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Topic: **Plasmid Replication**

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Plasmid Replication

✓Plasmids replicate autonomously because they have their own replication origins.

✓The enzymes involved in plasmid replication are normal cell enzymes particularly in case of small plasmids.

✓But, some large plasmids carry genes that code for enzymes that are specific for plasmid replication.

✓Plasmids are replicated by the same machinery that replicates the bacterial chromosome.

✓Some plasmids are copied at about the same rate as the chromosome, so a single cell is apt to have only a single copy of the plasmid.

✓Other plasmids are copied at a high rate and a single cell may have 50 or more of them.

✓Plasmids possess relatively few genes, generally less than 30, and the genes are concerned primarily with control of the replication initiation process and with apportionment of the replicated plasmids between daughter cells; the genetic information carried in plasmid genes is not essential to the host because the bacteria that lack them usually function normally.

Plasmid Replication

1. Plasmid replication requires host DNA replication machinery.

2. Most wild plasmids carry genes needed for transfer and copy number control.

3. All self replication plasmids have a *oriV*: origin of replication

4. Some plasmids carry and *oriT*: origin of transfer. These plasmids will also carry functions needed to be mobilized or *mob* genes.

5. Plasmid segregation is maintained by a *par* locus-a partition locus that ensures each daughter cells gets on plasmid. Not all plasmids have such sequences.

6. There are 5 main "incompatibility" groups of plasmid replication. Not all plasmids can live with each other.

7. Agents that disrupt DNA replication destabilize or cure plasmids from cells.

8. However, some plasmids of gram-negative bacteria replicate by unidirectional method. Most plasmids of gram-positive bacteria replicate by a rolling circle mechanism similar to that used by phage φx174. Most linear plasmids replicate by means of a mechanism that involves a protein bound to the 5′-end of each DNA strand that is used in priming DNA synthesis.

Plasmid replication mechanisms

There are three types of plasmid replication namely :

1. Rolling circle,
2. Col E1 type and
3. Iteron type replication

Replication initiation depends on a sequence – plasmid origin of replication (ori).

Rep proteins – plasmid encoded initiators of replication.

Rolling circle

✓Rolling circle replication mechanism is specific to bacteriophage family m13 and the fertility F factor which encodes for sex pili formation during recombination by means of conjugation.

✓Fragments smaller than 10 kilo base usually replicate by this replication mechanism as reported in some gram positive bacteria.

✓ It allows the transfer of single stranded replication product at a faster rate to the recipient cell through pilus as in case of fertility factor or to the membrane in case of phage

Mechanism:

Rolling circle occurs to a covalently closed circular piece of double-stranded DNA. A nick is produced in one of the strands by enzyme nickases creating a 5’ phosphate and a 3’ hydroxyl.

Free 3’ hydroxyl will be used by DNA polymerase to make new DNA pushing the old nicked strand off of the template DNA

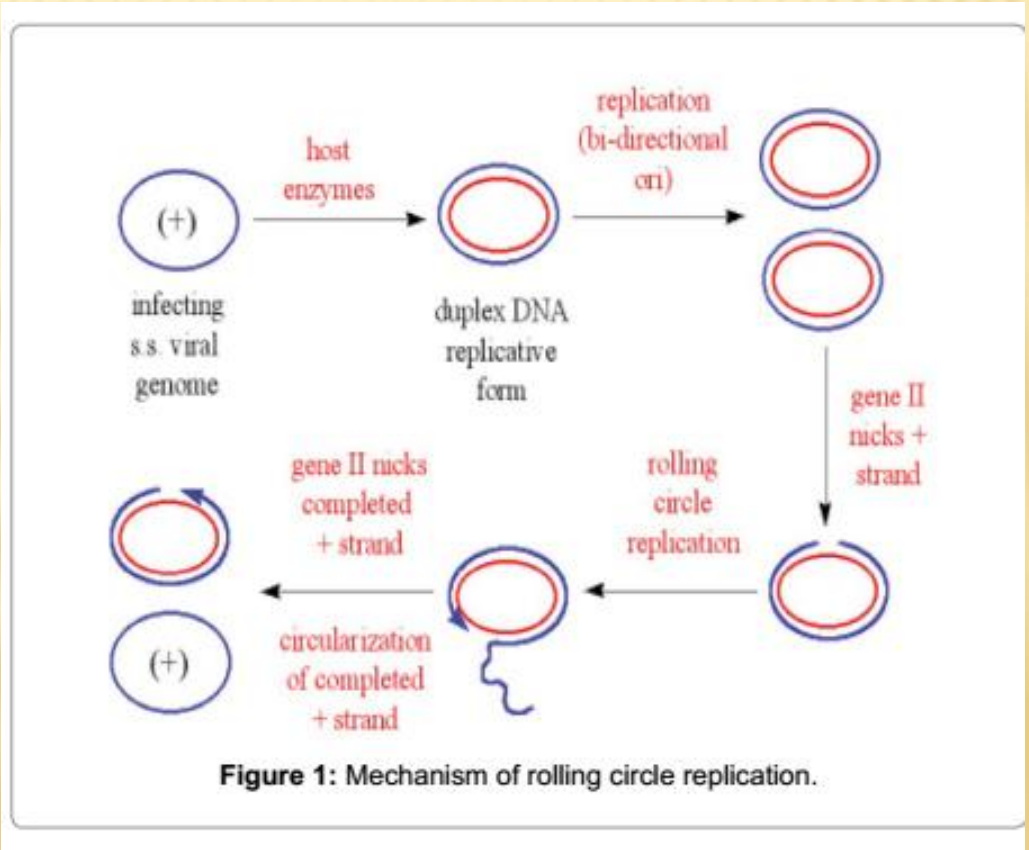

Figure 1: Mechanism of rolling circle replication.

Col E1 type replication

✓ Col E1 replication is a negative regulation mechanism which enables the plasmid to control its own copy numbers by involving RNA type I, RNA type II, Rom protein, and the plasmid itself.

✓Col E1 replication is initiated by means of RNA-RNA interactions and does not rely on replication initiation protein encoded by the plasmid to regulate its copy number

Mechanism: RNA type II that originates 555 base pairs upstream from the replication origin of Col E1 plasmid is transcribed which marks the start of Col E1 replication.

A determined hybrid with the DNA strand is formed by a loop enriched in G nucleotide positioned 290 of RNAII and a C-rich region on the template strand positioned 20 nucleotides upstream from the origin.

A DNA/RNA hybrid is recognized by enzyme RNase and dissociates the RNA hybrid to the 3' end of RNAII.

The resultant RNA primer is linked to the plasmid with a free 3' hydroxyl group.

This RNA enables replication of DNA to begin by providing DNA polymerase a specific site to initiate nucleotides synthesis.

Consequently DNA synthesis is commenced with the leading strand is happening

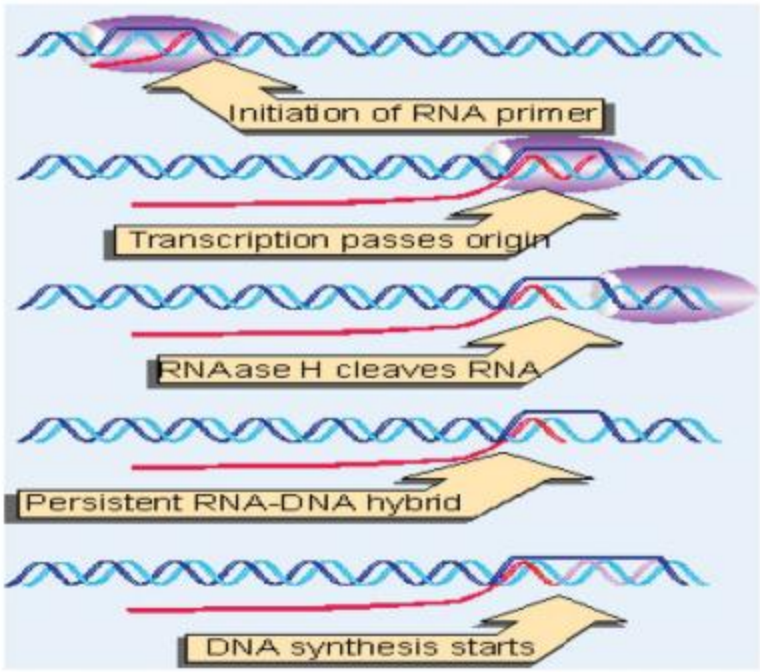

Figure 2: Mechanism of Col E1 type replication.

Iteron-containing replicons

✓Iterons are directly repeated DNA sequences which play an important role in regulation of plasmid copy number in bacterial cells.

✓ It is one among the three negative regulatory elements found in plasmids which control its copy number

✓This replicon consists of a gene that encodes Rep protein for plasmid replication initiation, set of direct repeat sequences called iteron, adjacent AT-rich region and Dna boxes which is a protein required for bacterial chromosome replication initiation.

✓However length of adjacent AT-rich region and number of iterons and DnaA boxes differs in a replicon.

Mechanism: Iteron contain replication begins with the binding of Rep proteins to the iteron being organized in the same orientation of the DNA helix.

✓And by binding to the DnaA boxes in the replicon the Rep-DnaA-DNA assembly promotes melting of the strand at the nearby AT-rich region to which host replication factors subsequently gain access and promote leading and lagging strand synthesis in a manner

analogous to initiation of replication at the chromosomal origin, oriC

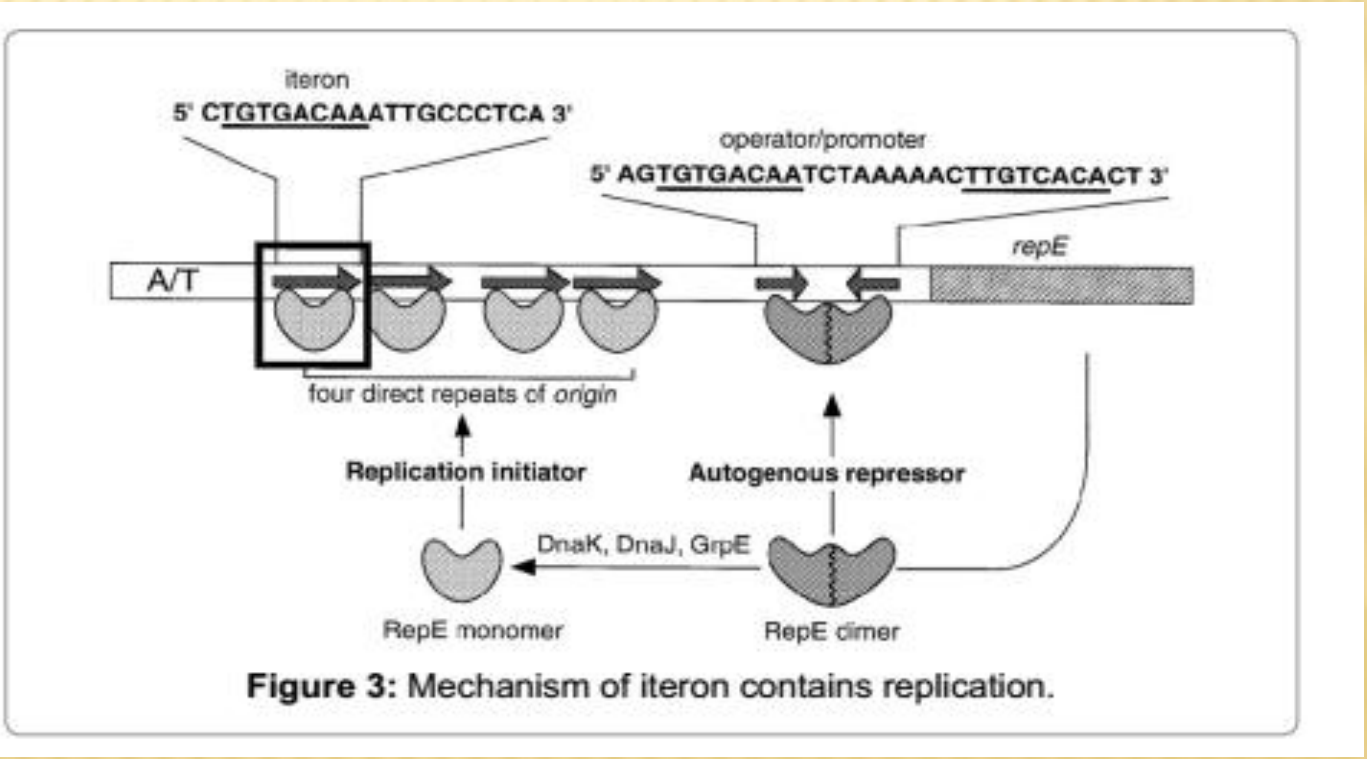

Figure 3: Mechanism of iteron contains replication.