Plasmids & Transposable Elements

Lecture 1

BY

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Genetic Engineering

- **Genetic Engineering** : Is the manipulation of genetic material .
- - **specific fragments** of DNA may be **isolated** (may come from other species , even from eukaryotic cells – since the genetic code is universal for all cells).

- **cut** into pieces by the action of **restriction endonuclease** .

- **rejoined** with **a vector** by the action of **DNA ligase** to create new genetic structure.

- **-introduce** foreign genes into **bacterial cells (genetically engineered).**

- Genetic Engineering (technology): allows scientists to study the activity of genes to understand their function.

- One of the **applications** of this technology is the potential to **treat genetic disease** ,such as cancers , by gene replacement.

Vectors

- **Plasmid vectors**
- **Bacteriophage vectors**
- **Cosmid vectors**
- **BACs and YACs :(*BACs* : Bacterial Artificial – Chromosomes**

  ***YACs* : Yeast Artificial – Chromosomes)**

What determines the choice vector?

- insert size
- vector size
- restriction sites
- Copy number
- cloning efficiency
- ability to screen for inserts

plasmids

How did plasmids get their name?

- In 1952, Joshua Lederberg set out to clarify the classification of these cytoplasmic inheritance factors. He proposed the catch-all term “**plasmid**” derived as a hybrid of "**cytoplasm**" and "**id**" (Latin for '**it**'), as “a generic term for any extrachromsomal hereditary determinant”.
- His proposal, however, was basically ignored.

- A separate term, "**episome**", defined as "**a non-essential genetic element which could exist either autonomously or integrated into the chromosome**" was proposed a few years later by Élie Jacob and François Wollman and became the widely adopted name for these elements.

- At the time, the use of episome seemed appropriate, especially since the **Fertility**, or **F-factor** discovered by Ester Lederberg in 1952 was noted to integrate into the *E. coli* chromosome in some cases.

- This terminology held until the 1960s when scientists began to study other extrachromosomal particles, particularly Resistance or **R-factors**.

- Like F-factors, **R-factors** could be transferred between bacteria via cell-to-cell contact;
- however, scientists noted that, unlike F-factors, the evidence did not support the idea that **R-factors** could integrate into the chromosome.

- Thus the term “episome” was eventually dropped and we’ve been using "**plasmid**" ever since!

- Since their discovery in the 1950s, plasmids have greatly impacted many areas of biological research and have been key in advancing our knowledge in areas such as
- bacterial conjugation and recombination,
- replication and topology,
- cloning and gene expression.

- **A plasmid** is a short, usually circular , and supercoiled double-stranded DNA is found in the cytoplasm separate from the main bacterial chromosome and can replicate independently of it (extrachromosomal genetic elements).

-Note : Supercoiling allows plasmids to renature quickly after they are denatured

Occurence :

- Procaryote organisms
- Eukaryotic organisms like *Entamoeba histolytica*, yeast etc.

Shuttle vectors have origins of replication and selectable markers for propagation in both bacteria and yeast .

- Their size varies from 1 kbp to over 400 kilobase pairs (kbp).

- ***Advantages:***
  - Small, easy to handle
  - Straightforward selection strategies
  - Useful for cloning small DNA fragments (< 10kbp)

- ***Disadvantages:***
  - Less useful for cloning large DNA fragments (> 10kbp)

- **Plasmids** aren't essential for bacterial life , but its presence give bacteria an additional properties enabling it to live in an unusual conditions .

Function

- Act as vectors in genetic engineering.
- transporting genes to sites in gene therapy .

- In addition to the information necessary for their replication, a plasmid can carry virtually any other **gene** (plasmids can convey a selective advantage on the host bacterium).

- **For example, some plasmids carry genes encoding**

1 Resitance to Antibiotics: Such plasmids are termed **resistance or R factors**.

2 Bacteriocins production: (molecules that inhibit **bacterial growth** or kill the bacteria)

3 Enterotoxin production: The lipopolysaccharide endotoxins on Gram-negative bacteria cause fever, changes in blood pressure, inflammation, lethal shock, and many other toxic events.

4 Enhanced pathogenicity

5 Reduced Sensitivity to mutagens

6 Degrade complex organic molecules

7 resistance to various heavy metals

Types of plasmids

Plasmids are classified according to the :

1- plasmids copy number

Copy number : is the average number of plasmid molecules per bacterial cell.

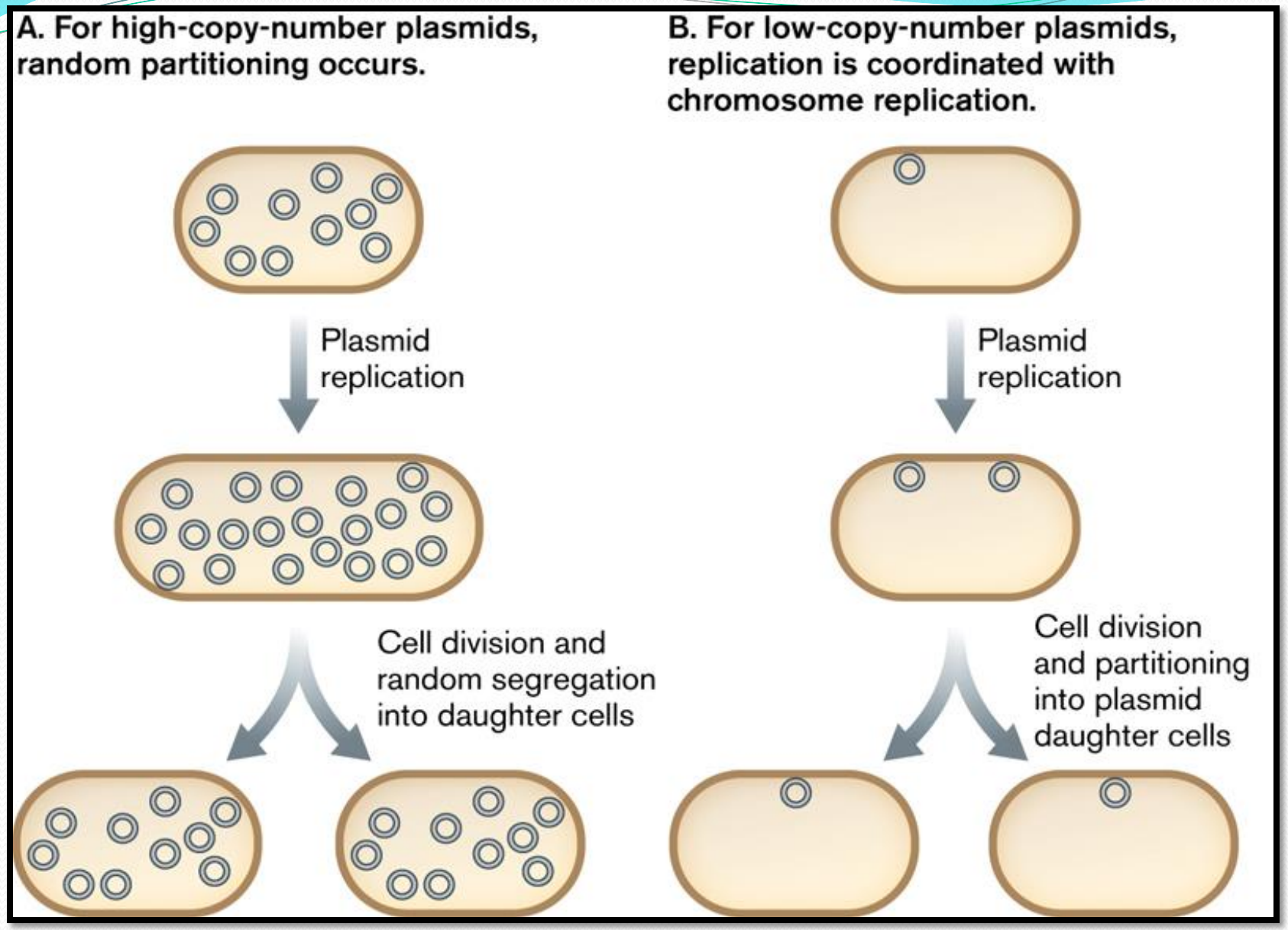
- **1- Relaxed (multicopy) plasmids**: Those are presnce in high copy number with low molecular weight(smaller plasmids) are generally non-conjugative .
- **2- Stringent plasmids(low copy number plasmids) :** Those are presnce in low copy number with high molecular weight(94.5 kbp for F-plasmid) are conjugative.

- A system in **low –copy number plasmid** ensures accurate and almost equal distribution of plasmid copies to daughter cells prior to division through carrying genes specific for the replication of those plasmids .

  It encode **partitioning (*par*) loci** that ensure ordered plasmid segregation prior to cell division. It forms dynamic filaments that segregate plasmids paired at mid-cell to daughter cells.

- **Smaller plasmids** make use of the host replicative enzymes to make copies of themselves.

- A few types of plasmids can also insert into the host chromosome, and these **integrative plasmids** are sometimes referred to as episomes in prokaryotes.


A. For high-copy-number plasmids, random partitioning occurs.
Plasmid replication
Cell division and random segregation into daughter cells
B. For low-copy-number plasmids, replication is coordinated with chromosome replication.
Plasmid replication
Cell division and partitioning into plasmid daughter cells

Low and High Copy Plasmids.

- Low copy (1-25): pBR322
- High copies (100 or more): pUC
- Copy number depends on:
  - Ori (relaxed or stringent control: specificity)
  - Size of the plasmid and
  - Associated insert

Plasmids	Origin of replication	Copy Number	Classification
pUC vectors	pMB1*	500–700	high copy
pBluescript® vectors	ColE1	300–500	high copy
pGEM® vectors	pMB1*	300–400	high copy
pBR322 and derivatives	pMB1*	15–20	low copy
pACYC and derivatives	p15A	10–12	low copy
pSC101 and derivatives	pSC101	~5	very low copy

2. by their ability to be transferred to other bacteria

- **Conjugative plasmids :**Plasmids with high molecular weight and limited copy number .The **sexual transfer** of plasmids to another bacterium through a pilus , those plasmids possess the 25 genes required for transfer called transfer or *tra* genes .

- **Non- conjugative plasmids:** Plasmids with low molecular weight and high copy number . Are incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids.

Mobilisable : An intermediate class of plasmids are mobilisable, and carry only a subset of the genes required for transfer. They can parasitize a conjugative plasmid, transferring at high frequency only in its presence.

Mobilisable plasmids are not able to promote their own transfer unless an appropriate conjugation system is provided by a helper plasmid. Mobilisable vectors contain a site for transfer initiation called origin of transfer , **oriT**, and have sequences encoding proteins (**Mob)** involved in the mobilization of the DNA during the conjugative process. Mob proteins alone are not sufficient to achieve the transfer of the genome. Additional proteins for transfer (**Tra**) are involved in the formation of a pore or pilus through which the genome passes to the recipient bacteria. Mobilisable plasmids do not encode Tra proteins and for this reason they require a helper plasmid providing the tra genes.

- **Incompatibility groups:** Several types of plasmids could coexist in a single cell. On the other hand, related plasmids are often 'incompatible', resulting in the loss of one of them from the cell line.

3. by function

- 1. **Fertility-(F) plasmids**,

  They are capable of conjugation (they contains the genes for the pili).

- **2. Resistance-(R) plasmids**,

  contain gene (s) that can build resistance against one or several antibiotics or poisons

- **3. Col-plasmids,**

  contain genes coding for colicines, proteins that can kill other bacteria.

- **4. Degradative plasmids,**

  able to digest unusual substances, e.g., toluene or salicylic acid.
- **5. Virulence plasmids,**

  turn a bacterium into a pathogen.
- **6. addiction system**,

  These plasmids produce both a long-lived poison and a short-lived antidote. Daughter cells that **retain a copy** of the plasmid **survive**, while a daughter cell that **fails to inherit** the plasmid **dies** or **suffers a reduced growth-rate** because of the lingering poison from the parent cell.

Plasmids in molecular biology

Minimum requirements for plasmids useful for recombination technology:

- **1. Origin of replication (ORI).** ORI enables a plasmid DNA to be duplicated independently from the chromosome

- **2. Selectable marker:** allow to select for cells that have desired plasmids.

  twin antibiotic resistance

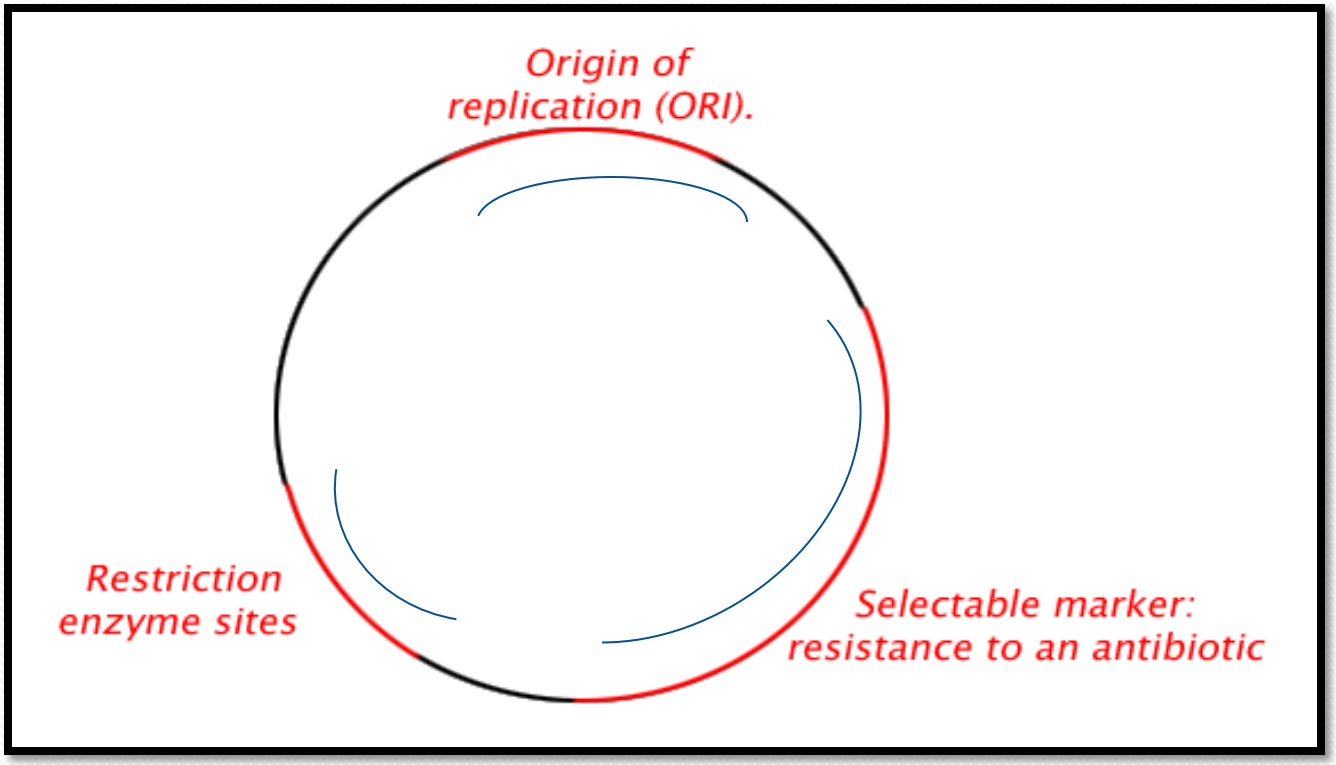
  blue-white screening

- 4. **Contains a multiple cloning site (MCS).**

- 3. **Restriction enzyme sites** in non-essential regions of the plasmid.

- **5. Easy to be isolated from the host cell.**

4
Division of host cells and selection of recombinant clones
Plate transformed and untransformed bacteria on selective medium
Agar containing ampicillin and x-gal
Numerous cell divisions resulting in clones (bacterial colonies)
Recombinant clone: transformed bacterial cells are resistant to ampicillin but do not produce blue color
Bacterial colony
Untransformed bacterial cells are sensitive to ampicillin and do not grow
Nonrecombinant clone: transformed bacterial cells are resistant to ampicillin and produce blue color
5
Amplification and purification of recombinant plasmid DNA
Pick a recombinant colony Inoculate liquid growth medium
Grow overnight
Harvest bacteria
Purify plasmid DNA


Origin of
replication (ORI).
Restriction
enzyme sites
Selectable marker:
resistance to an antibiotic

- 6. Small size(low molecular weight) :

  - easy to handling.

  - presence in high copy number(relax)

  - more efficient in bacterial transformation .

  - decrease the chance of the presence of many sensitive sites for one RE .

- 7. have the ability to speed replication in desired host cell for easily getting high numbers of recombinant molecules.

- 8. Should have well known characteristics respecting to genes sites , RE sites and nucleotides sequence .


Wishing
You
The Best Of Luck!!