Molecular biology and bacterial genetics

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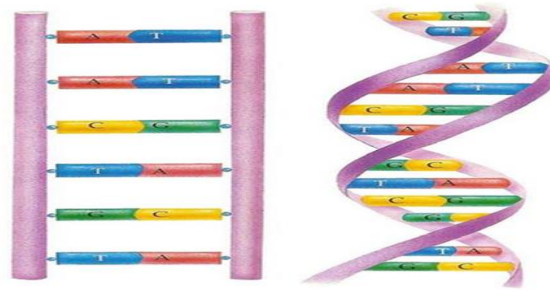

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The fidelity of DNA replication

Overall, replication results in an error rate of less than one mistake per billion nucleotides. How is this achieved? No single process could produce this level of accuracy**, a series of processes are required:**

1- first processes: DNA polymerases are very particular in pairing nucleotides **(nucleotide selection)** with their complements on the template strand. Errors in nucleotide selection by DNA polymerase arise only about once per 100,000 nucleotides.

2-second processes: Most of the errors that do arise in nucleotide selection are corrected in a second process called **proofreading.**

when a DNA polymerase inserts an incorrect nucleotide into the growing strand, the 3-OH group of the mispaired nucleotide is not correctly positioned for accepting the next nucleotide. The incorrect positioning stalls the polymerization reaction, and the 3→ 5 exonuclease activity of DNA polymerase removes the incorrectly paired nucleotide. DNA polymerase then inserts the correct nucleotide. Together, proofreading and nucleotide selection result in an error rate of only one in 10 million nucleotides.

3-A third process called **mismatch repair** corrects errors after replication is completed. Any incorrectly paired nucleotides remaining after replication produce a deformity in the secondary structure of the DNA; this recognized by enzyme that excise an incorrectly paired nucleotide and use the original nucleotide strand as a template to replace the incorrect nucleotide. **Mismatch repair requires the ability to distinguish between the old and the new strands of DNA** , because the enzymes need some way of determining which of the two incorrectly paired bases to remove.

Ex: In E.coli, methyl group (-CH3) are added to particular nucleotide sequences, but only after replication. Thus methylation lags behind replication: so, immediately after DNA synthesis, **only the old DNA strand is methylated.** Therefore it can be distinguished from the newly synthesised strand, and mismatch repair takes place preferentially on the unmethylated nucleotide strand.

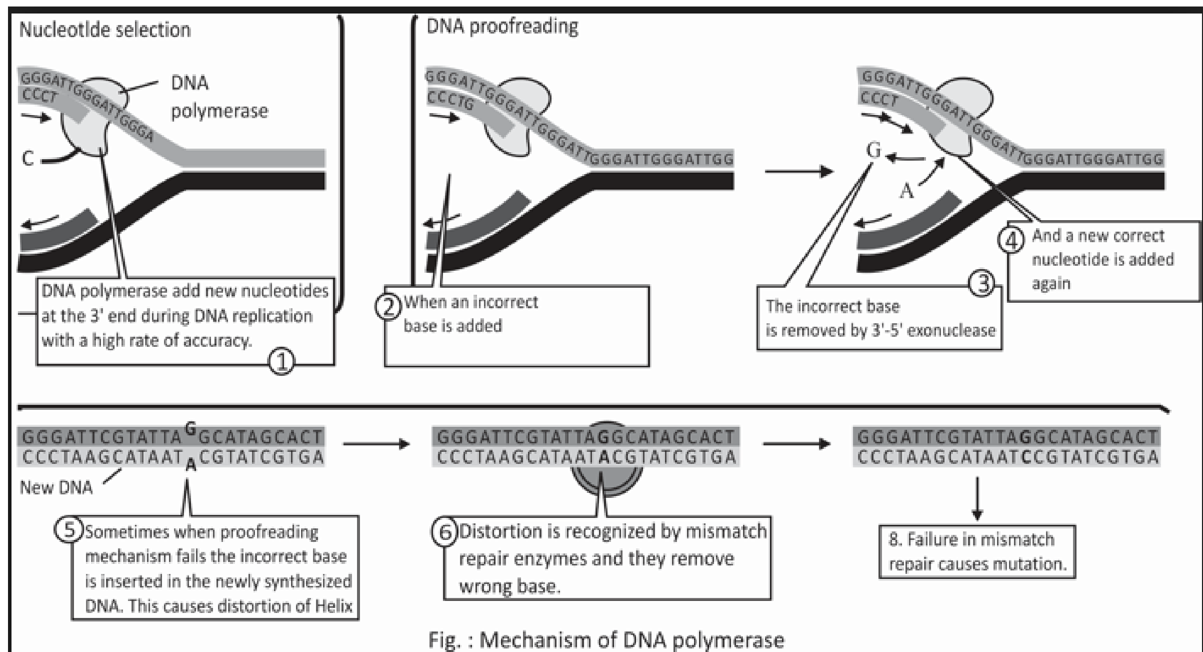


Fig. : Mechanism of DNA polymerase

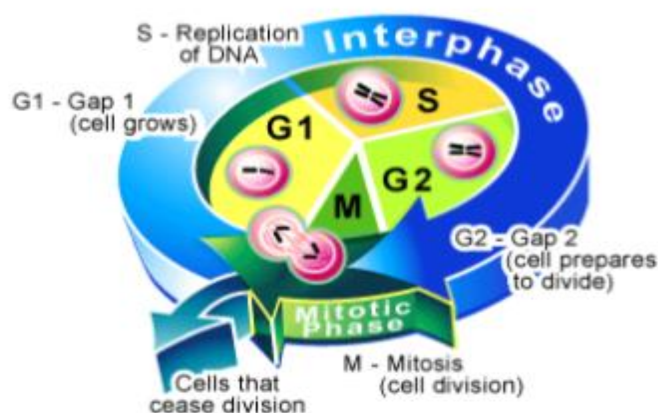
The differences between Eukaryotes and Prokaryotes

Replication is linked to cell division in all organisms; both prokaryote and eukaryote. Cell division in:

1-eukaryotes is carried out in the context of the cell cycle.

2- Unlike prokaryotes which can double under optimal conditions in as little as 20 min the eukaryotic cell cycle takes some 18 to 24 h to complete.

The cell cycle consists of a number of phases: G1, S, G2 and M.



• G1 = growth and preparation of the chromosomes for replication

• S = synthesis of DNA (and centrosomes), • G2 = preparation for M , • M = mitosis

G1, S and G2 are collectively referred to as interphase. If cells are no longer dividing, such as with terminally differentiated cells, they are considered to be in G0. As the cell passes through each phase of the cell cycle certain checks must be satisfied. At critical stages the level of special cytoplasmic proteins known as cyclins rise then subside once the cell has passed through the phase. There are also a group of enzymes known as cyclin dependent kinases (cdks) which phosphorylate protein targets involved in the control of the cell cycle.

Certain key indicators are used at each checkpoint. 1-A check for the completion of S phase (replication) is the presence of Okazaki fragments. If replication is complete there should be no Okazaki fragments.

2-DNA damage is checked for at G1 before the cell enters S**.**

3-Spindles are checked before the cell actually divides (M phase)

The similarities between prokaryotic and eukaryotic replication:

• Both are bi-directional processes

• DNA polymerases work 5' to 3'

• Leading and lagging strands

• Primers are required

The unique problems faced by eukaryotes that are not faced by prokaryotes:

1-Linear chromosomes with ends,

2- much more genetic material; the typical animal cell has 50 times more DNA than the average bacterium (*E. coli* has 4.6 million base pairs, humans have , 3 000 million base pairs or 650 times as much!!),

3- much more packaging; the nucleosomes and all higher order packing.

the DNA polymerases that are found in eukaryotes work much slower NOT faster!! At the rate they work it would take 30 days to copy the human genome if it was left to 2 replication forks

As with prokaryotes each origin of replication begins with the binding of a large **protein complex**; in the case of eukaryotes it is imaginatively named the Origin Recognition Complex (ORC). This complex will remain on the DNA throughout replication. **Other protein factors** then join the ORC and recruit proteins which coat the DNA. The next complex to associate is the **MCM complex,** This complex contains a helicase amongst other enzymes. This will be required to unbind the DNA. To facilitate the binding of the MCM complex, a helicase loader must also bind. once this has been completed we have a pre-replication complex.

These accessory proteins or licensing factors accumulate during G1 of the cell cycle. **The initiation complex** is now described as licensed to begin replication and also prevent re-initiation

The whole genome is not simultaneously replicated. It appears that clusters of 20 to 80 sites are initiated together. Forks extend from both sides of each initiation site and move in opposite directions until they fuse with an approaching fork from an adjacent site. New clusters are activated throughout S phase of the cell cycle and it takes several hours to copy the whole genome.

Summary of Proteins associated with DNA Replication in both Prokaryotes and Eukaryotes

Step in Replication	Prokaryotic cells	Eukaryotic cells
Recognition of origin of replication	Dna A protein	Unknown
Unwinding of DNA double helix	Helicase (requires ATP)	Helicase (requires ATP)
Stabilization of unwound template strands	Single-stranded DNA-binding protein (SSB)	Single-stranded DNA-binding protein (SSB)
Synthesis of RNA primers	Primase	Primase
Synthesis of DNA Leading strand Lagging strand	 DNA polymerase III DNA polymerase III	 DNA polymerase δ DNA polymerase α
Removal of RNA primers	DNA polymerase I (5 →3' exonuclease)	Unknown
Replacement of RNA with DNA	DNA polymerase I	Unknown
Joining of Okazaki fragments	DNA ligase (requires NAD)	DNA ligase (requires ATP)
Removal of positive supercoils ahead of advancing replication forks	DNA topoisomerase II (DNA gyrase)	DNA topoisomerase II

DNA synthesis at the ends of chromosomes

A fundamental difference between eukaryotic and bacterial replication arises because eukaryotic chromosomes are linear and thus have ends. As already stated, the 3-OH group needed for replication by DNA polymerases is provided at the initiation of replication by RNA primers that are synthesised by primase. This solution is temporary, because eventually the primers must be removed and replaced by DNA nucleotides.

In a circular DNA molecule, elongation around the circle eventually provides a 3-OH group immediately in front of the primer. After the primer has been removed, the replacement DNA nucleotides can be added to this 3-OH group.

In linear chromosome with multiple origins, the elongation of DNA in adjacent replicons also provides a 3-OH group preceding each primer. At the very of a linear chromosome, however, there is no adjacent stretch of replicated DNA to provide this crucial 3-OH group. Once the primer at the end of the chromosome has been removed, it cannot be replaced with DNA nucleotides, which produces a gap at the end of the chromosome, suggesting that the chromosome should become progressively shorter with each round of replication. The chromosome would be shortened each generation, leading to the eventual elimination of the entire telomere destabilization of the chromosome, and cell death. But chromosomes don't become shorter each generation and destabilize.

The ends of chromosomes (the telomeres),possess several unique features, one of which is the presence of many copies of a short repeated sequences. In the protozoan Tetrahymena, this telomeric repeat is CCCCAA, with the G-rich strand typically protruding beyond the C-rich strand.

The single stranded end of the telomere can be extended by **telomerase,** an enzyme with both a protein and an RNA component(also known as a ribonucleoprotein). The RNA part of the enzyme contains from 15 to 22 nucleotides that are complementary to the sequence on the G-rich strand. This sequence pairs with the overhanging 3— end of the DNA and provides a template for the synthesis of additional DNA copies of the repeats. DNA nucleotides are added to the 3— end of the strand one at a time and, after several nucleotides have been added , the RNA template moves down the DNA and more nucleotides are added to the 3-end. Usually, from 14 to 16 nucleotides are added to the 3-end of the G-rich strand.in this way the telomerase can extend the 3-end of the chromosome without the use of a complementary DNA template. How the complementary C-rich strand is synthesized . is not yet clear

It may be synthesized by conventional replication, with primase synthesizing an RNA primer on the 5- end of the extended (G-rich)template. The removal of this primer once again leaves a gap at the 5- end of the chromosome, but this gap does not matter, because the end of the chromosome is extended at each replication by **telomerase**; 1- no genetic information is lost; and

2- the chromosome does not become shorter overall. The extended single-strand end may fold back on itself, forming a terminal loop by nonconventional pairing of bases. This loop could provide a 3-OH group for the attachment of DNA nucleotides along the C-rich strand.

Telomerase is present in single-celled organisms, germ cells, early embryonic cells, and certain proliferative somatic cells (such as bone-marrow cells and cells lining the intestine), all of which must undergo continuous cell division. Most somatic cells have little or no telomerase activity, and chromosomes in these cells progressively shorten with each cell division.

These cells are capable of only a limited number of divisions; once the telomeres shorten beyond a critical point; a chromosome becomes unstable, has a tendency to undergo rearrangements, and is degraded. These events lead to cell death. The shortening of telomeres may contributes to the process of aging.

Note:

Genetically engineered mice that lack a functional **telomerase gene** (and therefore do not express telomerase in somatic or germ cells) experience progressive shortening of their telomeres in successive generations. After several generations, these mice show some sign of premature aging, such as graying , hair loss and delayed wound healing. Through genetic engineering , it is also possible to create somatic cells that express telomerase. In these cells, telomerase do not shorten, cell aging is inhibited, and the cells will divide indefinitely. **Telomerase** also appears to play a role in cancer. Cancer tumor cells have the capacity to divide indefinitely , and many tumor cells express the telomerase enzyme.