Peter Pristas

Molecular Biology

Mobile gene elements

Transposons, plasmids, phages

Transposable Elements

Transposable element **Mobile genetic elements** **Jumping Genes**

A discrete DNA segment which can translocate from one site to another (target) site in the same replicon or in a different replicon in the same cell.

This process of translocation is called transposition
- does not require sequence homology between TE and the target site

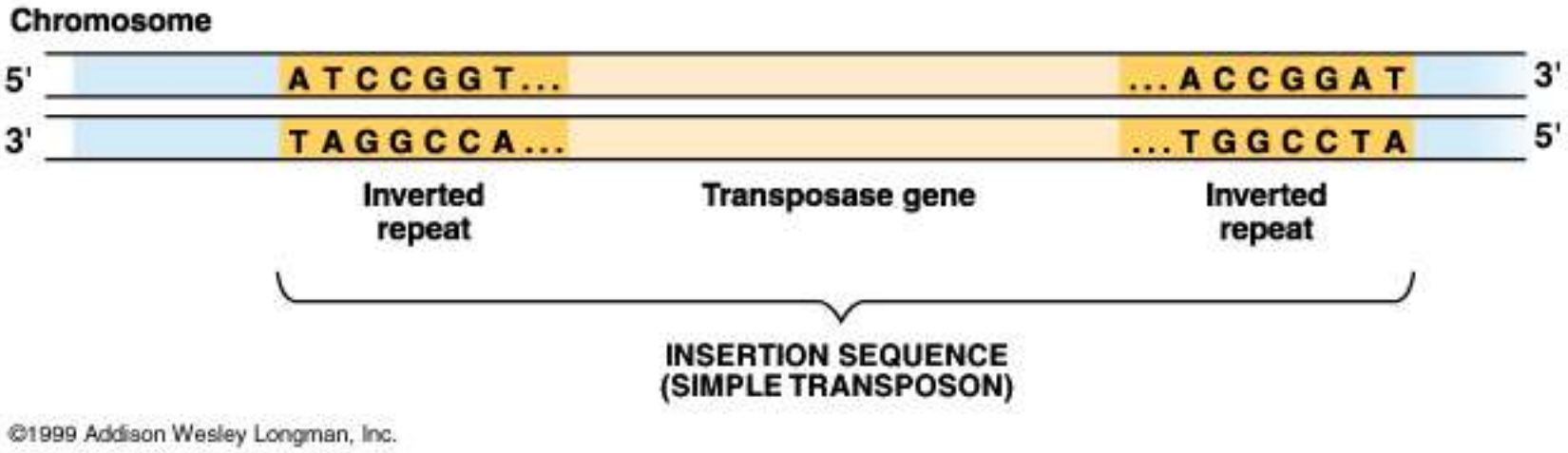
The enzyme involved - Transposase

TEs are normal components of chromosomes, plasmids, phages.

Transposons

Transposons are characterized by inverted repeats. Many tranposons encode a enzyme called a transposase which is responsible for catalysing DNA cutting and resealing.

Some transposons in bacteria carry antibiotic resistance genes. Multicoloured "Indian" corn is due to transposons.



©1999 Addison Wesley Longman, Inc.

Transposons discovery

Barbara McClintock discovered jumping genes in the 1940's and called them activator (Ac) and dissociation (Ds) elements.

Ds element disrupts the purple pigment

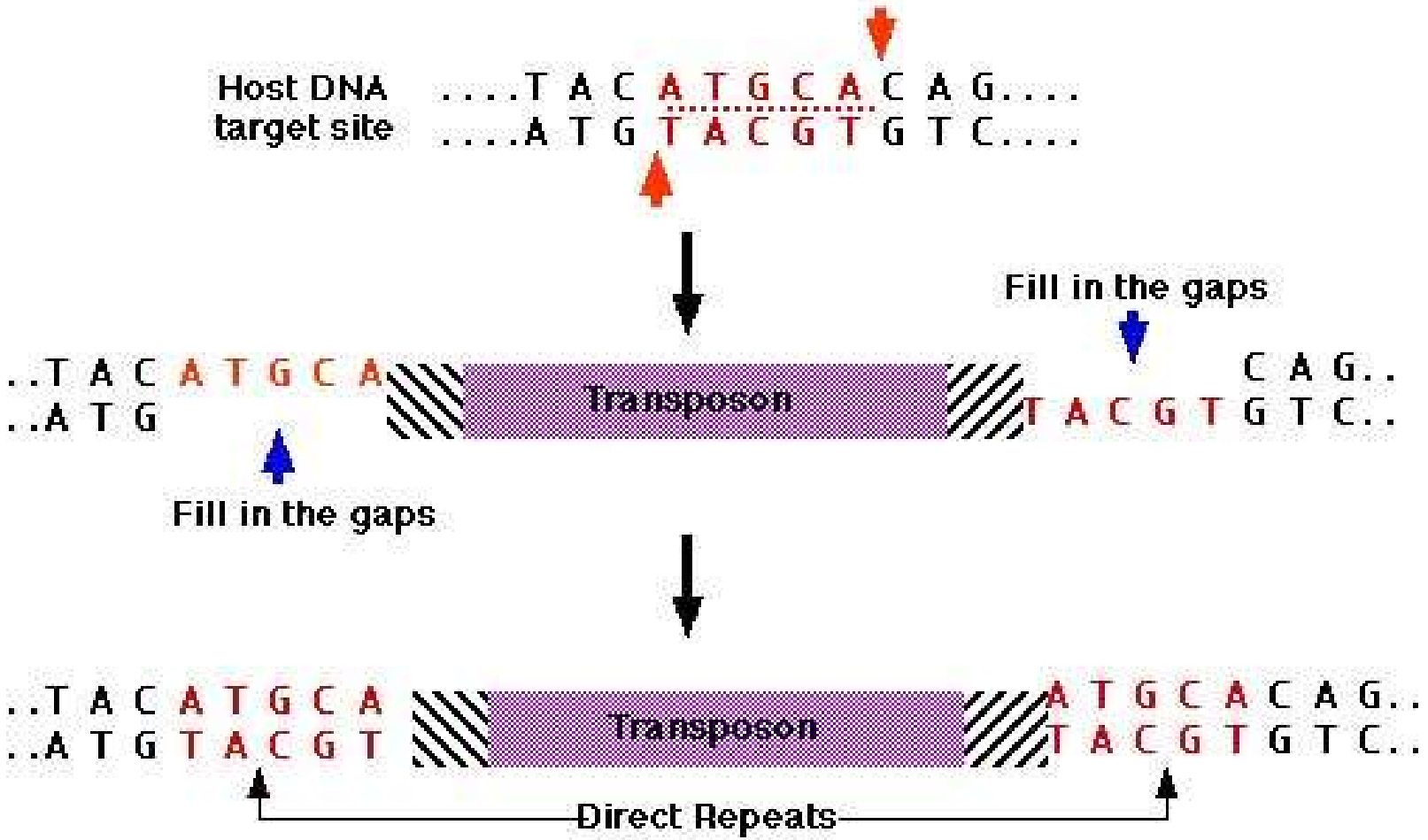
- Ds and Ac are now know to indeed be transposons.
- McClintock studied corn without molecular techniques, only genetic crosses.
- McClintock received the Nobel Prize in 1983 for this work.

Types of transposable elements

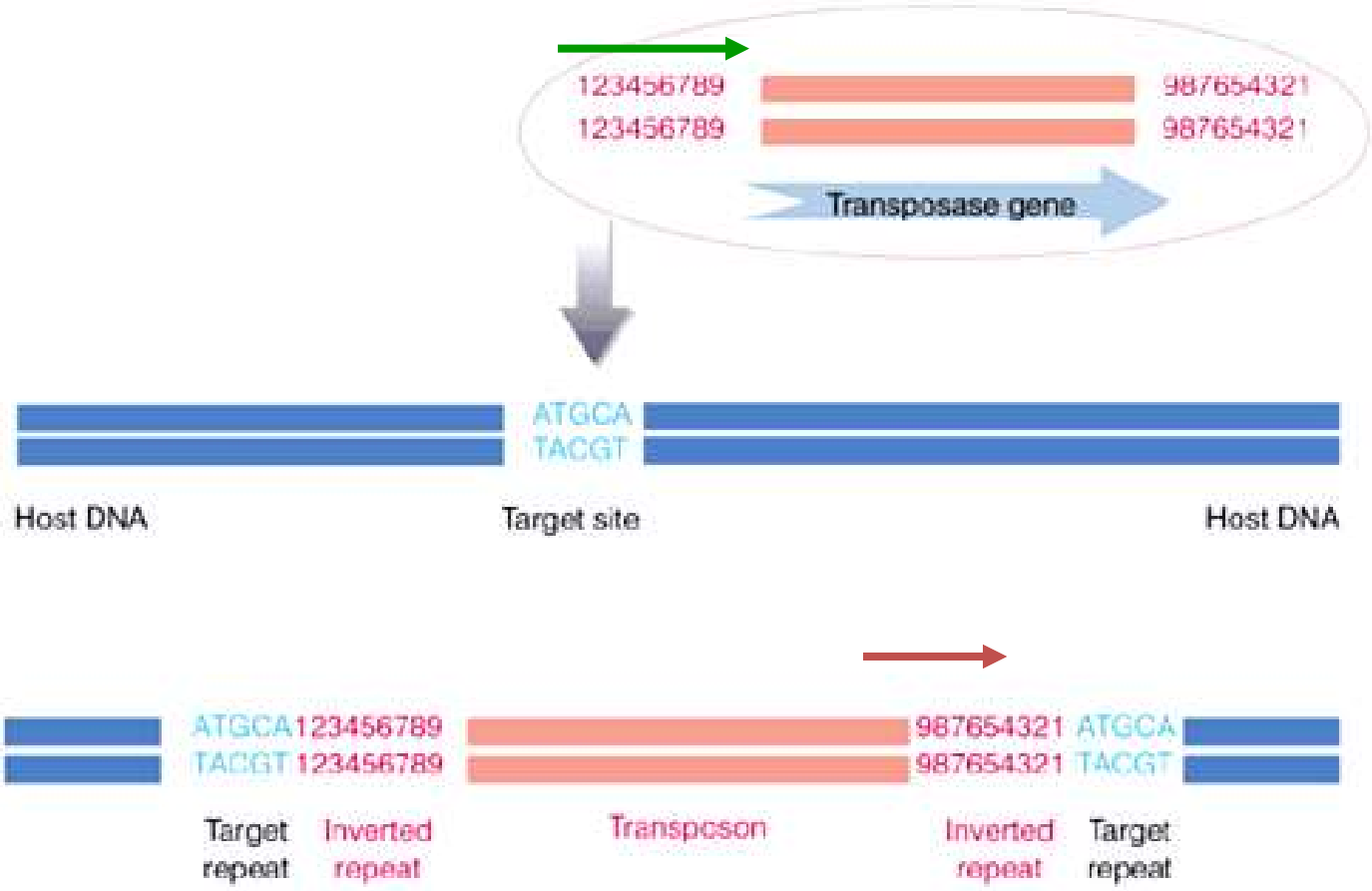
Type	Structural Features	Mechanism of Movement	Examples
DNA-Mediated Transposition			
Bacterial insertion sequences (IS elements)	≈50-bp inverted repeats flanking region encoding transposase and, in some, resolvase	Excision or copying of DNA and its insertion at target site	IS*1*, IS*10*
Bacterial transposons	Central antibiotic-resistance gene flanked by IS elements	Copying of DNA and its insertion at target site	Tn9
Eukaryotic transposons	Inverted repeats flanking coding region with introns	Excision of DNA and its insertion at target site	P element *(Drosophila)* Ac and Ds elements (corn)
RNA-Mediated Transposition			
Viral retrotransposons	≈250- to 600-bp direct terminal repeats (LTRs) flanking region encoding reverse transcriptase, integrase, and retroviral-like Gag protein	Transcription into RNA from promoter in left LTR by RNA polymerase II followed by reverse transcription and insertion at target site	Ty elements (yeast) *Copia* elements *(Drosophila)*
Nonviral retrotransposons	Of variable length with a 3′ A/T-rich region; full-length copy encodes a reverse transcriptase	Transcription into RNA from internal promoter; folding of transcript to provide primer for reverse transcription followed by insertion at target site	F and G elements *(Drosophila)* LINE and SINE elements (mammals) *Alu* sequences (humans)

Transpositons

duplication of DNA ends



Terminal Repeats

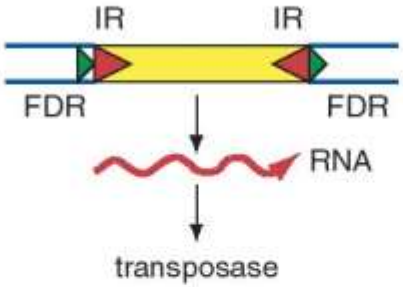


			Overall length	Target selection
IS1	9 bp	23 bp	768 bp	random
IS2	5 bp	41 bp	1327 bp	hotspots
IS4	11-13 bp	18 bp	1428 bp	$AAAN_{20}TTT$
IS5	4 bp	16 bp	1195 bp	hotspots
IS10R	9 bp	22 bp	1329 bp	NGCTNAGCN
IS50R	9 bp	9 bp	1531 bp	hotspots
IS903	9 bp	18 bp	1057 bp	random

Bacterial Transposons move via DNA Intermediates

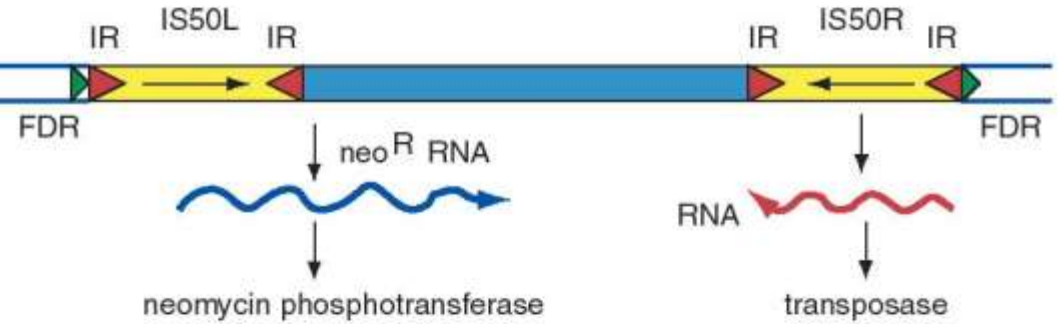
- **Insertion Sequences (IS)** are small non-coding transposons which randomly integrate into and excise from the genome
- IS have inverted repeats at their ends which can form stem-loop structures
- bacterial transposons contain long, terminal direct repeats at their ends
- bacterial transposons contain protein coding genes (e.g. antibiotic resistances)
- transposons contain a terminal duplication of their target site

Insertion sequences

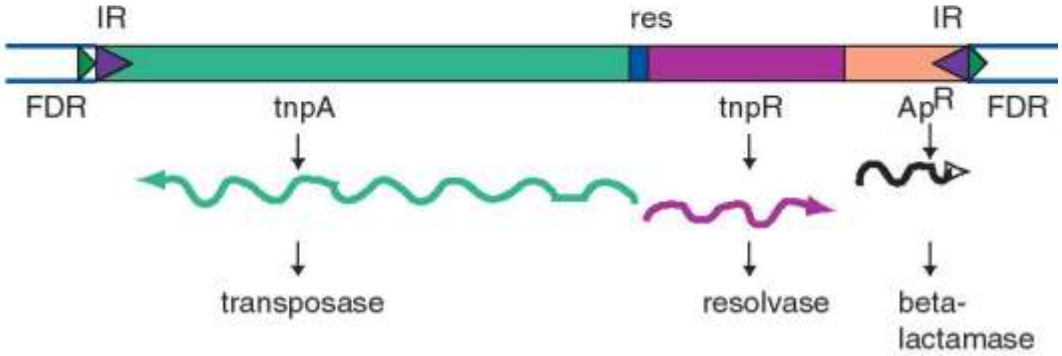


Transposons

Composite transposons, e.g. Tn5

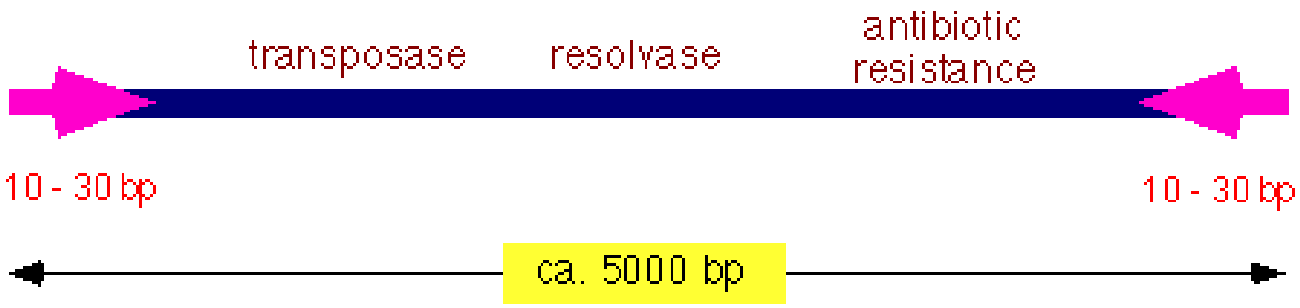


Transposons lacking terminal ISs, e.g. TnA



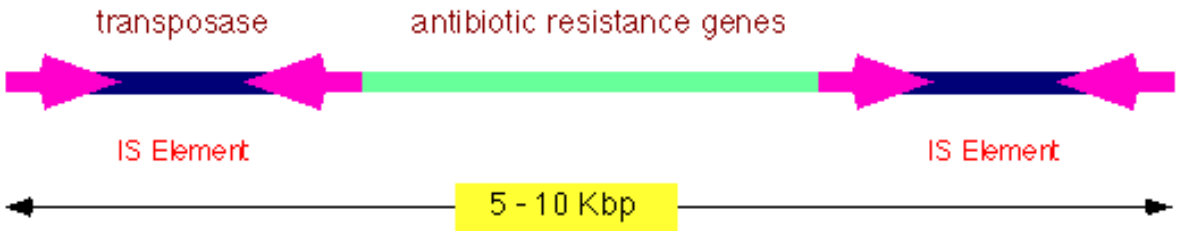
IS elements and transposons

Simple bacterial transposons



Transposon	Antibiotic or other resistance marker	Length (bp)	Inverted Repeat
Tn1	ampicillin	4957	
Tn3	ampicillin	4957	38 bp
Tn501	Hg resistance	8200	38 bp
Tn7	trimethoprim, spectinomycin & streptomycin	14000	30 bp
gamma-delta		6000	35 bp

Composite bacterial transposons



Transposon	Antibiotic or other resistance marker	Length (bp)	Inverted Repeat
Tn5	kanamycin	5700	IS50
Tn9	chloramphenicol	2638	IS1
Tn10	tetracycline	9300	IS10

Mechanism for DNA-mediated transposition

- Transposase nicks at ends of transposon (note cleavage is at the same sequence, since the ends are inverted repeats).
- Transposase also cuts the target to generate 5’ overhangs
- The 3’ end of each strand of the transposon is ligated to the 5’ overhang of the target site, forming a **crossover structure**.

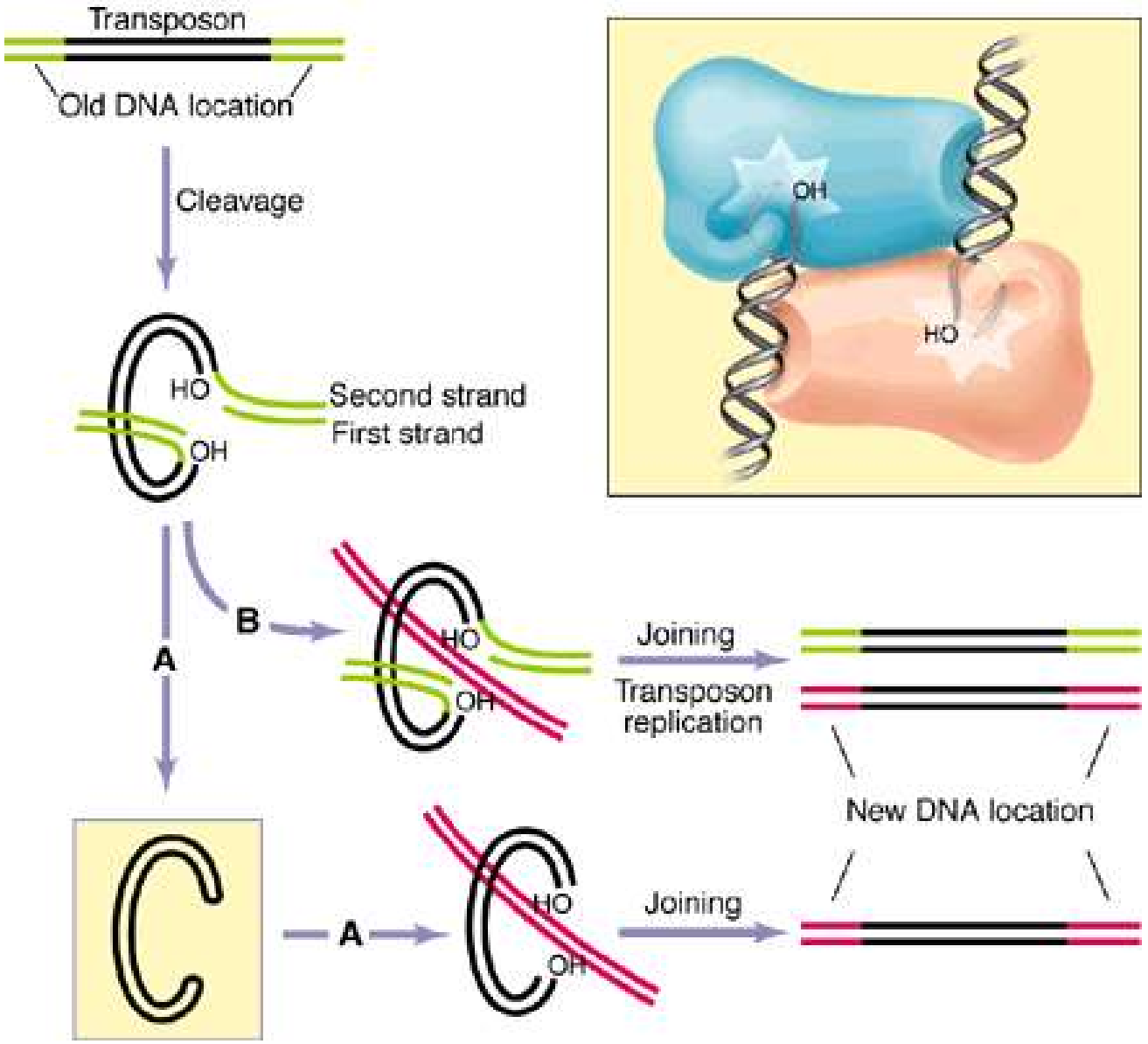
Replicative

- The 3’ ends of each strand from the staggered break (at the target) serve as primers for repair synthesis.
- Copying through the transposon followed by ligation leads to formation of a **cointegrate structure**.
- Copying also generates the flanking direct repeats.
- The cointegrate is resolved by recombination.

Non-replicative

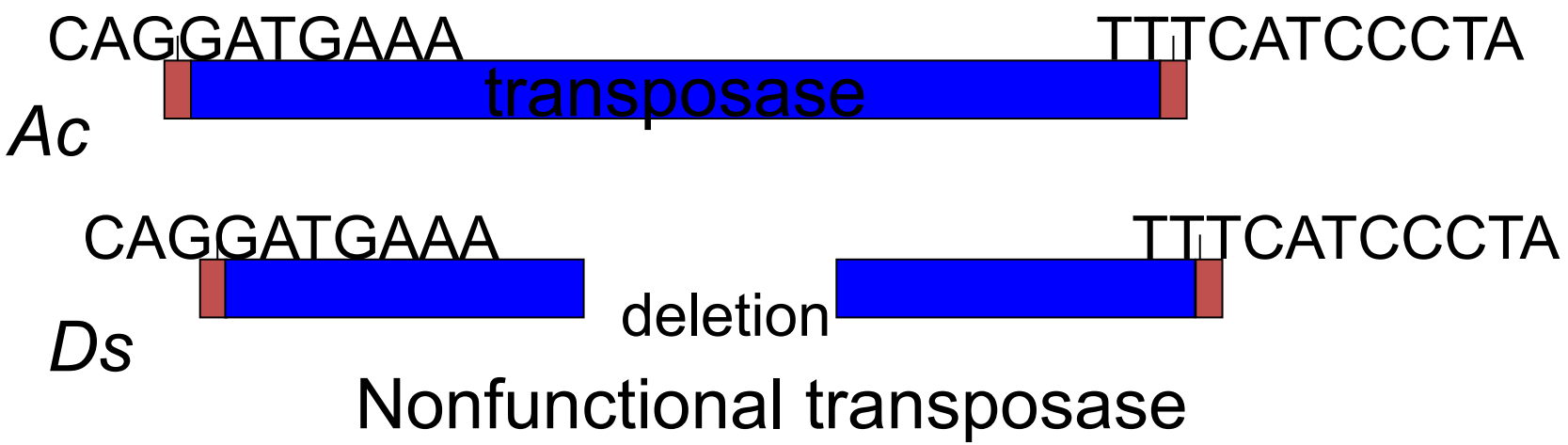
- Crossover structure is released by nicking at the other ends of the transposon (i.e. the ones not initially nicked).
- The gap at the target (now containing the transposon) is repaired to generate flanking direct repeats.

Transposition mechanisms

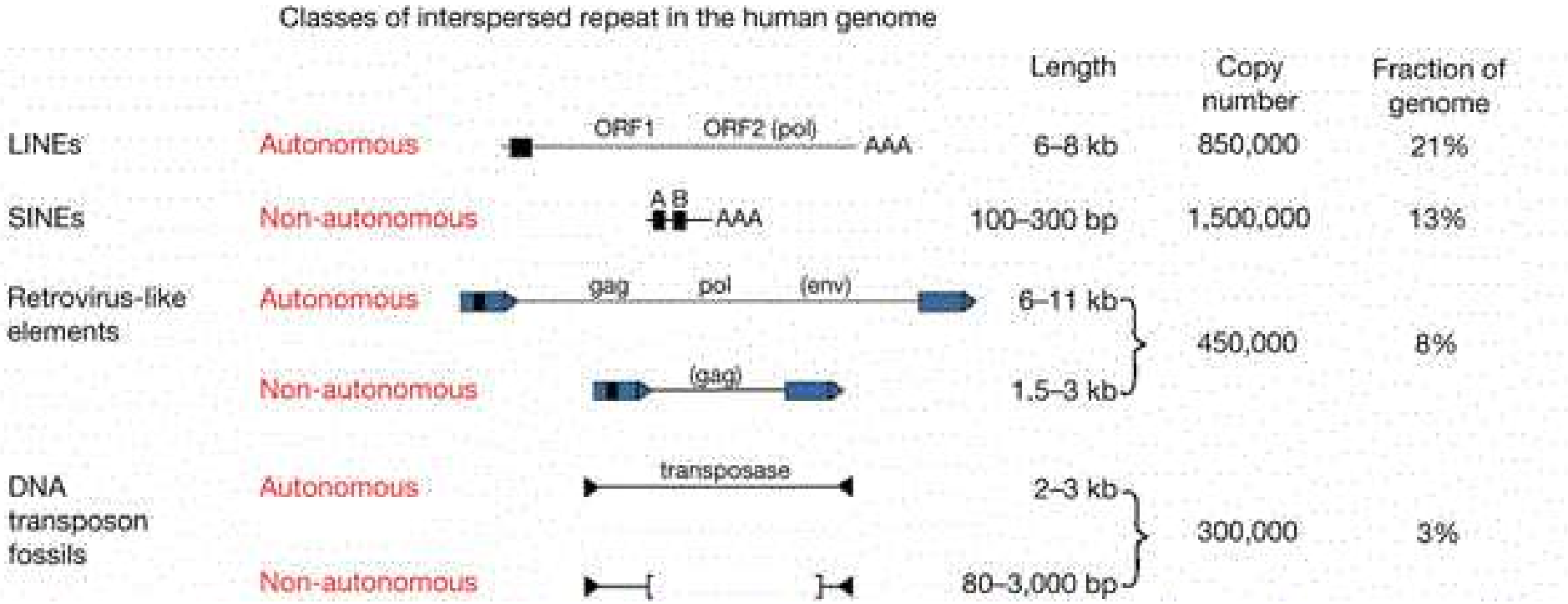


Ac/Ds elements

- **Described genetically by McClintock in maize:**
- ***Ds* - dissociation locus (chromosomal breaks), semi-autonomous element, its mobility depends on *Ac***
- ***Ac* - Activator, autonomous element**
- **Cloned from the waxy (*Wx*) locus, which encodes UDPglucose-starch transferase**



Almost all transposable elements in mammals fall into one of four classes

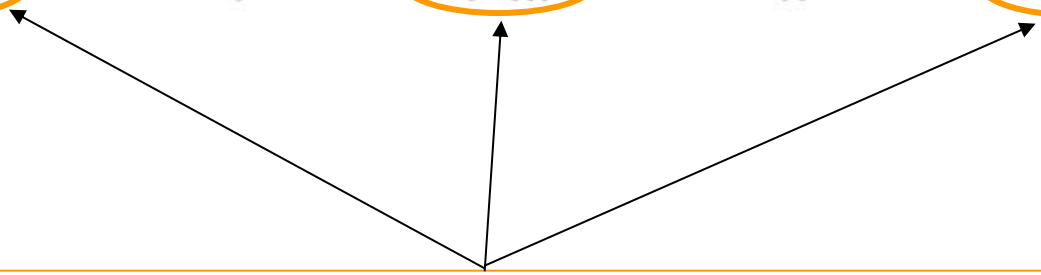


Human transposable elements

	Number of copies (× 1,000)	Total number of bases in the draft genome sequence (Mb)	Fraction of the draft genome sequence (%)	Number of families (subfamilies)
SINEs	1,558	359.6	13.14	
Alu	1,090	290.1	10.60	1 (~20)
MIR	393	60.1	2.20	1 (1)
MIR3	75	9.3	0.34	1 (1)
LINEs	868	558.8	20.42	
LINE1	516	462.1	16.89	1 (~55)
LINE2	315	88.2	3.22	1 (2)
LINE3	37	8.4	0.31	1 (2)
LTR elements	443	227.0	8.29	
ERV-class I	112	79.2	2.89	72 (132)
ERV(K)-class II	8	8.5	0.31	10 (20)
ERV (L)-class III	83	39.5	1.44	21 (42)
MaLR	240	99.8	3.65	1 (31)
DNA elements	294	77.6	2.84	
hAT group				
MER1-Charlie	182	38.1	1.39	25 (50)
Zaphod	13	4.3	0.16	4 (10)
Tc-1 group				
MER2-Tigger	57	28.0	1.02	12 (28)
Tc2	4	0.9	0.03	1 (5)
Mariner	14	2.6	0.10	4 (5)
PiggyBac-like	2	0.5	0.02	10 (20)
Unclassified	22	3.2	0.12	7 (7)
Unclassified	3	3.8	0.14	3 (4)
Total interspersed repeats		1,226.8	44.83	

Number and nature of transposable elements in human, fly and worm

	Human		Fly		Worm	
	Percentage of bases	Approximate number of families	Percentage of bases	Approximate number of families	Percentage of bases	Approximate number of families
LINE/SINE	33.40%	6	0.70%	20	0.40%	10
LTR	8.10%	100	1.50%	50	0.00%	4
DNA	2.80%	60	0.70%	20	5.30%	80
Total	44.40%	170	3.10%	90	6.50%	90



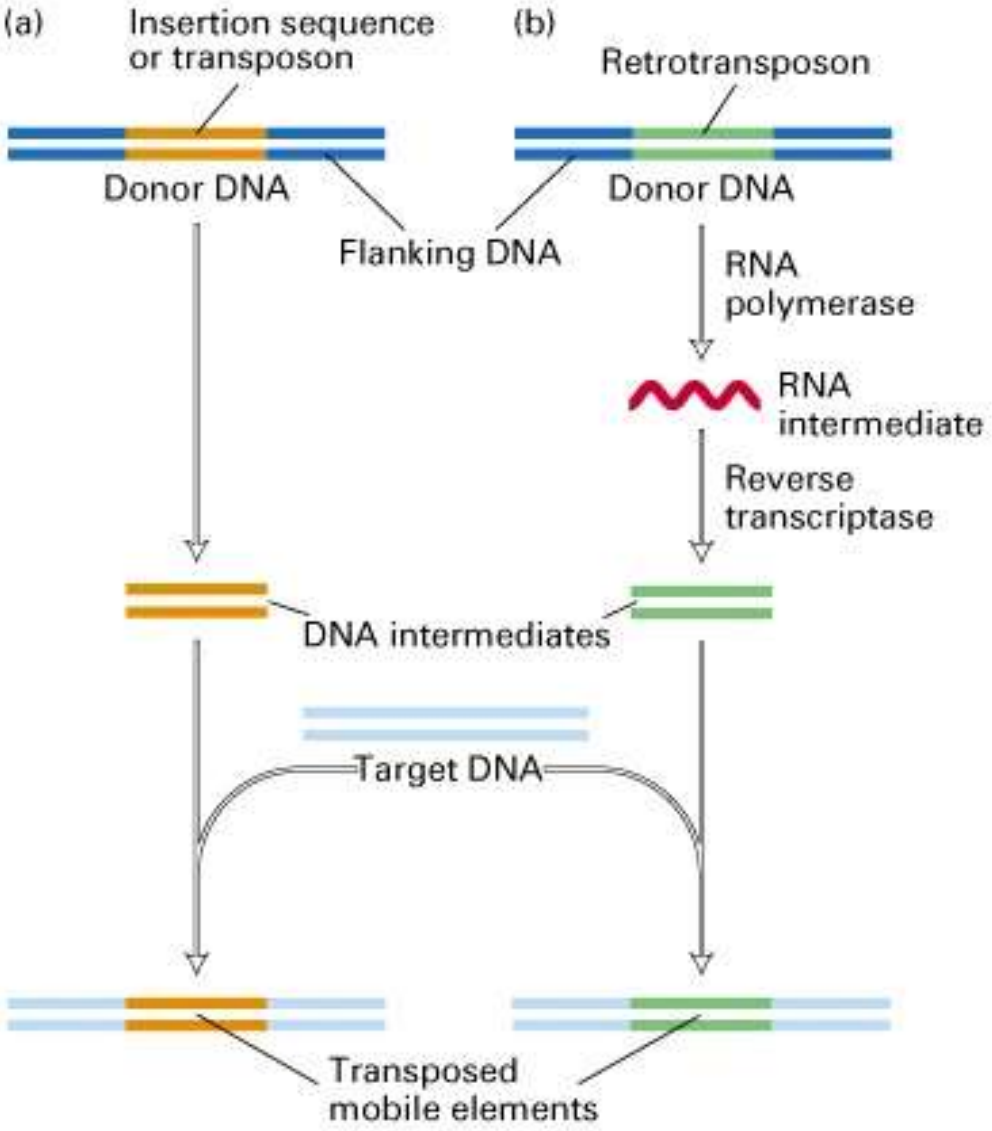
The euchromatic portion of the human genome has a much higher density of transposable element copies than fly and worm

Transposable elements that move by RNA intermediates

- Called **retrotransposons**
- Common in eukaryotic organisms
  - Some have long terminal repeats (LTRs) that regulate expression
    - Yeast *Ty-1*
    - Retroviral proviruses in vertebrates
  - Non-LTR retrotransposons
    - Mammalian LINE repeats (long interspersed repetitive elements, L1s)
    - Similar elements are found even in fungi
    - Mammalian SINE repeats (short interspersed repetitive elements, e.g. human *Alu* repeats)
    - *Drosophila jockey* repeats
    - Processed genes (have lost their introns). Many are pseudogenes.

Some transposons move via RNA Intermediates

- RNA-based transposition involves transcription by RNA polymerase, followed by reverse transcription by RNA-dependent DNA polymerase



Mechanism of retrotransposition

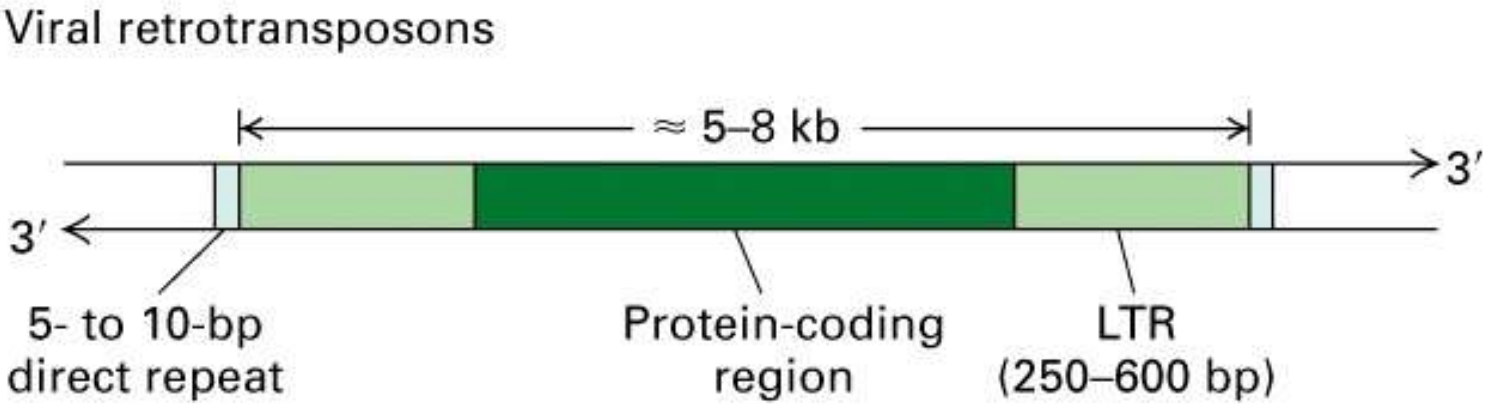
- The RNA encoded by the retrotransposon is copied by reverse transcriptase into DNA
- Primer for this synthesis can be generated by endonucleolytic cleavage at the target
- Both reverse transcriptase and endonuclease are encoded by SOME (not all) retrotransposons
- The 3’ end of the DNA strand at the target that is not used for priming reverse transcriptase can be used to prime 2nd strand synthesis

LTR (long terminal direct repeats) retrotransposons

- The LTR's (long terminal direct repeats) contain all of necessary transcriptional regulatory elements
- The autonomous also contain *gag* and *pol* which encode a protease, reverse transcriptase, RNaseH, and integrase.
- Reverse transcription occurs in the cytoplasm primed by a tRNA (in contrast to nuclear location and chromosomal priming of LINEs)

Many transposable elements (LTR) are structurally similar to retroviral DNA

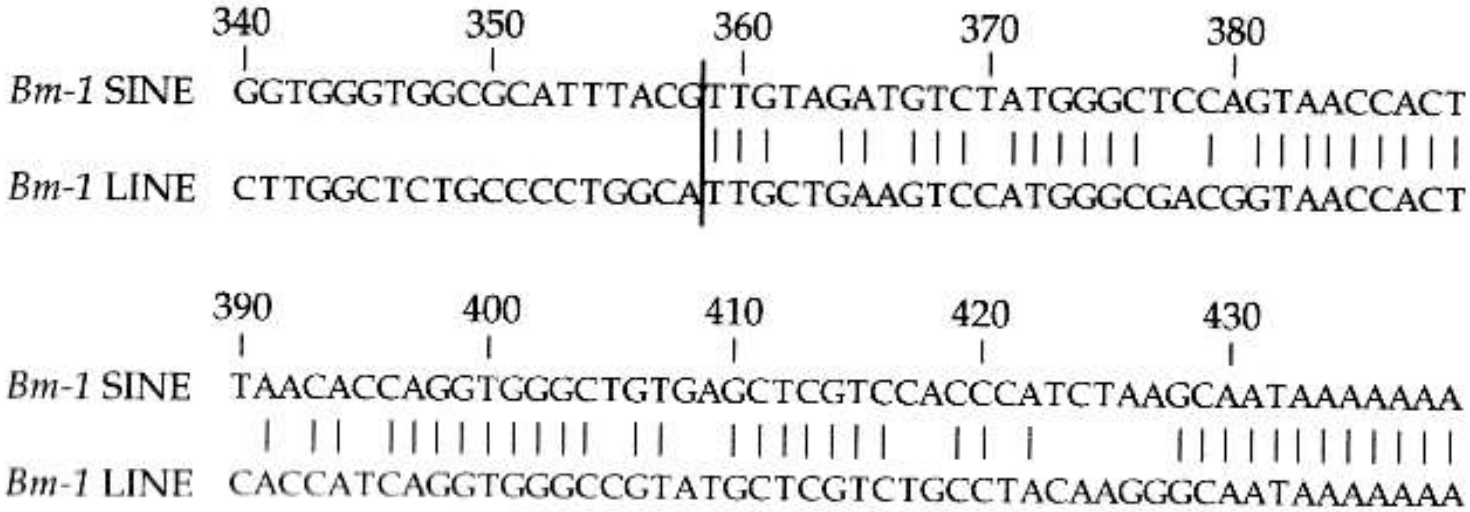
- yeast *Ty* and *Drosophila copia* elements contain *gag*, *pol, int, env*
  - transposition occurs via an RNA intermediate
  - Introns placed inside the retrotransposon by in vitro recombination is missing following transposition



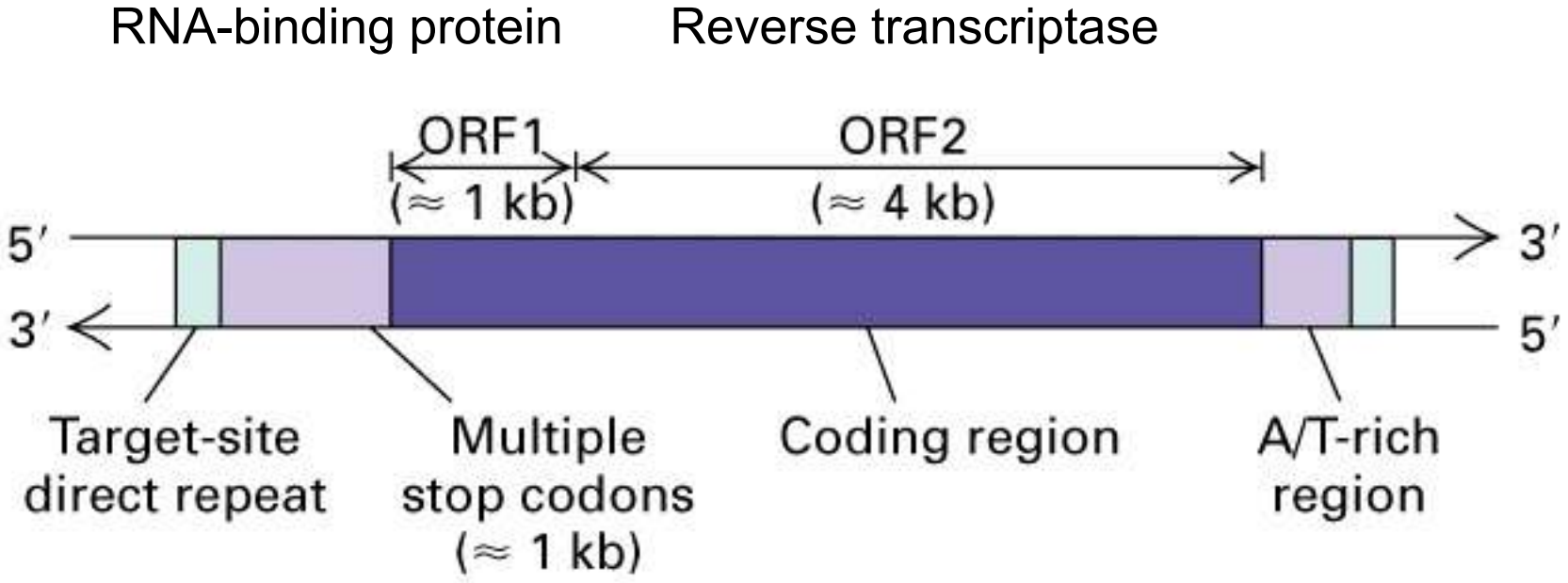
Non-viral retrotransposons

- Lack the long terminal repeats of viral transposons
  - Long interspersed elements (LINES)
    - 6-7 kb long
  - Short interspersed elements (SINES)
    - 300 bp long
- Transpose through RNA intermediates

SINEs are similar to LINEs in the 3’ end



L1 LINE element (nonviral retrotransposon)



- **600,000 copies in the human genome**
- **15% of total genomic DNA**

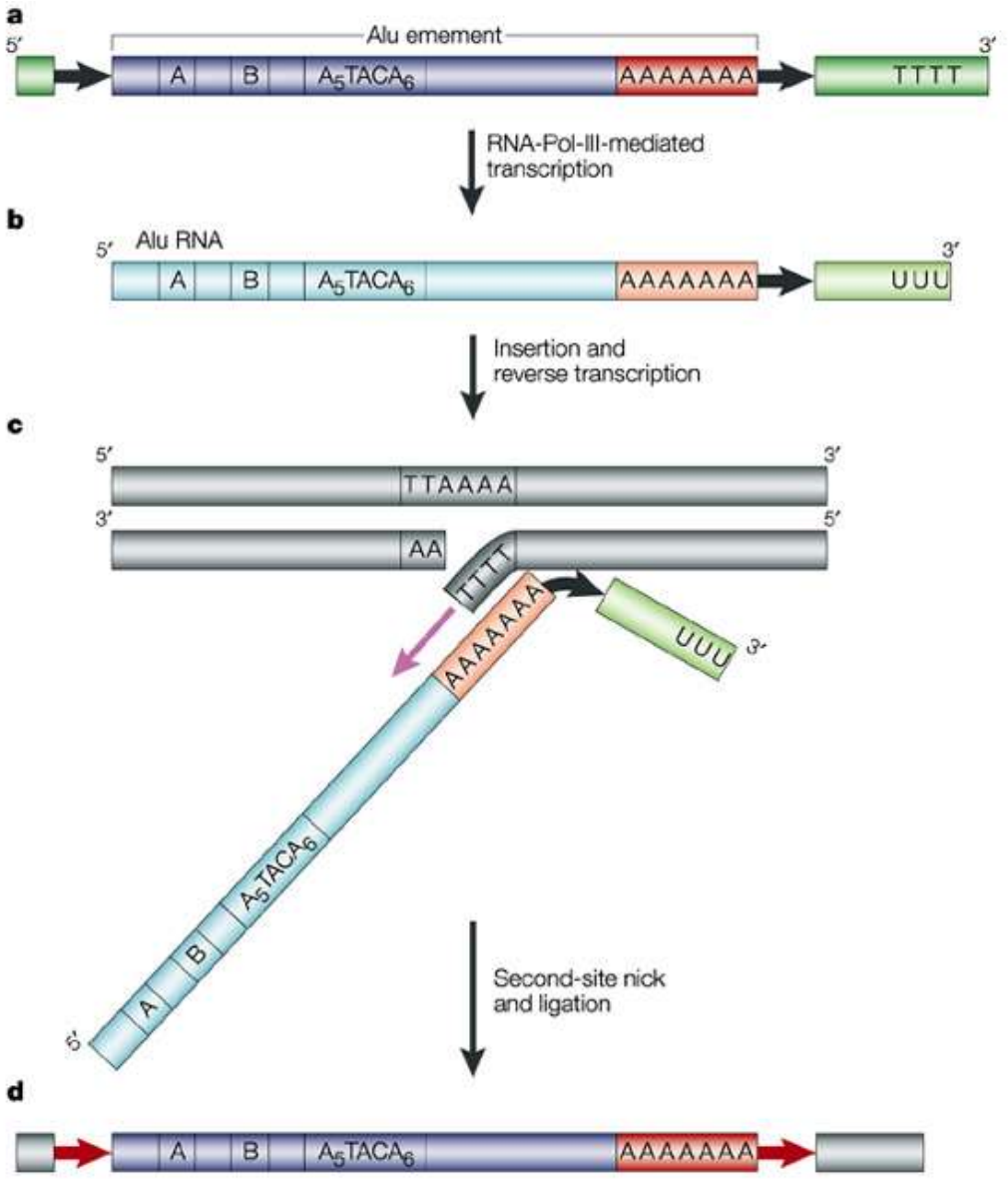
Comparison of LINEs and SINEs

- LINEs are autonomous
- 3 distantly related LINE families are found in the human genome, but only LINE1 is active.
- Human genome: ~515,000 copies of LINE1 (L1), ~365,000 L2, and ~37,000 L3 (most are truncated or rearranged)
  - Only ~30-60 are active
  - In mouse, ~3,000 are active.

- SINEs are short: 100-400 bp
- Promoter regions of all known SINEs are derived from tRNA sequences except for a single family derived from 7SL
- This latter family includes the only active SINE in the human genome: the Alu element.
- SINEs transpose by using the LINE machinery

Alu elements

The structure of each Alu element is bi-partite. The total length of each Alu sequence is 300 bp, depending on the length of the 3' oligo(dA)-rich tail. The elements also contain a central A-rich region and are flanked by short intact direct repeats that are derived from the site of insertion (black arrows). The 5' half of each sequence contains an RNA-polymerase-III promoter (A and B boxes).

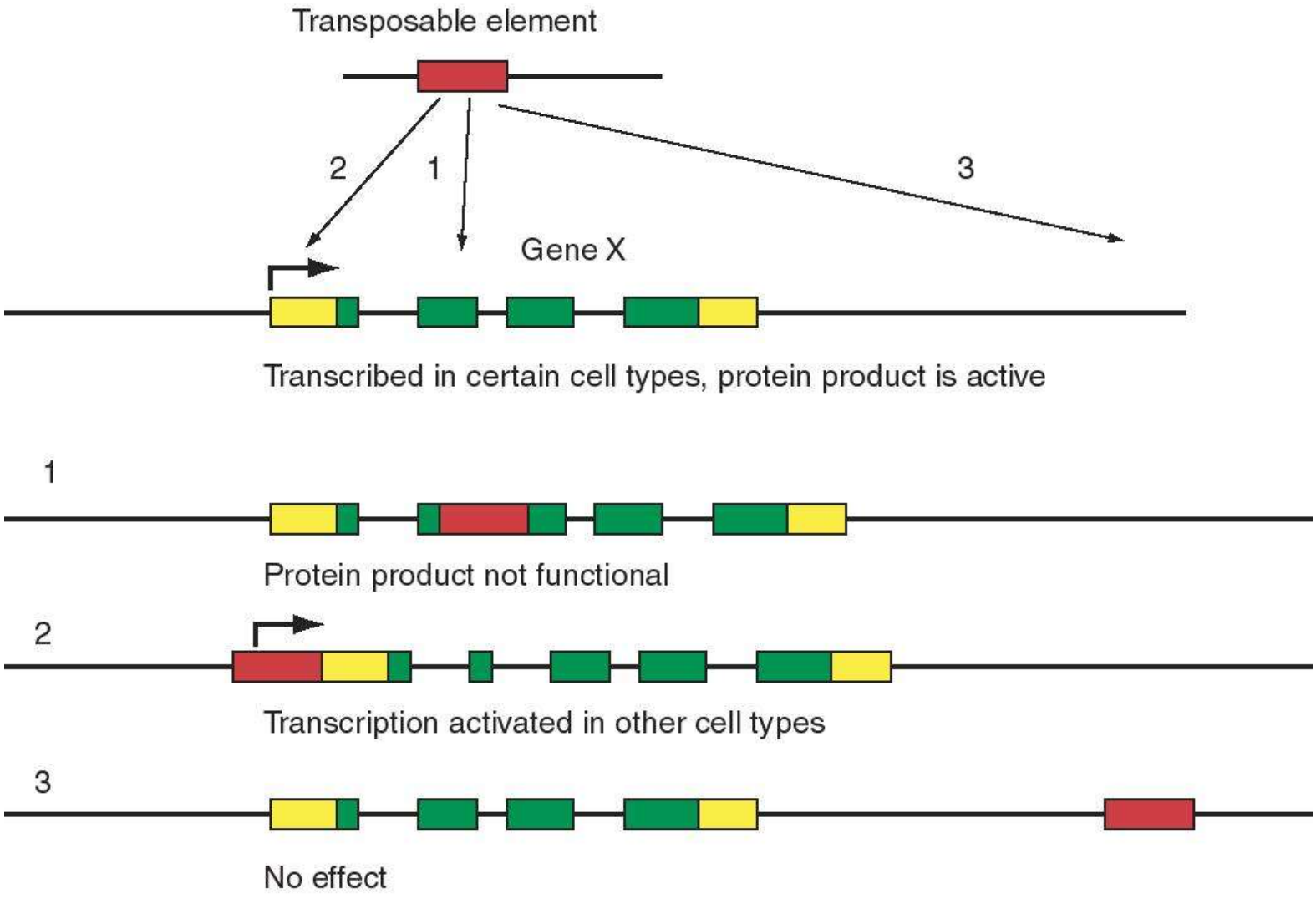


Nature Reviews | Genetics

Mobile Elements Are a Major Factor in Genome Evolution

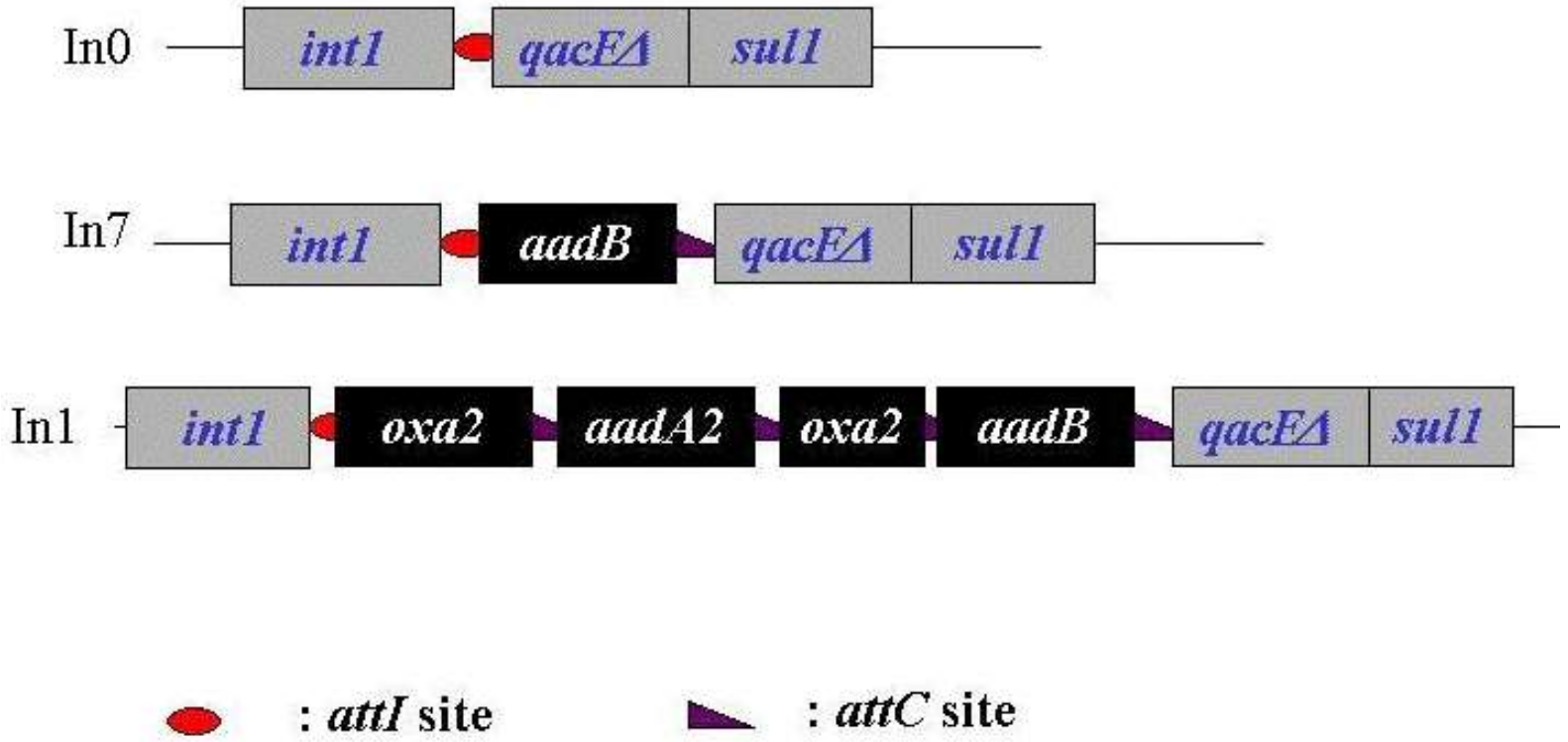
- rearrangement of genomes
- Rearrangement of exons, recombination of coding regions and control regions
- generation of excess amounts of DNA, additional gene copies
- Transposable elements employ different strategies for transfer
  - LINEs and SINEs rely on vertical transmission within the host genome and its progeny.
  - DNA transposons require frequent horizontal transfer
  - LTR retrotransposons use both strategies: some are long term residents of the genome and others only short-residence times.

Effects of transposable elements depends on their location



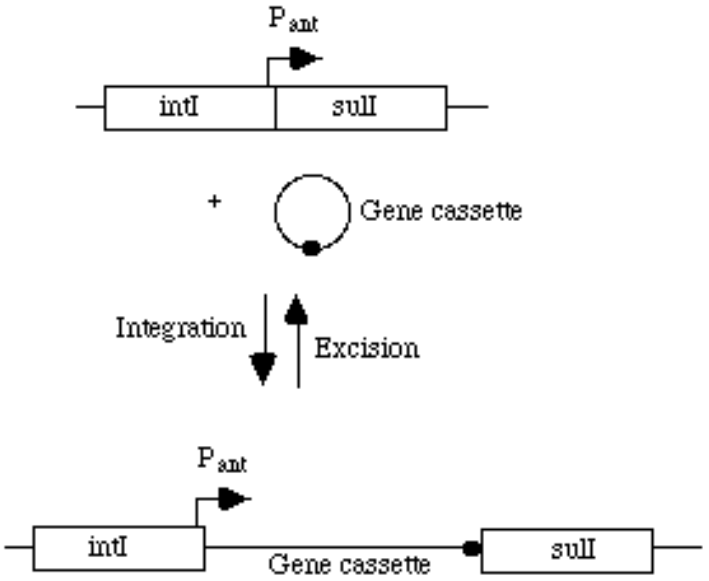
Integrons

- New class of transposable elements which capture (resistance) genes by site specific recombination

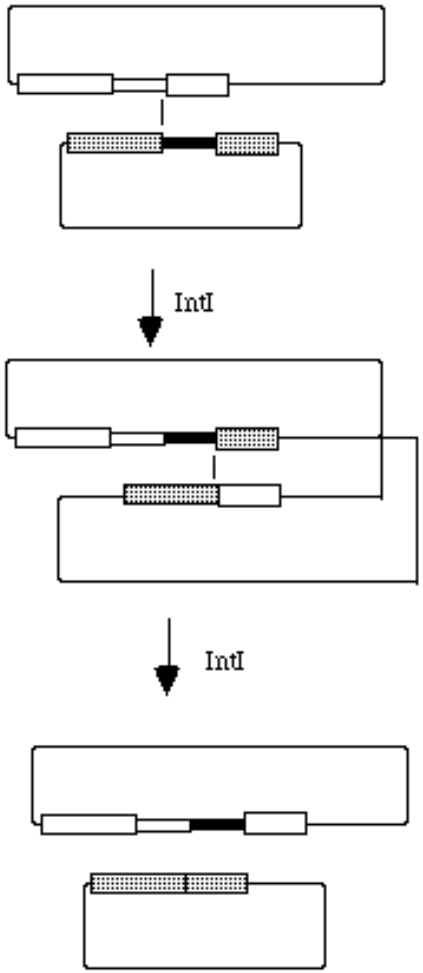


Integrons

- New class of transposable elements which capure (resistance) genes by site specific recombination



Insertion/excision of genes cassettes into integrons



Reciprocal aquisition and loss of a gene cassette by IntI-mediated cointegration and resolution

Viruses

- Viruses are obligate intracellular parasites
  - they only can reproduce with a host cell

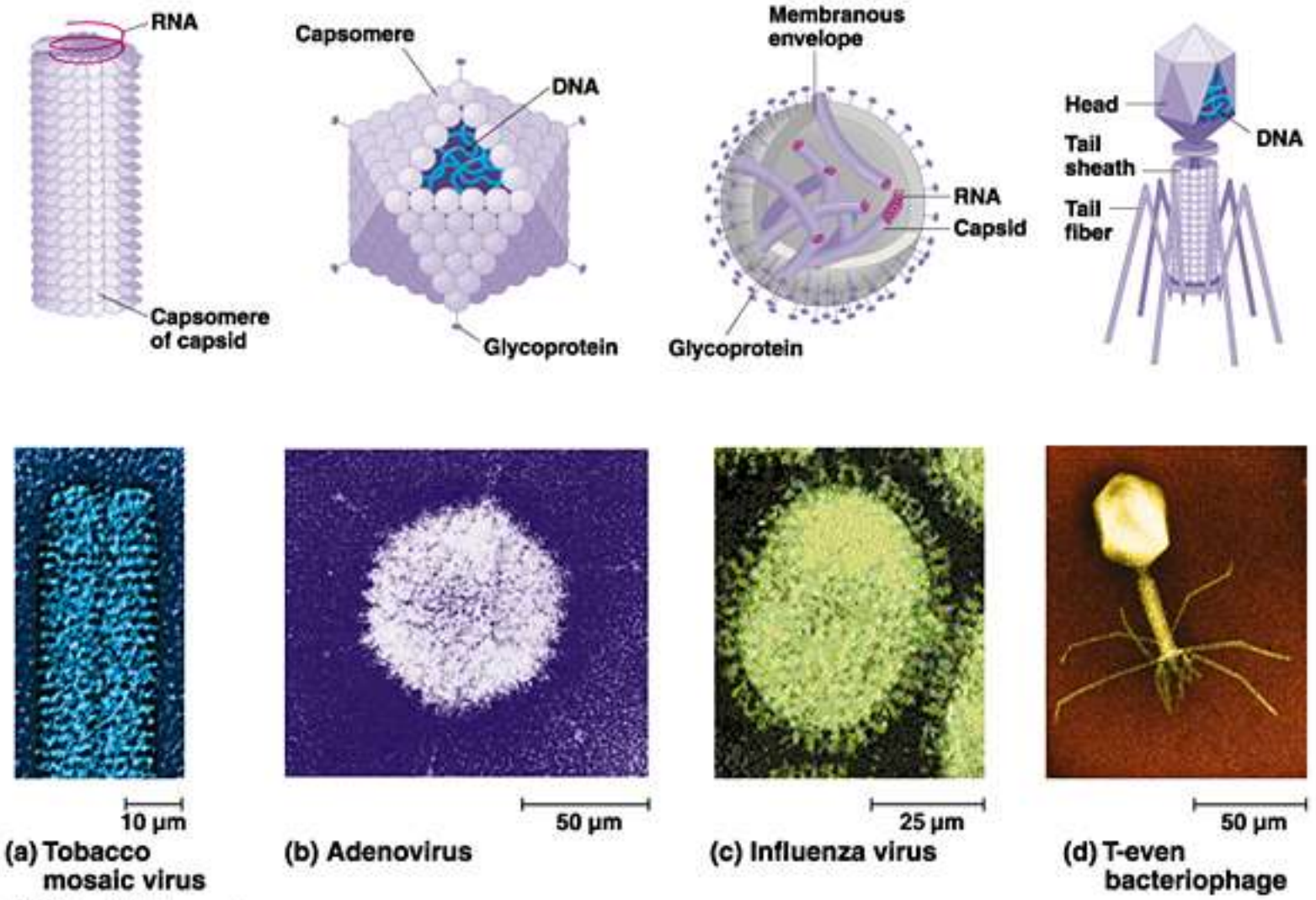
- Viruses lack many prerequisites for "life"
  - no enzymes for metabolism
  - no ribosomes -- absence of *de novo* protein synthesis

- basically, viruses are a set of encapulated genes that can be transmitted between a defined set of host cells.

- some viruses only infect humans whereas others may infect a variety of mammals
  - this is the "host range" of the virus

- viruses which replicate in bacteria are called bacteriophages, or phages for short

Viruses



©1999 Addison Wesley Longman, Inc.

Viruses

- to replicate, a virus must first have a means of placing its genetic material into the host cell

- once its genetic material is in, the host cell's normal metabolic processes are subverted by prexisting or newly synthesized viral proteins

- the host cell, once under viral control, is reprogrammed to manufacture new viral proteins and genetic material and a dramatic rate. The host cell provides all of the raw materials to manufacture new viral particles

    - amino acids
    - nucleotides
    - ATP (for energy)

- viruses are also well equipped to evade host cell defences

Viral genomes

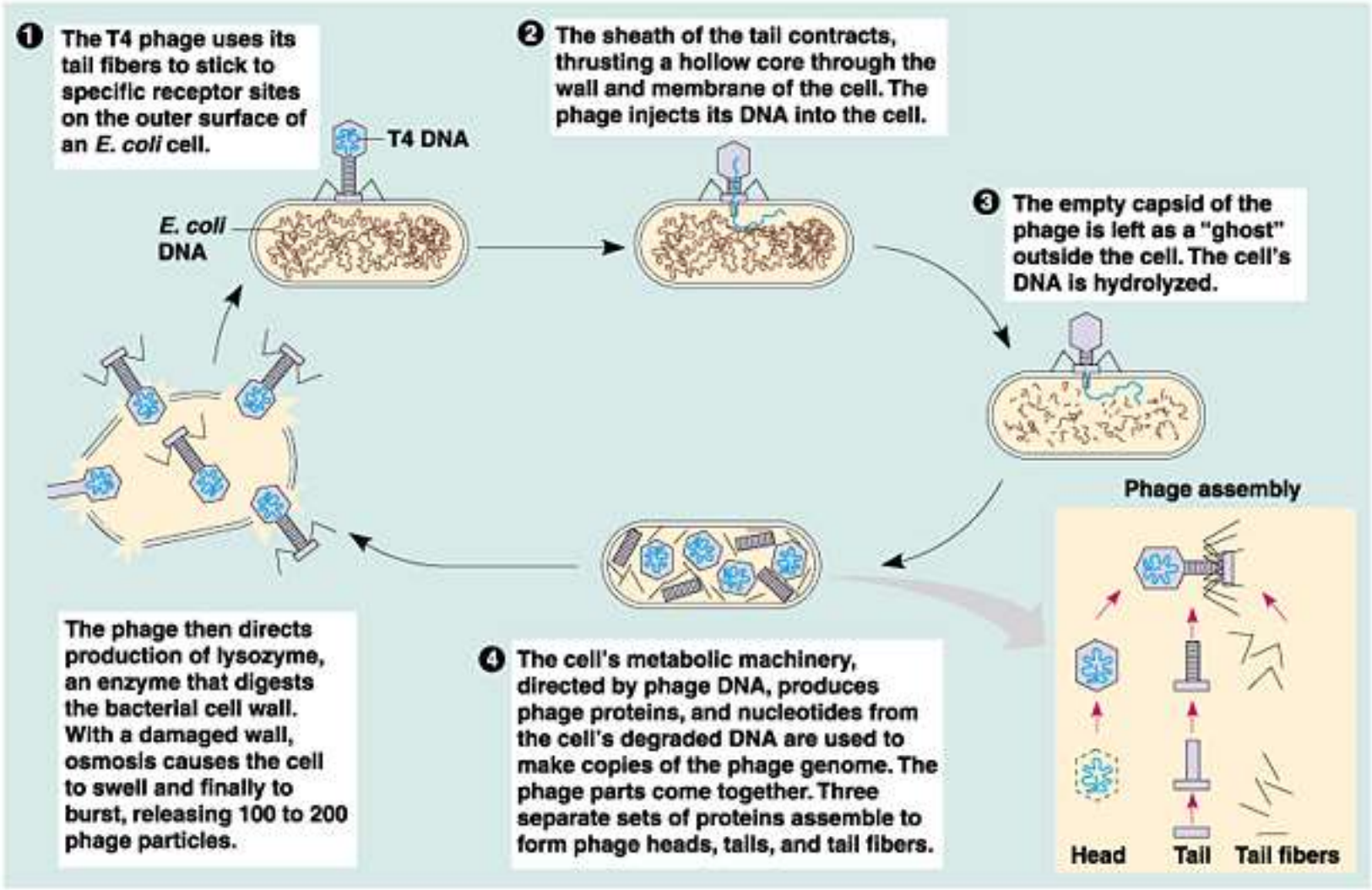
- **Composition & structure of virus genomes (i.e. nucleic acid which encodes the genetic information of the virus) more varied than in any other kingdom.**
- **DNA or RNA (bacterial viruses (bacteriophages - only DNA)**
- **single-stranded, ss; double stranded,ds; linear; circular; segmented.**
- **Ss may be positive (+)sense, i.e. the same polarity (nucleotide sequence) as mRNA**
- **negative (-)sense**
- **ambisense - a mixture of the two.**

Phage	DNA Properties	Host Dependency
M13 & Phi-X174	small ssDNA, only encodes capsid protein & assembly	High
Lambda	48 kb dsDNA, encodes capsid, some replicative proteins	Medium
T4	166 kb dsDNA, encodes 200 genes	Low

Comparison of Multiplication Cycles of Bacteriophage and Animal Viruses

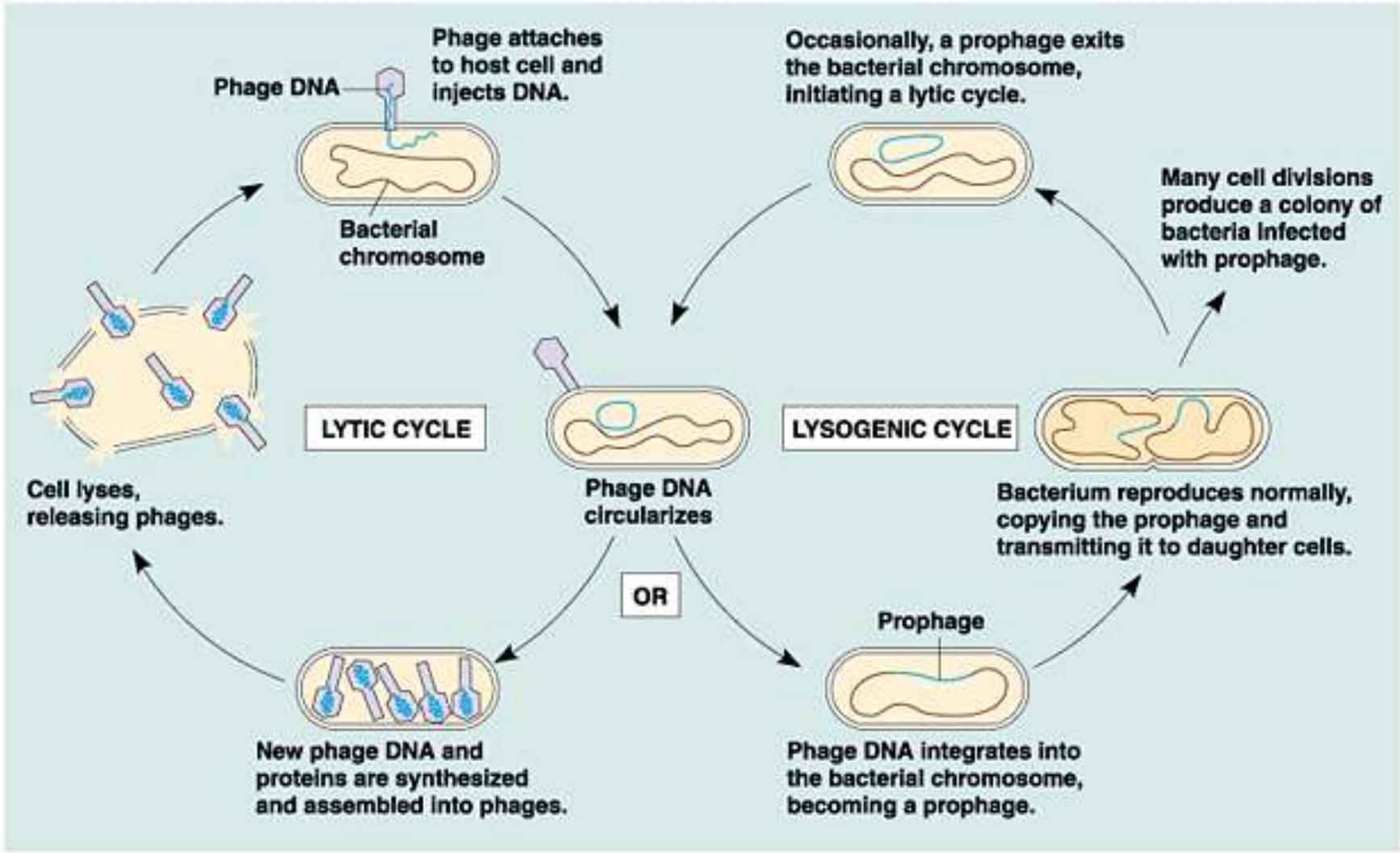
Stage	Bacteriophage	Animal Viruses
Attachment	Tail fibers attach to cell wall proteins	Attachment sites are plasma membrane proteins and glycoproteins
Penetration	Viral DNA injected into host cell	Capsid enters by endocytosis or fusion
Uncoating	Not required	Enzymatic removal of capsid proteins
Biosynthesis (Eclipse)	In cytoplasm	In nucleus (DNA viruses) or cytoplasm (RNA viruses)
Chronic infection	Lysogeny	Latency; slow viral infections; cancer
Release	Host cell lysed	Enveloped viruses bud out; nonenveloped viruses rupture plasma membrane

Reproduction of a bacteriophage



©1999 Addison Wesley Longman, Inc.

Lytic and Lysogenic Stages of a Viral Infection in Bacteria



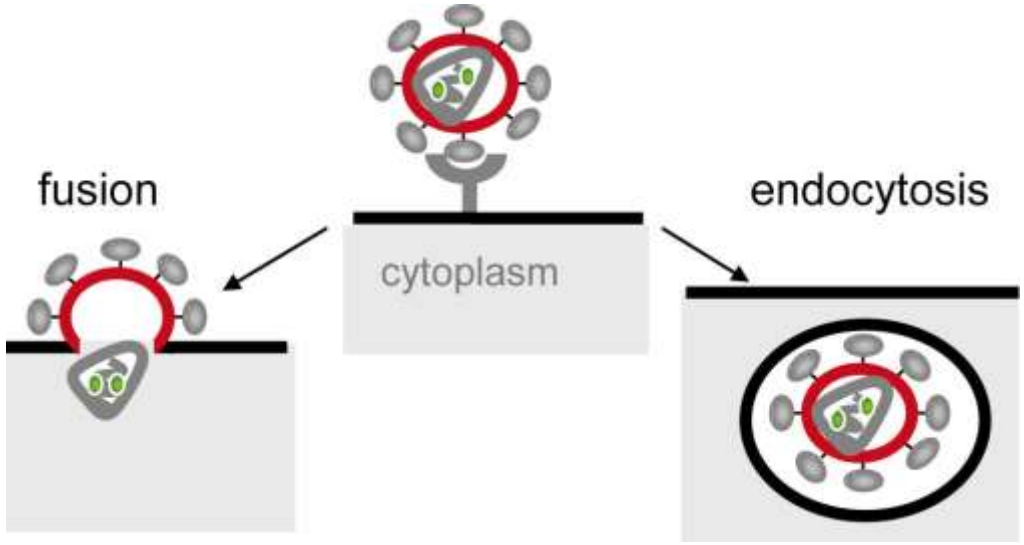
©1999 Addison Wesley Longman, Inc.

Entry of Viruses into Animal Cells

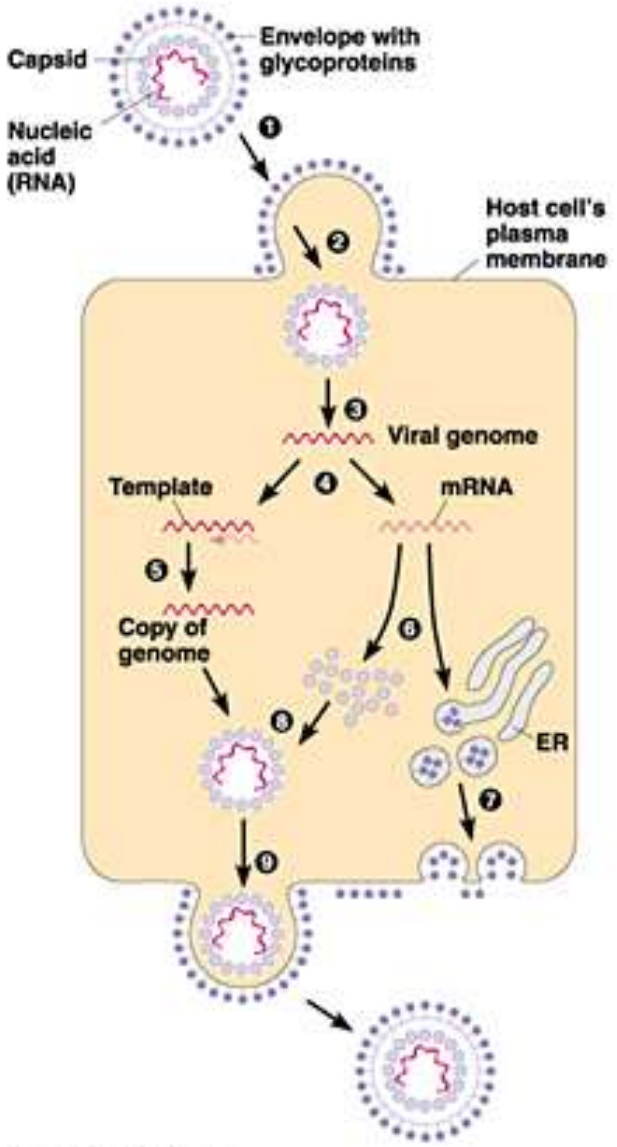
Not completely understood, but appears to be 3 methods:

- Direct penetration of naked virus *(viral genome enters cell, while capsid remains on cell's surface.)*
- Membrane fusion
- Endocytosis

  With membrane fusion and endoocytosis, the capsid is removed once inside the host cell.



Animal Cell Infection by an Enveloped Virus



©1999 Addison Wesley Longman, Inc.

Step 1:
The glycoproteins projecting from the viral envelope are recognized by cellular receptor proteins

Step 2:
The envelope dissolves and the capside/nucleic acid enters the host cell

Step 3:
Cellular enzymes remove the capsid into its components

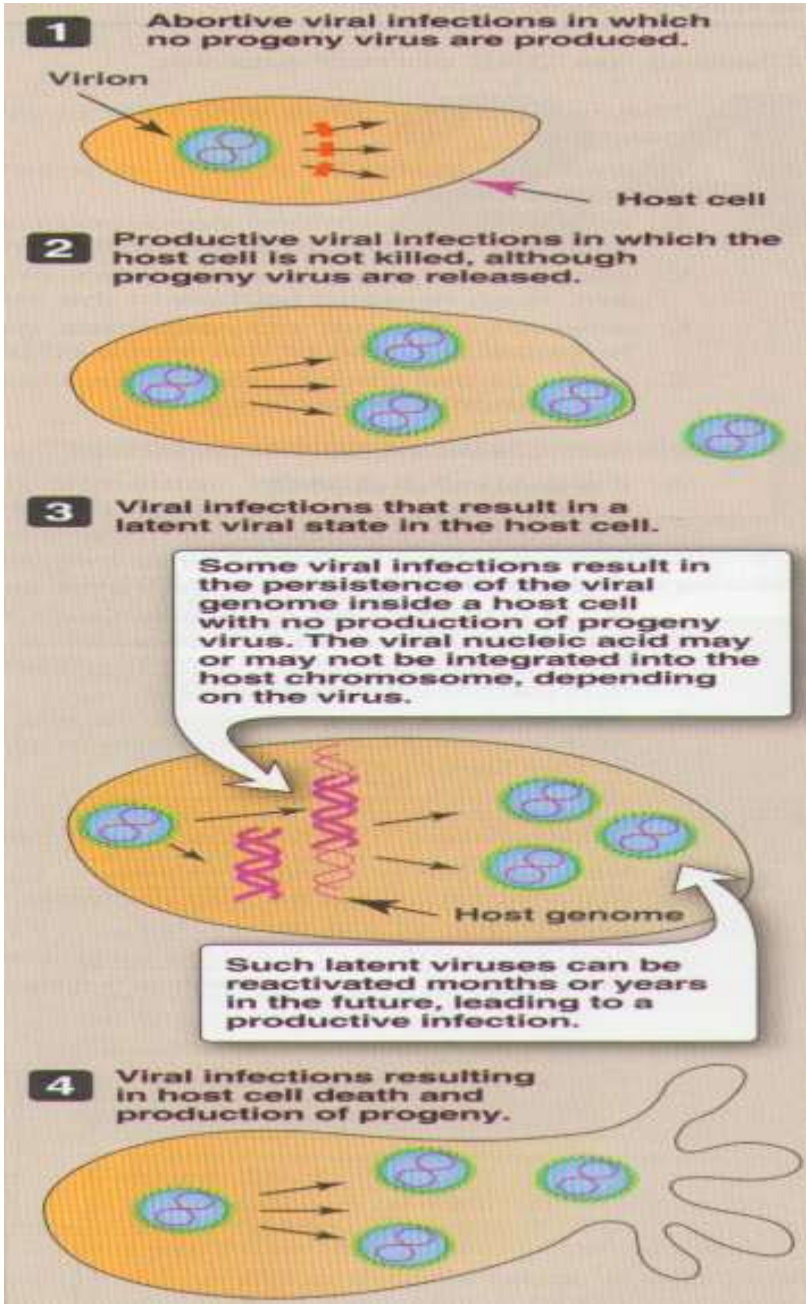
Steps 4 and 5:
New mRNA is transcribed by the host cell machinery

Step 6:
The mRNA is translated into a new viral proteins

Step 7:
The host cell also manufactures the glycoproteins which are transported to the cellular periphery

Steps 8 and 9:
The viral capsid reforms and the virus buds from the host

Effects of viral infection on a host cell

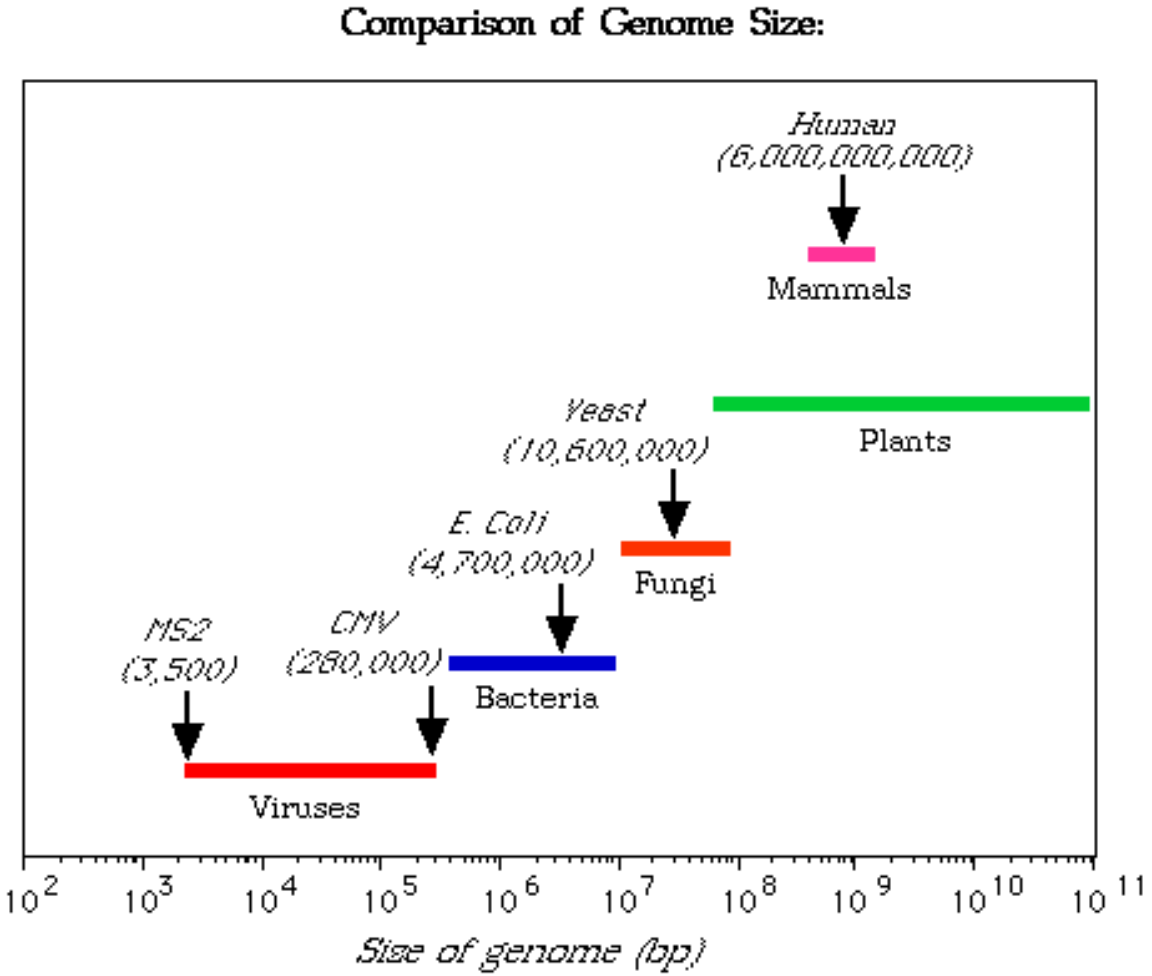


The Genetic Material Used by Viruses Comes in Many Forms

Class 1 — dsDNA viruses	
Papoavirus	cause papillomas (warts), cervical cancer, polyoma (tumours)
Adenovirus	respiratory diseases, tumours, can be a useful vector
Herpesvirus	cold sores (HSV-1), shingles, EBV induced malaise
Poxvirus	smallpox, cowpox
Class 2 — ssDNA	
Parvovirus	roseola (dependent on superinfection by other viruses)
Class 3 — dsRNA	
Reovirus	diarrhea, respiratory diseases
Class 4 — ssRNA serving as mRNA	
Picornavirus	polio, common cold, GI diseases
Togavirus	rubella, encepalitis, yellow fever
Coronavirus	SARS
Class 5 — ssRNA serving as template for mRNA	
Rhabdovirus	rabies
Paramxyovirus	measles, mumps
Orthomyxovirus	influenza
Class 6 — ssRNA serving as a template for DNA synthesis	
Retrovirus	leukemia, AIDS

Viral genomes

Virus genomes range in size from about 3,500 nucleotides (nt) e.g. bacteriophages of the family Leviviridae such as MS2) to about 280 kilobase pairs (kbp) e.g. the family Herpesviridae



Viral genomes

- RNA genomes are smaller. 30Kb maximum size. Corona viruses. Why? Viral RNA polymerases are error prone compared to DNA polymerases. Perhaps replication fidelity limits size. Often replicate in cytoplasm.
- DNA genomes? Up to 300kb. No necessity for a bigger size? Assembly, packaging problems? Perhaps bigger genomes do exist. Usually replicate in the nucleus.
- Genome type and size dictates replication.
- Very condensed genomes
- Bewildering number of strategies to maximise gene coding capacity, including:
- Overlapping genes; using different reading frames reading both strands

Viral genomes - constraints

- **The key event of viral replication is the synthesis of viral proteins by the host cell.**
- **The virus must present mRNA that the cell can translate and present its genome for packaging by viral proteins.**
- **Control signals must be appropriate to the host.**
- **Constraints for an RNA genome?**

  RNA made in nucleus. Template encoded RNA not made in cytoplasm. Cells cannot make DNA or RNA from an RNA template. So cannot replicate in nucleus or cytoplasm without other viral factors.
- **Constraints for a DNA genome?**

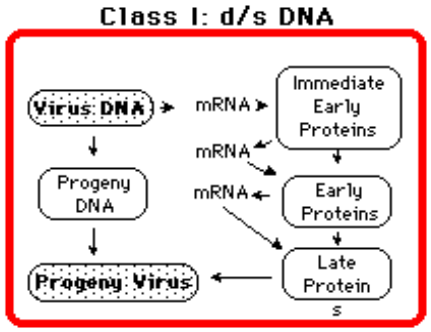
  Enzymes replicating and transcribing DNA are present in nucleus. So either must get the DNA genome to the nucleus or make their own transcriptases.

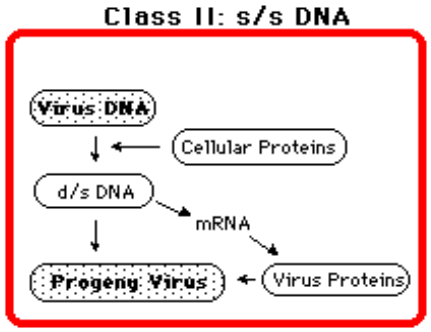
Generally eukaryotic cells only translates monocistronic messages because internal initiation sites are not recognised. Virus must therefore

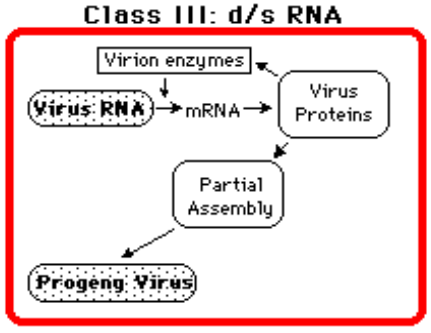
- **make a polyprotein which can be cleaved,**
- **or have a different message for each protein**
- **or make sure that internal initiation sites can be used.**

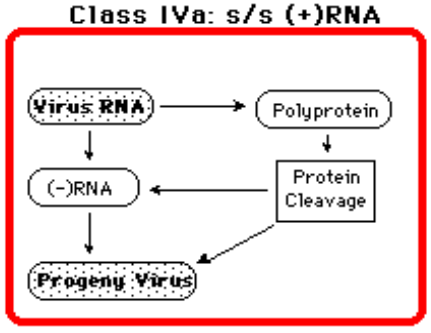
Summary

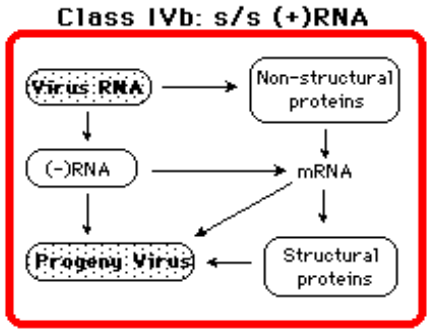
- DNA or RNA
- Ss, ds, linear, circular
- 3.5kb-300kb
- Positive, negative, ambisense
- Monopartite, multipartite, segmented.
- Examples.
- Host dictates gene expression strategy
- Genome type dictates replication strategy
- Genome sizes minimised, coding diversity maximised in a variety of ways.

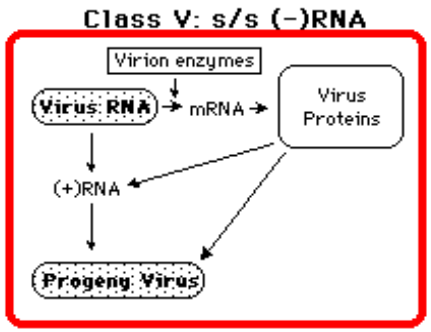


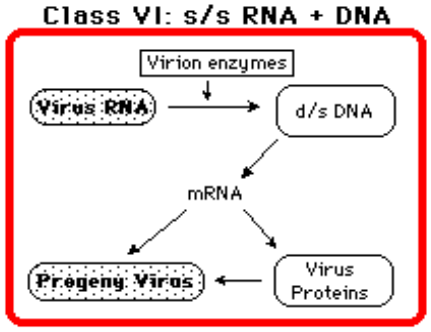


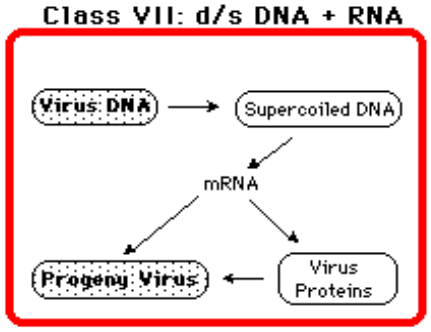








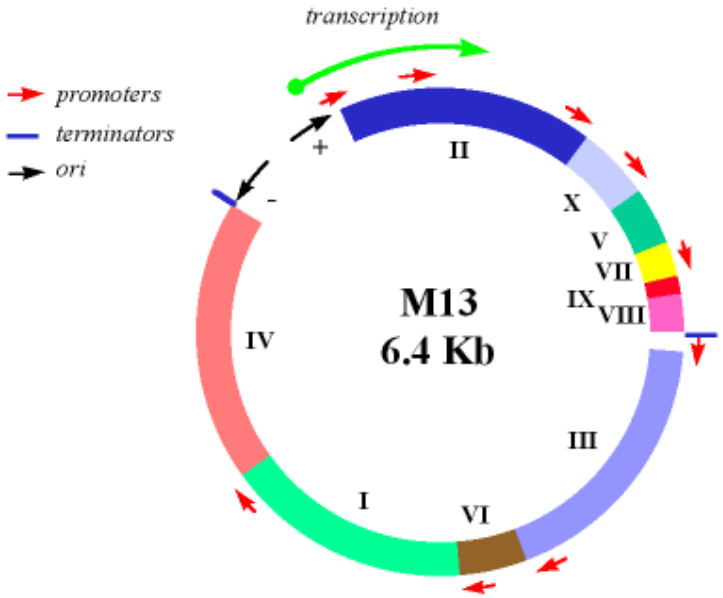


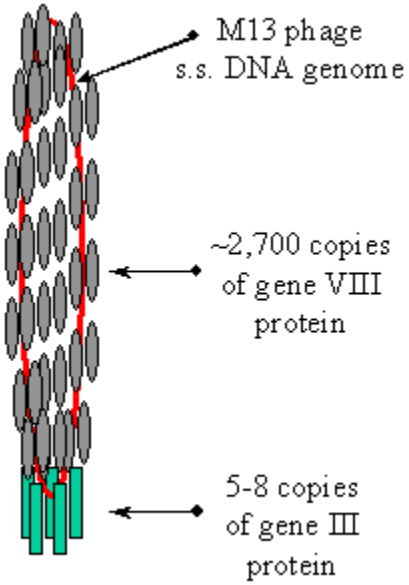


Overview of viral genomes

M13 phage

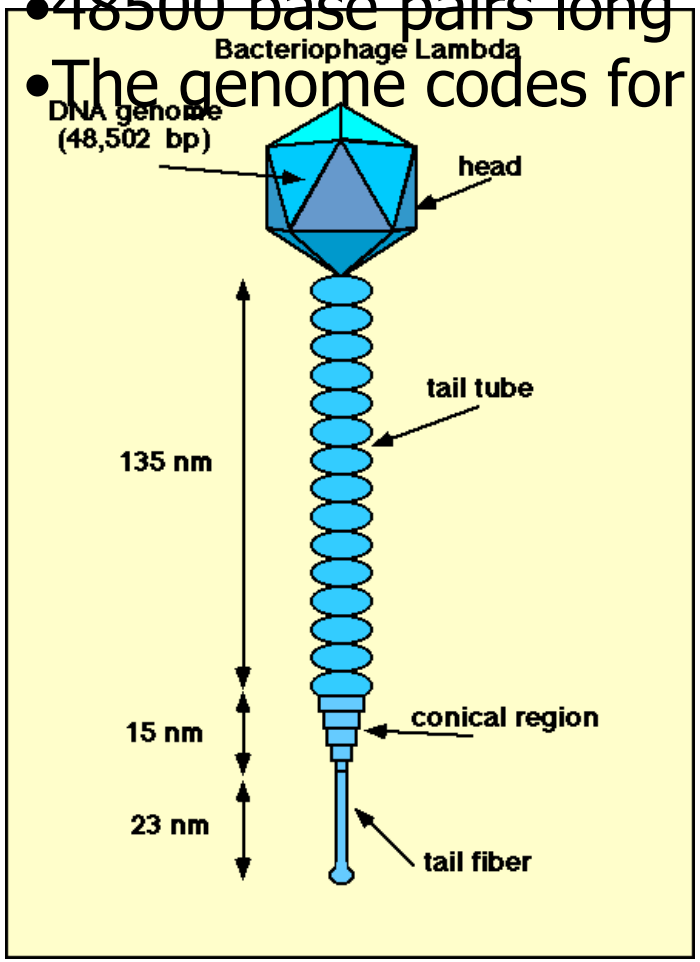
- Circular single-stranded DNA
- 6400 base pairs long
- The genome codes for a total of 10 genes

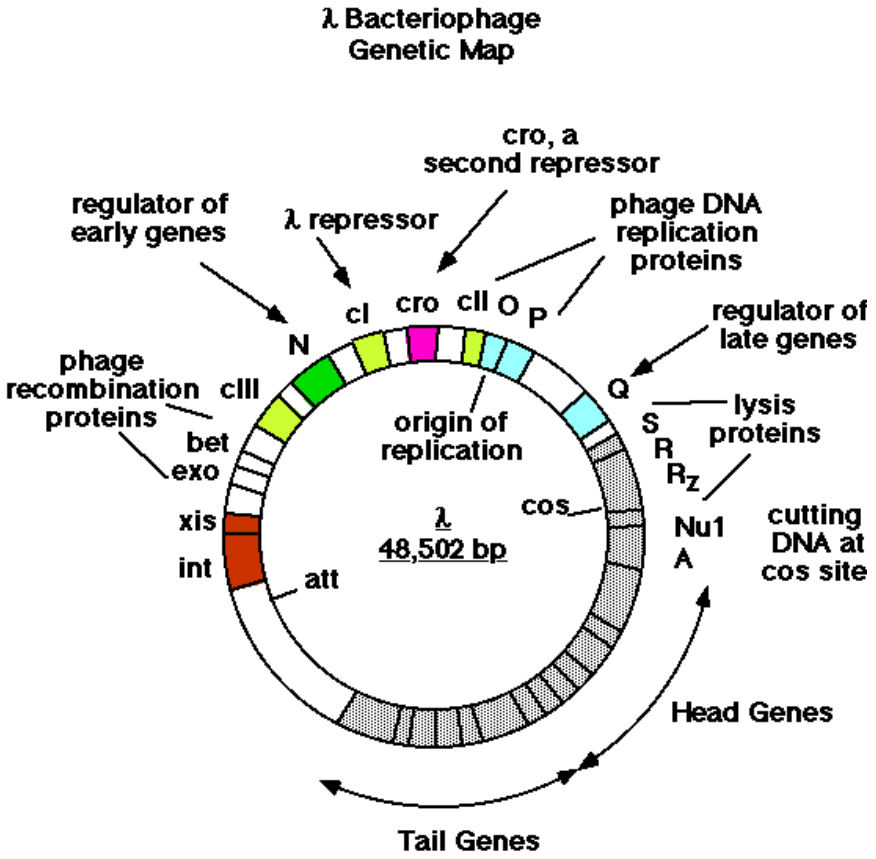




Lambda phage

- Linear double-stranded DNA (cohesive ends)
- 48500 base pairs long
- The genome codes for more than 40 genes

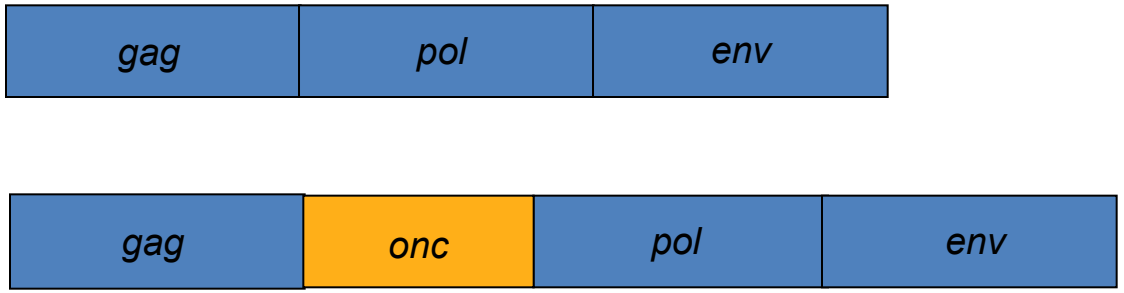




Some Viruses Can Cause Cancer

First "transforming" virus discovered by Peyton Rous in 1911

- **called Rous Sarcoma Virus**
- **caused leukemia and tumours in chickens**



Altered cellular protein which is now part of the viral genome — an oncogene

In humans:

- **herpesvirus infection**
- **HTLV-1 — leukemia**
- **papillomavirus (HPV-xx) — epithelial papillomae**

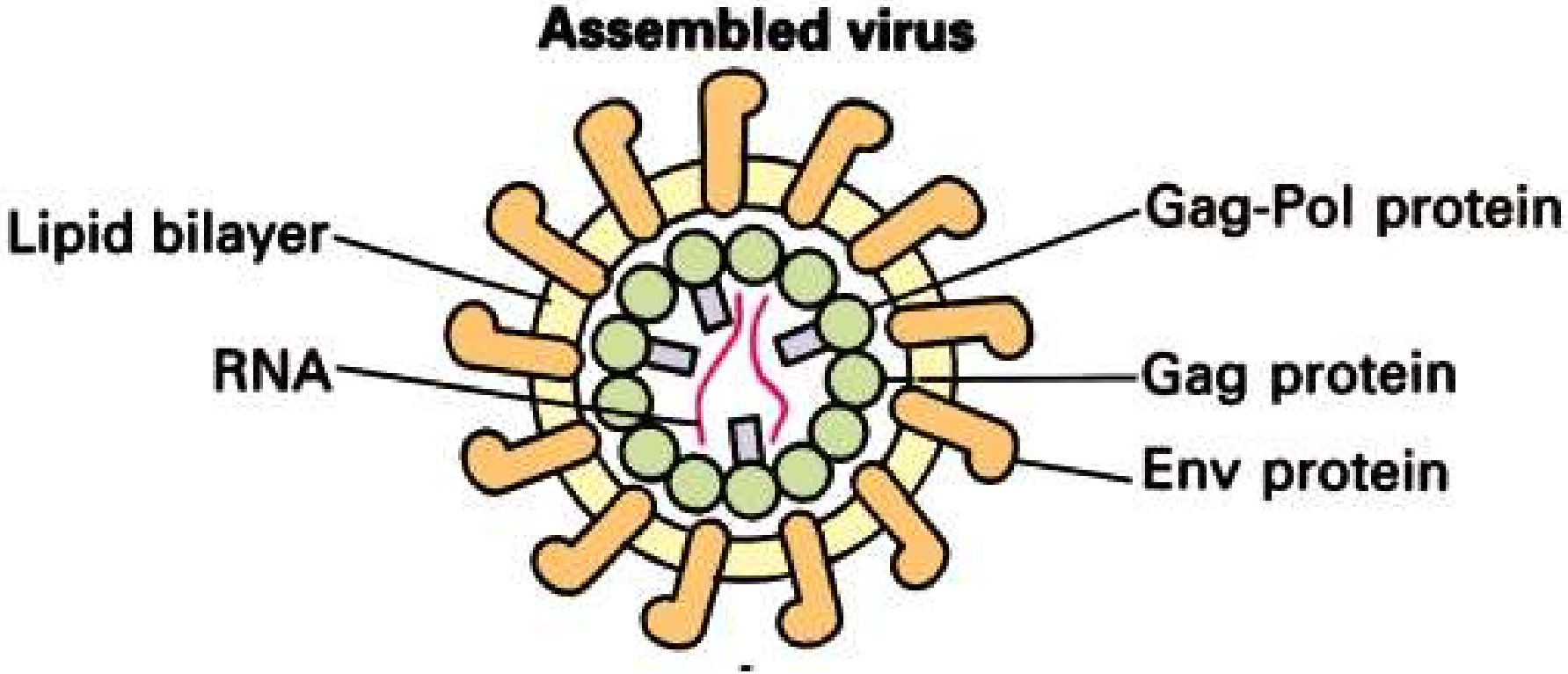
Oncogenic DNA Viruses

Virus	Disease	Cancer
Papovaviridae *Papillomavirus* (some)	Warts, including STD genital warts	Uterine (cervical) cancer
Herpesviridae *Lymphocryptovirus* (Epstein-Barr virus)	Infectious mononucleosis	Burkitt's lymphoma Nasopharyngeal carcinoma ??Hodgkin's disease
Hepadnavirus Hepatitis B virus(HBV)	Hepatitis B (infectious hepatitis)	Liver cancer
Adenoviridae	Acute respiratory disease; Common cold	Adenocarcinomas (cancer of glandular epithelial tissues)
Poxviridae	Smallpox; cowpox	Miscellaneous

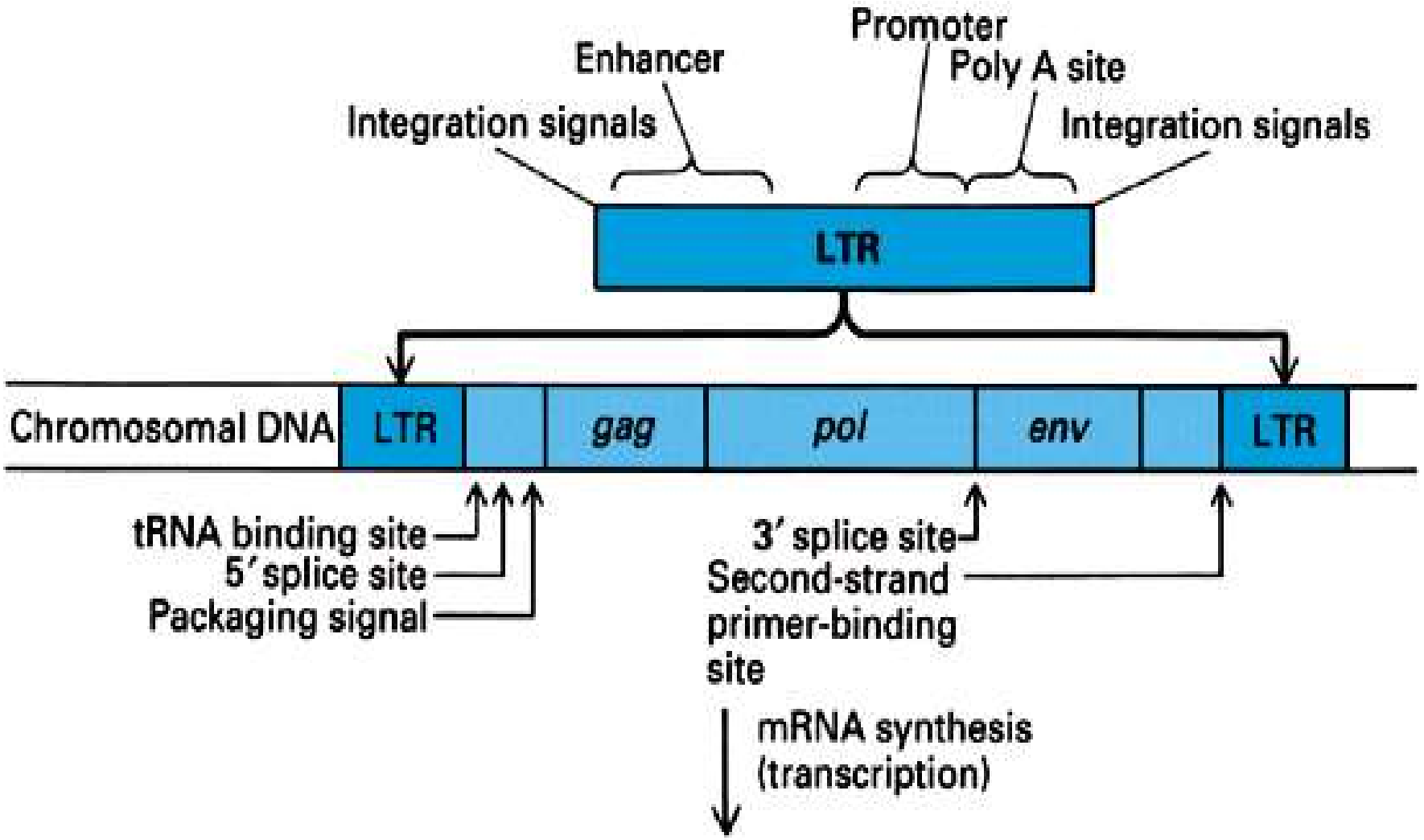
Main features of retroviruses

- integrate into the host genome via *integrase*
- RNA polymerase II makes the genomic RNA strand
- integration into the host genome occurs at random sites
- very strong promoter located within the left LTR

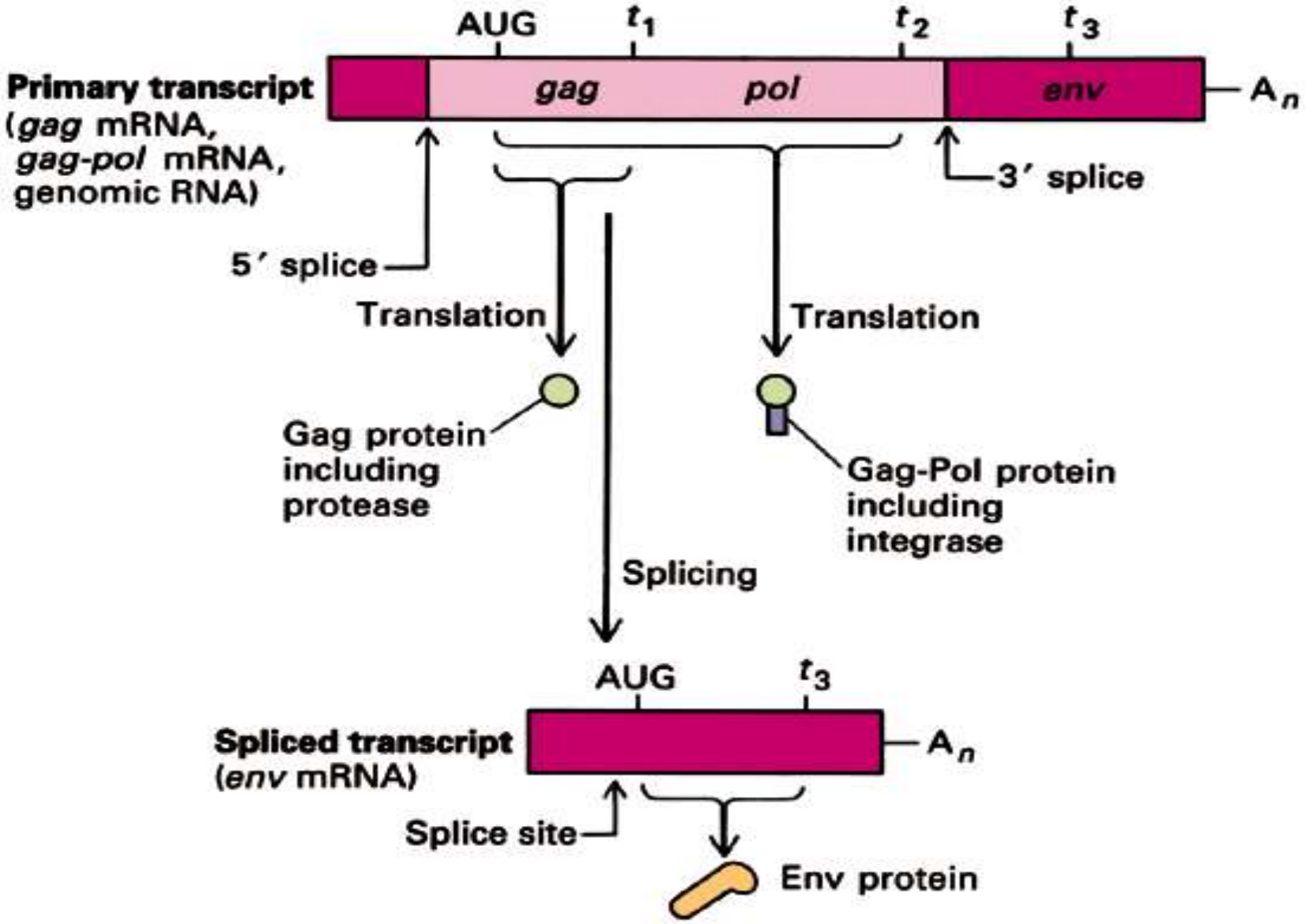
General Structure of an Enveloped Retrovirus

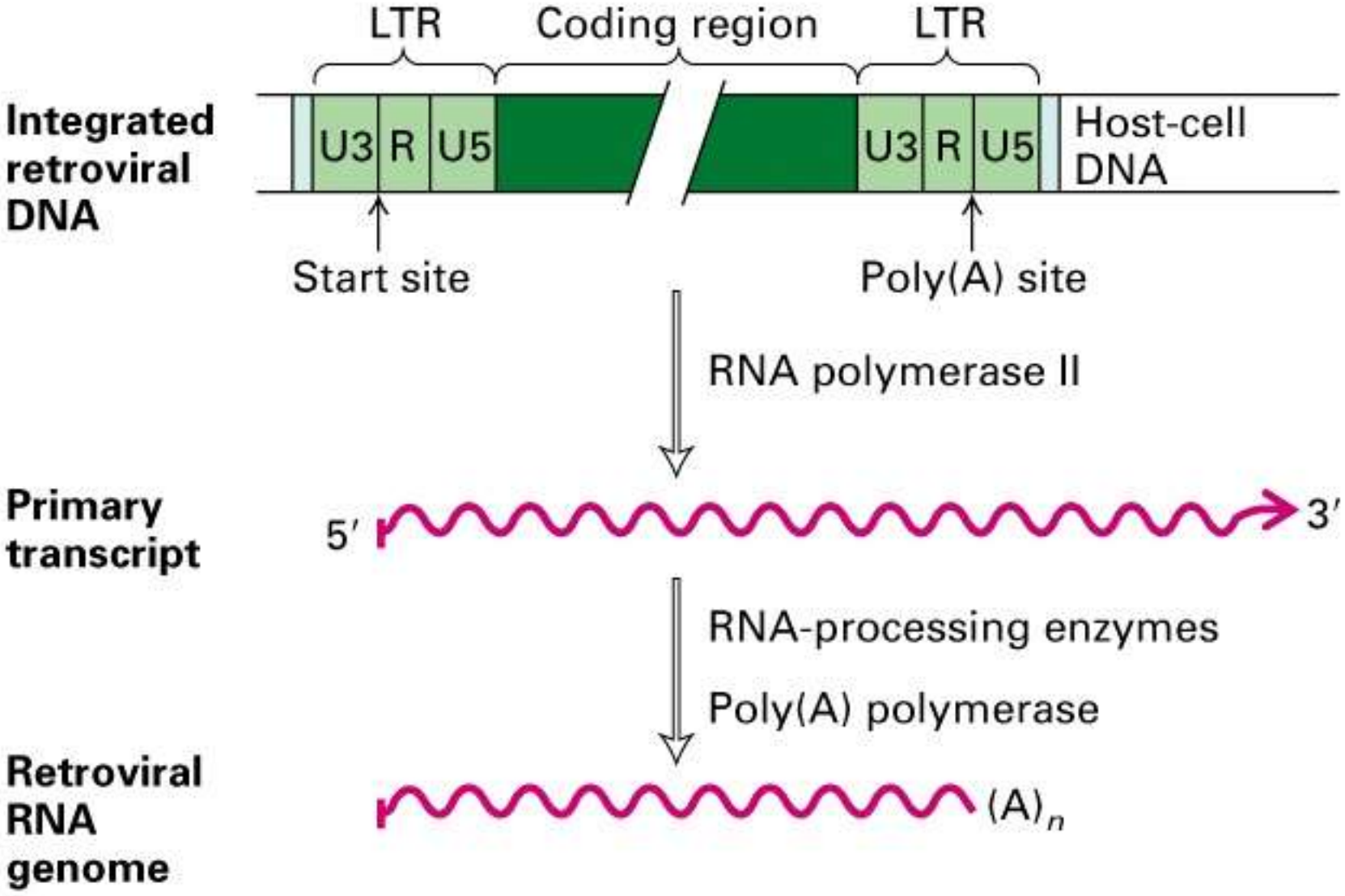


Retroviral genome organization

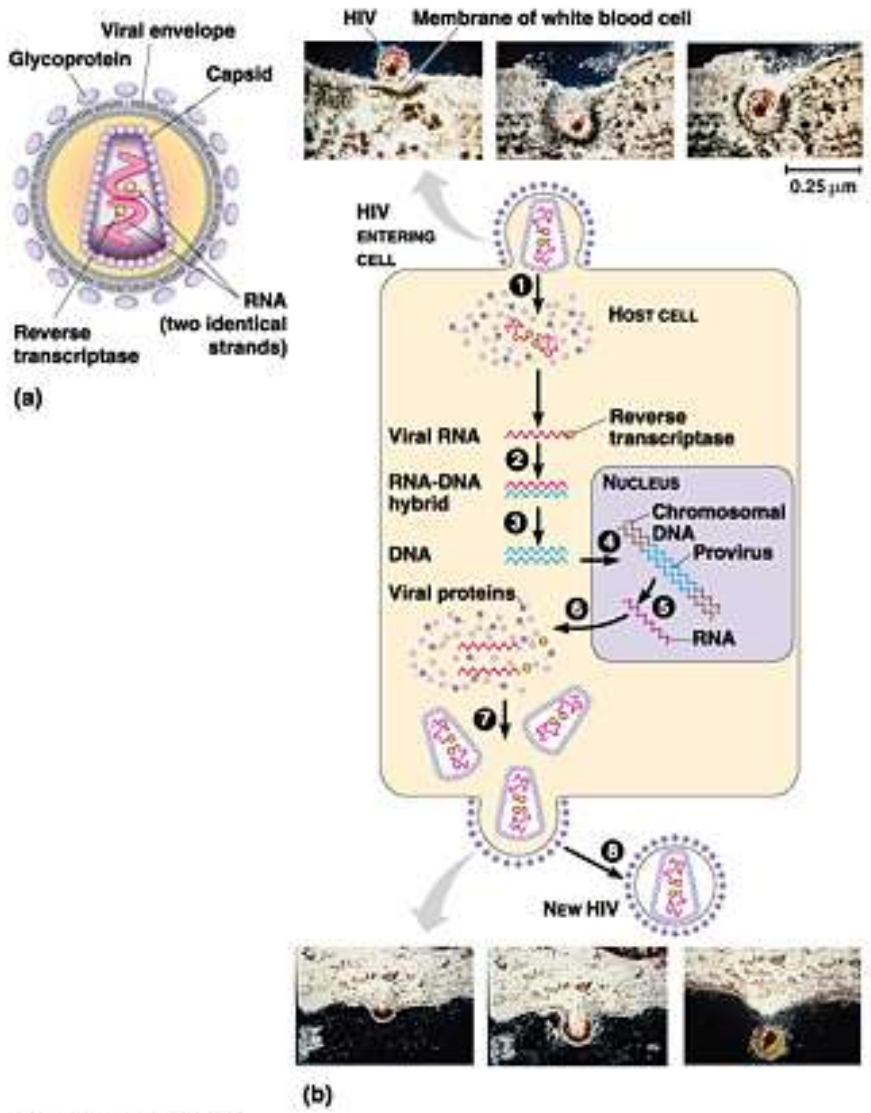


Synthesis of retroviral proteins




LTR
Coding region
LTR
Integrated retroviral DNA
U3
R
U5
U3
R
U5
Host-cell DNA
Start site
Poly(A) site
RNA polymerase II
Primary transcript
5′
3′
RNA-processing enzymes
Poly(A) polymerase
Retroviral RNA genome
(A)n

HIV reproduction



Step 1:
The genome enters a special T-cell variant through two cytokine receptors

Steps 2 and 3:
The endogenous reverse transcriptase converts the viral RNA genome into a DNA genomes. Cellular polymerase make a second DNA strand from the RNA/DNA hybrid

Step 4:
Special sequences and an integrase enzyme allow the viral dsDNA to incorporate into the host genome

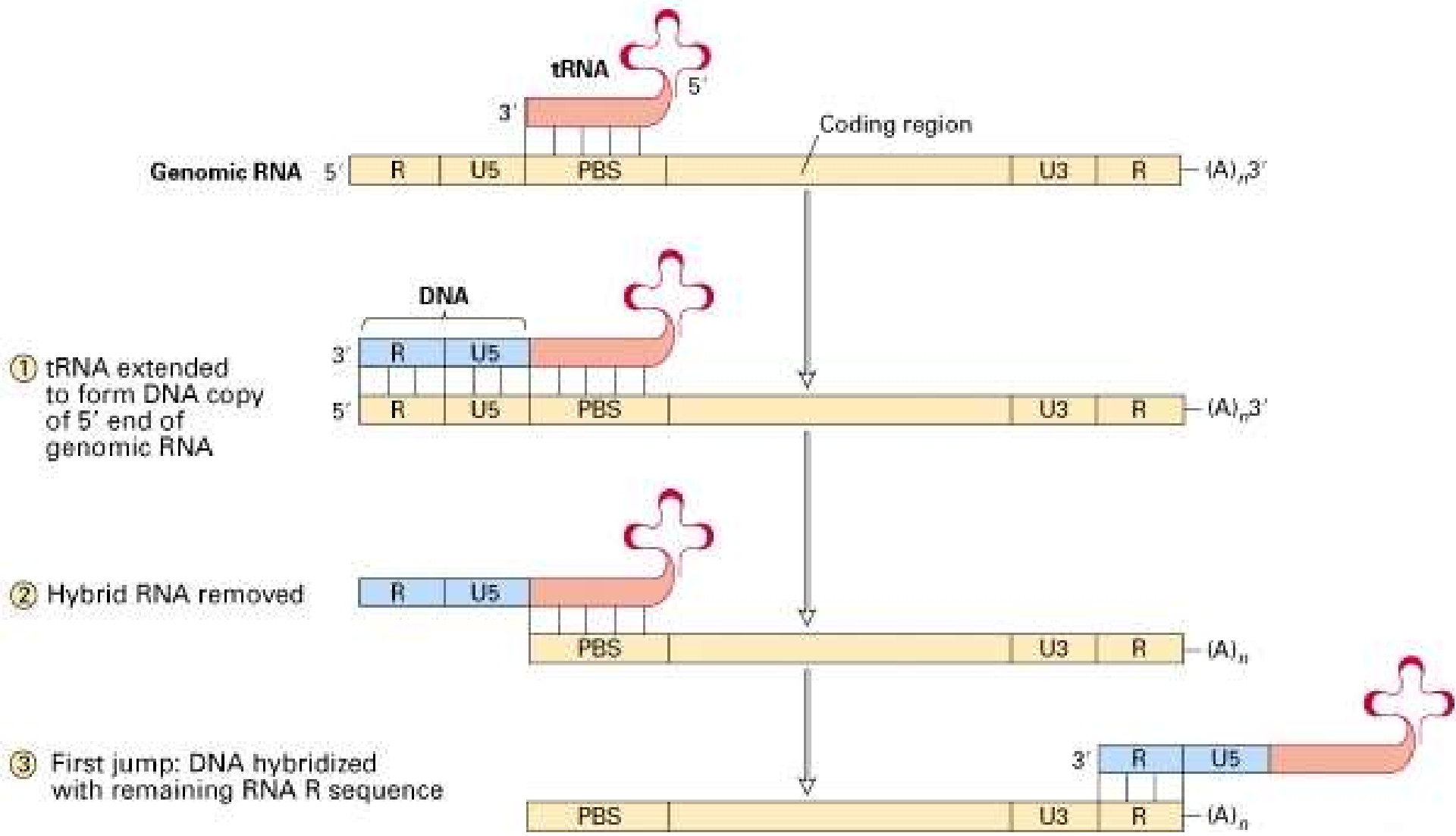
Steps 5 and 6:
From the viral genome, new viral protein components are made

Step 7 and 8:
At a right time, the capsid reforms and the viral ssRNA genome is reincorporated. New virus buds from the infected cell

HIV replication

- Reverse transcriptase copies ssRNA into DNA (RNA-dependent DNA polymerase)
- Primed by host-derived tRNA molecule
- polymerization from 3’ to 5’
- no proofreading capability = low fidelity or precision, HIV genes undergo a high rate of mutation
- RNAse H destroys the RNA template strand
- several “jumps” of the primed region are necessary to generate the promoter region
- terminal repeats (LTR) provide evidence of the jumps during replication

HIV replication



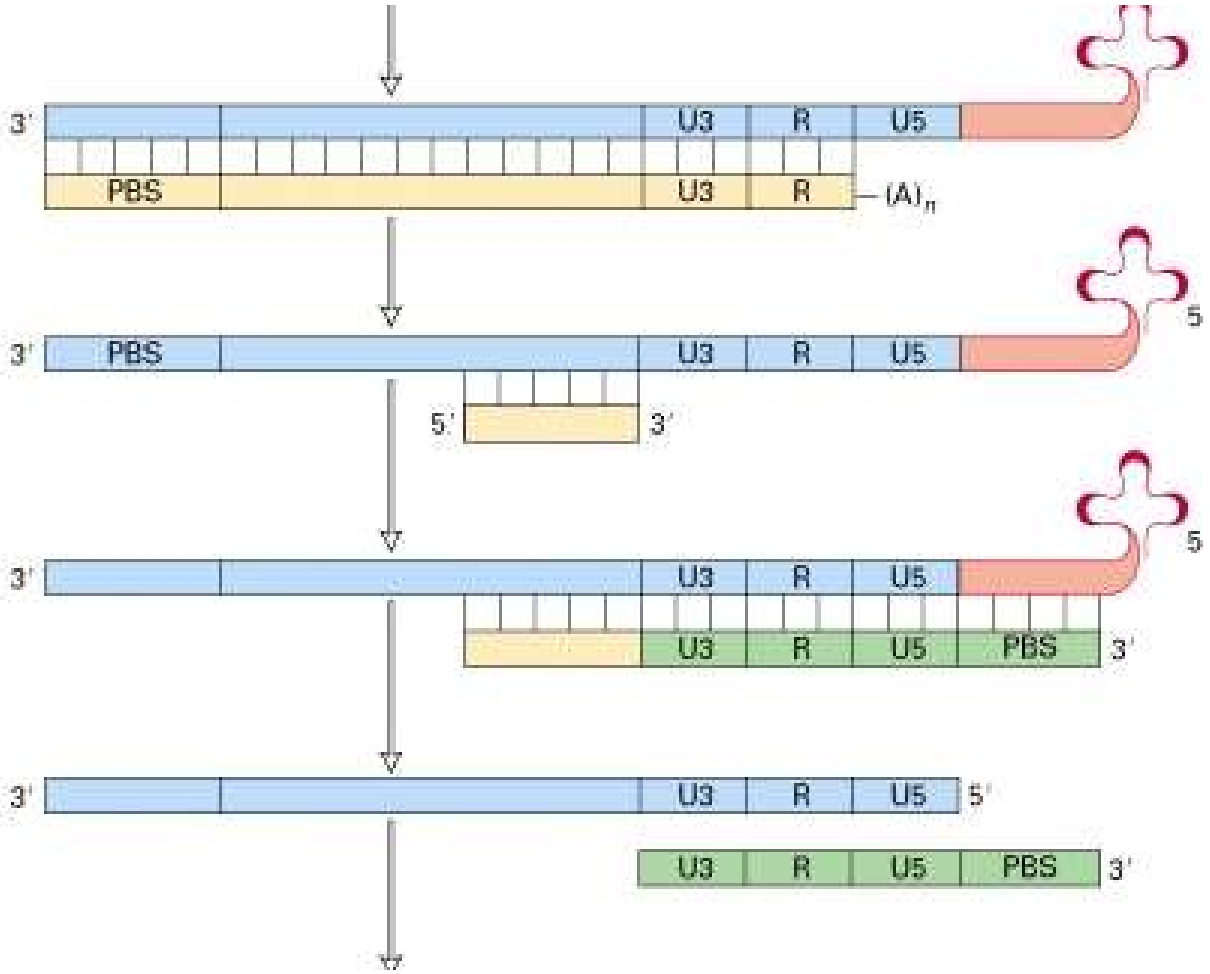
HIV replication

④ DNA strand extended from 3′ end

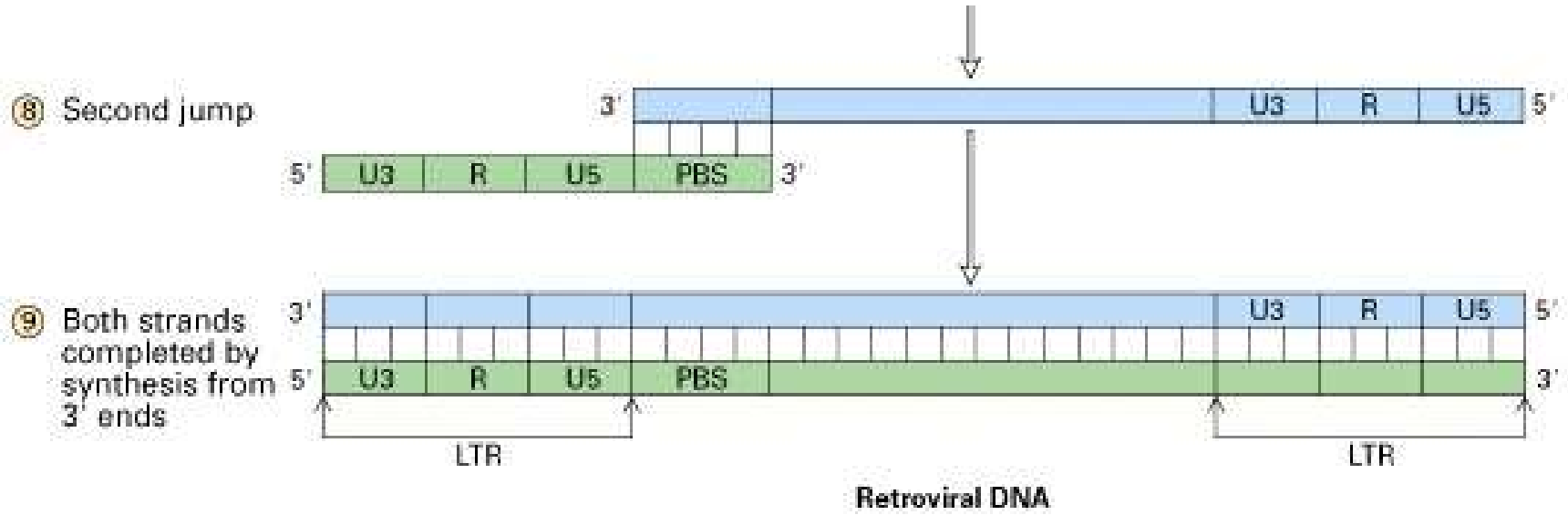
⑤ Most hybrid RNA removed

⑥ 3′ end of second DNA strand synthesized

⑦ Remaining hybrid RNA and tRNA removed



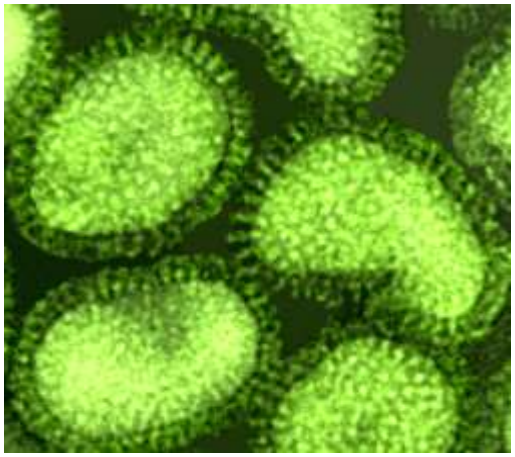
HIV replication



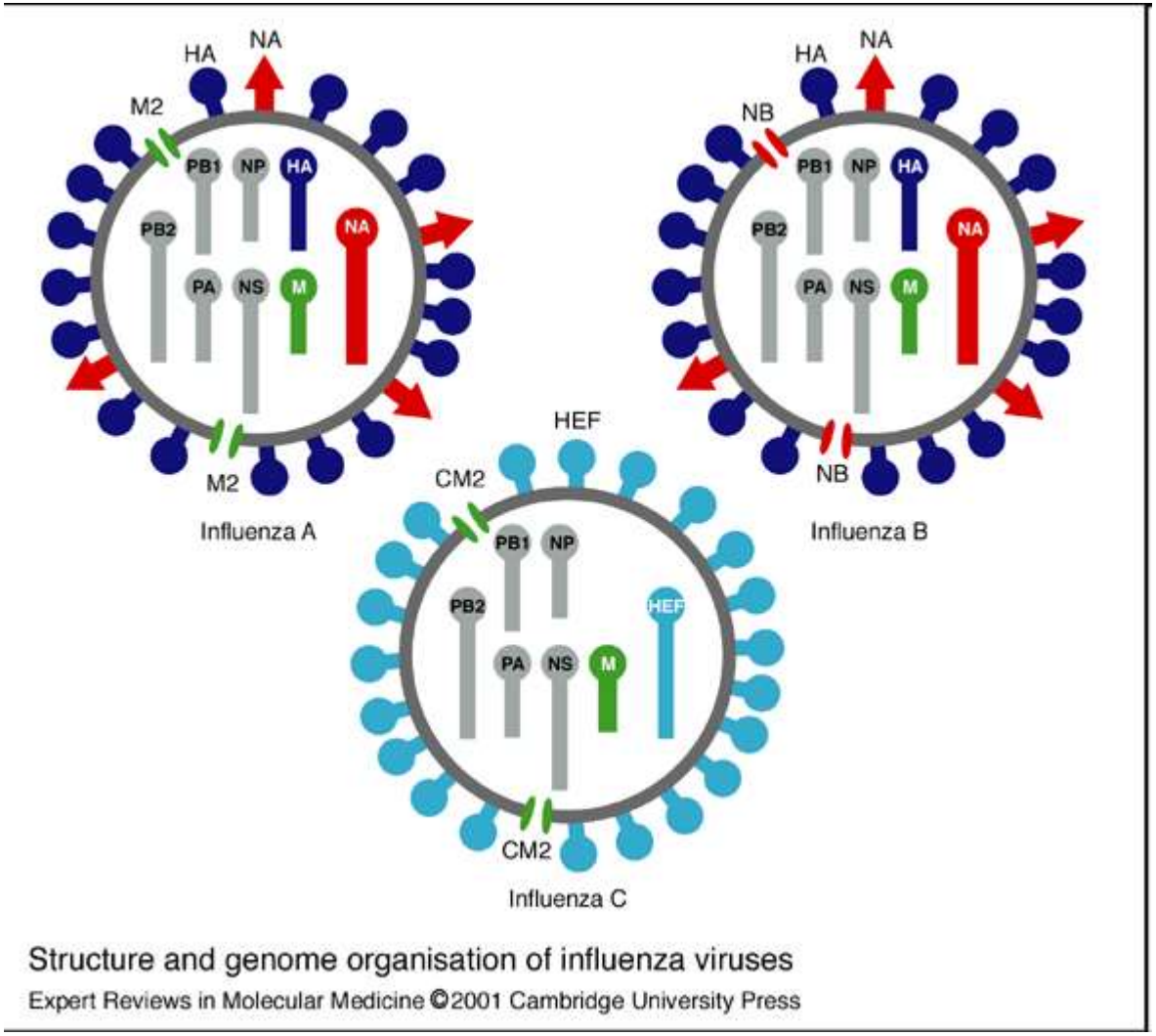
Flu virus

Orthomyxoviridae Species, Serotypes, and Hosts

type species	Serotypes	Hosts
Influenza A virus	H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7	Human, pig, bird, horse
Influenza B virus		Human, seal
Influenza C virus		Human, pig



Flu virus

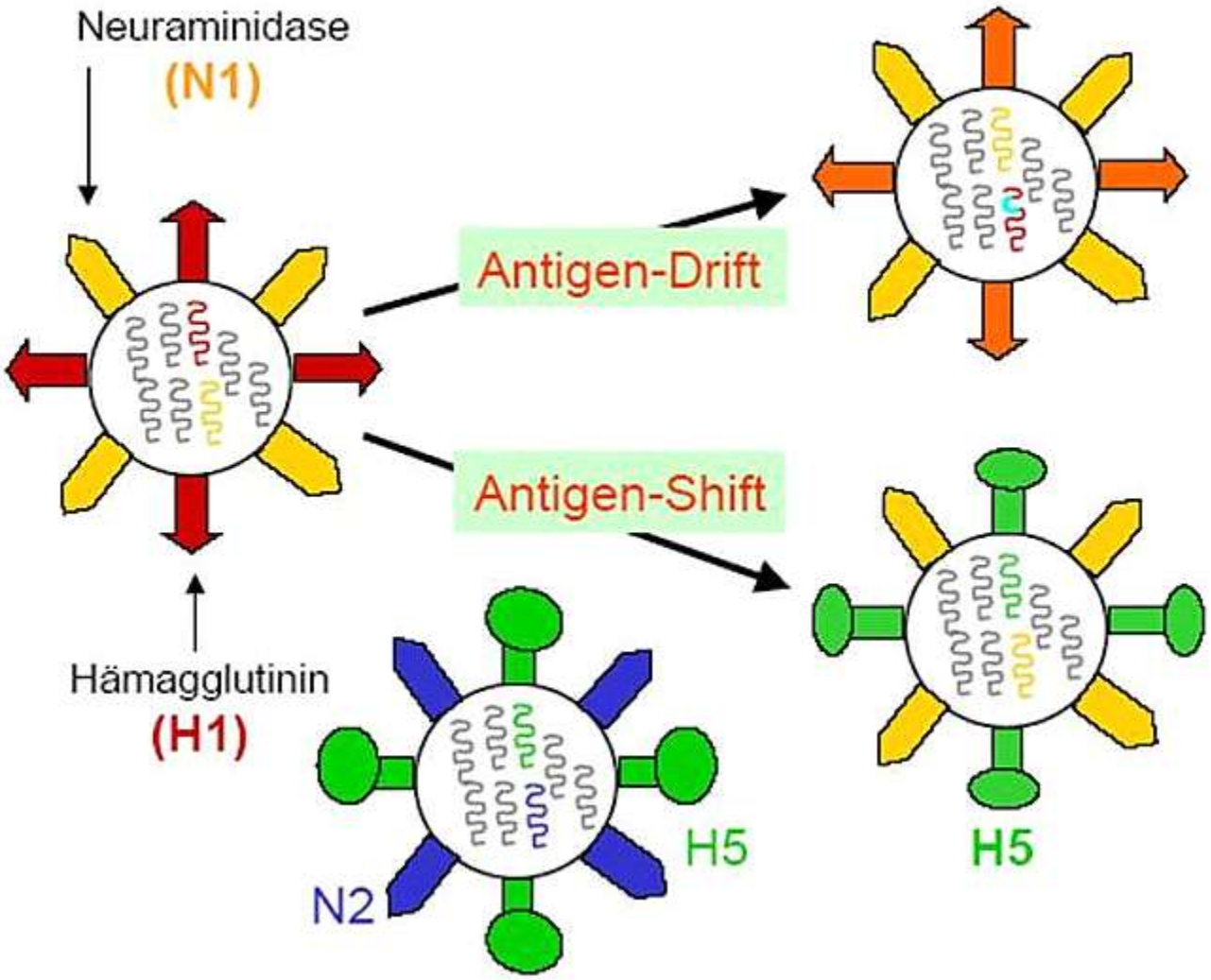


Structure and genome organisation of influenza viruses

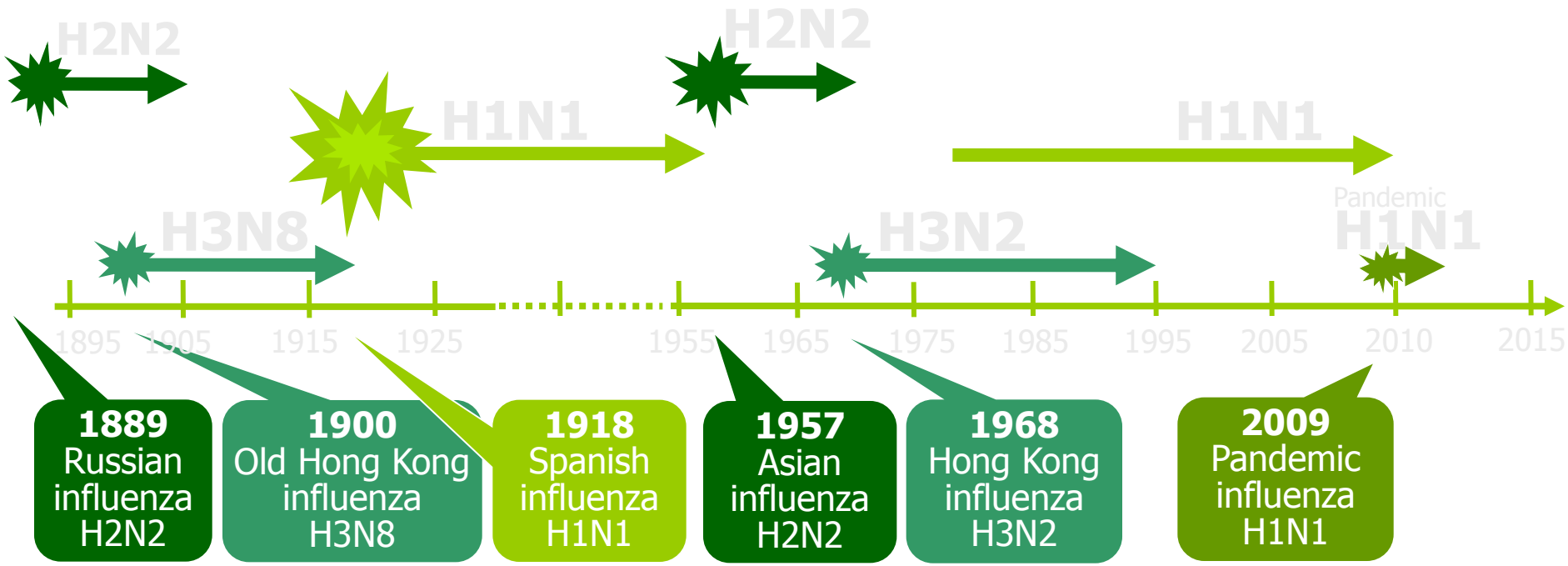
Expert Reviews in Molecular Medicine ©2001 Cambridge University Press

The surface proteins of each virus and their respective genes are shown in colour (blue, red and green); other genes are shown in light grey. The interior proteins, namely the matrix protein (M1), the nucleoprotein (NP) and the polymerases are not shown. Influenza A and B viruses contain eight RNA segments (genes), whereas influenza C viruses contain only seven RNA segments. Influenza C viruses contain a single surface glycoprotein (the haemagglutinin-esterase-fusion, or HEF, glycoprotein; shown in light blue), which functionally replaces the two surface glycoproteins that are found in influenza A and B viruses, namely haemagglutinin and neuraminidase [HA (shown in dark blue) and NA (shown in red)].

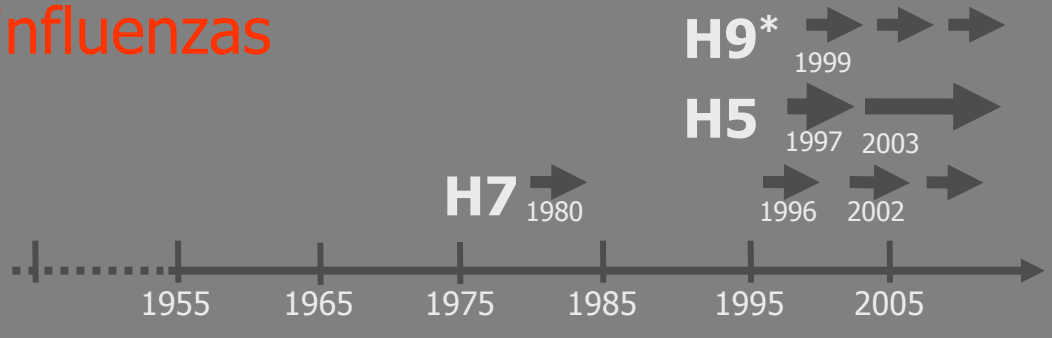
Flu virus


Neuraminidase
(N1)
Antigen-Drift
Antigen-Shift
Hämagglutinin
(H1)
N2
H5
H5

Flu epidemics

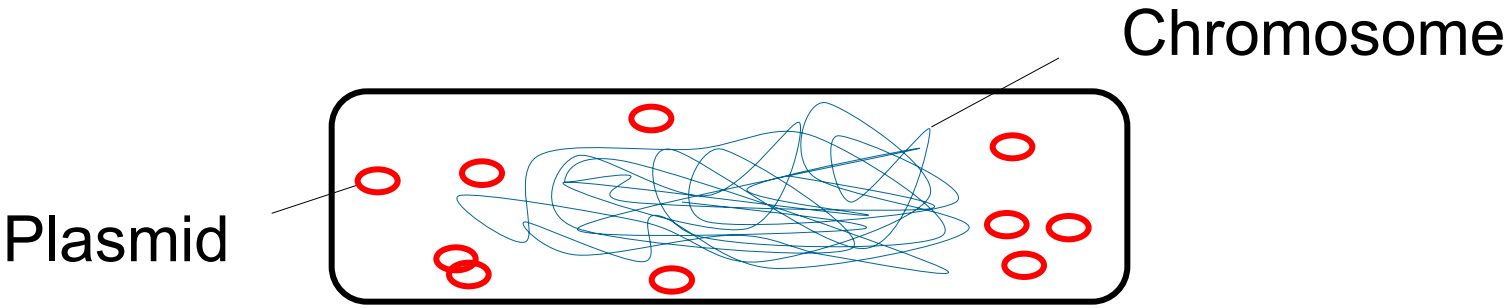


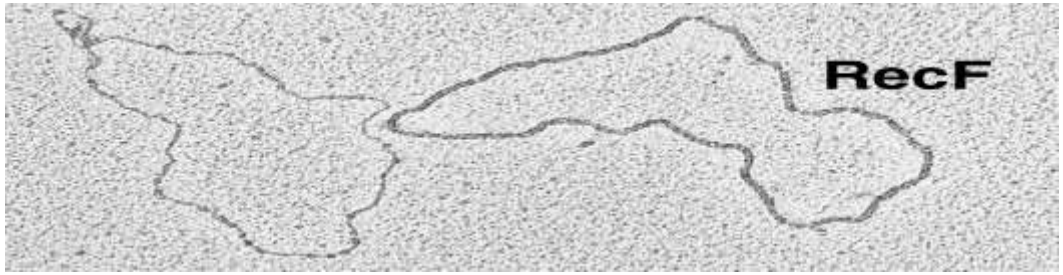
Recorded new avian influenzas



Plasmids

- Definition: Extrachromosomal genetic elements that are capable of autonomous replication (replicon)
- Episome - a plasmid that can integrate into the chromosome





Plasmids

- Extrachromosomal
- Circular or linear
- 2 kb to hundreds of kb in size
- Non-essential
- May carry ‘supplemental’ genetic information or may be cryptic
- Employ host functions for most of DNA metabolism
- Are plasmids parasites?

Why are they interesting?

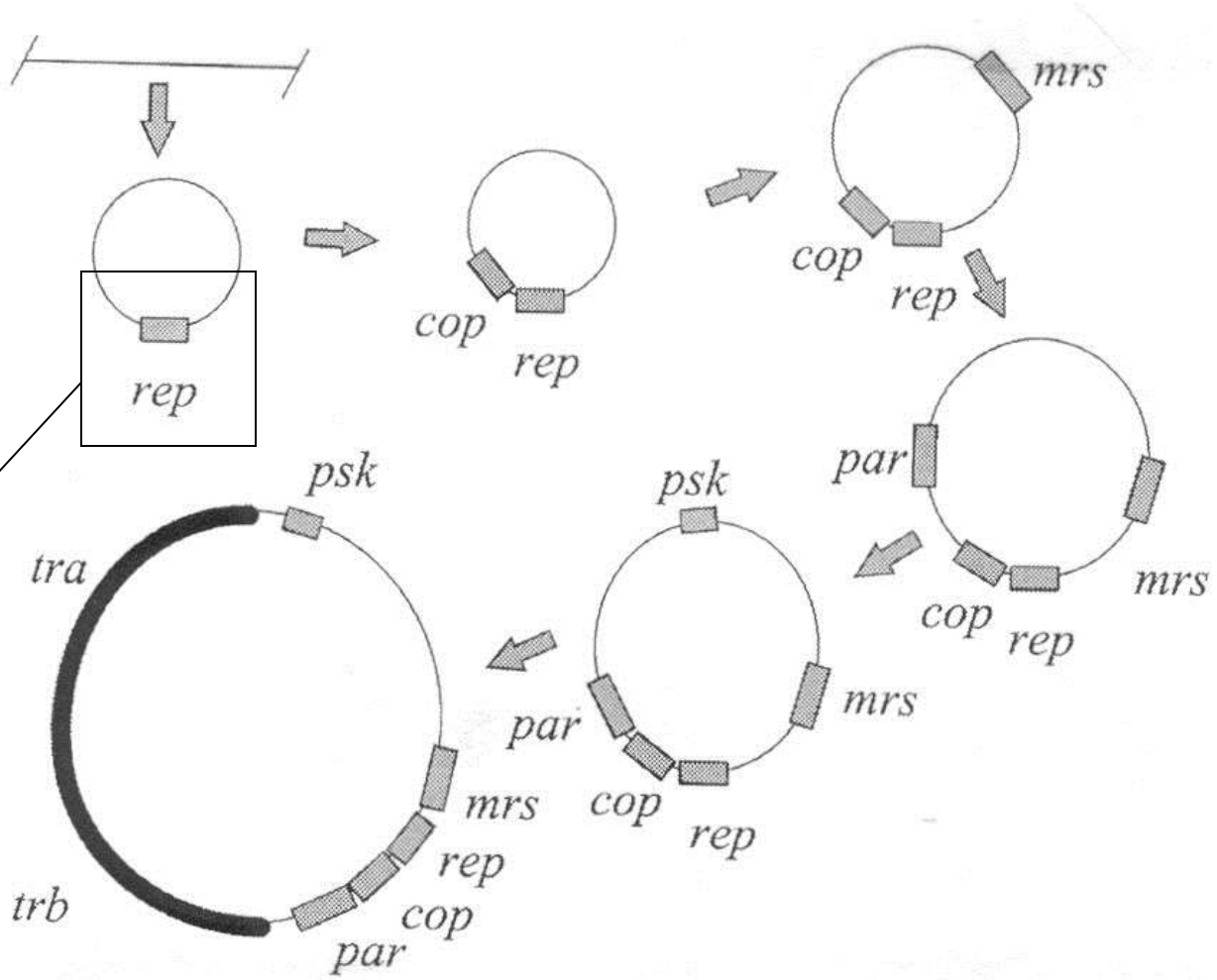
- One of the primary tools of recombinant DNA
- Genetic parasites of host genomes – selfish DNA
- A major route of gene flux – responsible for rapid evolution through horizontal transfer

Minimum components of a plasmid

1. Replication origin
2. Copy number control
3. Multimer resolution*
4. Partitioning functions*
5. Post-segregational killing (psk)*
6. Conjugal transfer*

* Not present for all plasmids, but some of these functions are found on most plasmids

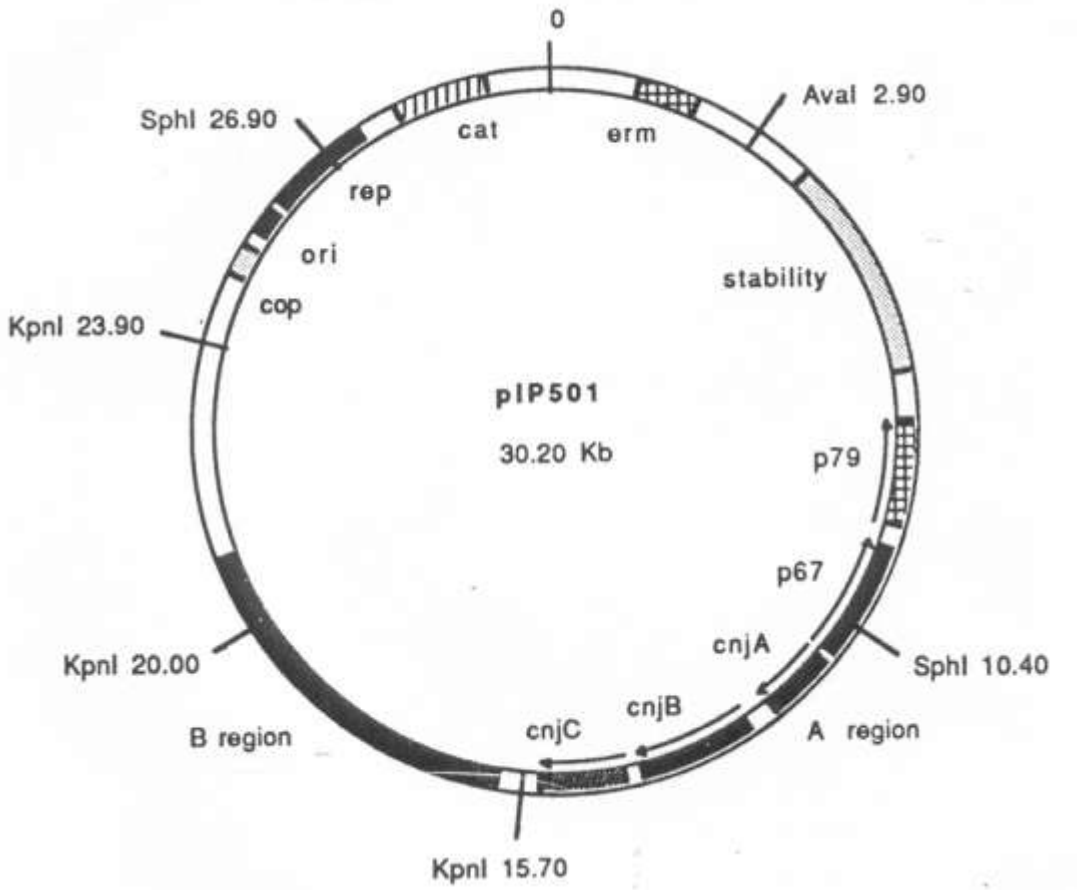
Evolution of a plasmid



Replication origin

C.M. Thomas, 2000, Mol. Microbiol. 37:485-91

Example of plasmid



Plasmid from *Streptomyces*

Genes carried

-plasmid maintenance
-antibiotic resistance
-exotic catabolism
-microcins
-host interaction
(symbiosis/virulence)
-mobile elements
(transposons/integrons/
pathogenicity islands

Classification of Plasmids

- Transfer properties
  - Conjugative
  - Nonconjugative
- Phenotypic effects
  - Fertility
  - Bacteriocinogenic plasmid
  - Resistance plasmid (R factors)

The F factor (F plasmid)

- **First conjugative plasmid identified (found in *E. coli* K12; many F-like plasmids found since).**
- **F is a 100 kb circular dsDNA, low copy number (1-2/cell).**
- **The F transfer system is encoded by a 34 kb region; expression is "derepressed" due to spontaneous mutation.**

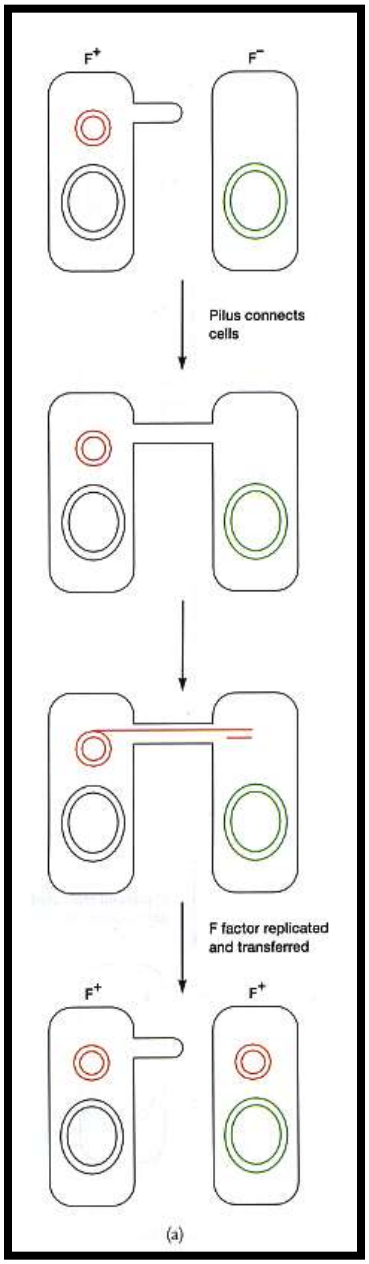
F-mediated Conjugation

F^+ cell contacts F^- cell

Cells are brought together

At point of apposition, DNA moves from F^+ cell into F^- cell

Full copy of F plasmid transmited to F^- cell; conversion to F^+



Transfer of non-conjugative plasmids

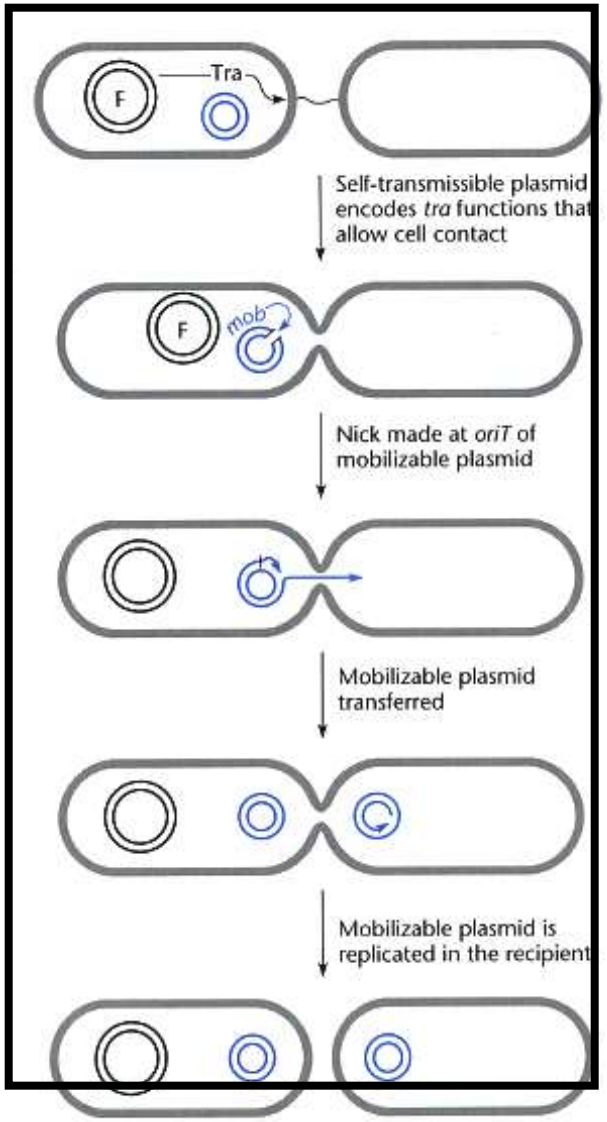
- **Conjugative elements can transfer with other plasmids:**
  - **1. By recombining with them to form one "cointegrate" plasmid. Cointegrate formation is often assimilated by IS sequences and transposons.**
  - **2. By : Many small plasmids are able to use the conjugative contact systems expressed by a transmissible plasmid in the same cell. Such plasmids include an *oriT* (or *mob*) site that can be recognized by the conjugative transfer system.**

Mobilization of plasmids via *mob* loci

F plasmid provides Tra proteins that are required for transfer of mobilizable plasmids with *oriT* sequences (sometimes called *mob* in non-F plasmids).

Nick at *oriT* results in transfer of mob containing plasmid to recipient (usually selected for by Ab^R determinant)

F plasmid may also transfer



Natural plasmids

Plasmid	Host	Plasmid size (kb)	Relevant feature
pT181	*Staphylococcus aureus*	4.4	Tetracycline resistance
pRN1	*Sulfolobus islandicus*[a]	5.4	-
2μ	*Saccharomyces cerevisiae*[b]	6.3	-
ColE1	*Escherichia coli*	6.6	Colicin production and immunity
pMB1	*Escherichia coli*	8.5	*Eco*RI restriction-modification system
pGKL2	*Kluyveromyces lactis*[b]	13.5	Killer plasmid
pAMβ1	*Enterococcus faecalis*	26.0	Erythromycin resistance
pSK41	*Staphylococcus aureus*	46.4	Multidrug resistance
pBM4000	*Bacillus megaterium*	53.0	rRNA operon

Natural plasmids

pBM4000	*Bacillus megaterium*	53.0	rRNA operon
pI258	*Staphylococcus aureus*	28.0	Metal ion resistance
pSLT	*Salmonella enterica* subsp. *typhimurium*	93.9	Virulence determinants
pMT1	*Yersinia pestis*	101.0	Virulence determinants
pADP-1	*Pseudomonas* sp.	108.8	Atrazine (herbicide) catabolism
pWW0	*Pseudomonas putida*	117.0	Aromatic hydrocarbon degradation
pBtoxis	*Bacillus thuringiensis* subsp. *israelensis*	137.0	Mosquito larval toxicity
pX01	*Bacillus anthracis*	181.7	Exotoxin production
pSOL1	*Clostridium acetobutylicum*	192.0	Solvent production
pSymB	*Sinorhizobium meliloti*	1683.3	Multiple functions associated with plant symbiosis

[a]Archaea; [b]Eukarya (yeast)

2µ DNA – plasmid from yeasts

The 2u circle is a 6.3 kb circular, extrachromosomal element found in the nucleus of most *Saccharomyces cerevisiae* strains. The 2u circle doesn't give cells that carry it any apparent selective advantage, but it is stably maintained at about 50 to 100 copies per haploid genome of the yeast cells. Like the host chromosomes, the 2u circle is coated with nucleosomes and replication is initiated by host replication enzymes once per cell cycle. The origin of bidirectional DNA replication is initiated at a specific site on the plasmid called an ARS sequence ("autonomous replication sequence").