
بسم الله الرحمن الرحيم

Lentivirus As Gene Transferring Agents

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Lentiviruses

- A genus of retroviruses
- Cause long-lasting and lethal diseases characterized by long incubation periods, in the mammalian species and human.
- The well-known **lentivirus** is the (HIV) Human Immunodeficiency Virus, which causes AIDS.
- Lentiviruses are also reported in goats, horses, cows, sheep and cats. Lentiviruses as well as their host have distribution worldwide.
- Lentiviruses can incorporate a major amount of viral cDNA into the DNA of the host cell and can infect non-dividing cells.
- So lentiviruses are the most capable technique for gene delivery.
- The lentivirus is a part of retroviridae family of viruses, with HIV-1 being the most widely studied.
  - Members of this family contain +ssRNA that is reverse transcribed into DNA and integrated into the host cell's genome.

The Lentivirus Genome Map and Structure

- HIV lentiviral RNA genome contains three major genes; *gag*, *pol* and *env*.

- The *gag* gene
  - encodes
    - matrix proteins which are necessary for virion assembly and infection of non-dividing cells.
    - Capsid (CA) proteins, which form the hydrophobic core of virion.
    - Nucleocapsid (NC) proteins, which protect the viral genome by coating and associating tightly with viral RNA in virions.
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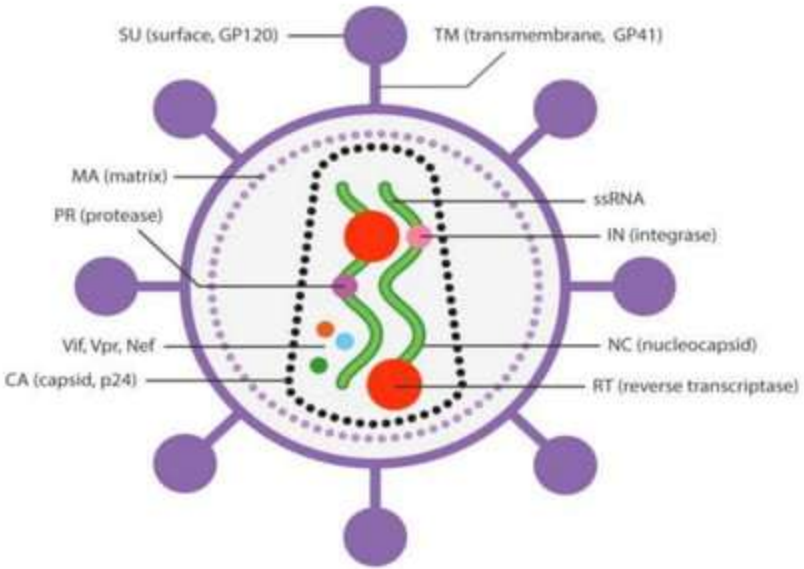
- The *pol* gene
  - encodes for
    - Viral protease (PRO), reverse transcriptase (RT) and integrase (IN), enzymes essential for viral replication.
- The *env* gene
  - codes for
    - surface glycoproteins that determine tropism and enable entry into the cell.

The Lentivirus Genome Map and Structure (Cont)

- The lentivirus genome also contains two important regulatory genes, *tat* and *rev*
  - that activate viral transcription as well as 4 accessory genes (*vif, vpr, vpu,* and *nef*),
  - these are less essential for virus replication in host cell.

The Lentivirus Genome Map and Structure (Cont)



The Recombinant Lentivirus System

- Integration, replication, and packaging of lentiviruses
  - are facilitated partially by
    - cis-actingRNA or DNA sequences.
    - These DNA sequences do not encode proteins.
    - Cis-acting elements such as the LTRs and ψ signals are essential in the design of recombinant lentiviral vectors
    - and are generally included in the transfer plasmid,
    - While the trans-acting viral elements encode regulatory, structural and accessory proteins.

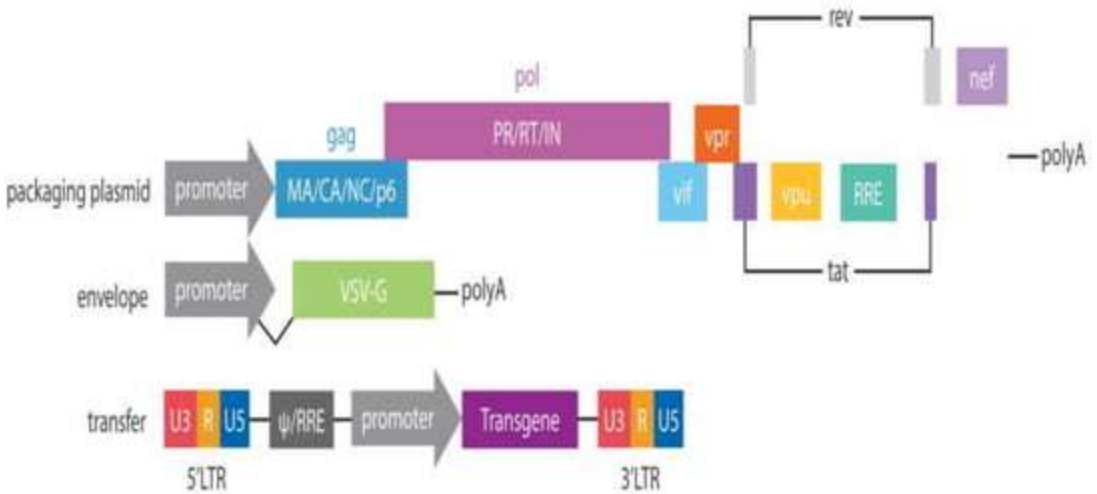
Generations of Recombinant Lentiviral Packaging System

- In effort to reduce the biosafety risk, three generations of recombinant lentiviral packaging system have been developed:

First generation recombinant lentiviral vectors

- The first generation recombinant lentiviral system splits the viral genome into three separate plasmids:
  - (a) a packaging plasmid;
  - (b) an Env plasmid encoding the viral glycoprotein; and
  - (c) a transfer vector genome construct.
- The transfer plasmid encodes for proteins necessary for packaging, reverse transcription and integration, but not for the expression of HIV proteins. In this way, the genomic components responsible for packaging the viral DNA are separated from the genomic components that activate them. Thus, the packaging sequences will not be integrated into the viral genome and the virus will not reproduce after it has infected the host cell.

First generation recombinant lentiviral vectors (Cont)



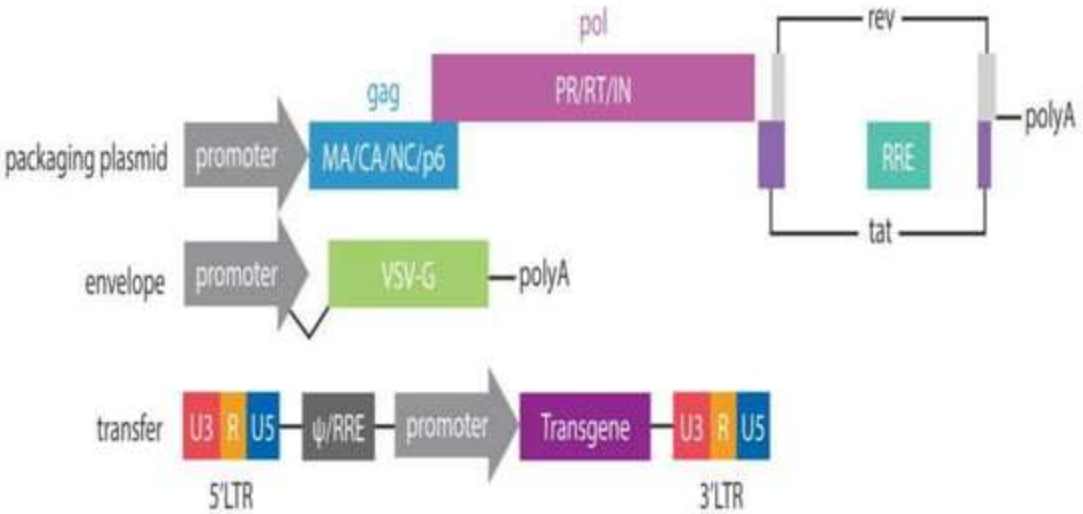
Second generation recombinant lentiviral vectors

- The first generation recombinant lentiviral vectors are no longer commonly in use due to biosafety risks.
- This encouraged the progress of a safer, second generation recombinant system.
- In this system, the genomic components encoding viral accessory proteins (*Vif, Vpu, Vpr or Nef*) are removed.
- These accessory proteins are important for HIV propagation in primary cells or in vivo but not essential for lentiviral vector functions.
- The second generation recombinant system splits essential components of the lentiviral system across three plasmids that are delivered separately for safety.

Second generation recombinant lentiviral vectors (Cont)

- The transfer plasmid;
  - encodes for
    - Trsansgene:
      - Contains cis-acting elements such as the 5' and 3' LTRs essential for promoting.
    - RNA polymerase II:
      - To begin transcription of viral mRNA and the ψ sequences;
      - Which signals genome packaging.
- The packaging plasmid:
  - is provided in trans and encodes only the essential trans-acting genes; *gag, pol, tat,* and *rev*
  - *that are required for entry and integration of viral genome.*
- *The envelop plasmid contains gene*
  - *encoding for envelop proteins.*

Second generation recombinant lentiviral vectors (Cont)



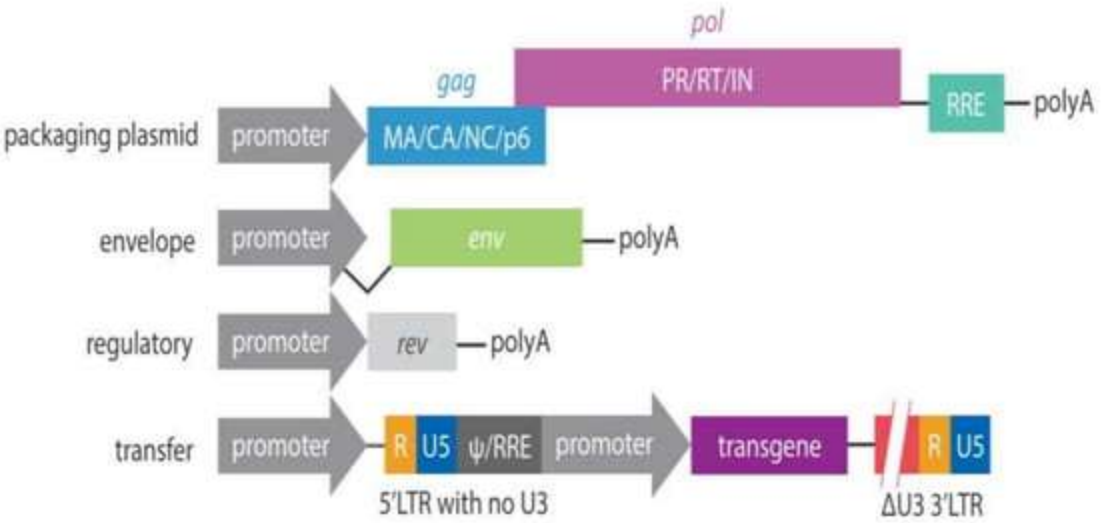
Third generation recombinant lentiviral vectors

- To almost completely eliminate dangerous lentiviral recombination events an even safer, third generation system was created.
- The third generation recombinant lentiviral vector system has four plasmids:
  - A packaging construct containing only *gag* and *pol* genes;
  - A plasmid expressing only *Rev*;
  - An *Env* plasmid;
  - A transgene plasmid.

Third generation recombinant lentiviral vectors (Cont)

- In addition, the LTR nearby the transgene are further modified as they contain enhancer and promoter region that can active adjacent cellular proto-oncogenes.
- By removing this promoter and enhancer region, U3, from the 3' LTR a replication incompetent "self-inactivating" (SIN) system is created as this deletion is transferred to 5' LTR after reverse transcription and integration. The U3 of the 5' LTR is also replaced with a CMV promoter to eliminate the need for the transcription trans activator, *Tat*.

Third generation recombinant lentiviral vectors (Cont)




THANK YOU