

Mechanisms of Plasmid Replication

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Abstract

Plasmids are small, circular extra fragments of DNA, commonly found in bacteria that are capable of replicating independent of the host genome, though plasmids are not required for survival of a living organism. But encodes essential genetic determinants that enable an organism to adapt and resist unfavourable conditions for better survival. Rolling circle, Col E1 type and iteron-containing replicons are the common modes through which plasmid replicates, each mechanism with unique significance to the organism.

Keywords: Rolling circle replication; Iteron- containing replication; Plasmid

Introduction

Double stranded, few kilo base self-replicating extra DNA fragments is known as “Plasmids” commonly recognized in different gram negative and positive bacterial strains as well as in some fungi including unicellular yeasts. Although plasmids are usually circular but linear plasmids have also been reported [1]. Though plasmid are not required for bacteria survival but encodes essential genetic determinants that enables bacteria to adapt and resist unfavorable conditions for better survival and to encounter external threats with other microbes occupying the same position in an ecological food chain. Replication mechanisms of plasmid are host specific and effects plasmid copy number. Plasmid replicons consists of one or more origin of replication (ori) and few regulatory elements such as Rep proteins, localized in the 4 kilo base region of the DNA fragment. In addition plasmid also possess few essential genes that assist in DNA replication. The molecular mechanism of bacterial plasmid replication is similar to the origin of replication of *E. coli* chromosome [2].

Plasmid Replication

Bacterial plasmid replication is not dependant on its nuclear genome replication with long intermissions between replication proceedings occurring during the course of cell division. Definite plasmid copy number depends on plasmid type, host organism and the growth conditions. Unintended aberrations from normal copy number are attuned. However dominant and recessive copy mutants to the wild type do exist [3].

Plasmid replication mechanisms

There are three types of plasmid replication namely rolling circle, Col E1 type and Iteron contain replication [3]

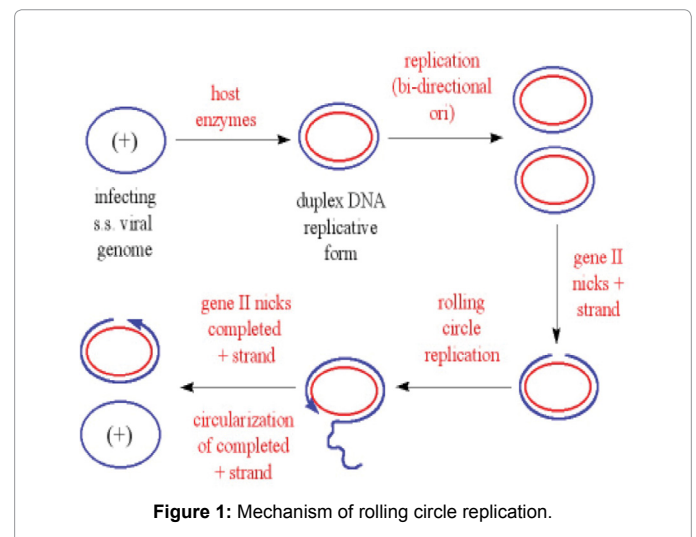
Rolling circle: Rolling circle replication mechanism is specific to bacteriophage family m13 and the fertility F factor which encodes for sex pili formation during recombination by means of conjugation. Fragments smaller than 10 kilo base usually replicate by this replication mechanism as reported in 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 [4].

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) [5,6].

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 [7].

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



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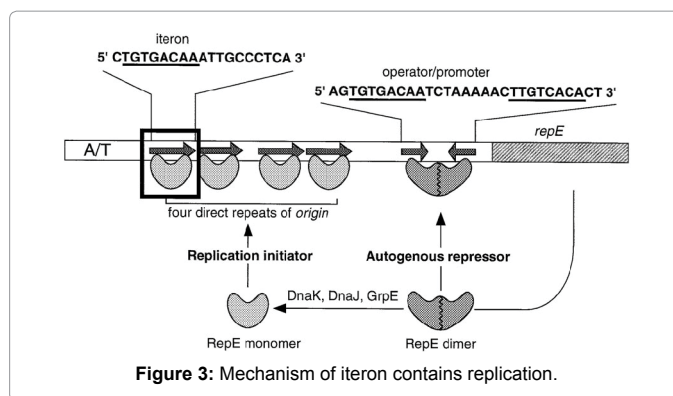
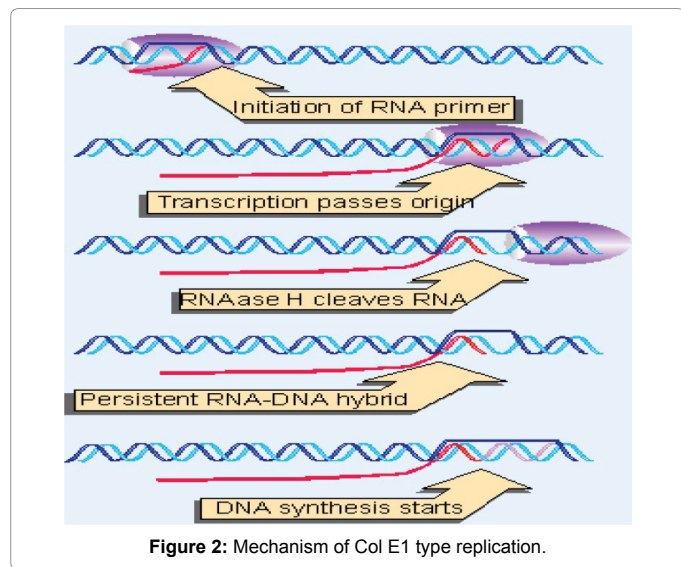
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290 of RNAII and a C-rich region on the template strand positioned 20 nucleotides upstream from the origin [8]. Several stems and loops are exhibited by the newly formed secondary structure. A DNA/RNA hybrid is recognized by enzyme RNase and dissociates 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) [9].

Iteron-containing replicons: 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 [10].

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) [11].

Plasmids copy number is controlled principally at the beginning of replication initiation. The frequency with which initiation of replication of iteron-containing plasmids occurs is modulated in part by sequestration of the origin region in nucleoprotein complexes and intermolecular pairing of complexes on different plasmids, which is referred to as "handcuff" [12].



Conclusion

Thus plasmid replication by means of rolling circle, Col E1 type and Iteron contain replication is an efficient way to control its copy number and compatibility in bacteria and other respective organisms.

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