Organellar DNA :-

Organelles like mitochondrion and chloroplasts have their own genome and proteome, describe as mitochondrial DNA (mtDNA) (Reich and Luck, 1966) and chloroplast DNA (cpDNA) (Ris and plant, 1962), which resemble bacterial genome in several respects. DNA located in cytoplasmic organelles is known to control hereditary traits exhibiting non-Mendelian inheritance (vide chapter on cytoplasmic Inheritance).

Chloroplast DNA (cpDNA) :-

Each chloroplast contains 20-200 copies of a circular double stranded DNA molecule, which may also be linear as found in maize. cpDNA is characterized by the presence of three regions - i) two inverted repeats (IR), each 10-24 kb long and carrying ribosomal ge

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ii) a short single copy (SSC) sequence, 18-20 kb long,
iii) a long single copy (LSC) sequence. There are some open reading frames (ORF) with coding sequences begining with a met codon and a stop codon at the end of the cpDNA. Chloroplast DNA is relatively large ranging from 120-210 kb, which is comparable to the size of a large bacteriophage (110 kb). Total cpDNA in a cell may make up to 14% of cellular DNA.

Most chloroplast genome appear to possess the same set of genes. Each molecule of cpDNA is encoded with 110-120 genes and code for about 125 proteins. In maize the genes on cpDNA contains both small and large rRNA genes and sequence for tRNA. It also synthesizes mRNA for some proteins involved in photosynthesis like enzyme rubisco. Two and eight genes respectively for the polypeptides of PSI and PSII have been identified. Some herbicide resistant genes have also been located in cpDNA. A circular genetic map was proposed by Ruth Sagar (1972) in chlamydomonas. Chloroplast genetics in higher plants including crops like pea, maize, rice etc has also been studied. Bendich (2004) emphasized that cpDNA in plants is generally found as multigenomic complex and branched linear DNA molecules and not as monogenomic circular molecules.

Mitochondrial DNA (mt DNA) :-

Like chloroplast, mitochondria also contain 5-100 copies DNA molecules which are usually circular, but may essentially be also linear. Remarkable variation exists in the size of mtDNA, ranging from 6-2500 kb. mtDNA contributes only about less than 1% of cellular DNA except yeast (18%).

Mitochondrial genomes display greater variability in genome content. The cloning and sequencing of the entire mtDNA have now been made in several organisms

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including human. It contains genes for rRNA, tRNA, ribosome associated proteins and enzymes. A substantial fraction of mtDNA in yeast represents unidentified reading frames containing introns of split genes and appear to code for proteins required for mRNA splicing, called maturases.

In higher plants, smaller circular (sometimes linear also) DNA molecules present in addition to the main circular mt DNA. The master circular contains repetitive sequences and due to extensive recombination between these repeats, smaller molecules originate. One interesting feature of mtDNA is its unidirectional and highly asymmetric replication. The daughter L-strand starts synthesis when two-third of H-strand is already synthesized. Another interesting point of plant mtDNA is that it can move between organelles which is called promiscous DNA. mtDNA is associated with the male sterility character of plants as in maize.

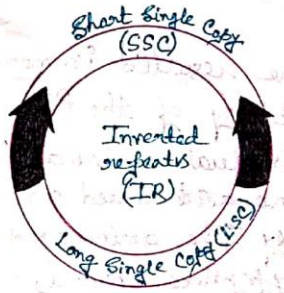


Fig → There characteristic regions in a cpDNA molecule.

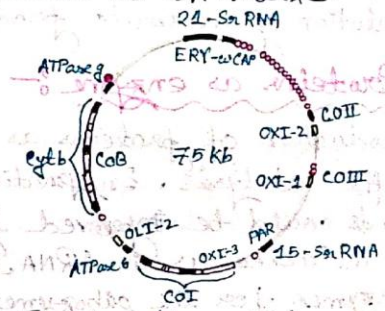


Fig → Genetic map of yeast (Saccharomyces cerevisiae) mitochondrial DNA. Black boxes show regions of mRNA or rRNA synthesis. Small circles are regions of tRNA synthesis (from P.K. Gupta)

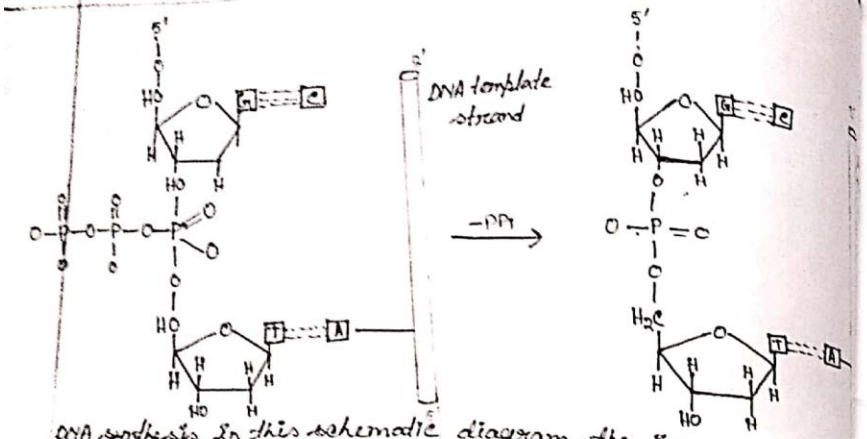
DNA Replication:-

- a DNA template is essential, to be copied by the DNA po-merase;
- a primer with a free 3'-OH that the enzyme can exte-

DNA polymerase-I is a template-directed enzyme, tha a recognizes the next nucleotide on the DNA template and th adds a complementary nucleotide to the 3'-OH to the primer creating a 3'5' phosphodiester bond, and releasing pyrophosphate The primer is extended in a 5'—3' direction.

DNA polymerase-I also corrects mistakes in DNA by re-oving mismatched nucleotides (i.e. it has proof-reading activ Thus, during polymerization, if the nucleotide that has just be incorporated is incorrect (mismatched), it is removed w a 3' → 5' exonuclease activity. This gives very high fidelity; error rate of less than 10^8 per base pair. DNA polymerase also has a 5' → 3' exonuclease activity; it can hydrolyze nuc acid starting from the 5' end of a chain. This activity p a key role in removing the RNA primer used during replic (see below). Thus, overall, DNA polymerase-I has three differ active sites on its single polypeptide chain; 5' → 3' poly-se, 3'-5' exonuclease and 5'-3' exonuclease. As well as role in DNA replication, DNA polymerase-I is involved i repair, for example, removing UV-induced alternations as pyrimidine dimers.

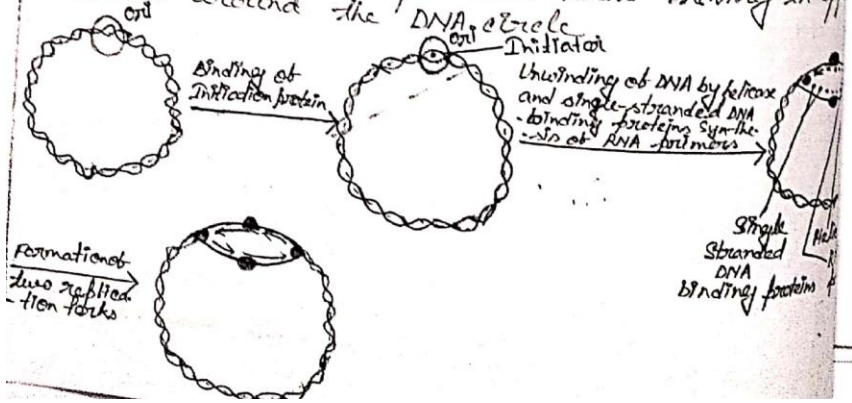
E. coli also contains two other DNA polymer DNA polymerase-II and DNA polymerase-III. As with polymerase-I, these enzymes also catalyze the tem-directed synthesis of DNA from deoxynucleotidyl 5'-osphates, need a primer with a free 3'-OH group, s-e DNA in the 5' → 3' direction, and have 3' → 5' exon activity. Neither enzyme has 5' → 3' exonuclease ac



DNA synthesis In this schematic diagram, the incoming dTTP bonds with the adenine on the template DNA strand and a 5'-3' phosphodiester bond is formed, releasing pyrophosphate.

Replication fork :-

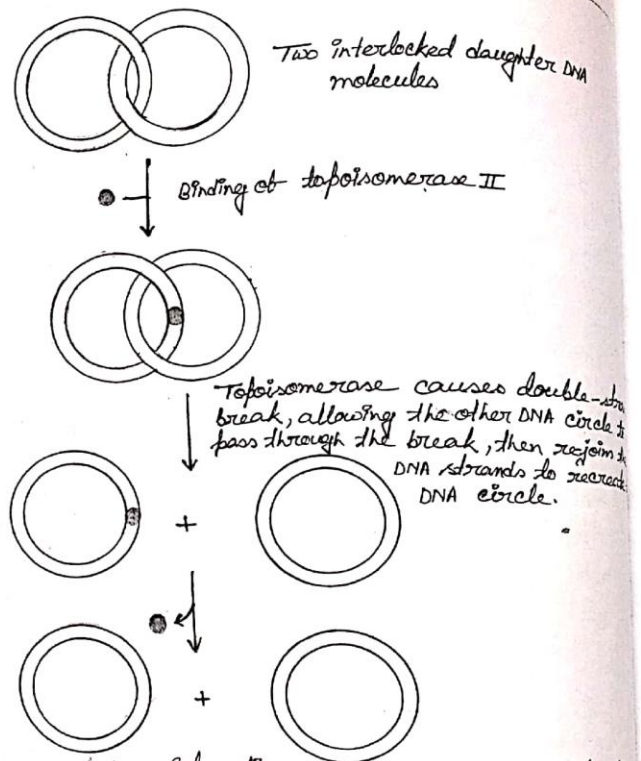
When the bacterial circular chromosome is replicated, replication starts at a single origin. The double helix open up and both strands serve as templates for the synthesis of new DNA. DNA synthesis then proceeds outward in both directions from the single origin (i.e.) it is bi-directional. The products of the process are two daughter double stranded DNA molecules each which has one original template strand and one strand of newly synthesized DNA. Thus replication is semi-conservative. The region of replicating DNA associated with the single origin is called a replication bubble or replication fork and consists of two replication forks moving in opposite directions around the DNA circle.



Accessory proteins :-

DNA polymerases I and III, primase and DNA ligase are not the only proteins needed for replication of the bacterial chromosome. The DNA template is a double helix with each strand wound tightly around the other and hence the two strands must be unwound during replication. How is this unwinding problem solved? A DNA helicase (Dna B helicase) is used to unwind the double helix (using ATP as energy source) and SSB (single-stranded DNA-binding) protein protects the single-stranded region from base pairing again so that each of the two DNA strand is accessible for replication. In principle, for a replication fork to move along a piece of DNA, the DNA helix would need to unwind ahead of it, causing the DNA to rotate rapidly. However, the bacterial chromosome is circular and so there are no ends to rotate. The solution to the problem is that an enzyme called topoisomerase I breaks a phosphodiester bond in one DNA strand (a single-strand break) a small distance ahead of the fork, allowing the DNA to rotate freely (swivel) around the other (intact) strand. The phosphodiester bond is then reformed by the topoisomerase.

After the bacterial circular DNA has been replicated, the result is two double stranded circular DNA molecules that are interlocked. Topoisomerase II separates them as follows. This enzyme works in a similar similar manner to topoisomerase I but causes a transient break in each strand (a double-strand break) of a double stranded DNA molecule. Thus topoisomerase II binds to one double-stranded DNA circle and causes a transient double-strand break that acts as a 'gate' through which the other DNA circle can pass. Topoisomerase II then reseals the strand breaks.



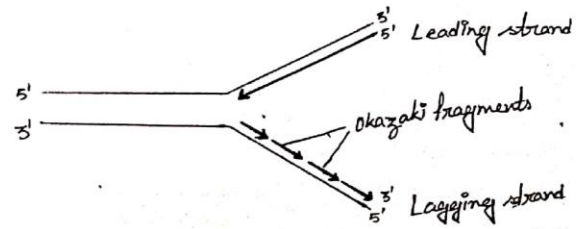
Multiple replicons:-

In eukaryotes, replication of chromosomal DNA occurs only in the S phase of the cell cycle. As for bacterial DNA, eukaryotic DNA is replicated semi-conservatively. Replication of each linear DNA molecule in a chromosome starts at many origins, one every 30-300 kb of DNA depending on the species and tissue, and proceeds bi-directionally from each origin. The use of multiple origins is essential in order to ensure that the large amount of chromosomal DNA in a eukaryotic cell is replicated within the necessary time period. At each origin, a replication bubble forms consisting of two replication forks moving in opposite directions. The DNA replicated under the control of a single origin is called a replicon.

-ichia coli cells

Okazaki fragments:-

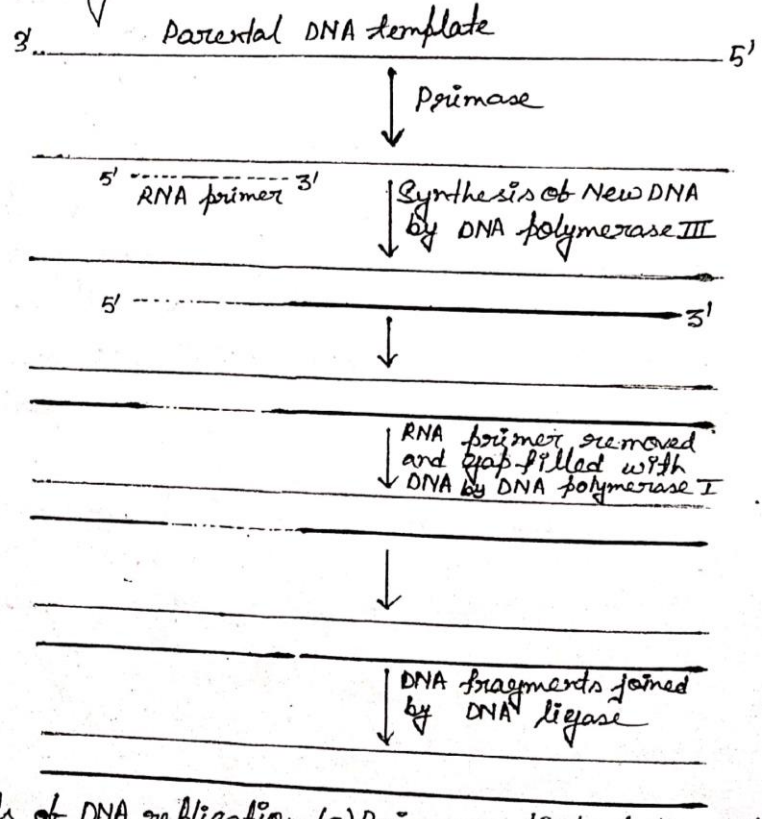
Double-stranded DNA is antiparallel. One strand runs 5'→3' and the complementary strand runs 3'→5'. As the original double-stranded DNA opens up at a replication fork, new DNA is made against each template strand. Superficially, therefore, one might expect new DNA to be made 5'→3' for one daughter strand and 3'→5' for the other daughter strand. However, all DNA polymerases make DNA only in the 5'→3' direction and never in the 3'→5' direction. What actually happens is that on the template strand with 3'→5' orientation, new DNA is made in a continuous piece in the correct 5'→3' direction. This new DNA is called the leading strand. On the other template strand (that has a 5'→3' orientation), DNA polymerase synthesizes short pieces of new DNA (about 1000-2000 nucleotides long) in the 5'→3' direction and then joins these pieces together. The small fragments are called Okazaki fragments after their discover. The new DNA strand which is made by this discontinuous method is called the lagging strand.



Synthesis of DNA at a replication fork. As the parental DNA (thin lines) opens up, each of the two parental strands acts as a template for new DNA synthesis (thick lines). The leading strand is synthesized continuously but the lagging strand is synthesized as short (Okazaki) DNA fragments that are then joined together.

RNA primer :-

DNA polymerase cannot start DNA synthesis without a primer. Even on the lagging strand, each Okazaki fragments requires an RNA primer before DNA synthesis can start. The primer used in each case is a short piece of RNA and is synthesized by an RNA polymerase called primase. Primase can make RNA directly on the single-stranded DNA template because, like all RNA polymerases it does not require a primer to begin synthesis. The primer made by primase is then extended by DNA polymerase III. DNA polymerase III synthesizes DNA for the leading and lagging strand. After DNA synthesis by polymerase III, DNA polymerase I uses its 5' → 3' exonuclease activity to remove the RNA primer and then fills the gap with new DNA. DNA polymerase III cannot carry out this task because it lacks the 5' → 3' activity of DNA polymerase I. Finally, DNA ligase joins the ends of the DNA fragments together.



Details of DNA replication. (a) Primase binds to the DNA template strand (solid line) and (b) synthesizes a short RNA primer (dotted line); (c) DNA polymerase III now extends the RNA primer by synthesizing new DNA (thick line).

DNA synthesis proceeds until replication bubbles merge together.

All of the regions of a chromosome are not replic-ted simultaneously. Rather, many replication eyes wi be found in one part of the chromosome and none in another section. Thus replication origins are activated in clusters, called <u>replication units</u>, consisting of 20 origins. During S phase, the different replication uni are activated in a set order untill eventually the whole chromosome has been replicated. Transcription-lly-active DNA appears to be replicated early in S ph whilst chromatin that is condensed and not transcri-tionally active is replicated later.

Five DNA polymerase:-

Eukaryotic cells contain five different DNA po-merases; α, β, γ, δ and ε. The DNA polymerase involved in replication of chromosomal DNA are α and δ. DNA polym-rases β and ε are involved in DNA repair. All of these polymerases except DNA polymerase γ are located in th nucleus. DNA polymerase γ is found in mitochondria an replicates mitochondrial DNA.

Leading and lagging strands:-

The basic scheme of replication of do-stranded chromosomal DNA in eukaryotes follows that bacterial DNA replication; a leading strand and a lagg strand are synthesized, the latter involving discontinuo synthesis via Okazaki fragments. The RNA primers require are made by DNA polymerase α which carries a <u>primas subunit</u>. DNA polymerase α initiates synthesis of the la-ng strand, making first the RNA primer and then exten-g it with a short region of DNA. DNA polymerase δ th synthesizes the rest of the Okazaki fragment. The lead strand is synthesized by DNA polymerase δ. The δ enz has 3'-5' exonuclease activity and so can proof-read