VIVEKANANDA COLLEGE,THAKURPUKUR

TOPIC:REPLICATION PROCESS

COURSE:GENE ORGANISATION,EXPRESSION AND REGULATION

SEMESTER:4-GE-4

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DEPARTMENT:BIOCHEMISTRY

Figure No. .....

PROCESSES INVOLVED IN REPLICATION

i) Initiation:

Initiation involves recognition of the position on a DNA molecule where replication will begin, a site known as the origin of replication (Ori C).

ii) Elongation:

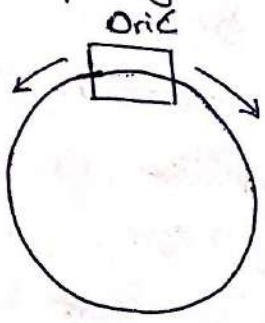
Elongation involves the events occuring at the replication fork, where the parent polynucleotide is copied.

iii) Termination:

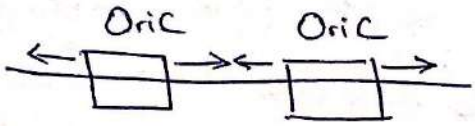
Termination occurs when the parent molecule has been fully replicated.

Initiation

It is a random process and begins at the same position or positions known as OriC. Once initiated the 2 replication forks progress in opposite direction along DNA, replication is ∴ bidirectional. For bacteria, there is a single origin of replication but for eukaryotes there are multiple origin of replication, here replication forks progress for shorter distances.



prokaryotic



eukaryotic

The Ori C of E. coli spans approx 245 bp of DNA. There are 2 short repeat motifs. One of 9 nucleotide repeated 4 times at the OriC and the other 13 nucleotides repeated 3 times.

245bp.

Step 1 :

DnaA protein (52KD) recognizes and binds upto four 9bp repeats in OriC to form a complex of negatively supercoiled OriC wrapped around ~30 molecules of DnaA protein monomers. This process is facilitated by HU or Integration host factor, histone like protein that helps DNA bending.

Step 2:

The DnaA protein subunits then successively melt 3 tandemly repeated 13 bp AT-rich segments located near OriC left boundary.

Figure No. .....

Step 4:
In the presence of SSB and gyrase, DnaB, a helicase, further unwinds the DNA in the pre-priming complex in both the direction allowing the entry of primase and RNA polymerase. The participation of both these enzymes facilitates primer synthesis in the leading strand, together with limitation of this process to Ori C site, suggest that RNA polymerase activates primase to synthesize the primer

SEMIDISCONTINUOUS SYNTHESIS

Elongation:

Once replication has been initiated the replication fork progress along the DNA. Here 2 events occur

i) During DNA replication both strands are copied. DNAP can only synthesize from 5' → 3' direction. This leads to synthesis of one strand continuously known as the leading strand and the other copied in a discontinuous manner known as the lagging strand or okazaki fragments.

ii) For DNA pol primers are needed to initiate complementary strand synthesis on the leading polynucleotide and one for each discontinuous DNA segment on the lagging strand.

Lagging Strand Synthesis

Although the leading strand DNA Polymerase can replicate as soon as it is exposed, synthesis of the lagging strand must wait for the movement of the replicating fork to expose a substantial length of template before it can be replicated. Each time a substential length of template is exposed, DNA synthesis is initiated and continues until it reaches the 5' end of the previously newly synthesized lagging strand of DNA. The initial products of lagging strands are shorter fragment called okazaki fragments, first isolated by Okazaki and okazaki from E. coli, there

are ≃1000 - 2000 nt in length. In eukaryotes they are much shorter >200 nt, indicating that a single nucleosome is replicating, 140 - 150 bp wound around a core particle within 50-70bp as the linker DNA. For synthesis of lagging strand RNA primers is required for each strand synthesized by primase which is about 5nt in length (primer). It is a repeated process unlike leading strand priming.

In eukaryotes primase is an integral part of DNA pol α, the polymerase synthesizes the primer of about 10nt and then adds ≃30nt to DNA. The rest of strand synthesis is completed by DNA pol δ.

Replication fork:

The helicase binds to the origin forming the pre-priming complex, primase on binding with this complex forms the primosome which initiates replication of leading strand synthesis. DnaB unzips the DNA generating replication fork in conjuction with DNA topoisomerases and the lagging strand is spooled out as ssDNA which is maintained by SSB protein. After 1000-2000 nt of the leading strand is synthesized the first round of discontinuous lagging strand is synthesized.