REPLICATION

Replication is the process of copying of parent DNA to daughter DNA molecules having nucleotide sequence identical to those of parent DNA.

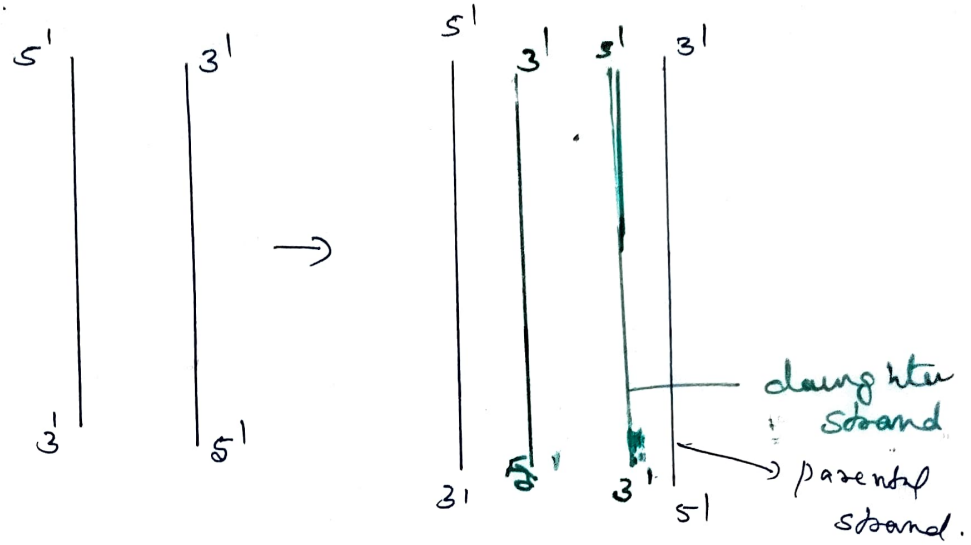
DNA molecule duplicates itself

Purpose of replication:

Hereditary transmission of genetic information. Progeny provided & genetic information possessed by the parent so it has to be completed & high fidelity (& out any error). This will maintain genetic stability in the organism.

Semi conservative : Each molecule of DNA replicates by a process called Semi conservative replication. 2 new DNA molecules exactly identical & parent DNA is formed. Since only one parental strand is conserved in each daughter DNA - the process is called Semi conservative - 50%.

Each single strand of double helix of DNA will serve as a template (mould) to form a new DNA complimentary to itself.



Base pairing rule for replication is very specific (By Edwin Chagraff)

$$A = T \qquad C \equiv G$$

ENZYMES FOR REPLICATION

Main enzyme is DNA POLYMERASE (SN)

DNA polymerase Type III is true replicating enzyme in bacteria & Eukaryotic cell

5 DNA polymerases

- α polymerase — main replicating enzyme
- β " — repair enzyme
- γ " — for mitochondrial DNA replication
- δ " — repair enzyme. (leading strand synthesis)
- Epsilon " — repair enzyme

DNA polymerase has unique features
(1) Complex structure (2) multiple catalytic activities like polymerising action, Editing (proof reading) exonuclease activity (3) requires a template strand & a primer strand (small fragment of pre formed RNA act as primer)

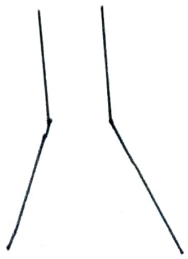
Polymerising action only in $5' \rightarrow 3'$ direction.
It utilises d ATP, d TTP, d GTP & d CTP as substrates (d = deoxy) for polymerisation.

STEPS OF REPLICATION

DNA replication requires more than 20 enzymes & protein factors collectively called DNA replicase System or replisome (SN)
Replication begins at specific site in DNA called origin of replication.

1st step

Unwinding or seperation of double helical parental DNA. This creates a replication fork at the point of seperation of 2 strands.



Un winding is initiated by a nick or break in phospho diester linkage of one strand. After replication this nick is re sealed by DNA Topoisomerases (out any energy input. In bacteria Gyrases & helicases help in Un winding.

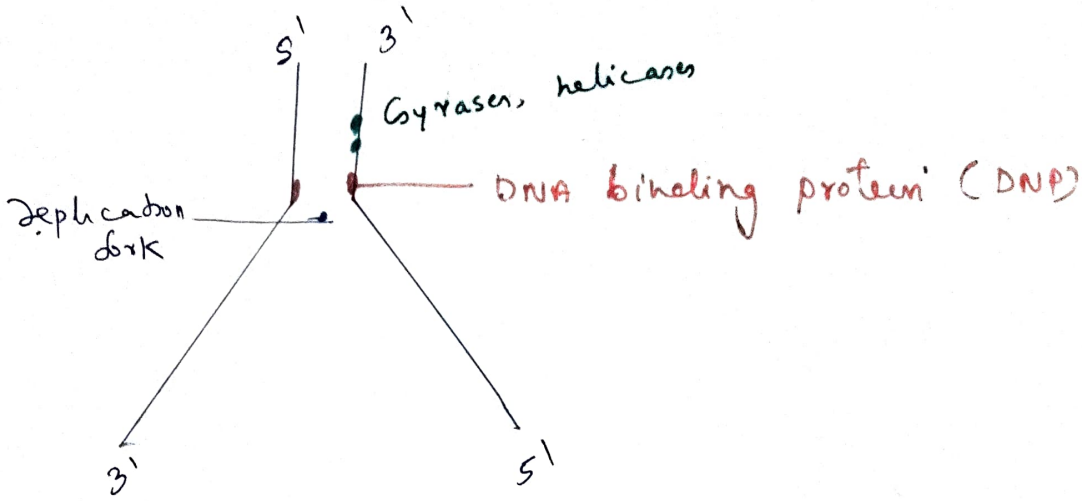
Once double helix is seperated Several molecules of DNP (DNA binding protein) bind tightly to the seperated strand & Prevent rebinding. Replication

Replication occurs in both strands Simultaneously only in 5' — 3' direction

Chemical reaction in polymerisation

$$(\text{Polynucleotides})_n + d\text{NTP} \overset{A\ G\ C}{\underset{\quad T}{\rightleftharpoons}} \longrightarrow (\text{polynucleotides})_{n+1} + PP_i$$

$$PP_i \xrightarrow[H_2O]{\text{Pyrophosphatase}} 2\,P_i$$



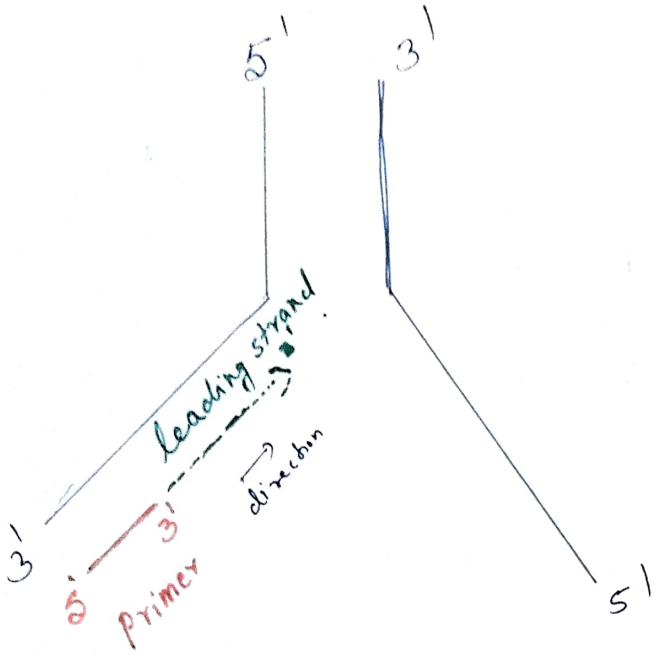
REPLICATION of 5'–3' Parental strand

RNA Primer Synthesised by Primase enzyme

S strand is replicated continuously in 5'–3' direction observing the base pairing rule.

A=T
C≡G

To the 3' OH end of RNA primer proper base paired deoxy ribo nucleotides are added and direction of movement of new strand is in same direction as movement of replication fork. This newly synthesised strand is leading strand In mammals leading strand is produced by delta DNA Polymerase enzyme.



Replication of 3'–5' Parental strand

is brought upon by alpha DNA polymerase enzyme at the same time.

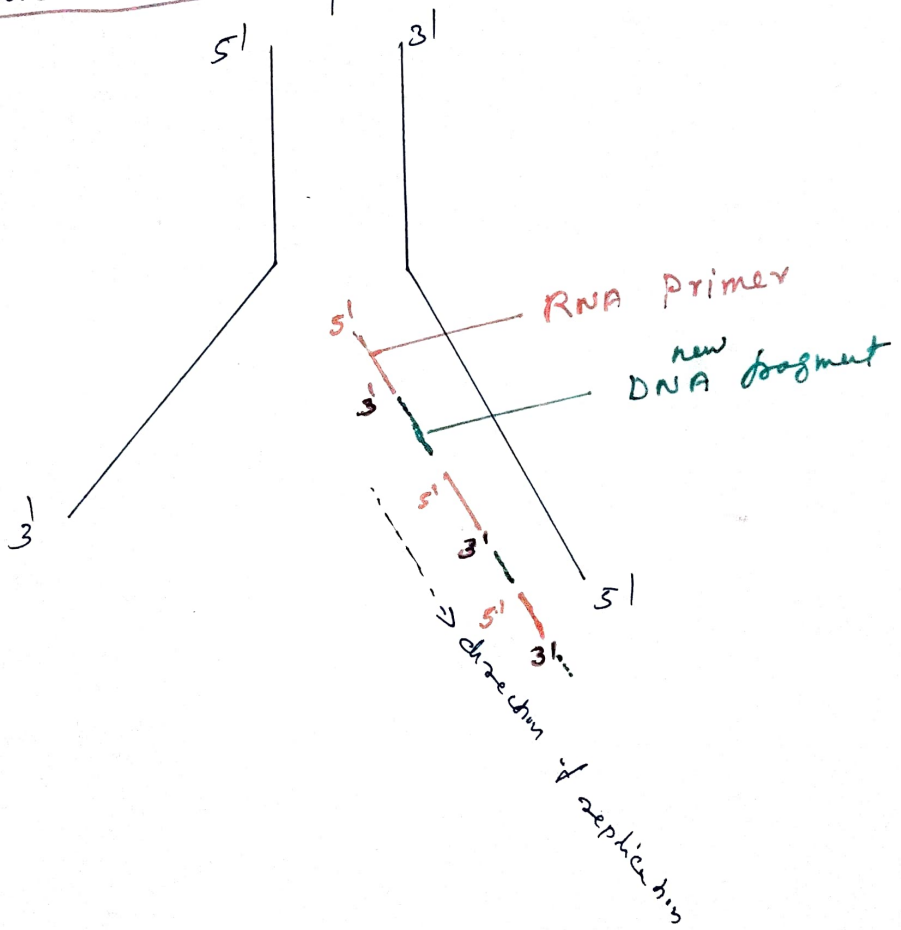
Process is slightly complicated

Initiation of this replication also occurs by the formation of a short RNA primer molecule. Primer RNA enters the nick site (formed by primase enzyme) to the 3' end of

To 3' end of Primer RNA molecule successive deoxyribonucleotides are added Complimentary to the template strand About 100-200 deoxyribo nucleotides are thus added. The pieces of DNA attached to RNA primer

are called Okazaki fragments. Several Okazaki pieces are produced.
(Okazaki is Japanese Biochemist who 1st isolated them)

RNA primers are then removed nucleotide by nucleotide by the same enzyme DNA polymerase which also has exonuclease activity.



Gap left by removal of RNA primer is sealed by proper base paired deoxyribonucleotides

no: of such newly synthesised DNA fragments (nascent DNA) are spliced together by DNA ligase

now a strand complimentary to the 3'-5' parental strand is produced. This is known as lagging strand.

Lagging strand is made

- thus continuously
- in short pieces
- in 5'-3' direction itself
- in direction opposite to the movement of replication fork.

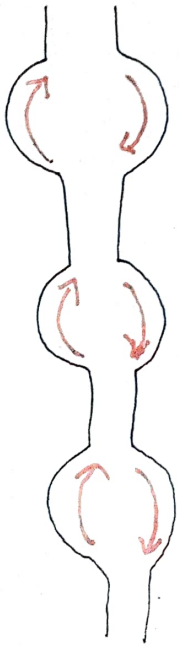
The fork opens further up & same process of replication can be repeated.

STEPS OF REPLICATION

1) Unwinding of parental DNA to form replication fork.
2) Synthesis of RNA primer (by primase enzyme) complementary to DNA template
3) Synthesis of leading strand in 5'—3' direction by delta polymerase enzyme
4) Synthesis of lagging strand as okazaki fragments in 5'-3' direction by alpha polymerase enzyme
5) Polymerisation completed by DNA ligase enzyme
6) Proof reading & Correction of replication errors.
7) Organisation of DNA into chromatin.

Replication bubble

Replication occurs in both direction along the chromosome & both strands are replicated simultaneously & this generates replication bubble.



Common replication errors.

1) Depurination
2) Alkylation of bases
3) formation of Pyrimidine Dimers C-C / T-C
4) Deamination of Cytosine & adenine bases (amino gp of cytosine & adenine removed)
5) Insertion or Deletion of nucleotides.

Proof reading or Editing function is done by DNA repair enzyme
mistakes recognised & Corrected.

repair enzymes are DNA polymerase I & II
in Prokaryotes
β DNA Polymerase
epsilon DNA polymerase } in Eukaryotes.

Inhibitors of replication

Nalidixic acid
Novobiocin
Ciprofloxacin } and bacterial agents inhibit replication in bacteria

6 mercapto purine
5 fluoro uracil (5FU)
Cytosine arabinoside } nucleoside analogues inhibit DNA replicating & used as anticancer agents in mammalian cells.