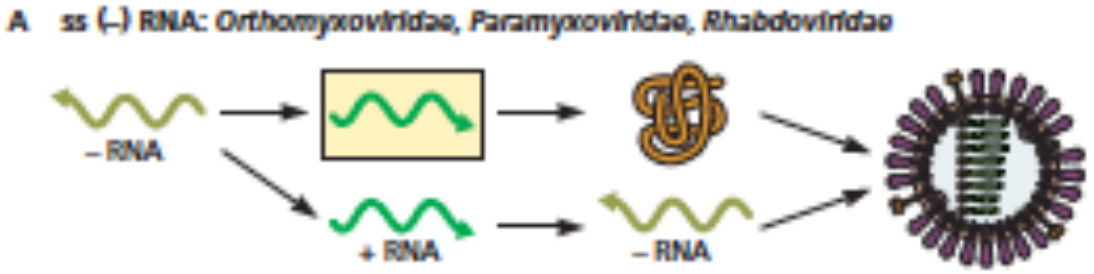
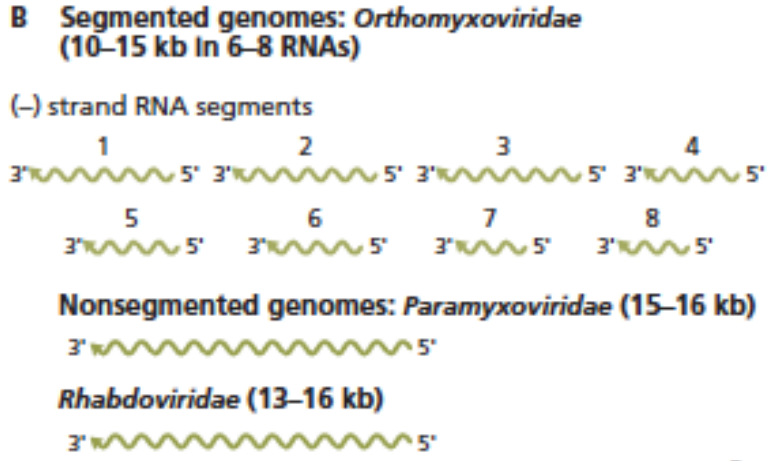
Class V: ssRNA (-), Expression-Replication

First step in virus multiplication is transcription by the virion RNA-dependent RNA polymerase



The genome of these viruses can be divided into two types

- Non-segmented genome (order *Mononegavirales*)
- Segmented genome (for example *Orthomyxoviridae*)



From Flint et al. *Principles of Virology* (2015), ASM Press

Class V: ssRNA (-), Expression-Replication

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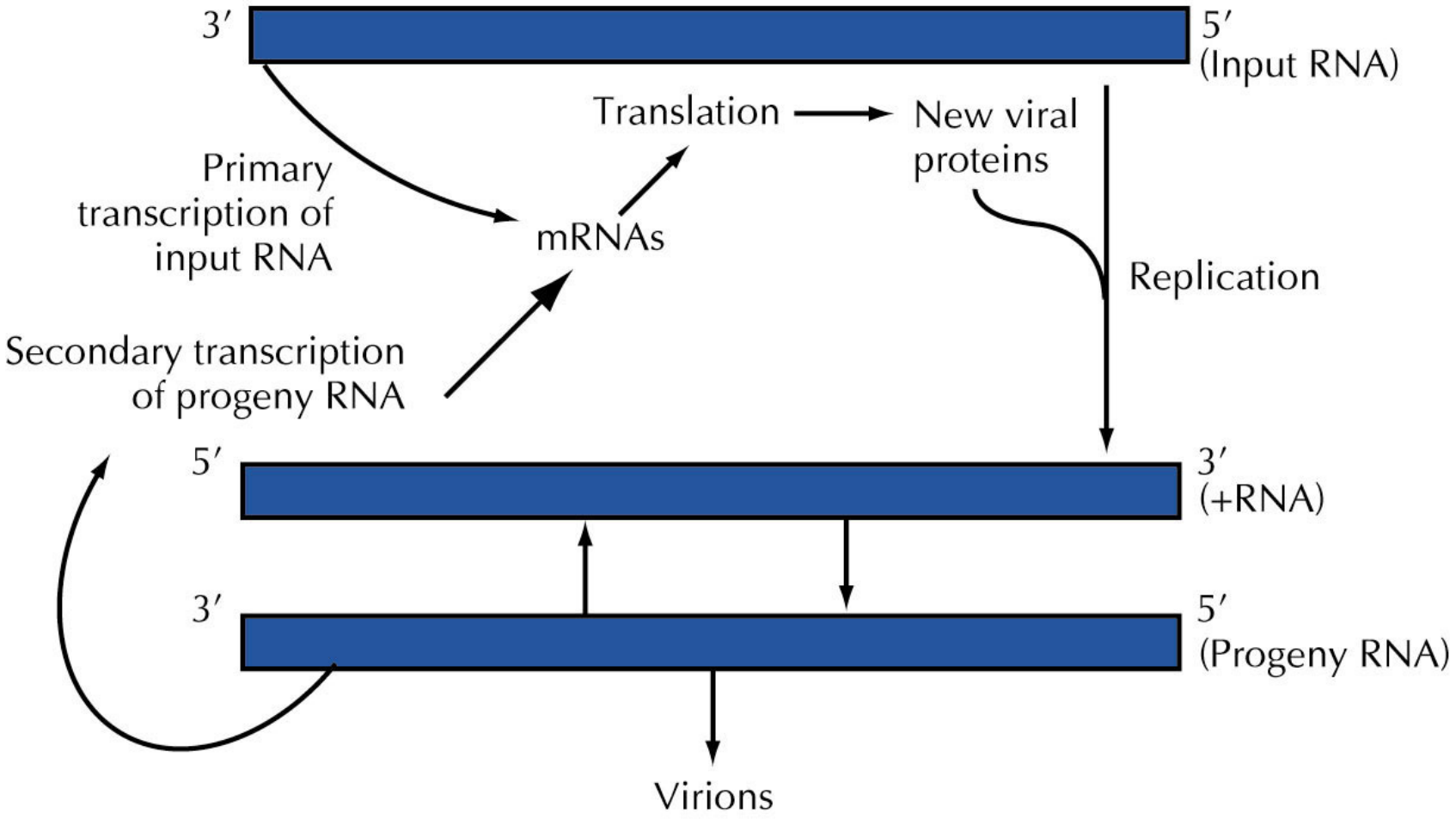
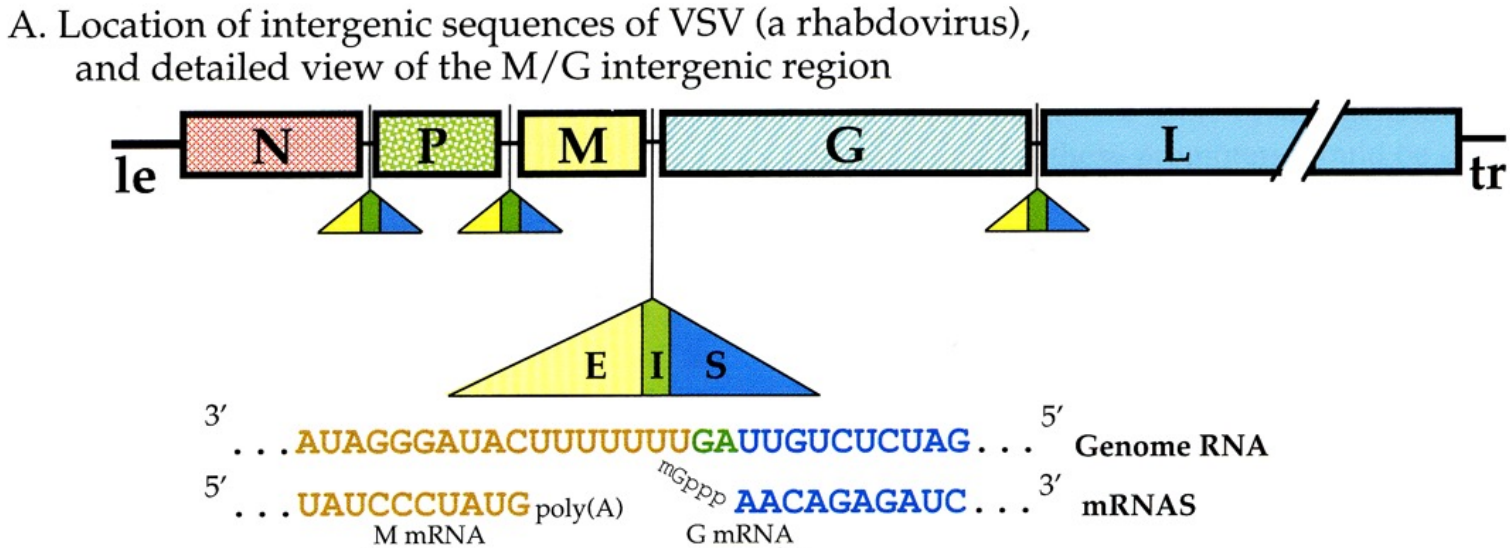


From Cann *Principles of molecular virology* (2001). Academic Press

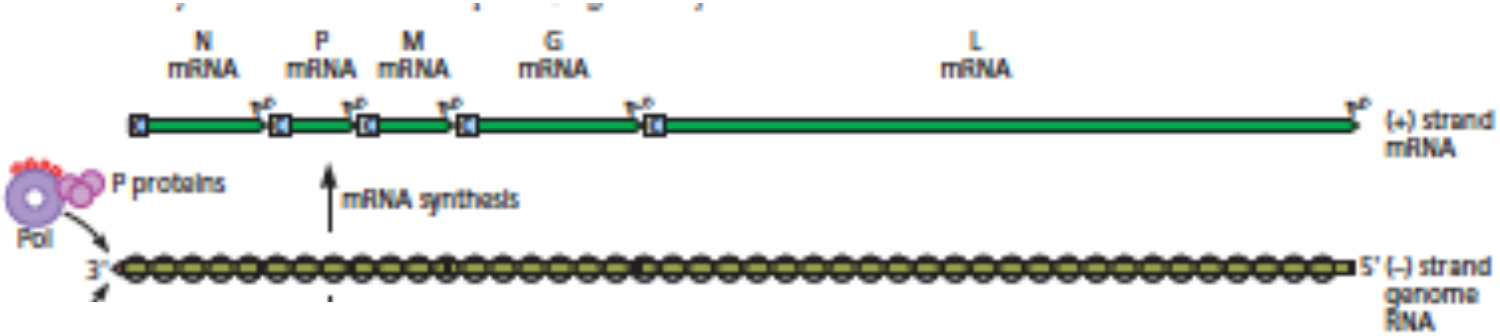
RNA (-), Rhabdoviridae (VSV)

Genome organization



E=end
I=intergenic
S=start

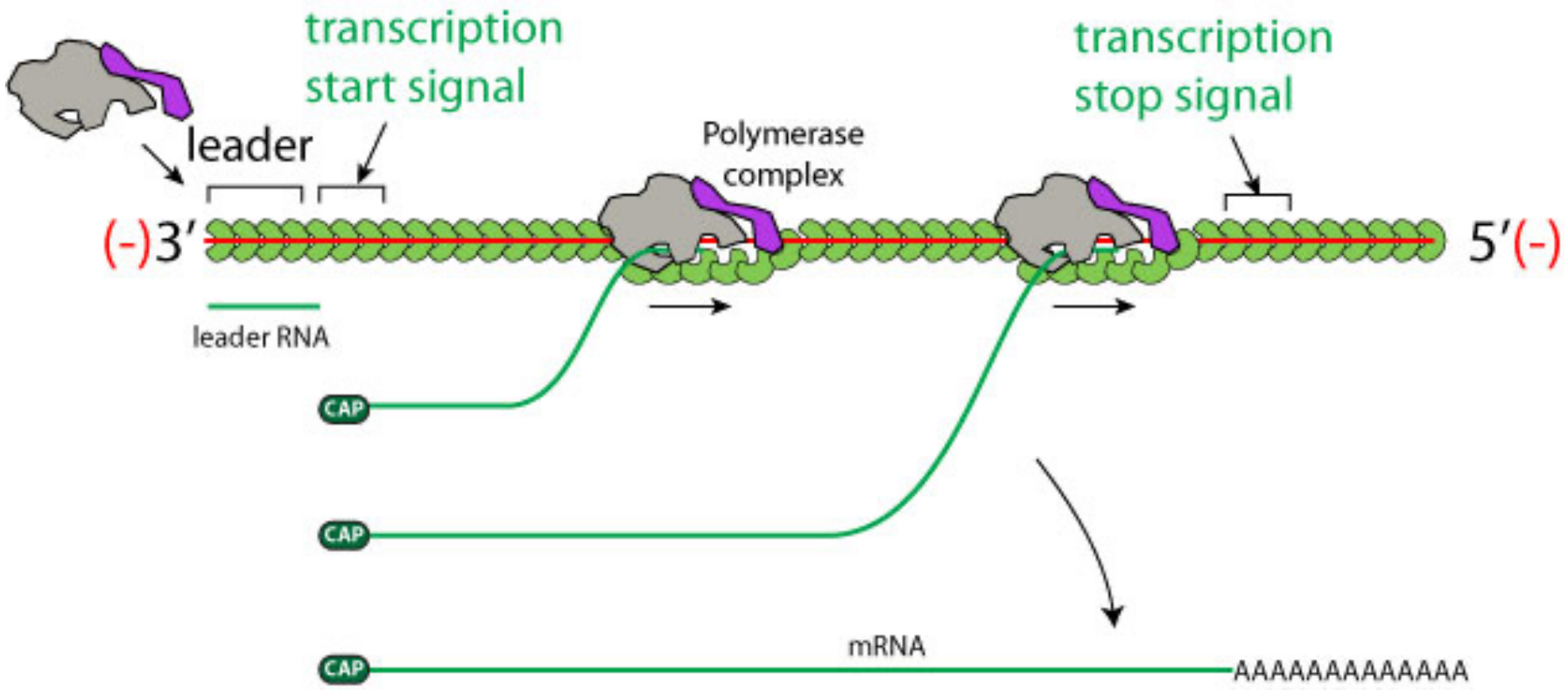
RNA (-), Rhabdoviridae (VSV)



Vesicular stomatitis viral RNA synthesis. Viral (-) strand genomes are templates for the production of either subgenomic mRNAs or full-length (+) strand RNAs. Once the nucleocapsid is released into the cytoplasm, the RNA genome is repetitively transcribed (primary transcription) by the virion transcriptase. N removal does not occur since the transcriptase only recognizes the RNA-N protein complex as template. The switch from mRNA synthesis to genomic RNA replication is mediated by two RNA polymerase complexes and by the N protein. mRNA synthesis initiates at the beginning of the N gene, near the 3' end of the viral genome. Poly(A) addition is a result of reiterative copying of a sequence of seven U residues present in each intergenic region. Chain termination and release occur after approximately 150 A residues have been added to the mRNA. The RNA polymerase then initiates synthesis of the next mRNA at the conserved start site 3'UUGUC . . . 5'. This process is repeated for all five viral genes.

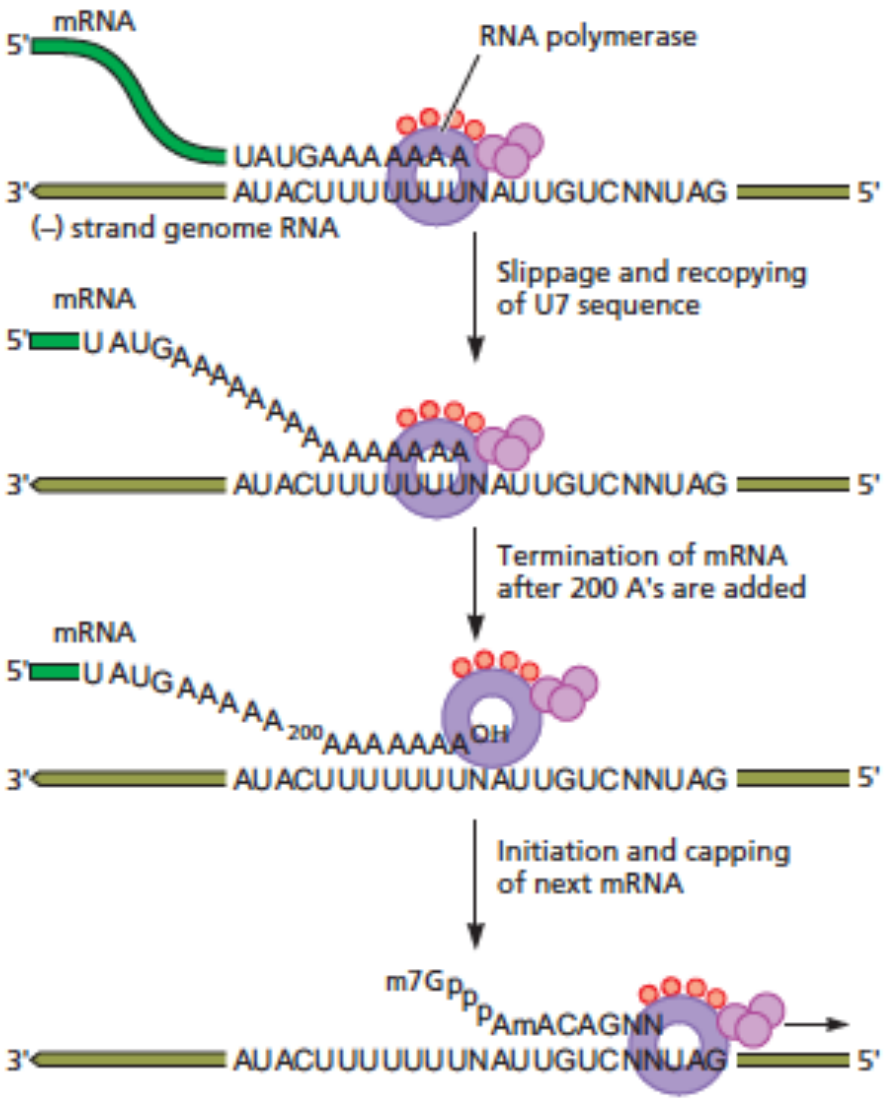
From Flint et al. *Principles of Virology* (2015), ASM Press

RNA (-), Rhabdoviridae (VSV)



The RNA dependent RNA polymerase (RdRp)complex initiates transcription by binding to the leader sequence in 3' of the genomic negative strand RNA. The RdRp transcribes a 5' triphosphate-leader RNA, then stop and restart on the transcription initiation signal of the N gene. The RNA initiated on this signal is capped.

RNA (-), Rhabdoviridae (VSV)



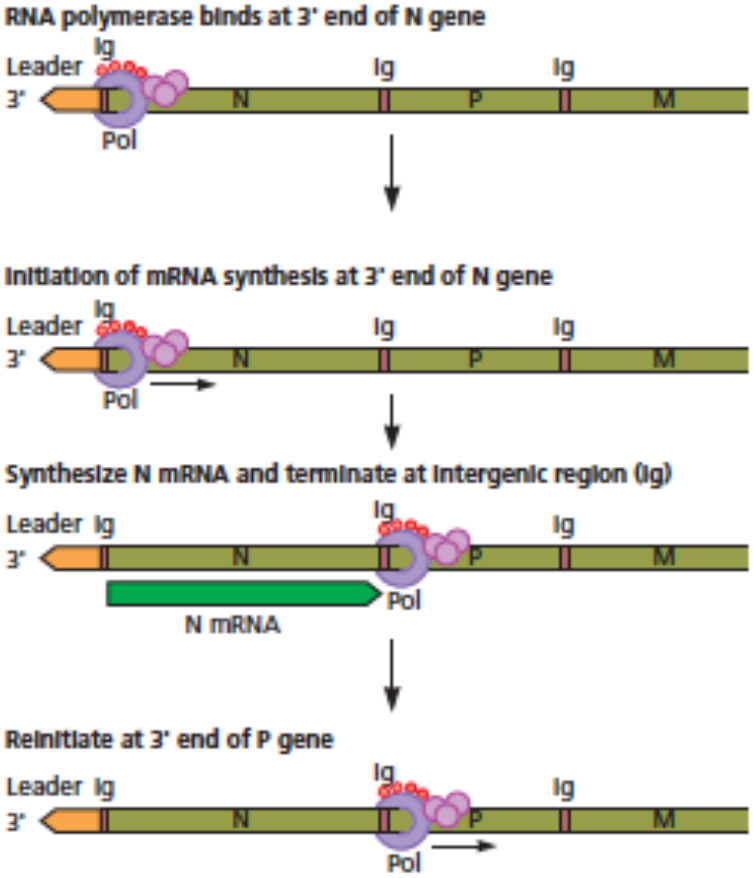
Poly(A) addition and termination at an intergenic region during vesicular stomatitis virus mRNA synthesis.

Copying of the last seven U residues of an mRNA-encoding sequence is followed by slipping of the resulting seven A residues in the mRNA off the genomic sequence, which is then recopied. This process continues until approximately 150 A residues are added to the 3’ end of the mRNA.

Termination then occurs, followed by initiation and capping of the next mRNA. The dinucleotide NA in the genomic RNA is not copied.

From Flint et al. *Principles of Virology* (2015), ASM Press

RNA (-), Rhabdoviridae (VSV)

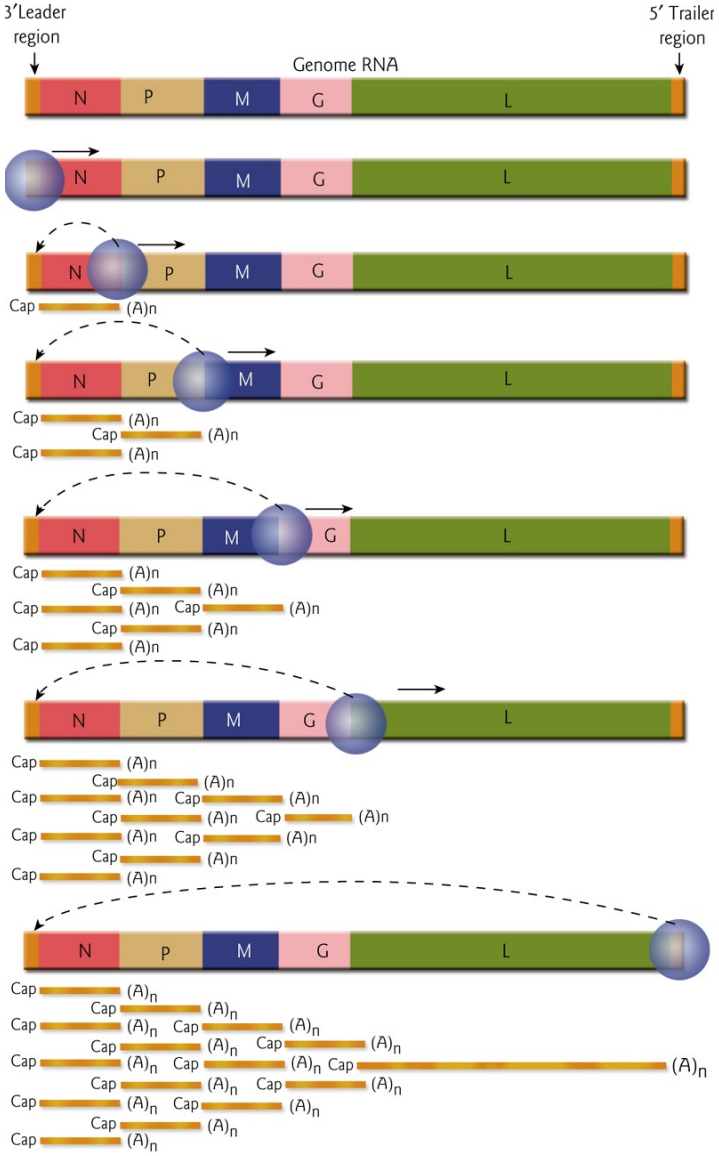


Stop-start model of vesicular stomatitis mRNA synthesis.

The RNA polymerase (Pol) initiates mRNA synthesis at the 3' end of the N gene. After synthesis of the N mRNA, RNA synthesis terminates at the intergenic region, followed by reinitiation at the 3' end of the P gene. This process continues until all five mRNAs are synthesized. Reinitiation does not occur after the last mRNA (the L mRNA) is synthesized, and, as a consequence, the 595-terminal nucleotides of the vesicular stomatitis virus genomic RNA are not copied. Only a fraction of the polymerase molecules successfully make the transition (70% of the time) from termination to reinitiation of mRNA synthesis at each intergenic region.

From Flint et al. *Principles of Virology* (2015), ASM Press

RNA (-), Rhabdoviridae (VSV)

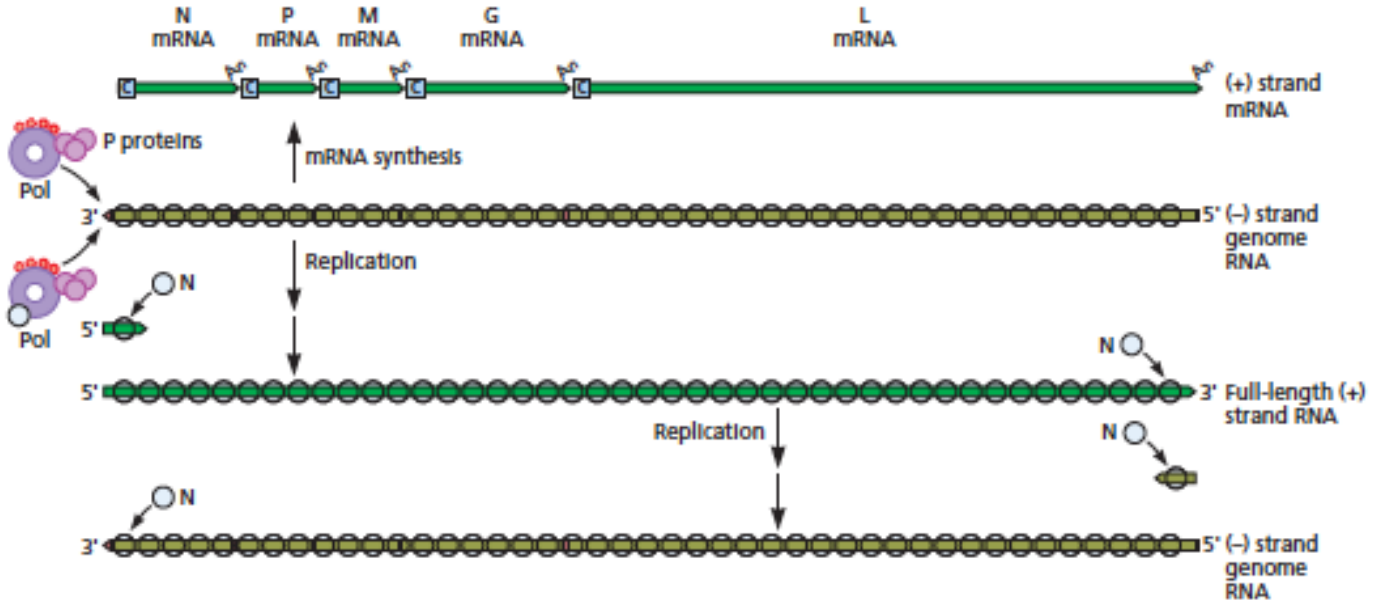


Genome is transcribed following a "stop and start" mechanism

mRNAs are synthesized sequentially in decreasing proportions as the polymerase moves toward the 5' terminus of the genome. The relative abundances of the proteins reflect those of the mRNAs

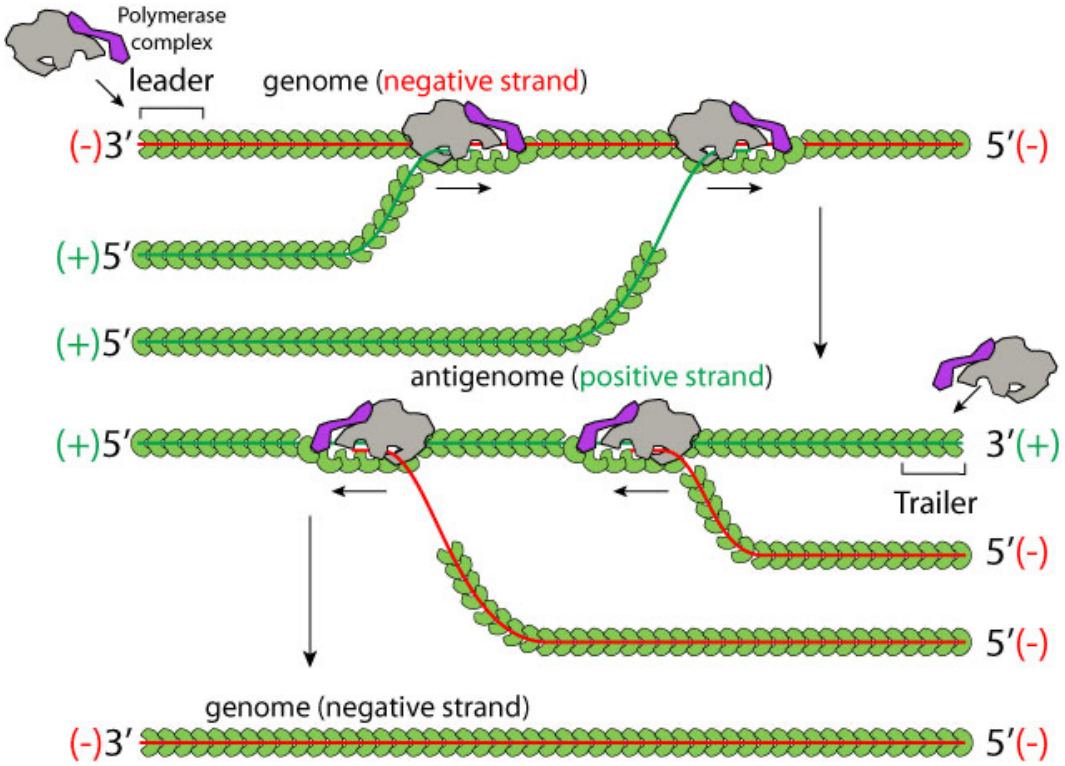
From Dimmock NJ, Introduction to Modern Virology, Blackwell, 2016

RNA (-), Rhabdoviridae (VSV) from transcription to genome replication



Synthesis of the full-length (+) strand begins at the exact 3’ end of the viral genome and is carried out by the RNA polymerase L-N-(P)4. The (+) strand RNA is bound by the viral nucleocapsid (N) protein, which is associated with the P protein in a 1:1 molar ratio. The N-P complexes bind to the nascent (+) strand RNA, allowing the RNA polymerase to read through the intergenic junctions at which polyadenylation and termination take place during mRNA synthesis.

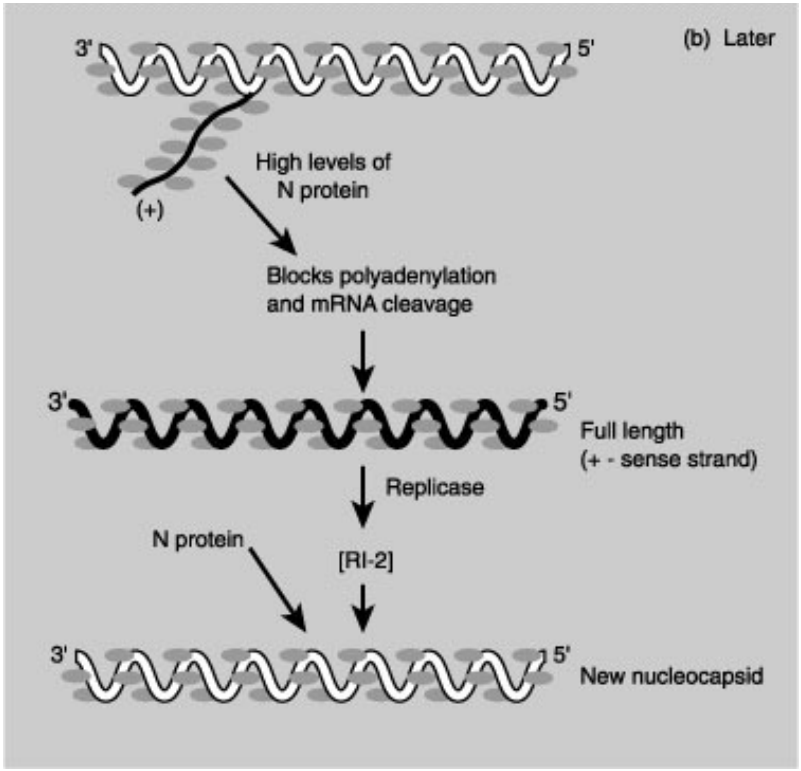
RNA (-), Rhabdoviridae (VSV) from transcription to genome replication



The RNA dependent RNA polymerase complex binds to the leader sequence on the RNA genome, and starts replication. The antigenome is concomitantly encapsidated during replication. The RNA dependent RNA polymerase complex ignores all transcription signals when in replication mode. The antigenome is then replicated (thus new genomes synthetised) under the same process, the viral polymerase complex binding first to the trailer sequence.

The genome/antigenome ratio is about 10 for many viruses. Presumably the trailer sequence is a stronger replication promoter that the leader sequence.

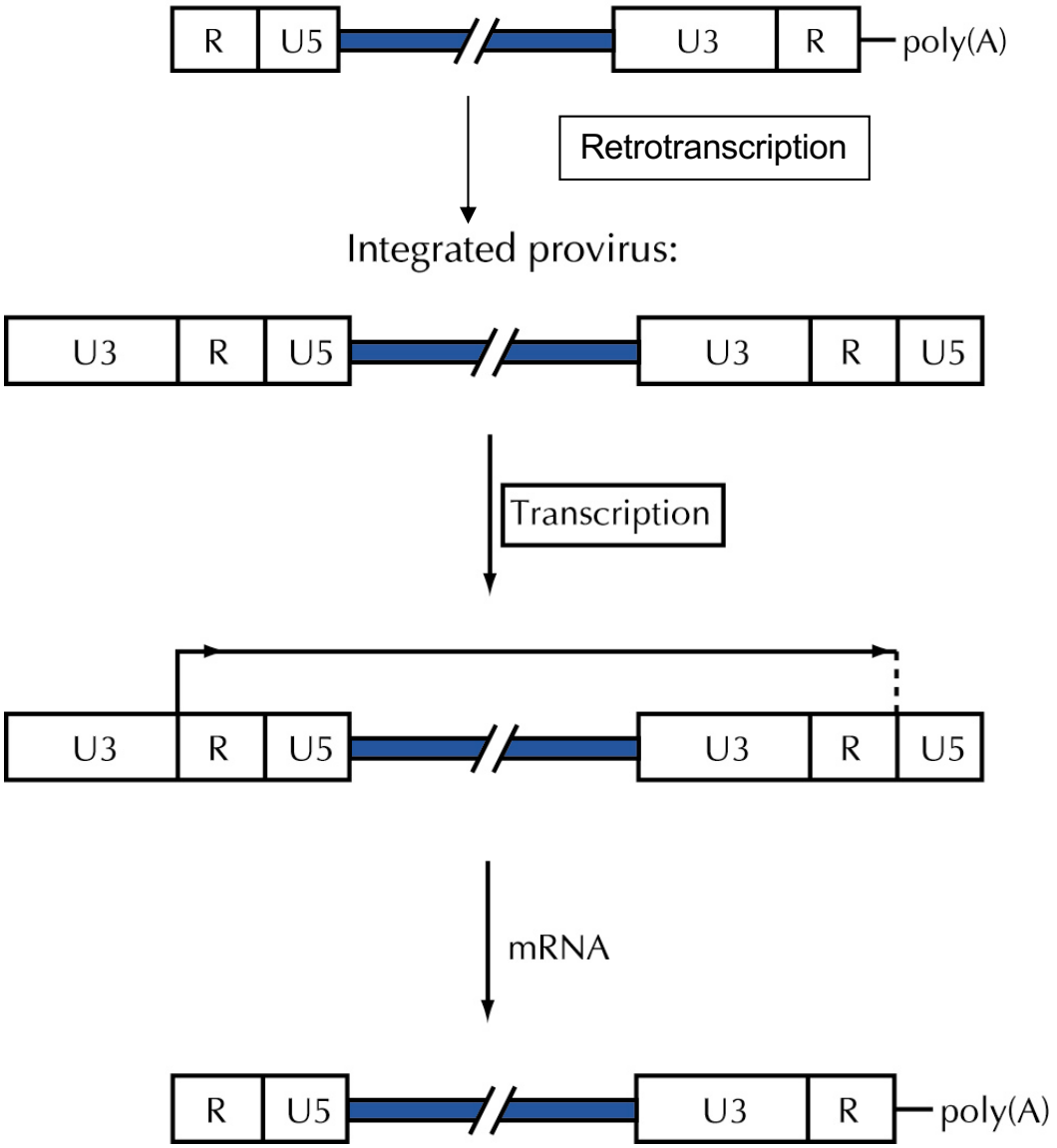
RNA (-), Rhabdoviridae (VSV), from transcription to genome replication



The abundance of the neosynthesized N and P proteins regulate the transition from transcription to the genome replication phase. To produce a full-length (+) strand RNA, the stop-start reactions at intergenic regions must be suppressed, a process that depends on the N and P proteins. The P protein maintains the N protein in a soluble form so that it can encapsidate the newly synthesized RNA. N-P assemblies bind to leader RNA and cause Antitermination. Additional N protein molecules then associate with the (+) strand RNA as it is elongated, and eventually bind to the seven A bases in the intergenic region. This interaction blocks reiterative copying of the seven U bases. Consequently, RNA synthesis continues through the intergenic regions. The number of N-P protein complexes in infected cells therefore regulates the relative efficiencies of mRNA synthesis and genome RNA replication. The copying of full-length (+) strand RNAs to (-) strand genomic RNAs also requires the binding of N-P protein complexes to elongating RNA molecules. Newly synthesized (-) strand RNAs are produced as nucleocapsids that can be readily assembled into progeny viral particles.

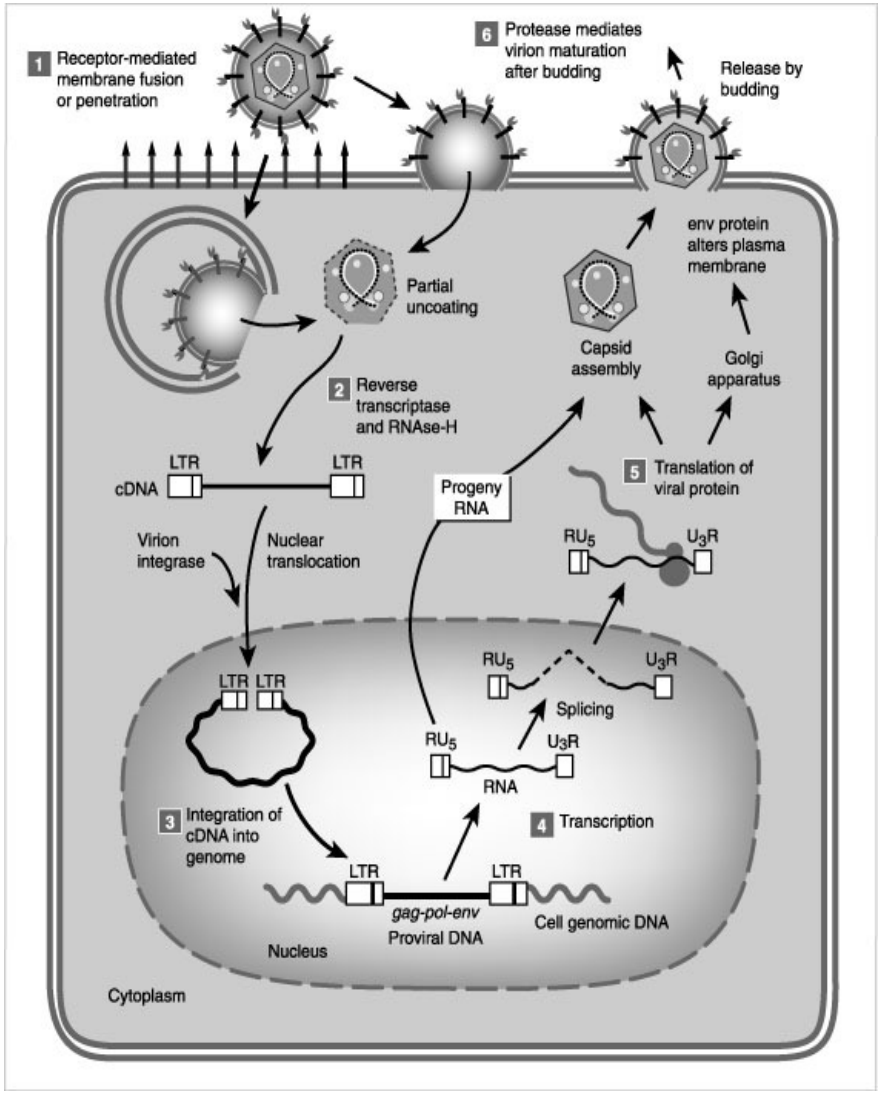
From Wagner and Hewlett *Basic virology* (2003) Blackwell Science Press

Class VI: Retrovirus



From Cann *Principles of molecular virology* (2001). Academic Press

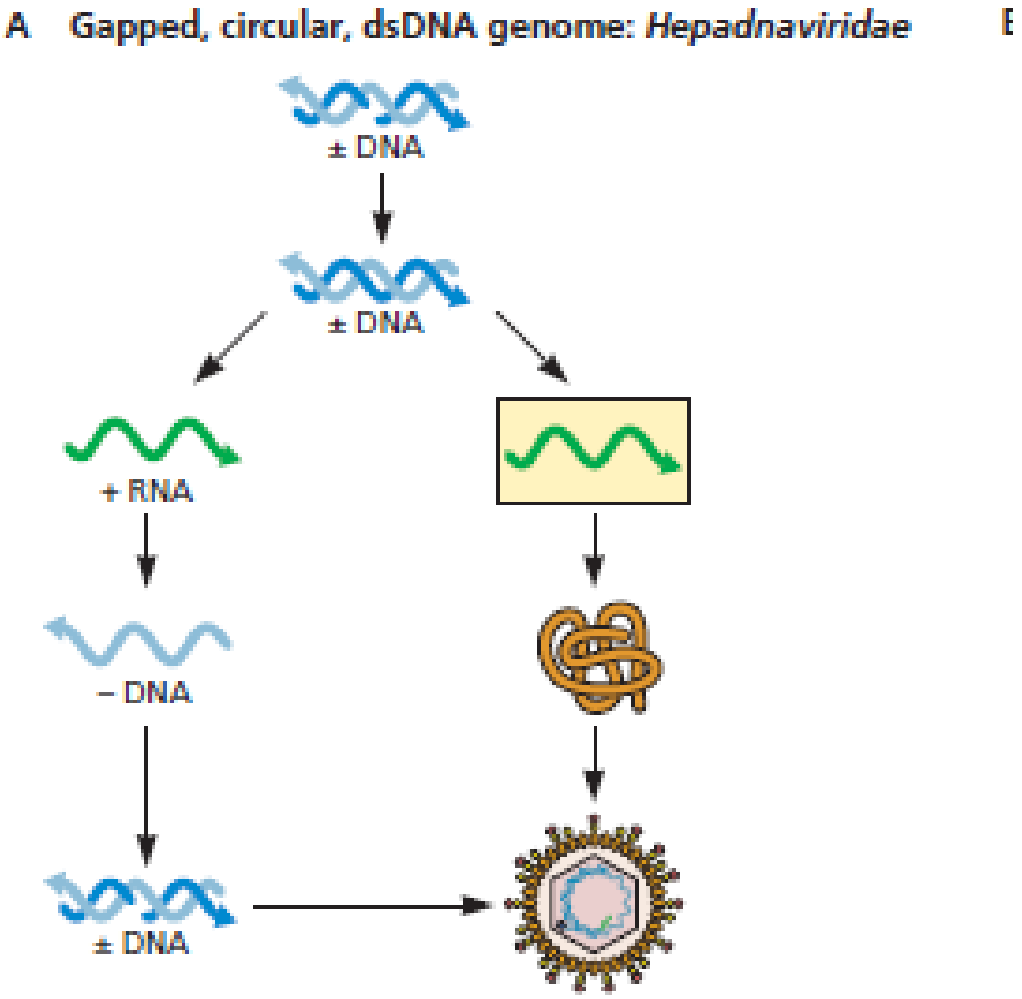
Class VI: ssRNA (+) with DNA intermediate



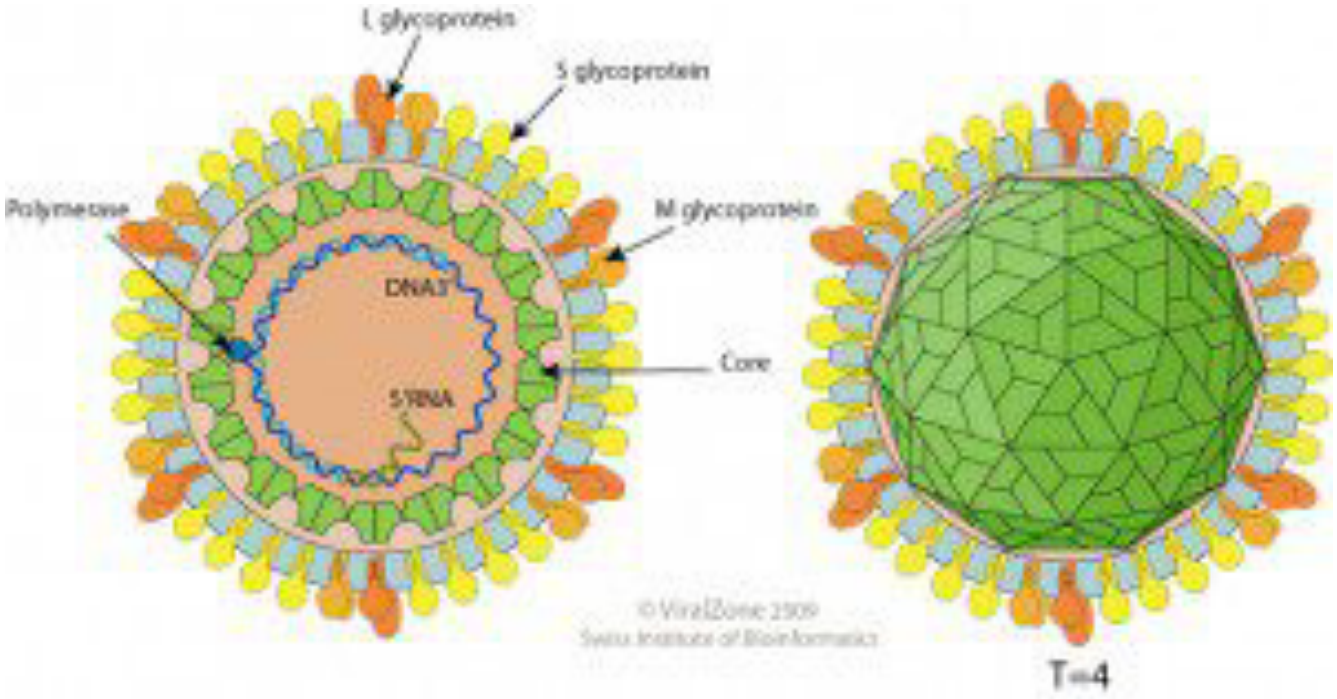
From Wagner and Hewlett *Basic virology* (2003) Blackwell Science Press

Class VII, Hepadnaviridae (HBV): dsDNA with RNA-intermediate

This group of viruses also uses reverse transcriptase
Unlike retroviruses, retro-transcription occurs within the maturing particle



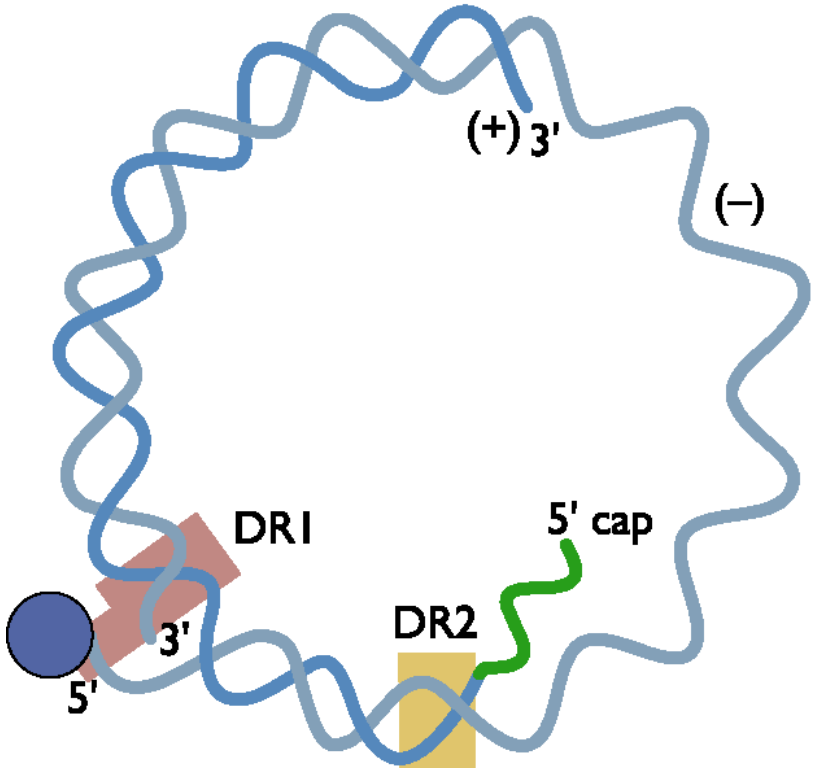
Class VII, Hepadnaviridae (HBV): dsDNA with RNA-intermediate

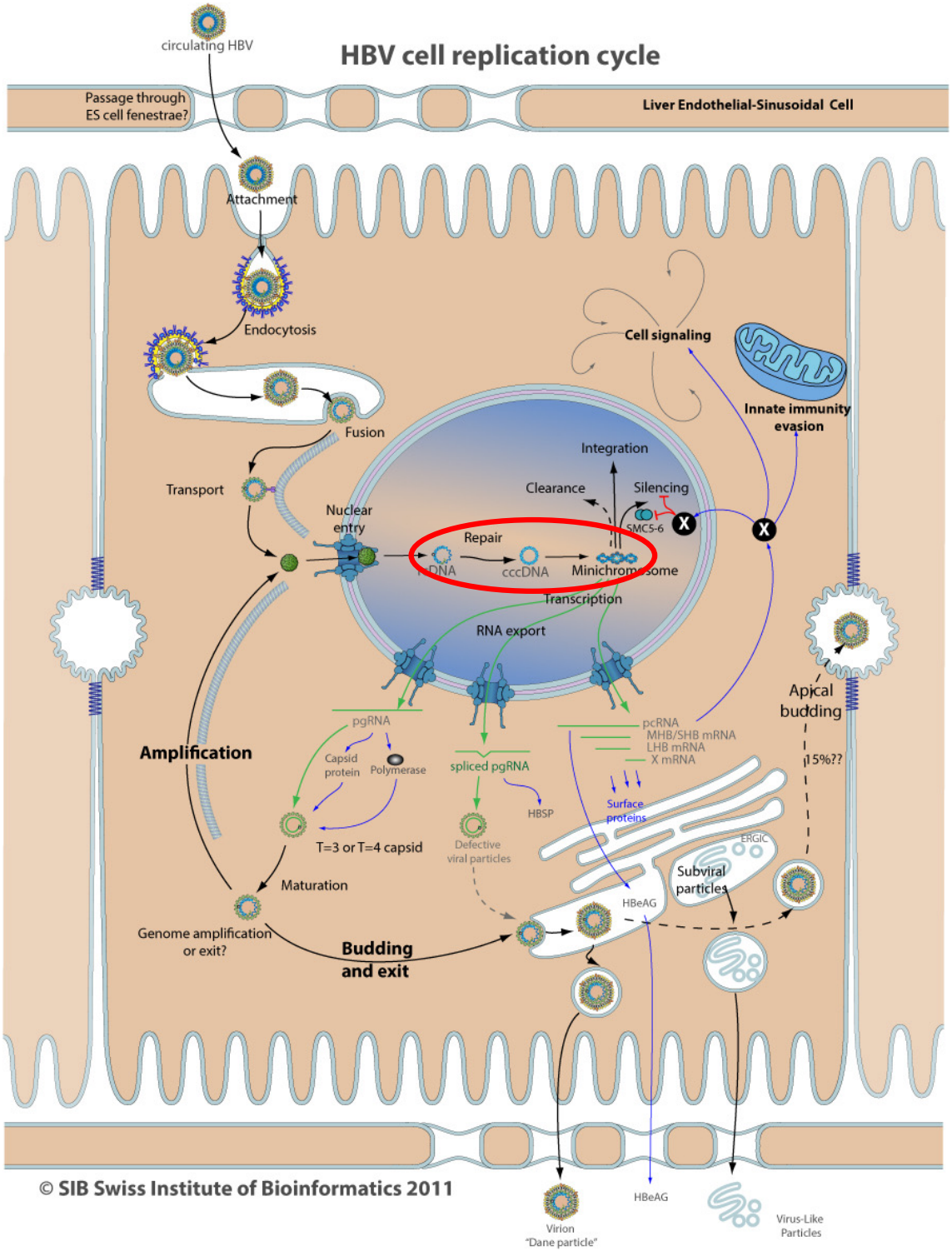


Class VII, Hepadnaviridae (HBV): dsDNA with RNA-intermediate

This group of viruses also uses reverse transcriptase
Unlike retroviruses, retro-transcription occurs within the maturing particle

The DNA in extracellular hepadnavirus particles is a partially duplex molecule of around 3.2 kb with circularity that is maintained by overlapping 5' ends. The (-) strand has the polymerase, shown as a blue ball, attached to its 5' end. The (+) strand has a capped RNA of 18 nucleotides at its 5' end. Direct repeats (10- to 12-bp) called DR1 and DR2 (colored purple and yellow, respectively) are present at he 5' ends . As in retroviruses, these repeat sequences play the critical role of facilitating template transfers during reverse transcription.
In mammalian hepadnavirus genomes, the (+) strand is shorter than the (-) strand and has heterogeneous ends.





HBV life cycle

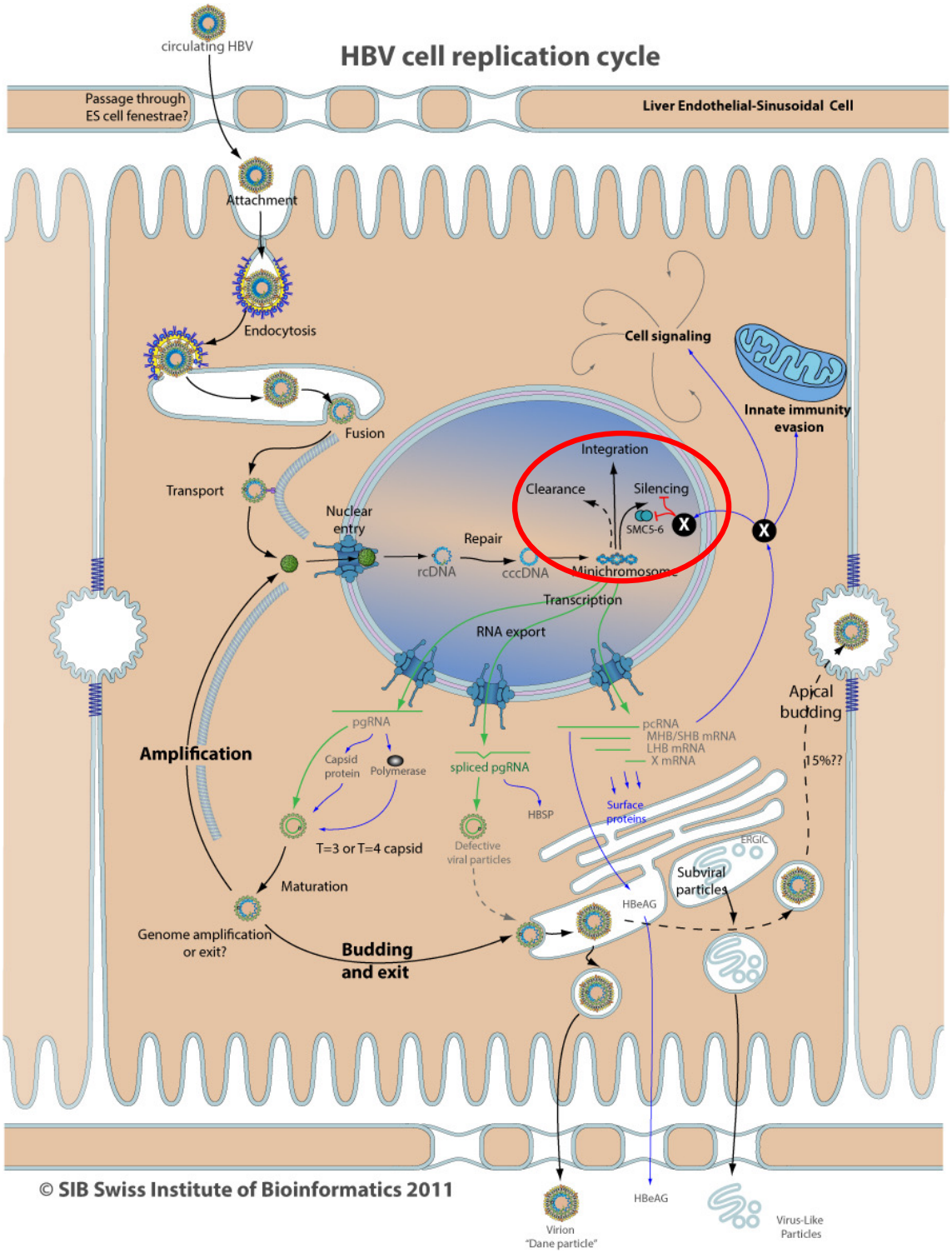
HBV life cycle-Minichromosome synthesis (rcDNA repair)

From rcDNA to cccDNA

The formation of the cccDNA from the rcDNA is incompletely understood, but several steps are essential for the synthesis:

- The removal of the polymerase covalently attached to the 5' end of the negative strand.
- The removal of the primer from the 5' plus strand.
- Fill in gaps in the positive strand
- Fill in gaps at the ends of both strands which must be ligated to yield closed circles

The cccDNA content in liver cells in natural infections has been reported to be low (between 5 and 50 copies per cell), and is very important for establishing and maintaining HBV replication



HBV life cycle

HBV life cycle-cccDNA clearance

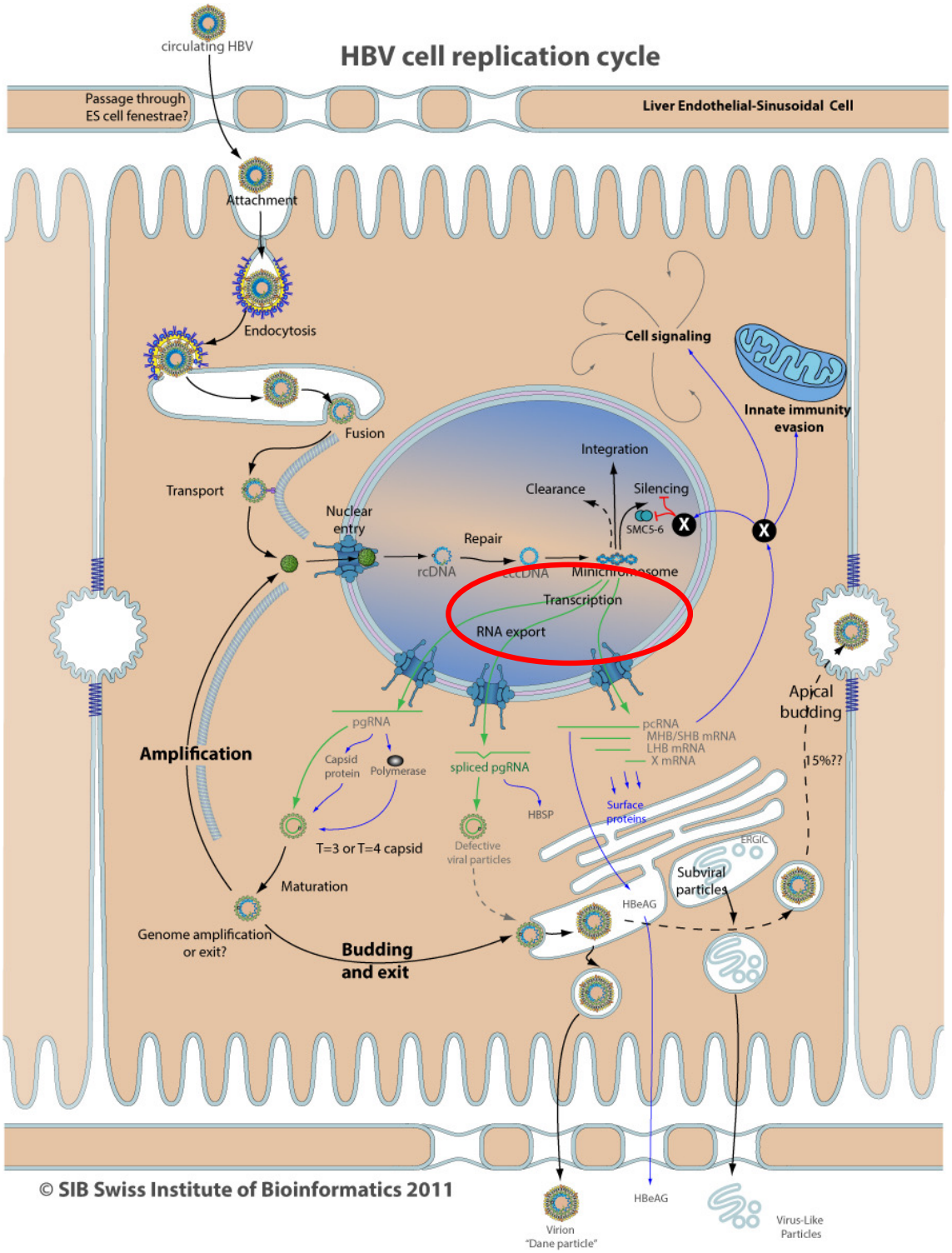
The minichromosome is very stable within the nucleus, and direct degradation within the infected cell of the cccDNA has never been reported. However, it is unclear how and to what extend the cccDNA localizes to new host nucleus after mitosis. In Epstein-Barr virus infection, EBNA1 maintains circular genomes in proliferating cells by tethering them to host chromosomes (Sears et al. 2004)

HBV life cycle-cccDNA integration

Persistent HBV replication is associated with a high frequency of integration of HBV sequences into the human host genome while a lower frequency is observed during acute hepatitis B infections (Murakami et al. 2004). They are present in over 85 to 90% of HBV related Hepatocellular carcinomas (HCCs).

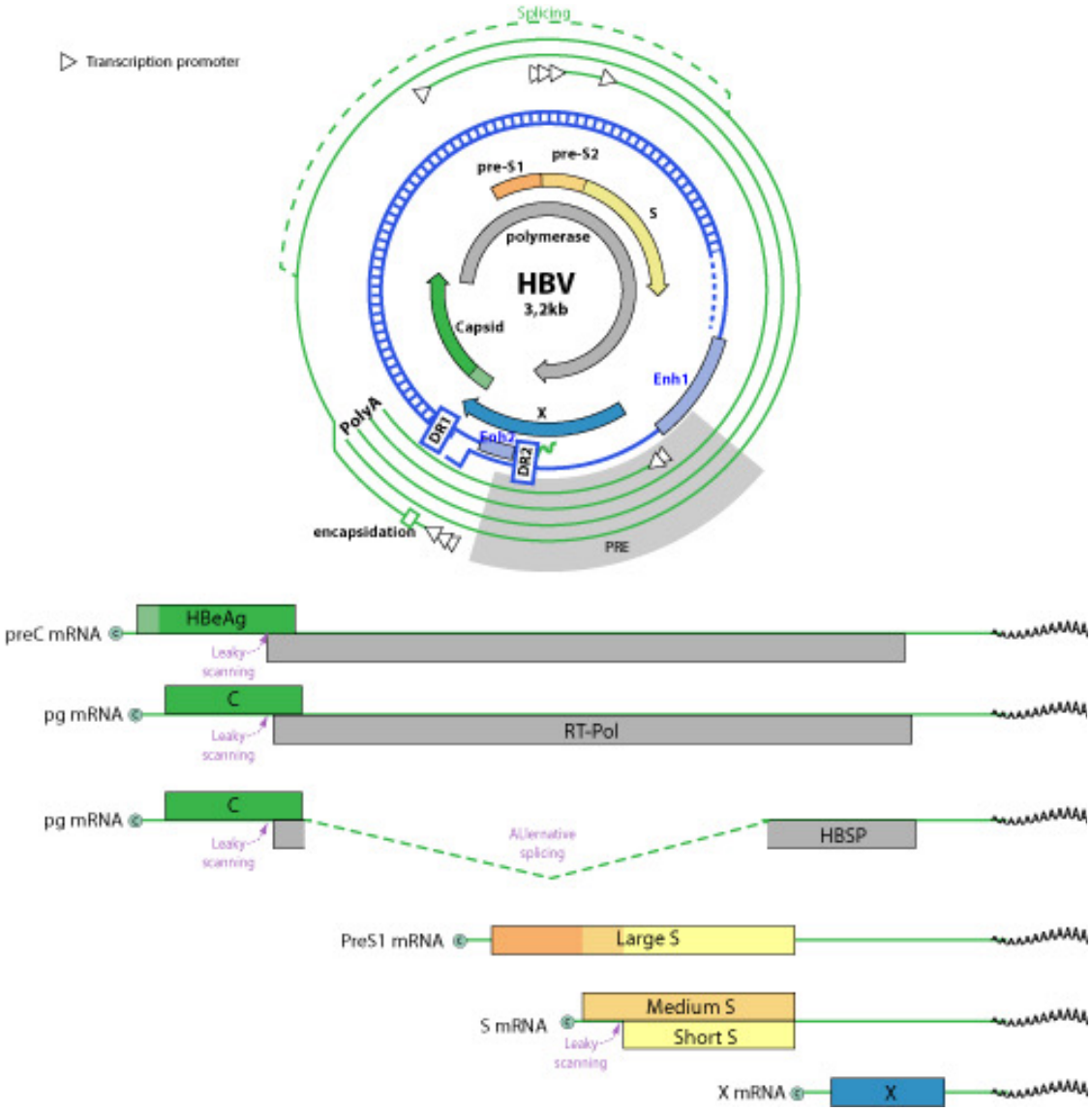
HBV life cycle-cccDNA silencing

The cellular machinery is able to down-regulate HBV minichromosome transcription, but it is still unclear if this happens in vivo. HBx might play a role in preventing this to happen. A study using HepaRG cells confirms that HBx expression is needed for epigenetic modifications of cccDNA initiating HBV RNA transcription



HBV life cycle

HBV life cycle-genome expression



The X mRNA seems to be transcribed early and transiently, whereas structural proteins mRNAs are on the contrary expressed later and constantly. The S promoter would thus be more active that the PreS1, maybe explaining the huge ammount of SHB and MHB proteins synthesized during infection.

Recent advances in basic science

Alternative splicing of viral transcripts: the dark side of HBV

Dina Kremsdorf,[1] Bouchra Lekbaby,[1] Pierre Bablon,[1] Jules Sotty,[1] Jérémy Augustin,[1] Aurélie Schnuriger,[1,2] Jonathan Pol ,[3,4] Patrick Soussan [1,2]

Kremsdorf D, *et al. Gut* 2021;**70**:

Spliced HBV mRNA

More than ten spliced products of pre-genomic RNA (pgRNA) have been identified. The major 2.2kb variant transcript is spliced between the positions 2447 and 489. Its translation produces the Hepatitis B spliced protein (HBSP protein) which contains the first 47 amino acids residues of polymerase. Its function is unknown.

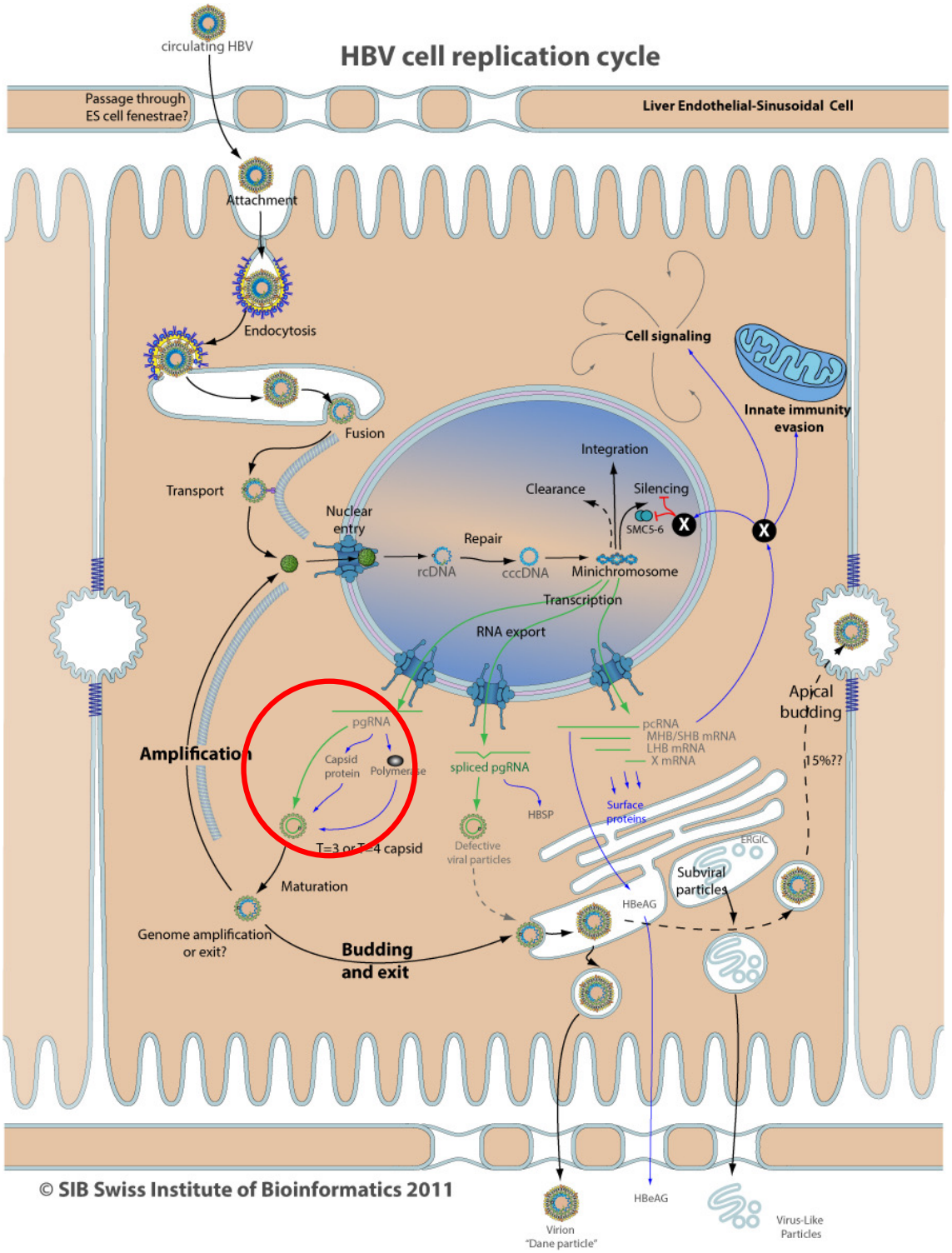
The spliced pgRNA can be encapsidated and reverse transcribed giving rise to defective particles (Soussan et al. 2008).

HBV life cycle-genome expression

RNAs export to the cytoplasm

Cellular nucleus retains unspliced or incompletely spliced RNAs where they are rapidly degraded, and this system has to be circumvented by some nuclear viruses. For example HIV-1 encodes for the Rev protein which promotes nuclear exit of HIV-1 unspliced genomic RNAs.

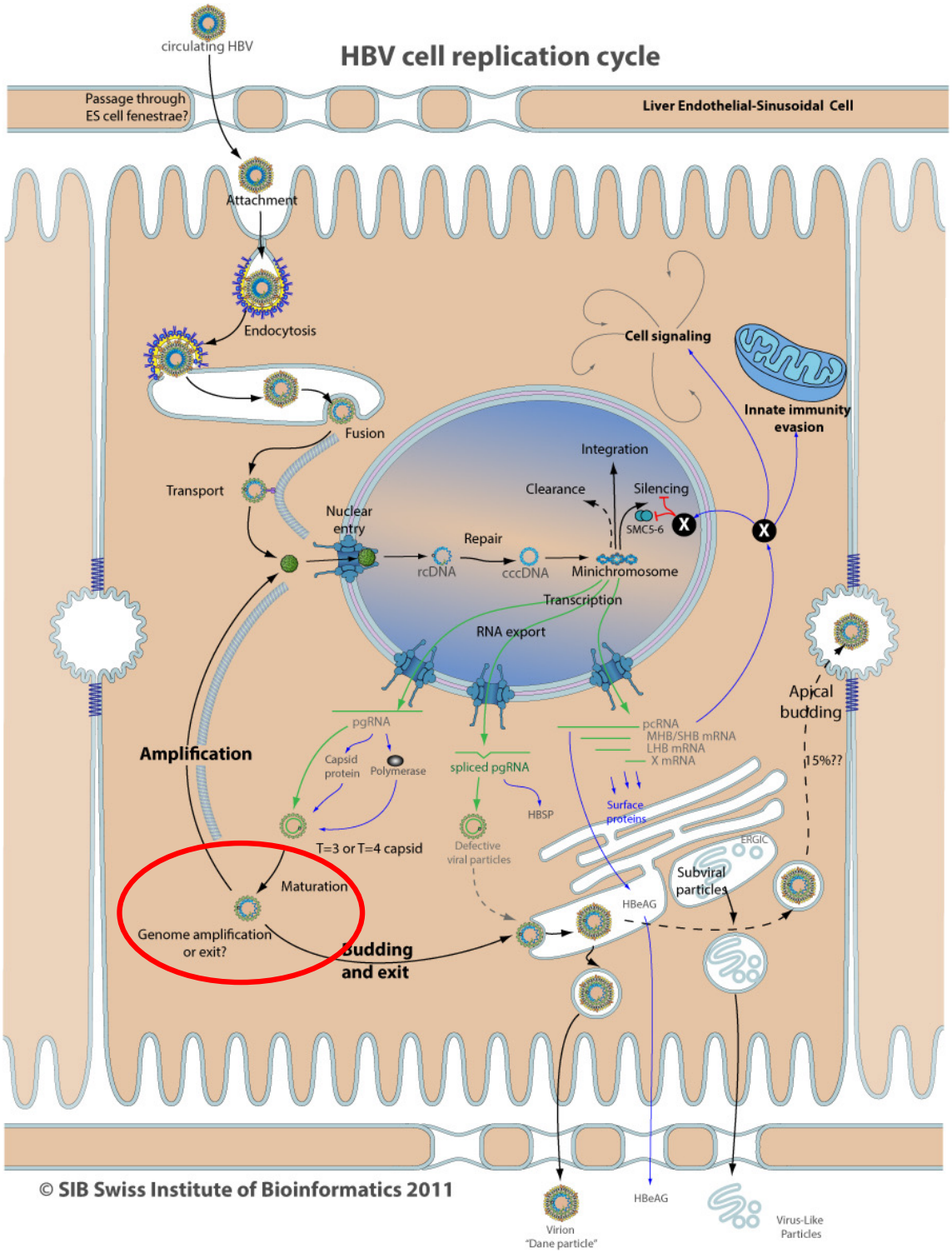
HBV genome encodes conserved splicing sites, but the majority of its mRNAs exits the nucleus unspliced. This is presumably because HBV mRNAs comprise a nuclear exit post-transcriptional regulatory element (PRE) (Huang et al. 1993). The PRE sequence shares similarities with the REV-responsive element (RRE) from HIV-1, although it does not utilize the same export pathway. Indeed, nuclear export inhibitor leptomycin prevents REV/RRE mediated export but not the PRE-dependent pathway (Otero et al. 1998). The host cellular factor PTB has been suggested to participate in PRE-dependent HBV RNA export (Zang et al. 2001).



HBV life cycle

HBV genome synthesis and packaging

The pregenome RNA is translated to produce capsid protein. The P protein, the viral reverse transcriptase, is also produced from pregenome RNA but at low efficiency; the ratio of capsid to P protein translation is 200 to 300 to 1. Following its synthesis, P binds to the packaging signal at the 5’ end of its own transcript, where viral DNA synthesis is eventually initiated. Concurrently with capsid formation, the RNA-P protein complex is packaged and DNA replication is primed from a tyrosine residue in the polymerase. Reverse transcription of the pregenome occurs within the capsid.



HBV life cycle

HBV genome amplification or exit (assembly of mature virions)

After completion of DNA synthesis, the newly assembled “cores” acquire the ability to interact with envelope proteins. However, at early times after infection, core particles are transported to the nucleus, where the viral genomes are deposited and give rise to additional copies of CCC DNA. Eventually, 5 to 50 molecules of CCC DNA accumulate, leading to a concomitant increase in viral mRNA concentrations.