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DNA Replication | Module – 1

Phase	Enzyme/ proteins	Role	Diagrams
Initiation	Initiator proteins	Recognises and joins to the ori region (usually AT rich), and recruits the rest of the replication machinery.	
	Helicase	Separates the two strands of the double helix (breaks hydrogen bonds)	
	Topoiso-merase (or gyrase in bacteria)	Relieves the mechanical stress due to supercoiling of DNA (that builds up by unwinding) by breaking backbone, passing the other strand through the break and resealing (covalent bonds) hence two coils become one. -Topoisomerase inhibitors prevent DNA rep. by accumulating supercoiling ahead of the rep. fork, and blocking it.	
	Single stranded binding protein	Keeps the DNA region unwound	
Elongation	Primase	Synthesis short RNA molecules (type of RNA polymerase) which contain 8-10 nucleotides homologous to the template strand. It is recruited to the region unwound by helicase and laid down 5' to 3' on both strands (provides 3' OH gp to attach to 5' C of DNA nucleotide)	
	DNA Polymerase I	Replaces RNA primers with DNA nucleotides (shows exonuclease property- cuts DNA nucleotides one at a time 5'-3')	
	DNA Polymerase III	-Main Replication polymerase that elongates the nucleotide strand from 3' OH group provided by RNA primer. - release pyrophosphate -Two molecules work simultaneously and interact with each other to ensure that two strands replicate together. -Proof reading mechanism	

	DNA Poly E	Leading strand of eukaryote, with proof reading mechanism	
	DNA pol d	Lagging strand of eukaryote, with proof reading mechanism	
	DNA Ligase	Joins Okazaki fragments by sealing the breaks in sugar- phosphate backbone of newly synthesised lagging strand.	
	Exonuclease	Cut DNA nucleotides one at a time from ends	
	Endonuclease	Cut DNA molecule internally	

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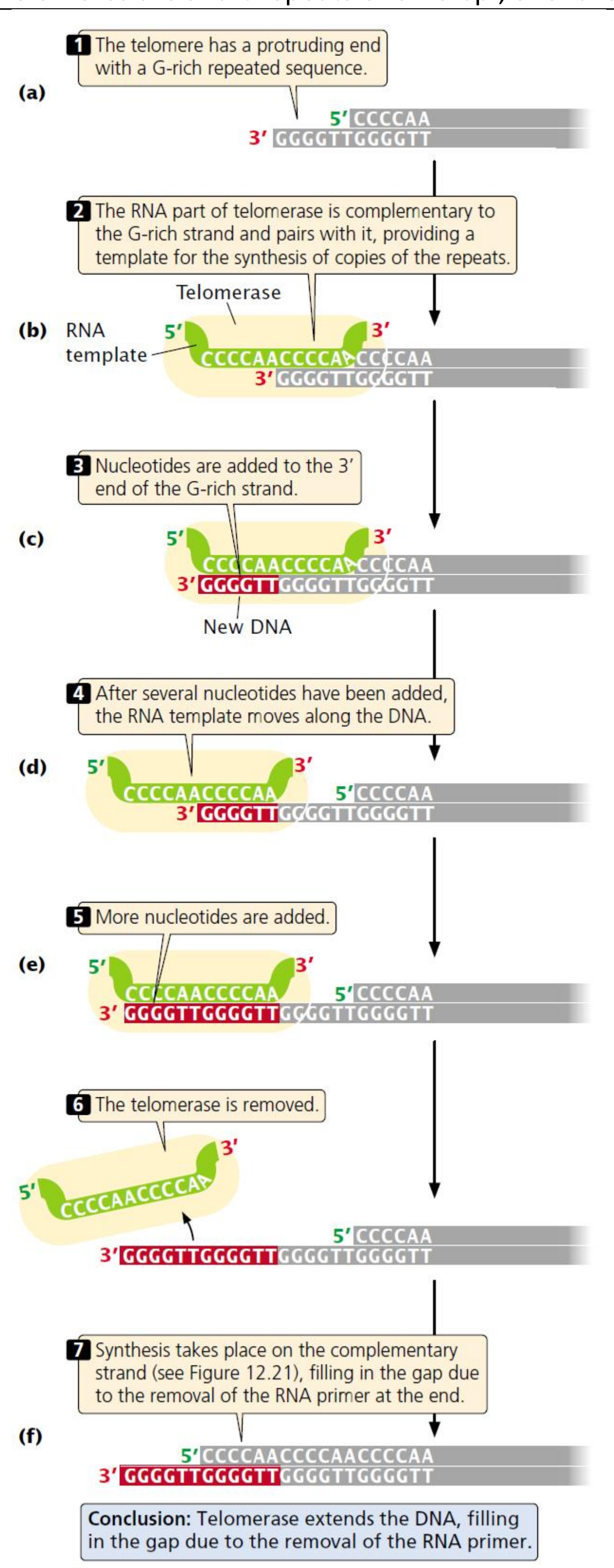
TELOMERASE activity:

DNA polymerase can only elongate from a free 3′ -OH end, the replication of the leading strand occurs continuously but for the lagging strand, it occurs by a back-stitching mechanism as RNA primers provide the 3′ -OH group at regular intervals as the replication fork opens. Hence, the last RNA primer will provide a free 3′ -OH group to synthesize DNA but since the primers are later removed, since no free 3′ -OH is present to replace the last primer in linear chromosomes, bits of its end are lost with every replication.

- The chromosome would be shortened each generation, leading to the eventual elimination of the entire telomere, destabilization of the chromosome, and cell death.

Telomeres are short repeats of 6-10 bp., 3′ end is G-rich repeats.



Telomerase: enzyme that extends chromosomal ends by adding telomere repeat sequences as a **reverse transcriptase**. It uses an RNA template to synthesize the complementary strand.

1. Telomerase recognizes the tip of an existing G-rich repeat system at the end of chromosome, and the RNA part of enzyme attaches to it.
2. Using the RNA template within the enzyme, it elongates the parental strand in 5′ → 3′ direction.
3. It adds additional repeats to the 3′ end as it moves down the leading strand.
4. The lagging strand is then completed by DNA polymerase α which carries a DNA primase as one of its subunit.
5. Its DNA Primase subunit produces an RNA Primer, which is then elongated and eventually removed.
6. A gap is left when this RNA primer is removed, however it does not matter because,
7. The original information at the ends of linear chromosomes is completely copied in the new DNA, no genetic information is lost.

However, this is not a problem for circular DNA.

- Somatic cells: little/ no telomerase activity

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.

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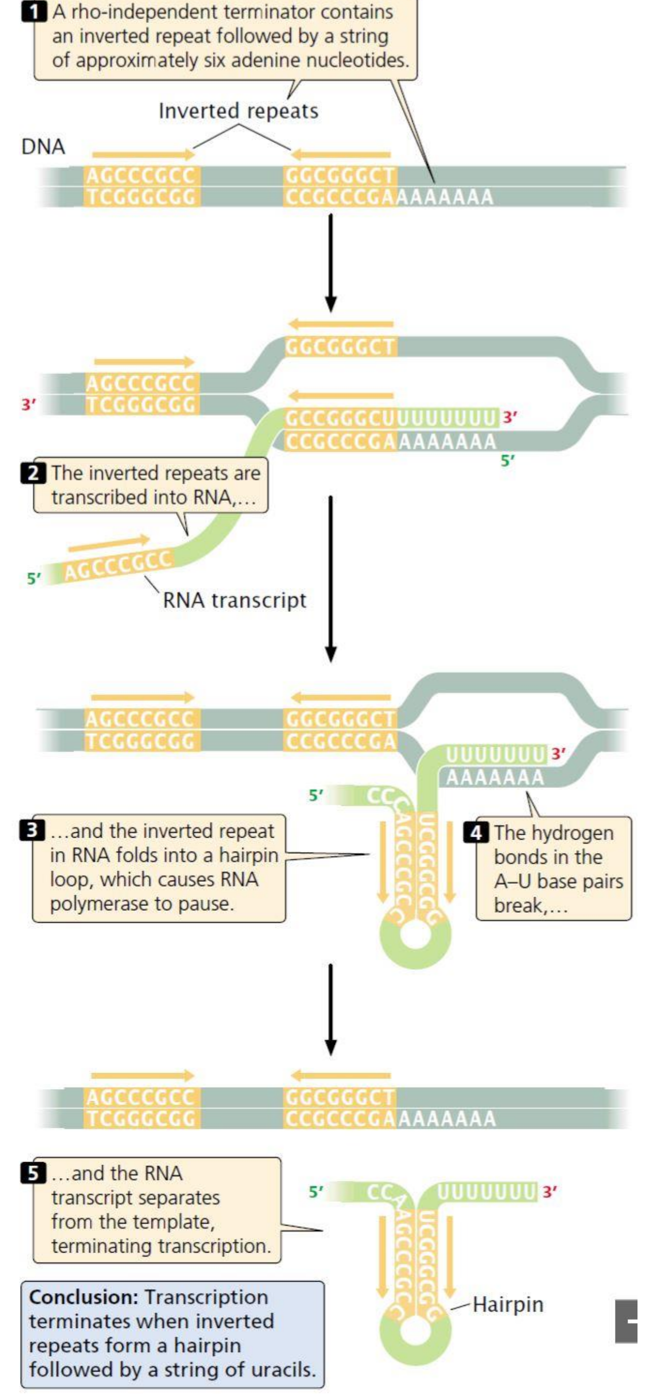
1.) contain inverted repeats, when inverted repeats have been transcribed into RNA, a hairpin secondary structure forms.
2.) string of adenines follows the second inverted repeat in the template DNA → transcribed to string of Uracils after the hairpin in synthesised RNA.
The hairpin loop:
- slows down RNA polymerase,
- destabilizes the DNA-RNA pairing, causing both to dissociate apart.
The A-U base-pairing downstream of the hairpin loop are relatively unstable and weaker bonds a.c.t. other pairs, therefore, they easily break apart.



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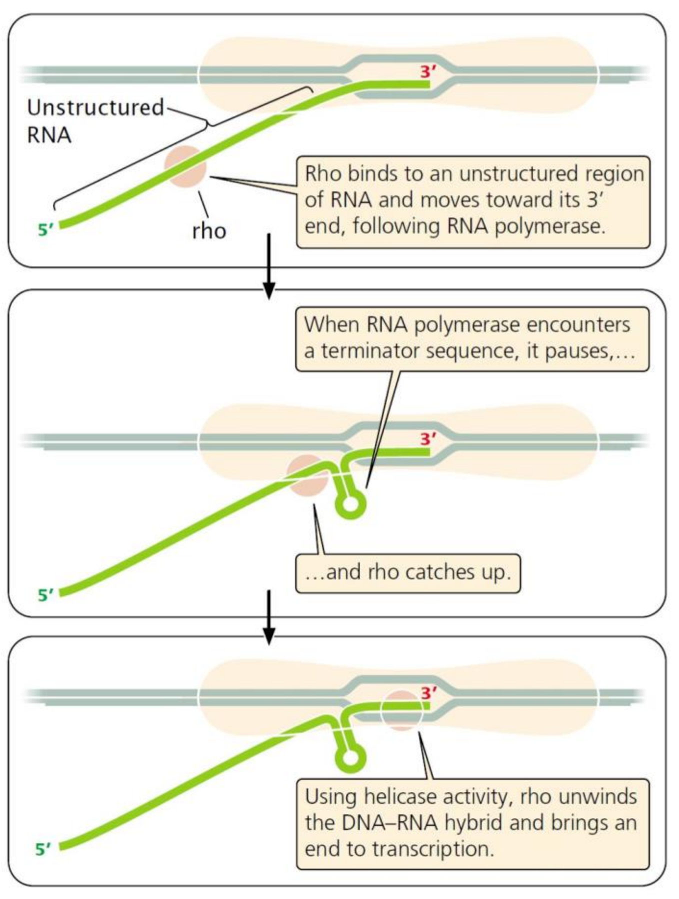
1.) DNA sequences that slows polymerase down and produces a pause in transcription;
2.) a DNA sequence that encodes a stretch of RNA upstream of the terminator that is devoid of any secondary structures. This
This Unstructured region is the binding site for rho protein on RNA.
This moves forward towards its 3’ region. Since RNA was paused by the terminator sequence, it allows rho protein to catch up.
The rho protein has helicase activity, which it uses to unwind the RNA–DNA hybrid in the transcription bubble, bringing an end to transcription.
NO hairpin structure necessary!!!



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