleic acid replicates as part of the host chromosome

- **Bacteriophage Lambda (λ) integration:**
  - Circularisation of DNA
  - *attP* (Phage site) & *attB* (Bacterial genome site)



  - Site specific recombination

RECOMBINATION, STRAND TRANSER



PHAGE TRANSDUCTION: TWO TYPES

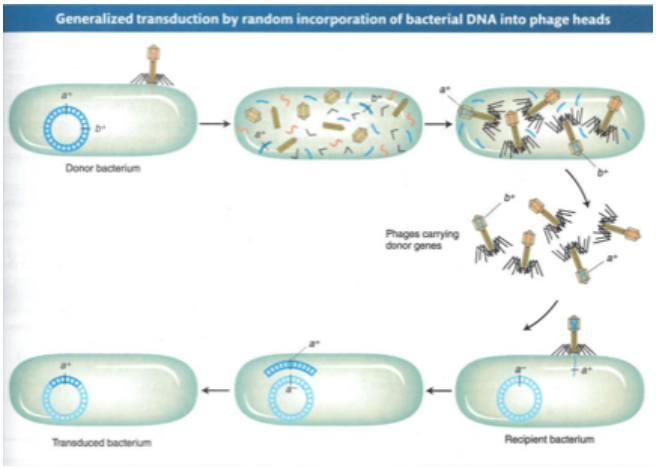
- **DNA transfer from donor bacteria to recipient bacteria via bacteriophage**

  - **Generalised transduction**
    - any part of the bacterial chromosome
    - occurs during lytic cycle
    - random packing of host DNA into phage particle
  - **Specialised transduction**
    - only transfers DNA adjacent to prophage insertion
    - occurs during **lysogenic to lytic conversion**
    - inaccurate excision of prophage from host chromosome

GENERALISED TRANSDUCTION

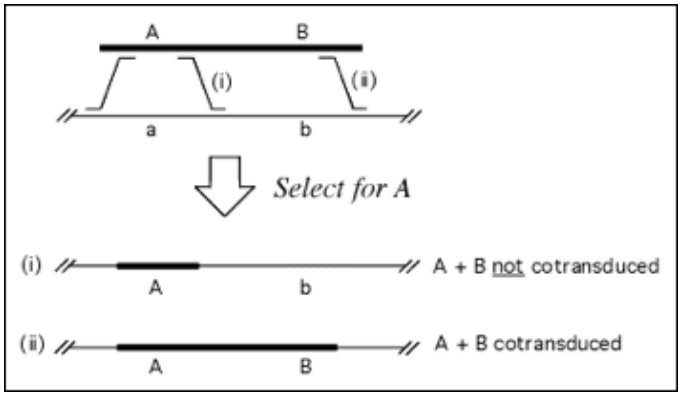
- **Bacteriophage**
  - must digest host DNA (nuclease)
  - approx. 1 in 10^4 phage particles carry host DNA
  - known as "transducing particle" when host DNA is present

- **Bacteria with Donor DNA recombined into chromosome: 'Transductant'**
  - DNA fragment integrates into chromosome



  - homologous recombination using host RecA enzyme

- **Generalised Transduction can provide linkage information**
- **About 50 additional genes can be carried in a phage**
- "2 mins" of chromosome
- Fine-scale mapping
- **'Cotransduction of genes'**
- depends on distance
- genes close together are more likely



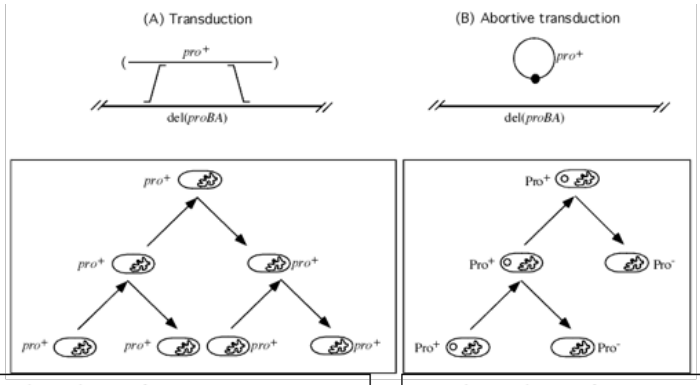
- to be packaged together, hence a higher cotransduction frequency

Two genetic markers (A,B) are packaged onto single generalised transducing fragment

- **Typically, phenotype of one genetic marker is selected**
  - transductants that inherited one marker will be screened for second inherited marker

- **Co-transduction frequency:**
  -ratio of transductants that co-inherited (A^+ and B^+) / by total number of A^+ transductants

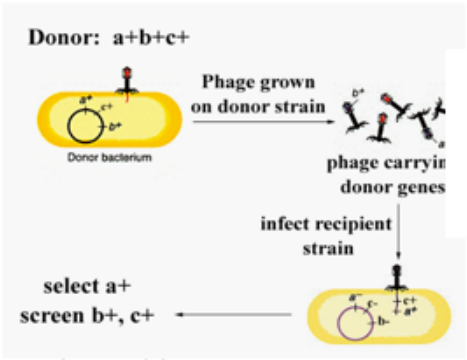
LINKAGE MAPPING OF BACTERIAL GENES BY TRANSDUCTION



Transduced DNA fragment:
- replicated along with chromosome
- propagated to daughter cells

Circularised DNA fragment:
- does not integrate into chromosome
- unable to replicate autonomously
- bacterial cells continue to divide,
- thus diluting out single bacterium with transduced DNA fragment

Linkage mapping of bacterial genes by transduction

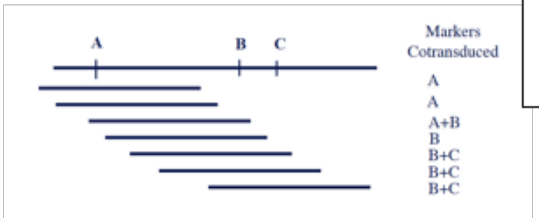


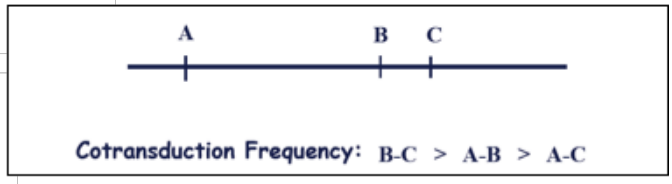
Done as a selection, then a screen

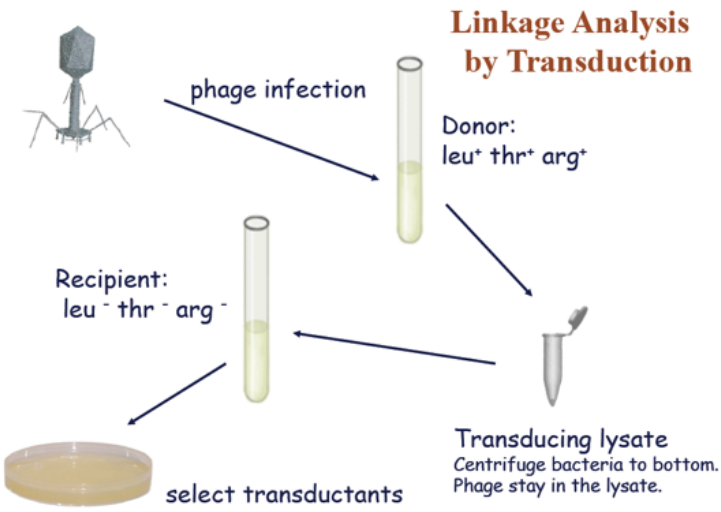
$$\text{A-B} = \frac{A^+B^+}{A^+} \times 100 = \%\ \text{cotransduction}$$

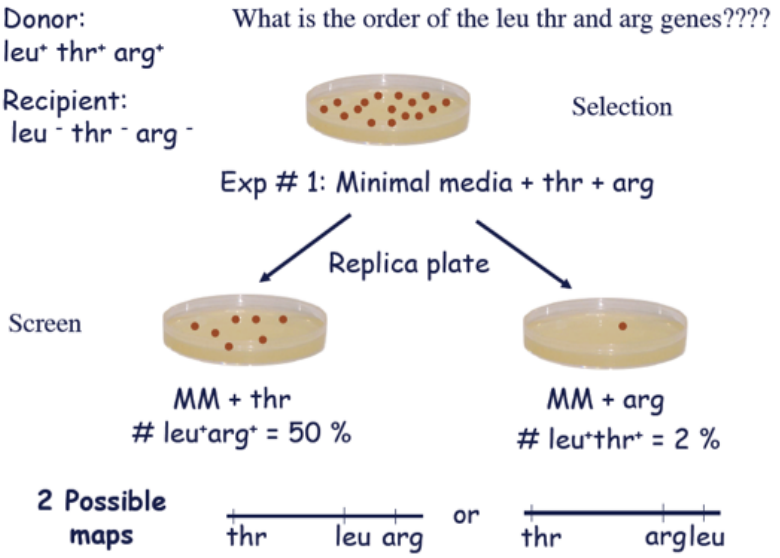
- The denominator is the marker that was selected.

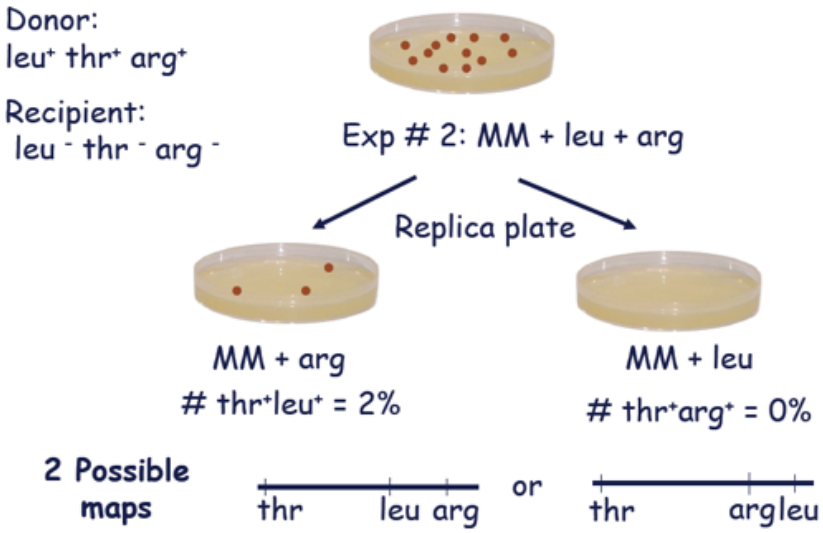
1/3 x 100 = 33% co-transduction of A-B





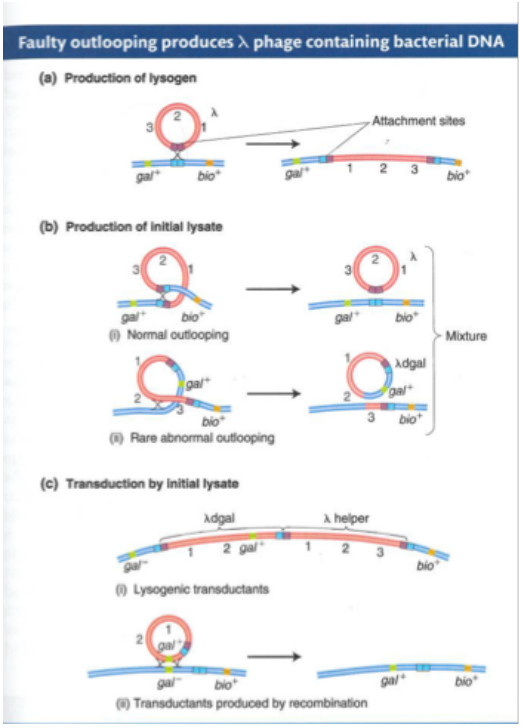

Linkage Analysis
by Transduction
phage infection
Donor:
leu+ thr+ arg+
Recipient:
leu - thr - arg -
Transducing lysate
Centrifuge bacteria to bottom.
Phage stay in the lysate.
select transductants


Donor:
leu+ thr+ arg+
What is the order of the leu thr and arg genes????
Recipient:
leu - thr - arg -
Selection
Exp # 1: Minimal media + thr + arg
Replica plate
Screen
MM + thr
leu+arg+ = 50 %
MM + arg
leu+thr+ = 2 %
2 Possible maps
thr leu arg
or
thr argleu


Donor:
leu+ thr+ arg+
Recipient:
leu - thr - arg -
Exp # 2: MM + leu + arg
Replica plate
MM + arg
thr+leu+ = 2%
MM + leu
thr+arg+ = 0%
2 Possible maps
thr leu arg
or
thr argleu

Final Map

SPECIALISED TRANSDUCTION



phage is integrated

normal excision (frequent)

abnormal excision (rare)

this lysate can be used to transduce *gal* and *bio* genes at a high frequency

When cell senses danger it produces a lot of RecA to protect its DNA. Phage can sense RecA and it excices ffrom the genome, but when it excices it does not do it always correctly, sometimes it takes with it the Gal or other genes.

Repression relieved:

- Lytic cycle kicks off
- Prophage excision

Excision of phage from chromosome:

- via a site-specific recombination process
- **'integrase'** *and* **'excisionase'**
- 1 excision per 10^6 iis incorrect and takes with it some host DNA

Result is 'specialised transducing phage'

- can still infect
- may have a defective genome
- might need a wild-type helper to infect
- carries genes adjacent to original site of integration

Only specific portions of bacterial chromosome are transduced

SURVIVING IN THE BACTERIAL JUNGLE

In their natural environment, bacteria have to **compete for space and nutrients** to survive. They do this by:

- production of antimicrobial compounds (e.g. bacteriocins) to kill other species
- secretion of enzymes to aid utilisation of nutrients e.g. b galactosidase to metabolise lactose
- modification of the physical environment e.g. production of polymers to aid attachment to surfaces
- using motility mechanisms to find new habitat
- fratricide (bacteria kill their siblings)

However bacteria can also **cooperate**

- **quorum sensing** – the entire population of a species produces molecules that enable the population to respond in a coordinated manner (although certain bacteria can "cheat")
- **mutualism –** production of compounds into the environment that benefit the entire community, e.g. siderophores that sequester iron which can then be taken up by bacteria

Uptake of DNA can be good for a cell!

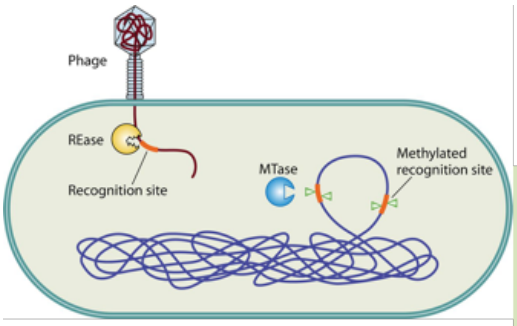
- increases gene pool
- increases metabolic diversity and functions the cell can carry out
- antibiotic resistance
- more genes, the more control a cell can have

Uptake of DNA can be bad for a bacterium!

- the more DNA in a cell, the more energy needed to replicate it
- cells have to ensure faithful replication
- errors can be costly, leading to cell death
- foreign DNA can be harmful
- mutant genes may replace functioning genes
- DNA might insert into functional genes or operons

Defense against invading DNA: R-M
Restriction and modification (R-M) of DNA

1. **Restriction endonuclease (RE)** – cleaves unmethylated DNA
2. **Methlytransferase (MTase)** – methylates the cells DNA at the recognition site of the RE to prevent restriction



A simple form of immunity; allows bacteria to protect themselves from invasion; recognition of "self" DNA because it is methylated

Restriction-Modification (R-M) Systems (R-M)
At least 3000 different restriction endonucleases (REs) exist,
with over 300 specificities
Many more – 1000s – probably exist
Found in Bacteria and Archaea
Almost all bacterial appear to produce
REs and MTases.
90% of sequenced genomes contains at least one R-M system

Sir Rich Roberts
- discovered introns in eukaryotes
- formed NEB, a company to sell Res.
Now a major biotech company
Look him up (later) on Wiki

Bacteria have evolved other ways to defend themselves:

- Switch ON and OFF phage receptors
- Secrete polysaccharides that limit access to a receptor
- Express membrane proteins that interfere with nucleic acid injection
- Suicide (abortive infection) – cell sacrifices itself to prevent spread of viral DNA
- Other mechanisms continue to be discovered

BUT – phages are persistent!
- they can overcome some of these mechanisms!
  use different receptors; produce degrading enzymes;
  mutate their proteins to avoid triggering abortive infection

PHAGE THERAPY

Discovered over 100 years ago
Phage as antimicrobials were investigated long before antibiotics were discovered
George Eliava (1892-1937) – a microbiologist from Georgia;
trained at Pasteur Institute, Paris with Felix D'Herelle
(co-discovered bacteriophage)
Founded an Institute in Tbilisi,
focusing on phage therapy.

However, as the golden era of antibiotics developed in the 1950-70s, interest in phage therapy declined.

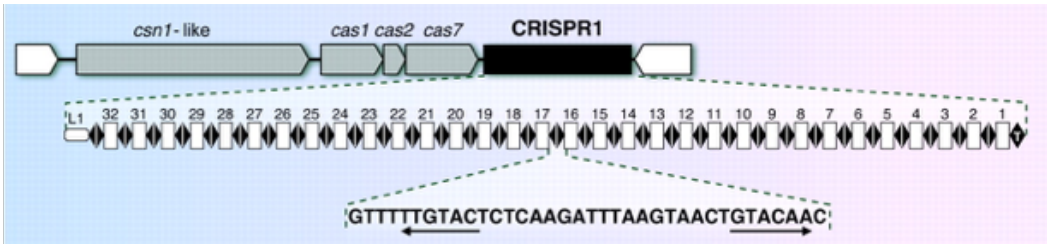
Points to consider:

- Antibiotics are chemicals, not life forms; they do not grow and mutate.
  Natural section can result in alterations to
  phage as well as to the bacterial host.
  Potentially, bacteria could become resistant to phage,
  but phage could then evolve to infect this "resistant" strain, and so on.

- Phage are highly specific to certain bacterial species, T4 infects *E. coli,*
  and will not kill off large numbers of our own microbiota.
  Nature has evolved phage to kill a specific strain of bacteria.
  Can we design phage to target specific strains of pathogens?

3. Genome metasequencing reveals many, many more phage
   in the environment than originally thought.
   e.g. sequencing of everything in the oral cavity reveals phage transcripts
   are phage active during periodontal disease?
   could such communities be a source of new phage for potential therapies?

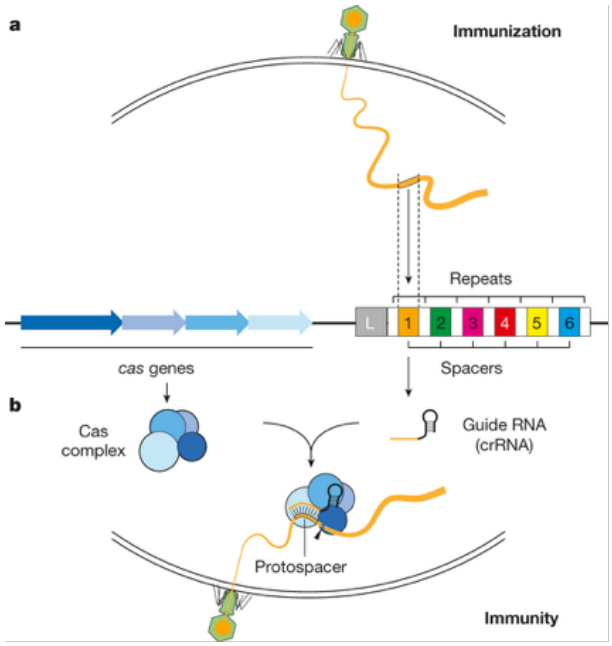
1990s – genome sequencing revealed
many bacterial and archaeal species had unusual repetitive sequences
Short (24-40 bp) repeat sequences; palindromic; inverted repeats at the end
Spacers are 20-50 bp; not repetitive
Arranged in **clusters** or **arrays**
Clustered Regularly Interspaced Short Palindromic Repeats



3 components:
DNA repeat sequences
Spacers regions , not all identical
Associated genes – **Cas genes**- contain nuclease and helicase domains

1. Spacer sequences match DNA from phage that normally infect the bacterium
e.g. spacers in *Streptococcus thermophilus* match phage that infect this species
2. Bacterial strains that have a spacer derived from a phage are **resistant** to that phage
3. The CRISPR locus is transcribed (RNA is produced)

Hypothesis: CRISPR is an immunity system of prokaryotes – prevents foreign DNA from invading



a. Capture of DNA sequences from invading phage

b. Invading phage DNA is recognised by bacterium and destroyed

1. Spacer DNA is transcribed to Guide RNA (crRNA)

2. Cas protein- crRNA-tracrRNA complex recognises invading DNA

3. Cas nucleases cleave invader DNA

CRISPR-Cas is an adaptive immunity system found in bacteria and archaea to defend form invading genetic material
It is an adaptive system - allows bacteria to adapt to new phage sequences
Mechanism is through:

- capture of foreign DNA and storage in arrays
- using guide RNAs to recognise invading DNA
- using nucleases to cleave invading DNA