Simple Retroviruses

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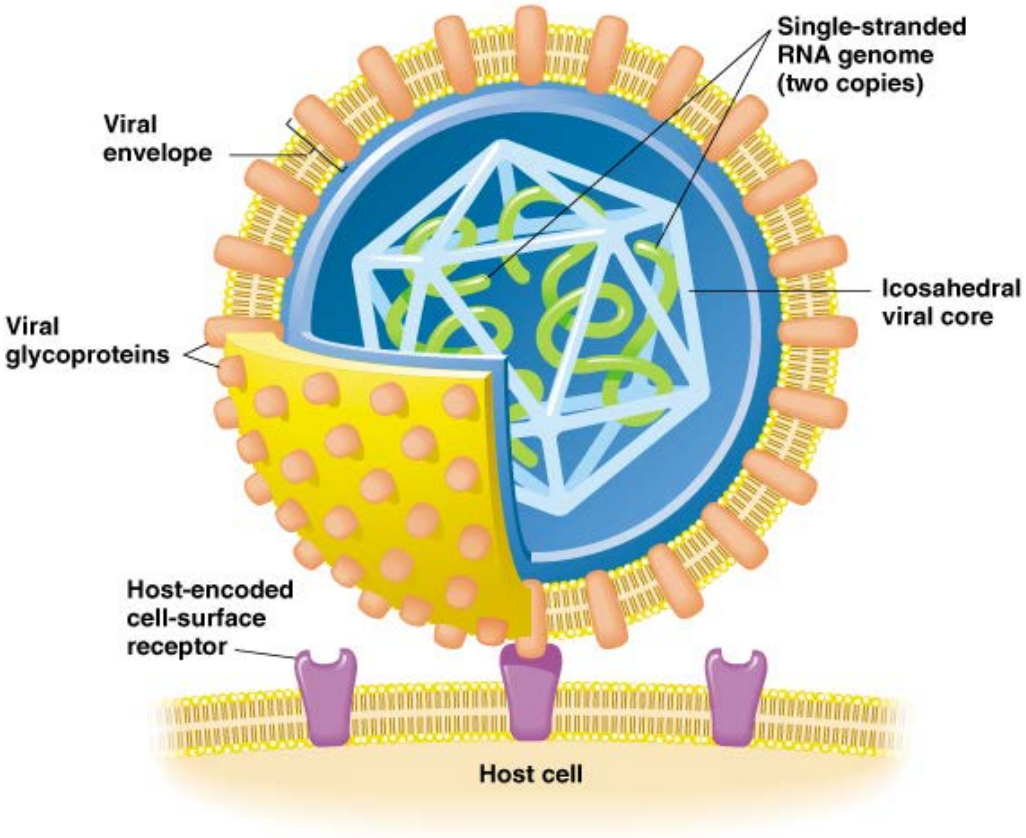
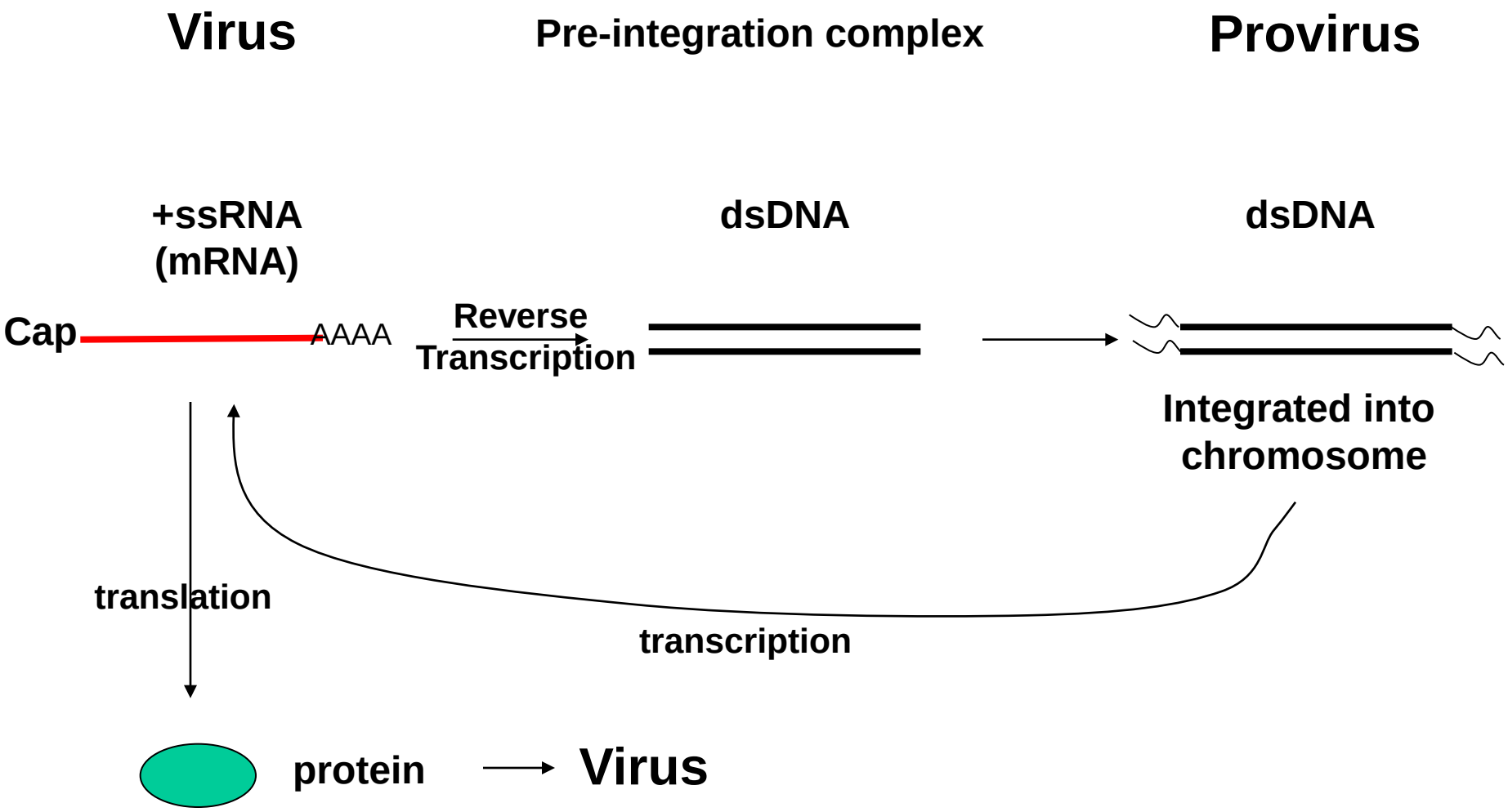
Retroviruses

- Single stranded (+) RNA Viruses
- Enveloped
- "Retro": Reverse transcription, ss RNA → ds DNA
(Cells: DNA →RNA)
- Integration to chromosome (genetic alteration)

SCHEMATIC OF A RETROVIRUS



Retroviral replicative forms



Milestones in history

Discovery of RNA tumor viruses

1911 Peyton Rous, induction of sarcoma by filtrated extracts of chicken sarcoma, isolation of RSV, (*Nobelprize 1966*)

1936 J.J. Bittner MMTVas causitive agent of mouse breast cancer

1970 Howard Temin, David Baltimore discover Reverse Transcriptase

(Nobelprize 1975)

Oncogenes

1976 H.E. Varmus, J.M. Bishop, P.K. Vogt, D.Stehelin discribe oncogenes in transforming retroviruses *(Nobelprize 1989)*

humane Retroviruses

1980 R.C. Gallo isolates HTLV I

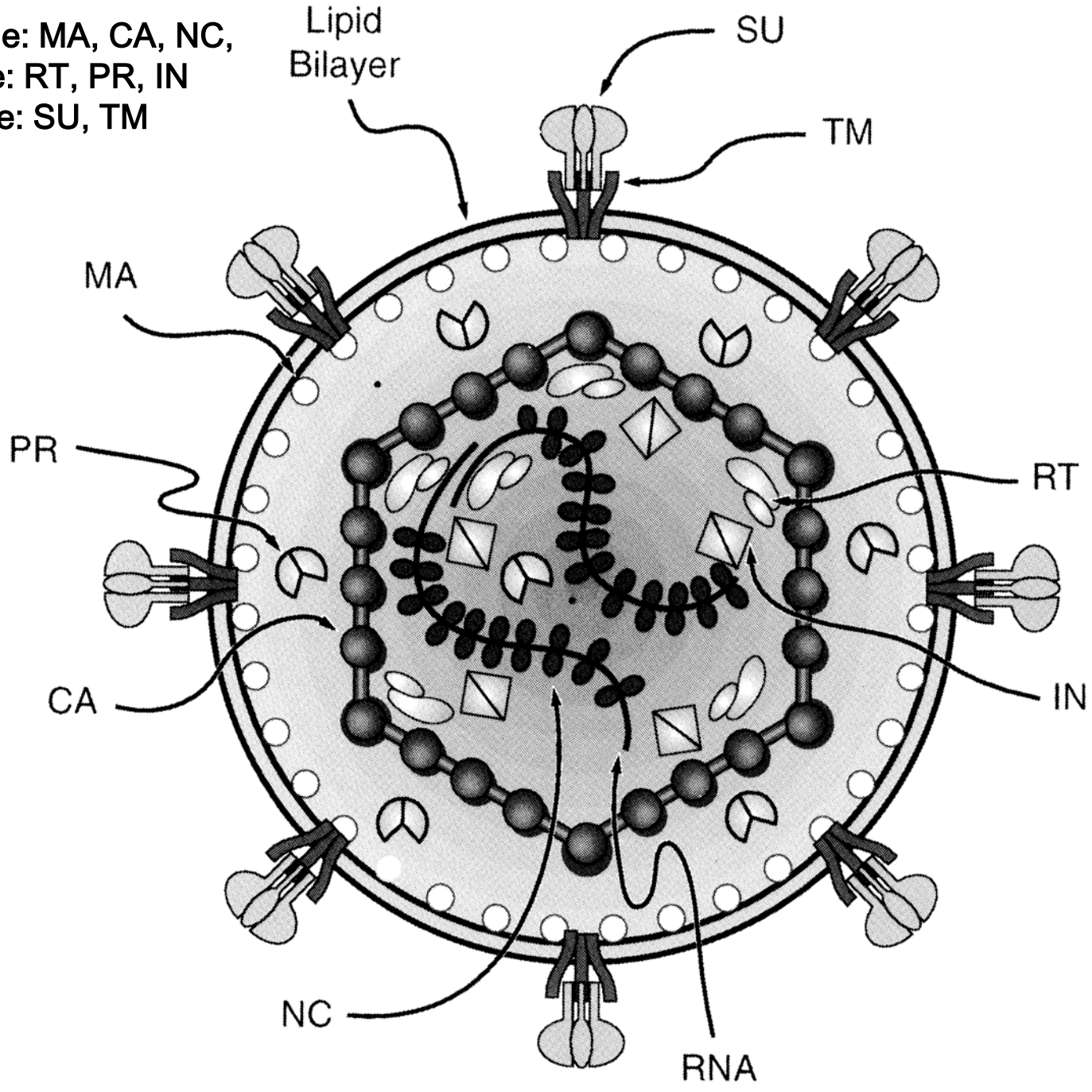
1983 R.C. Gallo, L.Montagnier und F. Barré-Sinoussi discribe HIV

(Nobelprize to Montagnier u. Barré-Sinoussi, 2008)

Retrovirusnomenklatur

Alpharetroviren	**aviäre Leukoseviren** **Rous Sarcomvirus** **aviäres Myeloblastomvirus**	**Exogen/ endogen**
Betaretrovirus	**Maus-Mamma-Tumorvirus** **Simianes Retrovirus**	**Exogen/ endogen**
Gammaretrovirus	**Felines Leukämievirus** **Mäuseleukämieviren** **Affenleukämieviren** **Murine Sarcomviren**	**Exogen/ endogen**
Deltaretroviren	**Humanes T-Zellleukämievirus** **BLV, STLV**	**Exogen**
Lentiviren	**Primaten Immundefizienzviren (HIV, SIV)**	**Exogen**
Spumaviren		**Exogen**

Gag Proteine: MA, CA, NC,
Pol Proteine: RT, PR, IN
Env Proteine: SU, TM



Life Cycle of Retroviruses

1) Early Steps:

a) Infection:
Viral attachment, entry and uncoating
(Interactions of Env with cell receptors)

b) Replication: Reverse transcriptase
(+) ss RNA → (-) strand DNA →
(+) strand DNA → ds proviral DNA

c) Integration:
Insertion of proviral DNA into chromosome

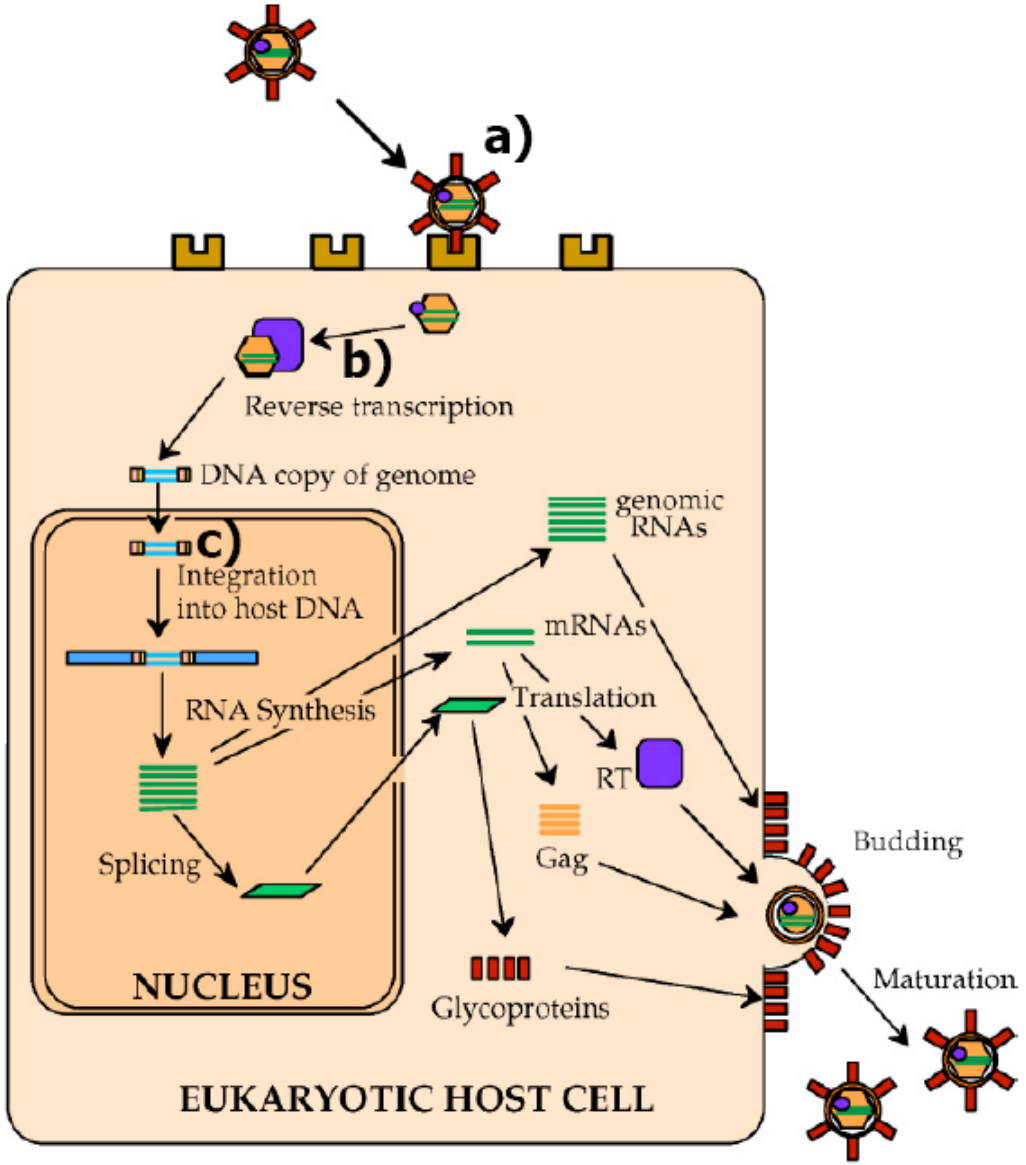


Fig. 1.11
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Life Cycle of Retroviruses

2) Late Steps:

a) Transcription:
Synthesis of viral RNA and mRNA

b) Translation:
Synthesis of viral polypeptides

c) Viral assembly/budding:
Packaging of viral polypeptides & viral RNA genomes

d) Maturation:
Viral release and proteolytic processing of viral polypeptides

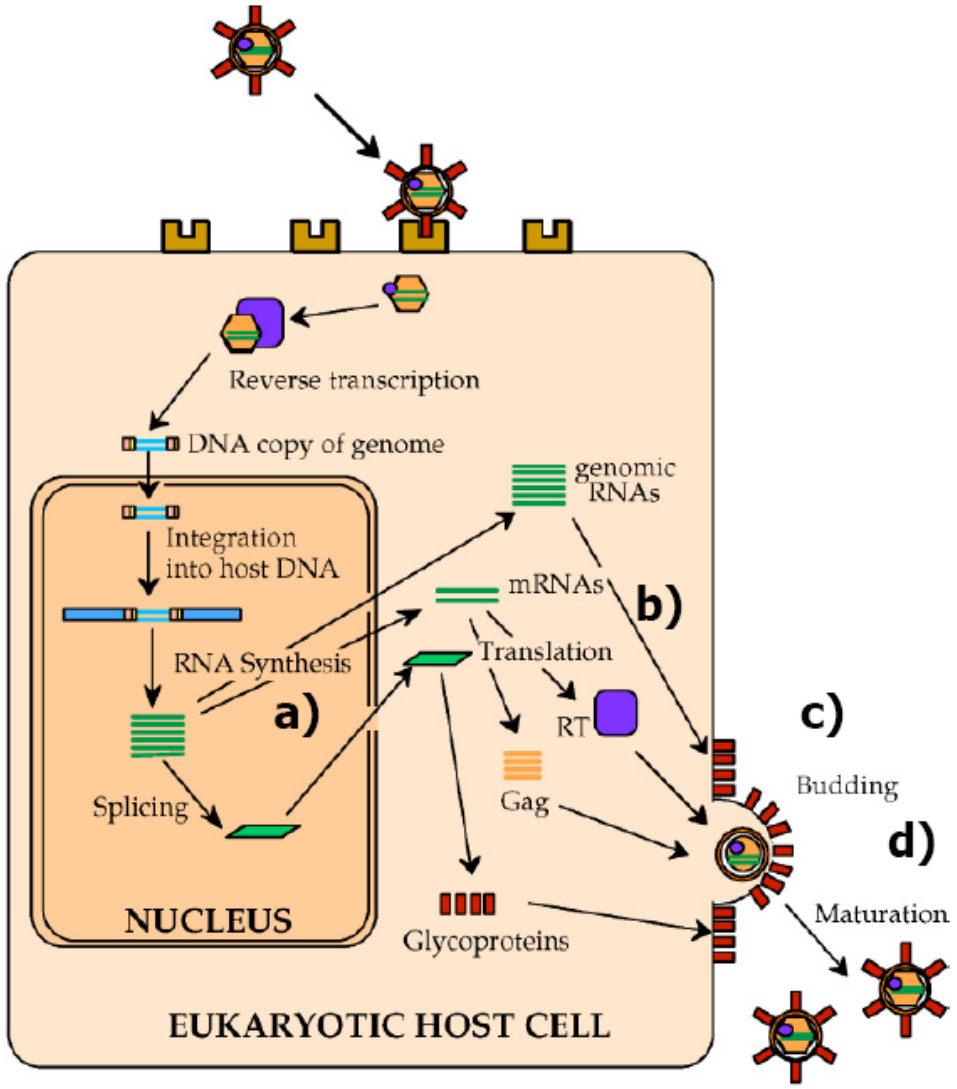

Fig. 1.11
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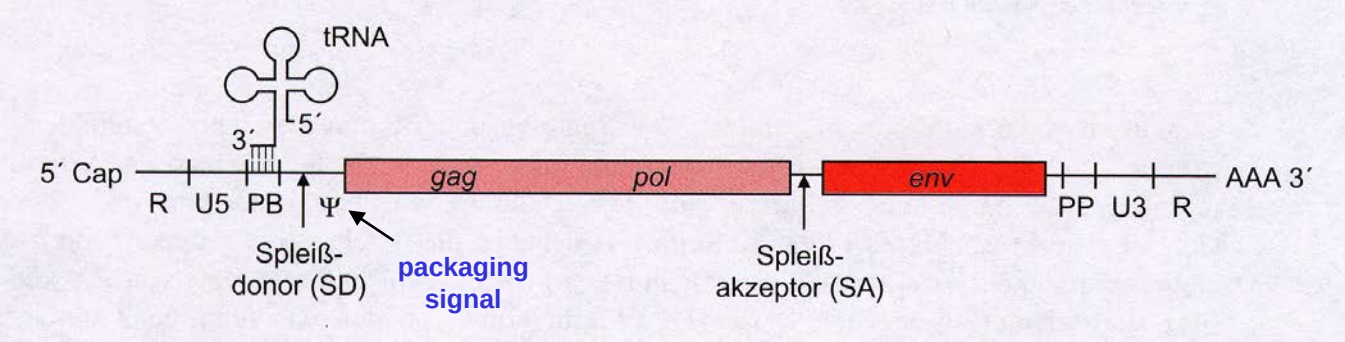
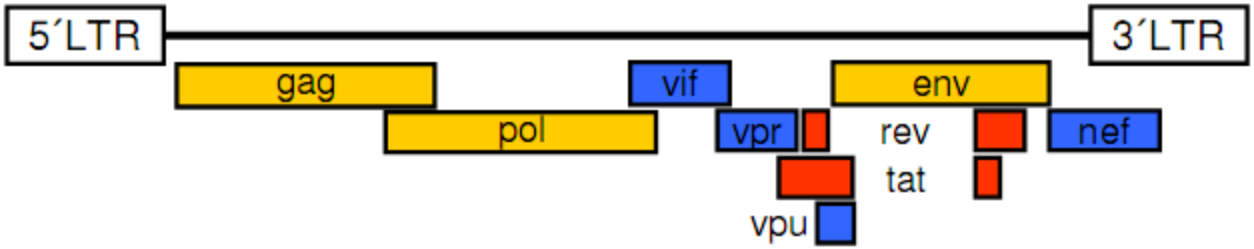
Examples of retroviral genomes



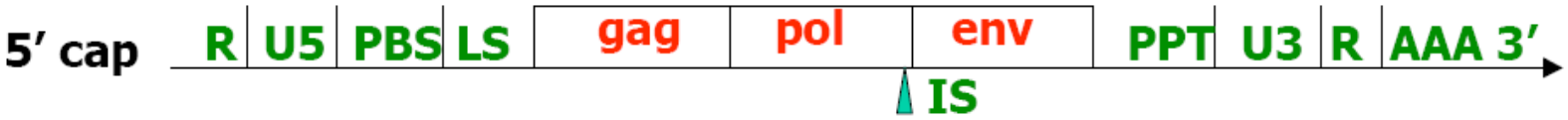
Two transcripts:

Simple retrovirus



complex retrovirus

Structure of Retroviral RNA genomes



(2) Coding Elements

(A) gag (group specific antigen):

- Proteins found in viral internal structure.
- Synthesized as one long peptides, and processed to 3 to 5 capsid proteins by virally encoded proteases

MA: Matrix protein

CA: Capsid protein

NC: Nucleocapsid proteins

(B) pol:

- Synthesized by "translational slip" causing –1 nt frame shift during gag gene translation.

PR: Protease, proteolytic processing of Gag and Pol polypeptides.

RT: Reverse transcriptase, replication of ssRNA to ds DNA.

IN: Integrase, insertion of ds viral DNA into host chromosome.

(C) env

-Synthesized from spliced mRNA, processed to 2 proteins by cellular proteases.

SU (surface protein): Recognition of cellular receptors, gp120 (HIV-1), glycosylated, targets for antibodies.

TM (transmembrane protein): Anchoring and fusion of virus-receptor complex, gp41 (HIV-1)

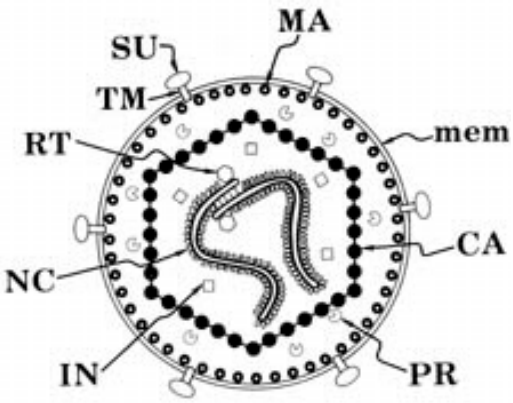
Gene products: Gag

- Gag = group specific antigens
- synthesis: Gag-precursor
- cleavage by viral protease:
  - MA = matrix (or *m*embrane-*a*ssociated)
  - CA = capsid
  - NC = nucleocapsid
- MA:
  - peripheral membrane protein
  - myristylation at the N-terminus

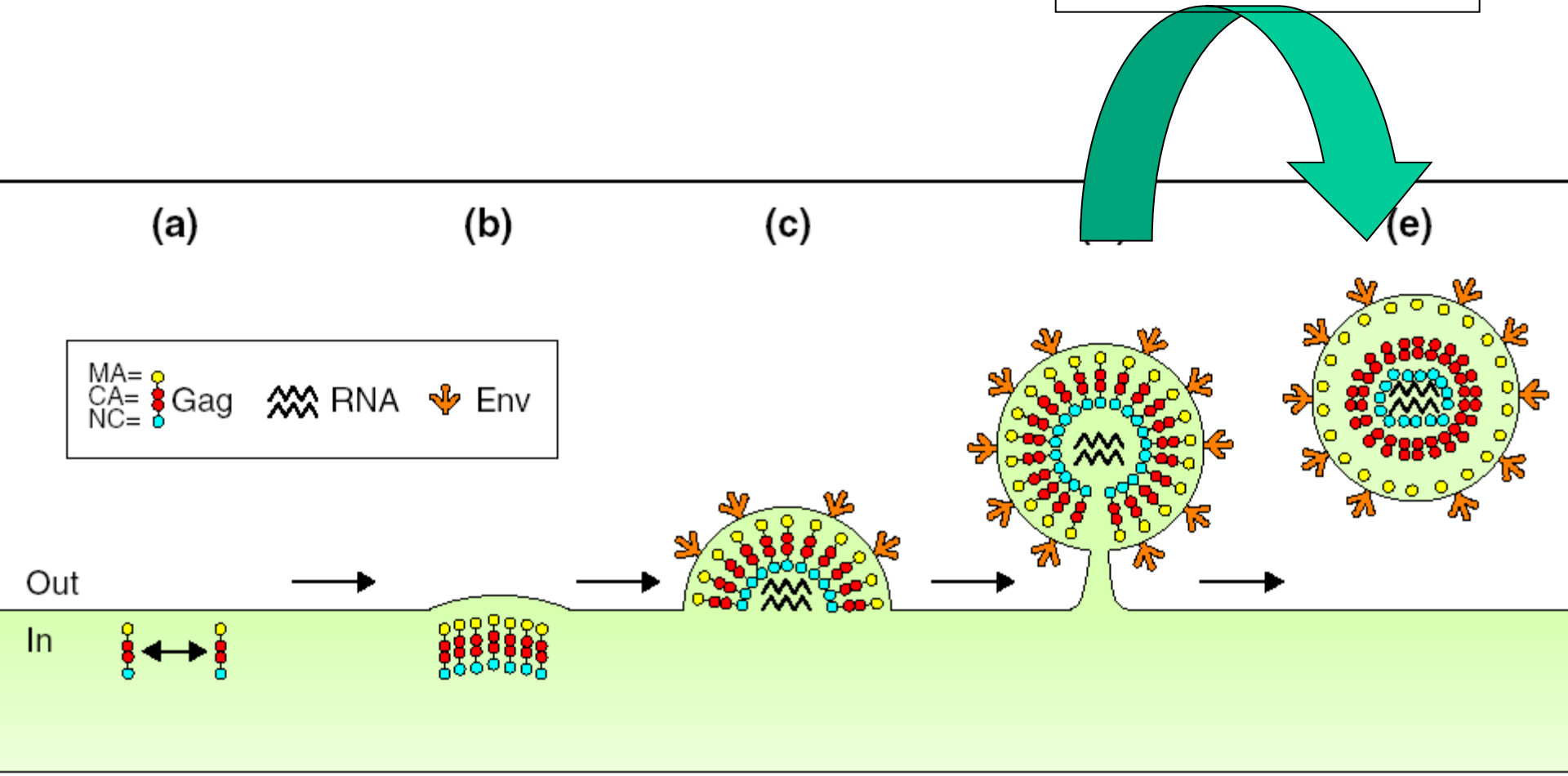
    (association of MA with membrane; essential for viral assembly)
- CA:
  - forming a shell around the RNA (core-stability)
  - important for an early productive infection
- NC:
  - tightly bound to genomic RNA;

    interaction with Ψ-site ➔ packaging

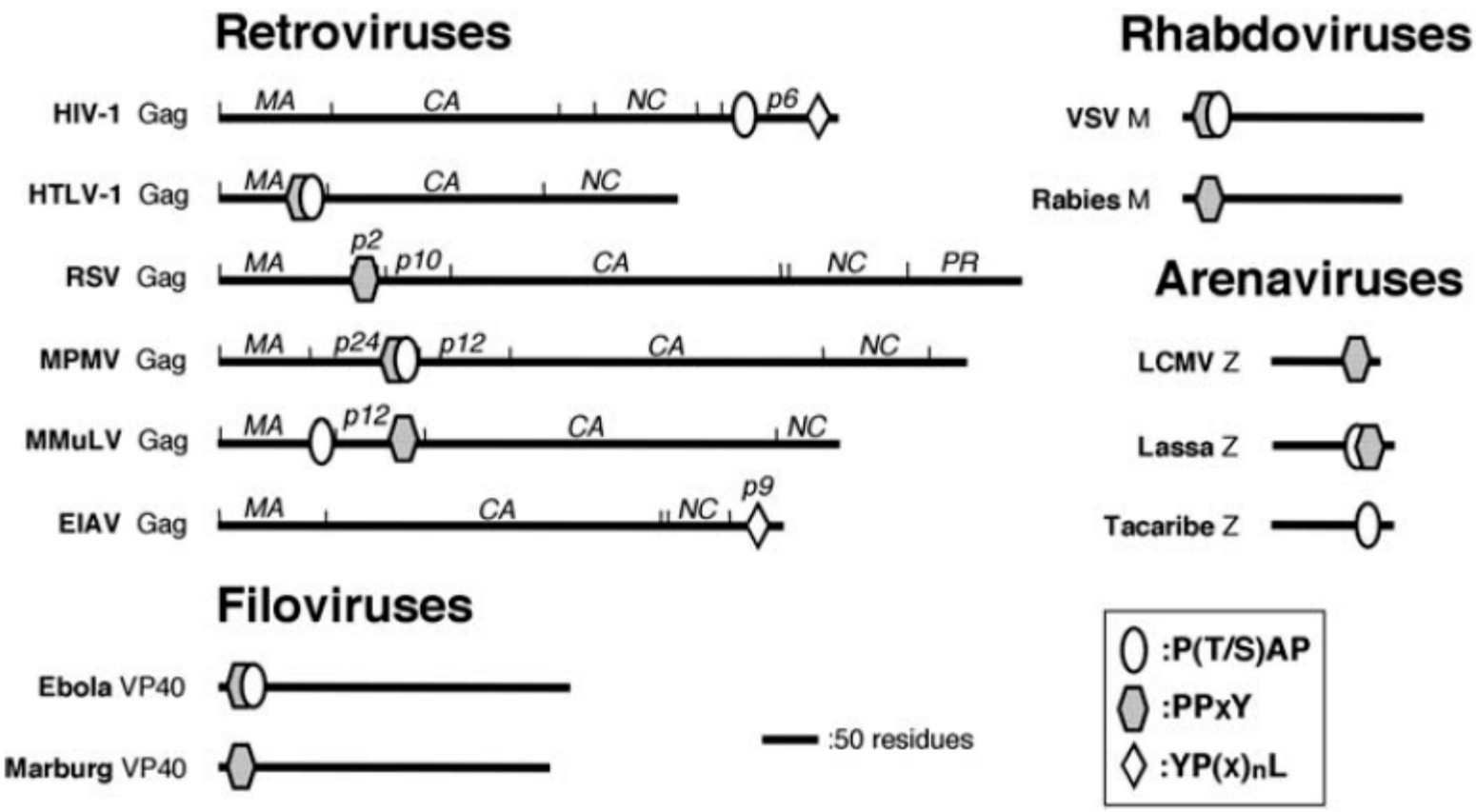
    Zn^{2+}-dependent (stabilization, dimerization)



Virus maturation



Viral Late Domains



Morita + Sundquist, Annu. Rev. Cell Dev Biol 2004

Gene products: Pro-Pol

- Pro-Pol = Protease-Polymerase
- synthesis: Gag-Pro-Pol-precursor
- cleavage by viral protease

- PR:
  - processing of Gag, Gag-Pro-Pol and Env polyproteins
  - converting virions into infectious particles

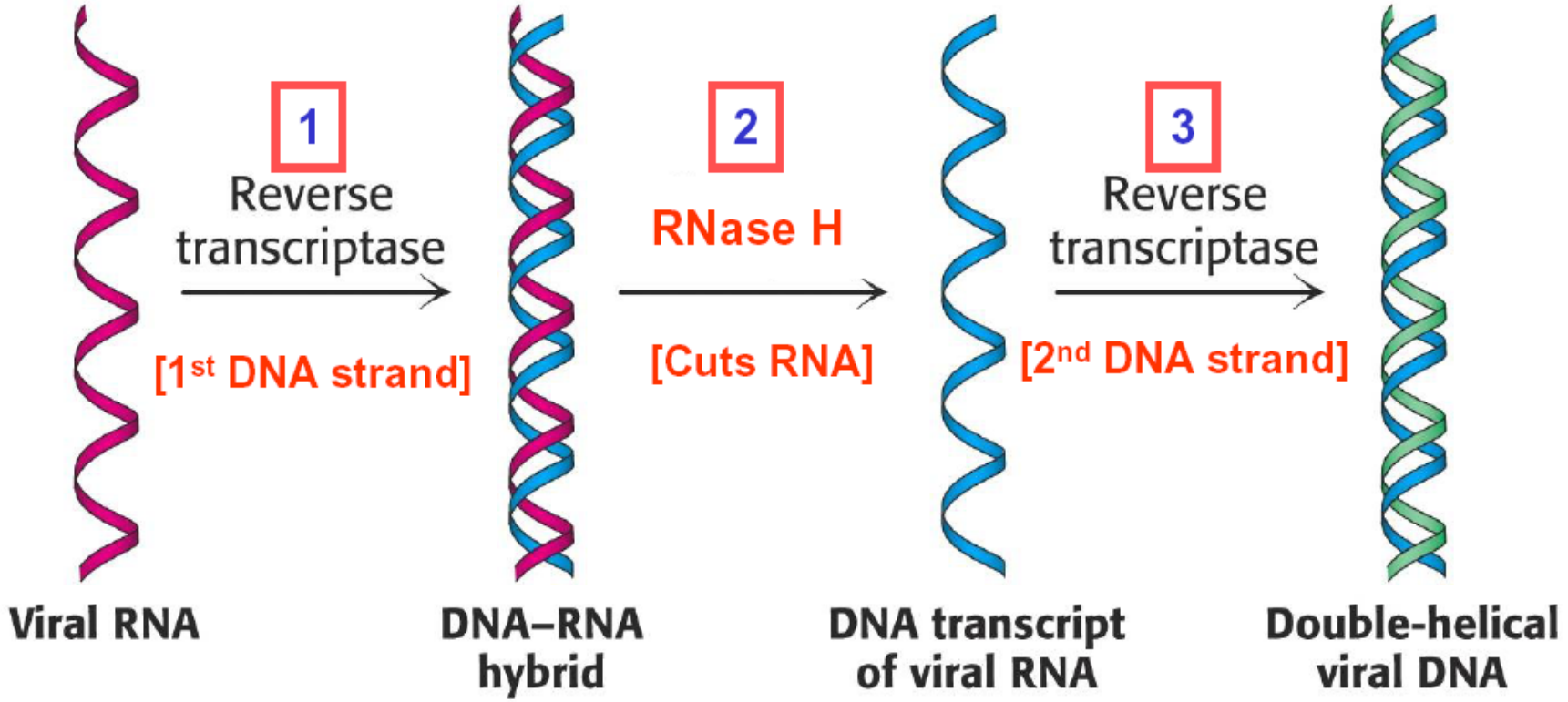
- RT:
  - RNA-dependent DNA-polymerase
  - highly conserved
  - RNaseH activity, specificity to degrade RNA/DNA hybrids

- IN
  - endonuclease,
  - integration of the dsDNA genome into the host cellular genome
  - no prevalent target site specificity

Enzymatic Proteins: Reverse Transcriptase

- **DNA Polymerase Activity**
  - Requires primer with 3’ OH termination
  - Template either RNA or DNA
  - Requires Mg^{++} (or Mn^{++})
  - Lacks proof-reading function; high error rate (10^{-4} errors per base)
- **RNase H Activity (Nuclease specific for RNA in RNA:DNA hybrids)**
  - Activity encoded in different domain than polymerase

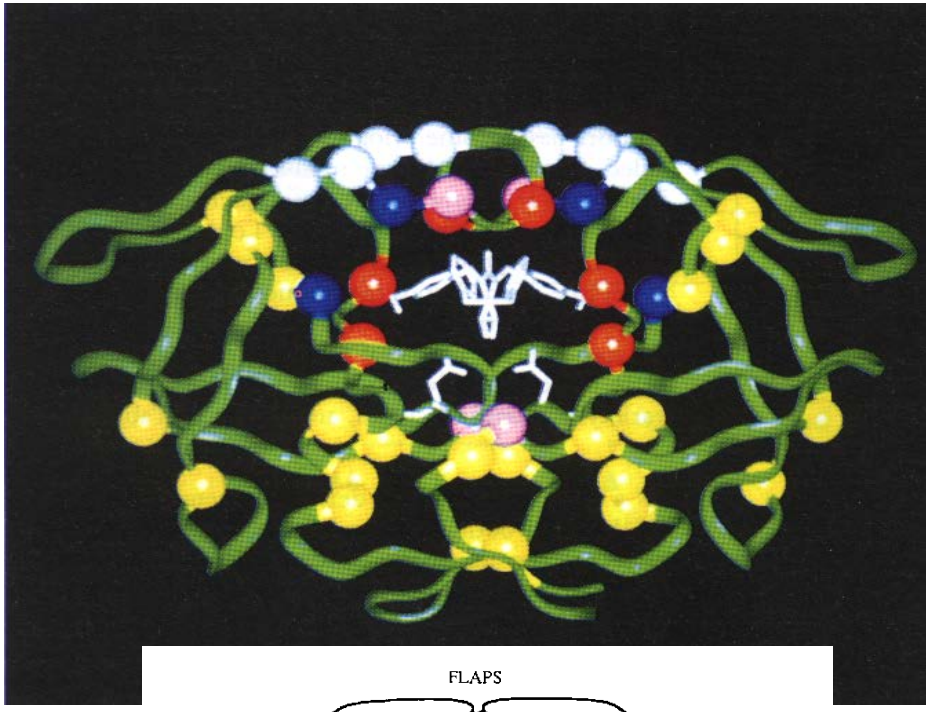
Retrovirus reverse transcriptase: Has 3 different enzyme activities

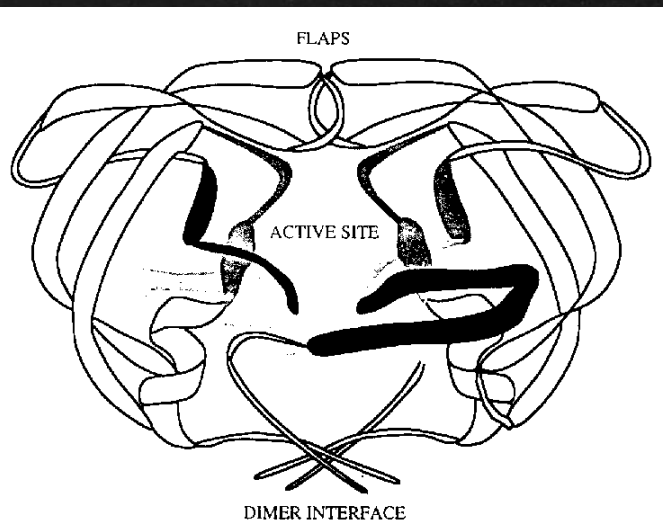


High error rate, cause of viral diversity

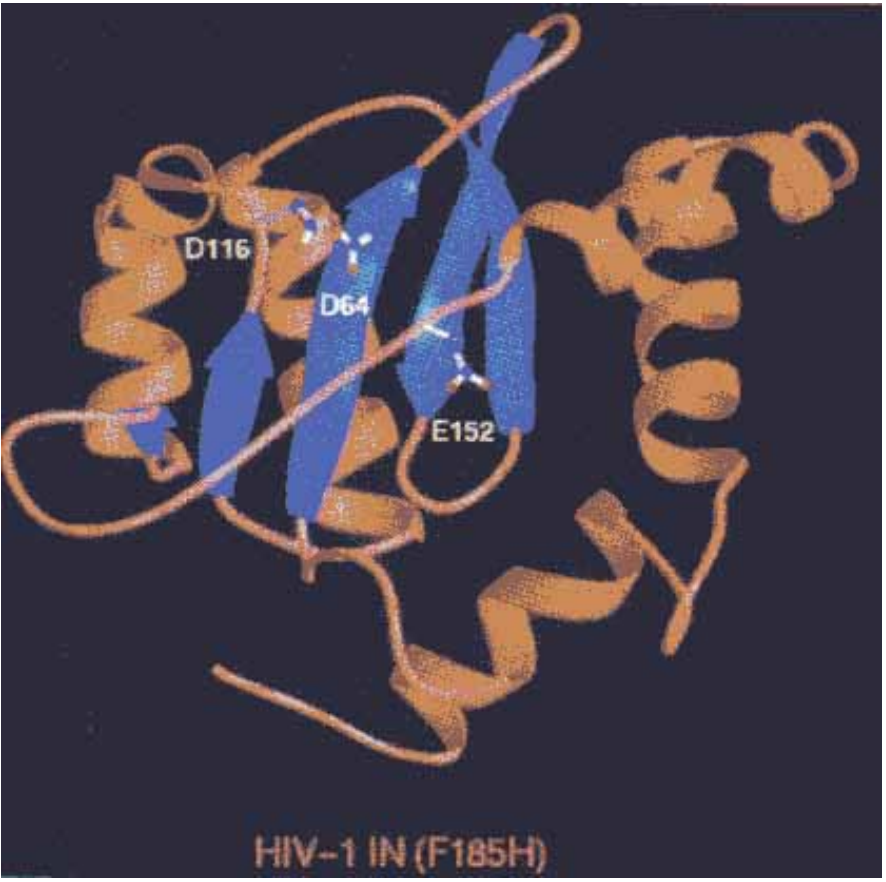
Enzymatic Proteins: Protease

- 10 kd, dimer
- Cuts Gag polyprotein to MA,CA,NC
- Aspartyl protease
- Exquisite cleavage specificity
- Major class of anti-HIV drugs are Protease Inhibitors



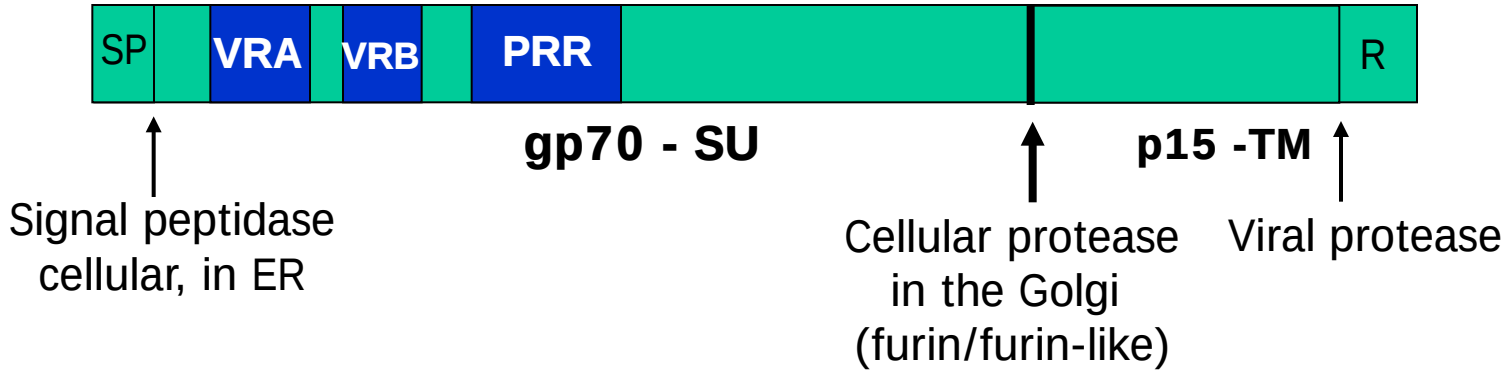


Enzymatic Proteins: Integrase



- **Integrates retroviral DNA into host genome**
- **Endonuclease activity**
- **Drugs available for HIV**

Gene product: Envelope



- **Env-precursor**: cleaved by cellular protease into SU and TM
- **SU (surface)**: - mostly invariant sequences,
  - interrupted by 2 regions of variable length: VRA and VRB
  - glycosylated in the ER
- **VRA, VRB**: - highly variable
  - VRA: major determinant for receptor choice
- **PRR**: (proline rich region) flexible, allow for stability of receptor binding region
- **TM (transmembrane protein)**: anchor in the membrane,

  R-peptide cleaved off in viral particle by viral protease ➔ fusogenic Env

Species Specificity in MLV-Tropism

	Infection of Species:		
	Murine	Non-Murine (Human)	Receptor
Ecotropic MLV	✓	-	mCAT-1
Amphotropic MLV	✓	✓	Pit2

Virus entry; cartoon of fusion process (1)

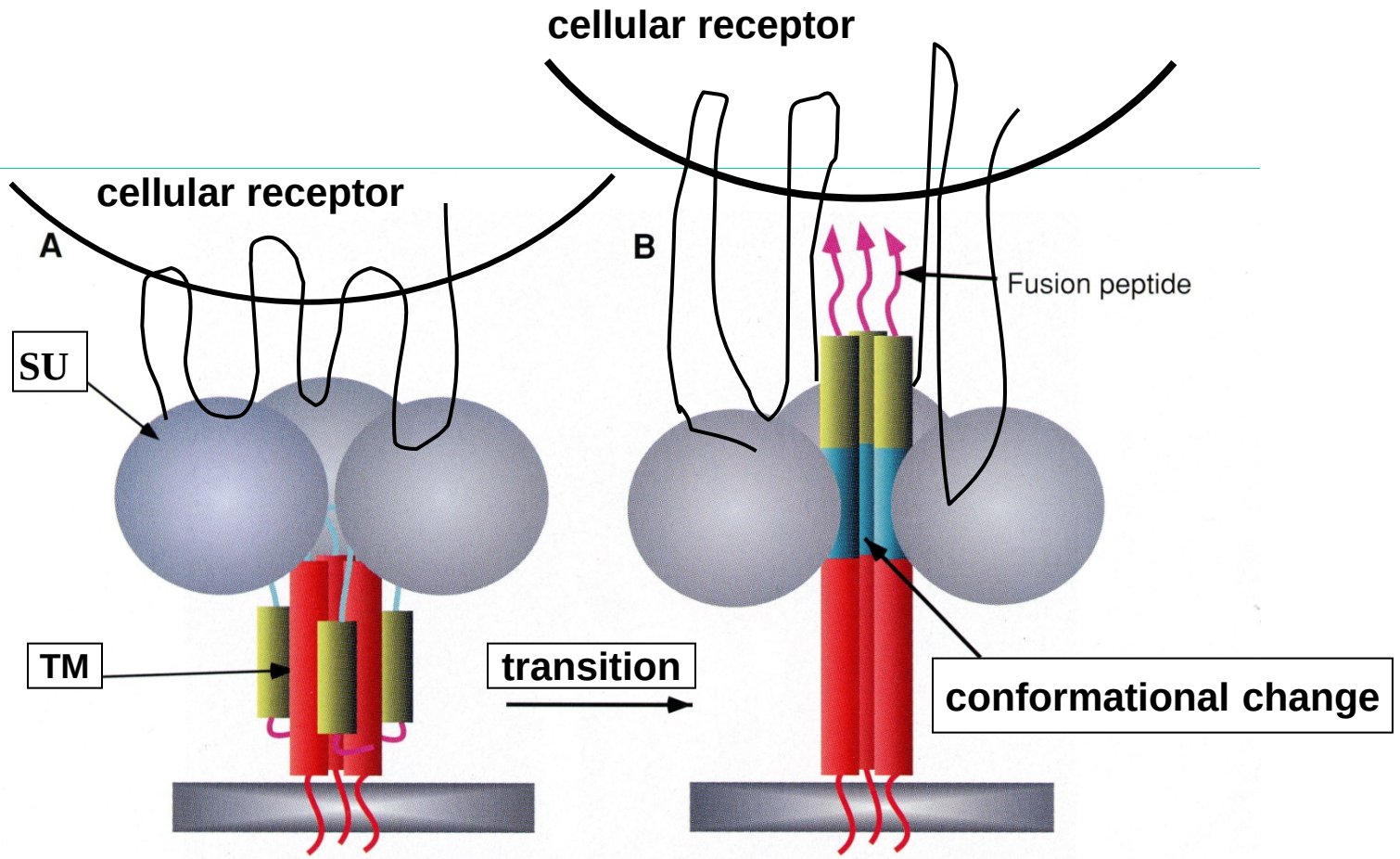
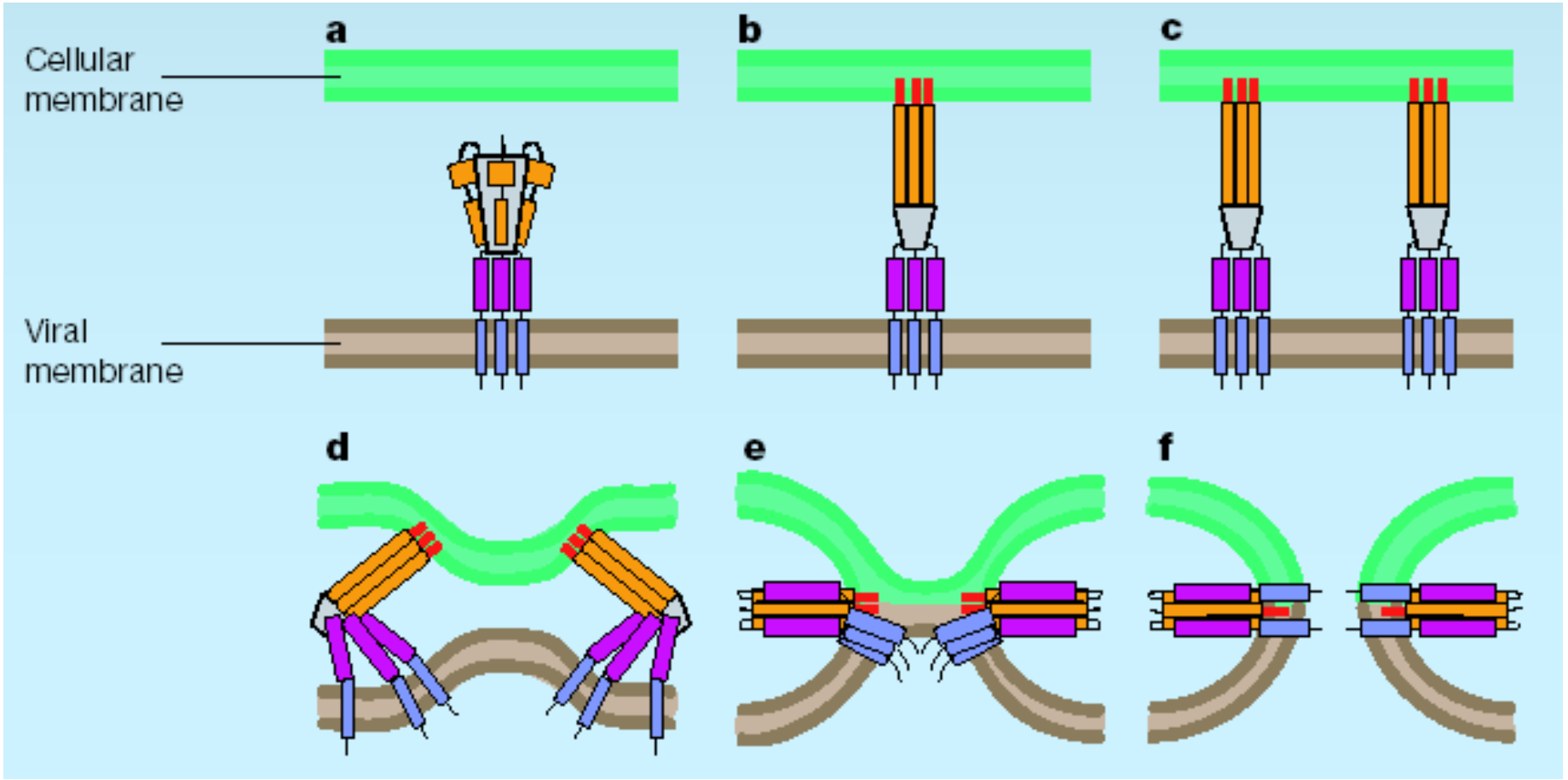


Figure 9 Schematic representation of the structural changes in the hemagglutinin trimer following acidification. (*A*) Neutral pH (virion-associated) form. (*B*) Acid pH (endosome-activated) form.

Virus entry; cartoon of fusion process (2)

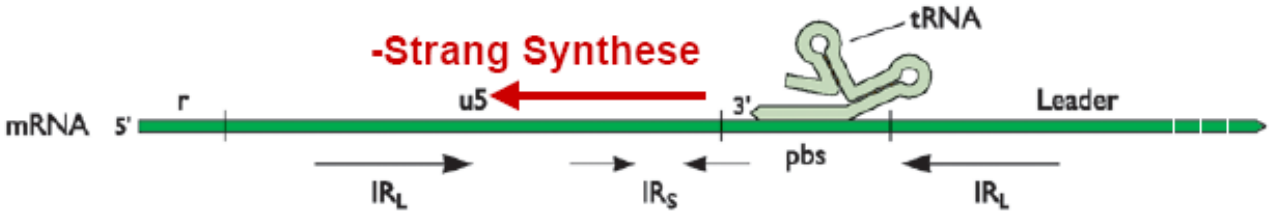


Retroviral replication

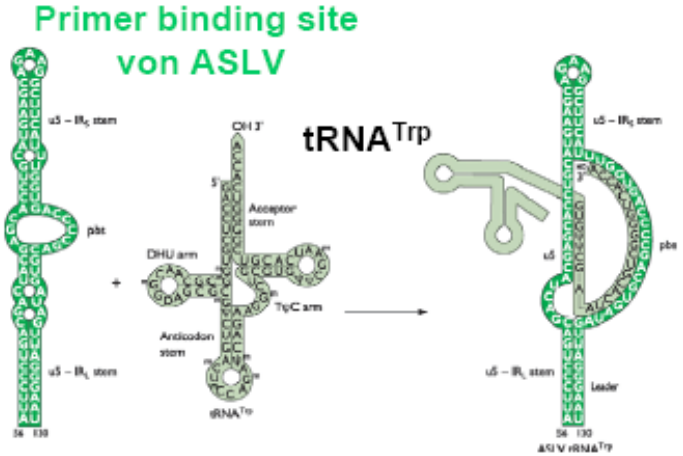
hiv-ltr-fn.mht

Eine tRNA der Wirtszelle dient als primer für die retrovirale RT

- Retroviren verpacken tRNA-Moleküle (ca. 100 Kopien; non-random)
- Die 3‘ terminalen 18 Basen einer bestimmten tRNA binden an das virale Genom

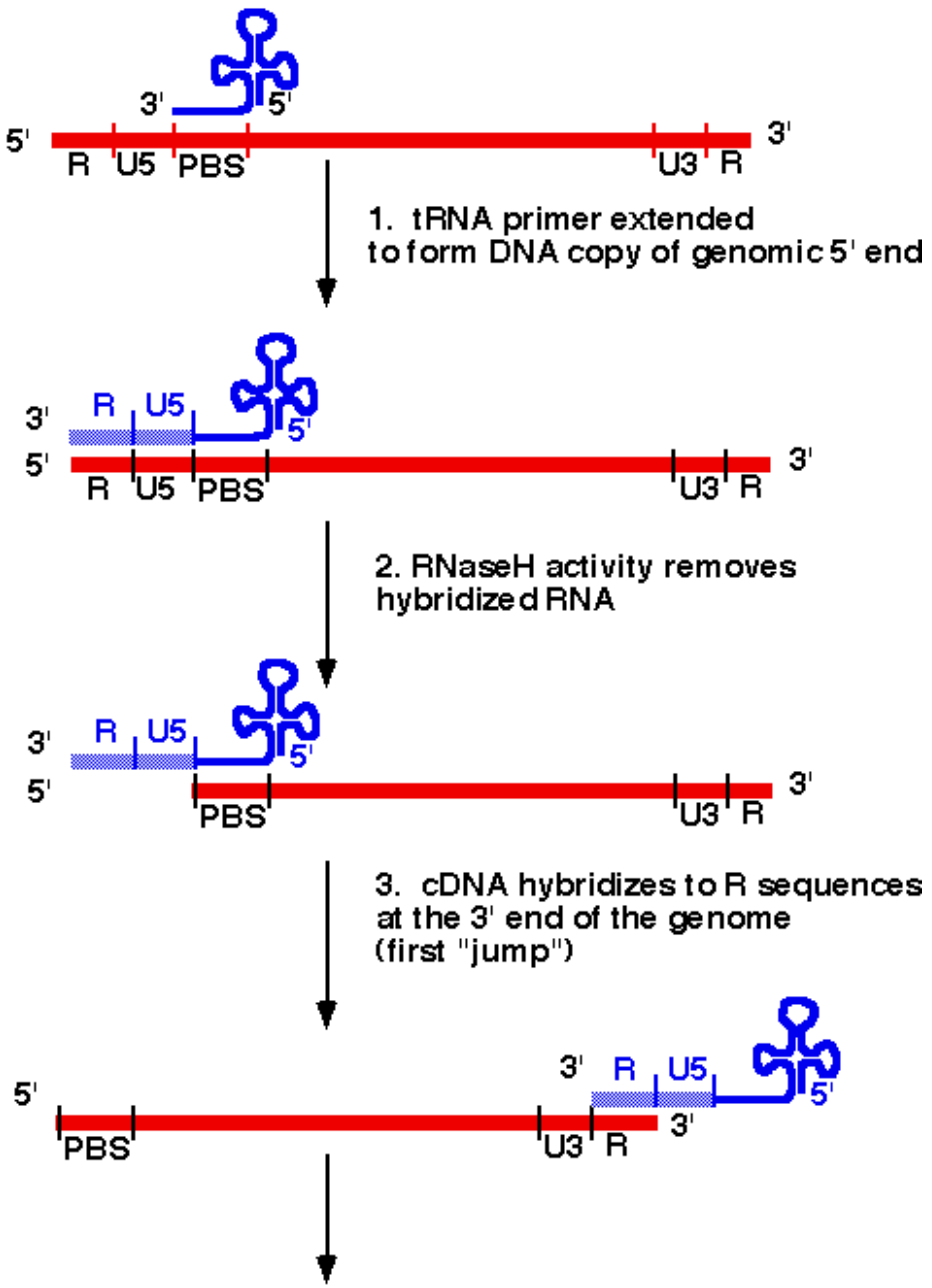


- Zur Bindung wird die tRNA-Struktur partiell entwunden (Beteiligung von NC?)
- Säuger-Retroviren: bevorzugt $tRNA^{Pro}$, $tRNA^{Lys3}$, $tRNA^{Lys1,2}$

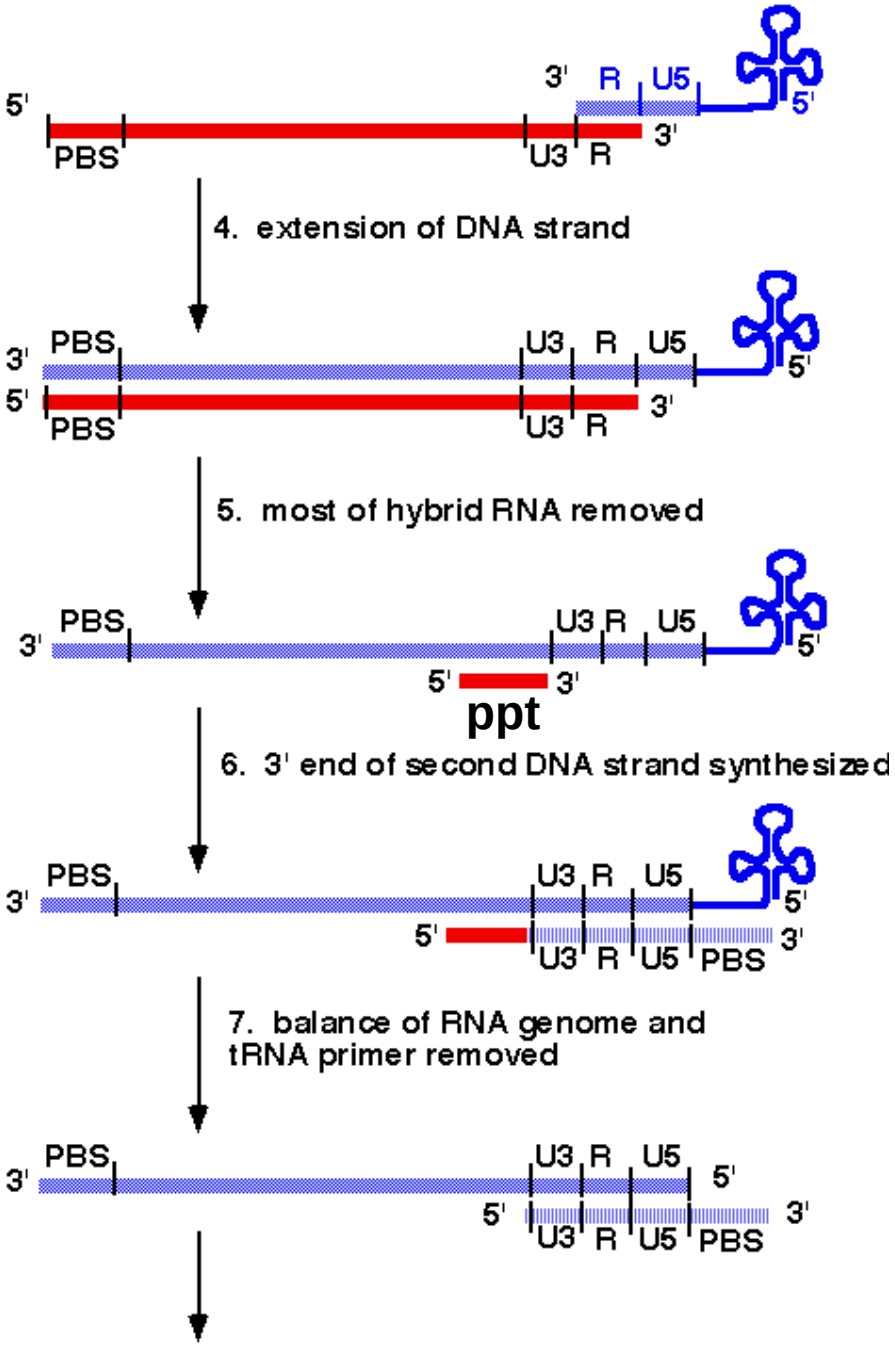


© Flint et al. Principles of Virology

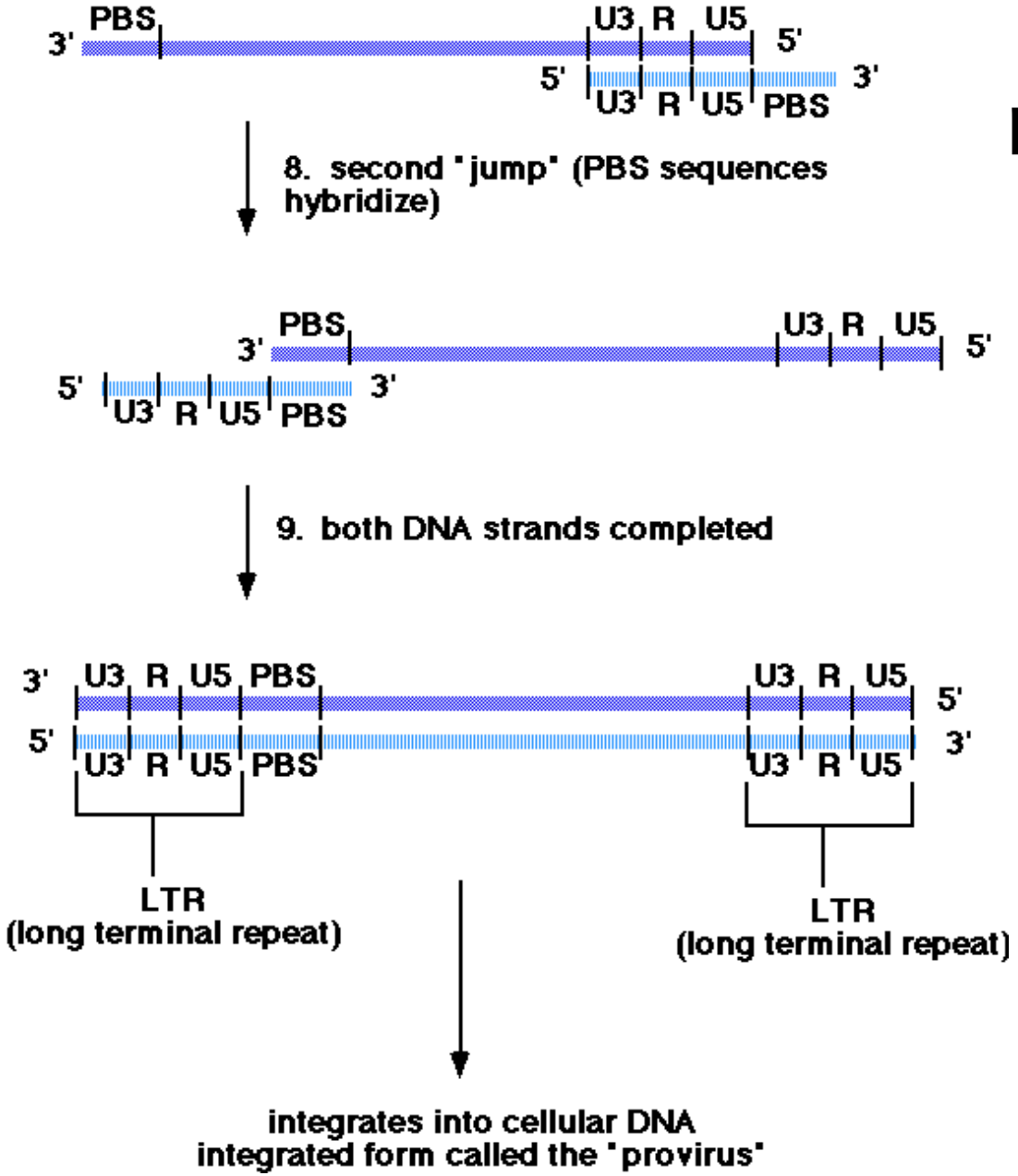
Retrovirus replication carried out by reverse transcriptase



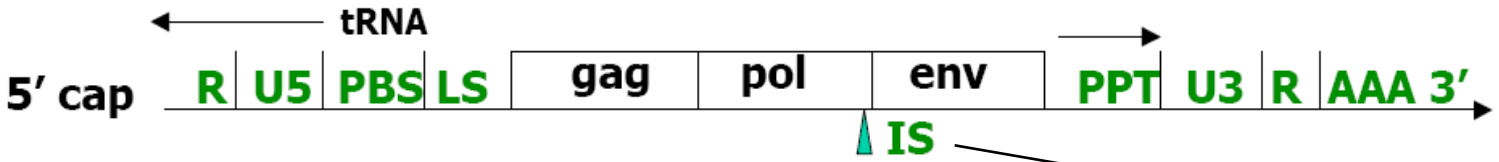
Retrovirus replication carried out by reverse transcriptase



Retrovirus replication carried out by reverse transcriptase



Structure of Retroviral RNA genomes



(1) Non-coding Elements

(A) 5′ region:

R: Repeat/redundancy (direct repeat), 150 to 800 bases at both 5′ and 3′ of RNA, **Transcription start**
Required for transferring synthesized viral DNA from one end to the other end of RNA.

U5: 70 to 200bases, the first region reverse transcribed and integration (att).

PBS (primer binding site): 18 base long complementary to 3′ end of tRNA,
Initiation of reverse transcription by RT for (-) strand synthesis.

Leader sequence: Splicing donor for sub-genomic mRNA (i.e. env),
packaging signal (E) to specify the incorporation of viral RNA into viruses.

(B) Middle regions:

Internal signals: Splicing acceptor for env and regulatory proteins.

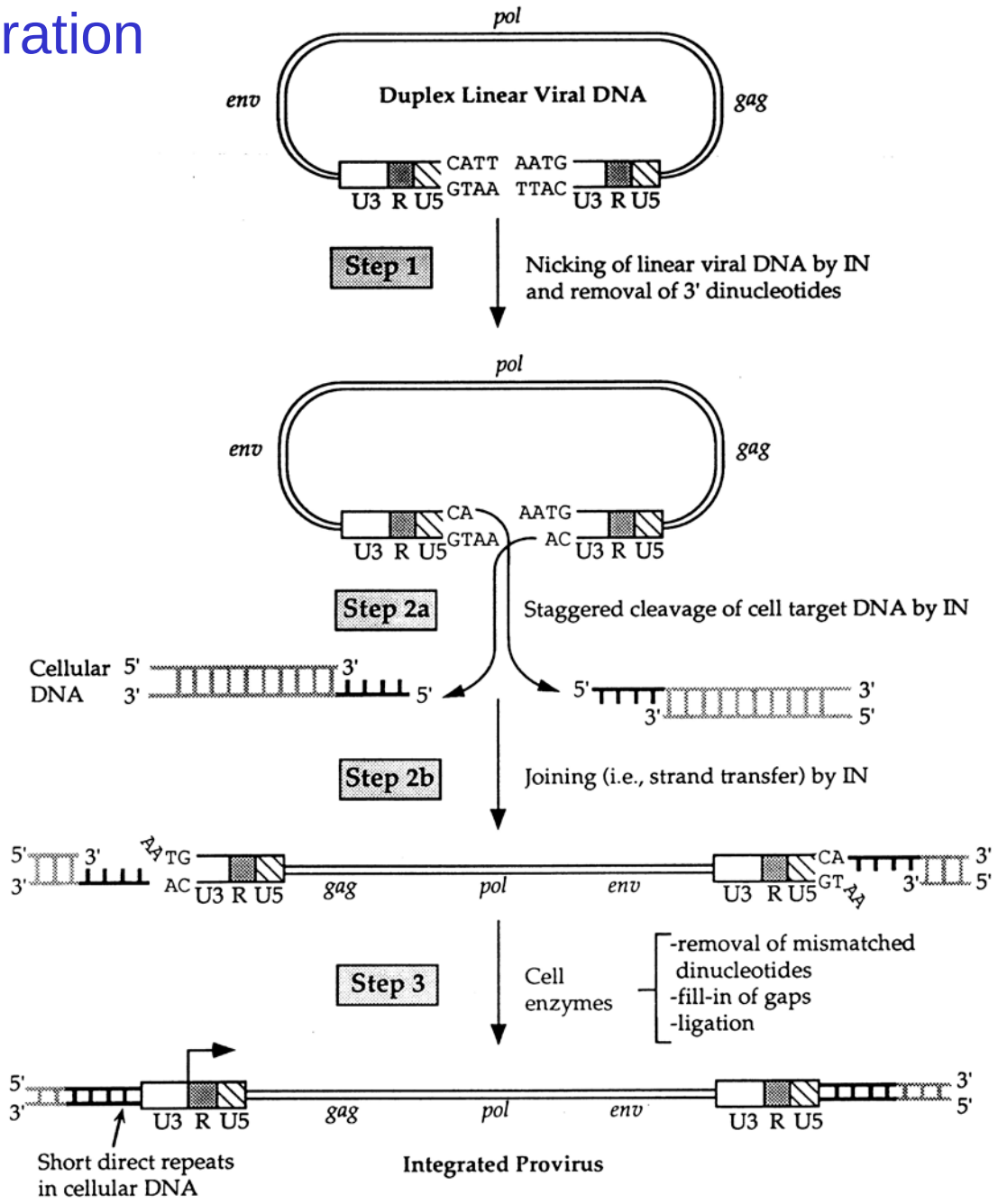
PPT (polypurine tract): Many A/G, initiation of (+) strand DNA synthesis.
Primer during reverse transcription

(C) 3′ regions:

U3: Inverted copy of U5 att,
Transcription initiation sequences: promotor, transcription activator binding sites, att site

R: Signals for polyadenylation.

Integration

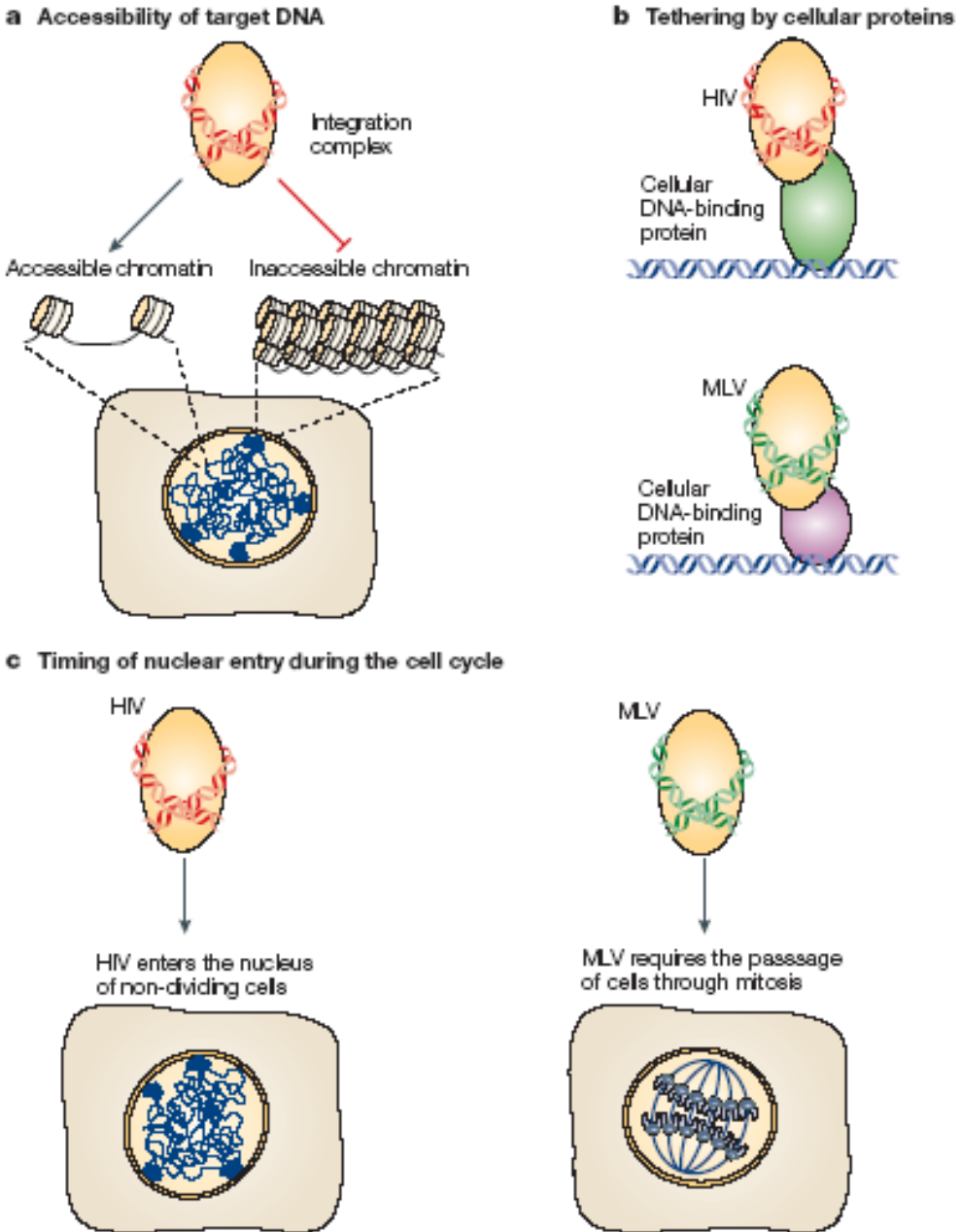


3′ processing/Endonuclease (Removal of 2 nts at the 3′ ends of proviral DNA)

3′ end transfer into chromosome

Mismatch removal
Gap filling
Ligation

Candidate mechanisms direct integration-site selection



Transcriptional start regions are favored targets for MLV and HIV integration

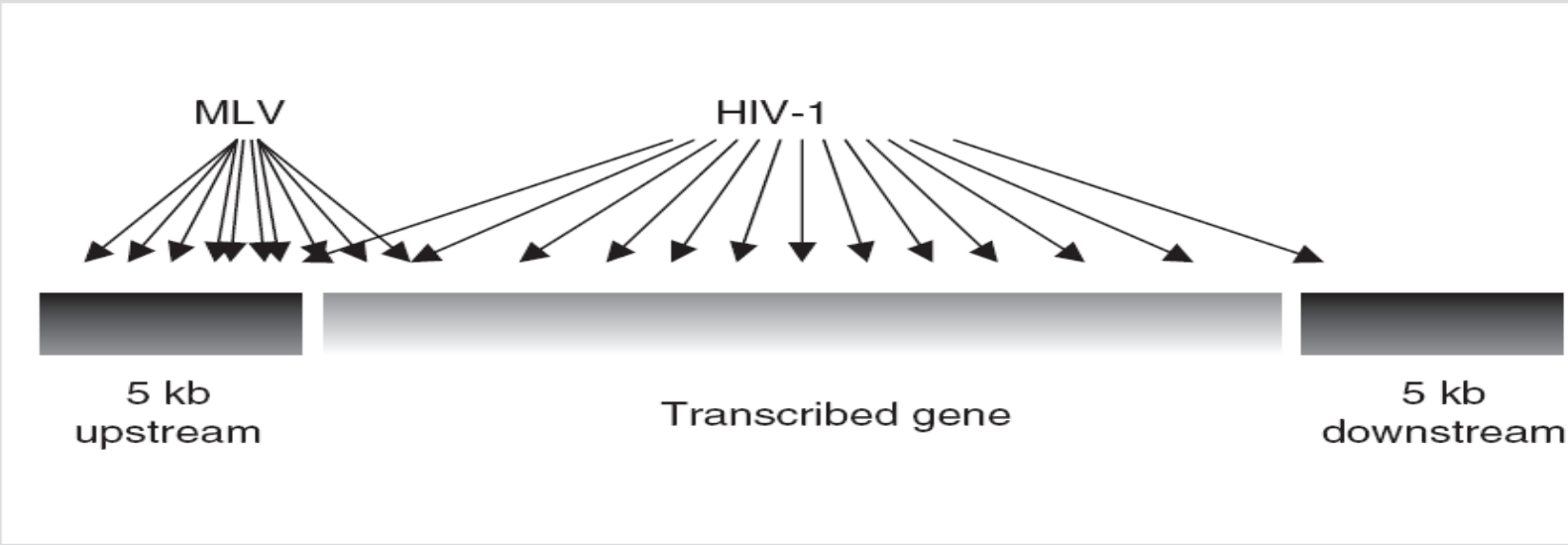
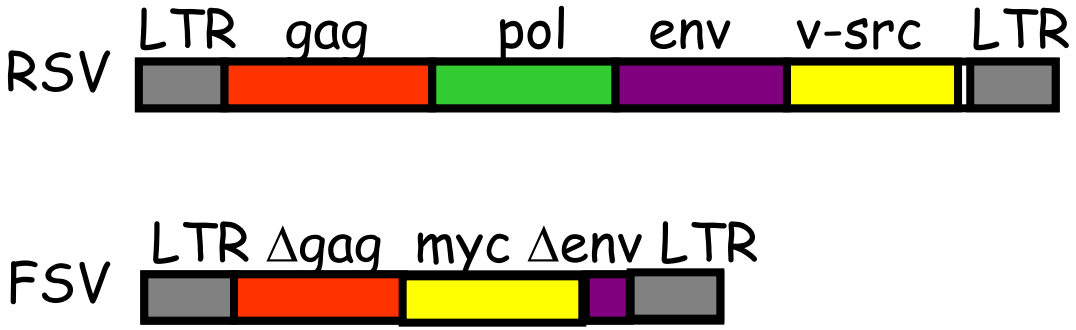


Figure 2. *Transcription start regions are favored targets for MLV and HIV-1 integration.*
Frequency of MLV and HIV-1 integration (indicated by arrows) in three separate regions of a gene (5 kb upstream, the transcribed region, and 5 kb downstream). (Modified from ref. 24.)

Pathogenese:
Krebs und andere Krankheiten

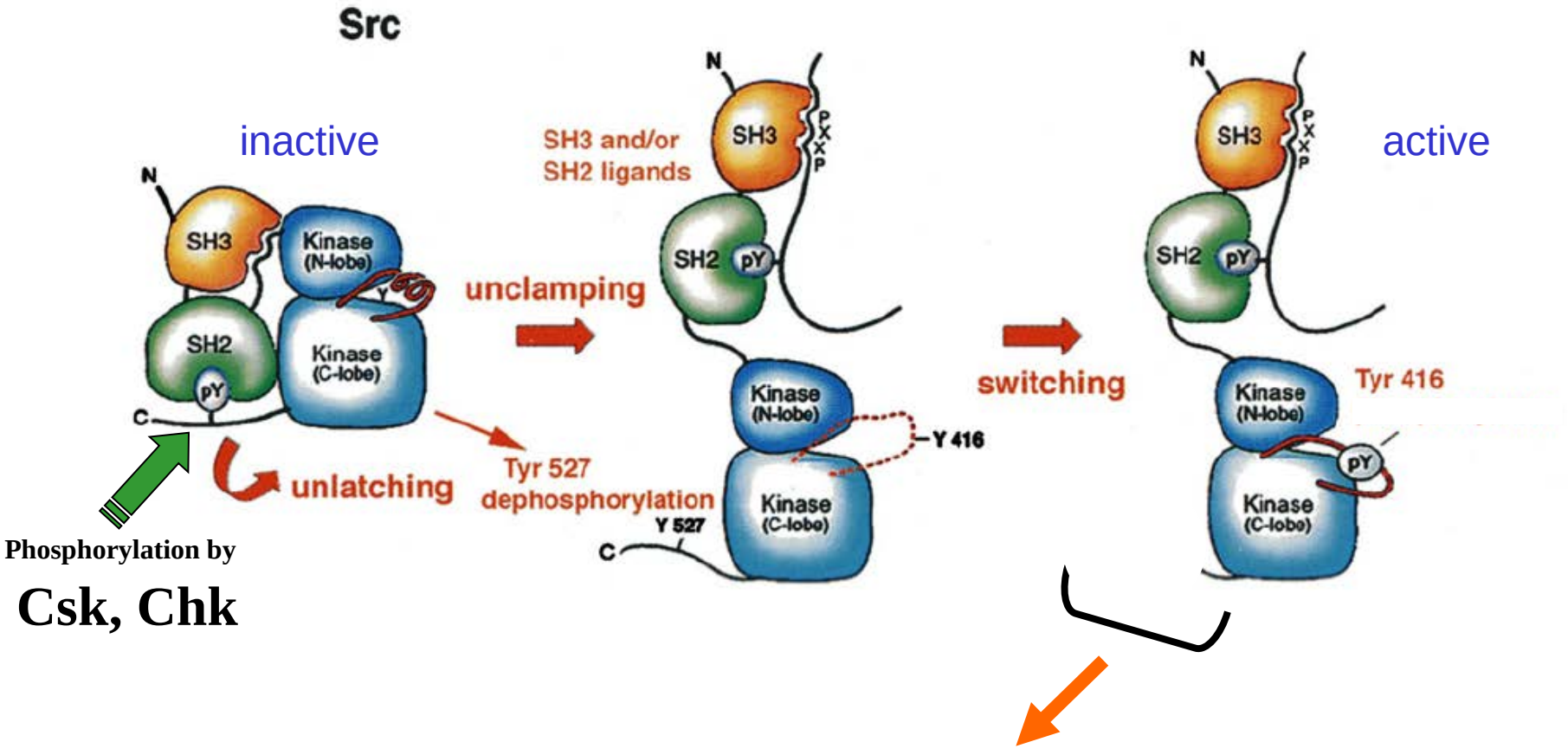
Oncoretroviruses
Acute transforming viruses



- gain of cellular genomic sequences:
  deleted, mutated, fusion with viral proteins
- replication incompetent; need of helper virus for replication
  (exception: RSV)

- e.g. Rous Sarcoma Virus (RSV): v-src (non-receptor tyrosine kinase)
  Murine sarcoma virus (MSV3611): v- raf (Serine/Threonine kinase)

Comparison proto-oncogene (cellular) - oncogene (viral)



Phosphorylation by

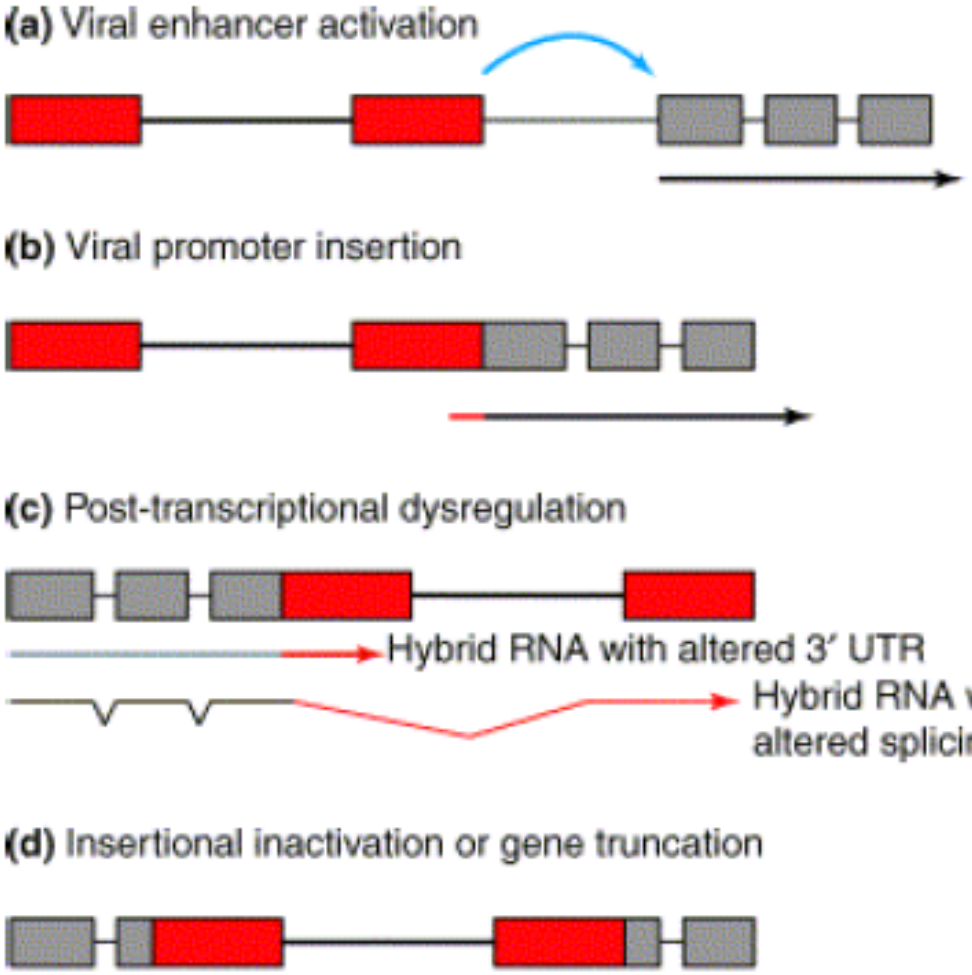
Csk, Chk

C-terminus not present in v-Src

Oncogenes

Oncogene Class	Oncogene Example	Retrovirus	Non-viral tumor	Normal Function
Class I: Growth Factors	sis	simian sarcoma		platelet-derived growth factor
Class IIA: Cell-surface Receptors	erbB erb B2	avain erythroblastosis	neuroblastoma	epidermal growth factor receptor
Class IIB: Intracellular Receptors		avian erythroblastosis		thryoid hormone receptor
Class IIIA: Intracellular Signal Transducers (tyrosine kinases)	src abl	Rous sarcoma virus Ableson murine leukemia virus	chronic myelogenous leukemia	tyrosine kinase
Class IIIB: Intracellular Signal Transducers (ser/thr kinases)	mos	Moloney murine sarcoma virus		serine/threonine kinase
Class IIIC: Intracellular Signal Transducers (G proteins)	H-ras	Harvey murine sarcoma virus	Bladder, mammary and skin carcinoma	guanine nucleotide binding protein (GTPase)
Class IV: Nuclear Transcription Factors	jun fos myc	avian sarcoma virus FBJ sarcoma virus avian MC29 myelocytomatosis virus		transcriptional regulators
Class V: Cell Cycle Control Proteins	RB p53		retinoplastoma most human cancers	tumor suppressors

Insertional activation of proto-oncogenes



Viral enhancer acts on a nearby gene (dominant)

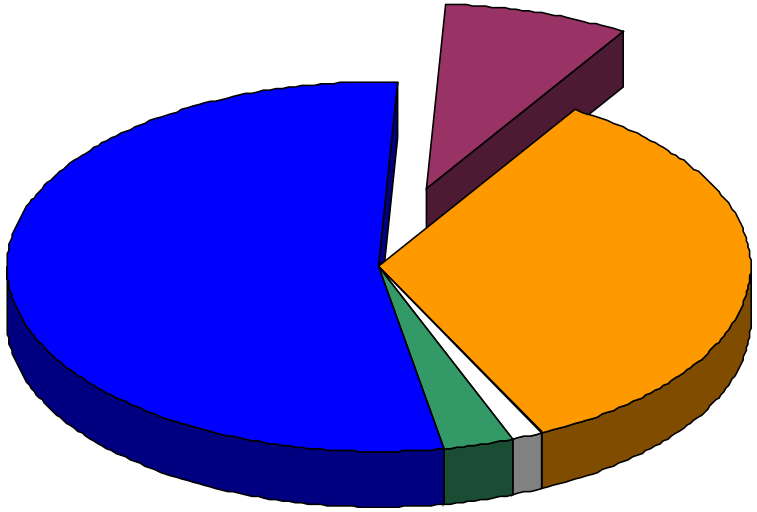
Viral promoter transcribes a nearby oncogene (dominant)

Altered transcription, processing, or stability (dominant)

Inactivate a gene (recessive mutation)

Fig. 2 from Trends in Mol. Medicine 2:43-45 (2003)

Composition of the human genome



8% LTR-Retrotransposons

34%
Non-LTR-Retrotransposons

54%
Miscellaneous
(Introns, regulatory regions, telomeres, microsatelites, etc.)

3%
DNA Transposons

1-1.5 %
Exons of functional host genes

Endogenous / Exogenous Retroviruses

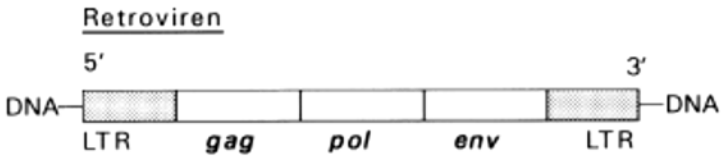
- existence in somatic/germ cells and route of transmission

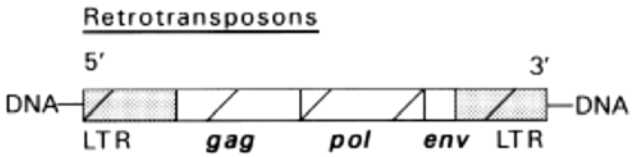
- **Exogenous Retrovirus**
  - ➔ transmission from outside of the body; horizontally
  - ➔ functional genome

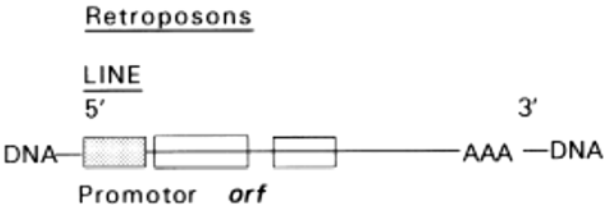
- **Endogenous Retrovirus**
  - ➔ integration into germ cell line; in each cell of the body
  - ➔ transmission from mother to child; vertically
  - ➔ complete or disrupted genome; non-functional, partial expression (up to 8% of the human genome)

Genome structure of retroelements

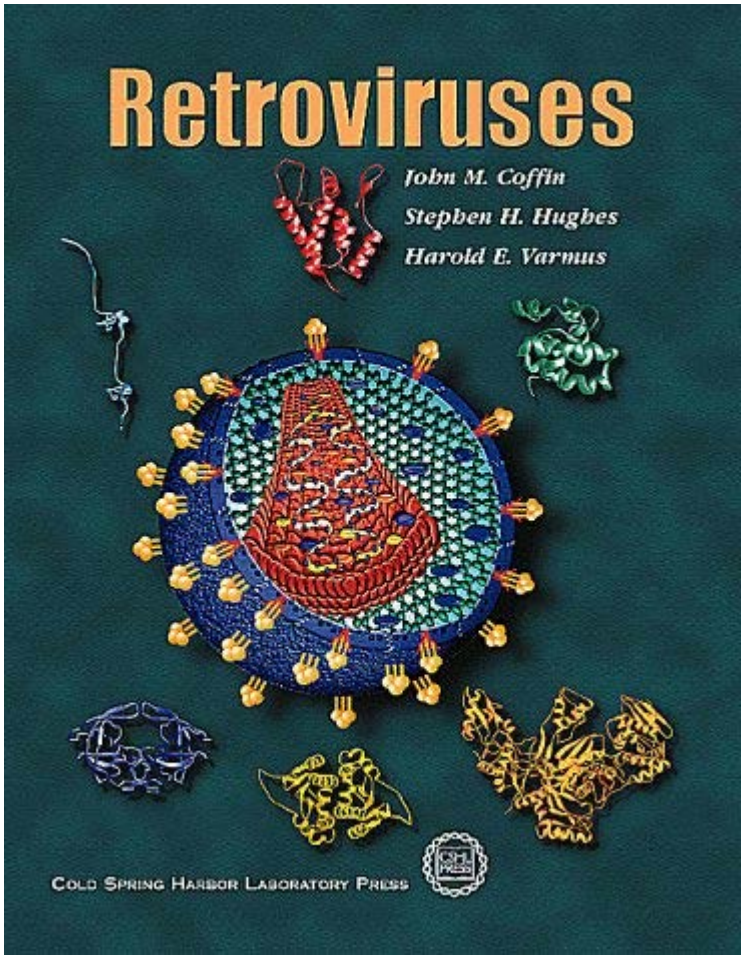
45% of our genome consists of transposed or retrotransposed sequences
(pseudogenes, SINE, LINE, HERV)

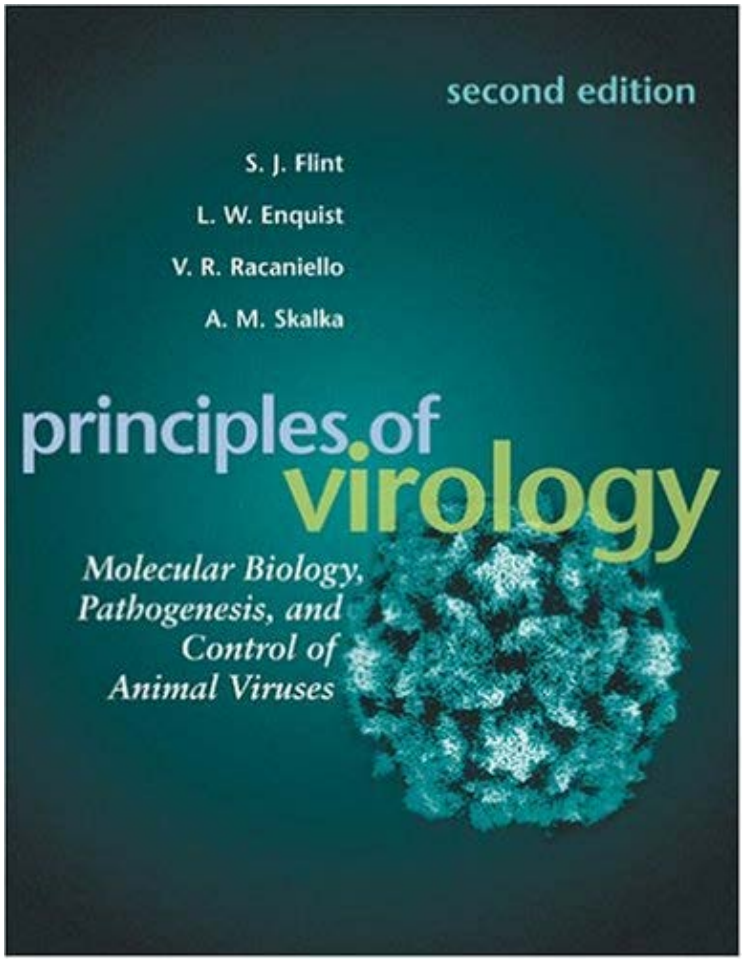






	Size	Examples	Copys/Genomes
Retroviren	ca. 7-9 kb	**HERVs (human)** **IAP (mouse)Size**	**1-100**
Retrotransposons	ca. 6-8 kb	**Ty3 (Yeast)**	**10^2 to 10^4**
LINE	ca. 6 kb	**LINE 1 (human)**	**10^4 to 10^5**
SINE	ca. 0.4 kb	**Alu (human)**	**10^5 to 10^6**





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