Reverse transcription and integration

Session 9

Virology Live

Fall 2021

"One can't believe impossible things," said Alice. "I dare say you haven't had much practice," said the Queen. "Why, sometimes I've believed as many as six impossible things before breakfast."
--Lewis Carroll, *Alice in Wonderland*

Tumor virus history

- 1908 - Discovery of chicken leukemia virus, Bang & Ellerman
- 1911 - Discovery of Rous sarcoma virus, Peyton Rous (Nobel Prize 55 years later)
- Called tumor viruses
- Found to have RNA genomes


Vilhelm Ellerman


Oluf Bang


Peyton Rous

Howard Temin's insight

- RNA tumor viruses caused permanent changes in cells (transformation)
- Viral DNA was integrated into host genome
- Became permanent part of host DNA
- Provirus hypothesis

David Baltimore's insight

- (+) RNA viruses: No RdRp in particle
- (-) RNA viruses: RdRp in particle
- An enzyme to copy (+) RNA to DNA must be in virus particle

Baltimore and Temin independently discovered RT in RNA tumor virus particles (Nobel Prize, 1975)

RNA-dependent DNA Polymerase in Virions of Rous Sarcoma Virus

INFECTION of sensitive cells by RNA sarcoma viruses requires the synthesis of new DNA different from that synthesized in the *S*-phase of the cell cycle (refs. 1, 2 and unpublished results of D. Boettiger and H. M. T.); production of RNA tumour viruses is sensitive to actinomycin D[3,4]; and cells transformed by RNA tumour viruses have new DNA which hybridizes with viral RNA[5,6]. These are the basic observations essential to the DNA provirus hypothesis—replication of RNA tumour viruses takes place through a DNA intermediate, not

RNA-dependent DNA Polymerase in Virions of RNA Tumour Viruses

DNA seems to have a critical role in the multiplication and transforming ability of RNA tumour viruses[1]. Infection and transformation by these viruses can be prevented by inhibitors of DNA synthesis added during the first 8–12 h after exposure of cells to the virus[1–4]. The necessary DNA synthesis seems to involve the production of DNA which is genetically specific for the infecting virus[5,6], although hybridization studies intended to demonstrate virus-specific DNA have been inconclusive[1]. Also, the formation of virions by the RNA tumour viruses is sensitive to actinomycin D and therefore seems to involve DNA-dependent RNA synthesis[1–4,7]. One model which explains these data postulates the transfer of the information of the infecting RNA to a DNA copy which then serves as template for the synthesis of viral RNA[1,2,7]. This model requires a unique enzyme, an RNA-dependent DNA polymerase.

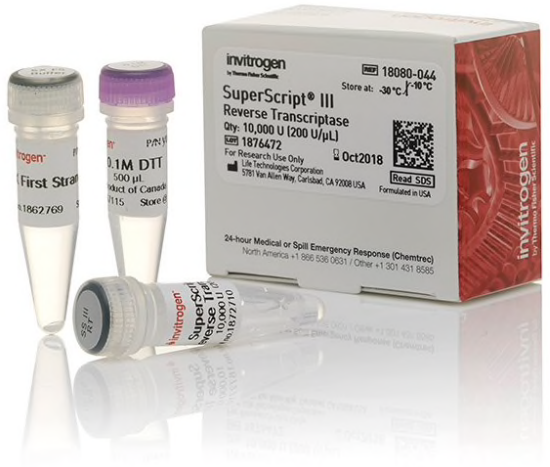
Listen to TWiV #100 (Baltimore) for more insight

Reverse transcriptase

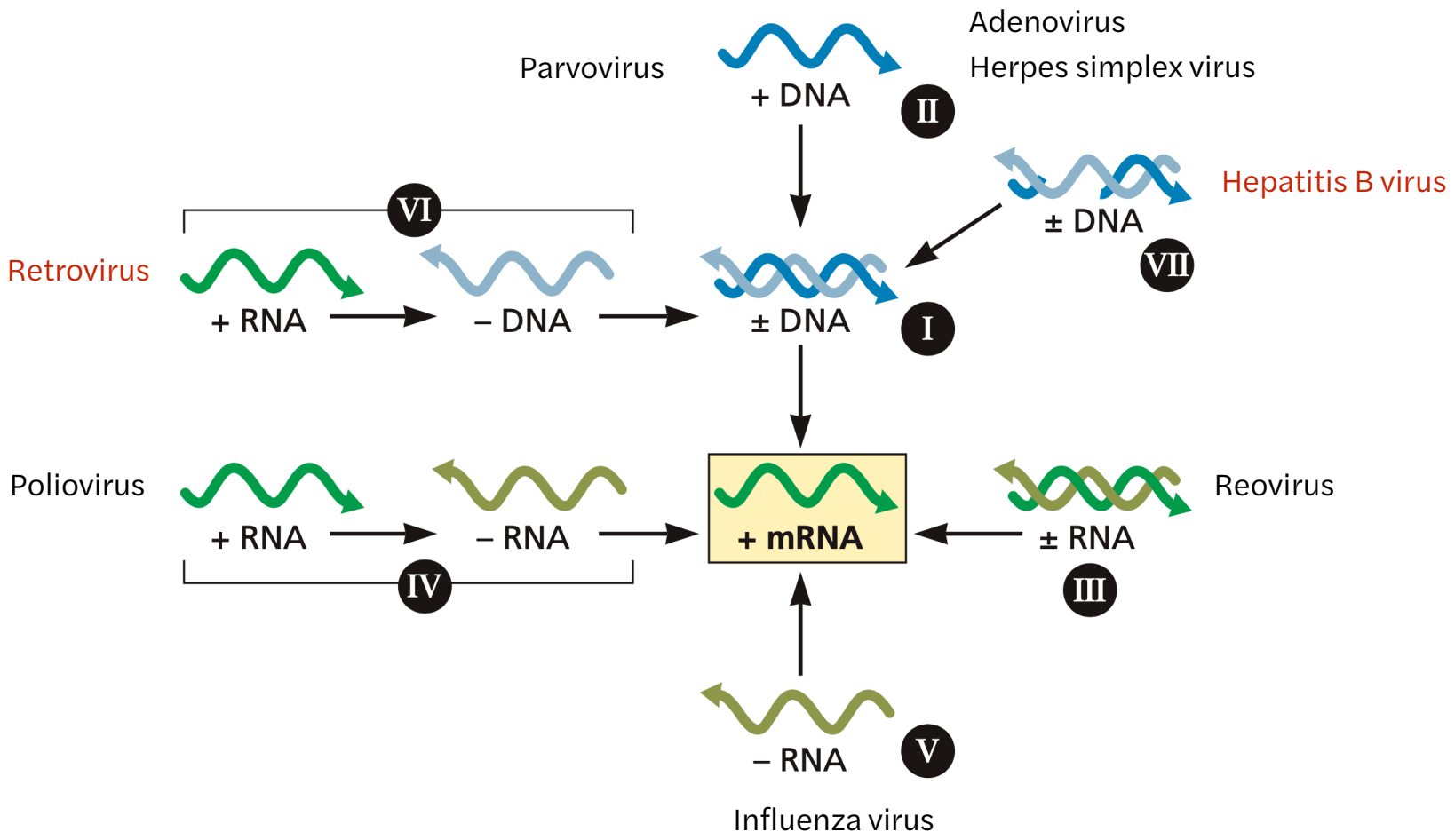
- Retroviruses got their name because of their ability to reverse the flow of genetic information

DNA => RNA => protein

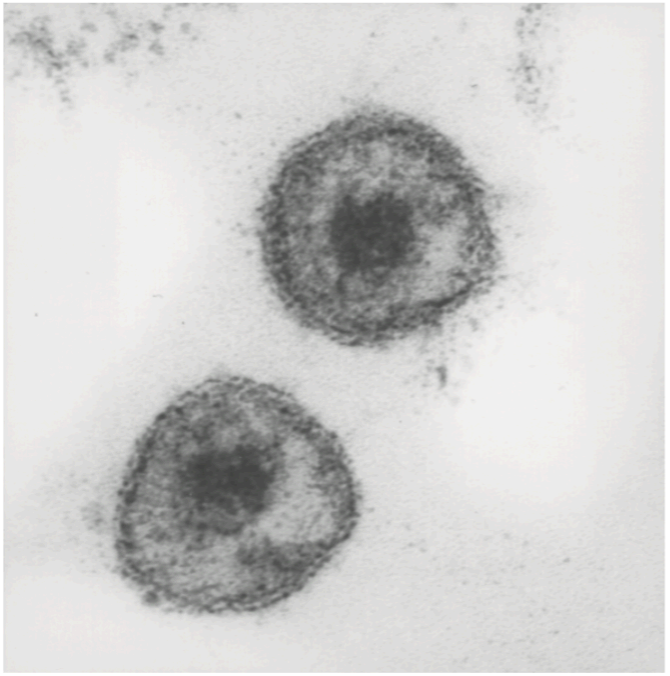
- RT discovery revolutionized molecular biology (e.g. SARS-CoV-2 diagnosis may utilize RT-PCR)

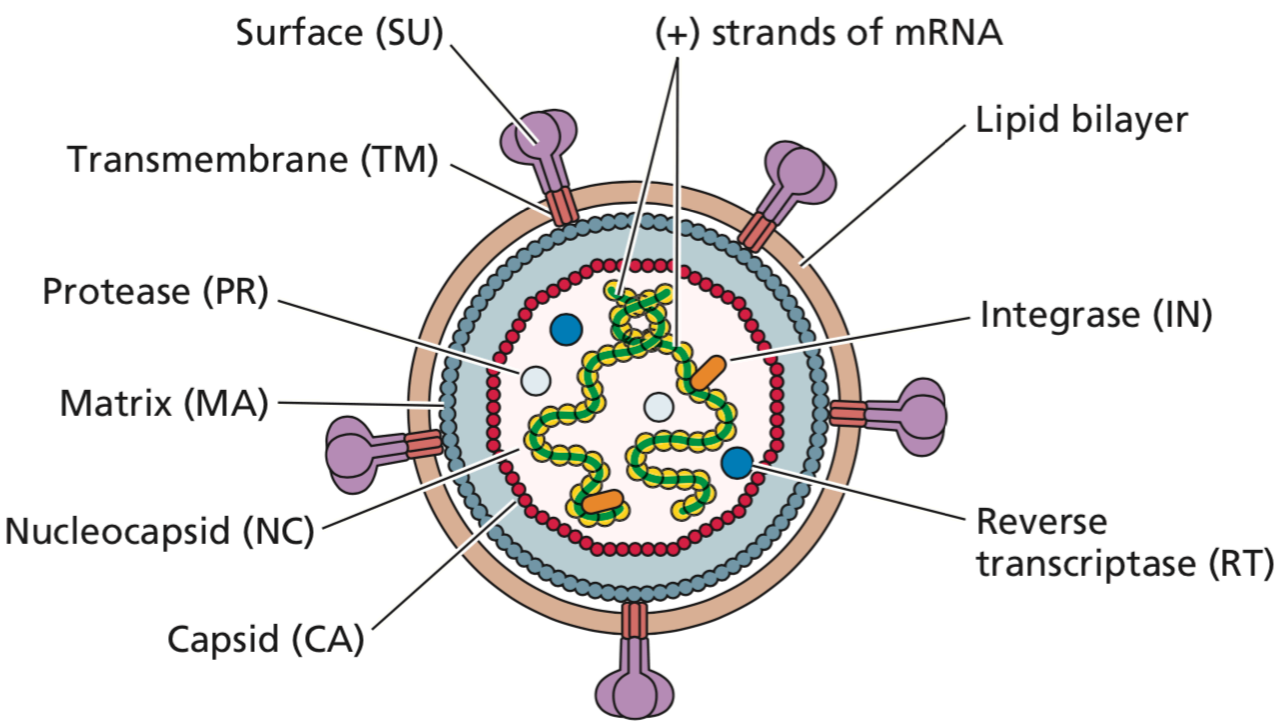


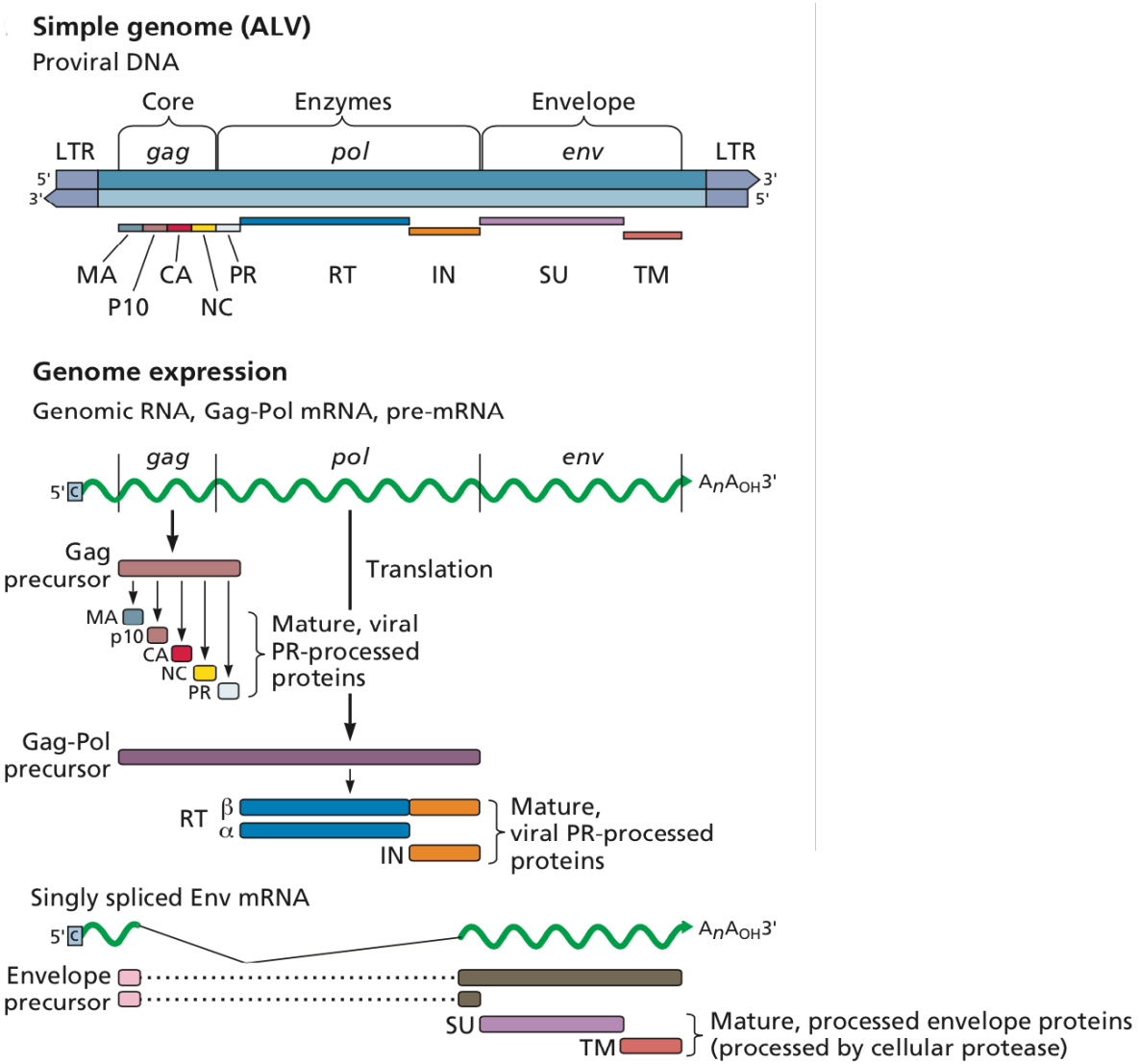
Viruses with RT

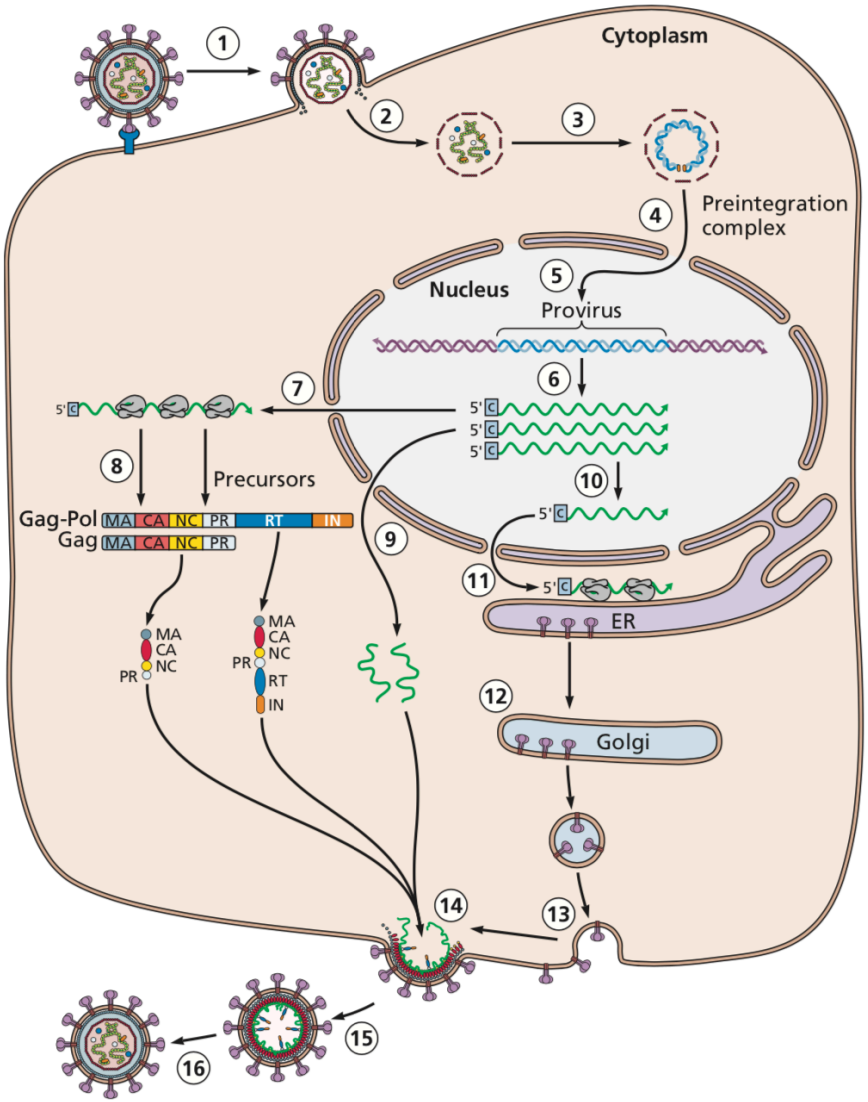


Rous sarcoma virus, a retrovirus









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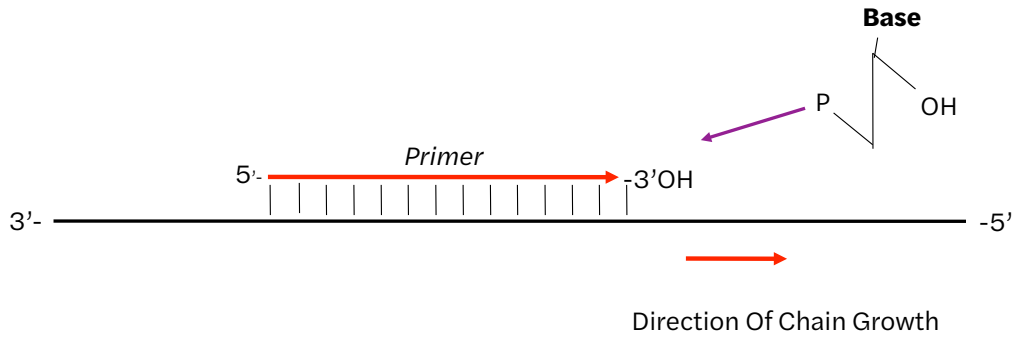
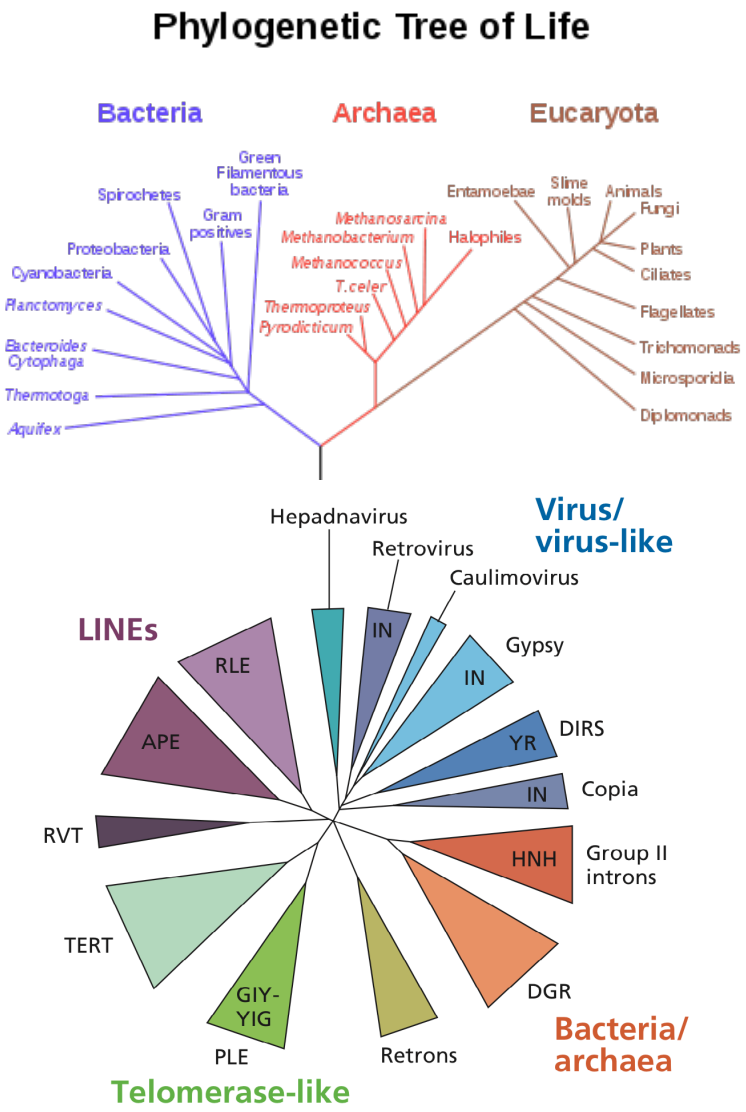
Reverse transcriptase



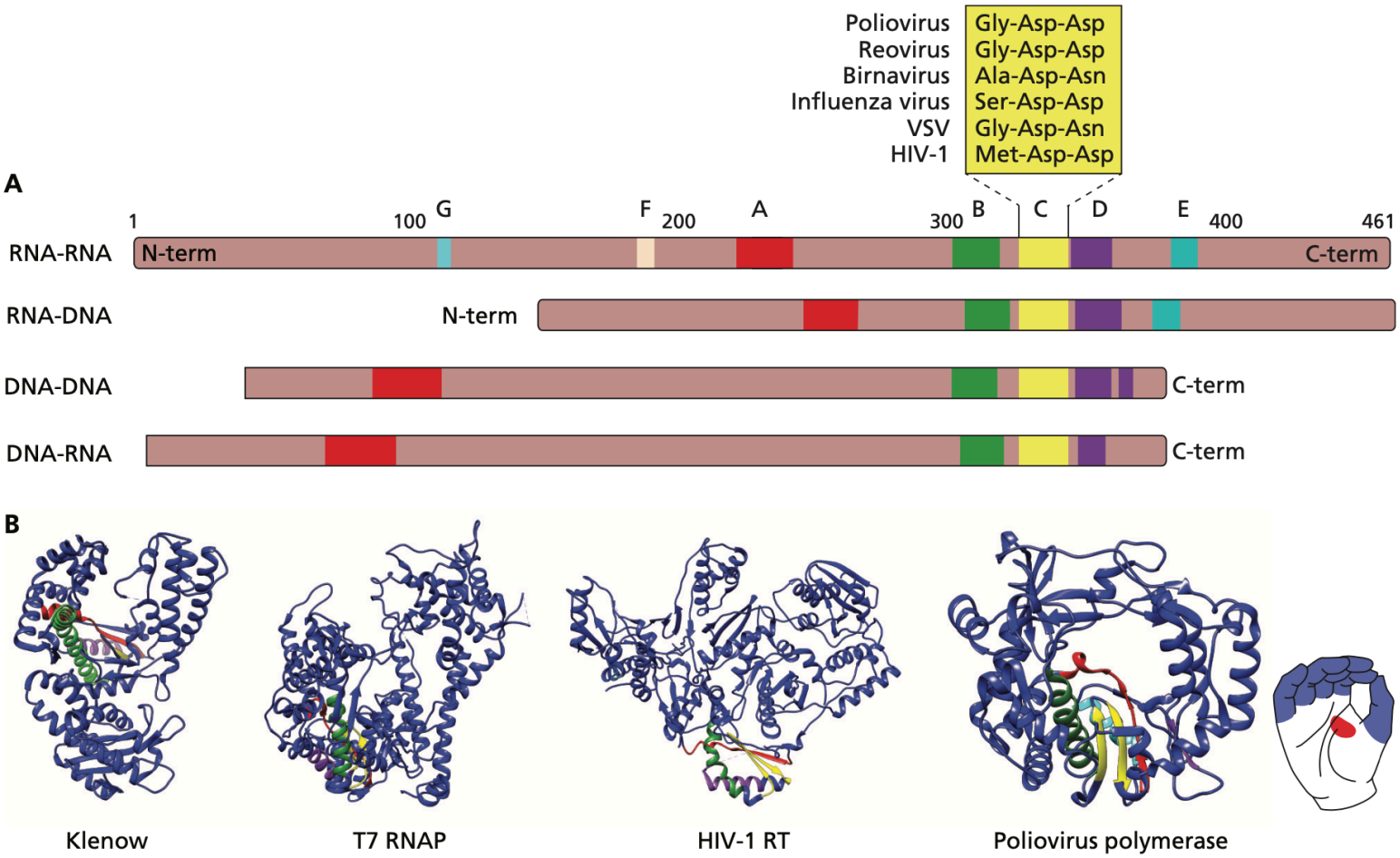
- Primer can be DNA or RNA
- Template can be RNA or DNA
- Only dNTPs, not rNTPs, are incorporated

RT

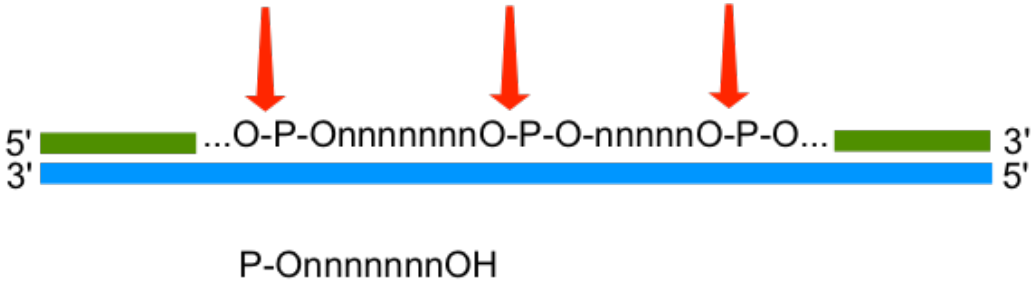
- Bacteria, Archaea, eukaryotes have RT activity
- Therefore RT evolved before the separation of Archaea, bacteria, and eukaryotes
- RT might be the bridge between early RNA world and modern DNA world
- RT also in HBV, *Caulimoviridae*



Sequence relationships among polymerases

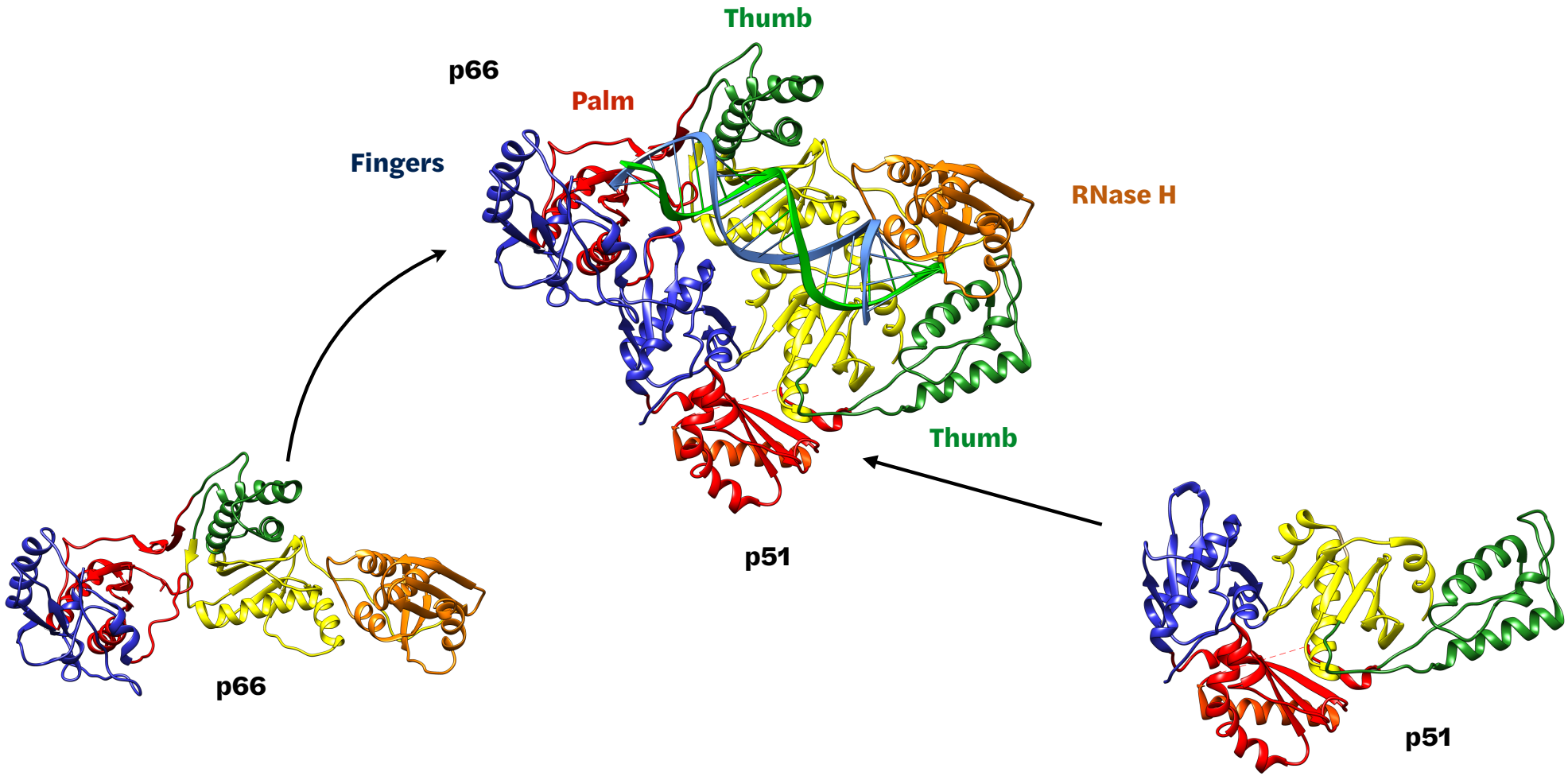


RNAse H: A second activity of RT

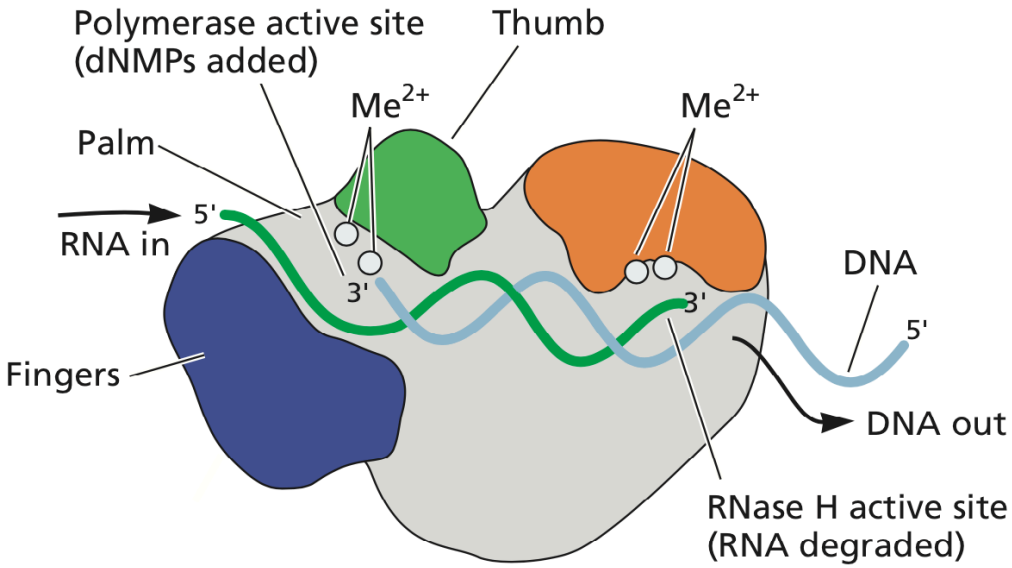


- Cleaves RNA only when in duplex form
- RNA can be in RNA:RNA or RNA:DNA duplexes
- Makes endonucleolytic cleavages
- Produces short oligonucleotides with 5’-phosphate, 3’-OH

HIV-1 Reverse transcriptase



HIV-1 Reverse transcriptase



DNA synthesis is slow (4 h per 9 kb genome) and error prone (1 misincorporation per 10^4 to 10^6 nt)

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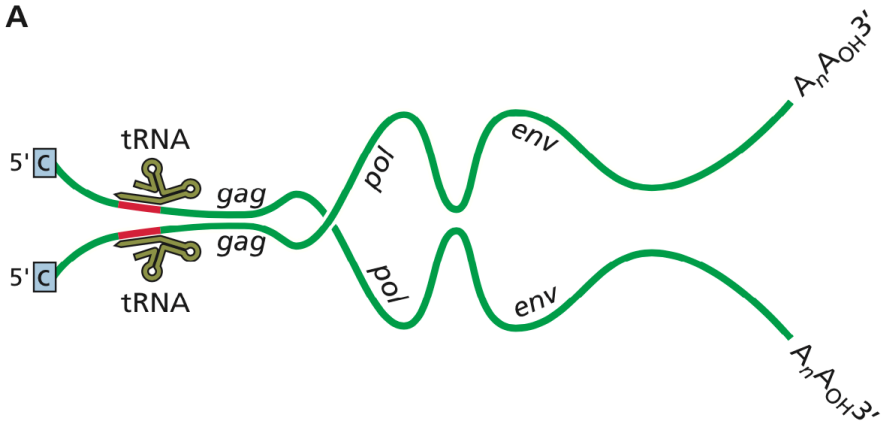
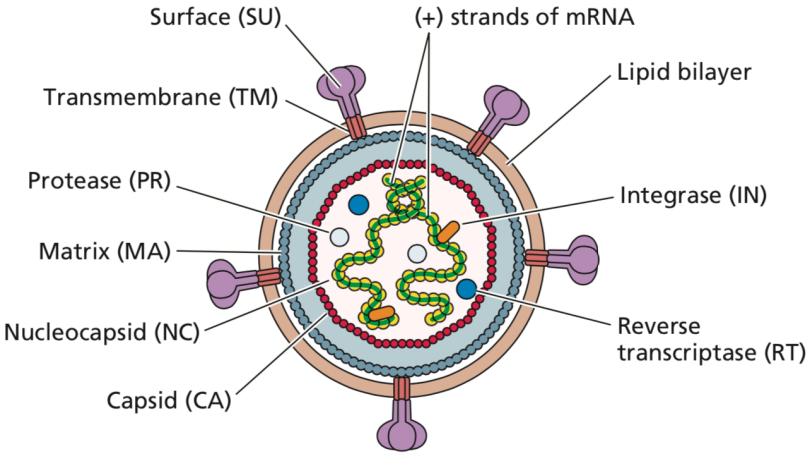
Reverse transcriptase has revolutionized molecular biology. Which statement about the enzyme is not correct?

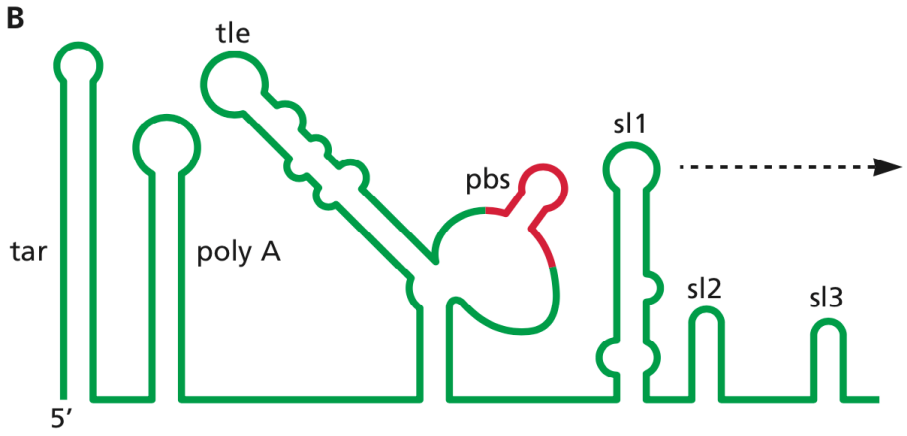
A. RT is unique to retroviruses
B. RT is packaged in the retrovirus particle
C. The RT protein also has RNAse H activity
D. The name of the enzyme comes from its ability to reverse the flow of genetic information
E. Might have bridged the ancient RNA world and the DNA world

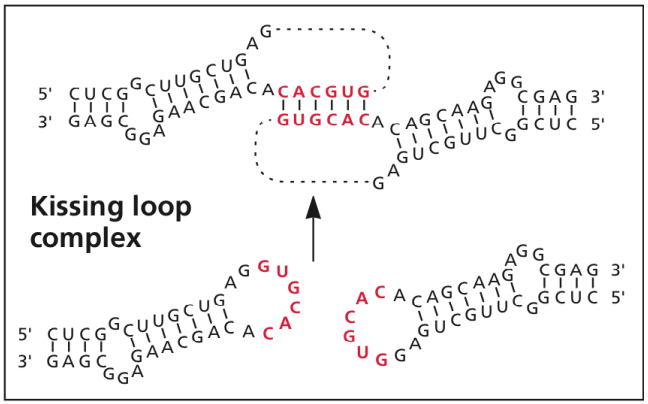




RNA coated with NC protein

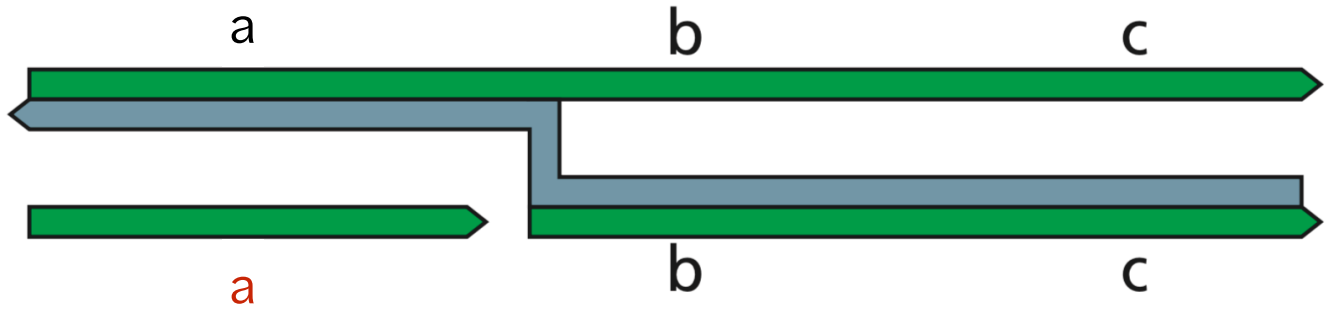
50-100 molecules RT per virus particle



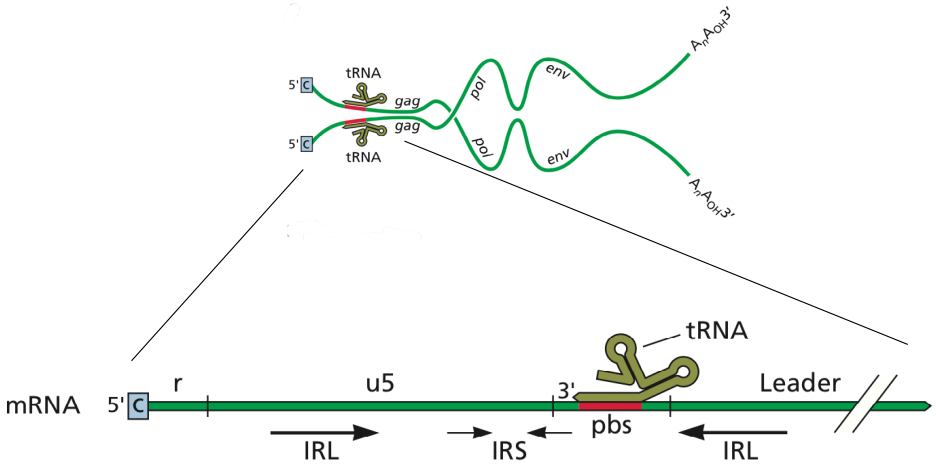


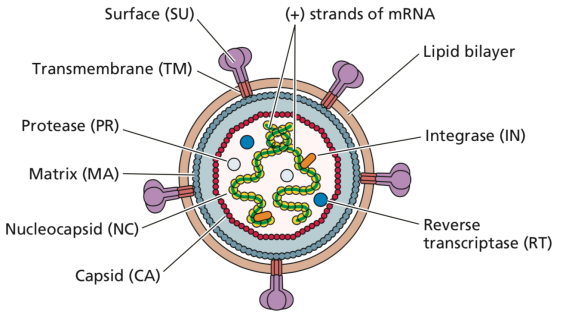
RNA dimer

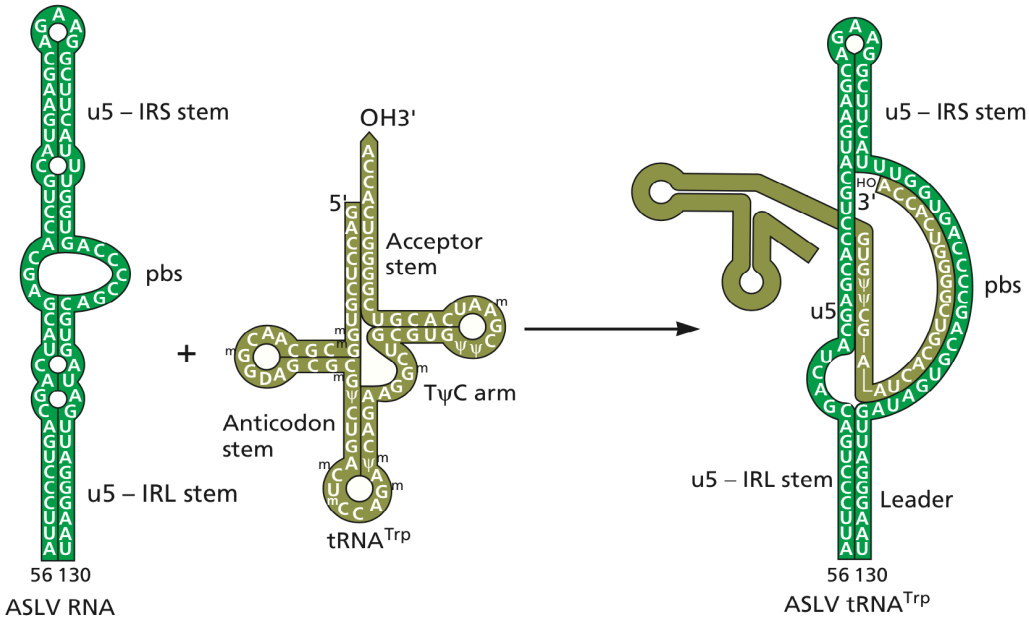
- Explains why retroviruses are relatively resistant to UV and ionizing radiation
- Two copies of all genes
- Recombination (copy-choice) during reverse transcription builds one functional genome



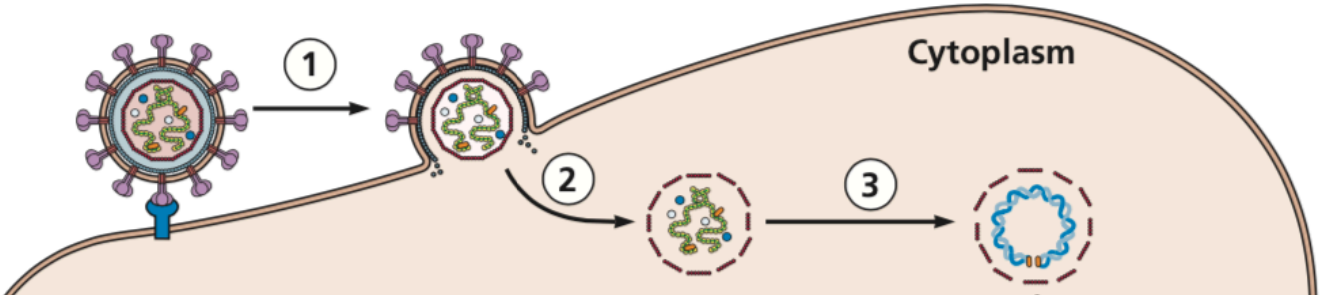
Primer tRNA binding to retroviral genome





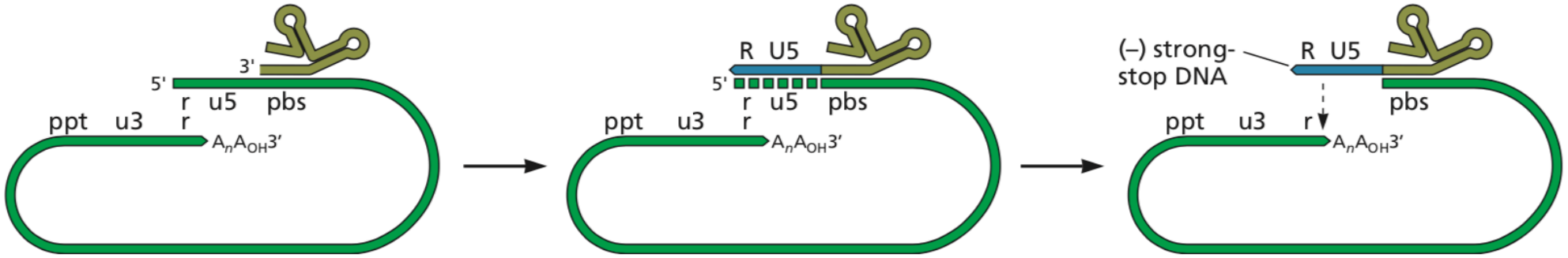


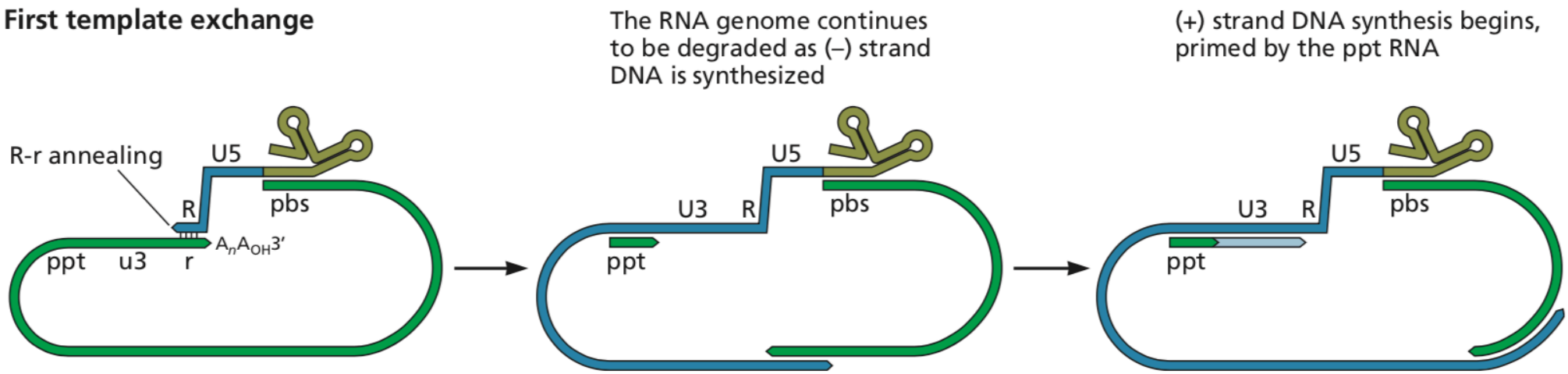
DNA synthesis: cytoplasmic



Initiation of (–) strand DNA synthesis

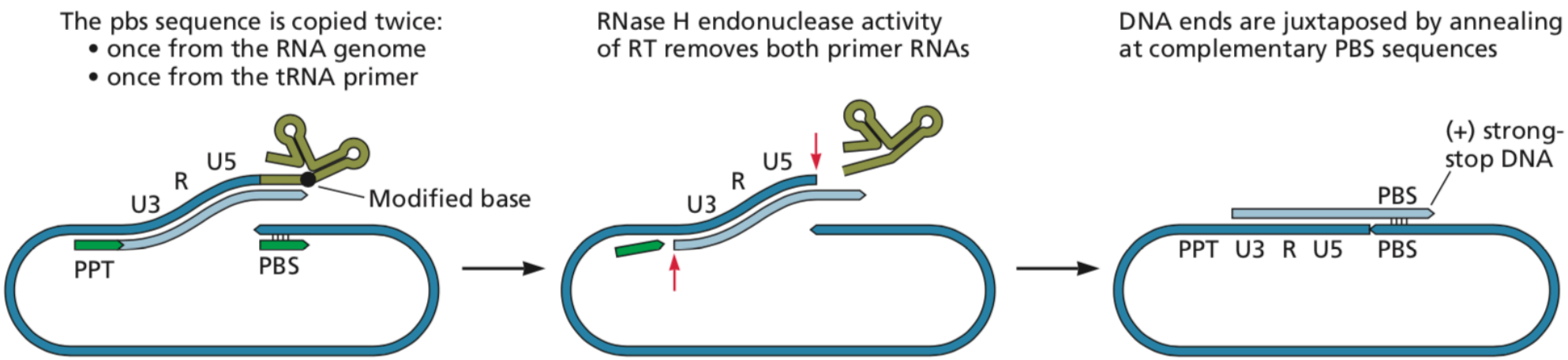
The 5' end of the viral RNA genome is degraded by the RNase H activity of RT as the (–) strand DNA is synthesized.

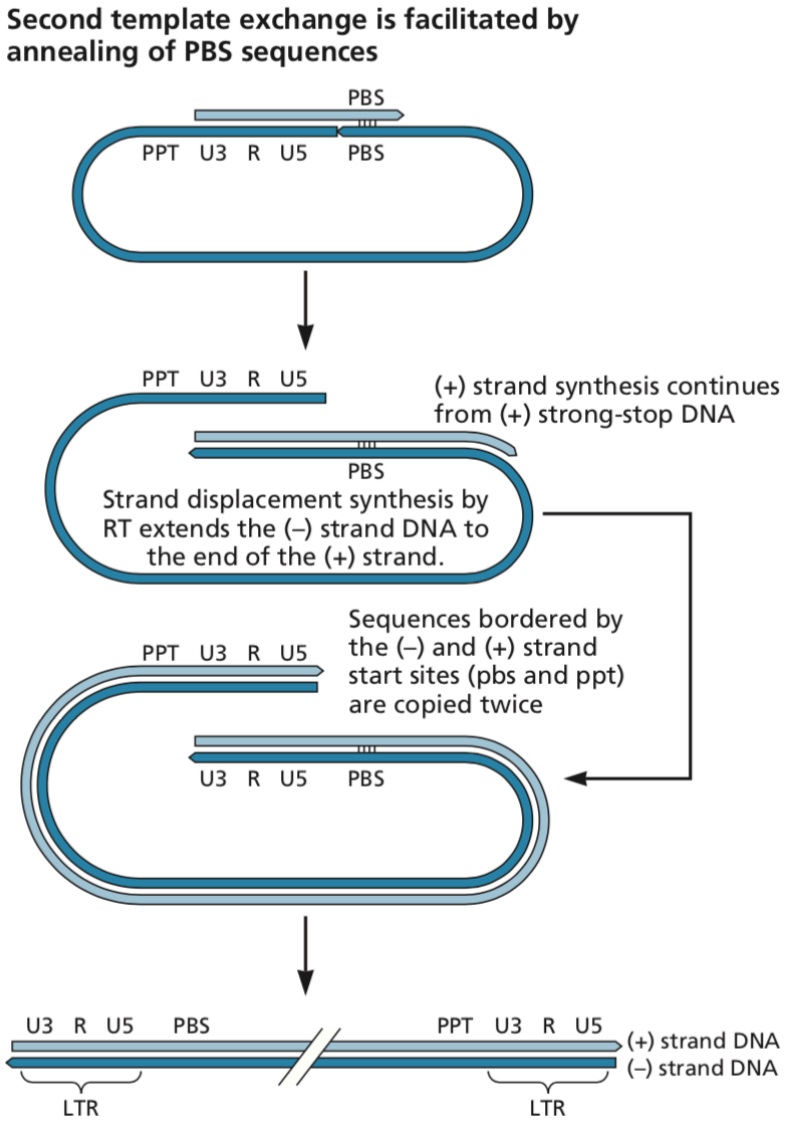


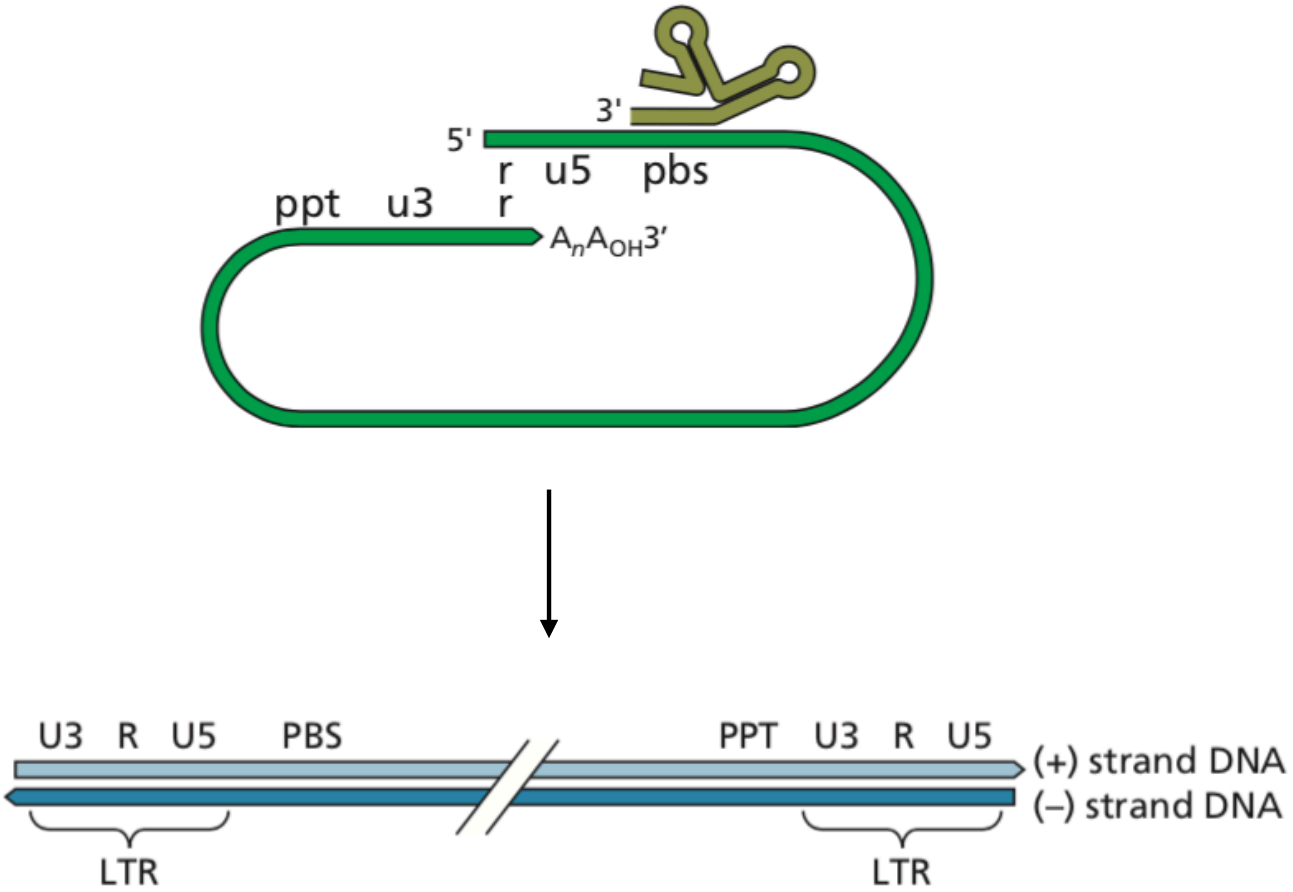


ppt = polypurine tract

(+) strand DNA synthesis







Animation of reverse transcription: https://youtu.be/RYwVnzYf4V8

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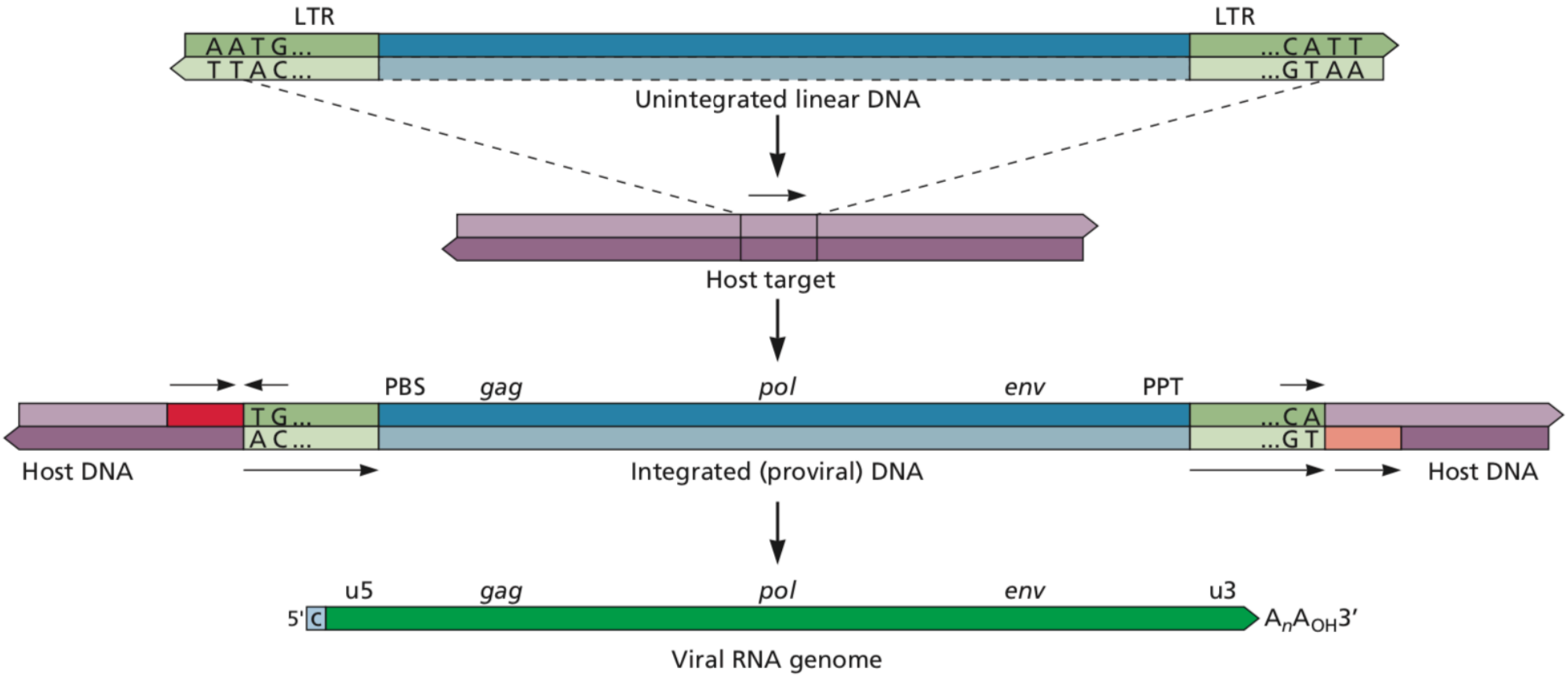
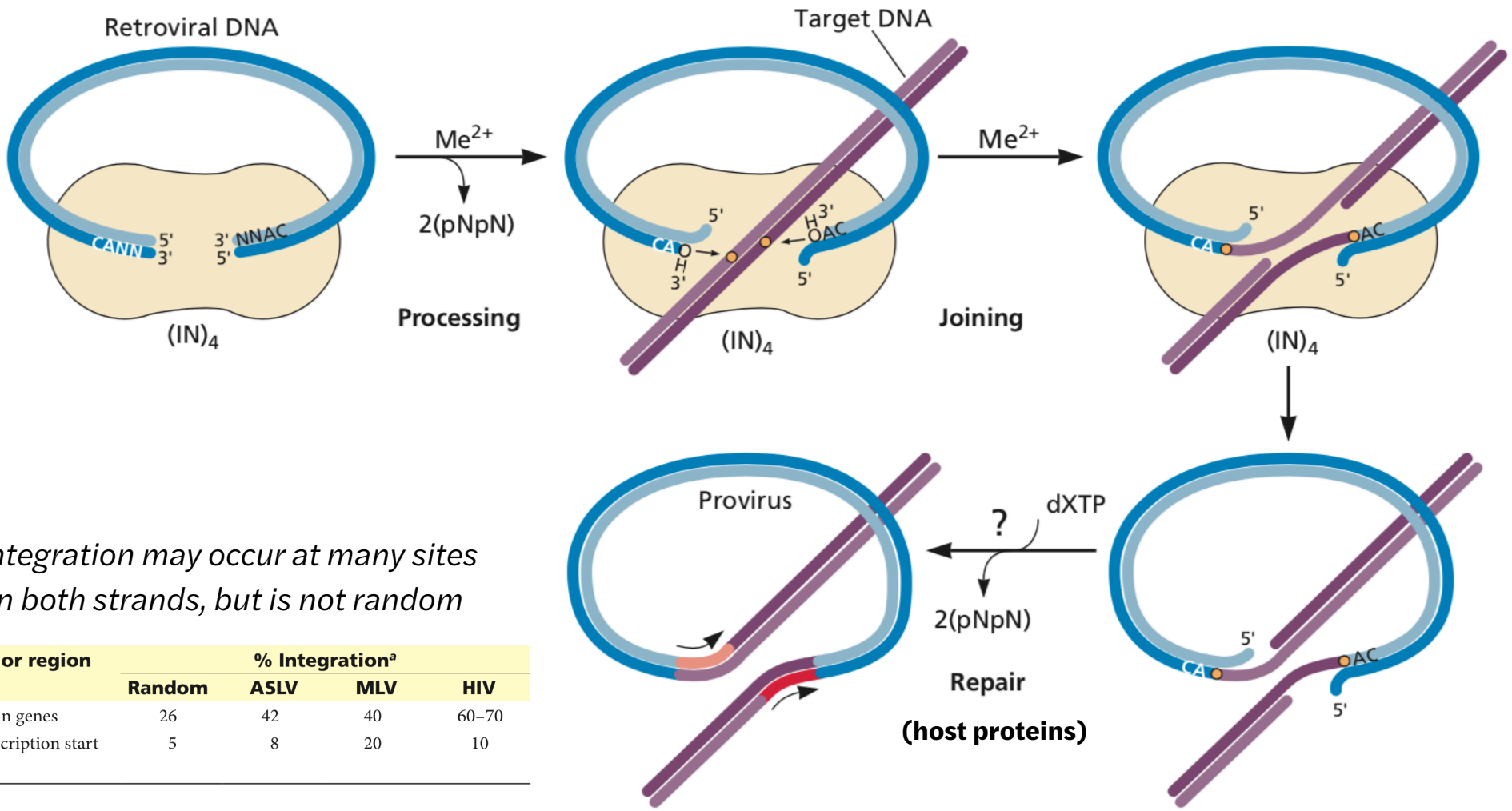
Which of the following steps occur during reverse transcription of retroviral genomic RNA?

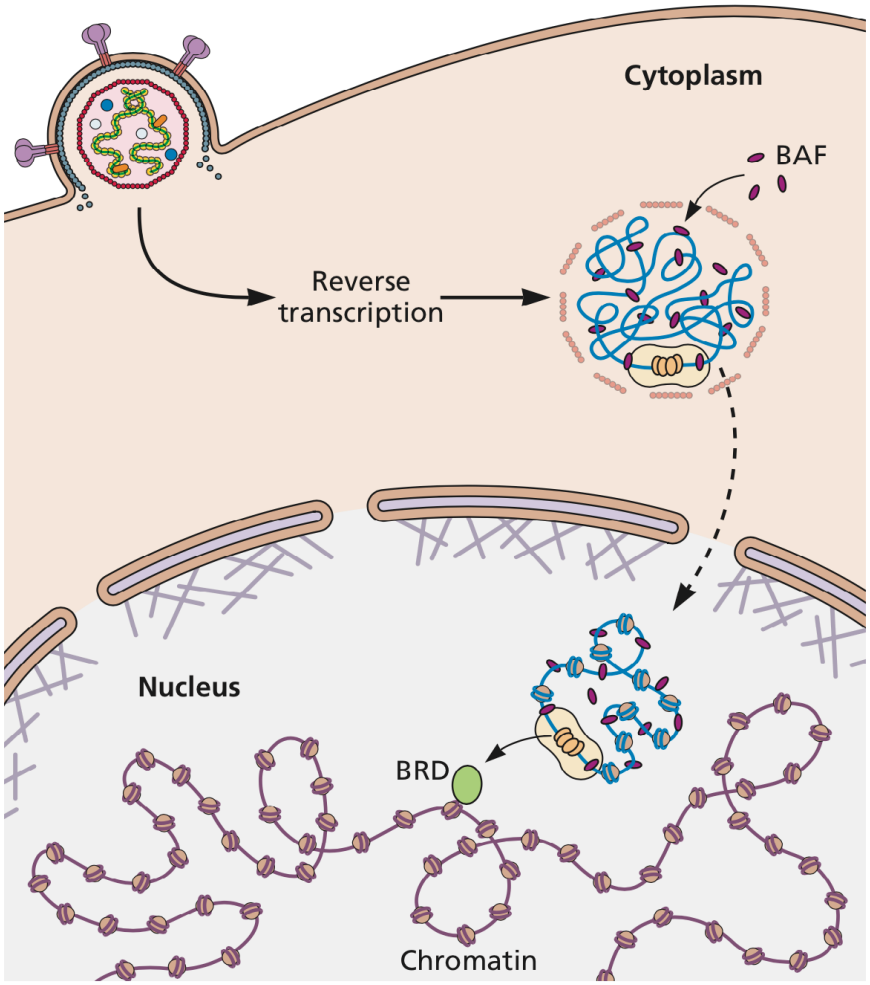
A. Priming of (-) DNA synthesis by tRNA
B. Two template exchanges
C. Degradation of the viral RNA by RNAse H
D. Generation of two LTRs
E. All of the above



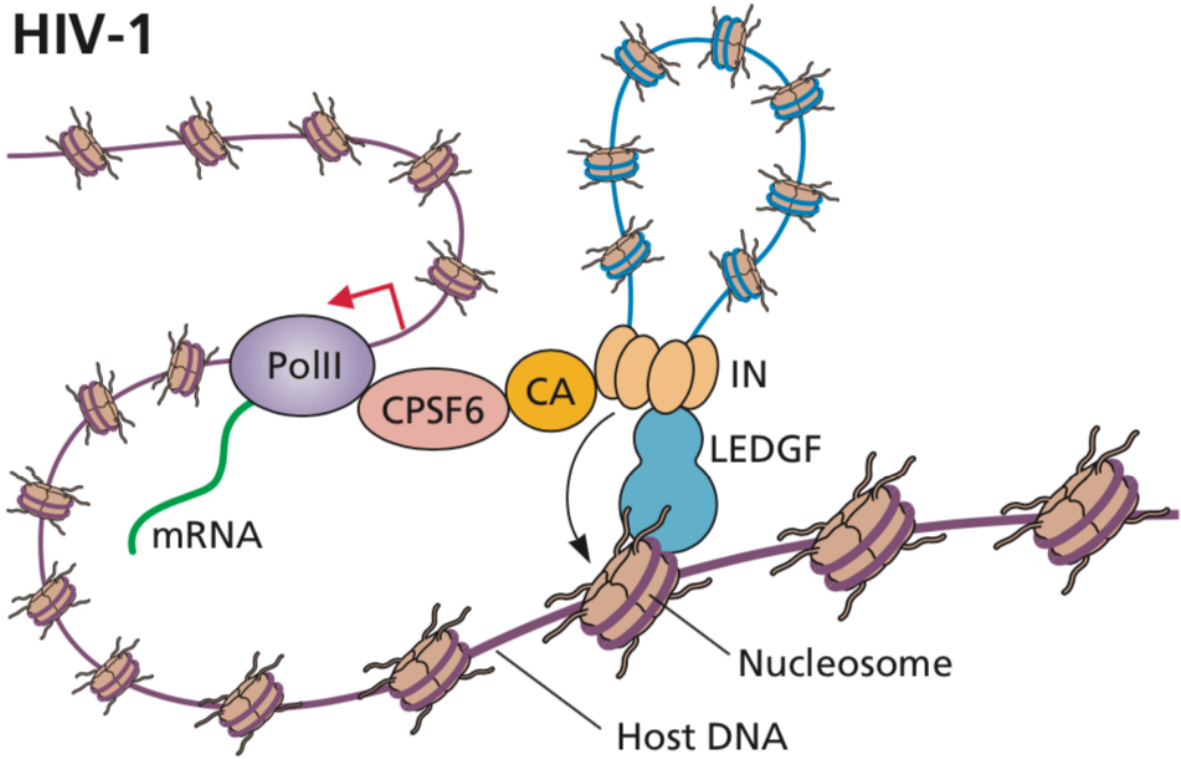


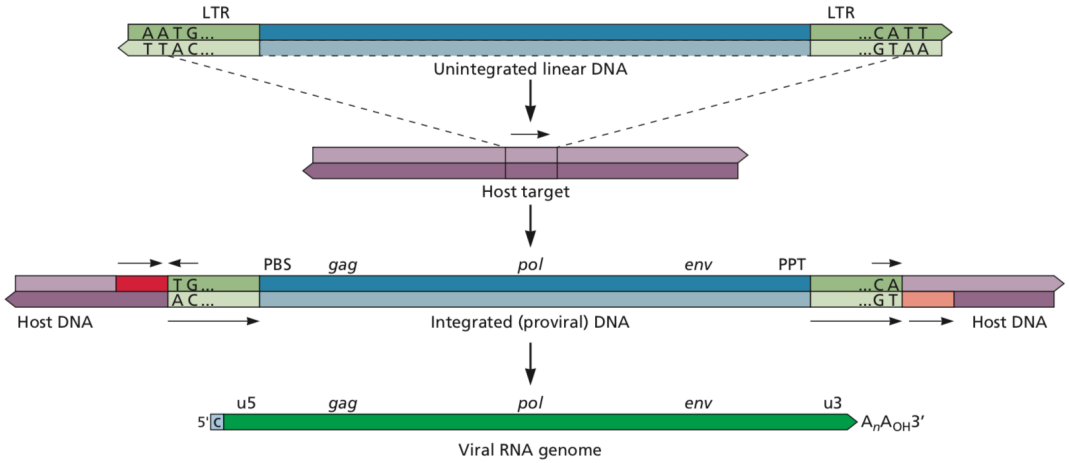
Integration may occur at many sites on both strands, but is not random

Site or region	% Integration[a]			
	Random	ASLV	MLV	HIV
Within genes	26	42	40	60–70
Transcription start sites	5	8	20	10



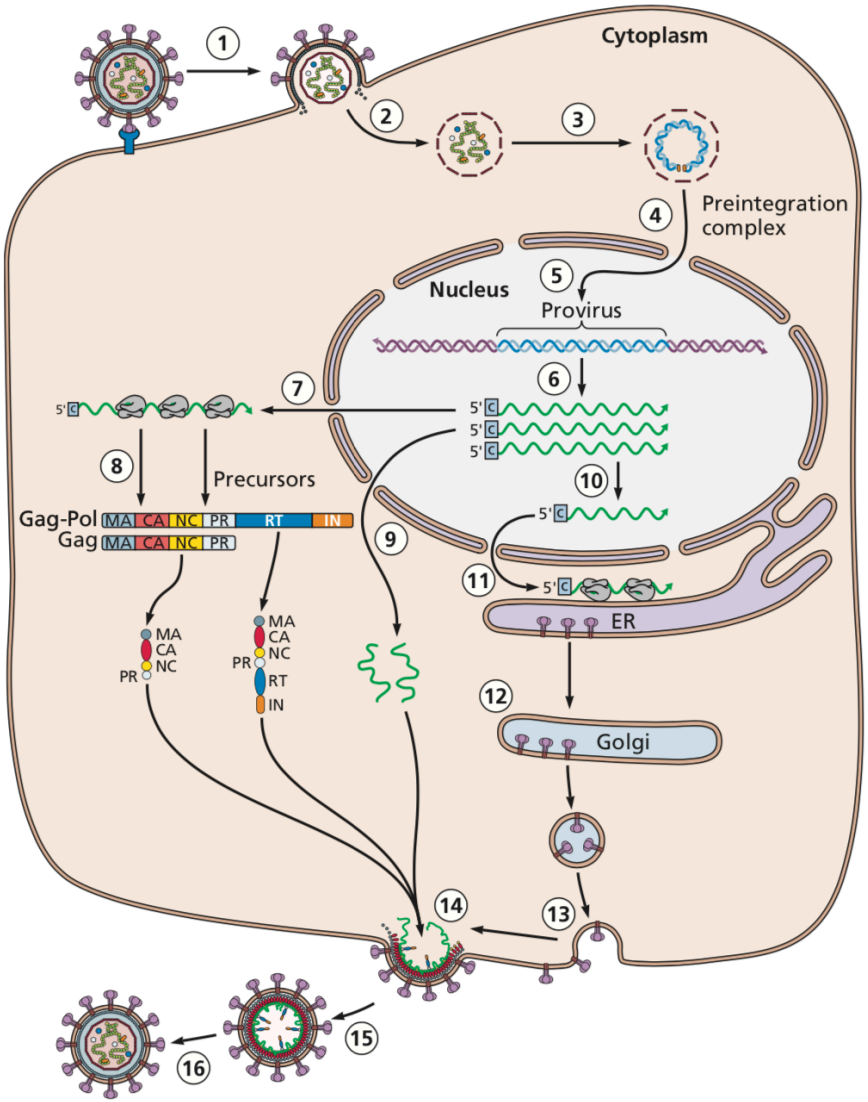
BAF binding prevents auto-integration





- One DNA produced from two RNAs by RT
- Strong promoter (the LTR) built during RT
- Proviral DNA directs the host transcription machinery to synthesize many copies of viral mRNA
- Viral mRNA is translated into viral proteins OR encapsidated into virus particles

There is *no viral DNA replication* and *no viral RNA replication*



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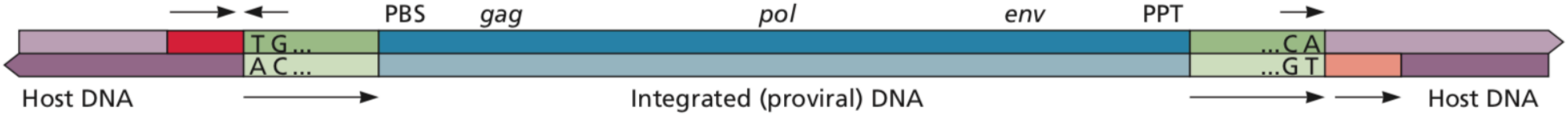
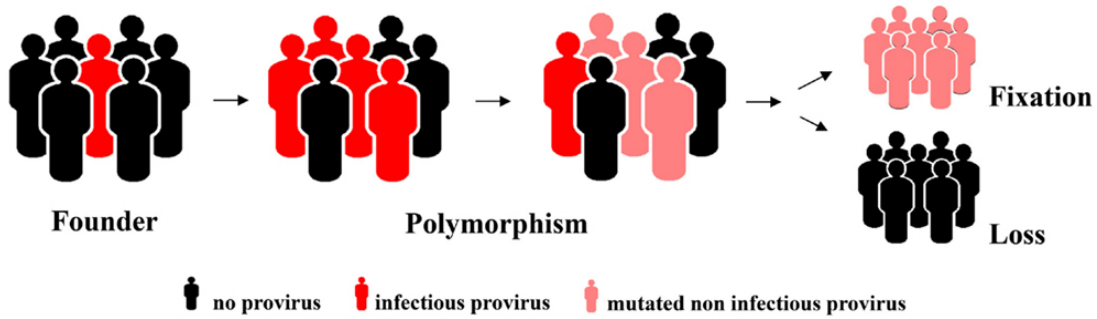
Provirus is a permanent part of host genome

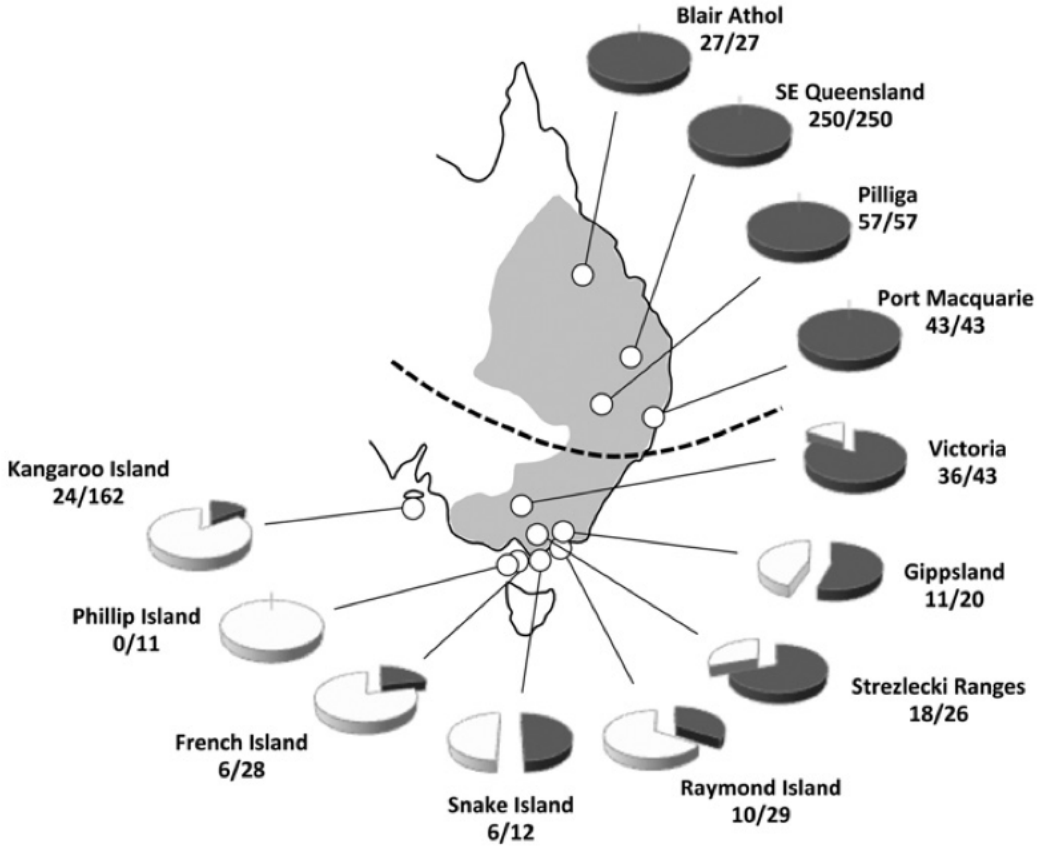


- No mechanism for precise excision of integrated provirus
- Only way out of genome is transcription by host RNA pol II
- Cell genomes are littered with ancient and modern retroelements - via endogenization



Contemporary endogenization in Koalas

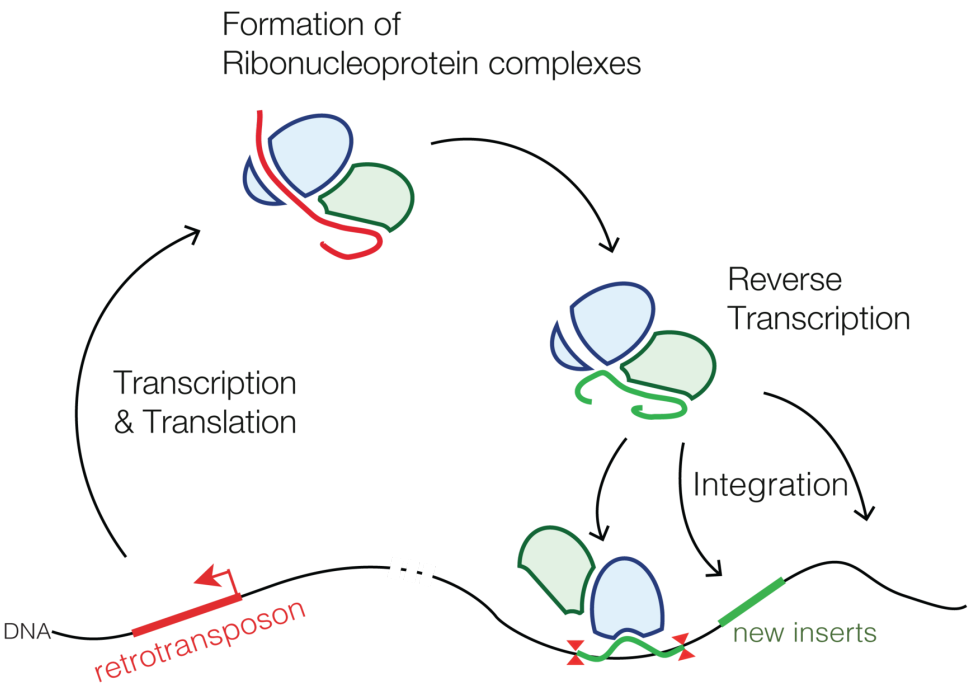
~50,000 years ago, cross-species transmission from rodents



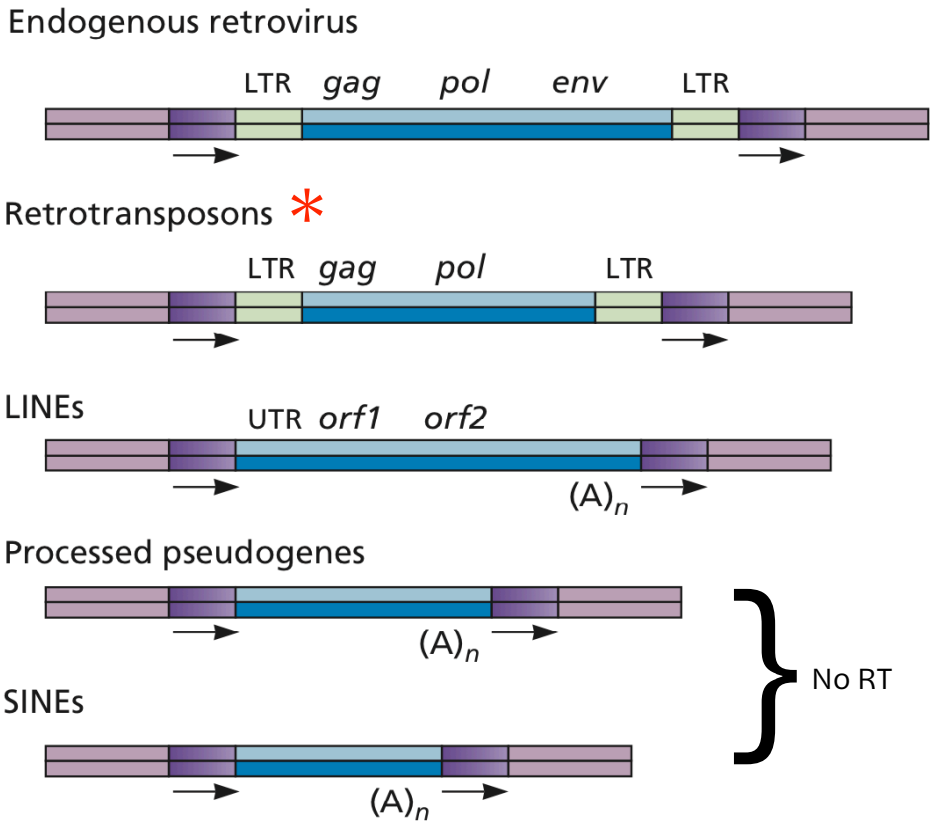
doi: 10.1111/j.1751-0813.2012.00964.x

Retroelements

- Sequences that move in the genome via RT
- Proviral DNA integrated into the germline = endogenous retroviruses, ERV
- Often replication-defective (all are defective in humans)
- ~42% of human genome comprises mobile genetic elements, including endogenous proviruses and other retroelements



Retroelements in the human genome



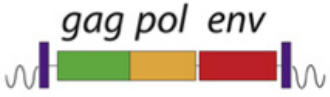
Retroelements in the Human Genome
42.2%; 2.7 X 10^6

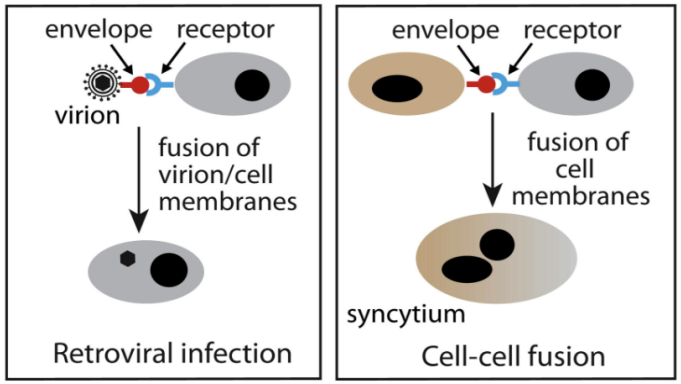
Non-LTR 33.9%; 2.4 X 10^6			**LTR-Containing** 8.3%; 0.3 X 10^6		
LINEs	(L1)	16.9%	Endogenous Retroviral sequences	(ERVs)	7.7%
	(L2)	3.2			
SINEs	(Alu)	10.6%	Others including Retrotransposons		0.6%
	(MIR)	2.5			
Processed pseudogenes		<1.0			

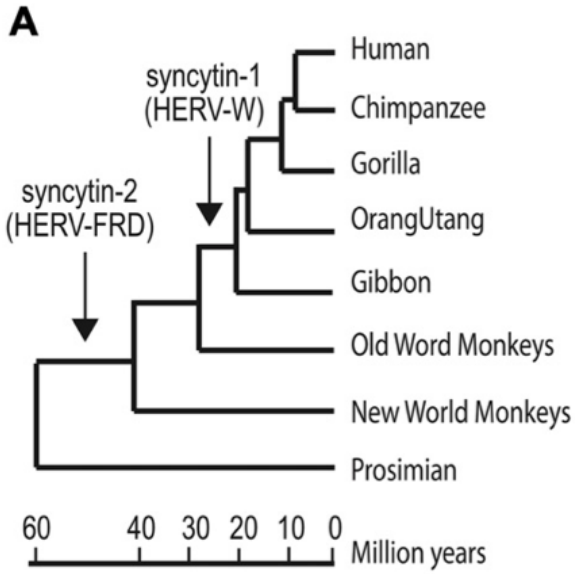
<0.05% are active in human genome today

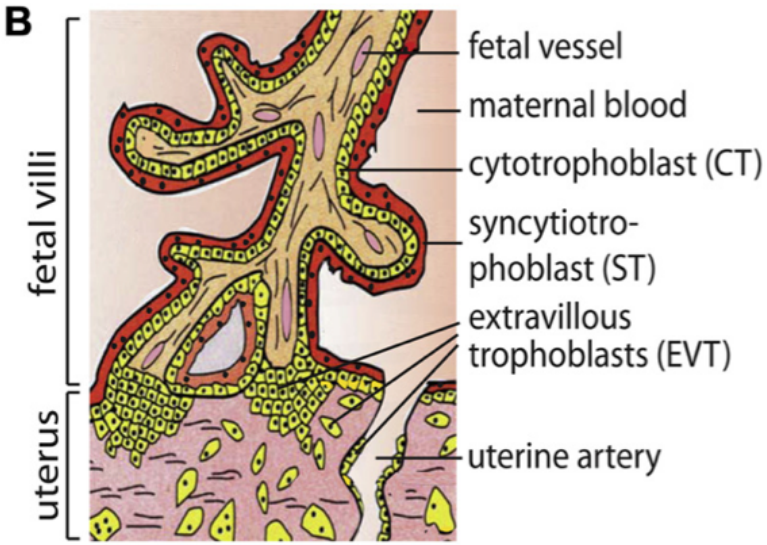
*Likely progenitors of retroviruses

Syncytins: Exapted retroviral env





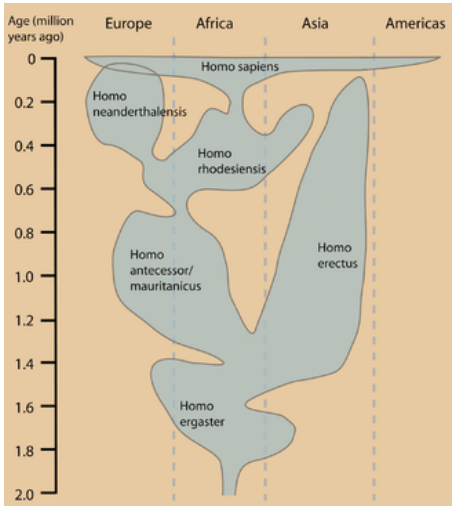


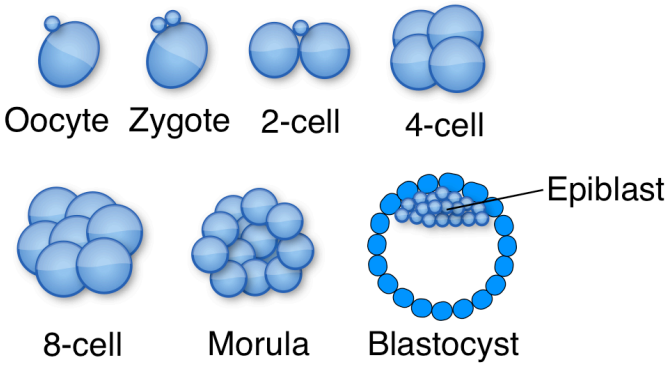


Placenta 33 (2012) 663-671

Retroviral influence on human embryonic development

- HERV-K, infected human ancestors ~200,000 years ago
- HERVs do not product infectious virus
- HERV-K mRNAs are produced during normal human embryogenesis
- From 8 cell stage to epiblasts
- Virus-like particles observed
- Induces an antiviral protein, IFITM1





A retrovirus makes chicken eggshells blue

http://www.virology.ws/2013/09/11/a-retrovirus-makes-chicken-eggshells-blue/

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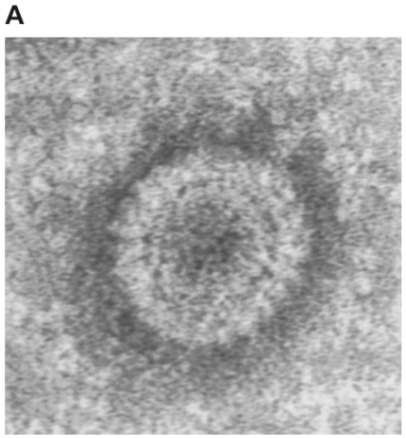
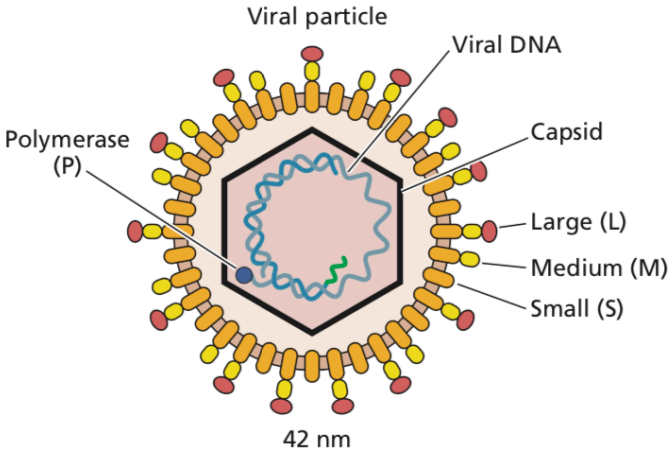
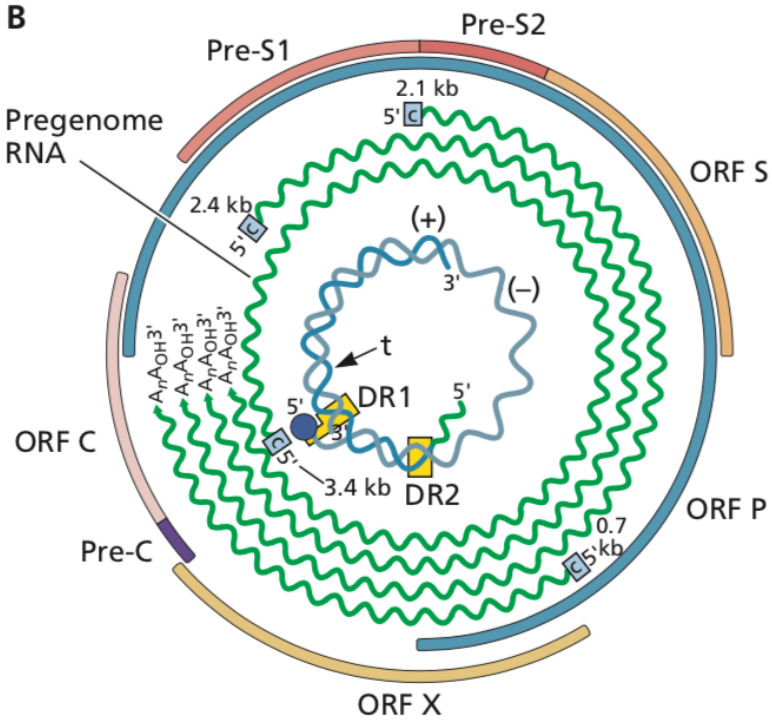
Which of the following statements about retroelements is not correct?

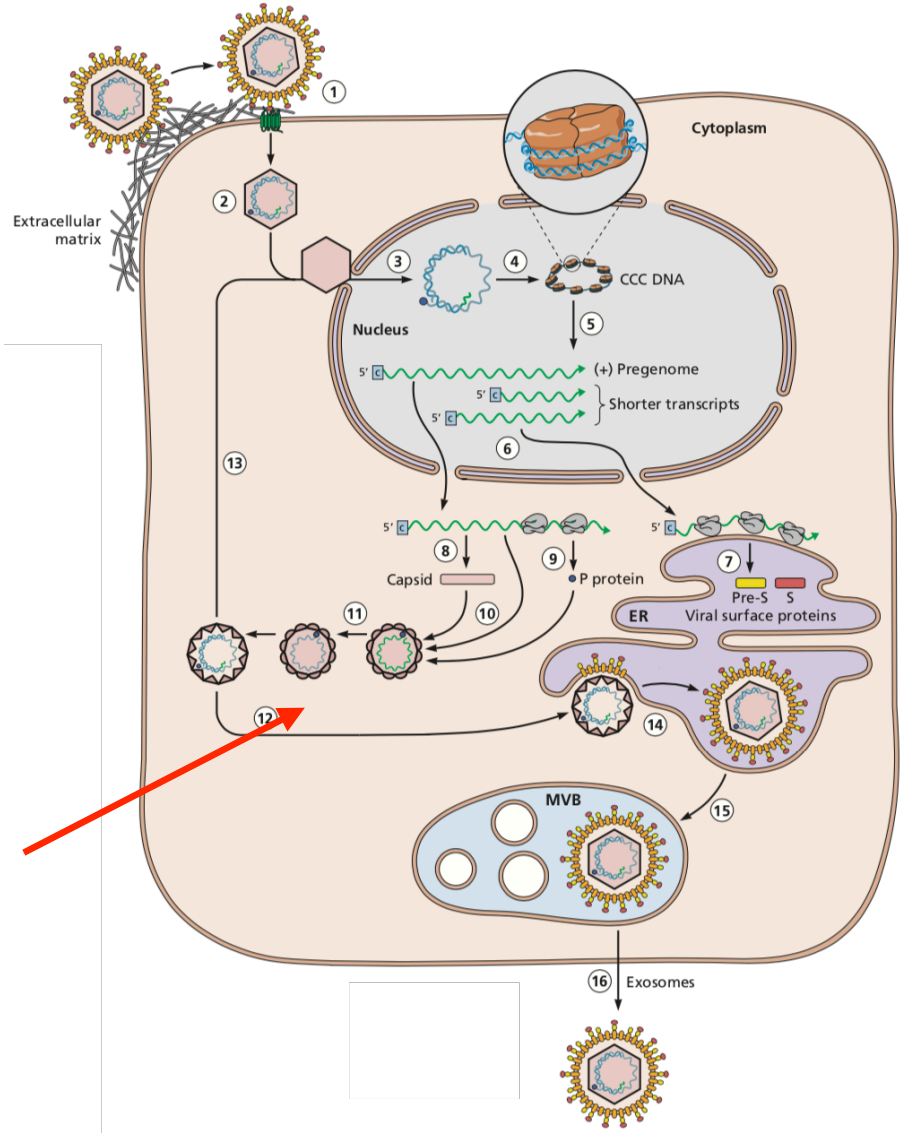
A. There are many copies in eukaryotic genomes
B. They are currently entering the Koala germline
C. Those in the human genome produce infectious viruses
D. They can be beneficial
E. None of the above

Hepadnaviridae - DNA viruses that encode RT



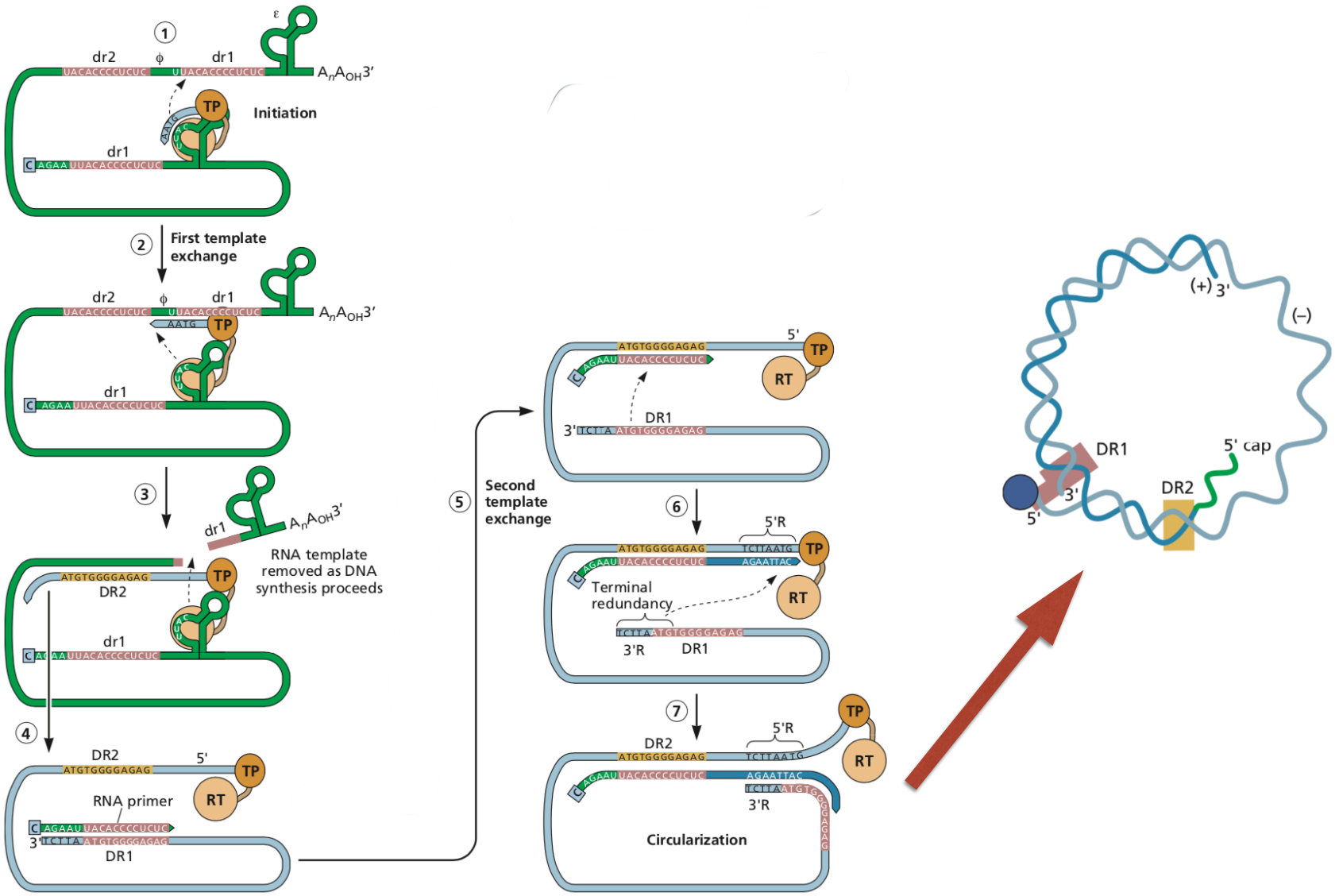


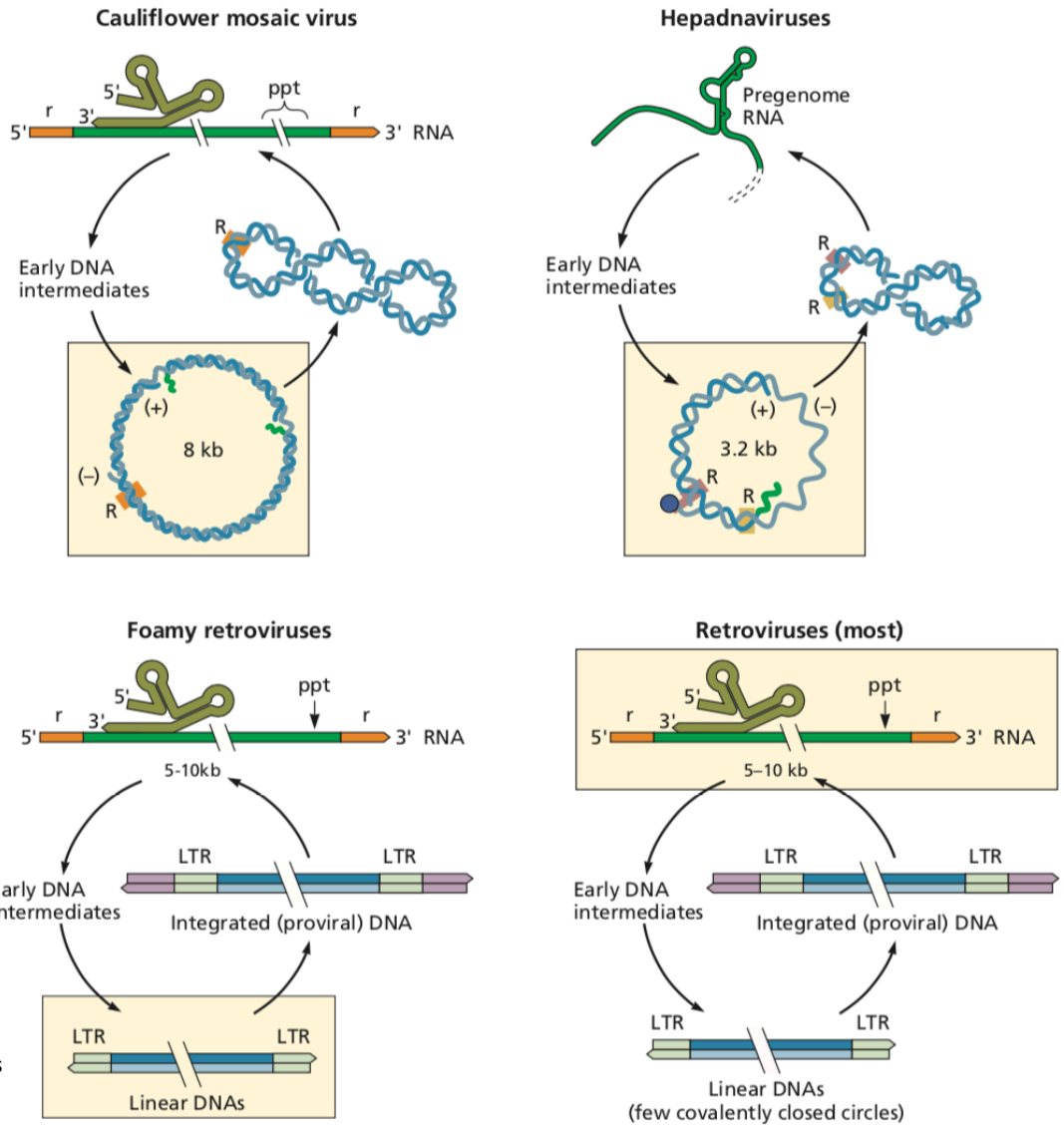




No genome integration

RT commences in the cytoplasm!





Yellow = in virus particle

DNA in 5-20% of virus particles

Next time: Assembly