# VIVEKANANDA COLLEGE
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## NAAC ACCREDITED 'A' GRADE




Topic:Replication of RNA genome

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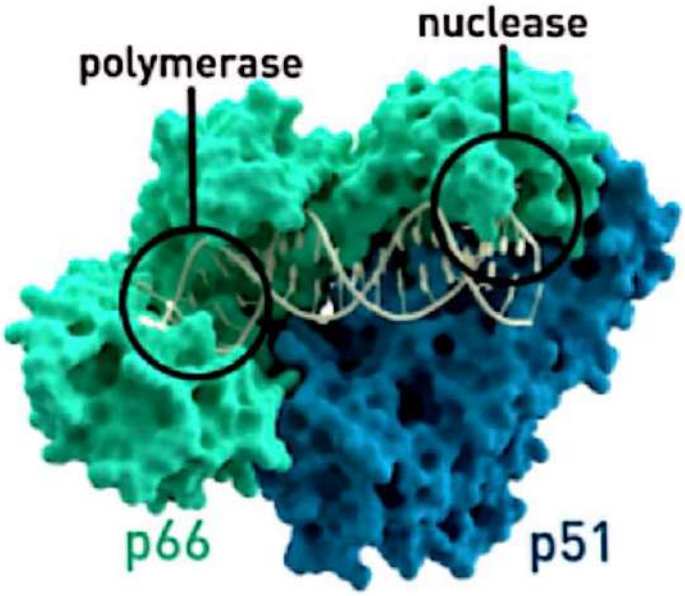
## The Replication of RNA Genomes

Viruses also reproduce, but they cannot do so on their own. That is why they cannot be called "alive" in the strictest sense of the word. They use the replication apparatus of the host cells, and have additionally developed a number of special characteristics. Scientists differentiate viruses according to the genome type — there are DNA and RNA viruses: viruses may have single-stranded or double-stranded linear RNA, single-stranded or double-stranded linear DNA, single-stranded or double-stranded circular DNA and other variations. Some viruses contain some of the enzymes required for their replication, for example the influenza virus, whose envelope not only contains an RNA genome but also an RNA polymerase. When the virus enters the host cell, the enzyme RNA polymerase starts to replicate the viral genome. The synthesis of the genome of DNA viruses usually begins at a replication origin that binds specific initiator proteins, which recruit replication enzymes of the host cell which then replicate the viral genome.

The HI virus is a retrovirus and thus a very exotic case. The virus got its name due to the fact that it reverses the normal process of transcribing DNA into RNA (transcription) during reproduction. The virus has a single-stranded RNA genome and an enzyme called reverse transcriptase. This enzyme copies the single-stranded RNA genome into a complementary DNA molecule, thereby enabling the integration of the viral genome into the host DNA. Once the viral genome is integrated into the host genome, it can be transcribed into RNA by the host enzymes at which point it can reproduce. Since viruses are able to use a broad range of replication mechanisms to reproduce, scientists working on the development of anti-viral drugs need to specifically investigate the individual viruses one by one. Many of the currently used drugs interfere with viral replication, for example the so-called nucleoside analogues which are used against the hepatitis B virus.

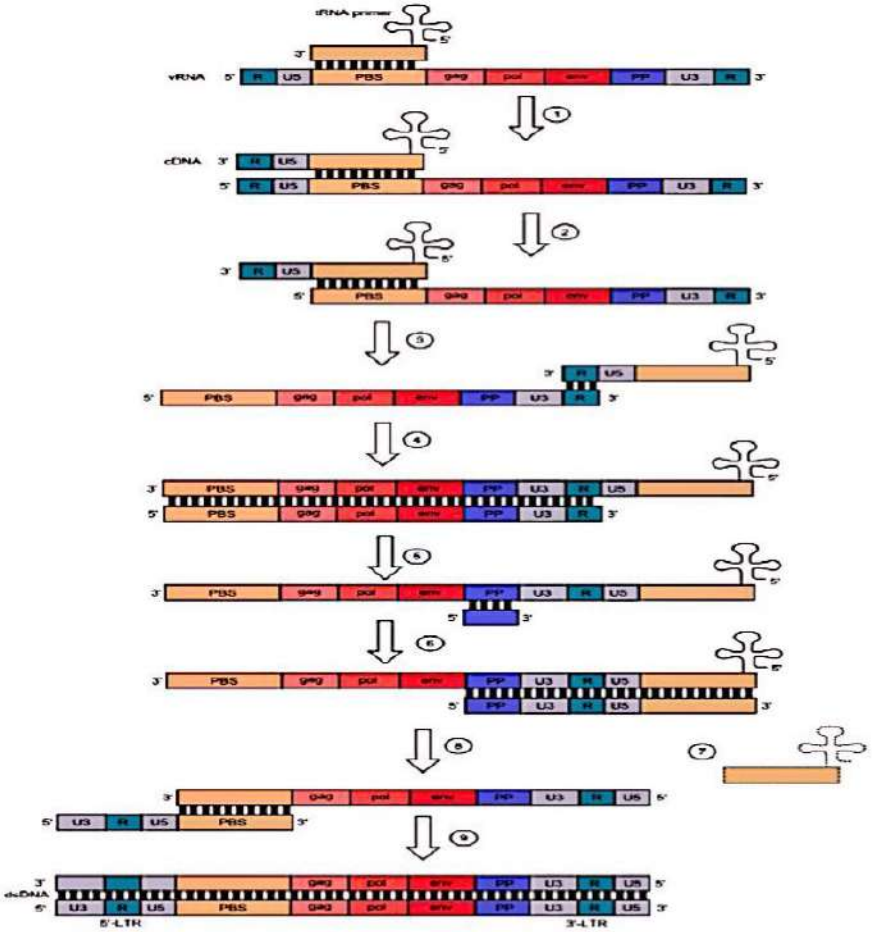



A reverse transcriptase (RT) is an enzyme used to generate complementary DNA (cDNA) from an RNA template, a process termed reverse transcription. Reverse transcriptases are used by retroviruses to replicate their genomes, by retrotransposon mobile genetic elements to proliferate within the host genome, by eukaryotic cells to extend the telomeres at the ends of their linear chromosome, and by some non-retroviruses such as the hepatitis B virus, a member of the Hepadnaviridae, which are dsDNA-RT viruses.
Retroviral RT has three sequential biochemical activities: RNA-dependent DNA polymerase activity, ribonuclease H (RNAse H), and DNA-dependent DNA polymerase activity. Collectively, these activities enable the enzyme to convert single-stranded RNA into double-stranded cDNA. In retroviruses and retrotransposons, this cDNA can then integrate into the host genome, from which new RNA copies can be made via host-cell transcription. The same sequence of reactions is widely used in the laboratory to convert RNA to DNA for use in molecular cloning, RNA sequencing, polymease chain reaction (PCR), or genome analysis.

The enzymes are encoded and used by viruses that use reverse transcription as a step in the process of replication. Reverse-transcribing RNA viruses, such as retroviruses, use the enzyme to reverse-transcribe their RNA genomes into DNA, which is then integrated into the host genome and replicated along with it. Reverse-transcribing DNA viruses,

# Process of reverse transcription or retrotranscription

Reverse transcriptase creates double-stranded DNA from an RNA template.
In virus species with reverse transcriptase lacking DNA-dependent DNA polymerase activity, creation of double-stranded DNA can possibly be done by host-encoded DNA polymerase δ, mistaking the viral DNA-RNA for a primer and synthesizing a double-stranded DNA by similar mechanism as in primer removal, where the newly synthesized DNA displaces the original RNA template.
The process of reverse transcription, also called retrotranscription or retrotras, is extremely error-prone, and it is during this step that mutations may occur. Such mutations may cause drug resistance.




Retroviral reverse transcription: Mechanism of reverse transcription in HIV. Step numbers will not match up.

Retroviruses, also referred to as class VI ssRNA-RT viruses, are RNA reverse-transcribing viruses with a DNA intermediate. Their genomes consist of two molecules of positive-sense single-stranded RNA with a 5' cap and 3' polyadenylated tail. Examples of retroviruses include the human immunodeficiency virus (HIV) and the human T-lymphotropic virus (HTLV). Creation of double-stranded DNA occurs in the cytosol[6] as a series of these steps:

- Lysyl tRNA acts as a primer and hybridizes to a complementary part of the virus RNA genome called the primer binding site or PBS.
- Reverse transcriptase then adds DNA nucleotides onto the 3' end of the primer, synthesizing DNA complementary to the U5 (non-coding region) and R region (a direct repeat found at both ends of the RNA molecule) of the viral RNA.
- A domain on the reverse transcriptase enzyme called RNAse H degrades the U5 and R regions on the 5' end of the RNA.
- The tRNA primer then "jumps" to the 3' end of the viral genome, and the newly synthesised DNA strands hybridizes to the complementary R region on the RNA.
- The complementary DNA (cDNA) added in (2) is further extended.
- The majority of viral RNA is degraded by RNAse H, leaving only the PP sequence.
- Synthesis of the second DNA strand begins, using the remaining PP fragment of viral RNA as a primer.
- The tRNA primer leaves and a "jump" happens. The PBS from the second strand hybridizes with the complementary PBS on the first strand.
- Both strands are extended to form a complete double-stranded DNA copy of the original viral RNA genome, which can then be incorporated into the host's genome by the enzyme integrase.

Creation of double-stranded DNA also involves strand transfer, in which there is a translocation of short DNA product from initial RNA-dependent DNA synthesis to acceptor template regions at the other end of the genome, which are later reached and processed by the reverse transcriptase for its DNA-dependent DNA activity.

Retroviral RNA is arranged in 5' terminus to 3' terminus. The site where the primer is annealed to viral RNA is called the primer-binding site (PBS). The RNA 5'end to the PBS site is called U5, and the RNA 3' end to the PBS is called the leader. The tRNA primer is unwound between 14 and 22 nucleotides and forms a base-paired duplex with the viral RNA at PBS. The fact that the PBS is located near the 5' terminus of viral RNA is unusual because reverse transcriptase synthesize DNA from 3' end of the primer in the 5' to 3' direction (with respect to the newly synthesized DNA strand). Therefore, the primer and reverse transcriptase must be relocated to 3' end of viral RNA. In order to accomplish this reposition, multiple steps and various enzymes including DNA polymerase, ribonuclease H(RNase H) and polynucleotide unwinding are needed.[8][9]

The HIV reverse transcriptase also has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA, as well as DNA-dependent DNA polymerase activity that copies the sense cDNA strand into an antisense DNA to form a double-stranded viral DNA intermediate (vDNA)