Reverse transcription and integration

Lecture 9
Biology 3310/4310
Virology
Spring 2017

“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

Temin's insight

- Retroviruses caused permanent changes in cells (transformation)
- Retroviral DNA was integrated into host genome
- Became permanent part of host DNA
- Provirus

Baltimore and Temin independently discovered RT in RNA tumor virus particles

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requires the synthesis of new DNA different from that synthesized in the *S*-phase of the cell cycle (refs. 1, 2 and unpublished results of D. Boettiger and H. M. T.); production of RNA tumour viruses is sensitive to actinomycin D[3,4]; and cells transformed by RNA tumour viruses have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[1,3,7]. This model requires a unique enzyme, an RNA-dependent DNA polymerase.

Listen to TWiV #100 (Baltimore) for more insight

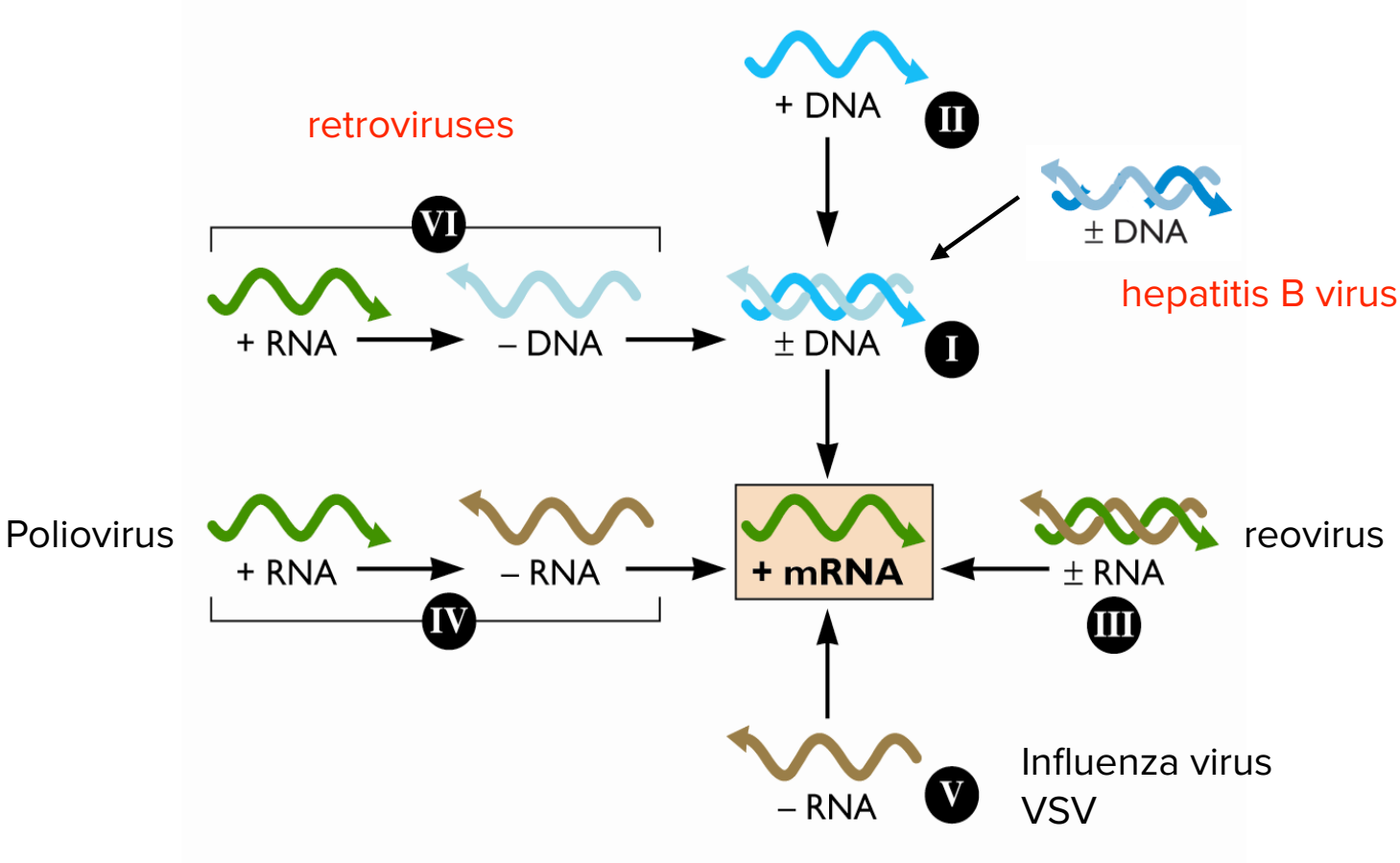
Reverse transcriptase

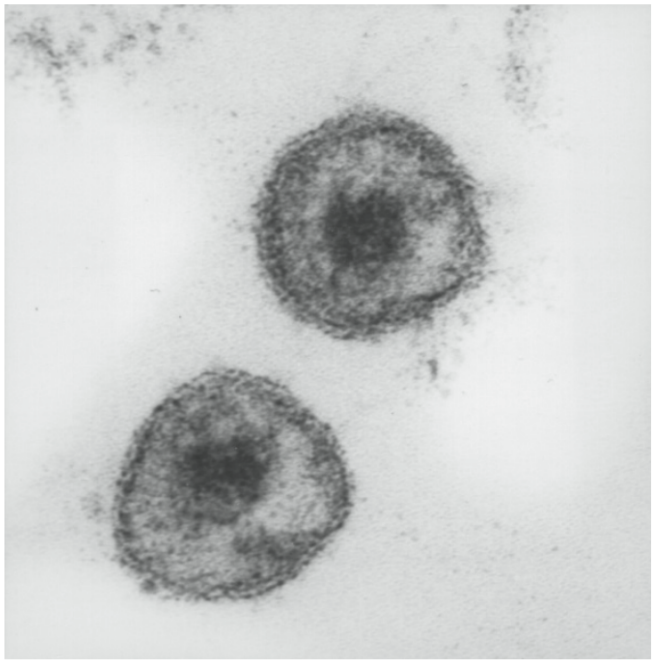
- Enzyme that countered *Central Dogma*:

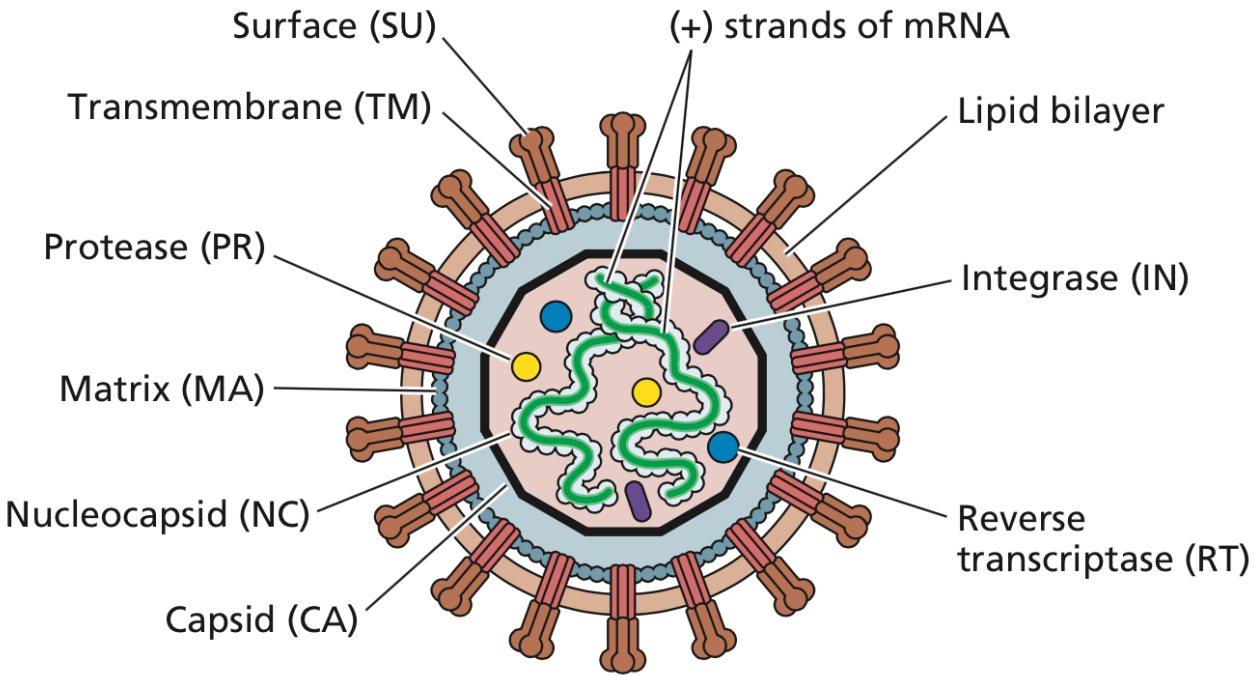
  DNA => RNA => protein

- Retroviruses got their name because of their ability to reverse the flow of genetic information
- RT discovery revolutionized molecular biology

Viruses with RT

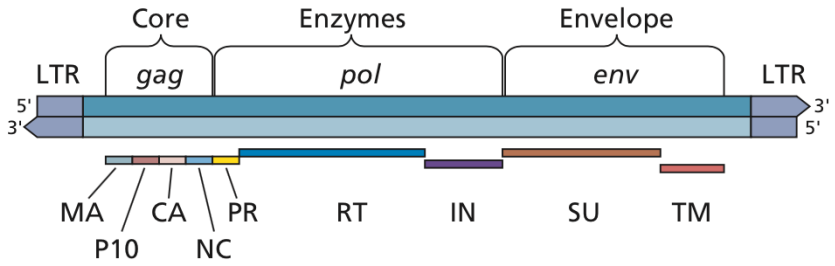






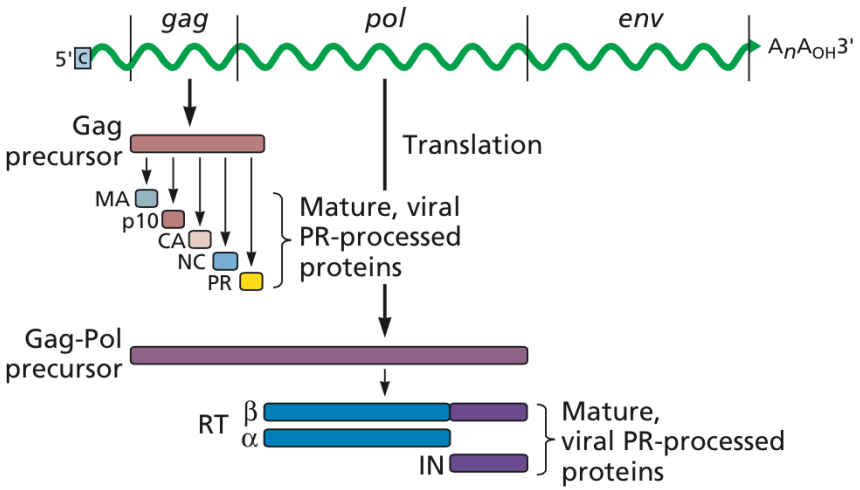
Simple genome (ALV)

Proviral DNA

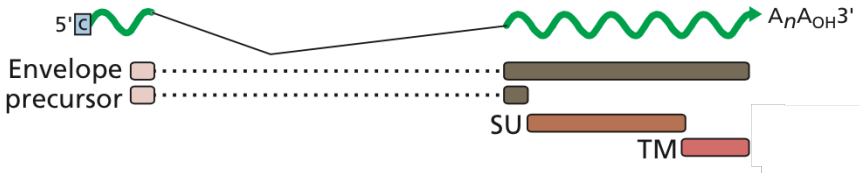


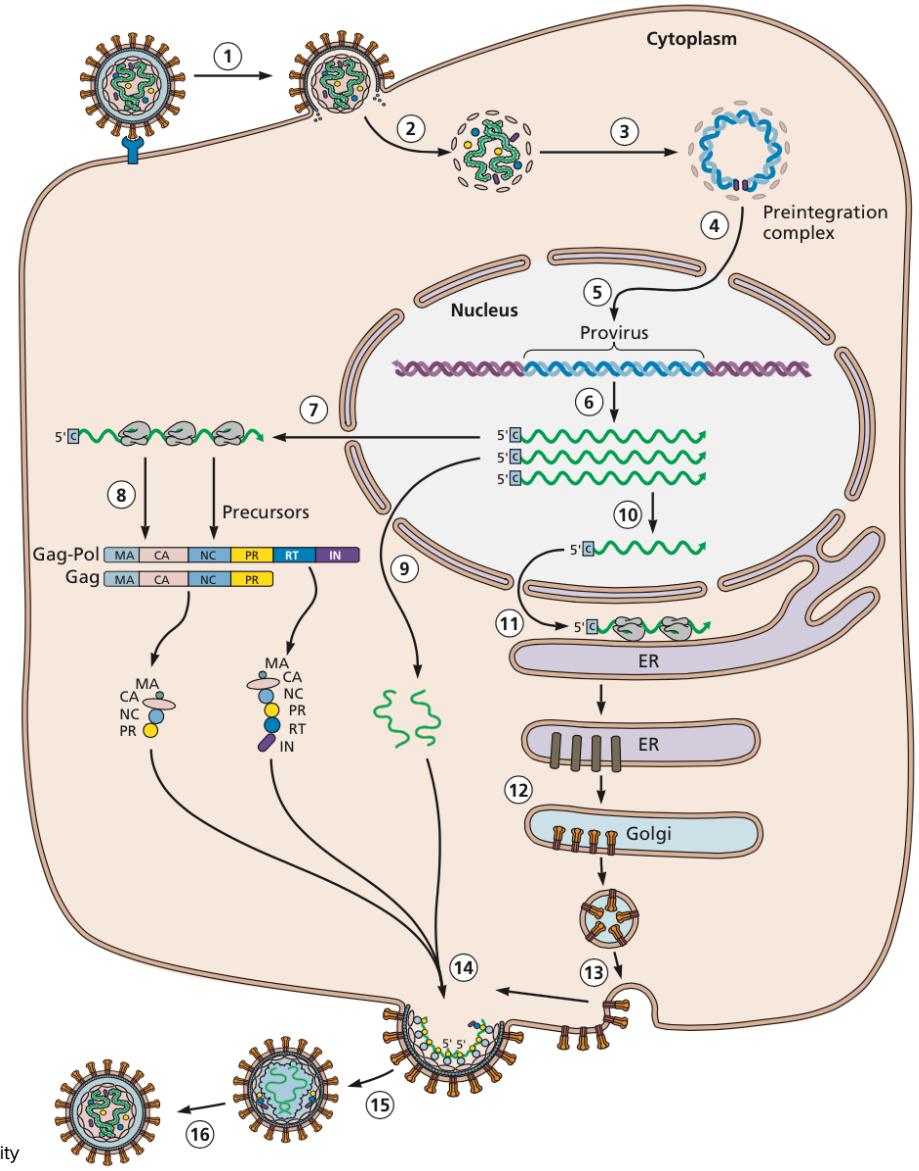
Genome expression

Genomic RNA, Gag-Pol mRNA, pre-mRNA

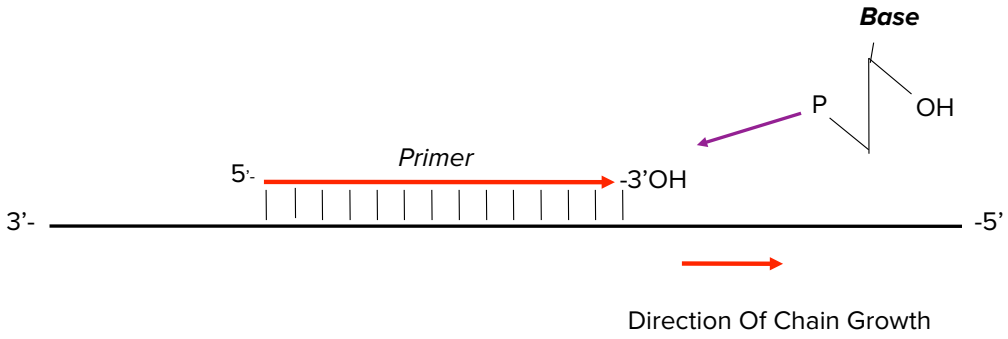


Singly spliced Env mRNA





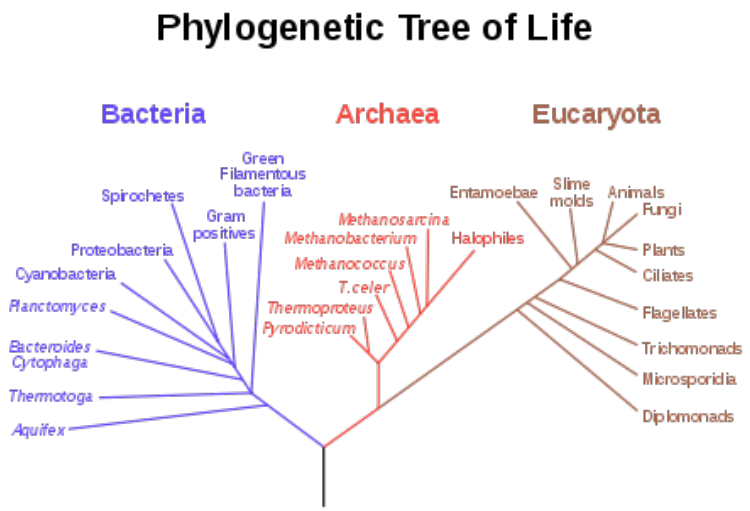
Reverse transcriptase



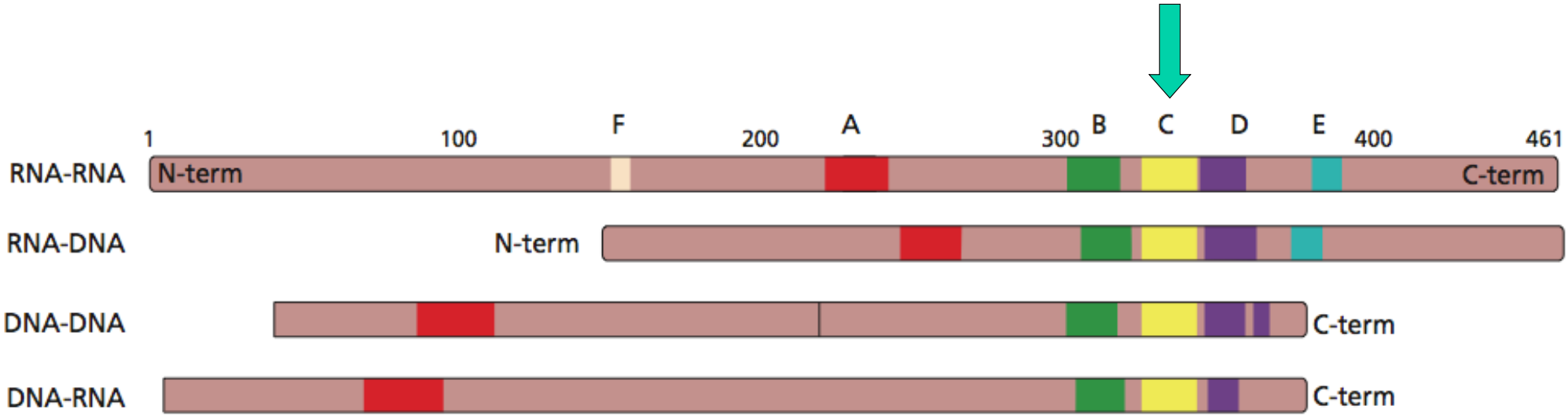
- Primer can be DNA or RNA
- Template can be RNA or DNA
- Only dNTPs, not rNTPs, are incorporated

RT

- Bacteria and Archaea 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*

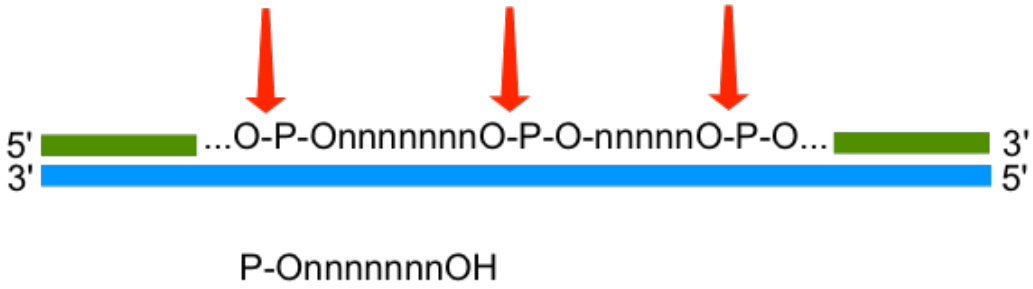


Sequence relationships among polymerases

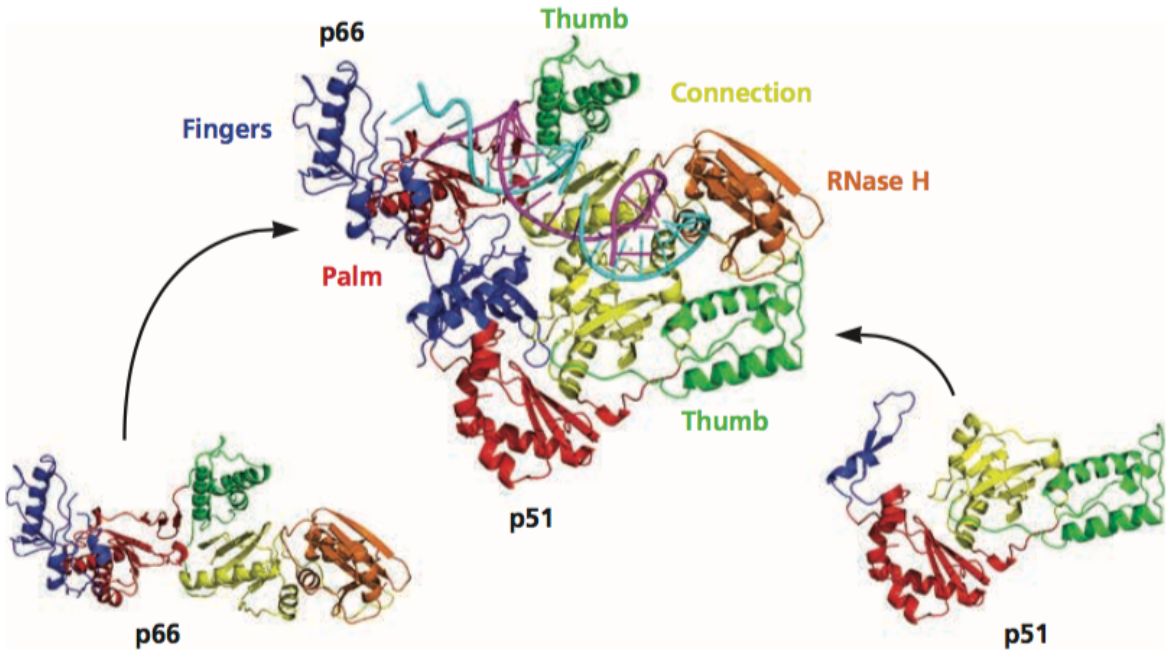


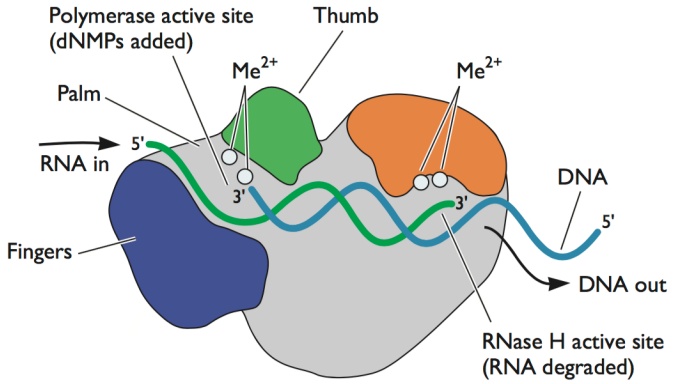
- Gly-Asp-Asp in (+) strand RNA polymerases
- Asp-Asp in RT, segmented (-) strand polymerases
- Gly-Asp-Asn in nonsegmented (-) strand polymerases

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





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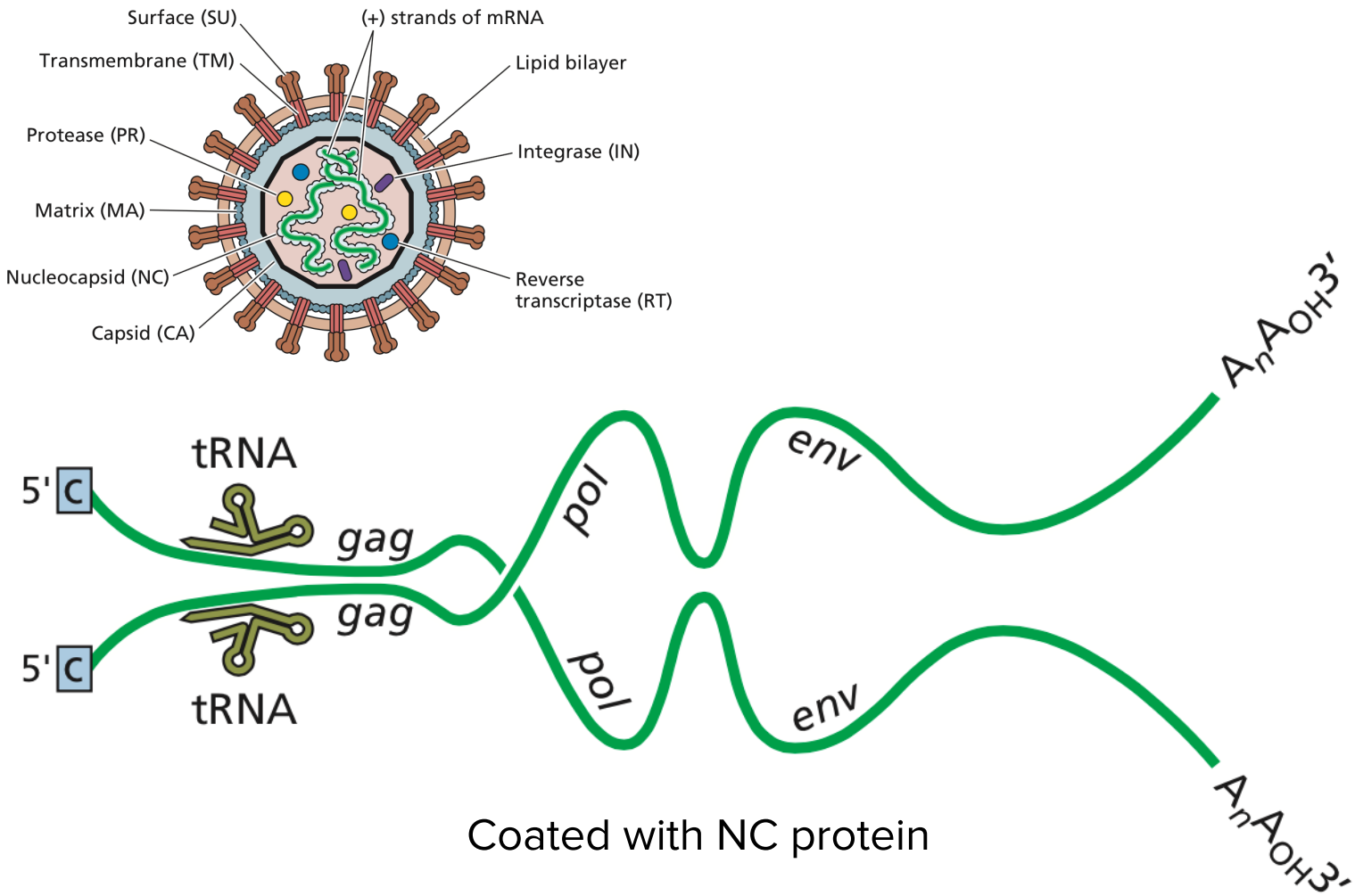
Reverse transcriptase has revolutionized molecular biology. Which statement about the enzyme is not correct?

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

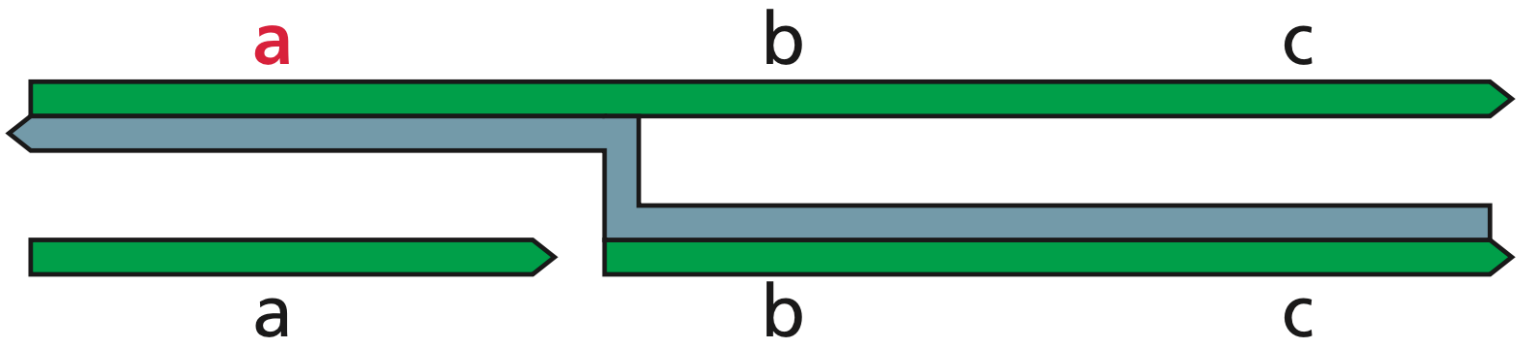


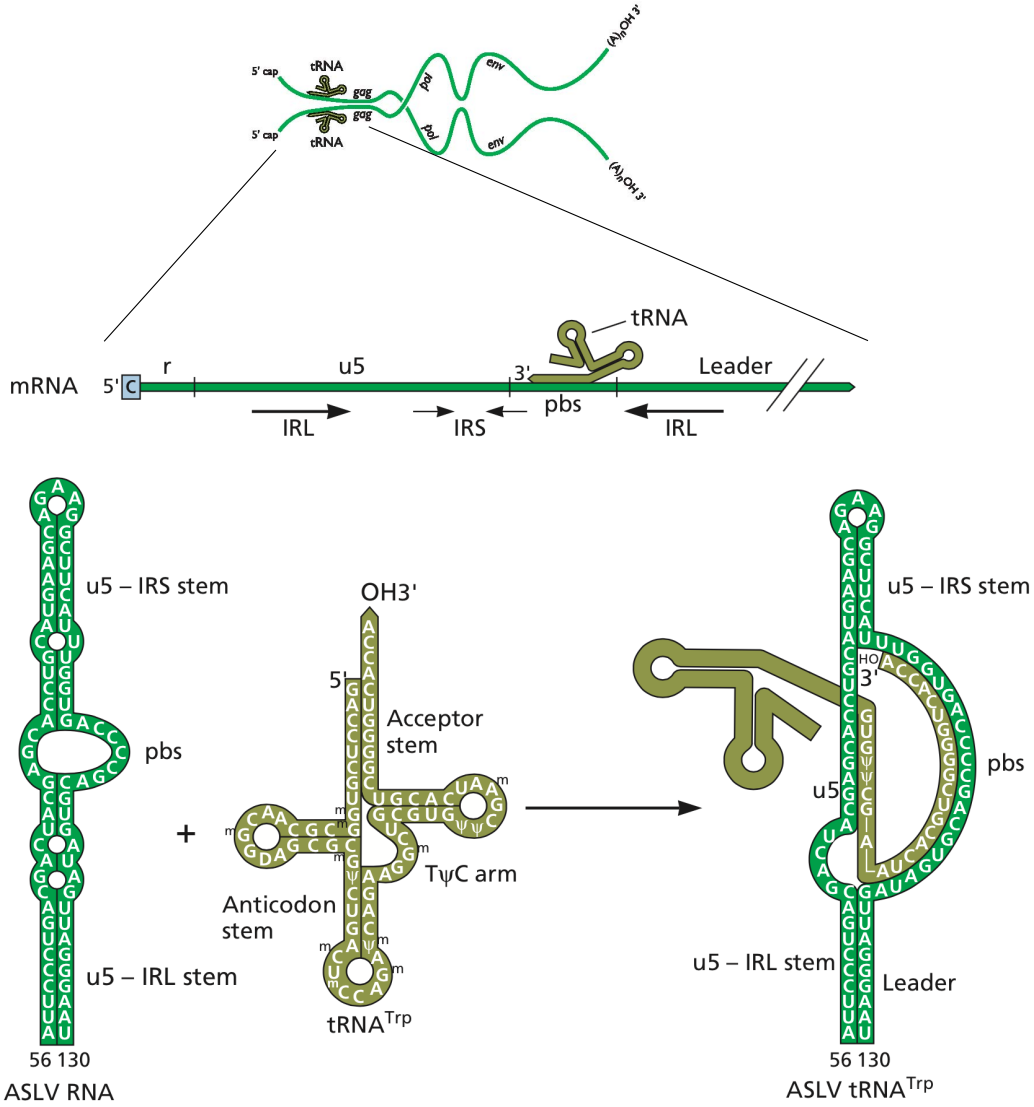
Coated with NC protein

50-100 molecules RT per virus particle

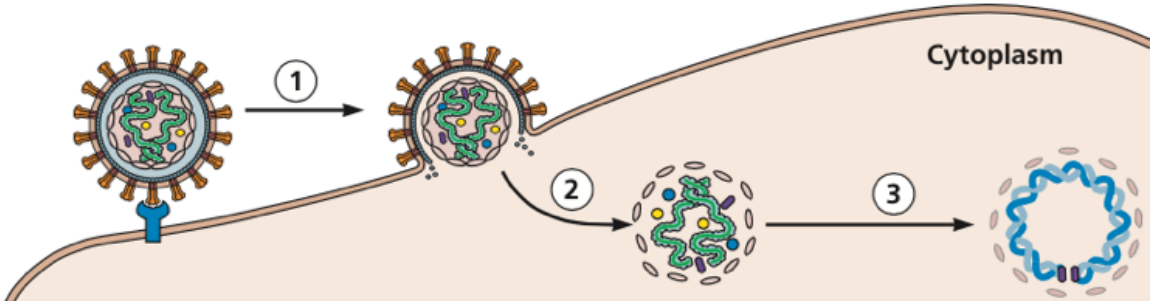
RNA dimer

- Explains why retroviruses are relatively resistant to UV and ionizing radiation
- Two copies of all genes
- Copy-choice rebuilds one functional genome

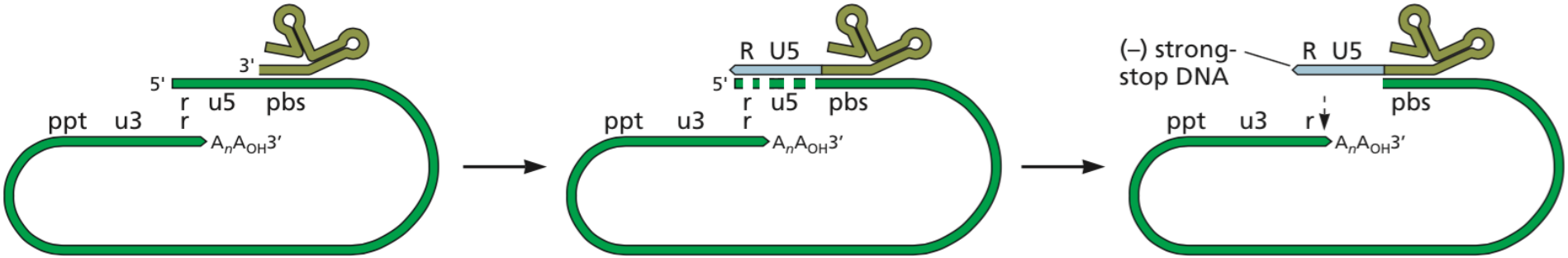




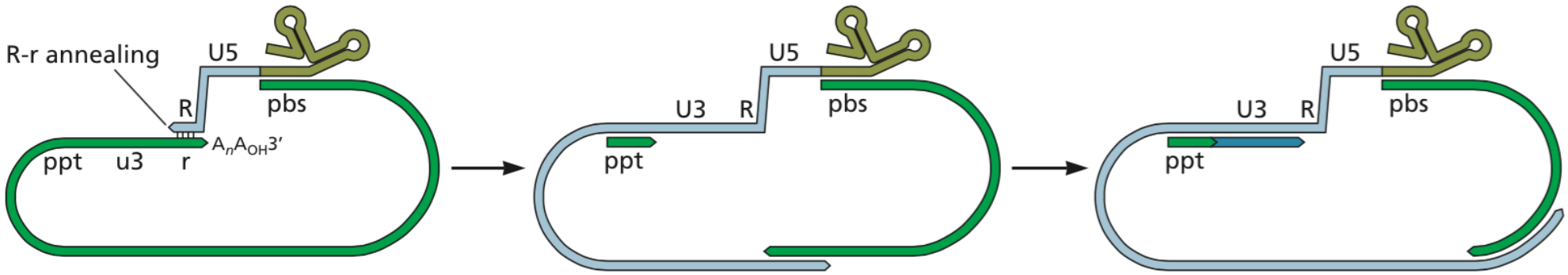
DNA synthesis: cytoplasmic



Initiation of (–) strand DNA synthesis

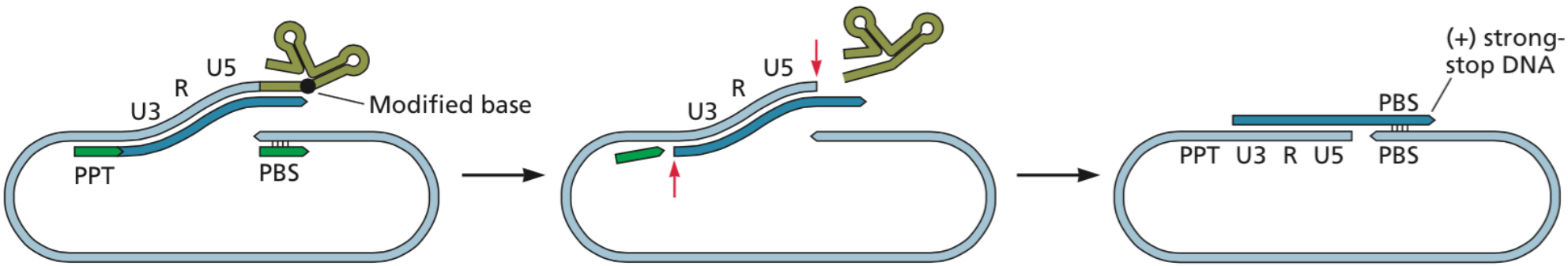


First template exchange

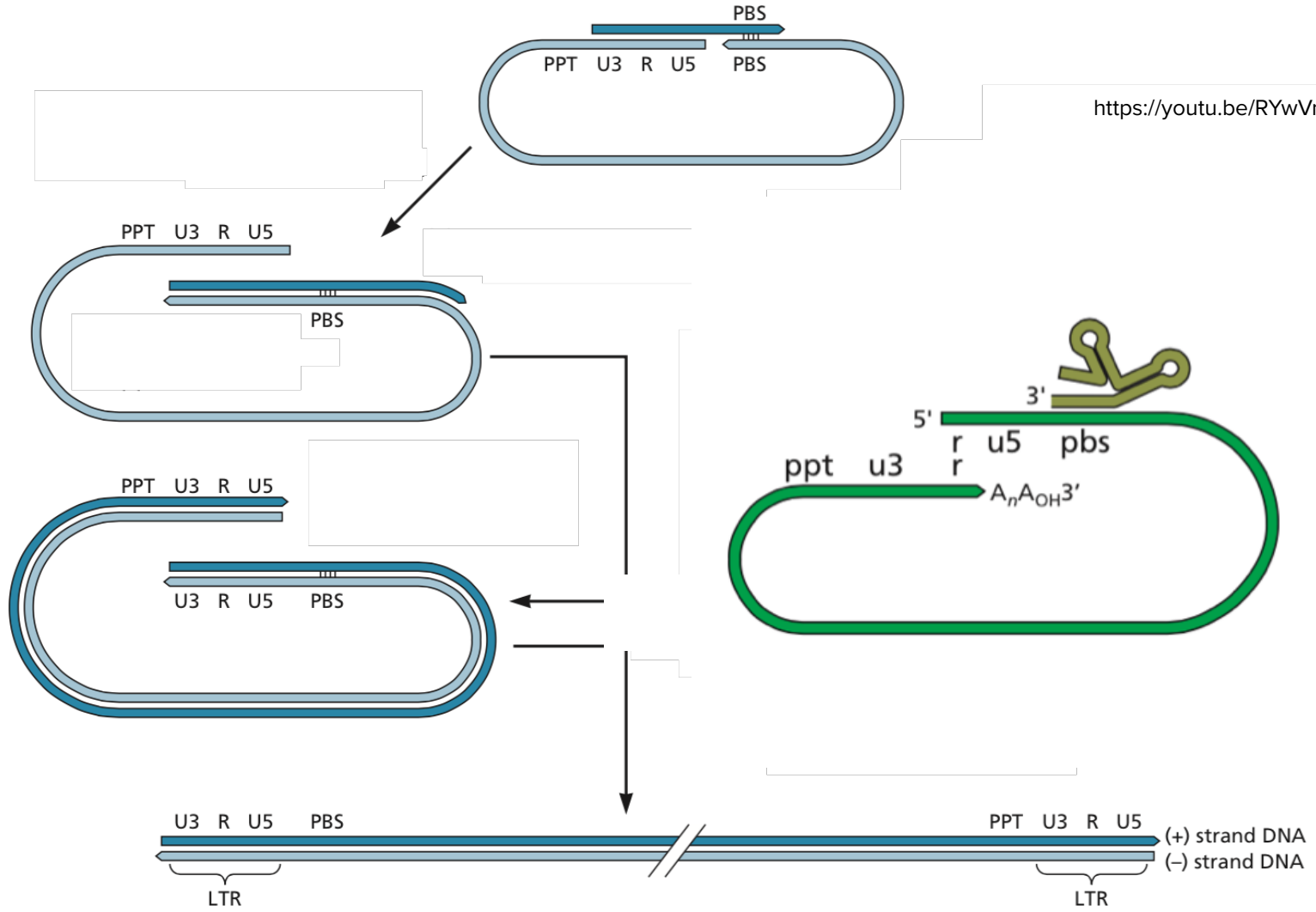


ppt = polypurine tract

(+) strand DNA synthesis



Second template exchange is facilitated by annealing of PBS sequences



https://youtu.be/RYwVnzYf4V8

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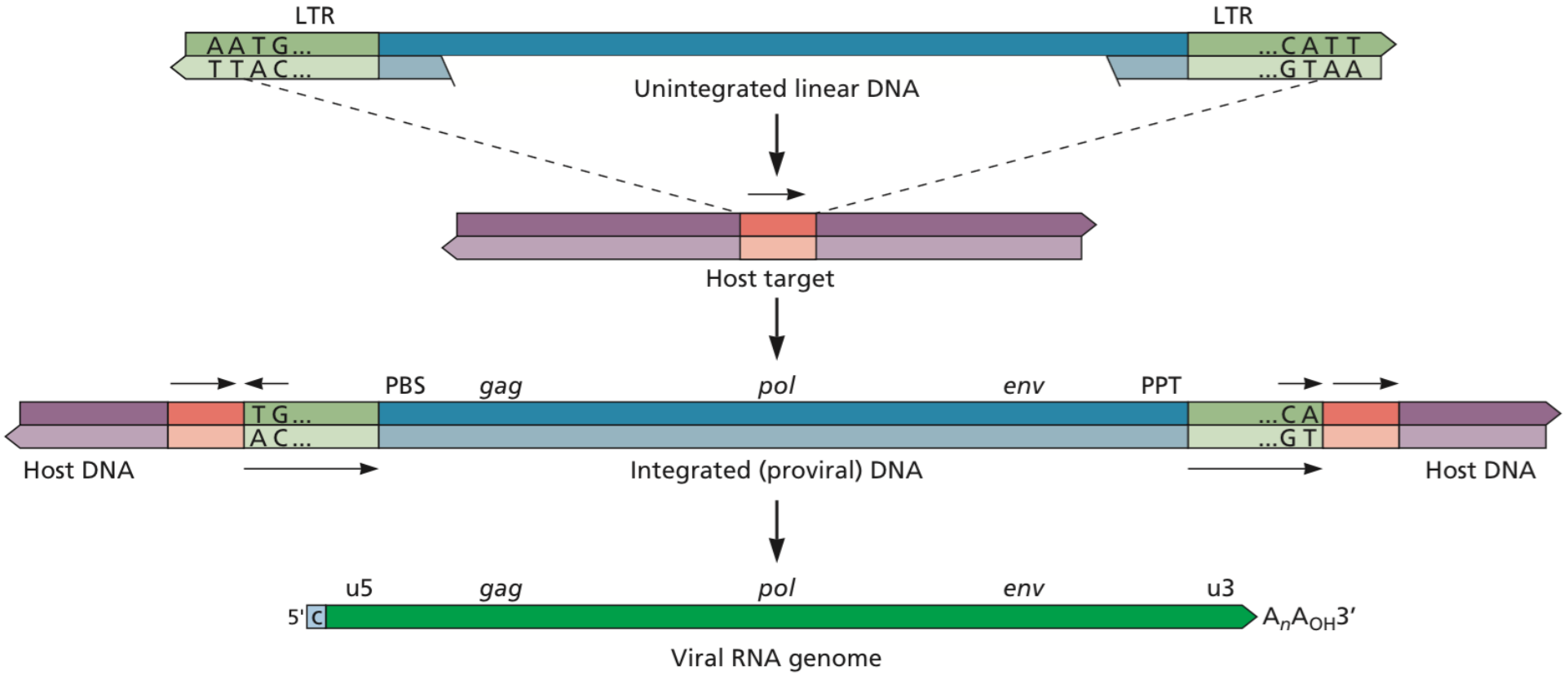
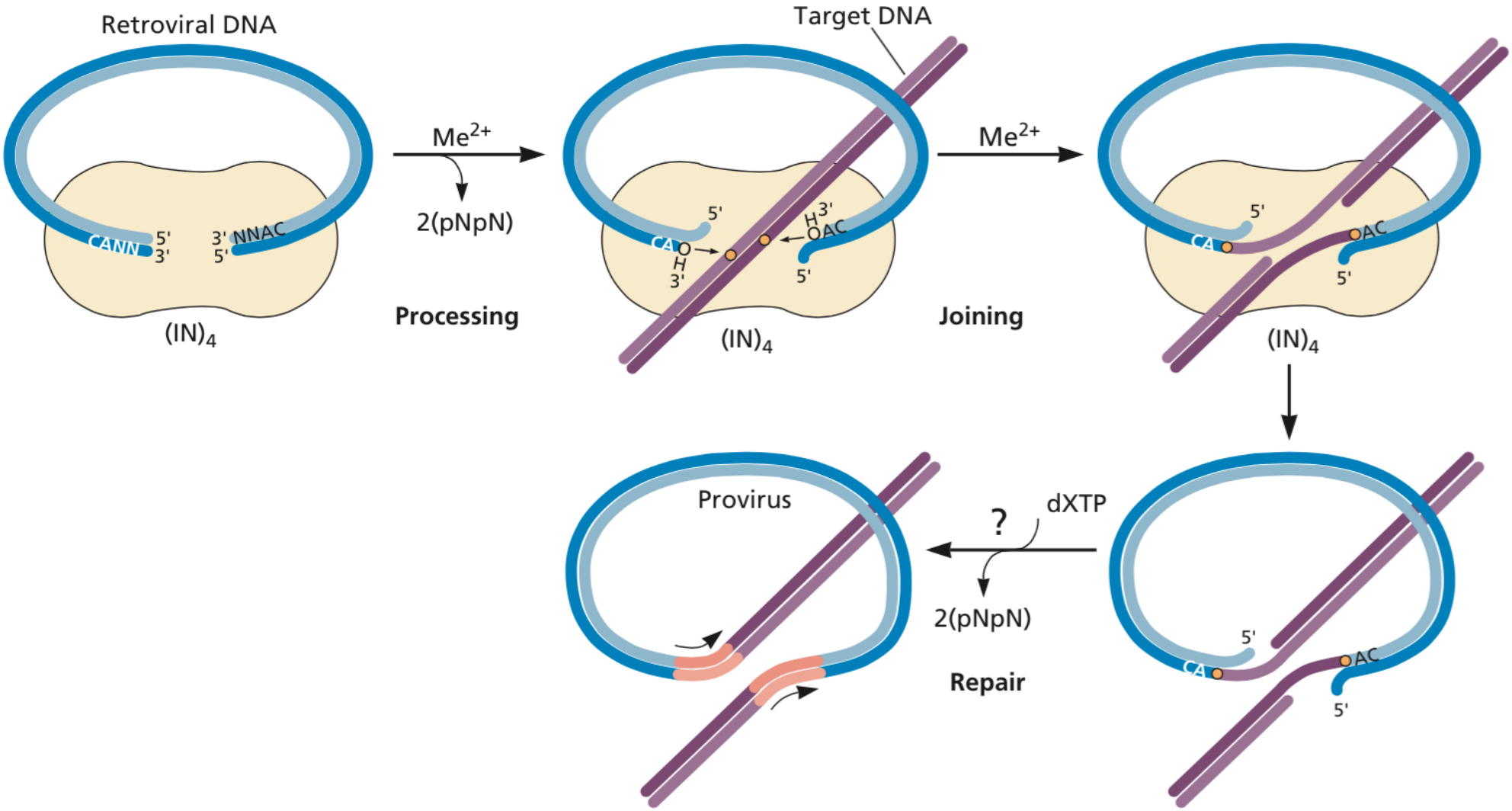
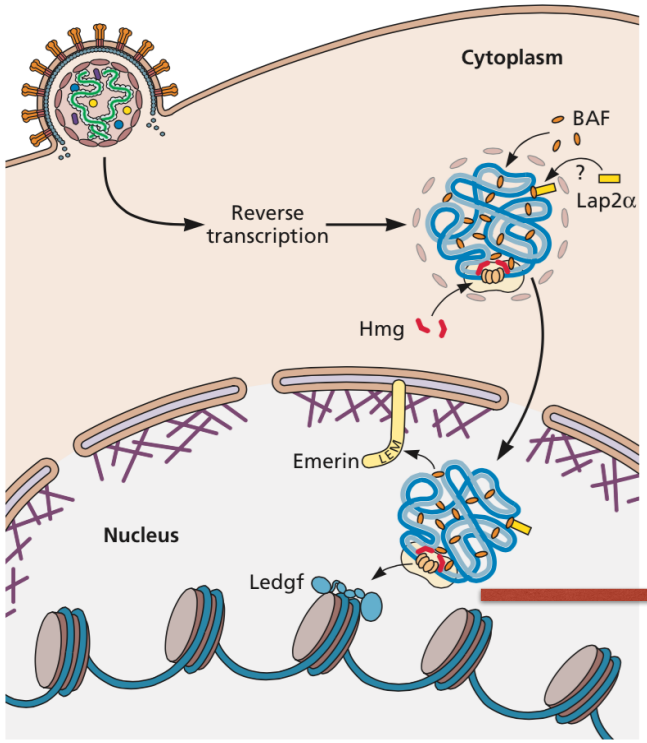
Which of the following steps occur during reverse transcription of retroviral genomic RNA?

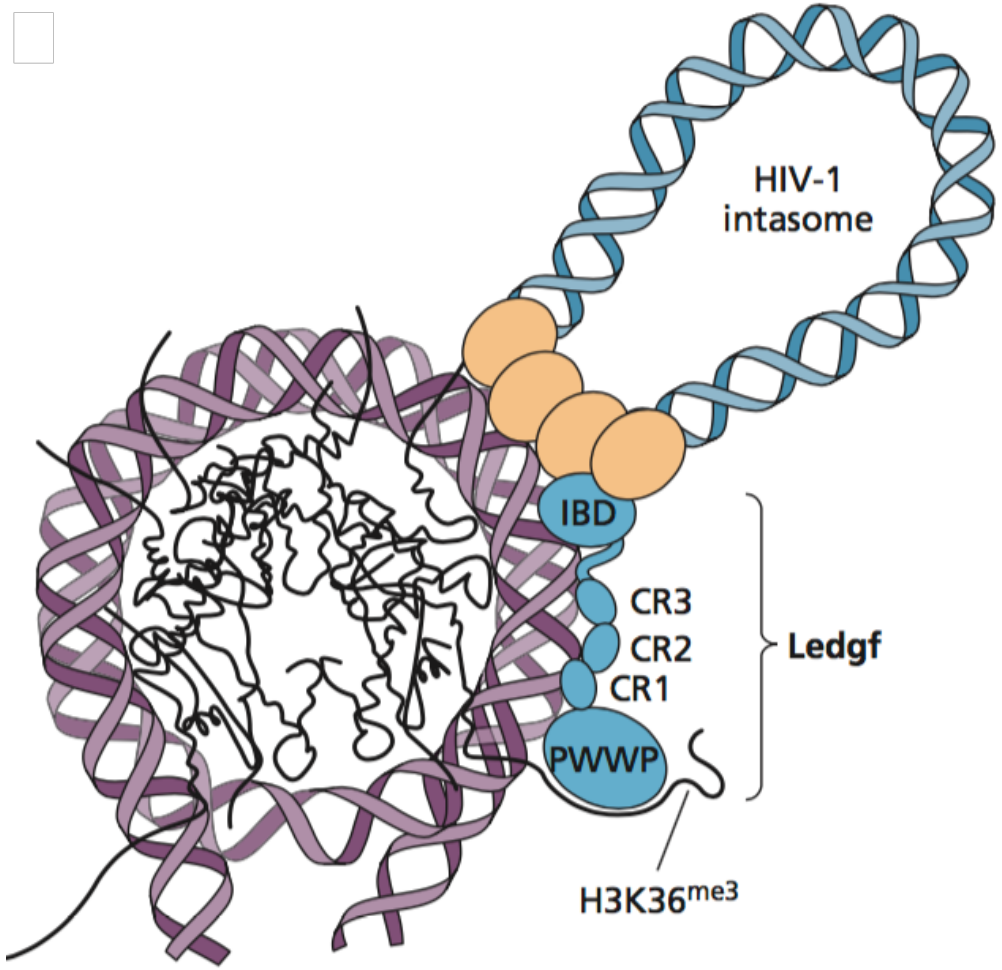
A. Priming of (-) DNA synthesis by tRNA
B. Two template exchanges
C. Degradation of the viral RNA by RNAse H
D. Generation of two LTRs
E. All of the above

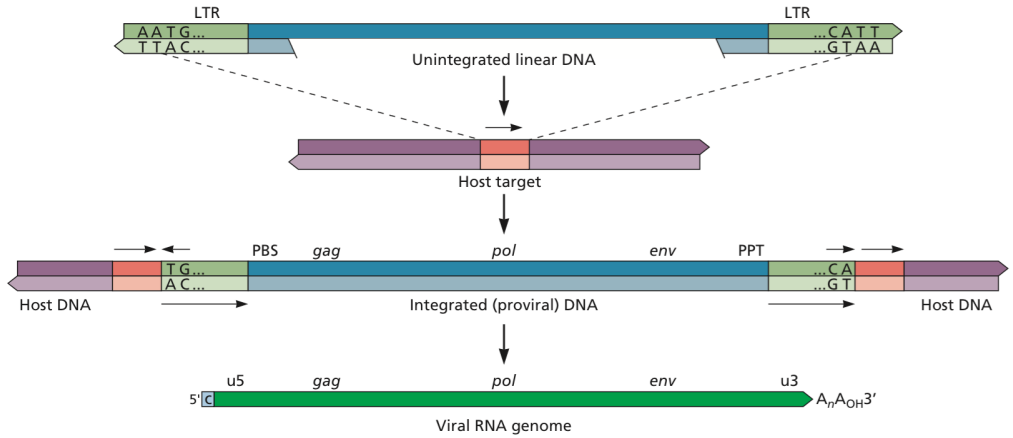






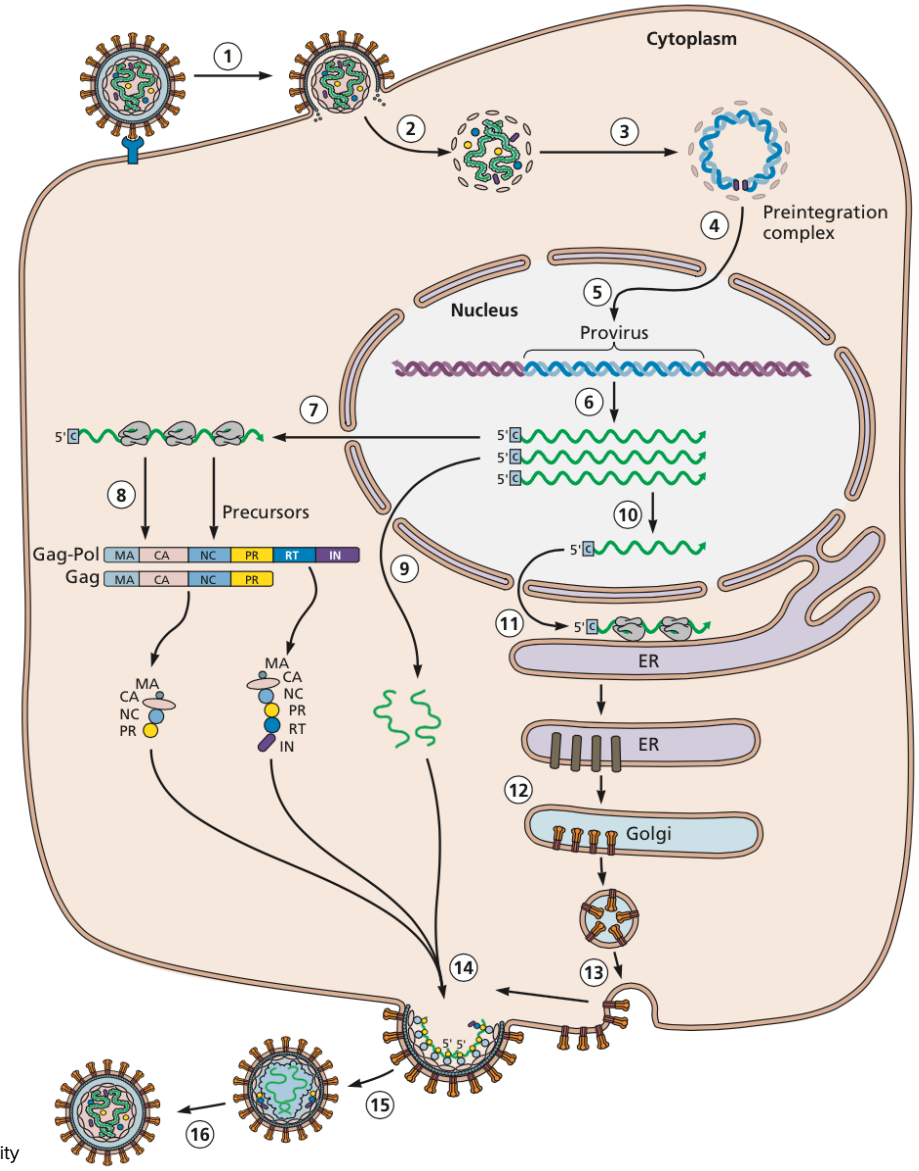
Preference for integration into DNA sequences that are wrapped around a nucleosome

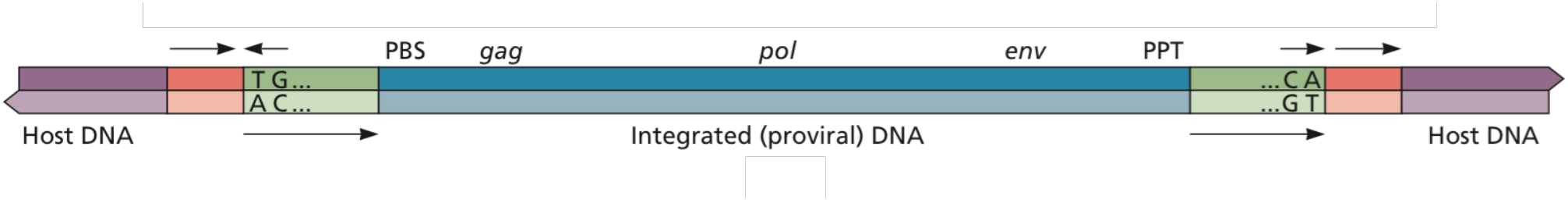




- One DNA produced from two RNAs by RT
- Strong promoter (the LTR) built during RT
- Proviral DNA directs the host transcription machinery to synthesize many copies of viral mRNA
- Viral mRNA is translated into viral proteins OR encapsidated into virus particles

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

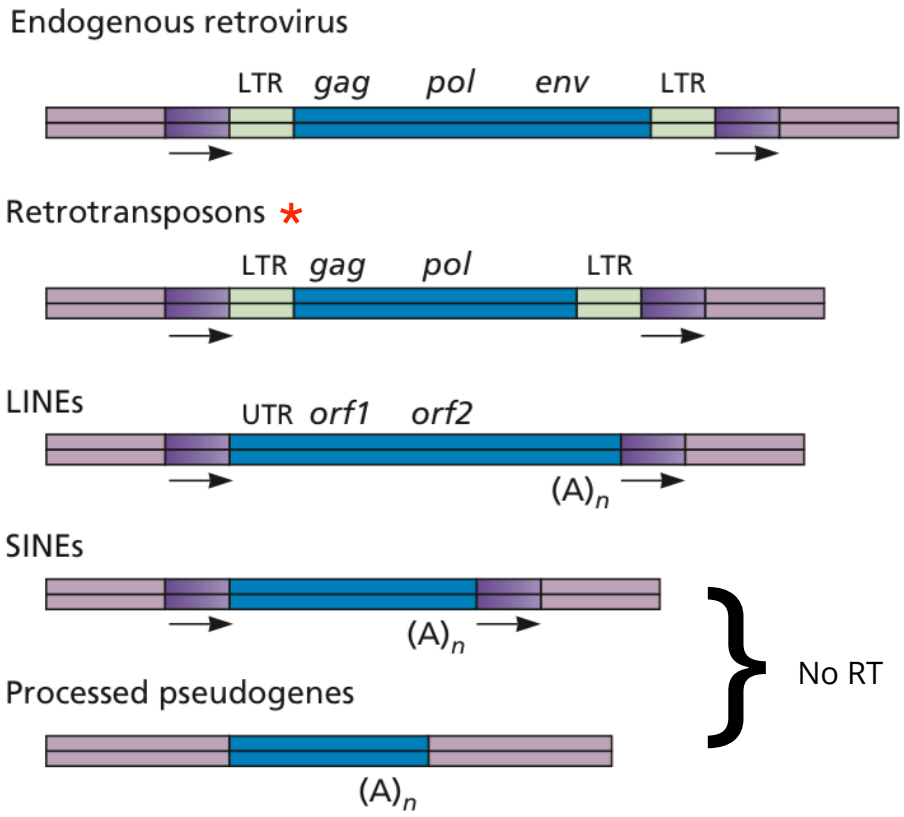




- No mechanism for precise excision of integrated provirus
- Only way out of genome is transcription by host RNA pol II
- Genomes are littered with ancient and modern retroelements

Retroelements

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



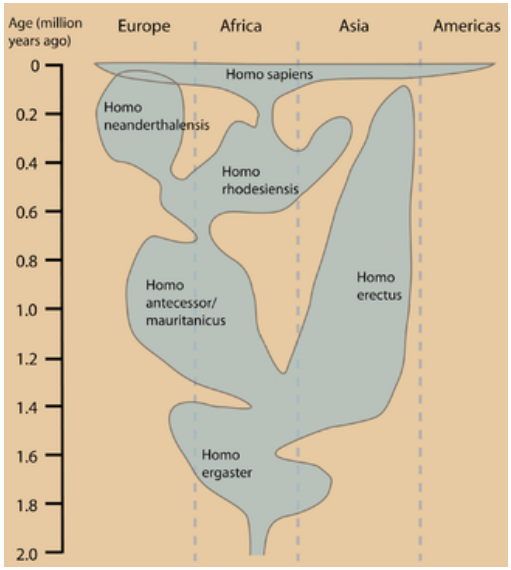
Retroelements in the Human Genome
42.2%; 2.7 X 10^6

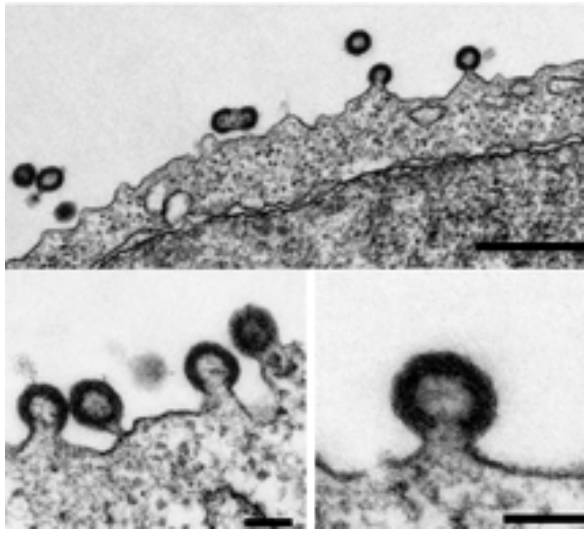
Non-LTR 33.9%; 2.4 X 10^6		**LTR-Containing** 8.3%; 0.3 X 10^6	
LINEs (L1)	16.9%	Endogenous Retroviruses (ERVs)	7.7%
(L2)	3.2		
SINEs (Alu)	10.6%	Others including Retrotransposons	0.6%
(MIR)	2.5		
Processed pseudogenes	<1.0		

* Likely progenitors of retroviruses

Rescue of an endogenous human retrovirus

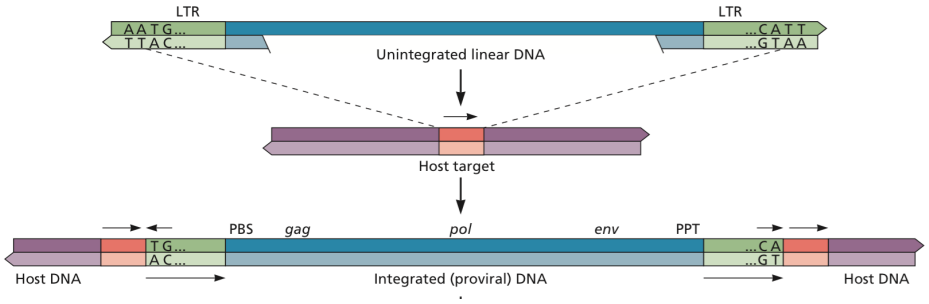
- HERV-K, infected human ancestors <1 Myr ago
- Repaired mutations - known HERVs are not infectious

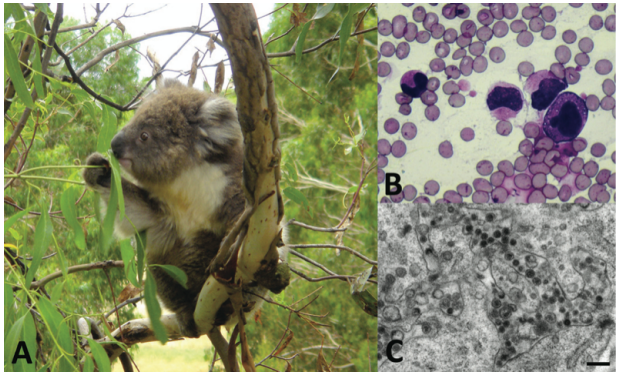


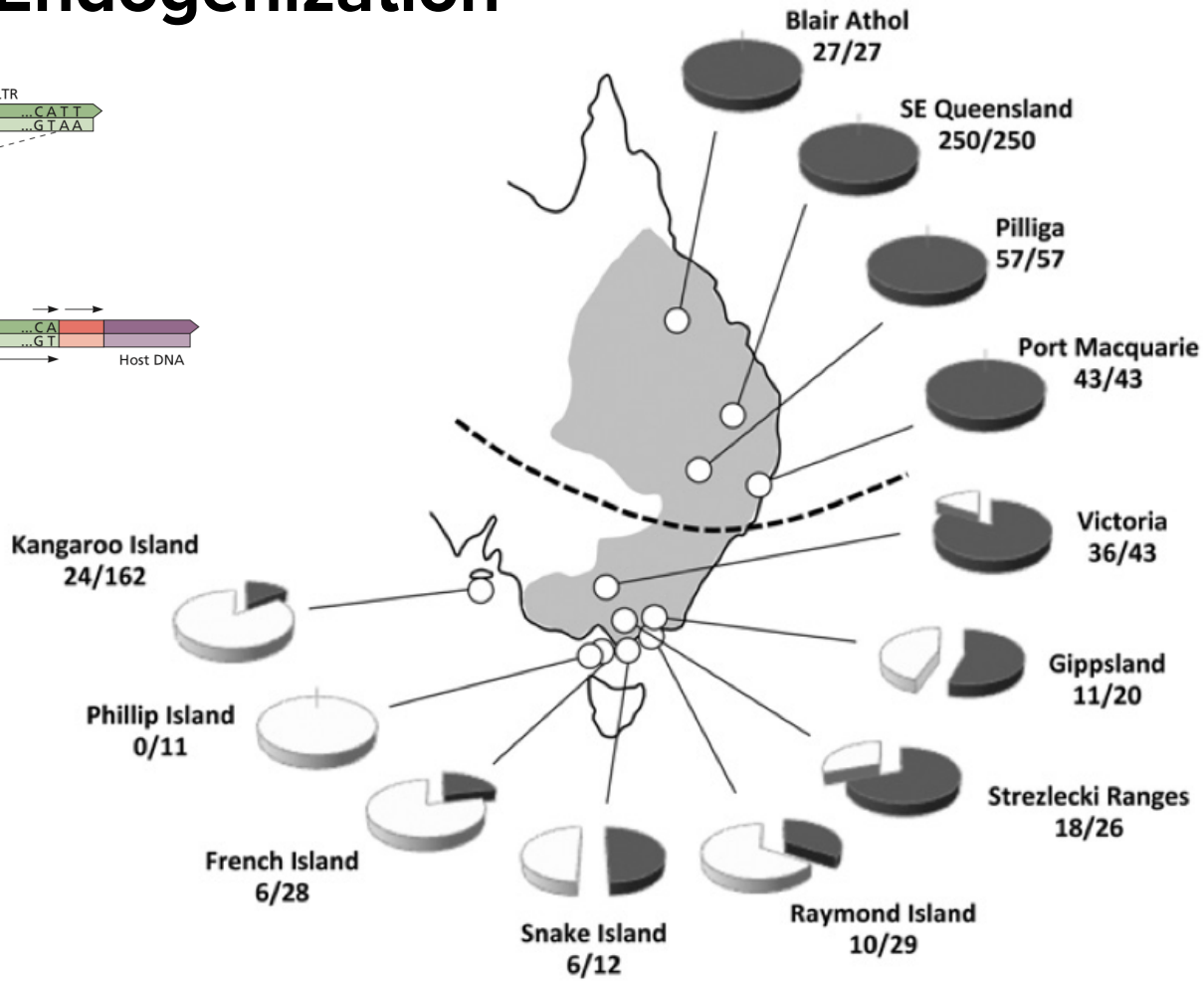


Reconstitution of an infectious human endogenous retrovirus.
Lee YN, **Bieniasz** PD.
PLoS Pathog. 2007 Jan;3(1):e10.

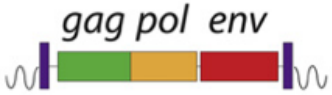
Endogenization

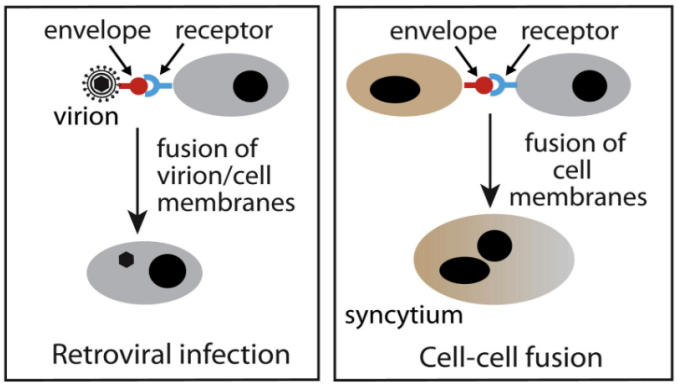


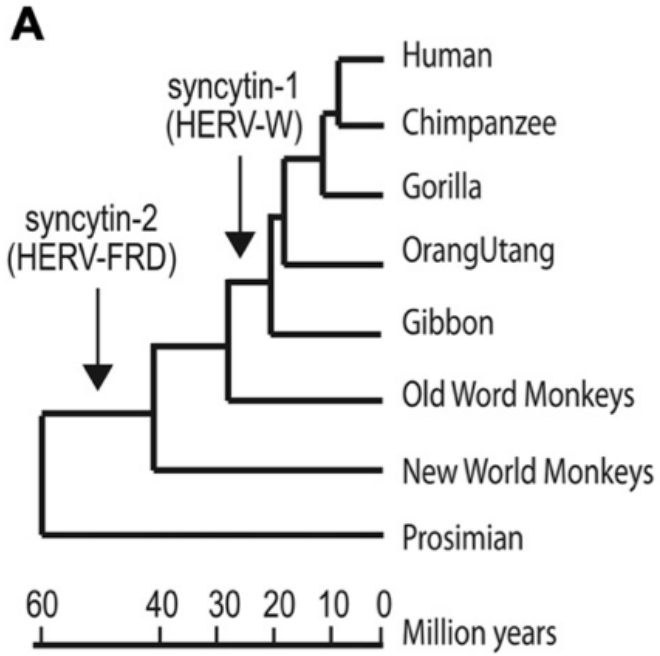


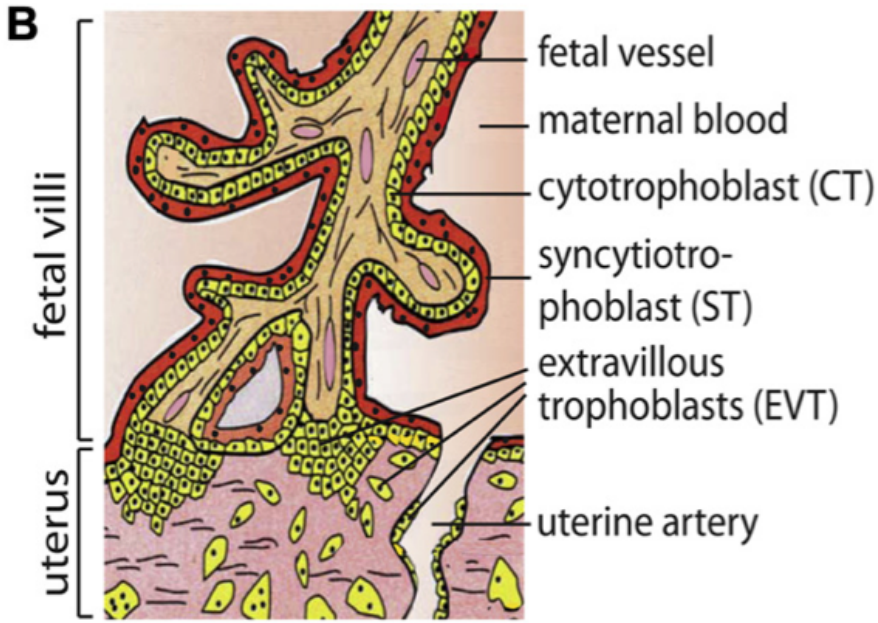


http://www.virology.ws/2012/10/11/museum-pelts-help-date-the-koala-retrovirus/









Placenta 33 (2012) 663-671

A retrovirus makes chicken eggshells blue

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

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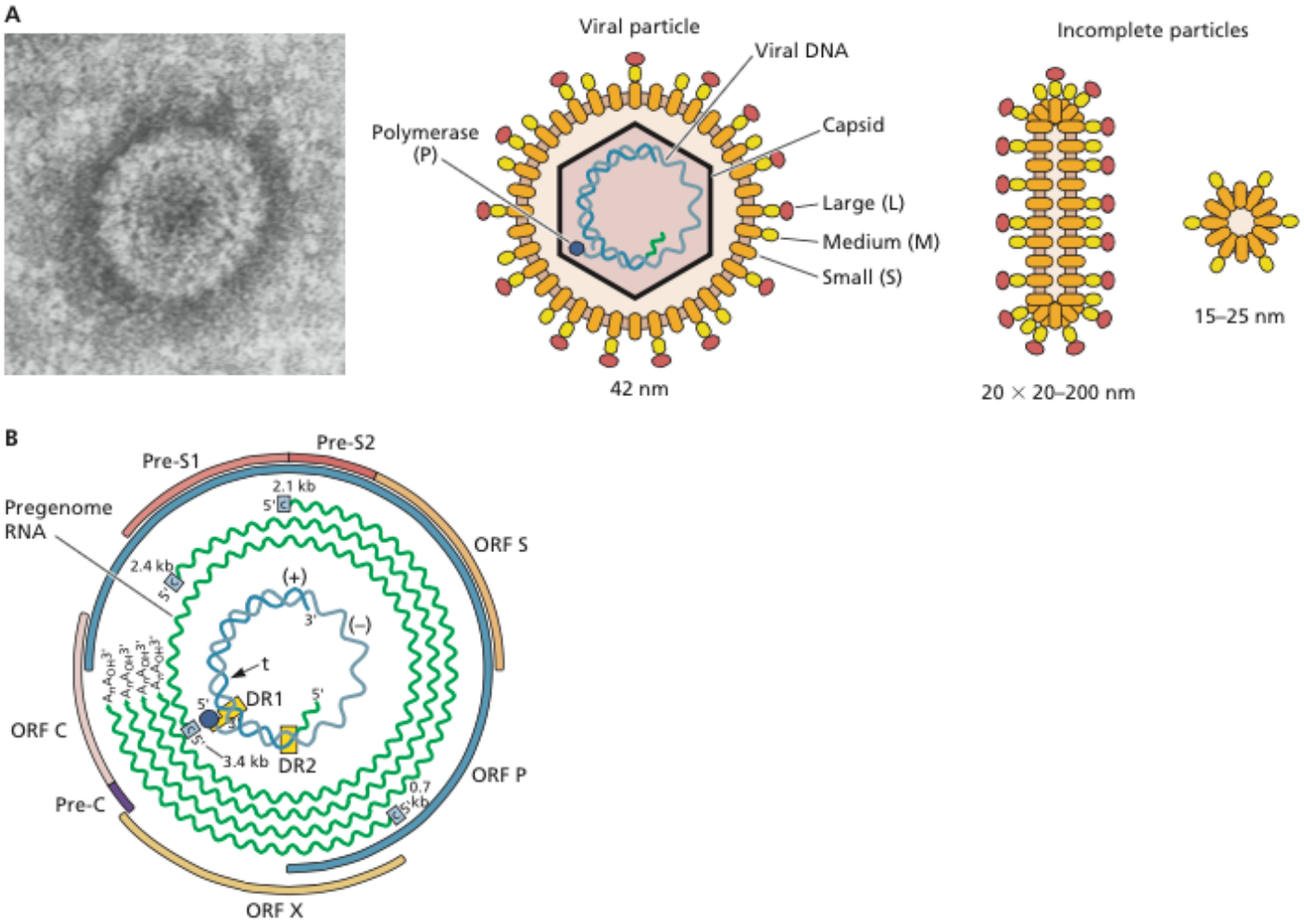
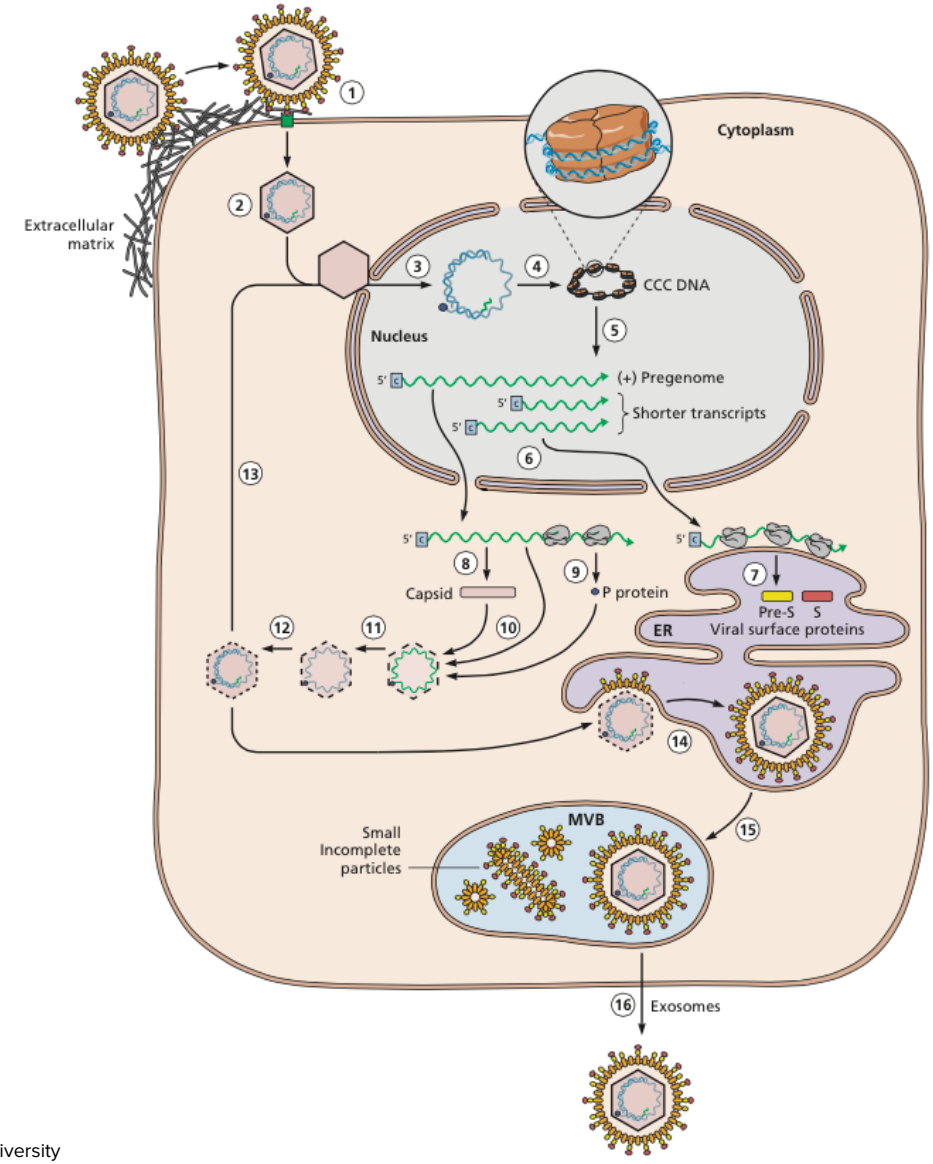
Which of the following statements about retroelements is not correct?

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





No genome integration