Extra Chromosomal Elements

By

Heba Al Sayed Al Degla

Lecturer of Medical Microbiology and Immunology
Mansoura Faculty of Medicine
All the DNA material present in a cell other than chromosomal DNA, the most famous elements are:

- Plasmid.
- Transposon.
- Bacteriophage (Virus infecting bacteria)
Plasmids
Def: pieces of DNA that exist separate from the chromosome containing the origin of replication, so they independently replicate from the chromosome.

Classification:
1. According to the size of plasmid:
   - from few hundred base pairs up to 3000 Kbp
2. According to copy number per cell:
   - Stringent plasmids: 1-2 copies/cell like F-plasmid and phage-plasmid hybrid (P1).
   - Low copy number plasmids: 10-15 copies/cell.
   - High copy number plasmid: up to 50 copies/cell.
   - Extremely high copy number plasmid: up to 100-200 copies/cell (specifically engineered plasmids).

A. For high-copy-number plasmids, random partitioning occurs.

B. For low-copy-number plasmids, replication is coordinated with chromosome replication.
3-1. According to the compatibility of plasmids:

- **Compatible plasmids:** 
  - The cell can maintain more than one plasmid in the same cell (if they carry different origins of replication).

- **Incompatible plasmids:** 
  - Inability of two plasmids with the same origin of replication to be maintained in the same cell.

*Escherichia coli* diagram:

- Diagram showing the interaction between plasmids and the chromosome ΔcyoA.

- Illustration of ATP and CAMP regulation processes.
4-Shape of plasmids:

- **Covalently closed circular (CCC) form:** the most common form of plasmid in bacteria, present as a ds completely closed circular form (as in *E. coli*).

- **Semi-circular form:** present as a transient form, naturally present as one strand is completely closed, the other strand is opened.

- **Linear:** double strand linear DNA present in some bacteria, unstable because it is attacked by exonucleases.
5-According to the host range:

- Broad host range plasmids: Can replicate and maintained in a wide range of bacteria.
- Narrow host range plasmids: only replicate and maintained in one or few closely related bacterial species.
Moving plasmids from cell to cell:

- **Conjugative plasmids:** which have the *tra* genes that can mobilize the plasmid from one cell to another of the same species or different species by conjugation.

- **Shuttle vector:** plasmids which propagate in two different host species (yeast and bacteria or mammalian cells).

- **Non-conjugative plasmids:** cannot be mobilized under any known conditions.
Artificial and natural plasmids:

Natural plasmids are present naturally in bacterial and some yeast cells carrying genes for its own replication and genes for some functions of the cells like F-plasmid (F-pili during conjugation) and some R-plasmids for drug resistance.

Artificial plasmids are naturally present plasmid but designed artificially by adding some markers (genes) like antibiotic resistant markers or DNA sequence to be target of restriction endouncleases. They are used as vectors in gene cloning and genetic engineering.

![Diagram of PBR 322 plasmid](image-url)
Importance of plasmids

- Resistance:
  - Antibiotic resistance, Heavy metals
  - (metal reductase, DNA repair enzymes).
- Conjugation.
- Production:
  - Toxins & enzymes, Bacteriocin.
- Biochemical reactions:
  - Sugar fermentation.
- Molecular biology:
  - As a vector
Transposons and transposition
Def: extra chromosomal small pieces of DNA that are capable of moving itself from one location in DNA to another, (movable elements or jumping genes). The mechanism of transposition can be either "copy and paste" or "cut and paste".

Structure: there are 3 forms of transposable elements:
(a) Insertion sequence (IS)
(b) Composite transposons (Tn)
(c) Non-composite transposons
(a) Insertion sequence (IS)

- The simplest form as small as 750 to 2000 bp.
- They encode only proteins needed for its own transposition.
- Carry the same repeats at their ends (either direct repeats or inverted repeats).
- Examples: IS 1, 3, and 10.
(b) Composite transposons (Tn)

- Contain 2 IS at both ends and central piece of DNA which encode for antibiotic resistance or virulence factors
- Examples:
  - **Tn 5** – Has IS 50 at both ends encodes for kanamycin resistance.
  - **Tn 10** – has IS 10 at both ends encodes for tetracycline resistance.
Transposon, Tn10

- **IS10L**: Inverted repeats of IS element
- **Tetracycline resistance gene\( (Tc^R) \)**
- **IS10R**: Inverted repeats of IS element

- **1,400 bp**
- **6,500 bp**
- **9,300 bp**
- **1,400 bp**

Inverted IS elements
(C) Non – composite transposons

- Have no IS at their ends but encodes for transposition proteins.
- They carry genes for antibiotic resistance, virulence factors and catabolic enzymes.
- Examples:
  - Tn 3: carry Ampicillin resistance gene
  - Tn 7: carry streptomycin and trimethoprim resistance
  - Bacteriophage MU.
- N.B: the most complex transposons known are bacteriophage
Transposon, Tn3

- tnpA
- tnpB
- bla

Transposase

β-lactamase

Left inverted repeat (38 bp)

Resolvase

Right inverted repeat (38 bp)

mRNAs
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<th><strong>Plasmids</strong></th>
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<td>Resistance, Production, Biochemical Conjugation</td>
<td>AB resistance</td>
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Direct repeats are generated by insertion

Target site

Staggered nicks made at target site

Transposon joined to single-stranded ends

Gaps at target site filled in and sealed

Target repeats
Replicative transposition creates a copy of the transposon, which inserts at a recipient site. The donor site remains unchanged, so both donor and recipient have a copy of the transposon.
Nonreplicative transposition allows a transposon to move as a physical entity from a donor to a recipient site. This leaves a break at the donor site, which is lethal unless it can be repaired.
Effect of transposition

Transposable elements can cause genetic changes, and have been involved in the evolution of both prokaryotic and eukaryotic genomes. Transposons may:

a. Insert into genes and disrupt its function.
b. Increase or decrease gene expression by insertion into regulatory sequences.
c. Produce chromosomal mutations through the mechanics of transposition.
Gene Transfer
Gene Transfer

Definition:
The process of moving a piece of DNA (either chromosomal or plasmid) from one cell to another using different means.

Types (Mechanisms):
- Conjugation: by direct cell-to-cell contact
- Transduction: by bacteriophage
- Transformation: by direct transfer of isolated DNA
A) Conjugation

- **Def:** a form of gene transfer in which **two cells** come in contact and DNA is transferred from one cell (donor) to the other (recipient).

- **Requirements:**
  - Donor cell should contain F plasmid, which encodes for F pili needed for conjugation.
  - Donor plasmid should contain tra gene (conjugative type) to mobilize the plasmid.
  - Occurs most frequently occurs in Gram negative bacilli but also in Gram positive organisms.
**Steps**

- The F pili of the donor cell (F⁺) attach to specific receptor on the recipient cell (F⁻).
- The F pili contract the recipient cell to be in close contact and then canalization occurs through the F pili between the two cells.
- Once canal formed F plasmid start to mobilize one strand of its double strand DNA to the recipient cell.
- New double strand formed to the single strand in both donor and recipient cells to from complete plasmid so the recipient now contain F⁺ plasmid and changed to F⁺ cell which will act as a donor cell.
Transfer of chromosomal DNA by conjugation (Hfr):

- If the F plasmid integrates into loci in the chromosome, this integrated F factor creates a high frequency of recombination cell (Hfr cell).
- If this integrated plasmid is transferred to another cell by conjugation, it can transfer a segment (locus) of chromosome during excision and can transfer this locus (gene or genes) from the donor cell (Hfr) to a recipient chromosomal cell.

**Diagram:**

- **F+ cell**: Recombination between F factor and chromosome, occurring at a specific site on each.
- **Hfr cell**: Insertion of F factor into chromosome, creating an integrated F factor.

(b) When an F factor becomes integrated into the chromosome of an F+ cell, it makes the cell a high frequency of recombination (Hfr) cell.
Conjugation in Gram +ve organisms:

Because pili are not present in Gram +ve organisms, conjugation can occur by other means, the most recorded is the pheromone-mediated conjugation in which recipient cells secrete signaling molecules (pheromone) to stimulate plasmid in the donor cell to synthesize adhesive cell surface components to act as pili. All steps are like in Gram -ve organisms.

Recipient cells secrete high concentrations of the oligopeptide pheromone cCF10.

Donor cells respond by upregulating conjugation factors such as ‘aggregation substance’ (or Asc10).

pCF10 transferred to recipient cell.
N.B:

- Conjugation between prokaryotes (E. coli) and eukaryotes (yeast cells) has been established.
- Plasmid–independent conjugation done through conjugative type of transposons especially in Gram +ve organisms.
B) Transduction

- **Def:** a form of gene transfer mediated by bacteriophage.
- **Types:**
  1. *Generalized transduction*
  2. *Specialized transduction*
Generalized transduction occurs with the lytic cycle of bacteriophage. When the phage infects a bacterial cell and replicates inside the cell, during assembly of the phages, sometimes chromosomal DNA (of the infected bacterial cell) can be packaged inside the phage head. This DNA is called generalized transducing DNA. By cell lysis, the released phages can infect another cell and transfer the chromosomal DNA to the recipient bacterial cell.
Specialized transduction occurs with the temperate or lysogenic bacteriophage (i.e. non-lytic cycle): the bacteriophage DNA will be integrated inside the bacterial DNA at a specific region (so-called specialized). When the integrated phage is activated, it will be excised from with a piece of bacterial chromosome and infect another bacterial cell, transferring this DNA into the recipient cell.

Diagram:
- Phage DNA integrated in bacterial DNA.
- Excision of phage & part of bacterial DNA.
- Infection of gal. -ve bacterium, it becomes gal.+ve.
C-Transformation

- **Def:** a method of gene transfer in which direct uptake of DNA by recipient cell occurs either naturally or artificially in the laboratory

1. *Natural transformation* (rare occasion):
2. *Laboratory induced competence*
3. *Transformation by electroporation*
- **Natural transformation** (rare occasion)
- Some bacteria are naturally transformable only in the presence of competence factors.
- E.g., *H. influenza*, *N. gonorrhoea*, *S. pneumoniae* and *B. subtilis*. 

![Diagram of transformation process]
Laboratory induced competence: Competence can be induced in non-competent cells (E. coli) by increasing the permeability of the cell envelope by adding calcium chloride solution and chilled on ice then heat-shocked (42°C for 2 min).
**Transformation by electroporation:**

- by exposing a mixture of recipient cells and plasmids to electrical field.
- These electro-competent cells have high level transformation efficiency.