Replication of Negative Strand RNA Viruses (VSV)

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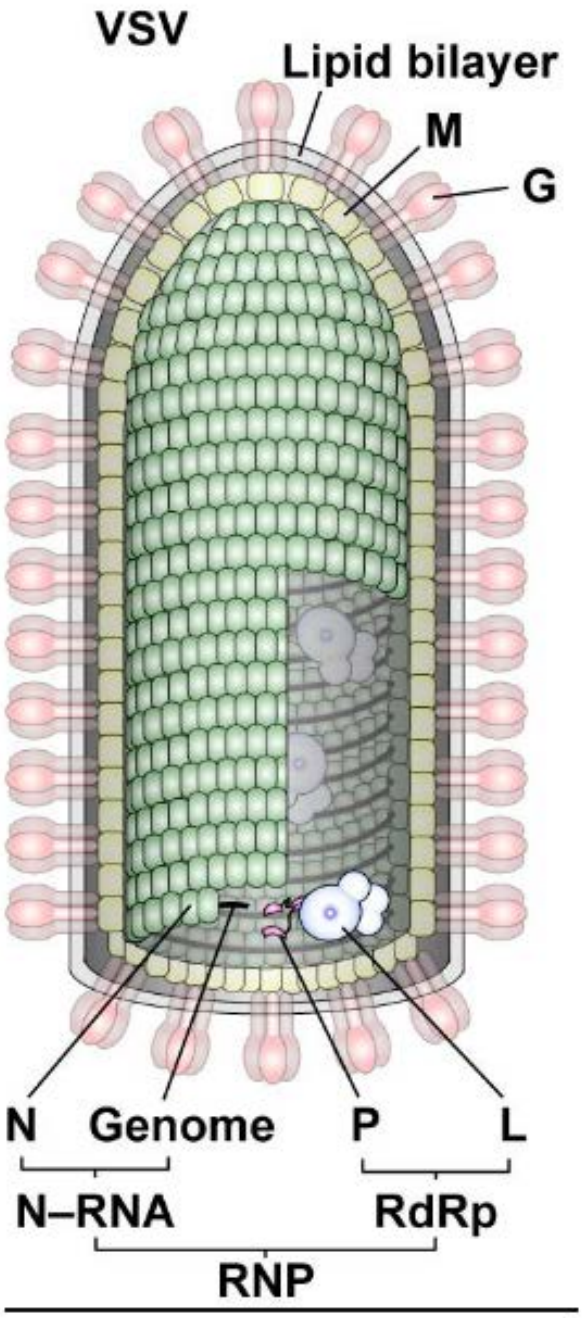
Vesicular stomatitis virus (VSV)

- Negative-strand RNA (NS RNA) viruses, which may have segmented or nonsegmented genomes, are to be blamed for plentiful of grave viral diseases such as measles, rabies, influenza, Crimean-Congo hemorrhagic fever, Ebola, and Lassa fevers in both humans and animals.
- Over the past 40 years, vesicular stomatitis virus (VSV), closely related to rabies virus, has served as a paradigm to study the fundamental molecular mechanisms of transcription and replication of Non-segmented negative strand (NNS) RNA viruses.
- Vesicular stomatitis Indiana virus [hereafter simply called vesicular stomatitis virus (VSV)] is an arthropod-borne animal virus belonging to the *Vesiculovirus* genus in the *Rhabdoviridae* family.
- A bullet-shaped VSV particle contains a single-strand RNA genome of 11,161 nucleotides (nt), which is encapsidated with the nucleo- (N) proteins to form a helical nucleocapsid (called the N–RNA complex/template).
- An RNA-dependent RNA polymerase (RdRp) complex is composed of the catalytic large (L) protein and its co-factor phospho-(P) protein, and is associated with the N–RNA complex to assemble a ribonucleoprotein (RNP) complex.
- In the virus particle, the RNP complex is coated with a layer composed of the matrix (M) proteins, which is further wrapped by a lipid bilayer envelope studded with the glyco- (G) proteins.

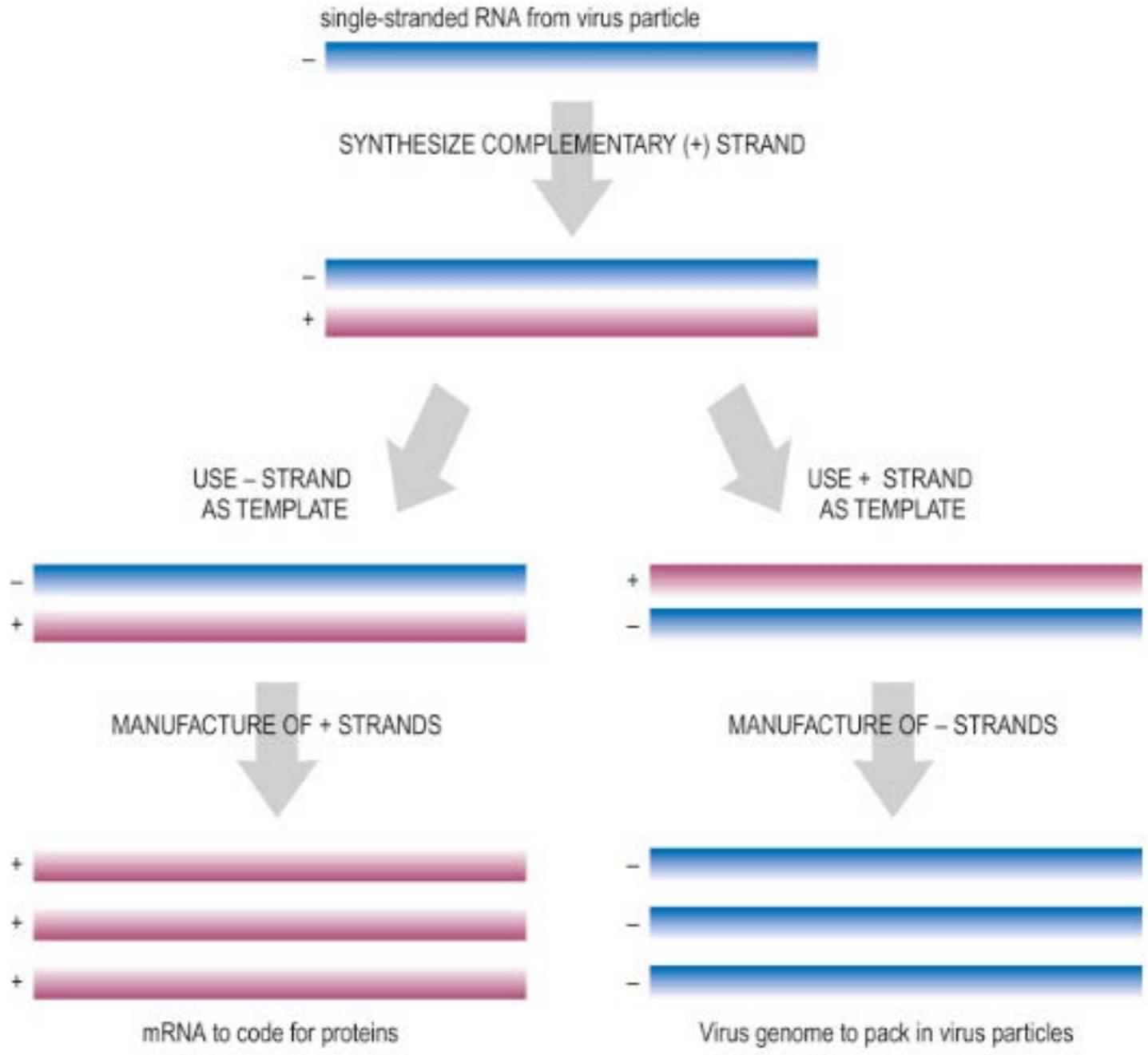

VSV
Lipid bilayer
M
G
N
Genome
P
L
N–RNA
RdRp
RNP

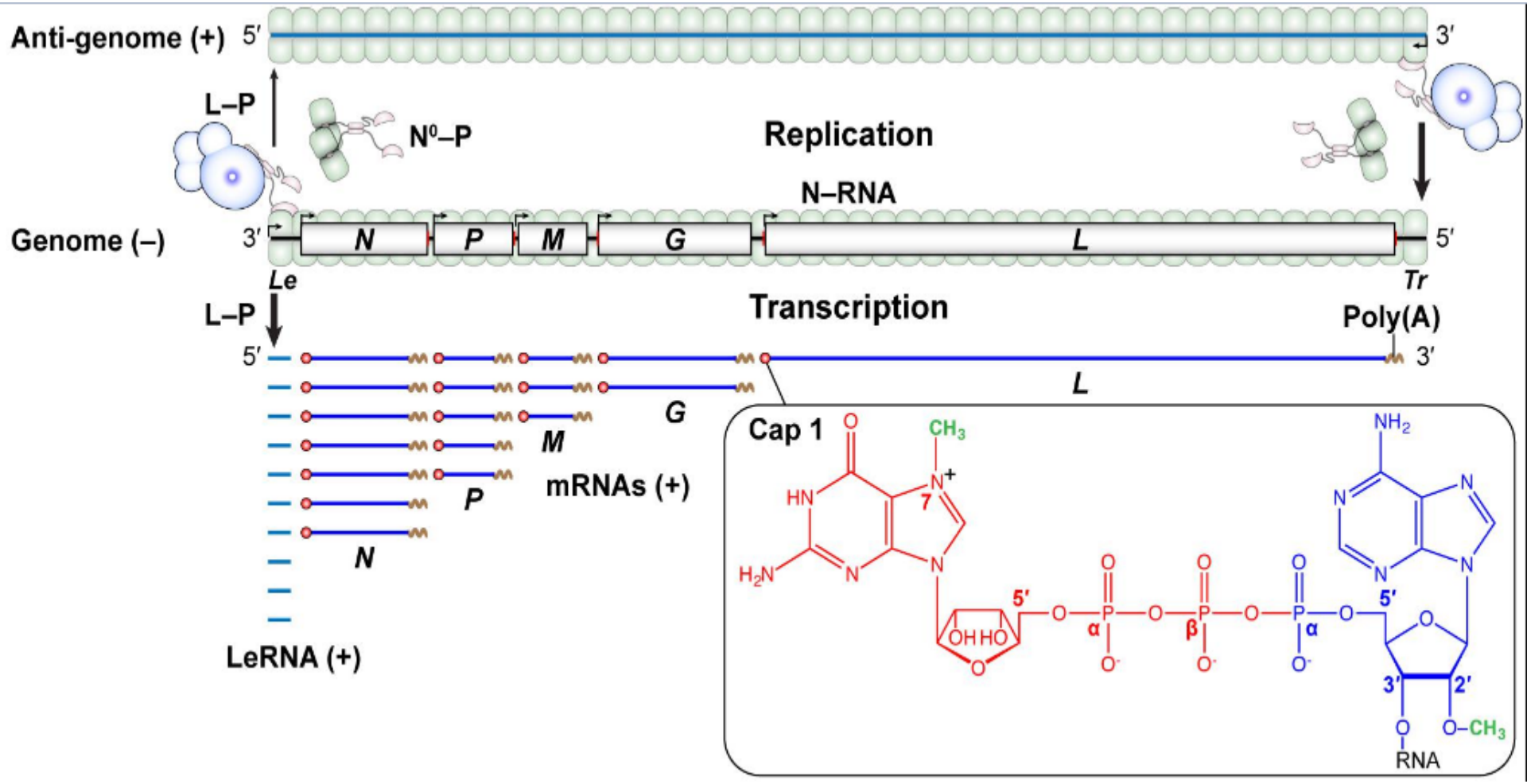
Transcription and Replication of the VSV Genome

- The VSV genome consists of five structural genes, *N, P, M, G*, and *L*, which are arranged in tandem from the 3′- to 5′-end.
- Other NNS RNA viruses share the same gene organization with VSV, but have diversified their structural genes and often acquired additional structural and/or non-structural genes during evolution.
- Despite vastly different primary structures of P proteins and their counterparts , these RdRp co-factors may play similar roles in transcription and replication.
- L proteins are the most conserved proteins among NNS RNA viruses, and catalyze all enzymatic reactions required for viral RNA synthesis and processing.
- VSV packages all enzymes required for primary transcription into virions, including RdRp, capping enzyme, Mtases (methyltransferase), and poly(A) polymerase activities.
- The negative-strand VSV genome begins and ends with the short 3′-leader (*Le*) and 5′-trailer (*Tr*) sequences, respectively, and contains the five internal genes that are tandemly connected via intergenic regions.

...Transcription and Replication of the VSV Genome

- The negative strand of RNA has a sequence complementary to the coding strand.
- Therefore, viruses that use this type of genome must synthesize the complementary plus strand upon entry into the host cell.
- The plus RNA strand first transcribed by viral RdRp, can then be used as a template to manufacture more viral genomes (right side).
- The negative RNA strand is then free to manufacture more copies of the plus strand (left side). These act as mRNA and direct viral protein synthesis.
- Each gene begins with the gene-start sequence and ends with the gene-end sequence, which play critical roles in transcription initiation/capping and termination/polyadenylation, respectively.
- A series of mRNAs made from the various genes of the virus.
- Once mRNA for viral RdRp is made in this pri transtription process, leads to the formation of many positive strand RNA mols which translates to viral proteins.


single-stranded RNA from virus particle
–
SYNTHESIZE COMPLEMENTARY (+) STRAND
–
+
USE – STRAND AS TEMPLATE
USE + STRAND AS TEMPLATE
–
+
+
–
MANUFACTURE OF + STRANDS
MANUFACTURE OF – STRANDS
+
+
+
–
–
–
mRNA to code for proteins
Virus genome to pack in virus particles


Anti-genome (+)
5′
3′
L–P
N⁰–P
Replication
N–RNA
Genome (–)
3′
N
P
M
G
L
5′
Le
Tr
L–P
Transcription
Poly(A)
5′
3′
L
G
M
P
N
mRNAs (+)
LeRNA (+)
Cap 1
CH3
NH2
HN
H2N
7
OH HO
5′
α
β
α
3′
2′
O–CH3
RNA

Encapsidation

- To replicate the VSV genome, the VSV RdRp needs to switch its mode from transcription to replication.
- During replication, the VSV RdRp ignores the termination signal at the end of the *Le* region, and throughout the genome to generate the full-length antigenome, which should be co-replicationally encapsidated with the N proteins.
- Selective encapsidation of LeRNA with the N protein may trigger switching from transcription to replication coupled with nucleocapsid assembly.

Envelope synthesis

- Envelope protein possess hydrophobic amino acid leader sequence at their amino-terminal ends.
- Sugar residues are added leading to the formation of glycoproteins.
- Glycoproteins transported to cytoplasmic membrane and replace host membrane proteins, followed by removal of leader sequence.
- Nucleocapsids then migrate to the area on the cyoplasmic membrane where these viral specific glycoprotein exist, recognizing viral glycoproteins with great specificity.
- Nucleocapsids then become aligned with glycoproteins and bud through them.