الوراثة الجزيئية

Molecular Genetics

برنامج التكنولوجيا الحيوية الزراعية(الوراثة)

المستوى الرابع

(أجبارى)

المحاضرة: (7)

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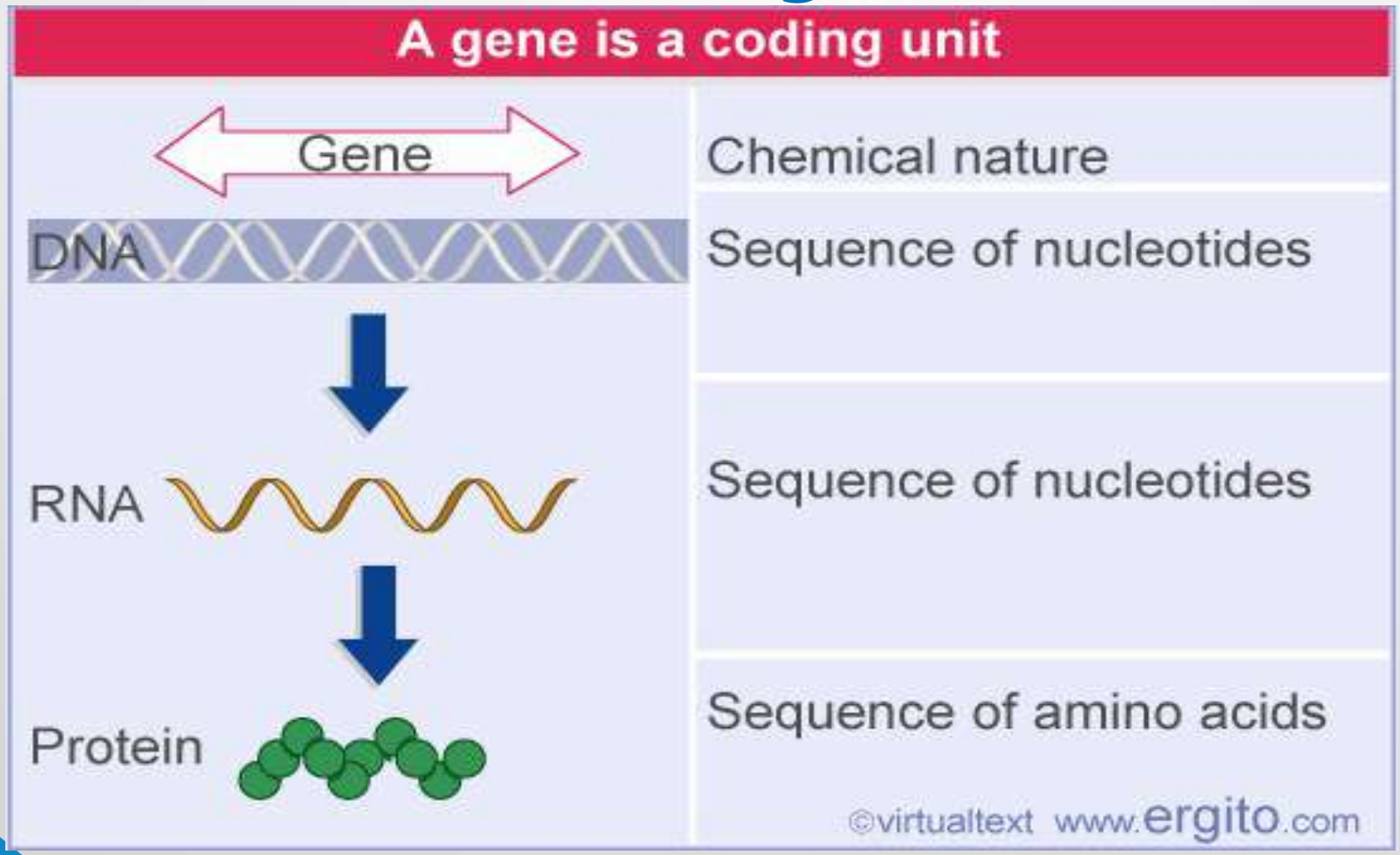
Reverse Transcription

Part-4

Central Dogma

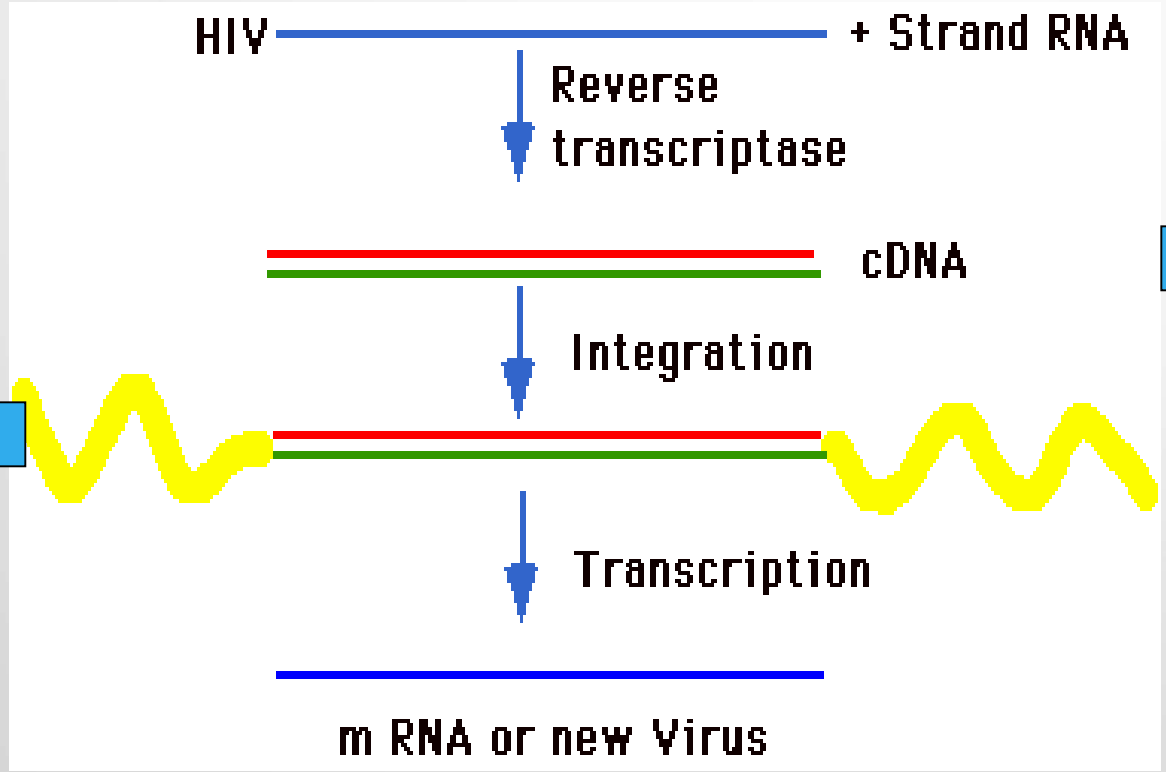


- Normal transcription involves synthesis of RNA from DNA.
- Reverse transcription is the transcription of single stranded RNA into double stranded DNA.
- With the help of the enzyme Reverse Transcriptase.

- Reverse Transcriptase also known as RNA directed DNA Polymerase.
- - DNA Nucleotidyl transferase (RNA directed)
- - Revertase.
- Reverse Transcriptase was discovered by Howard Temin and Baltimore in 1970 independently .
- - shared Nobel Prize in Physiology or Medicine in 1975 for their discovery.

- Reverse transcriptase common in Retrovirus.
- - HIV.
- M-MLV (Moloney Murine Leukemia Virus).
- AMV (Avian Myeloblastosis Virus).
- Reverse Transcriptase enzyme includes two activity: DNA polymerase and RNase H .

Retrovirus Replication Cycle



Packaging into virus ; budding

Antiviral drug

- Zidovudine
- Lamivudine
- Tenfovir

- Typical retrovirus has three or four genes.
- Gag-pol- env.
- Retrovirus are called + strand because viral RNA itself code for protein products.
- Reverse transcriptase enzyme code for proteins are called – strand.
- R : Redundant or repeating seq – 10-80.
- U5: 80 – 100.
- U3: 170-1350.
- Like DNA polymerase, Reverse transcriptase requires primers.
- tRNA of the host is Primer.

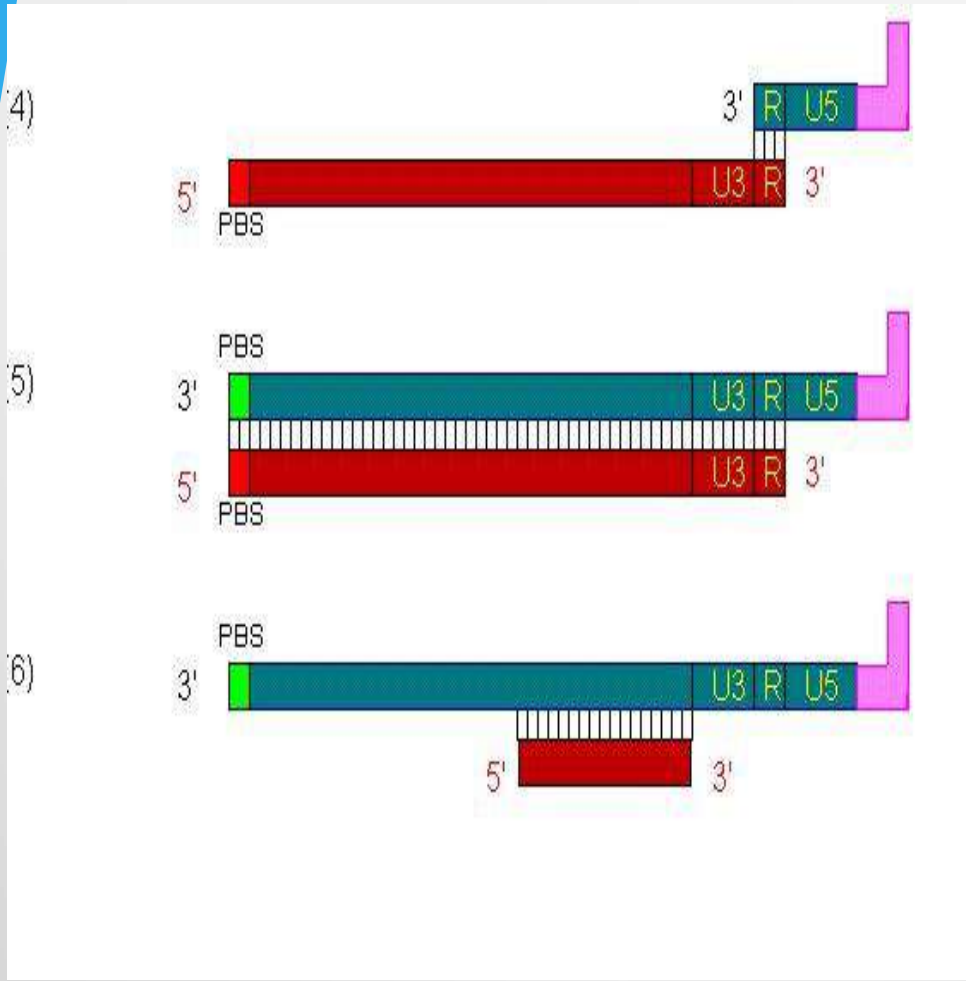
MECHANSIM OF RETROVIRUS REPLICATION

1. A Retrovirus specific cellular **tRNA** hybridizes with a complementary region called **PBS** (Primer Binding Sites)

2. **Reverse Transcriptase (RT) starts at this binding site and copies RNA into a single strand of complementary DNA. A DNA segment is extended from tRNA based on the sequence of the retroviral genomic RNA**

3. The viral R and U5 sequences are removed by **RNase H.**

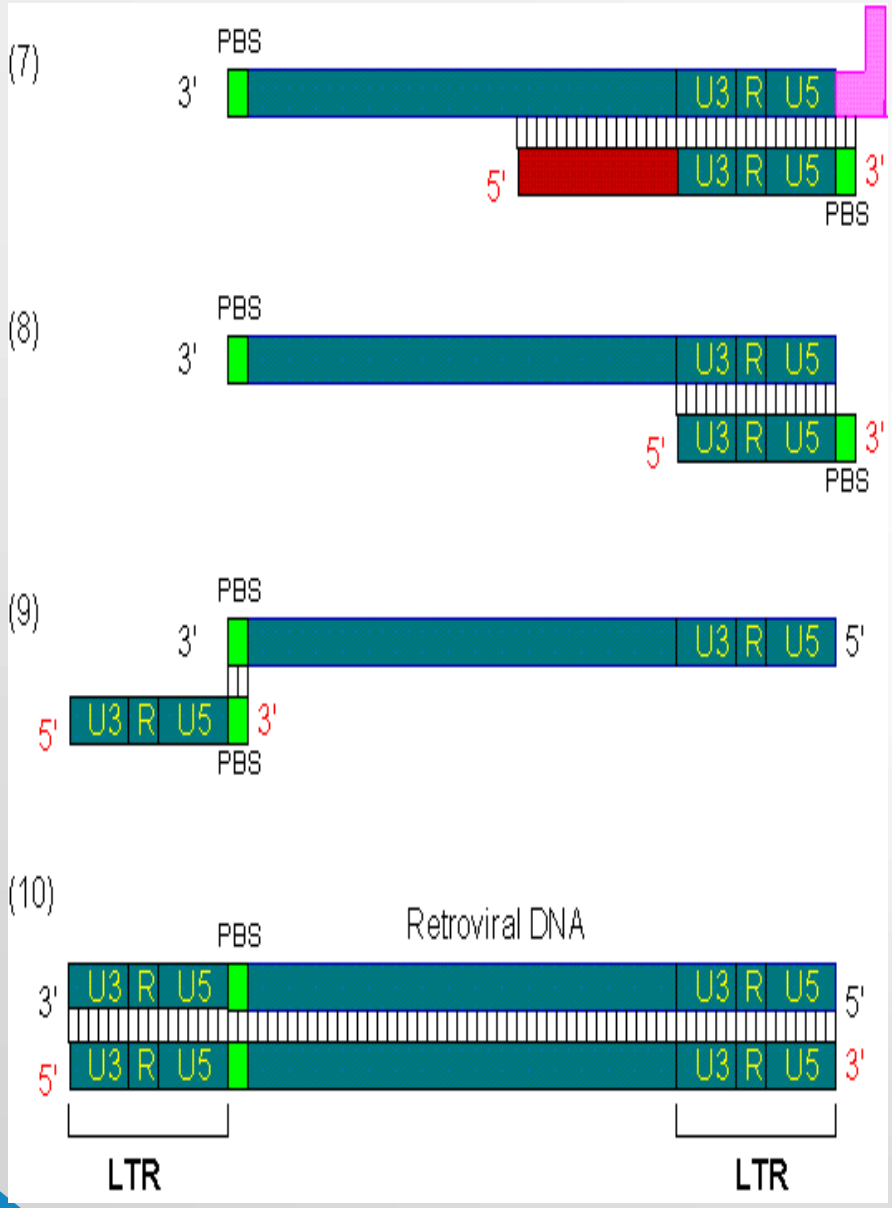
LTR – Long Terminal Repeat
Left LTR - Redundant sequence [R]+ 5′ untranslated region (U5)
Right LTR - Redundant sequence [R]+ 3′ untranslated region (U3)



4. **First jump**: DNA hybridizes with the remaining R sequence at the 3' end.

5. A DNA strand is extended from the 3' end.

6. Most viral RNA is removed by **RNase H**



7. A second DNA strand is extended from the viral RNA.

8. Both tRNA and the remaining viral RNA are removed by RNase H.

9. **Second jump**: The PBS region of the second strand hybridizes with the PBS region of the first strand.

10. Extension on both DNA strands.

- Reverse transcriptase has a high error rate when transcribing RNA into DNA as unlike DNA Polymerase, since it has no proof reading ability.
- This high error rate allows mutations to accumulate.
- The commercially available RT produced by *Promega* are quoted high error rates in range of one in 17,000 bp for AMV and one in 30,000 bases for M-MLV.

cDNA

- Reverse Transcriptase can use single stranded RNA into DS DNA – cDNA.
- cDNA library: A set of clones representing as many as possible of the mRNAs in a given cell type at given time.
- In the preparation cDNA library, mRNA is extracted, purified, and treated with the enzyme reverse transcriptase.
- Complementary DNA (cDNA) analogs of the isolated mRNA are thereby obtained.
- Since mature mRNA contains no introns or regulatory regions, a cDNA library composed of coding regions.

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