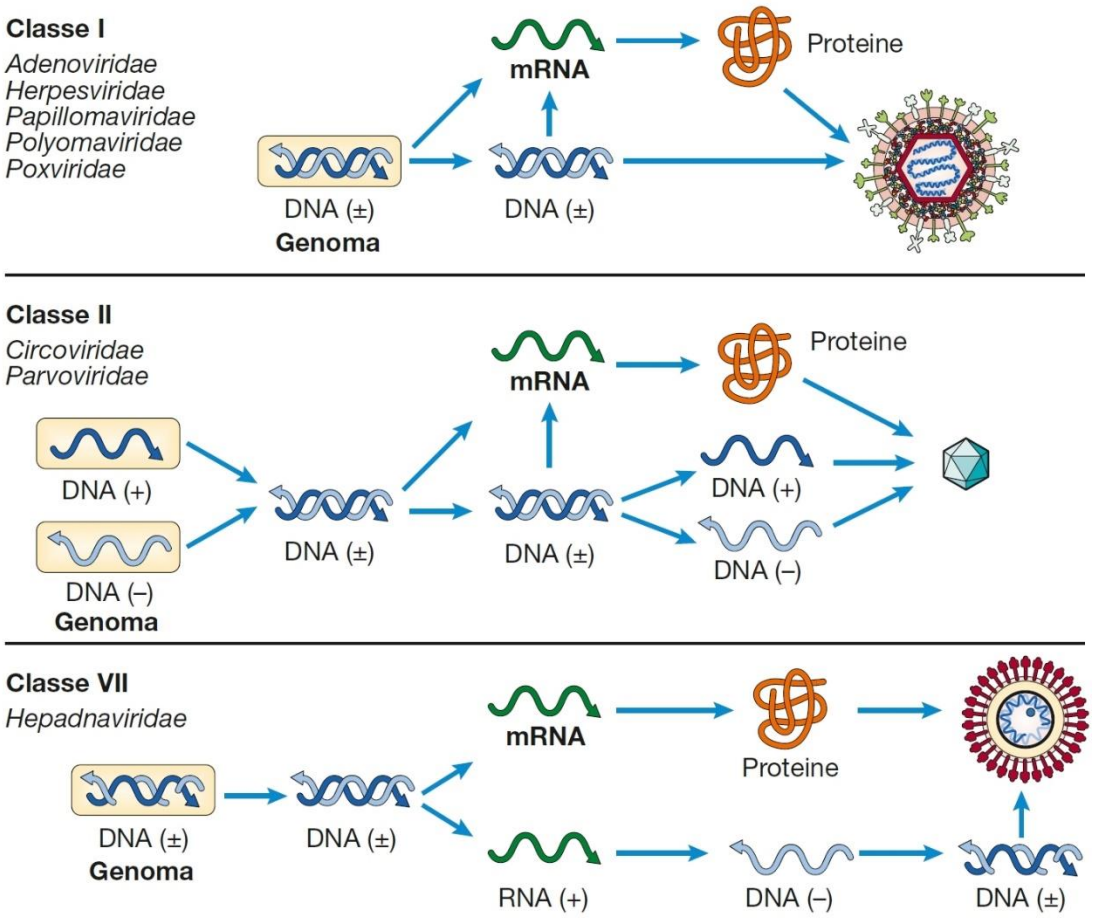
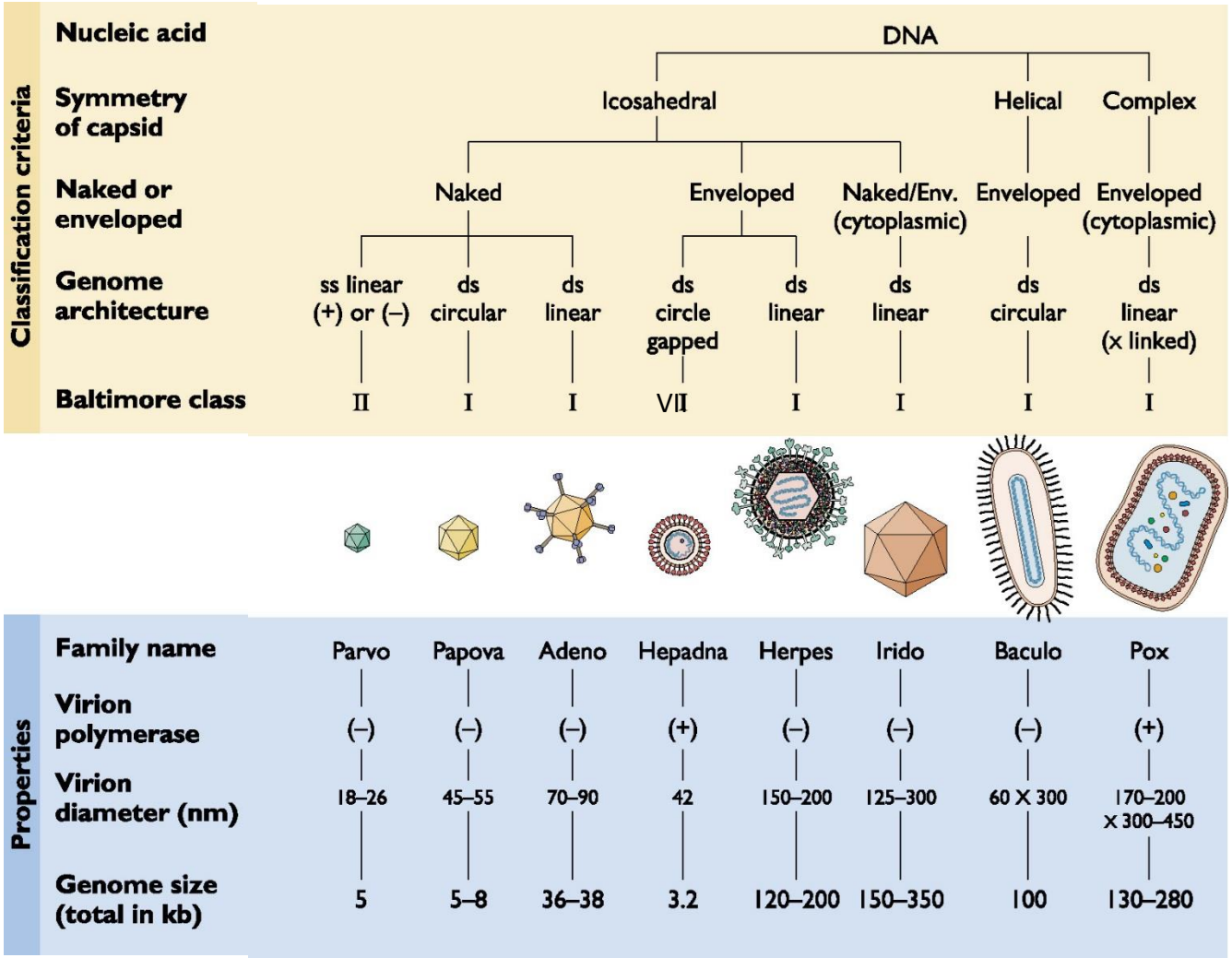
Figura 14.8 RAPPRESENTAZIONE SCHEMATICA DEL FLUSSO DEGLI EVENTI NELLA REPLICAZIONE DEI VIRUS ANIMALI A DNA DELLE CLASSI I, II E VII SECONDO LA CLASSIFICAZIONE DI BALTIMORE.



DNA viruses



The size of the genome reflects the complexity of the virus and its replication, which is more evident in DNA viruses than in RNA viruses.

From Flint et al. *Principles of Virology* (2000), ASM Press

DNA Viruses

DNA viruses follow different replication strategies and fall into three different Baltimore classes (I: dsDNA; II: ssDNA; VII: dsDNA with an RNA intermediate).

Within **Class I**, several viral families can be identified that exhibit different replication strategies, which can be grouped into two main categories.

1) Herpesviruses, adenoviruses, papillomaviruses, and polyomaviruses

These viruses have a DNA genome that migrates into the nucleus of the host cell, where they use the host cell's DNA-dependent RNA polymerases (RNA polymerase II) for transcription.

The genome of papilloma and polyoma viruses is transcribed in two phases, while that of herpesviruses and adenoviruses is transcribed in three phases, producing respectively two and three distinct groups of mRNAs, the last of which is destined for the production of the structural proteins of the virion.

DNA Viruses

2) Poxviruses

In these viruses, the entire replication cycle takes place in the cytoplasm, despite the fact that they are DNA viruses.

For transcription, they use a DNA-dependent RNA polymerase that is carried within the virion.

THE ONLY DNA VIRUSES THAT COMPLETE THEIR REPLICATION CYCLE IN THE CYTOPLASM!

Class II viruses (e.g., parvoviruses)

They possess a single-stranded DNA genome which, through the action of cellular enzymes, must direct the synthesis of a complementary DNA strand and the transcription of mRNA.

These events occur in the nucleus of the cell.

DNA Viruses

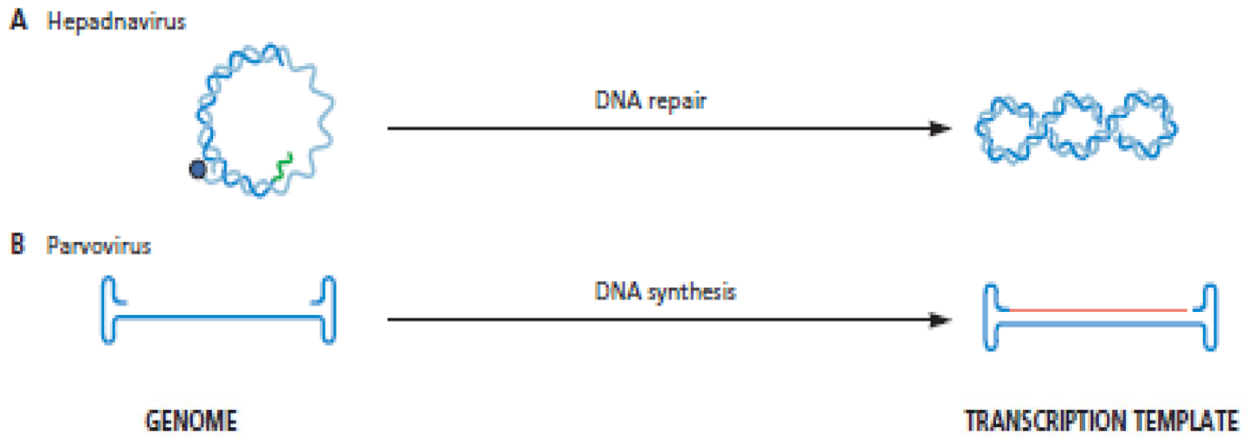
Class VII viruses (Hepadnaviruses):They have a partially double-stranded, open circular DNA genome, with an associated RNA-dependent DNA polymerase.

Hepadnaviruses: Of these viruses, the only one pathogenic to humans is the hepatitis B virus (HBV). These viruses have a circular genome composed of double-stranded DNA with a gap in the positive-polarity strand. Through the action of nuclear cellular repair enzymes, the missing segment is synthesized, producing a fully double-stranded molecule that becomes supercoiled.

Subsequently, through the action of nuclear cellular enzymes, a series of subgenomic mRNAs are transcribed, which code for the structural proteins, as well as a **genomic RNA** which, once incorporated into the nucleocapsid, will be reverse-transcribed into DNA by the viral RNA-dependent DNA polymerase, followed by synthesis of the complementary DNA strand.

Template for RNA pol II

To create a template suitable for transcription, incomplete double-stranded DNA genomes (Class VII) or single-stranded DNA genomes (Class II) are converted into dsDNA molecules by cellular enzymes.

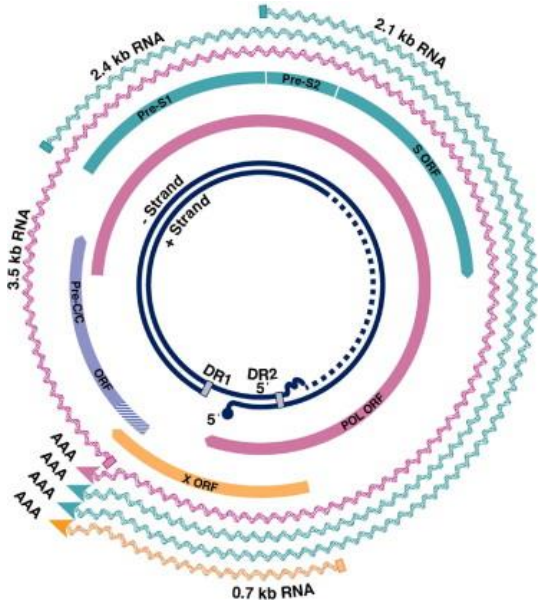


From Flint et al. *Principles of Virology* (2015), ASM Press

Viral DNA genome Replication

- Herpesvirus, adenovirus e poxvirus code for a viral DNA polymerase.

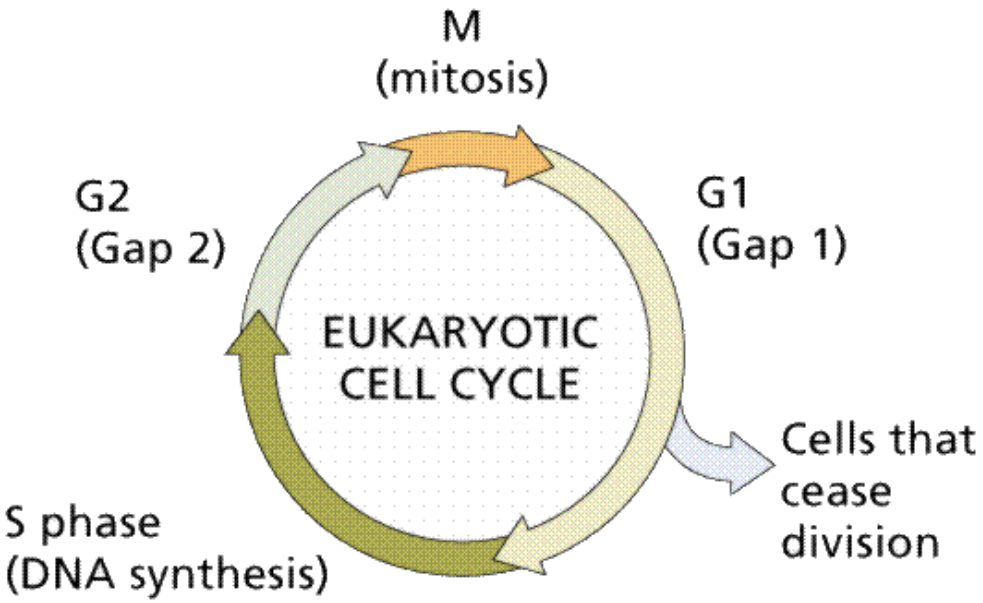
- HBV use a viral RNA-dependent DNA-polymerase (reverse transcriptase)



Viral DNA genome Replication

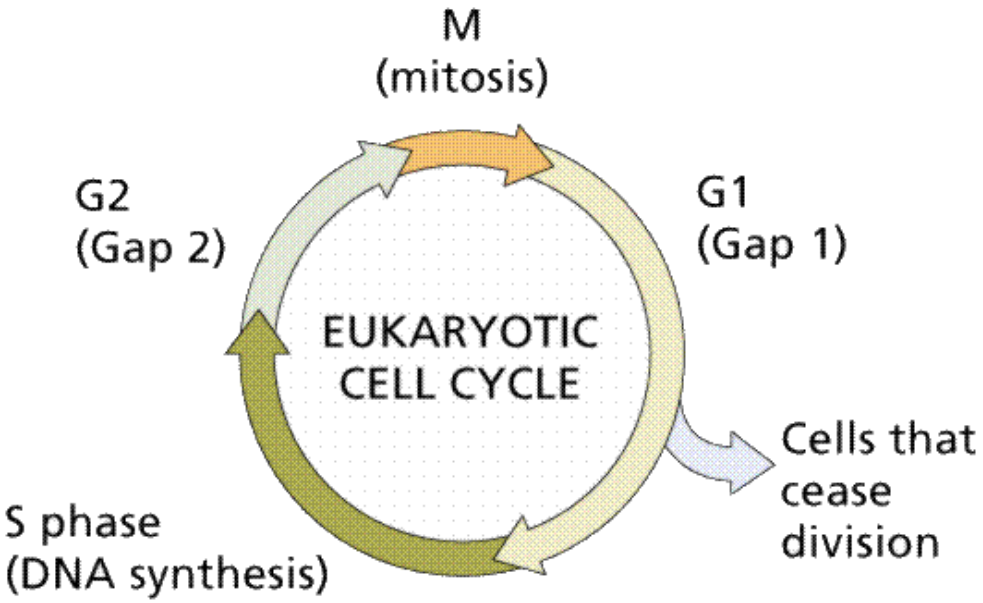
Parvovirus, Papilloma e polyomavirus use the cellular DNA polymerase,

DNA polymerase is produced during a specific phase of the cell cycle: the S phase, and is therefore found only in actively replicating cells.



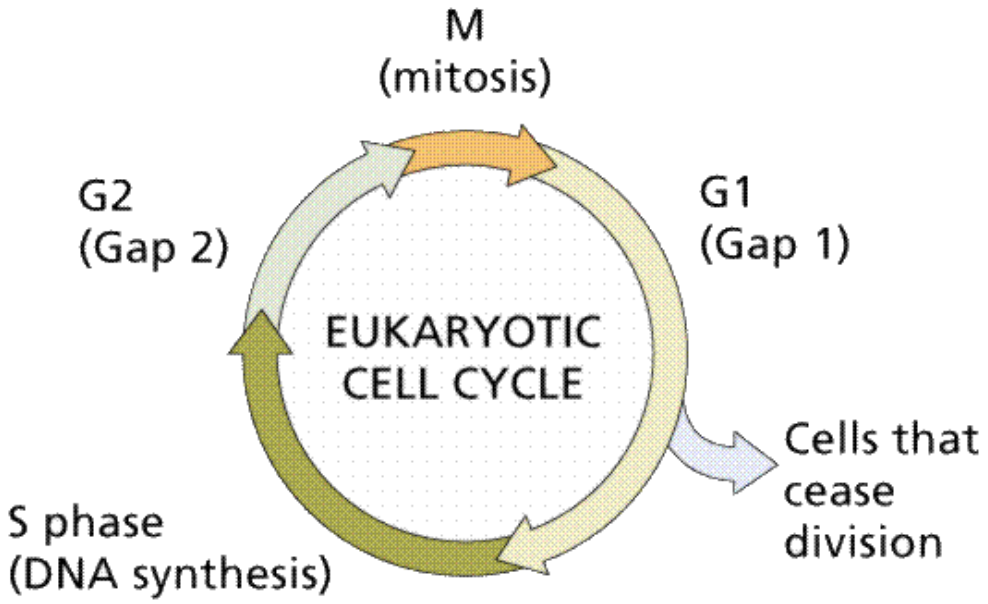
Viral DNA genome Replication

To use DNA polymerase, parvoviruses replicate only in actively proliferating cells: the precursors of red blood cells (erythroblasts).



Viral DNA genome Replication

To use cellular DNA polymerase, papillomaviruses and polyomaviruses induce progression of the cell cycle from the G1 phase to the S phase. This allows these viruses to infect even quiescent cells.



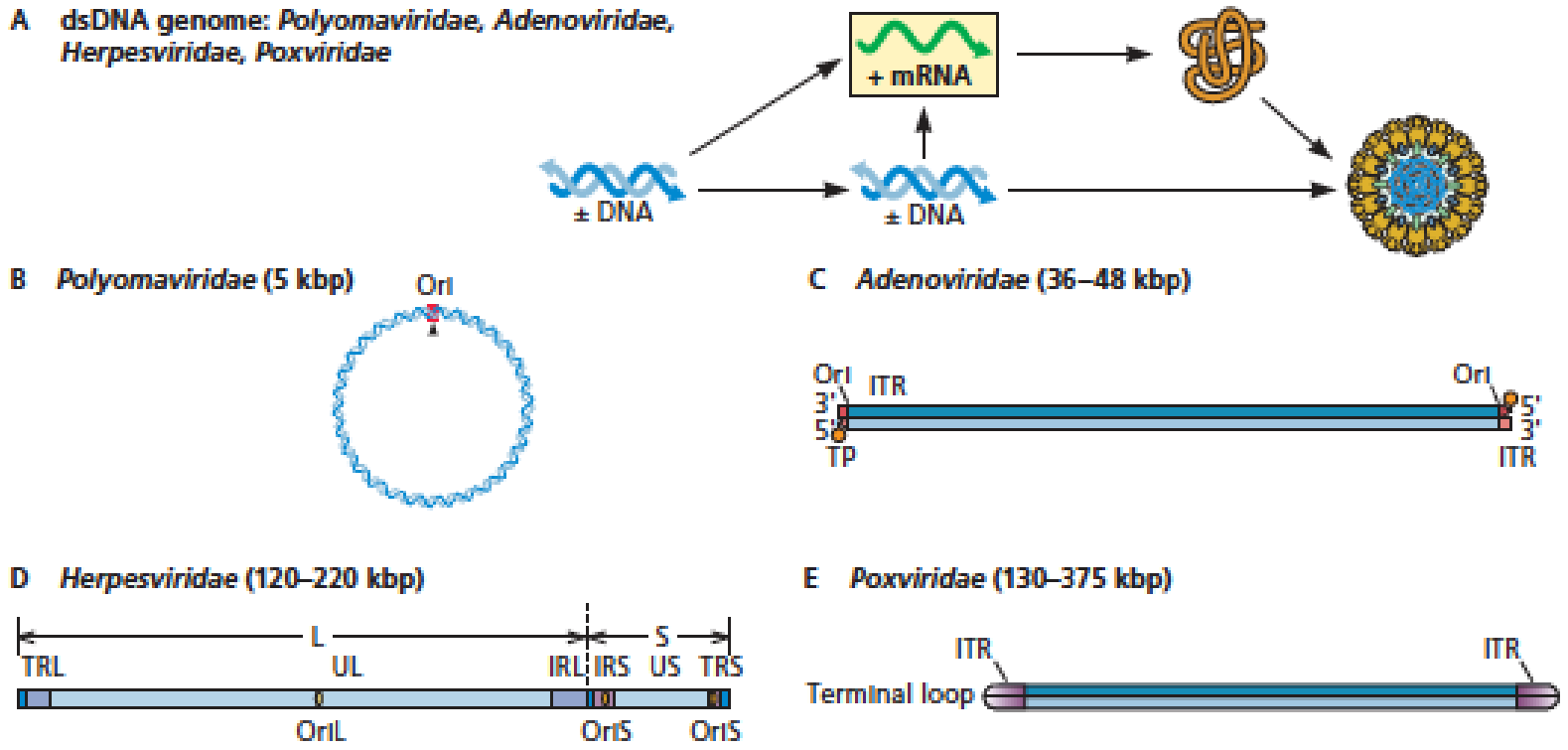
Class I: dsDNA

- Alcuni genomi di virus a DNA sono complessati ad istoni cellulari a formare una struttura simile alla cromatina all'interno dei virioni. Una volta all'interno del nucleo della cellula ospite si comportano come cromosomi satellite in miniatura seguendo i dettami degli enzimi cellulari e del ciclo cellulare
- Lo studio di questi virus ha portato all'identificazione degli elementi che guidano la sintesi di pre-mRNA e mRNA (promotori e enhancer; SV40)
- I primi mRNA poliadenilati al 3'osservati sono stati quelli di *Vaccinia virus*
- Geni composti da introni non codificanti e esoni codificanti, così come spliced mRNA, sono stati scoperti per la prima volta in Adenovirus da Roberts & Sharp in 1977.

Class I: dsDNA, Expression-Replication

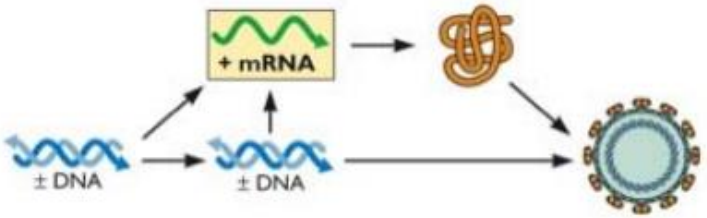
This class can be divided into two further groups

- Replication is exclusively nuclear. The replication of these viruses is relatively dependent on cellular factors. (Most of class I viruses)
- Replication occurs in cytoplasm (*Poxviridae*). These viruses have evolved all the necessary factors for transcription and replication of their genome and are therefore largely independent of the cellular machinery



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

Double stranded DNA (dsDNA) genomes



Genomes use host DNA polymerase

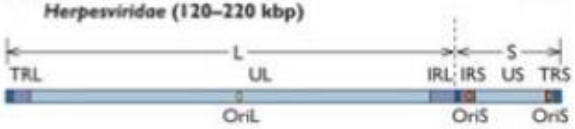
Polyomaviridae (5 kbp)

Papillomaviridae (8 kbp)

Genomes encode DNA polymerase

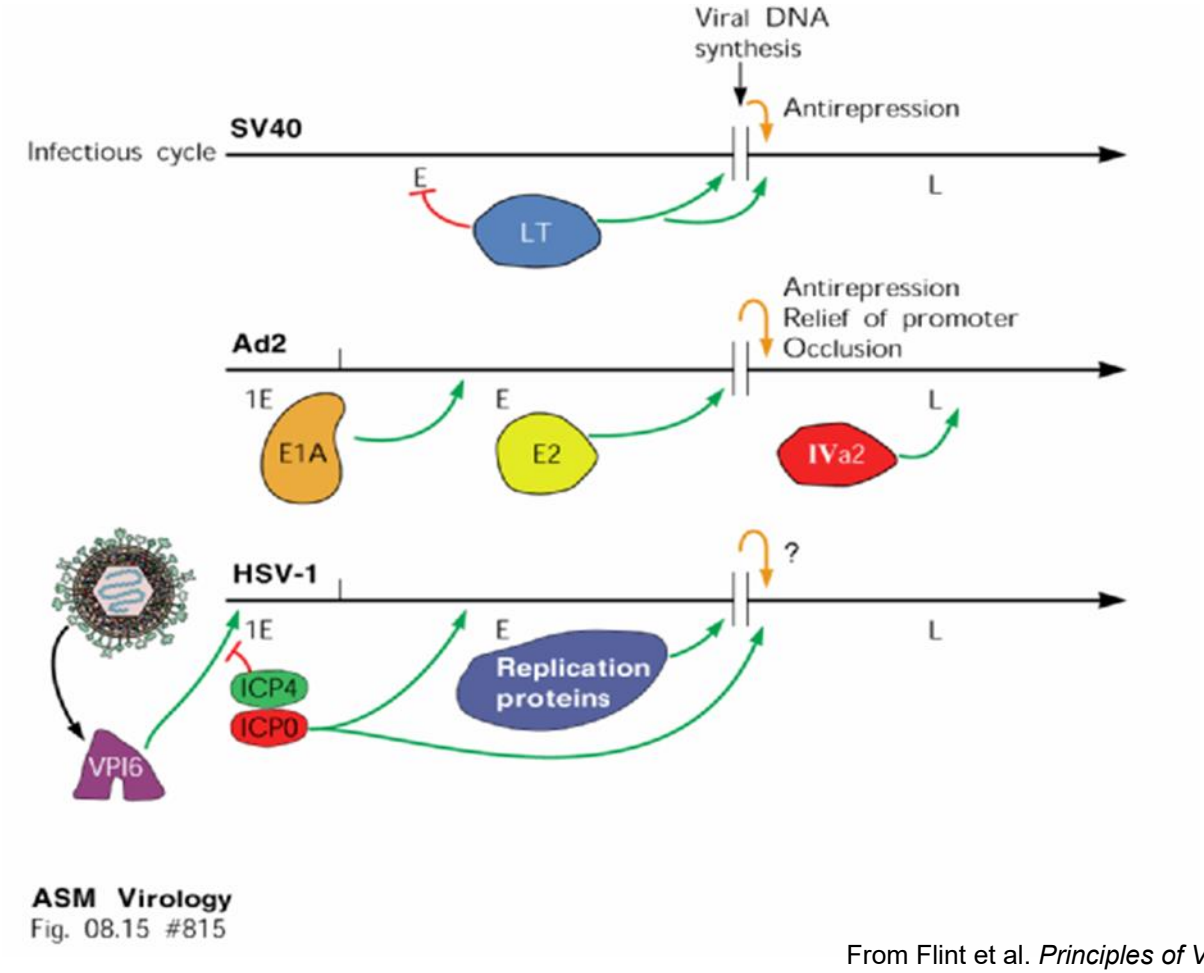
Adenoviridae (36–48 kbp)

Herpesviridae (120–220 kbp)



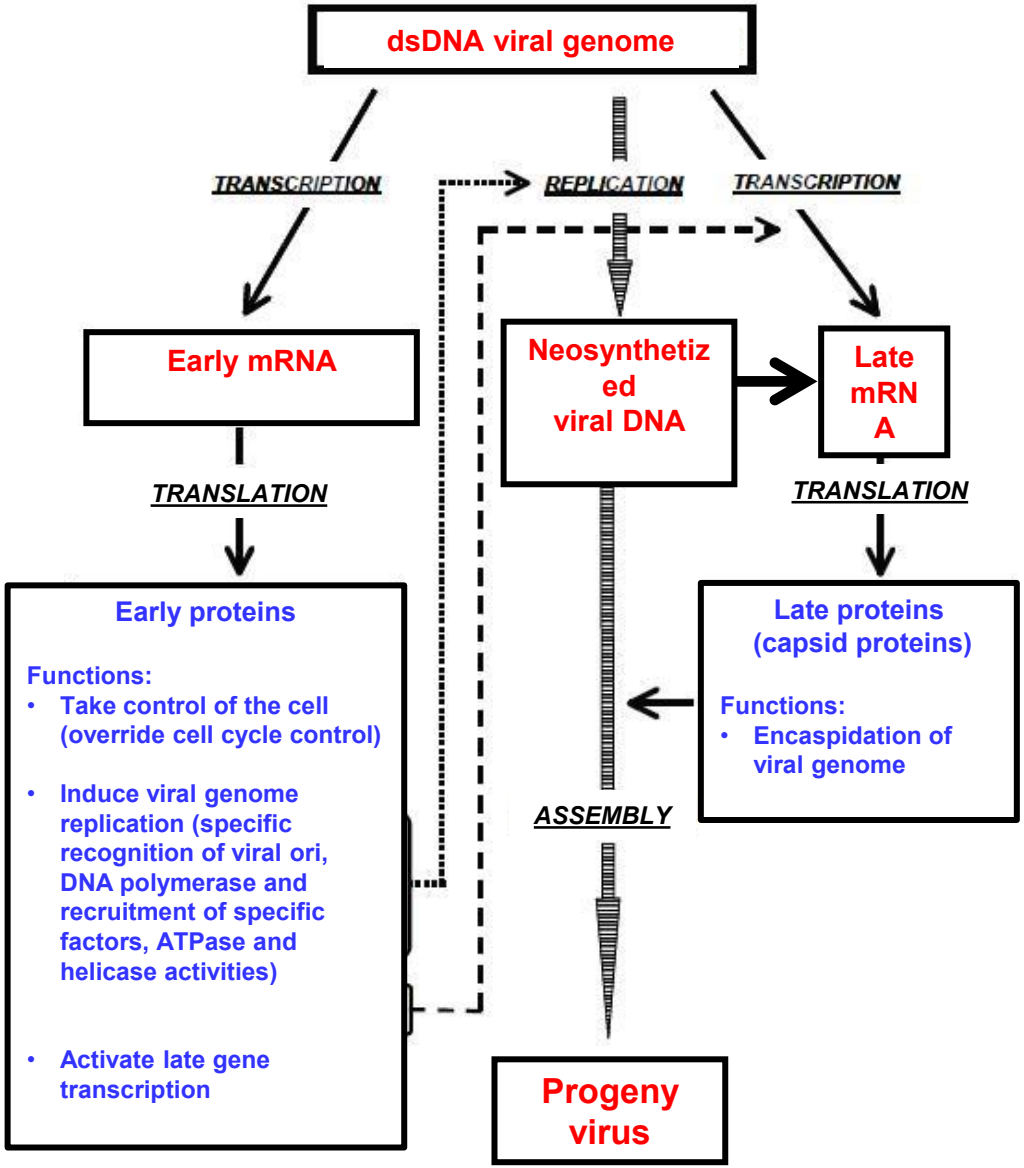
Poxviridae (130–375 kbp)

Class I viruses impose a temporal phasing on their genome expression

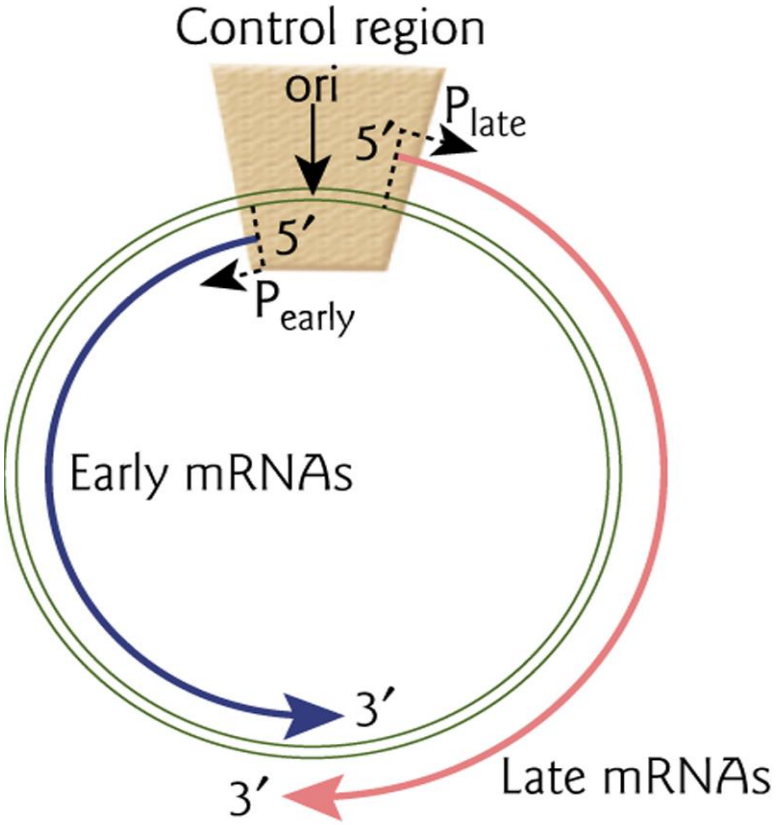


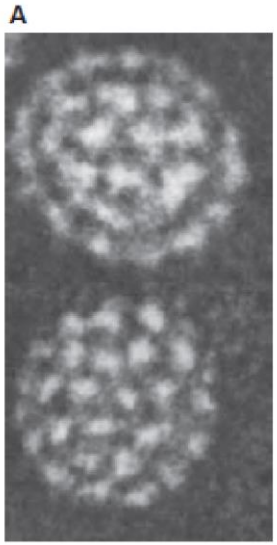
From Flint et al. *Principles of Virology* (2015), ASM Press

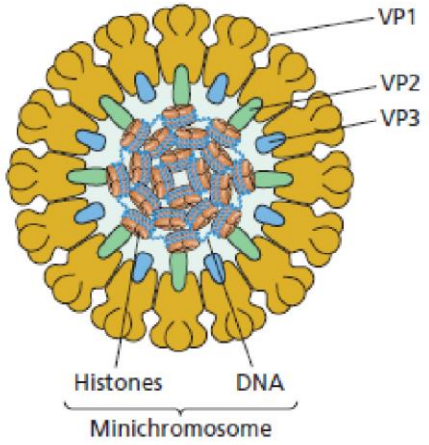
EXPRESSION-REPLICATION of GR I VIRUSES



Simian Virus 40 (SV40)







Il genoma virale è complessato agli istoni cellulari H2A, H2B, H3 e H4 a formare un minicromosoma

Contiene due unità trascrizionali: precoce e tardiva

Proteine precoci: Large T e small t

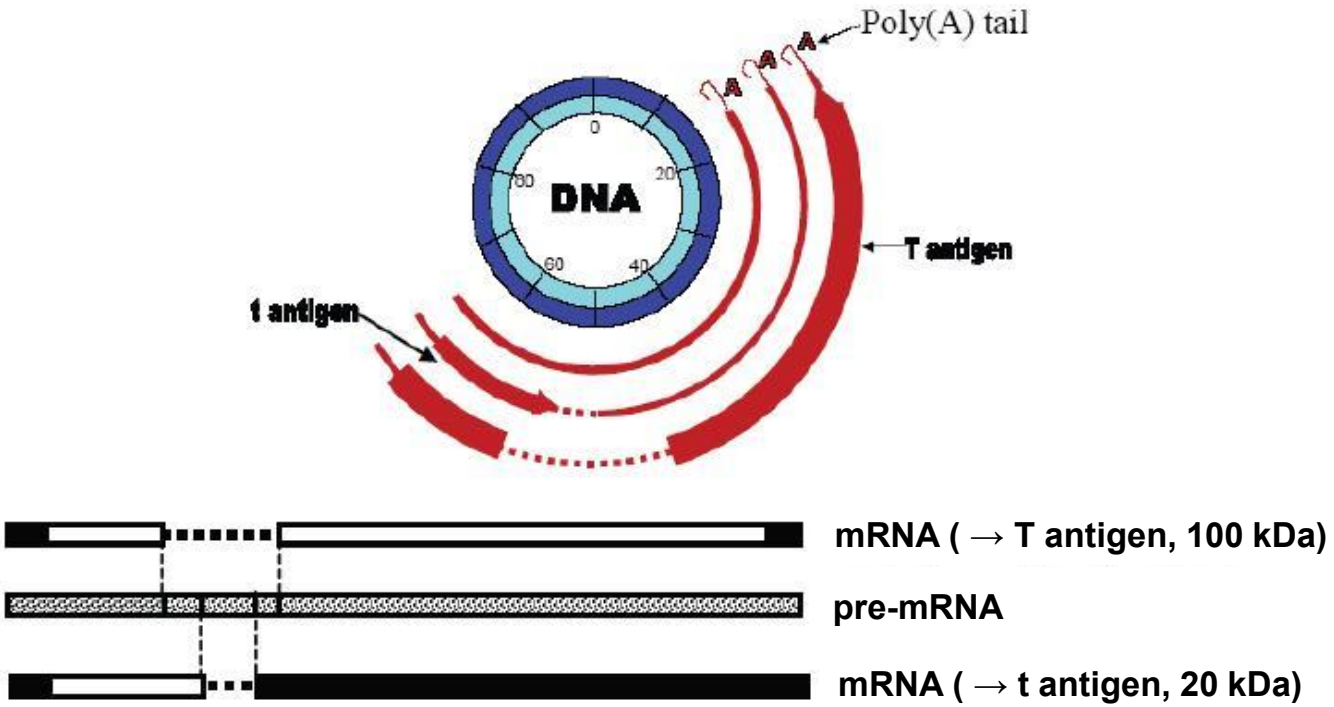
Replicazione del genoma

Proteine tardive: **VP1, VP2, VP3 e Agnoproteina**

SV40 virus

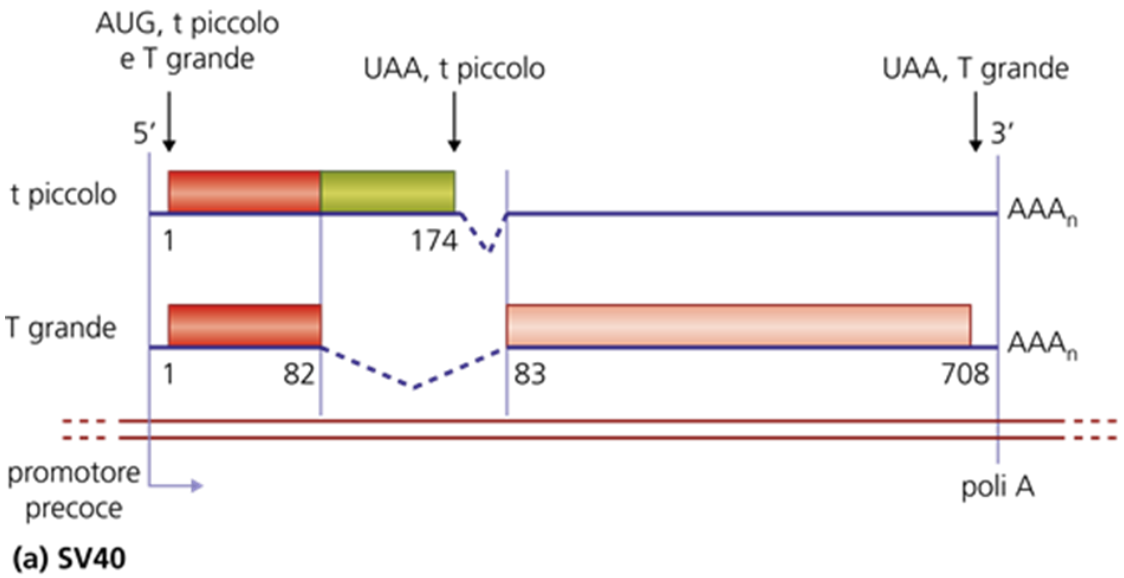
Poliomaviridae, Circular dsDNA genome, naked, icosahedral capsid

Early phase of SV40 ***: Synthesis of mRNA encoding early proteins (T and t antigens)***



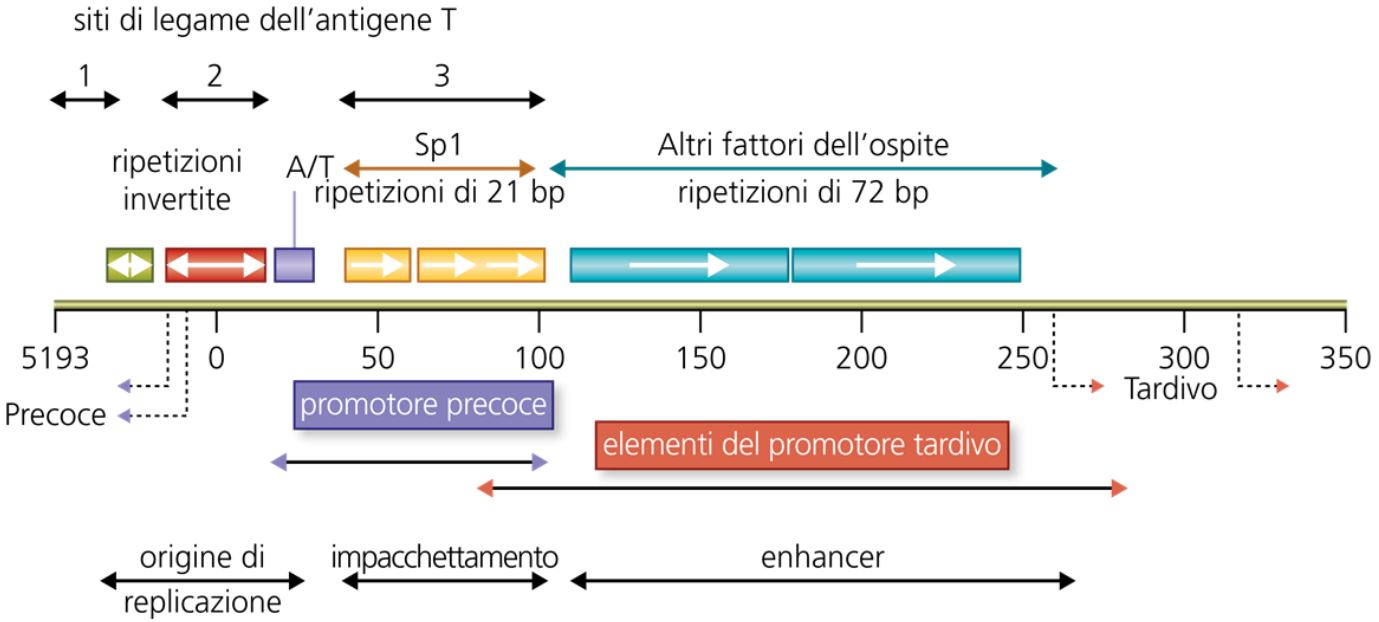
Alternative splicing → 2 viral mRNA from 1 pre-mRNA

Espressione precoce in SV40



(a) SV40

Espressione: SV40



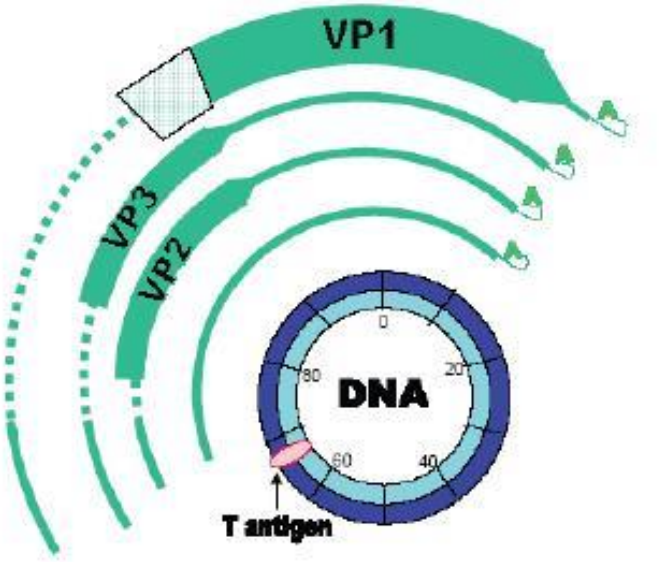
SV40 LT Ag

- Si lega a pRb e p53: attivazione sintesi DNA nell'ospite
- Blocco dell'apoptosi grazie all'interazione con p53
- Si lega ad ori per iniziare la sintesi del DNA virale (elicasi ATP dipendente)
- Spegne la trascrizione precoce legandosi al proprio promotore
- Attiva la trascrizione tardiva
- E' coinvolta nell'assemblaggio del virione

Nigel J. Dimmock, Andrew J. Easton, Keith N. Leppard
Introduzione alla Virologia moderna *Casa Editrice Ambrosiana. 2017*

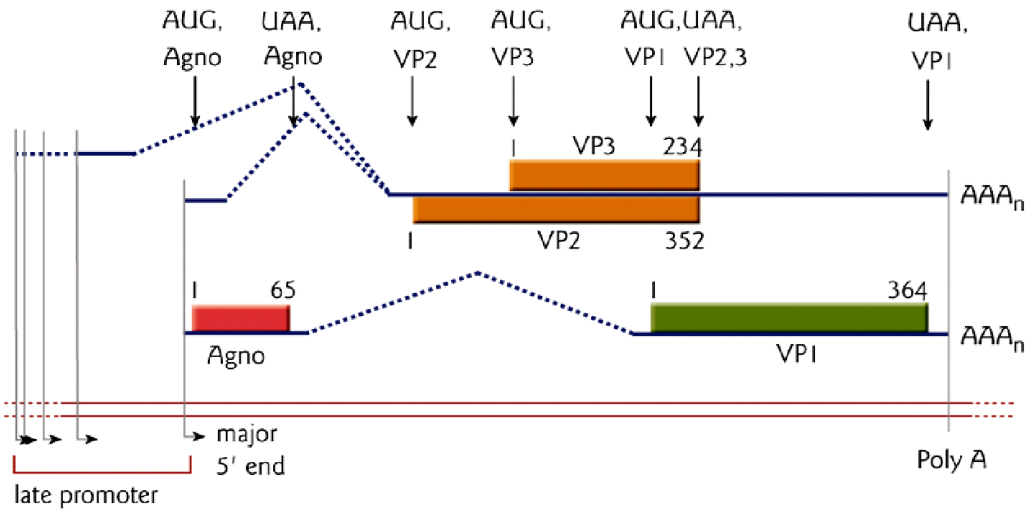
Fase tardiva

Late phase of SV40 ***: synthesis of late mRNA encoding structural viral proteins VP1, VP2 & VP3***



Alternative splicing → 3 viral mRNA from 1 pre-mRNA

Espressione tardiva di SV40



Gli mRNA tardivi contengono estremità 5‘ eterogenee dovute alla mancanza di una TATA box nel promotore tardivo, che serve normalmente a stabilire la posizione precisa dell’inizio della trascrizione.

SV40 Replication cycle

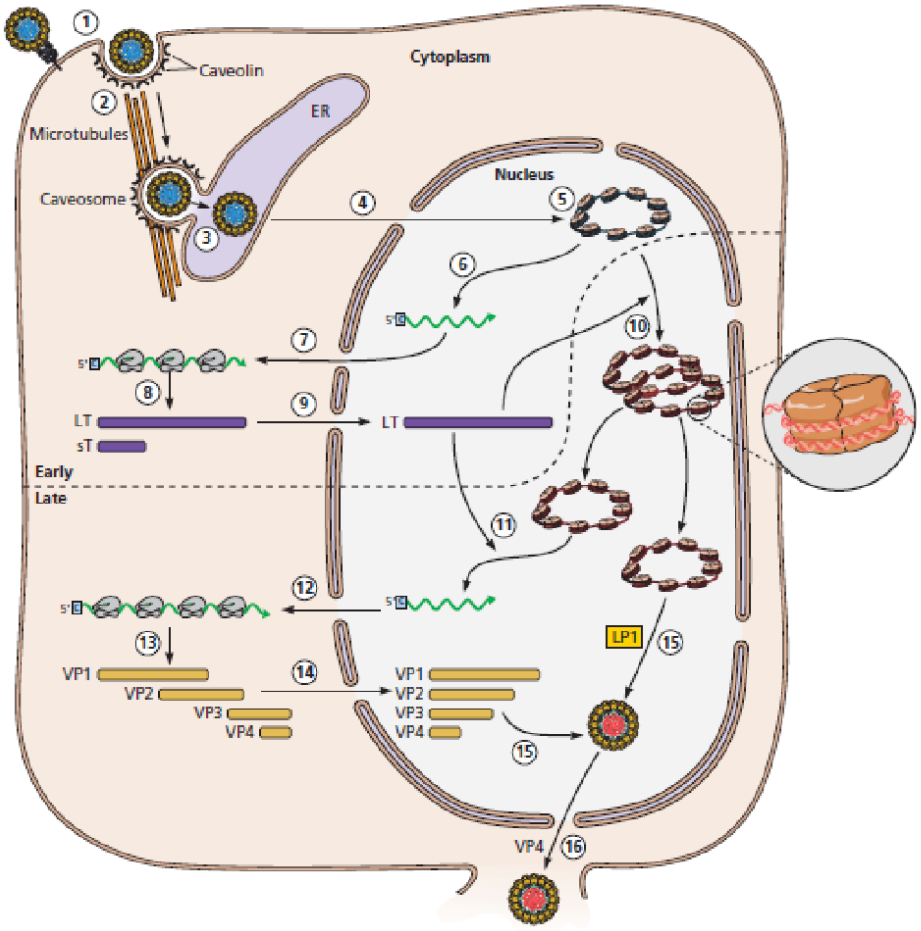
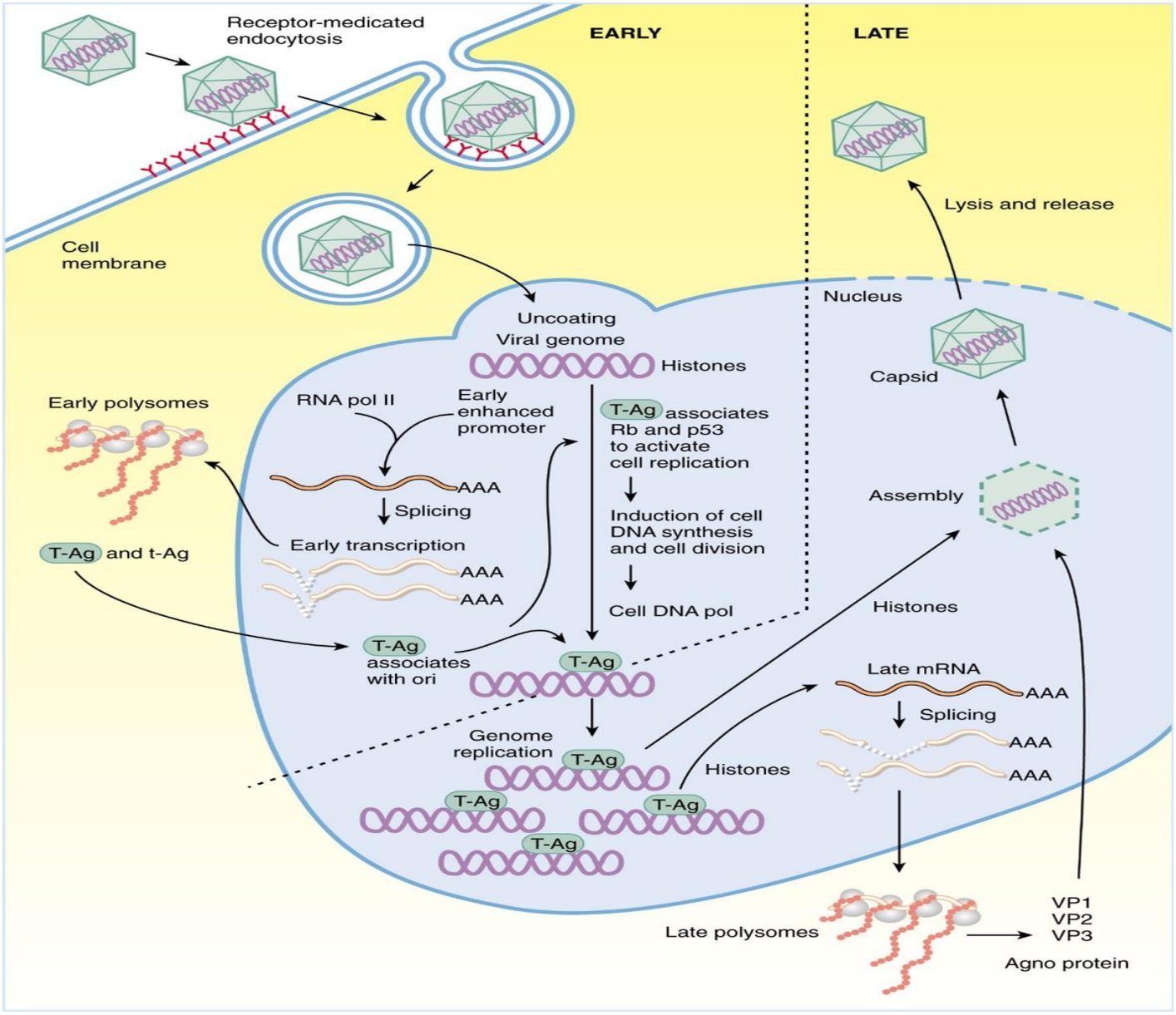


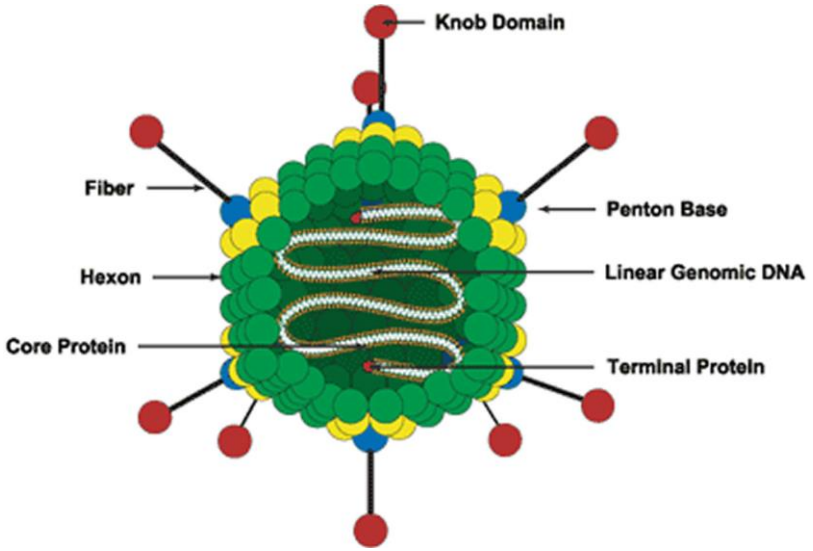
Figure 24 Single-cell reproductive cycle of simian virus 40. **(1)** The virus particle attaches to susceptible monkey cells upon binding of VP1 to the ganglioside Gm1 (a glycolipid) on the surface. **(2)** The particle is then endocytosed in caveolae, transported to the endoplasmic reticulum, and **(3)** enters that organelle. **(4)** Subsequently, it is transported to the nucleus and uncoated by unknown mechanisms. **(5)** The viral genome packaged by cellular nucleosomes is found within the nucleus. **(6)** The early transcription unit is transcribed by host cell RNA polymerase II. **(7)** After alternative splicing and export to the cytoplasm, **(8)** the early mRNAs are translated to produce the early proteins LT and sT. **(9)** The former is imported into the nucleus, **(10)** where it binds to the origin of replication to initiate DNA synthesis. Apart from LT, all components needed for viral DNA replication are provided by the host cell. As they are synthesized, daughter viral DNA molecules associate with cellular nucleosomes to form the viral nucleoproteins often called minichromosomes. **(11)** LT also stimulates transcription of the late gene from replicated viral DNA templates. **(12)** Processed late mRNAs are exported to the cytoplasm and **(13)** translated to produce the structural proteins VP1, VP2, and VP3, as well as VP4. **(14)** The structural proteins are imported into the nucleus and **(15)** assemble around viral minichromosomes to form virus particles. **(16)** Release of progeny virus particles is facilitated by VP4.

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


Receptor-medicated endocytosis
EARLY
LATE
Cell membrane
Lysis and release
Nucleus
Uncoating
Viral genome
Histones
Capsid
Early polysomes
RNA pol II
Early enhanced promoter
T-Ag associates Rb and p53 to activate cell replication
AAA
Splicing
Induction of cell DNA synthesis and cell division
Early transcription
AAA
AAA
Cell DNA pol
T-Ag and t-Ag
Assembly
T-Ag associates with ori
T-Ag
Histones
Late mRNA
AAA
Splicing
Genome replication
T-Ag
AAA
AAA
Histones
T-Ag
T-Ag
T-Ag
Late polysomes
VP1
VP2
VP3
Agno protein

Adenoviridae

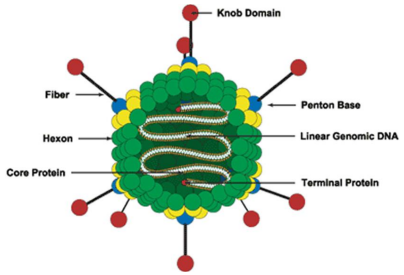
- Gli adenovirus sono stati isolati per la prima volta nel 1953 in una coltura cellulare adenoide umana. Da allora, sono stati riconosciuti circa 100 sierotipi, almeno 57 dei quali infettano l'uomo.
- Tutti i sierotipi umani sono inclusi in uno dei cinque generi della famiglia degli Adenoviridae: i **Mastadenovirus**.
- Esistono 7 sottogruppi per gli adenovirus umani (da A a G).
- Disturbi comuni causati dagli adenovirus includono infezione del tratto respiratorio, faringite, congiuntivite, cistite emorragica e gastroenterite.
- Gli Adenovirus vengono anche utilizzati nelle come vettori genici per terapie geniche e vaccini e studiati per la terapia oncolitica.



Adenoviridae

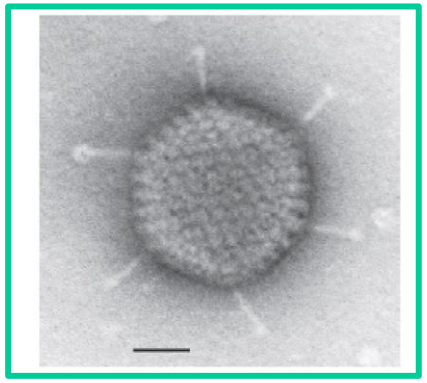
Group	Serotype	Associated Disease or Infections	References
A	12, 18, 31, 61	gastrointestinal, respiratory, urinary, cryptic enteric infection, linked to obesity, meningoencephalitis	[7,41–44]
B	3, 7, 11, 14, 16, 21, 34, 35, 50, 55, 66	conjunctivitis, gastrointestinal, respiratory, urinary, pneumonia, meningoencephalitis, cystitis	[7,41,42,44–47]
C	1, 2, 5, 6, 57	respiratory, gastrointestinal, obesity, pneumonia, hepatitis	[7,41,42,45]
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51, 53, 54, 56, 58-60, 63-67	conjunctivitis, gastrointestinal, linked to obesity, meningoencephalitis	[7,42,43,45,48]
E	4	conjunctivitis, respiratory, pneumonia	[7,41,47]
F	40, 41	gastrointestinal, infantile diarrhea	[7,42,49]
G	52	gastrointestinal	[7,42]

Brenetta et al, Biomedicine 2019



Adenoviruses are capable of causing lytic infections (for example in mucoepithelial cells), latent infections (for example in lymphoid cells and in the adenoids), and transforming infections (in hamster cells).

Adenoviridae



Doppio filamento lineare di 30-38 kbp, contenente 30-40 geni

La sequenza terminale di ciascun filamento è una ripetizione invertita di 100-150 bp (ITR), importante durante la replicazione. Le ITR contengono le origini di replicazione.

Al 5' di ogni filamento è associata una proteina di 55 kDa nota come proteina terminale (TP)

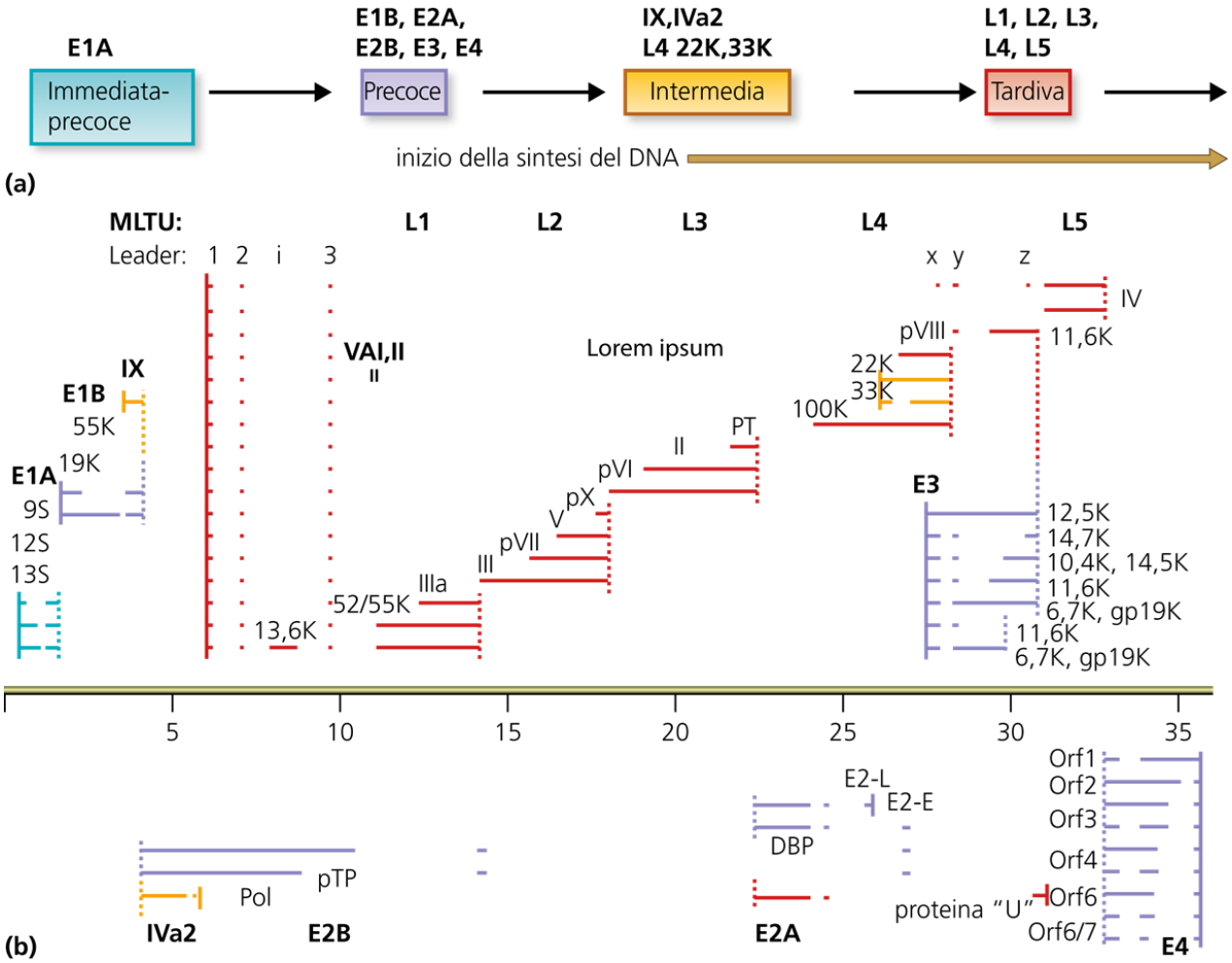
I geni sono organizzati in cluster e sono espressi a partire da un ridotto numero di promotori; da ogni promotore sono espresse più proteine (splicing alternativo, poliadenilazione differenziale)

In questi virus sono trascritti entrambi i filamenti del genoma

Tutti gli mRNA sono prodotti dalla RNApol II, due piccoli ncRNA (VA RNAs) dalla RNApol III

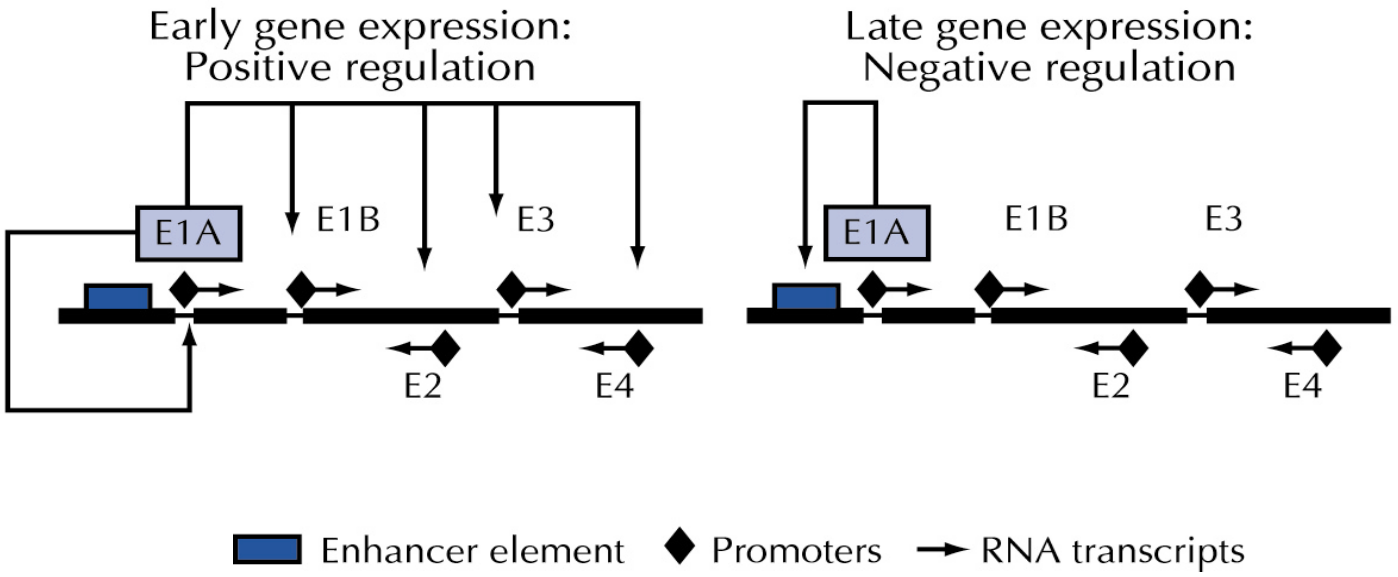
From Flint et al. *Principles of Virology* (2015), ASM Press

Genome expression in Adenovirus

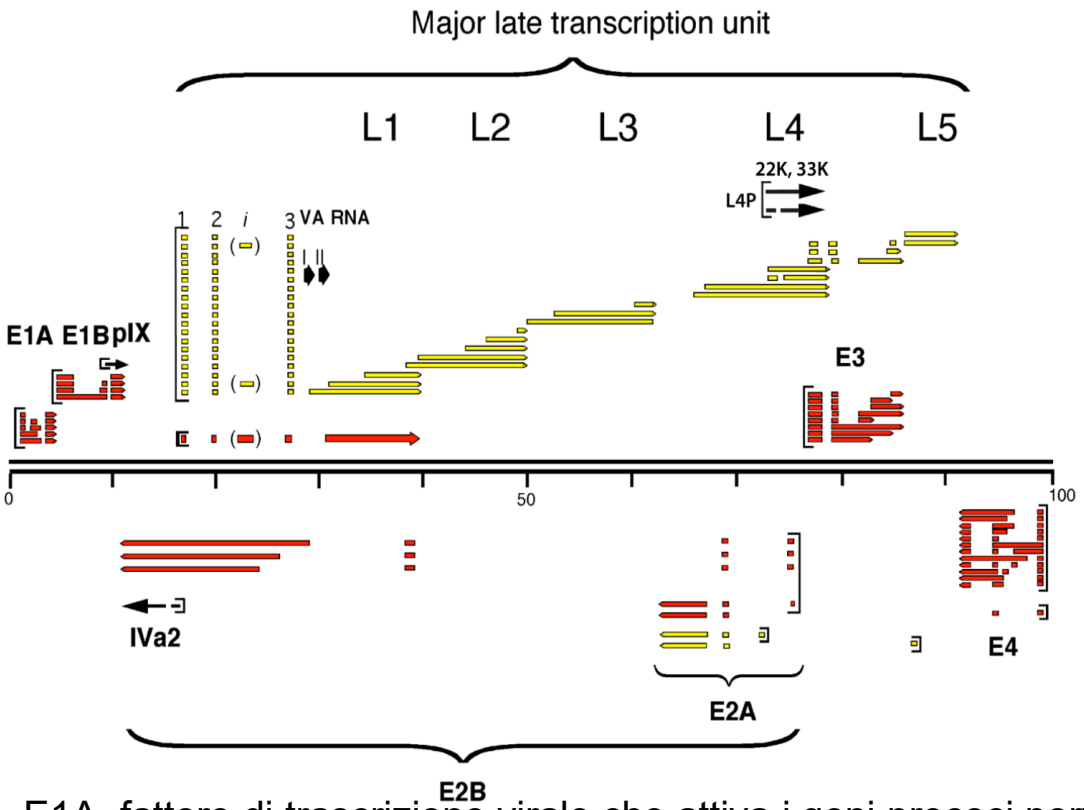


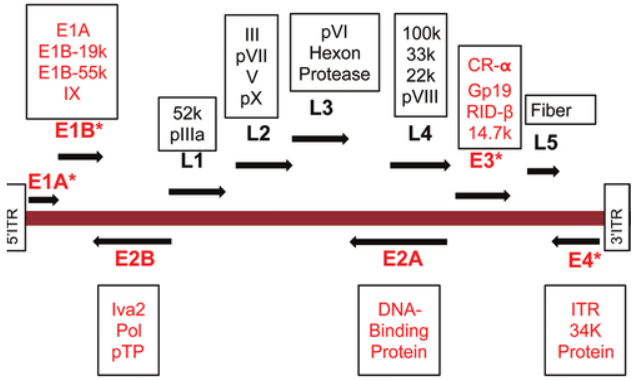
Nigel J. Dimmock, Andrew J. Easton, Keith N. Leppard
Introduzione alla Virologia Moderna *Casa Editrice Ambrosiana. 2017*

Genome expression in Adenovirus



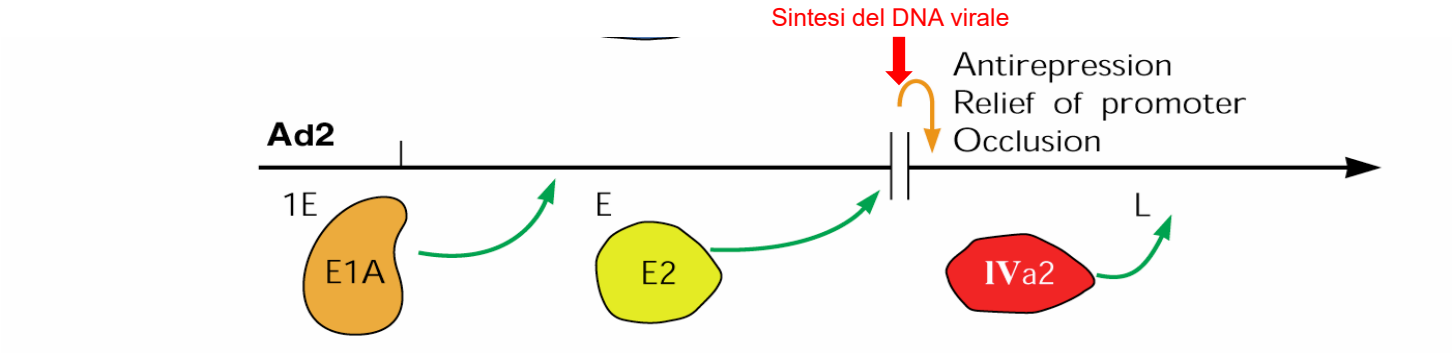
From Cann *Principles of molecular virology* (2001). Academic Press





- E1A, fattore di trascrizione virale che attiva i geni precoci portando alla produzione dei prodotti di E1B
- È inoltre fondamentale per la riprogrammazione della cellula ospite (inibisce il repressori del ciclo cellulare pRb),
- E1B inibisce l'apoptosi (p53),
- E1A induce poi i geni della E2 che codificano per la DNApol, la Proteina terminale Pte una DBP che, raggiunte elevate concentrazioni, reprime i geni della regione E2 ad eccezione del gene E2A (DBP).
- La proteina E4 interferisce con la funzione del sito di poliadenilazione a valle di L1, si trascrive un lungo mRNA che attraverso processi di splicing permette l'espressione delle L1-5.

Sequenza temporale degli eventi in Adenovirus



Le Proteine immediato-precoci E1A, alle quali sono associate molteplici funzioni, sono necessarie per l'attivazione dell'espressione delle unità trascrizionali precoci.
Tra queste c'è la regione E2, che codifica proteine richieste per la sintesi del DNA virale.
L'accumulo dei nuovi genomi virali a DNA porta alla de-repressione della trascrizione del gene che codifica la proteina di legame al DNA IVa 2 e conseguente attivazione della trascrizione a partire dal principale promotore tardivo. Questo promotore controlla la sintesi della maggior parte delle proteine strutturali.

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

Major Adenovirus Proteins

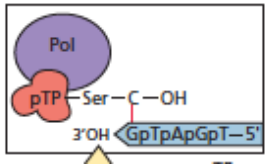
Gene	Number	Molecular Mass (kDa)	Functions of Proteins
*E1A**			Activates viral gene transcription Binds cellular growth suppressor (p105RB) to promote cell growth and transformation Deregulates cell growth Inhibits activation of interferon response elements
E1B			Binds cellular growth suppressor (p53) to promote cell growth and transformation Blocks apoptosis
E2			Activates some promoters Terminal protein on DNA DNA polymerase
E3			Prevents TNF-α action; MHC I expression
E4			Limits viral cytopathologic effect
VA RNAs			Inhibits interferon response
Capsid	II	120	Contains family antigen and some serotyping antigens
	III	85	Penton base protein Toxic to tissue culture cells
	IV	62	Fiber Responsible for attachment; contains some serotyping antigens
	VI	24	Hexon-associated proteins
	VIII	13	Penton-associated proteins
	IX	12	"Capsid cement" nonessential
	IIIa	66	"Facilitates assembly"
Core	V	48	Core protein 1: DNA-binding protein
	VII	18	Core protein 2: DNA-binding protein

*Early genes encode several messenger RNAs and proteins by alternative splicing patterns.

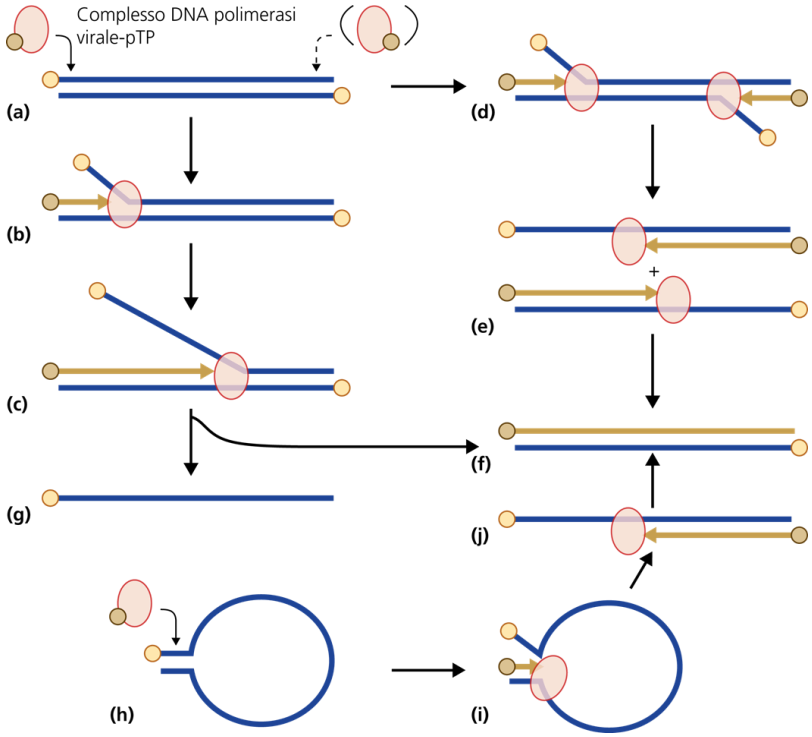
E, Early; *MHC I*, major histocompatibility complex I; *RB*, retinoblastoma gene product; *TNF-α*, tumor necrosis factor-α; *VA*, virus-associated.

Replicazione genoma di Adenovirus

L'assemblaggio della DNA polimerasi (Pol) e della proteina preterminale (pTP) a livello dell'origine di replicazione è seguito dal legame covalente di dCMP (desossicitidina 5'-monofosfato) ad uno specifico residuo di serina di pTP catalizzato da Polimerasi virale.

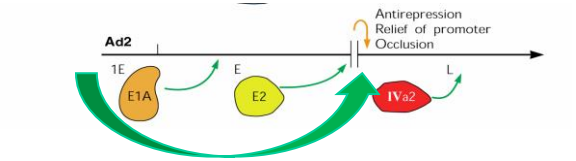


Il 3' OH fornito dal complesso pTP-dCMP che funge da innesco per la sintesi continua del DNA virale.



Nigel J. Dimmock, Andrew J. Easton, Keith N. Leppard
Introduzione alla Virologia Moderna *Casa Editrice Ambrosiana. 2017*

Replicazione genoma di Adenovirus

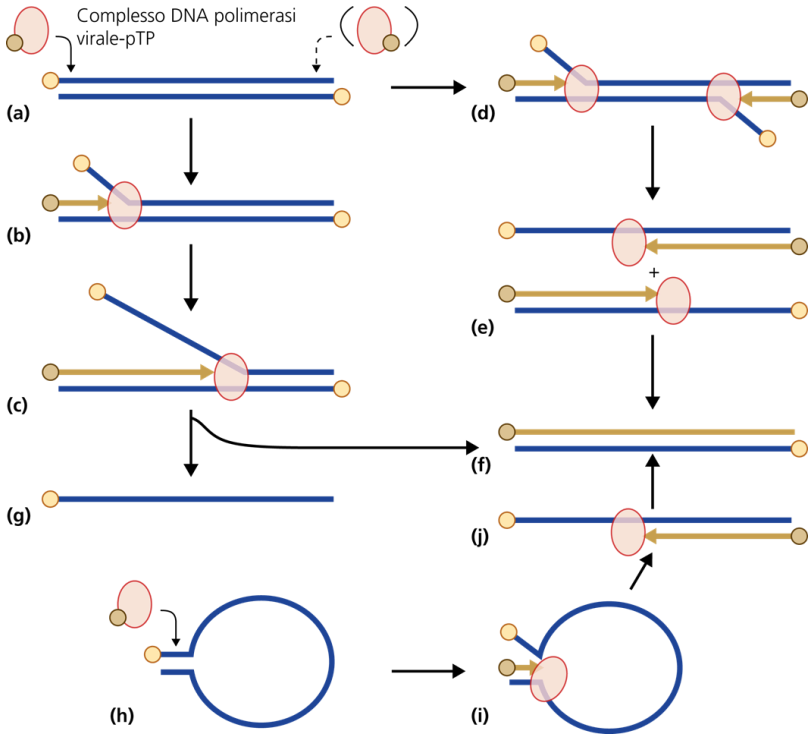


Il 3' OH fornito dal complesso pTP-dCMP innesca la sintesi continua del DNA virale.
La sintesi dunque procede in direzione 5'-3' scalzando il filamento non usato come templato.

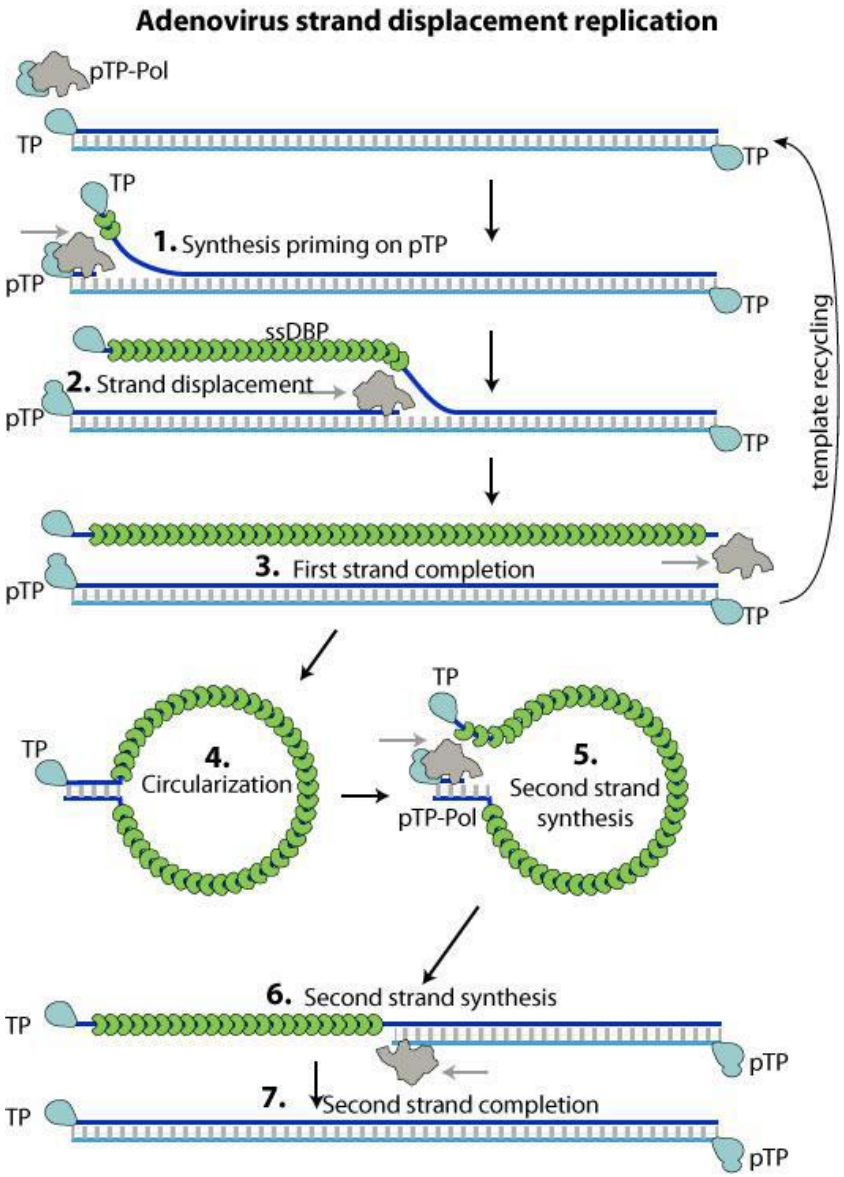
Questo processo può cominciare su uno solo o contemporaneamente sui due filamenti

Quando un solo filamento viene replicato ad ogni round, il filamento scalzato, grazie alla presenza di sequenze ripetute invertite terminali può formare per appaiamento un breve duplex terminale (manico di padella) e può essere poi replicato con lo stesso meccanismo

Per intervento di una proteasi si formano così i nuovi genomi provvisti di una TP associata al 5' di ogni filamento

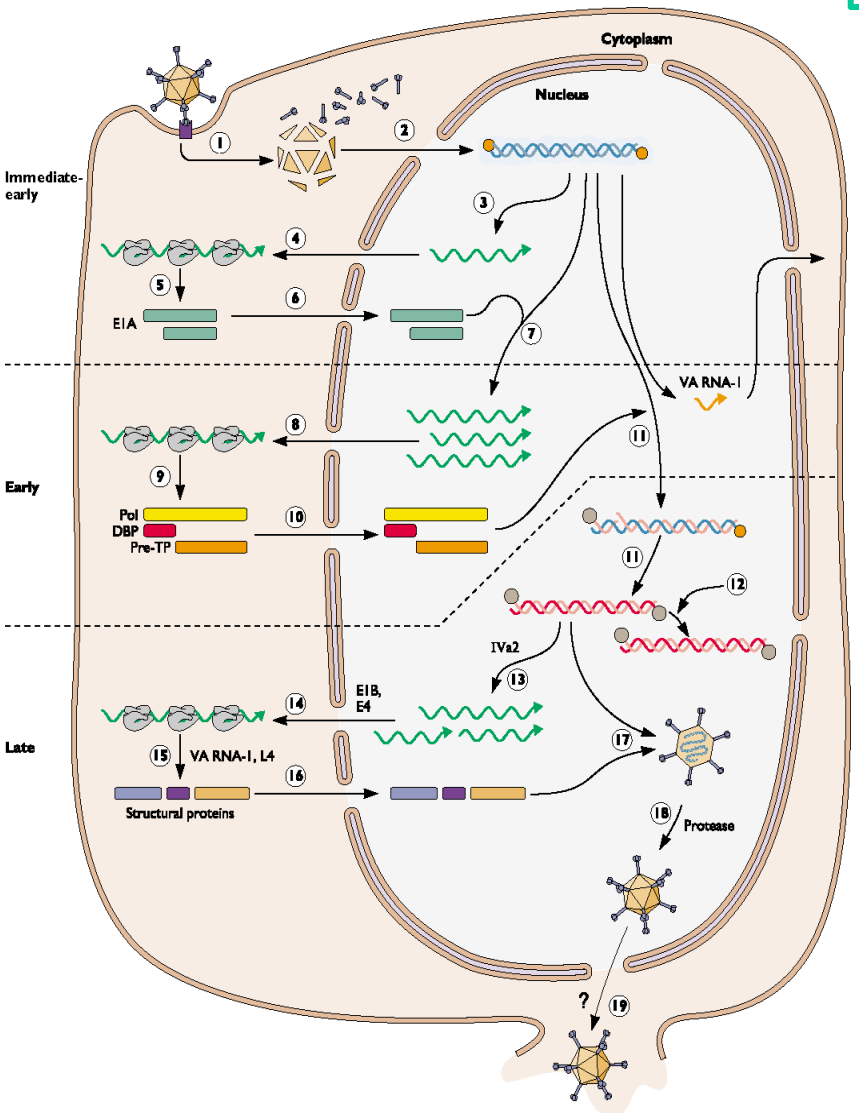


Nigel J. Dimmock, Andrew J. Easton, Keith N. Leppard
Introduzione alla Virologia Moderna *Casa Editrice Ambrosiana. 2017*



Replicazione del genoma di Adenovirus

Adenovirus



- La replicazione del DNA virale avviene nel **nucleo** ed è mediata dalla **DNA polimerasi virale**
- La trascrizione dei geni tardivi inizia dopo la replicazione del DNA. La maggior parte dei singoli mRNA tardivi sono generati da un ampio (83% del genoma) trascritto primario che viene elaborato in singoli mRNA.
- Le proteine del capside vengono prodotte nel citoplasma e quindi trasportate nel nucleo per l'assemblaggio virale. I procapsidi vuoti prima si assemblano, quindi il DNA virale e le proteine del nucleo entrano nel capside attraverso un'apertura in uno dei vertici.
- I processi di replicazione e assemblaggio sono inefficienti e soggetti a errori, producendo solo un'unità infettiva per 2300 particelle. DNA, proteine e numerose particelle difettose si accumulano nei corpi di inclusione nucleare.
- Il virus rimane nella cellula e viene rilasciato quando la cellula degenera e lisa.

Herpesviridae

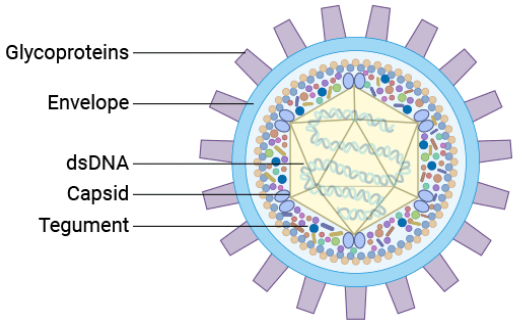
Classe di virus a **dsDNAlineare (immediatamente dopo l'infezione diventa circolare)** con un genoma di grandi dimensioni (130-240 Kb con 60-120 geni) e una particella virale dotata di pericapside.

La regione tra nucleocapside di tipo icosaedrico e rivestimento è detta «tegumento» e contiene da 15 a 20 proteine differenti.

Tutti gli herpesvirus sono in grado di stabilire una infezione latente nei loro ospiti incluso l'uomo.

Subfamily	Taxonomic name	Common name
Alpha-herpesvirinae	HHV-1	Herpes simplex virus 1 (HSV-1)
	HHV-2	Herpes simplex virus 2 (HSV-2)
	HHV-3	Varicella-zoster virus (VZV)
Beta-herpesvirinae	HHV-5	Human cytomegalovirus (HCMV)
	HHV-6	HHV-6 vairant A or B
	HHV-7	HHV-7
Gamma-herpesvirinae	HHV-4	Epstein-Barr virus (EBV)
	HHV-8	Kaposi's sarcoma-associated herpesvirus (KSHV)

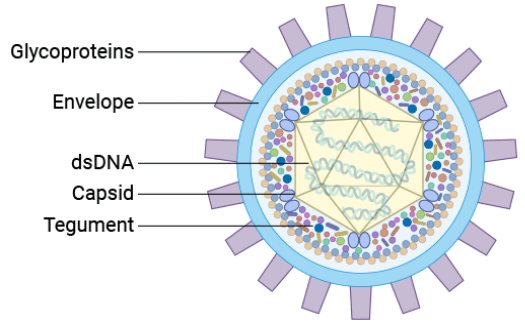
HHV, human herpesvirus.



Herpesviridae

Subfamily	Taxonomic name	Common name
Alpha-herpesvirinae	HHV-1	Herpes simplex virus 1 (HSV-1)
	HHV-2	Herpes simplex virus 2 (HSV-2)
	HHV-3	Varicella-zoster virus (VZV)
Beta-herpesvirinae	HHV-5	Human cytomegalovirus (HCMV)
	HHV-6	HHV-6 vairant A or B
	HHV-7	HHV-7
Gamma-herpesvirinae	HHV-4	Epstein-Barr virus (EBV)
	HHV-8	Kaposi's sarcoma-associated herpesvirus (KSHV)

HHV, human herpesvirus.



Properties Distinguishing the Herpesviruses

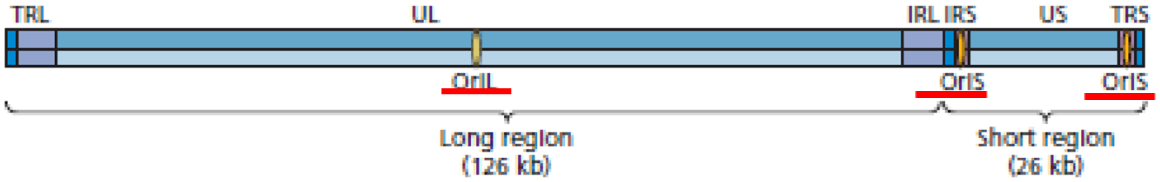
Subfamily	Virus	Primary Target Cell	Site of Latency	Means of Spread
Alphaherpesvirinae				
HHV-1	Herpes simplex type 1	Mucoepithelial cells	Neuron	Close contact (STD)
HHV-2	Herpes simplex type 2	Mucoepithelial cells	Neuron	Close contact (STD)
HHV-3	Varicella-zoster virus	Mucoepithelial and T cells	Neuron	Respiratory and close contact
Gammaherpesvirinae				
HHV-4	Epstein-Barr virus	B cells and epithelial cells	B cell	Saliva (kissing disease)
HHV-8	Kaposi sarcoma–related virus	Lymphocytes and other cells	B cell	Close contact (sexual), saliva?
Betaherpesvirinae				
HHV-5	Cytomegalovirus	Monocytes, granulocytes, lymphocytes, and epithelial cells	Monocyte, myeloid stem cell, and ?	Close contact (STD), transfusions, tissue transplant, and congenital
HHV-6	Herpes lymphotropic virus	Lymphocytes and ?	T cell and ?	Saliva
HHV-7	HHV-7	Like HHV-6	T cell and ?	Saliva

HHV, Human herpesvirus; *STD*, sexually transmitted disease.

Herpesviridae

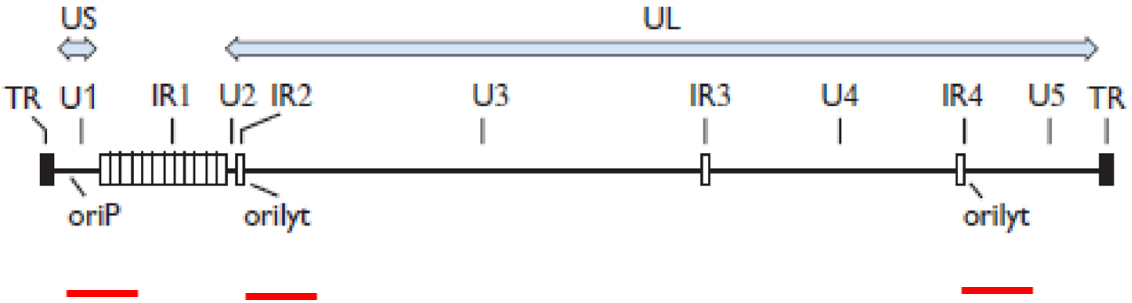
I vari membri sono piuttosto diversi in termini di sequenza genomica, ma condividono una struttura e organizzazione comune: il genoma (lungo da 130 kbp 240 kbp) contiene due regioni uniche (UL e US) fiancheggiate da ripetizioni invertite (TR e IR)

HSV-1



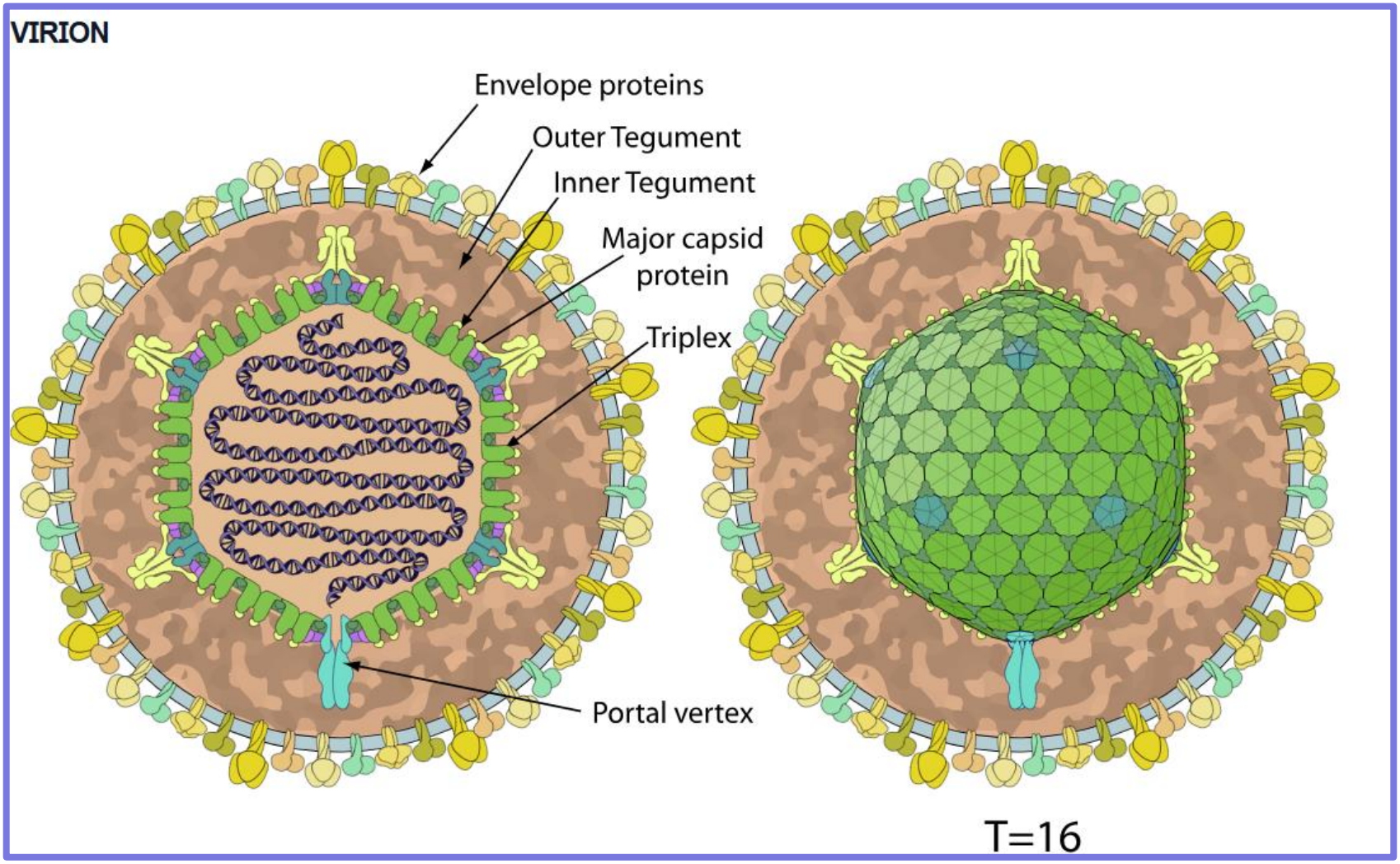
152 Kbp

EBV

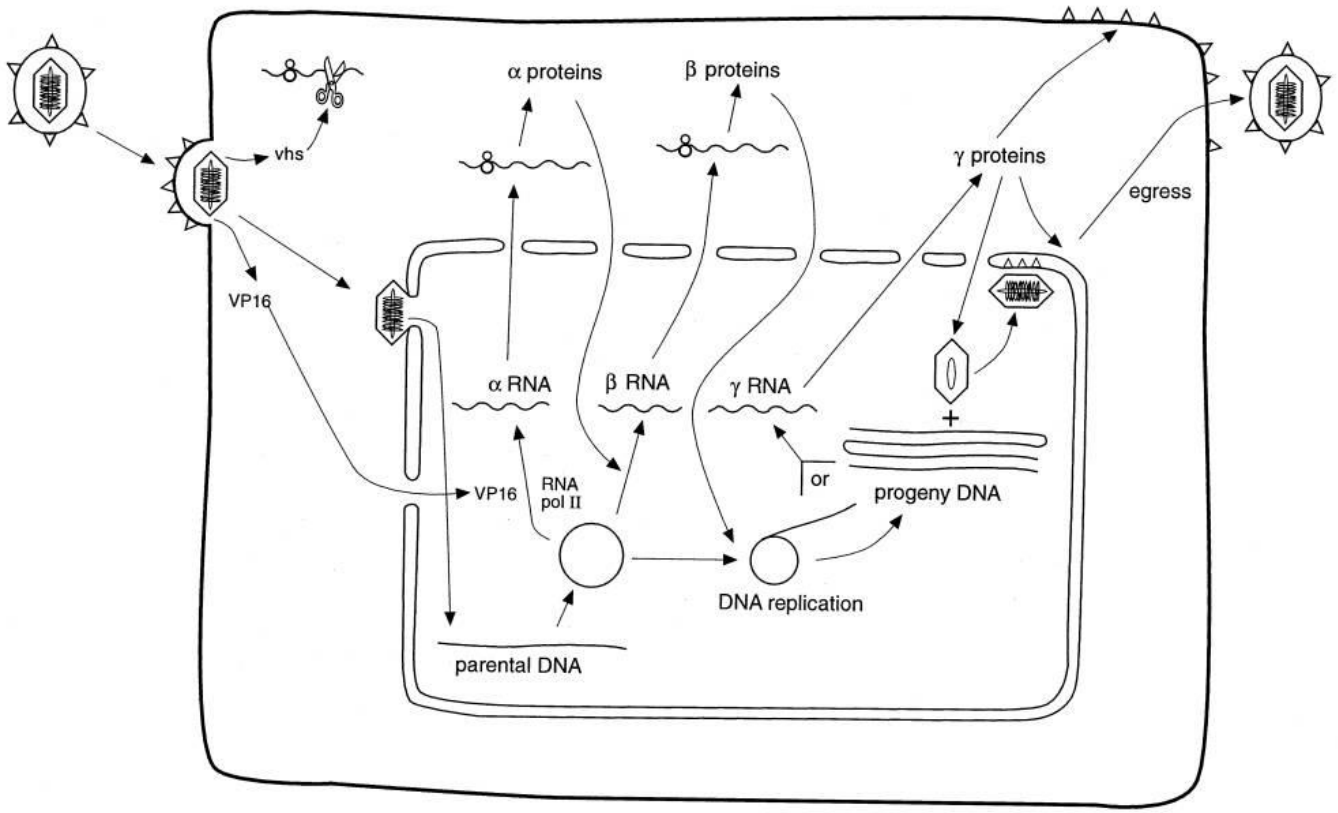


172 Kbp

Contiene tra i 60 e i 120 geni, a questo corrispondono virioni complessi contenenti circa 35 proteine. Poiché tutti i virus appartenenti a questa famiglia condividono le funzioni base per una infezione produttiva, la diversità nella complessità dei genomi è dovuta alla presenza di geni “dispensabili” per la moltiplicazione, ma coinvolti in aspetti specifici della patogenesi di ciascun tipo di virus.


VIRION
Envelope proteins
Outer Tegument
Inner Tegument
Major capsid protein
Triplex
Portal vertex
T=16

Herpesviridae



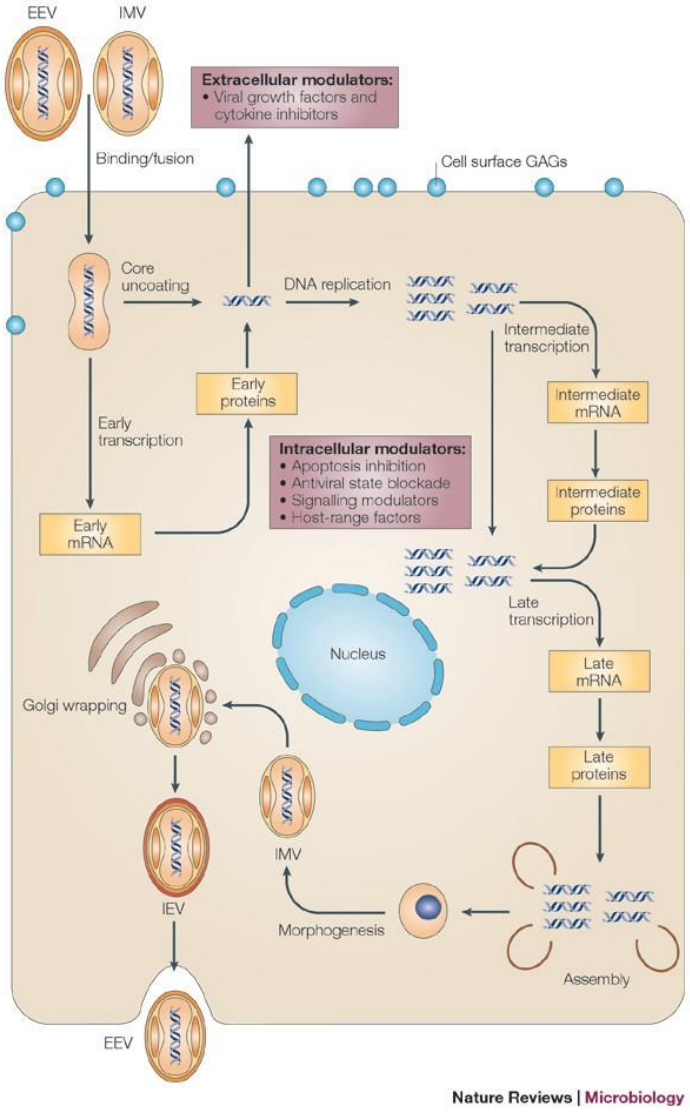
<u>Espressione gerarchica in tre fasi temporali:</u>
alpha (immediato precoce);
beta (precoce);
gamma (tardiva)

Poxvirus

Agenti eziologici del Vaiolo, del mollusco contagioso, del vaiolo delle scimmie (mpox) e di altre zoonosi (cowpox etc).

Nonché importanti agenti per la vaccinazione sia come Vaccinia virus sia come vettori virali

Questa classe di virus è caratterizzata da non avere una fase nucleare, tutto accade nel citoplasma



Poxvirus

Si distinguono due particelle virali:
il virus maturo (MV) e il virus rivestito (EV) entrambi dotati di envelope, ma il virus EV presenta uno strato di rivestimento esterno aggiuntivo che origina dal Golgi della cellula ospite e presenta le proteine di fusione

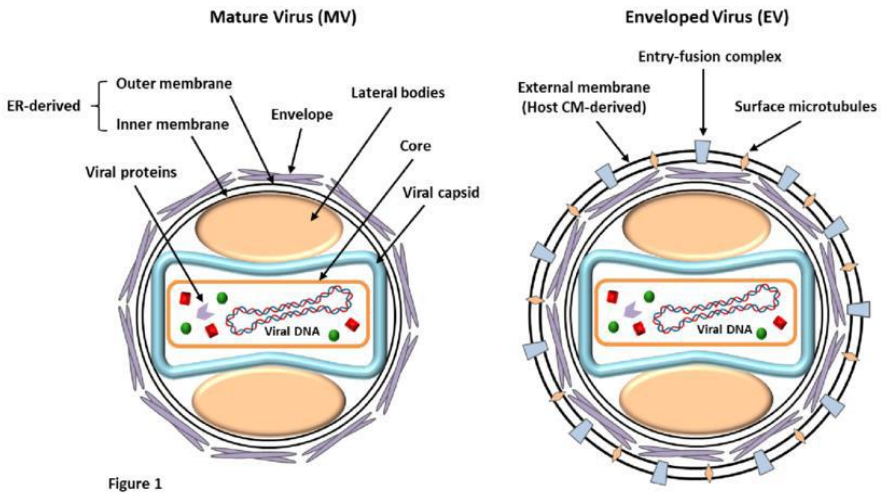
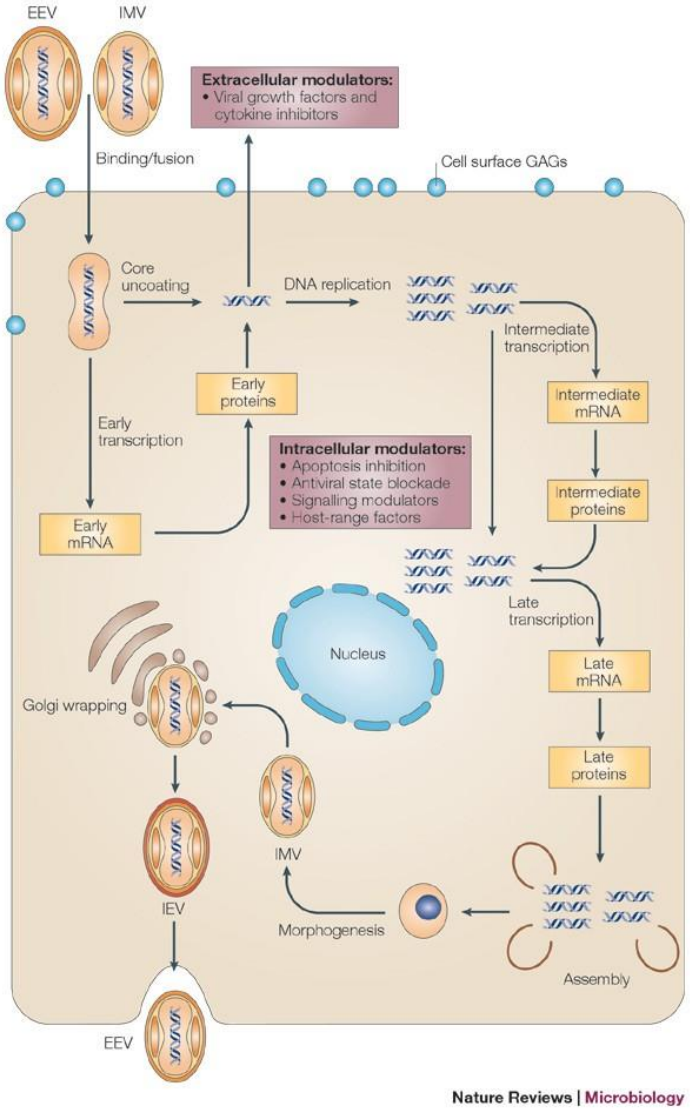
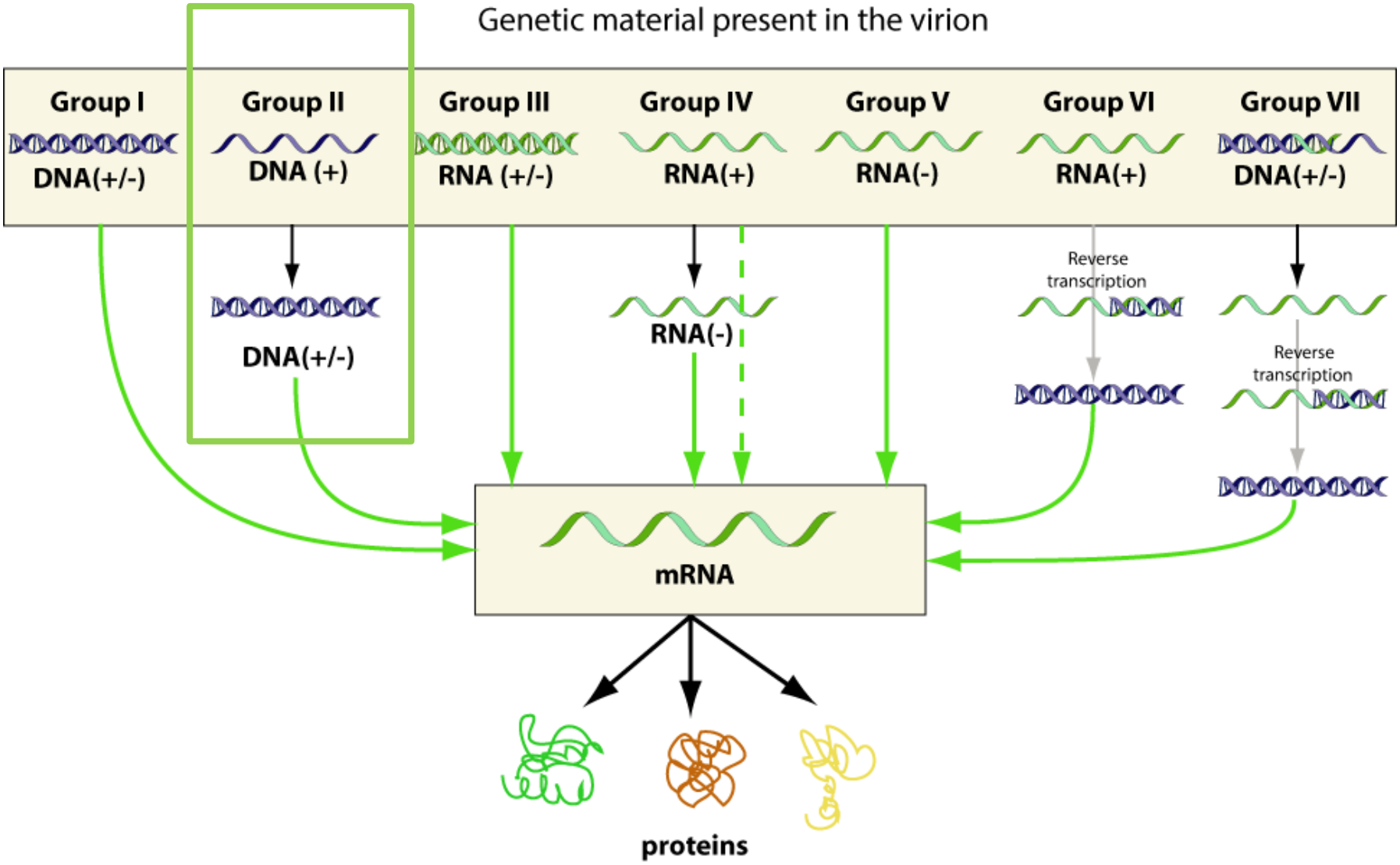


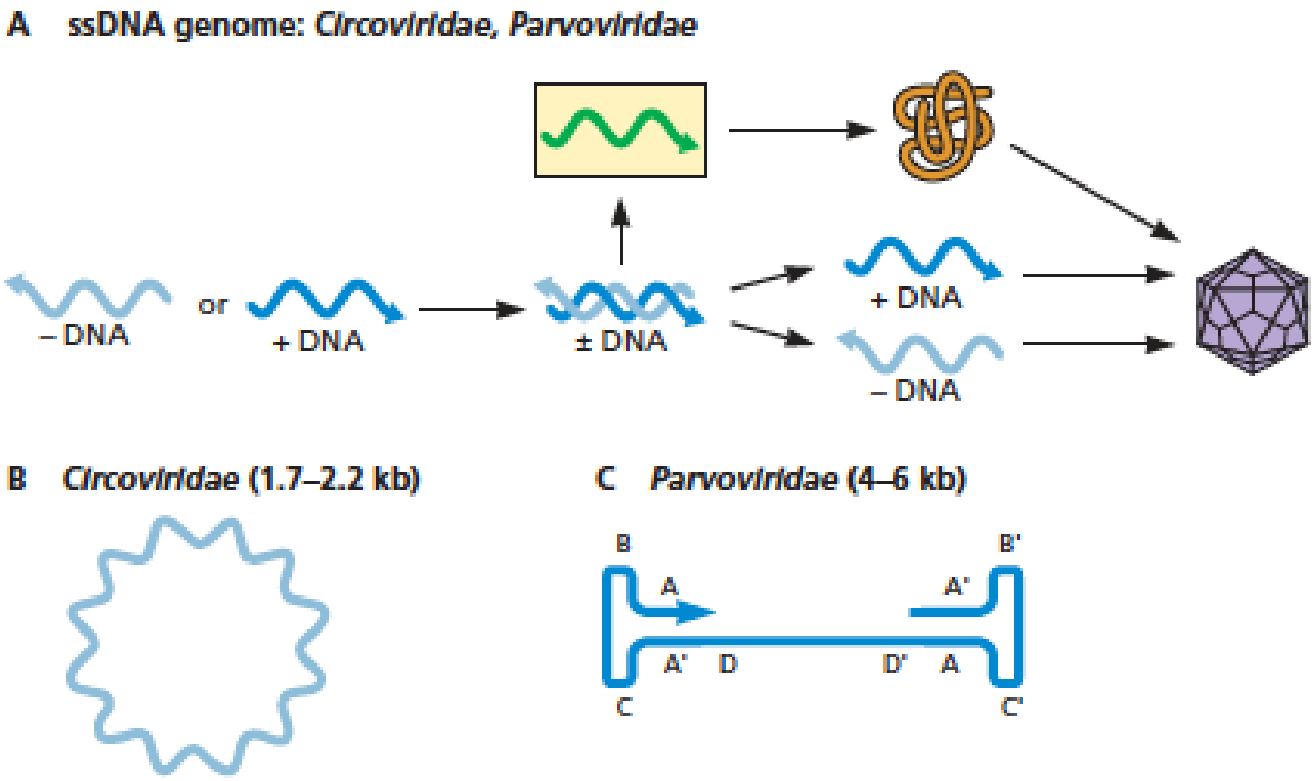
Figure 1




Genetic material present in the virion
Group I
DNA(+/-)
Group II
DNA (+)
Group III
RNA (+/-)
Group IV
RNA(+)
Group V
RNA(-)
Group VI
RNA(+)
Group VII
DNA(+/-)
DNA(+/-)
RNA(-)
Reverse transcription
Reverse transcription
mRNA
proteins

Class II: ssDNA, Expression-Replication

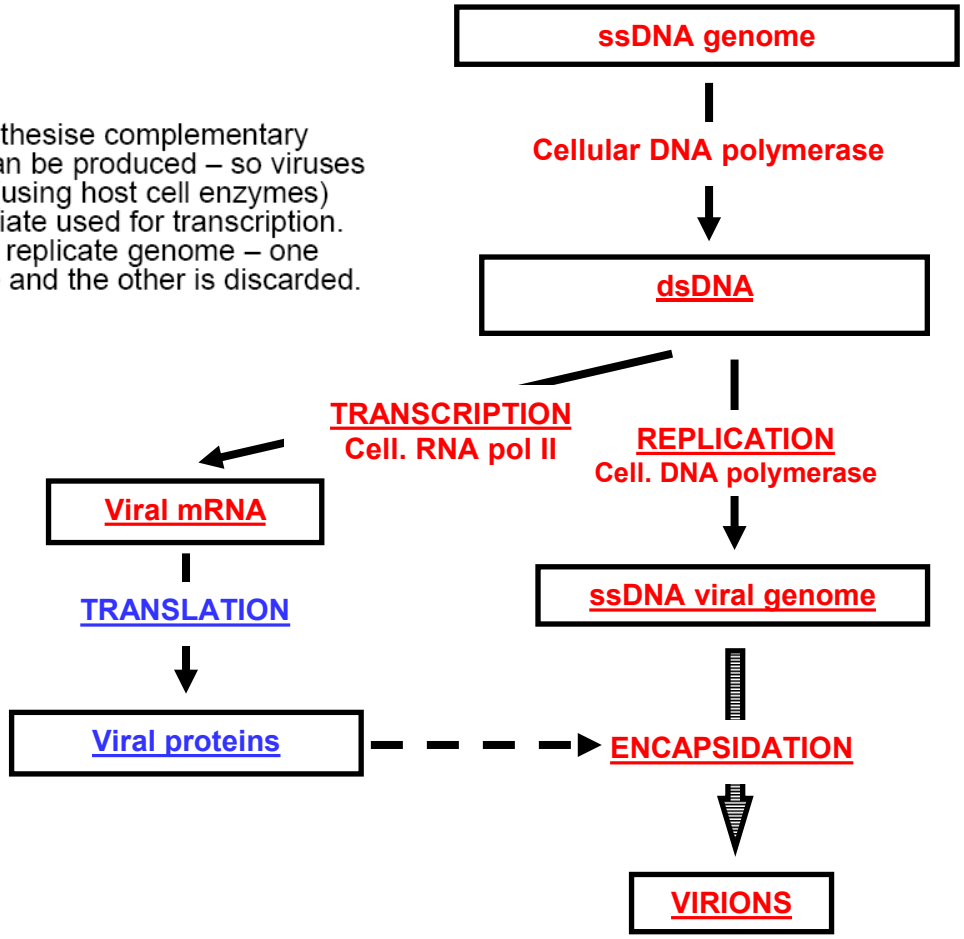
Replication occurs in the nucleus and involves the formation of a double-stranded intermediate which serves as a template for the mRNA transcription and for the synthesis of new viral genomes



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

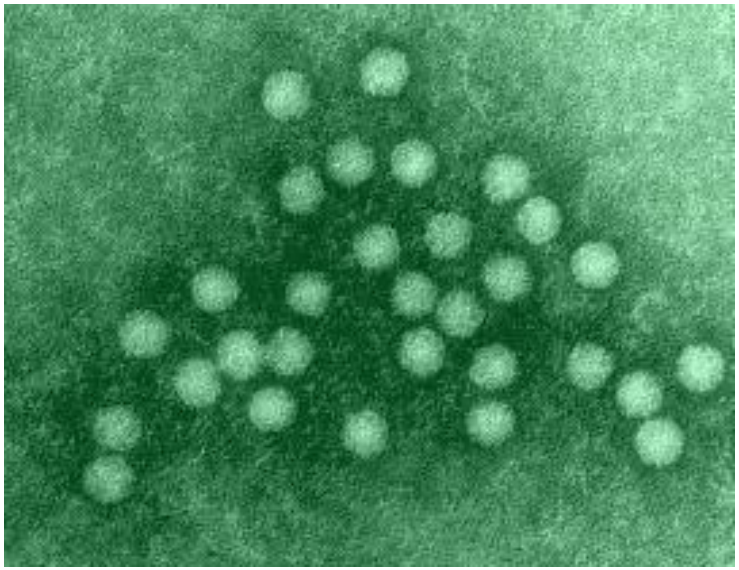
EXPRESSION-REPLICATION of GR II VIRUSES

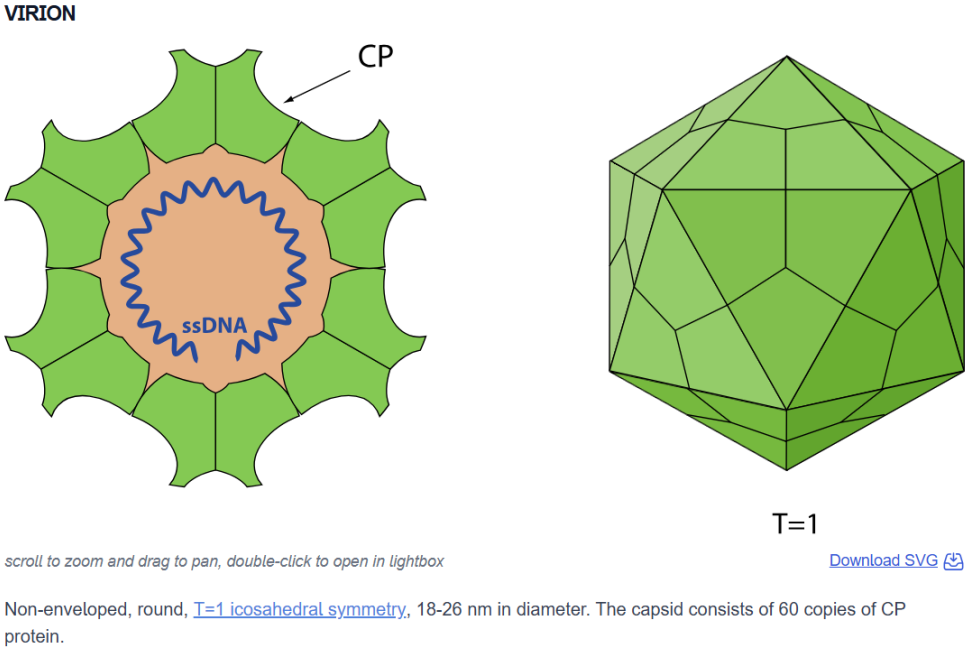
Class II – ssDNA – must synthesise complementary DNA strand before mRNA can be produced – so viruses form a dsDNA intermediate (using host cell enzymes) during replication – intermediate used for transcription. ds-intermediate also used to replicate genome – one strand becomes the genome and the other is discarded.



During S-phase

Parvoviridae family



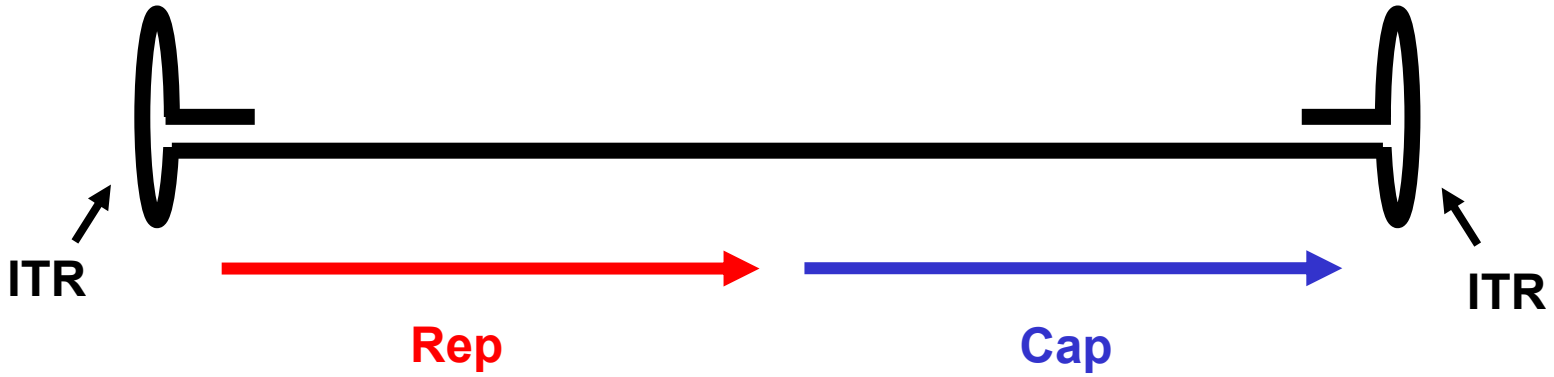


Non-enveloped icosahedral capsid (Ø 20 nm)

Sono eccezionalmente resistenti alle condizioni ambientali, spesso rimangono infettivi per mesi o anni.

A differenza dei virus a DNA più grandi, i parvovirus devono infettare le cellule mitoticamente attive perché non codificano per fattori in grado di stimolare la crescita cellulare o una polimerasi virale.

Parvovirus genome



Linear 5 kb ssDNA genome

ITR (inverted terminal repeat) : ORI + role in establishment of transcription complexes and packaging

Rep region : necessary for replication + cellular DNA polymerase recruitment

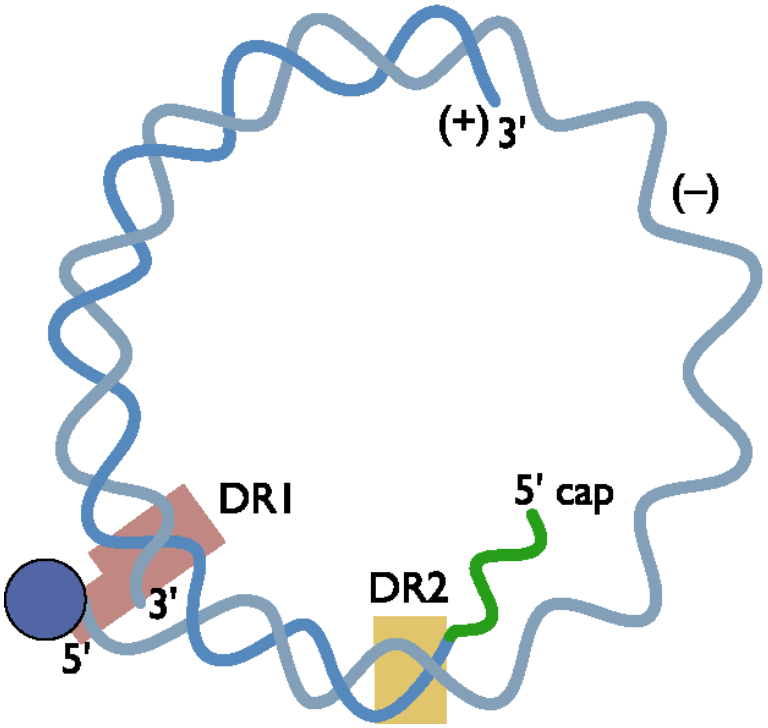
Cap region : encodes capsid proteins

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

The DNA in extracellular hepadnavirus particles is a partially duplex molecule of around 3.2 kb with circularity that is maintained by overlapping 5' ends.

The (-) strand has the polymerase, shown as a blue ball, attached to its 5' end.
The (+) strand has a capped RNA of 18 nucleotides at its 5' end. Direct repeats (10- to 12-bp) called DR1 and DR2 (colored purple and yellow, respectively) are present at he 5' ends .

As in retroviruses, these repeat sequences play the critical role of facilitating template transfers during reverse transcription.
In mammalian hepadnavirus genomes, the (+) strand is shorter than the (-) strand and has heterogeneous ends.



HBV GENOME ORGANIZATION

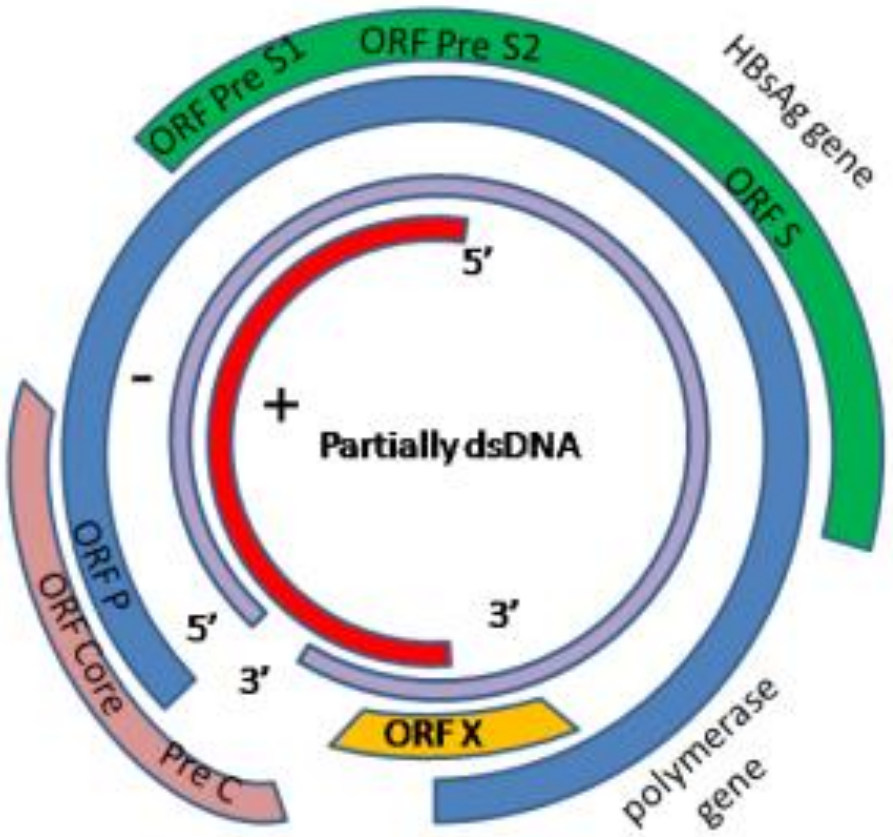
Overlapping genes organized in 4 regions :

•Region S ➔ envelope proteins : HBs, …

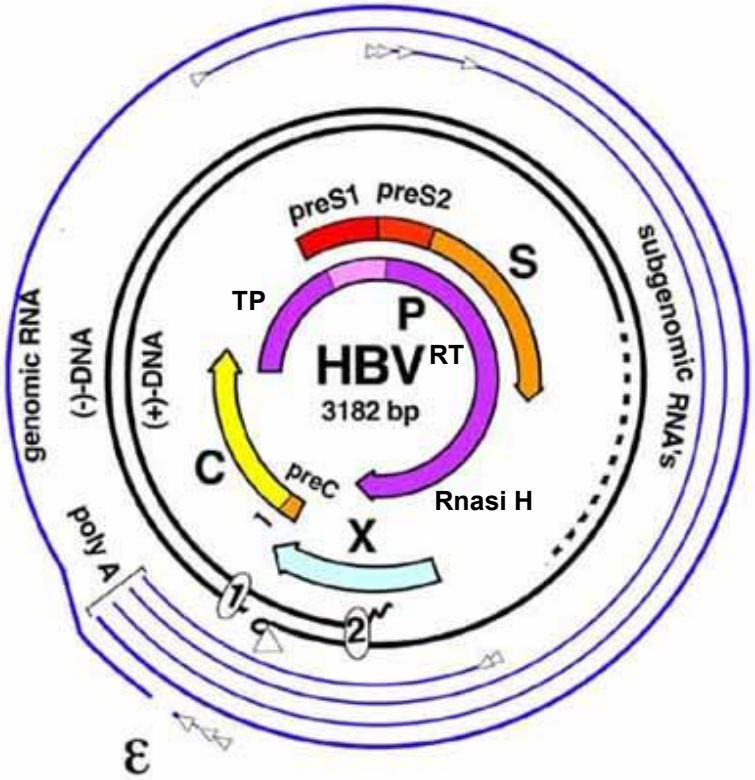
•Region C ➔ *core* proteins: HBc, HBe

•Region P ➔ viral Reverse transcriptase with 3 activities DNA dependent DNA polymerase + *Reverse Transcriptase* and *RNase H*

•Region X ➔ protein X, viral and cellular genome transactivator, associated with the development of liver cancer

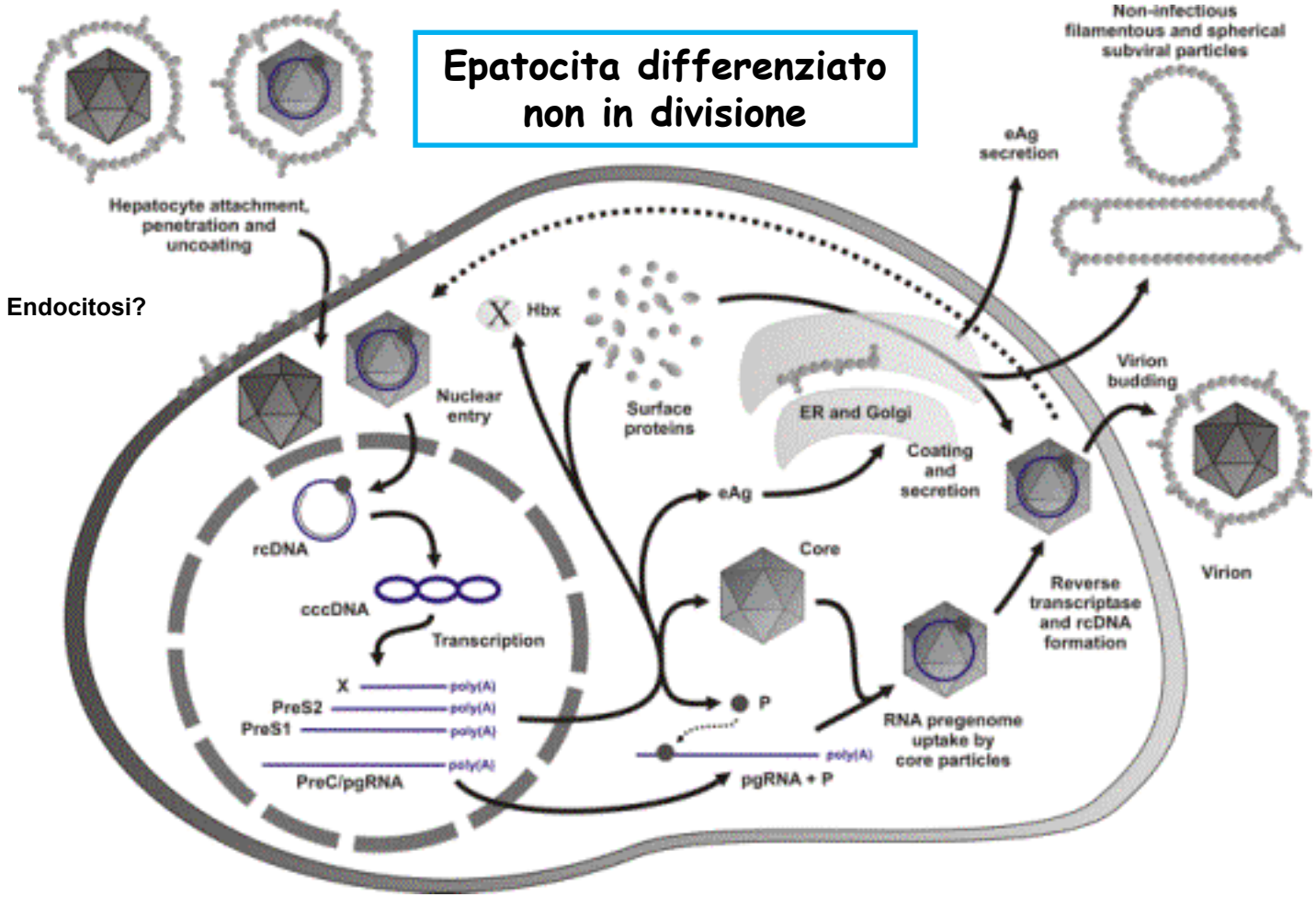


HBV Proteins



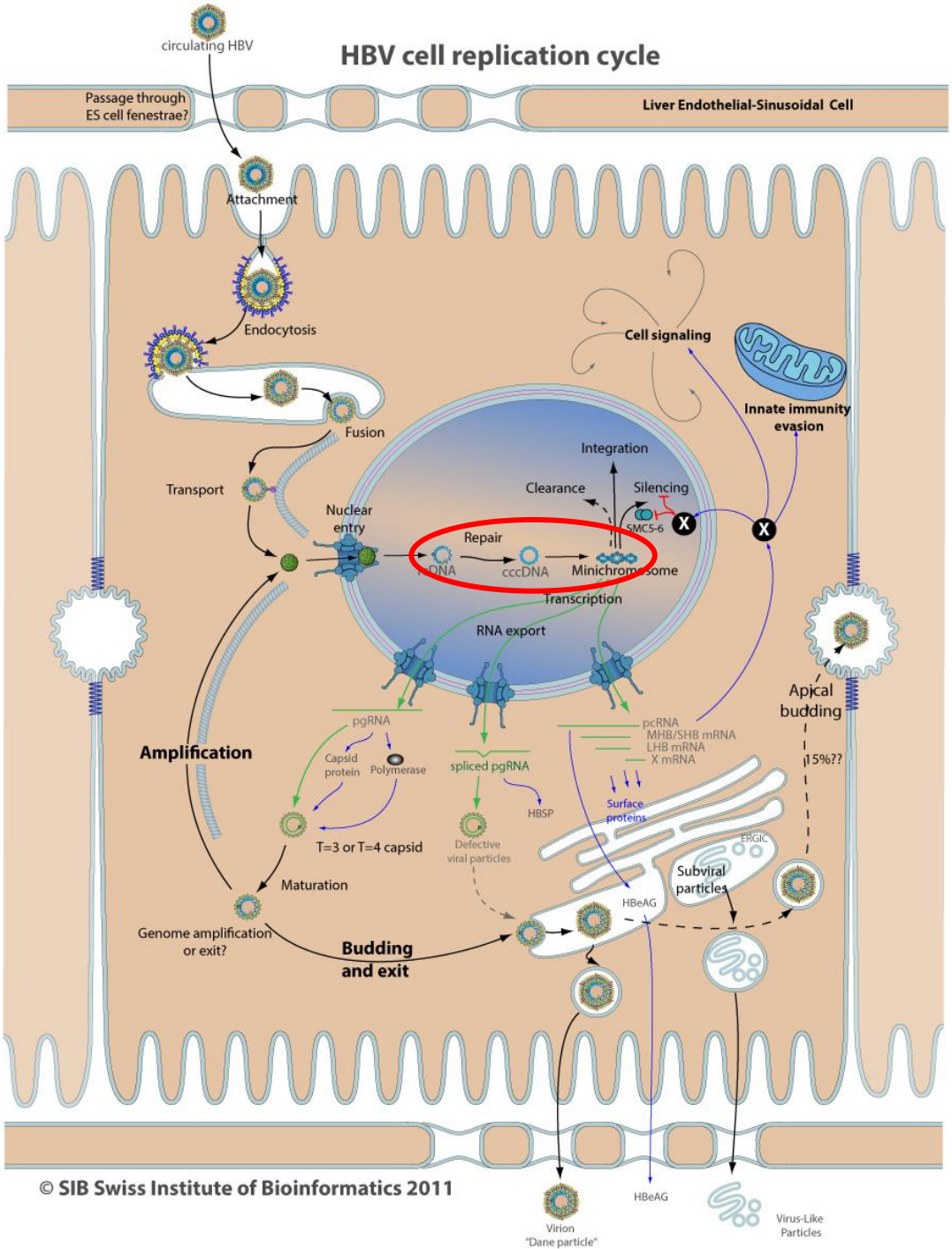
	Proteine	Funzione
S	Proteine dell'envelope: Piccola (HBsAg), Media e Grande	Glicoproteine di superficie
C	Proteina del core (HBcAg) antigene e (HBeAg)	Incapsida l'RNA pregenomico e il DNA genomico nel citoplasma Attività di immunomodulazione e inibizione della replicazione
P	Polimerasi	Trascrittasi inversa, Rnasi H (degrada RNA pregenomico durante la trascrizione inversa), DNA polimerasi
X	Proteina X	Transattivatore trascrizionale; cofattore per HCC

Epatocita differenziato non in divisione



L'integrazione non specifica di tratti di DNA di HBV non fa parte del ciclo virale, ma può avere un ruolo nello sviluppo di epatocarcinoma

HBV life cycle



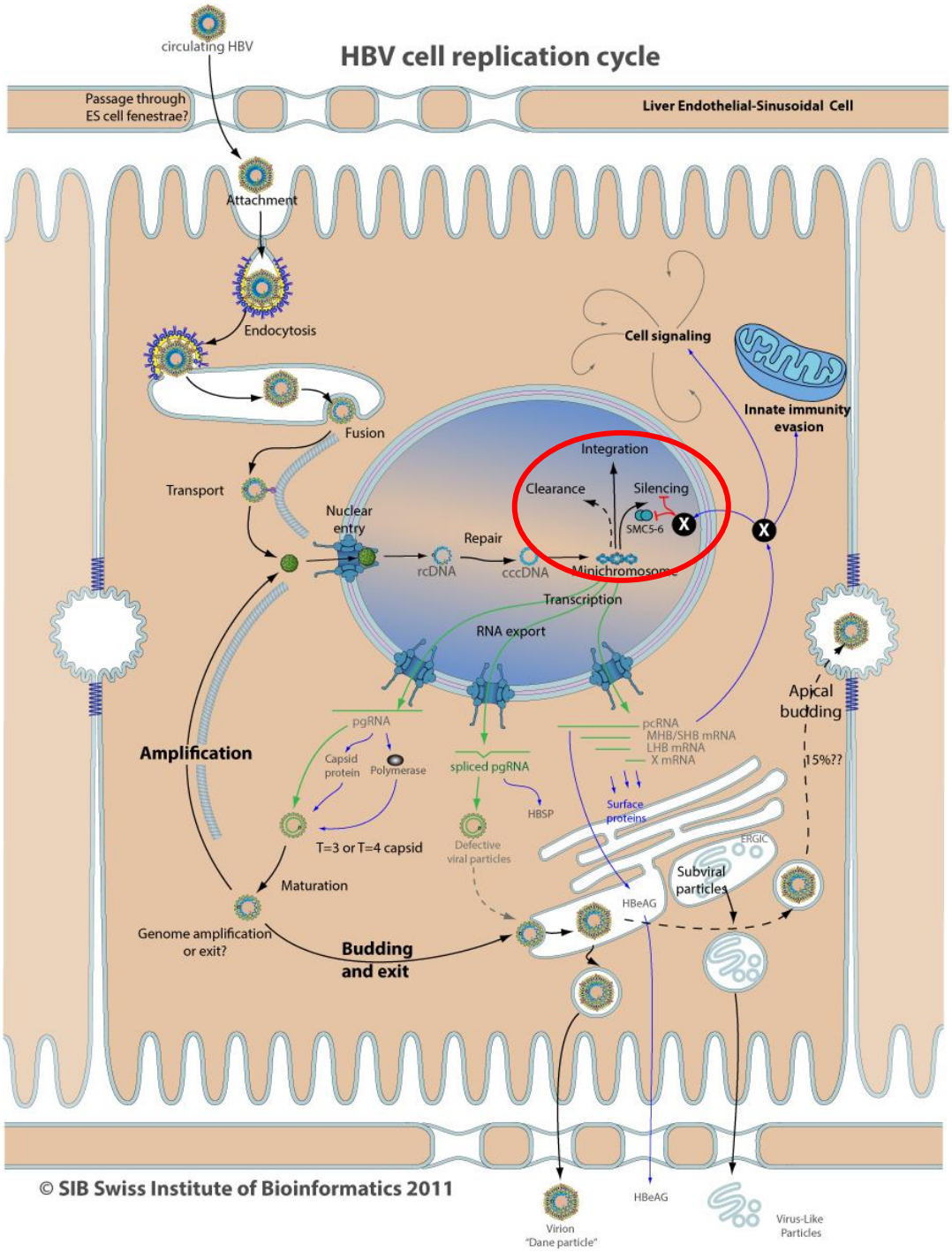
HBV life cycle-Minichromosome synthesis (rcDNA repair)

From rcDNA to cccDNA

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

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

HBV life cycle



HBV life cycle-cccDNA clearance

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

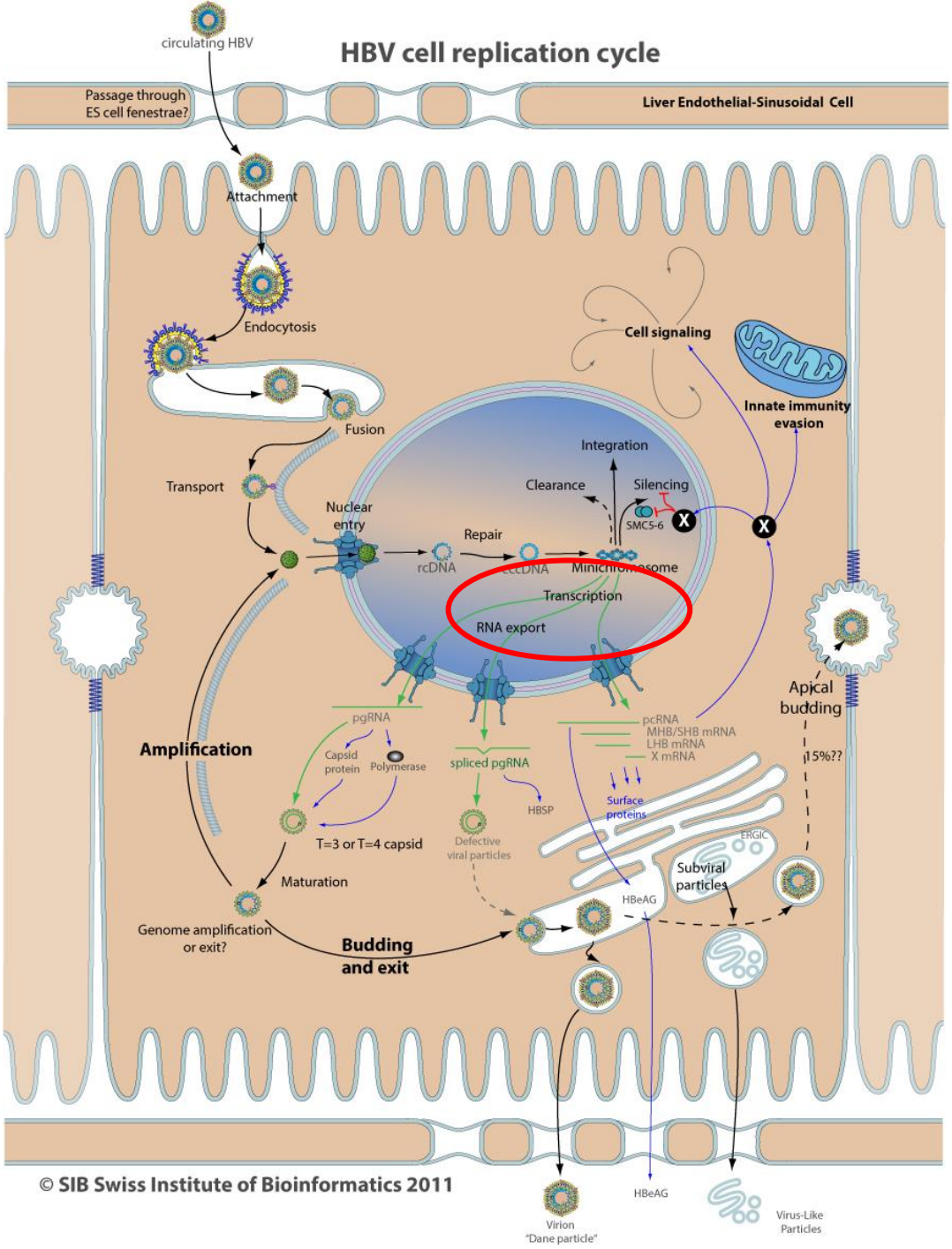
HBV life cycle-cccDNA integration

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

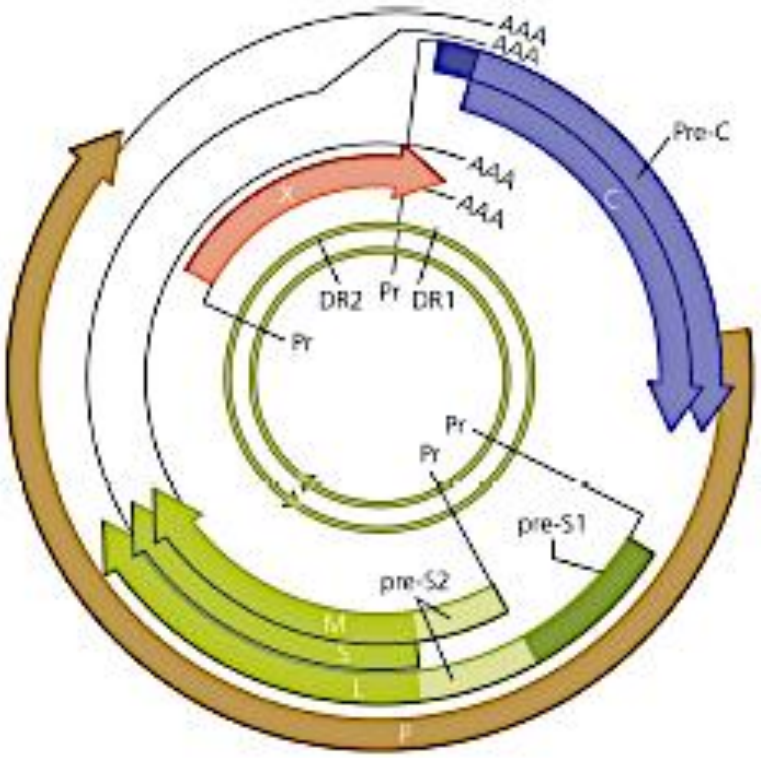
HBV life cycle-cccDNA silencing

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

HBV life cycle



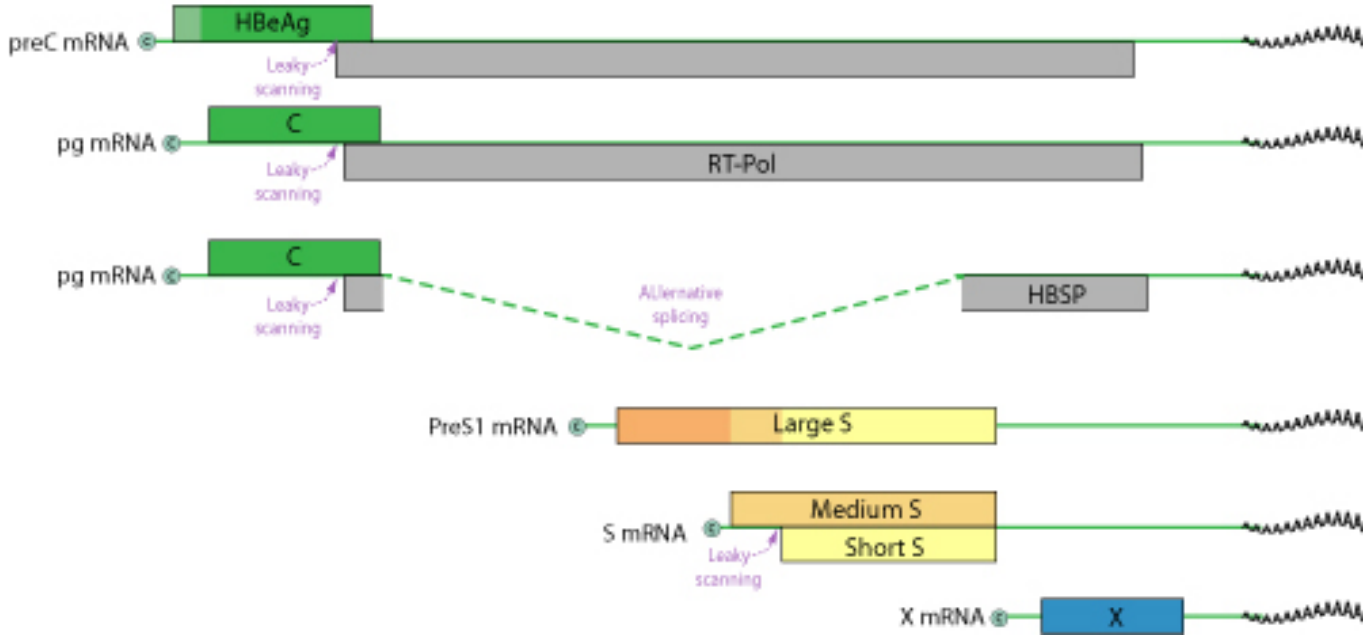
Class VII, Hepadnaviridae (HBV): Expression



- **Template: cccDNA (covalently closed circular DNA)**
- **RNA polimerase: cellular RNA pol II**
- **Transcripts without introns**

- Four transcript classes:
  - 3.5 kb (from pg/pc promoter)
  - 2.4 kb (from pre-S1 promoter)
  - 2.1 kb (from the S promoter)
  - 0.7 kb (from the X promoter)

Hepatitis B virus genome map.
The closed circular DNA is transcribed from four promoters (Pr); arrowheads indicate 3´ ends.



Spliced HBV mRNA

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

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

mRNA translation

meccanismo di 5' scanning (ribosomi iniziano dal primo AUG che incontrano)

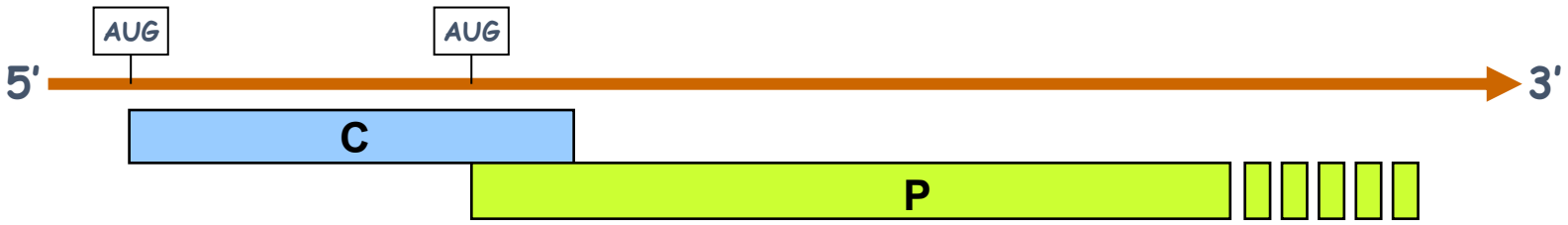
HBsAg (L, M, S)
HBcAg (C)
HBeAg
X

→ **traduzione dal 1° AUG**

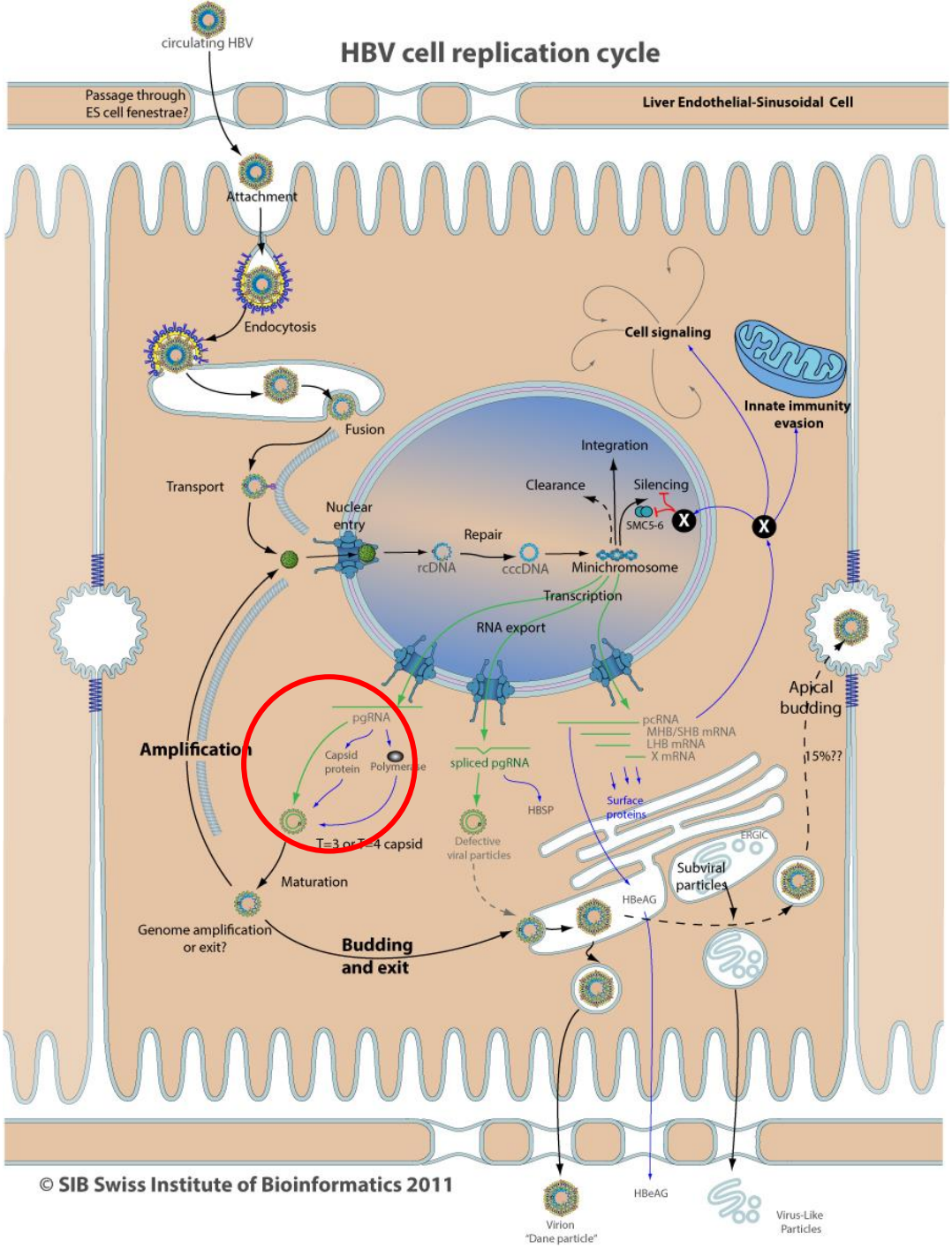
Eccezione:

Polimerasi

→ **traduzione con modalità ignote da AUG interno del pgRNA**



The pregenome RNA is translated to produce capsid protein.
The P protein, the viral reverse transcriptase, is also produced from pregenome RNA but at low efficiency; the ratio of capsid to P protein translation is 200 to 300 to 1.



HBV life cycle

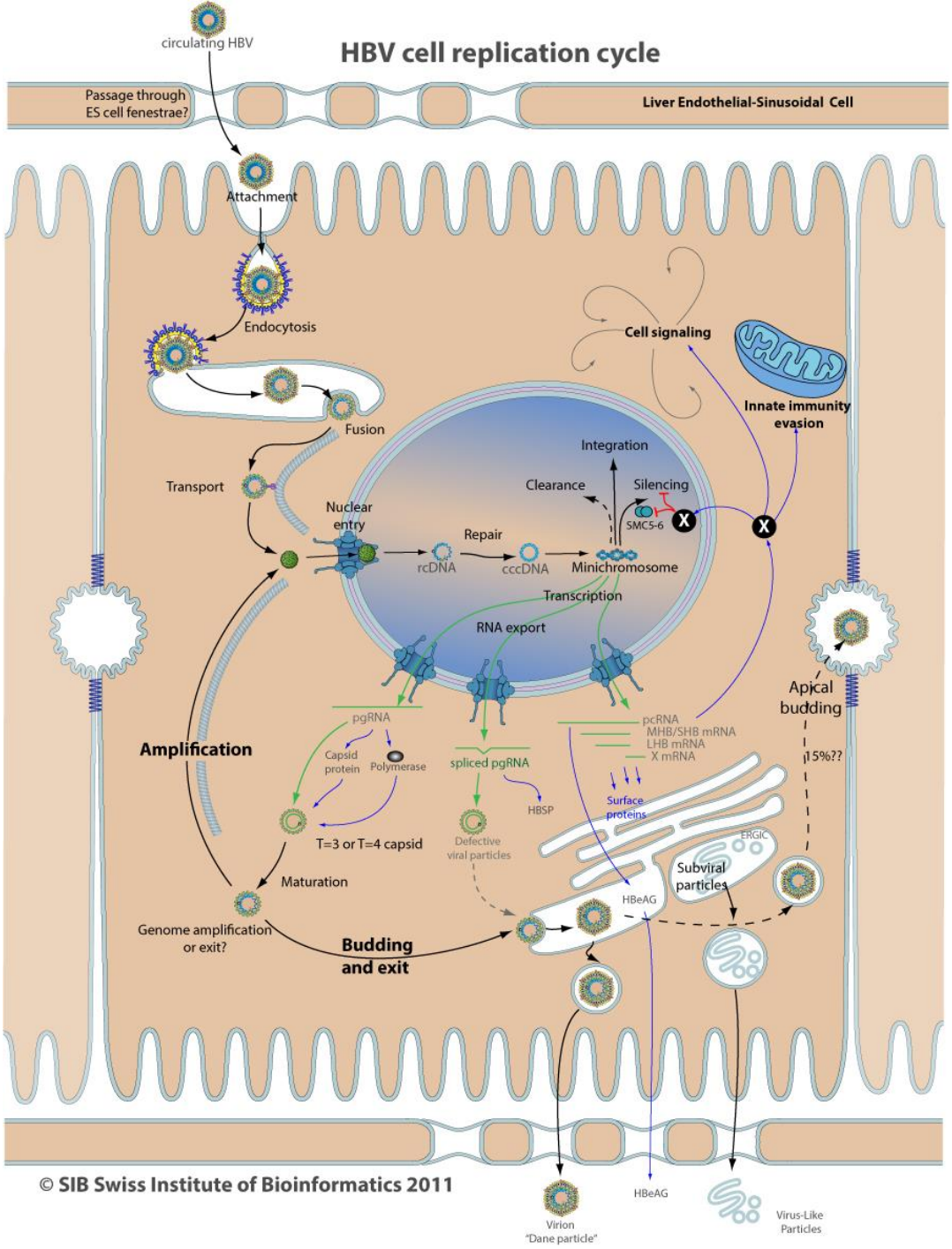
HBV genome synthesis and packaging

Following its synthesis, P binds to the packaging signal at the 5’ end of its own transcript, where viral DNA synthesis is eventually initiated.

Concurrently with capsid formation, the RNA-P protein complex is packaged and DNA replication is primed from a tyrosine residue in the polymerase.

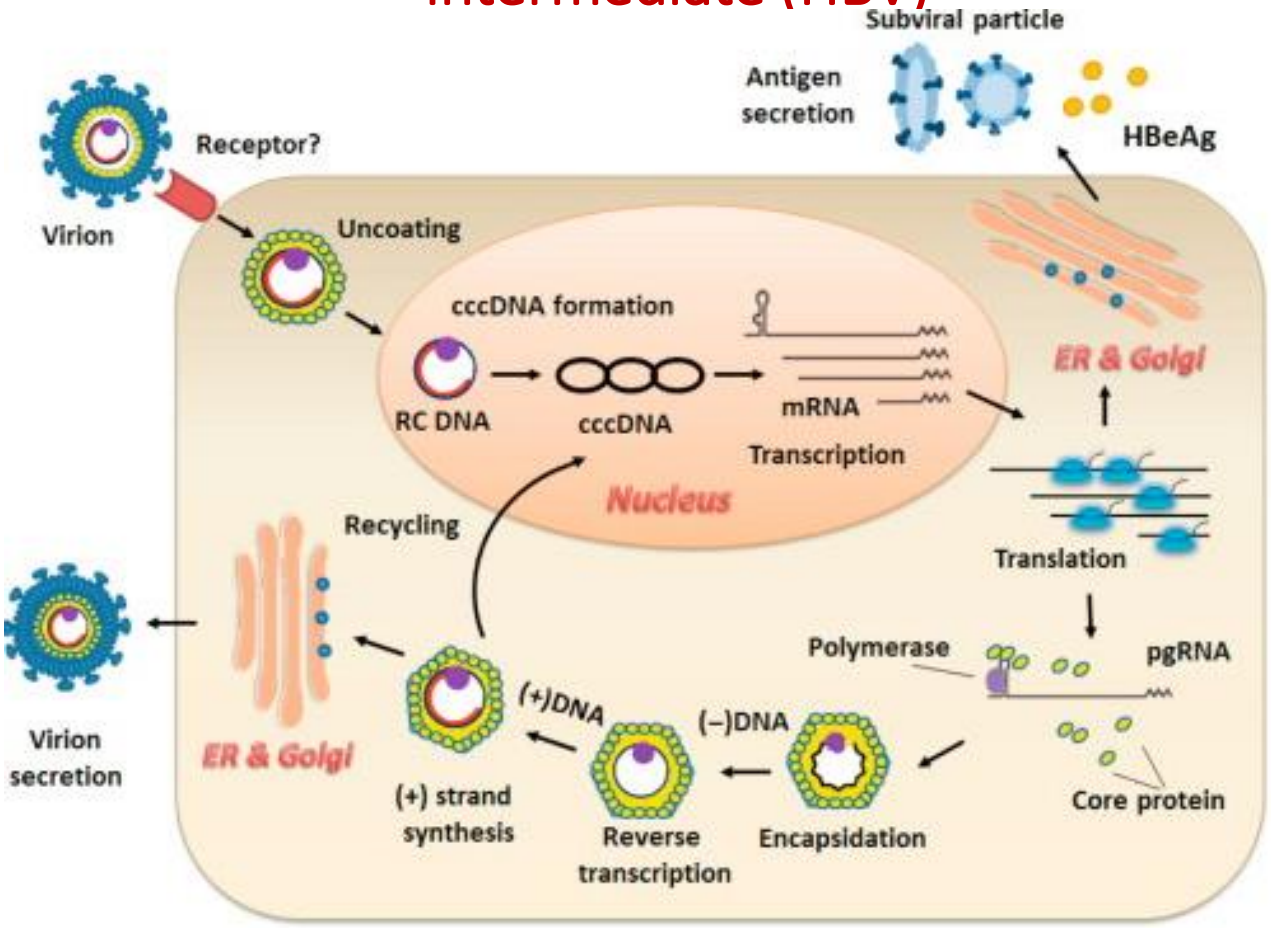
Reverse transcription of the pregenome occurs within the capsid.

After completion of DNA synthesis, the newly assembled “cores” acquire the ability to interact with envelope proteins.
However, at early times after infection, core particles are transported to the nucleus, where the viral genomes are deposited and give rise to additional copies of cccDNA. Eventually, 10 to 30 molecules of cccDNA accumulate, leading to a concomitant increase in viral mRNA concentrations.



HBV life cycle

Expression Replication Class VII: dsDNA with RNA intermediate (HBV)



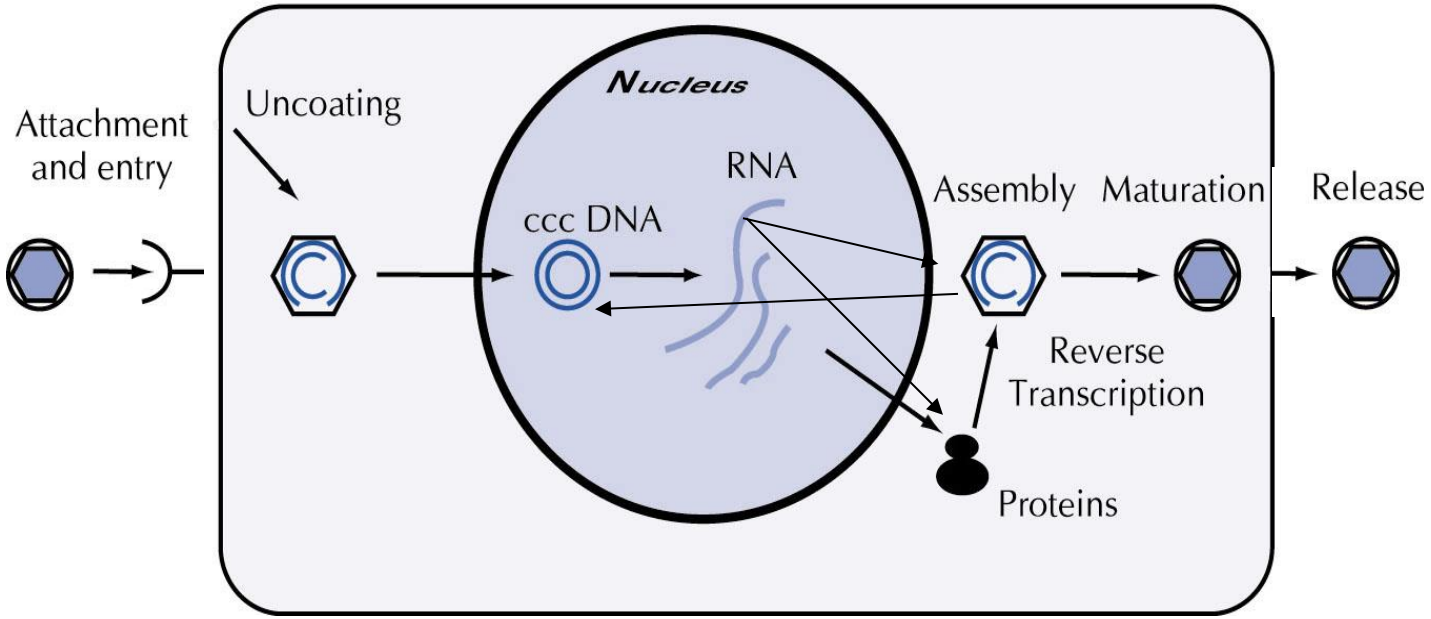
Once the viral genome is released into the nucleus, repair of the gapped (+) DNA strand is likely accomplished by cellular enzymes. The product is a covalently closed circular form called CCC DNA, which associates with histones to form a minichromosome. The (-) strand of CCC DNA is the template for transcription by cellular RNA polymerase II of a longer-than-genome-length RNA called the pregenome and shorter, subgenomic transcripts, all of which serve as mRNAs.

Class VII: dsDNA with RNA intermediate

This group of viruses also relies on reverse transcription

In contrast to retroviruses reverse transcription occurs inside the virus particle during maturation

On infection the first event is the completion of the DNA +strand.



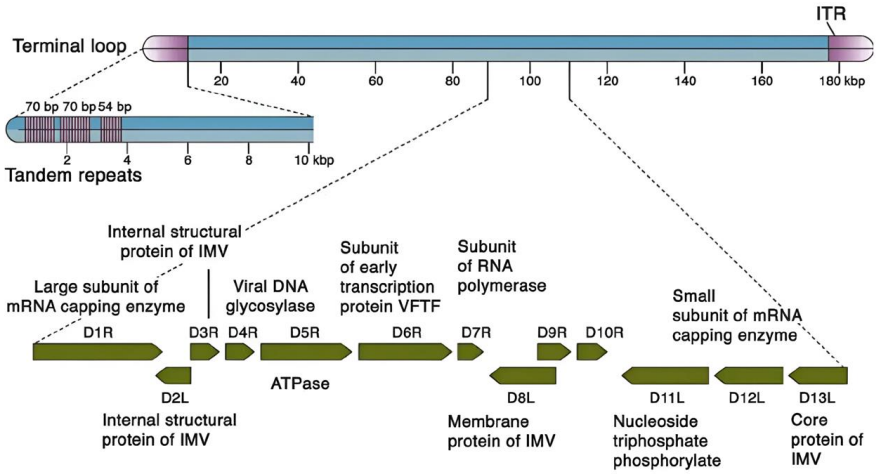
From Cann *Principles of molecular virology* (2001). Academic Press

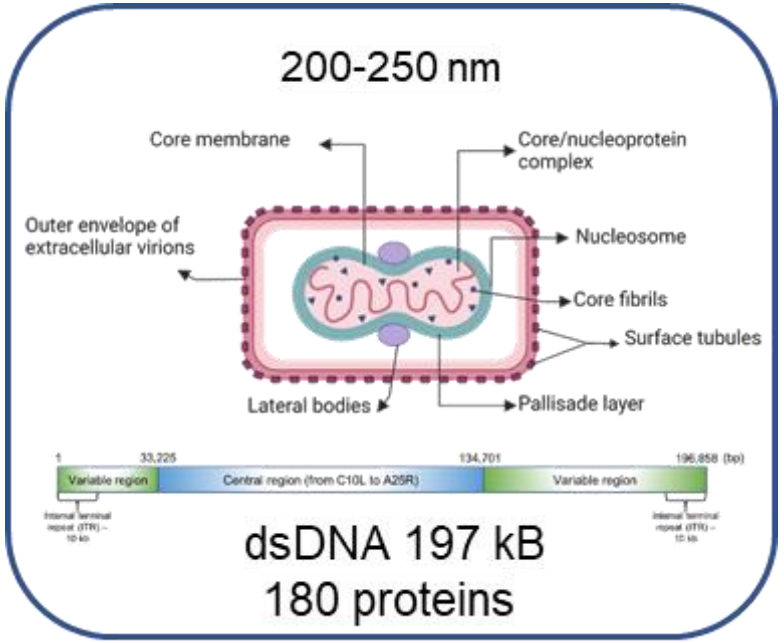
Poxvirus

Virus dalla struttura complessa e di grandi dimensioni (fino a 300x200nm)

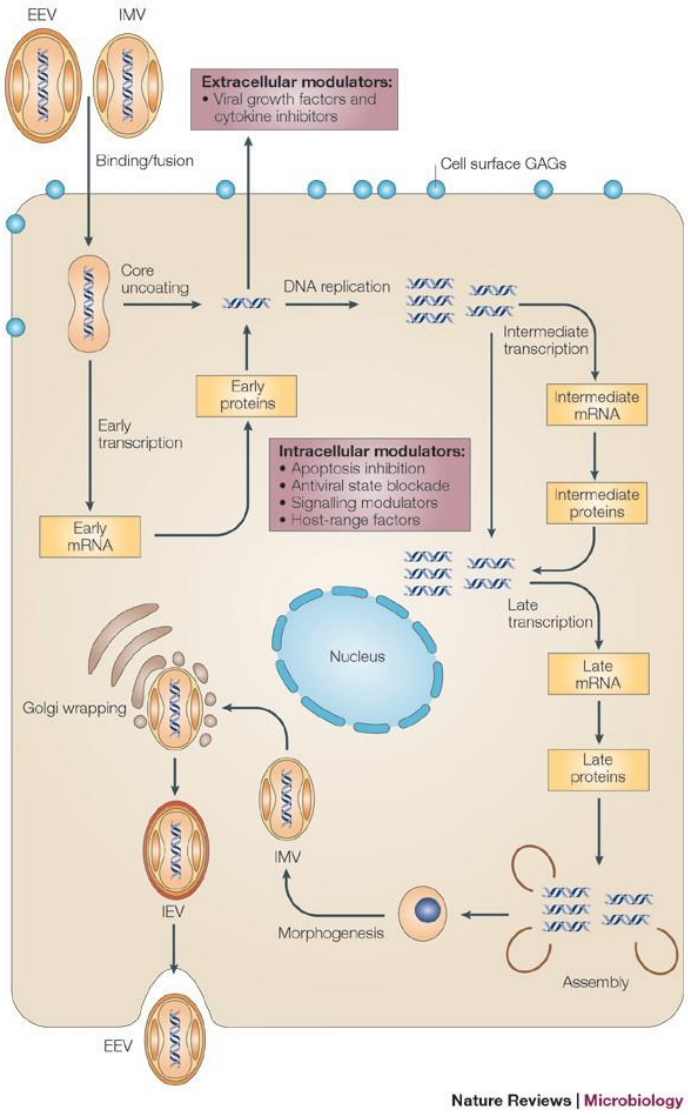
Dotati di un genoma **lineare** a **dsDNA** di grandi dimensioni (135 to 360 kbp) con **estremità chiuse** e contenenti **sequenze ripetute e invertite**

Il genoma codifica tutti gli enzimi necessari per la replicazione del DNA virale, trascrizione dei geni (intermedi e tardivi), regolazione e sintesi delle proteine strutturali del virus





Poxvirus



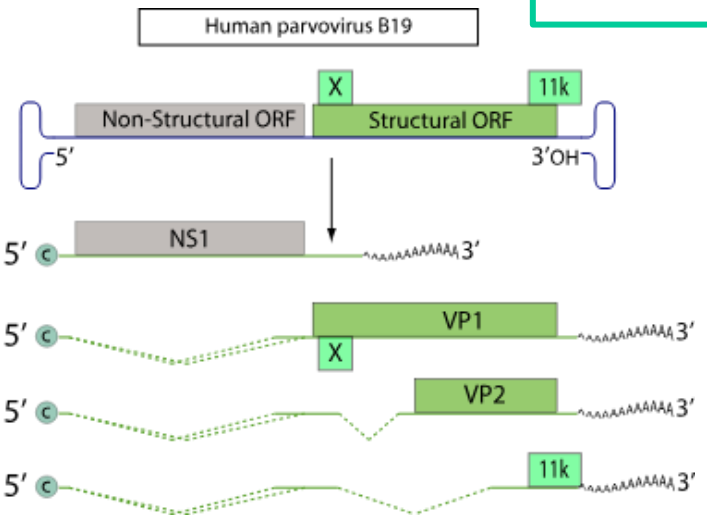
E' la famiglia di virus per i quali la dipendenza dall'ospite è tra le più limitate

Il fatto che l'intero ciclo avvenga nel citoplasma determina la necessità di portare nel virione le proteine necessarie ad iniziare il ciclo replicativo
E la necessità di codificare gli enzimi necessari alla loro espressione genica e replicazione

Anche nei Poxvirus riconosciamo una espressione gerarchica temporale dei prodotti genici suddivisi in geni
Immediato precoci (regolatori)
Precoci (trascrizione-replicazione)
Tardivi (strutturali)

The genome includes:
a highly conserved central region, encoding for viral transcription, replication, and virion morphogenesis factors,
and two peripheral regions containing immune-modulating and virulence genes implicated in determining the host range and pathogenesis

Parvovirus



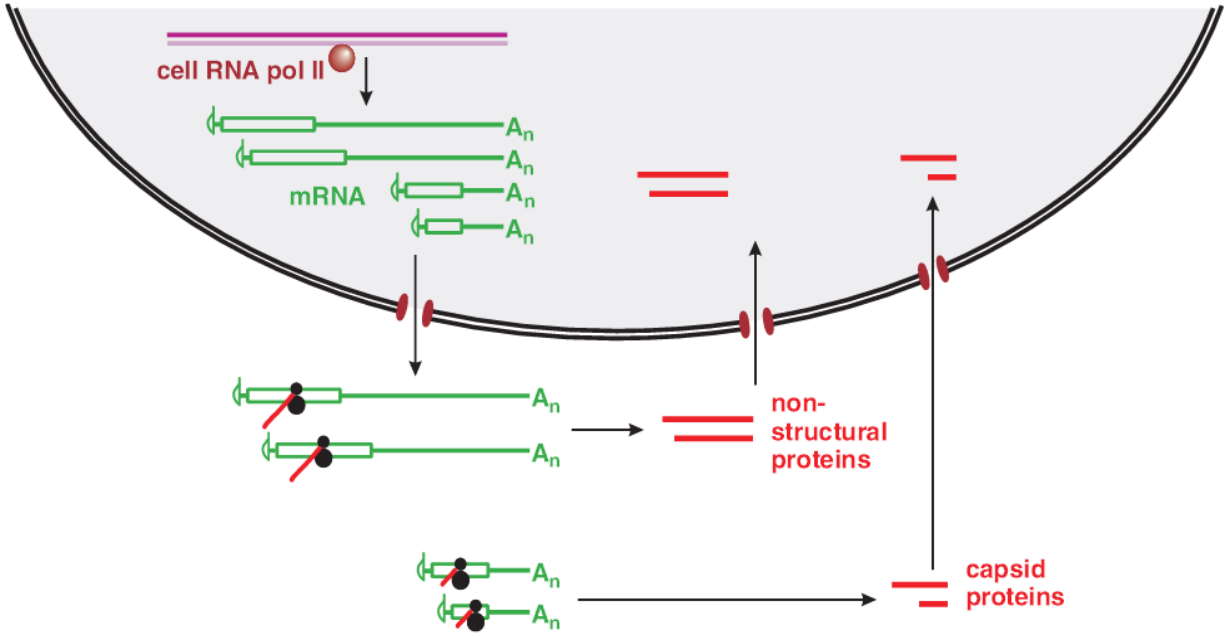
Depending on the virus there can be one (Erythrovirus and Iteravirus), two (Densovirus and Brevidensovirus) or three (Dependovirus) promoters for mRNA transcription. Alternative splicing allows expression of both structural and non-structural proteins. Leaky scanning is used as well by densoviruses.

Cellular RNA pol II transcribes genes and cellular transcription factor play key roles

1°transcript –various splicing events to make 2 classes based on size:

1. larger mRNAs –non-structural, phoshorylated and play role in control of gene expression and in DNA replication
2. smaller mRNAs –structural

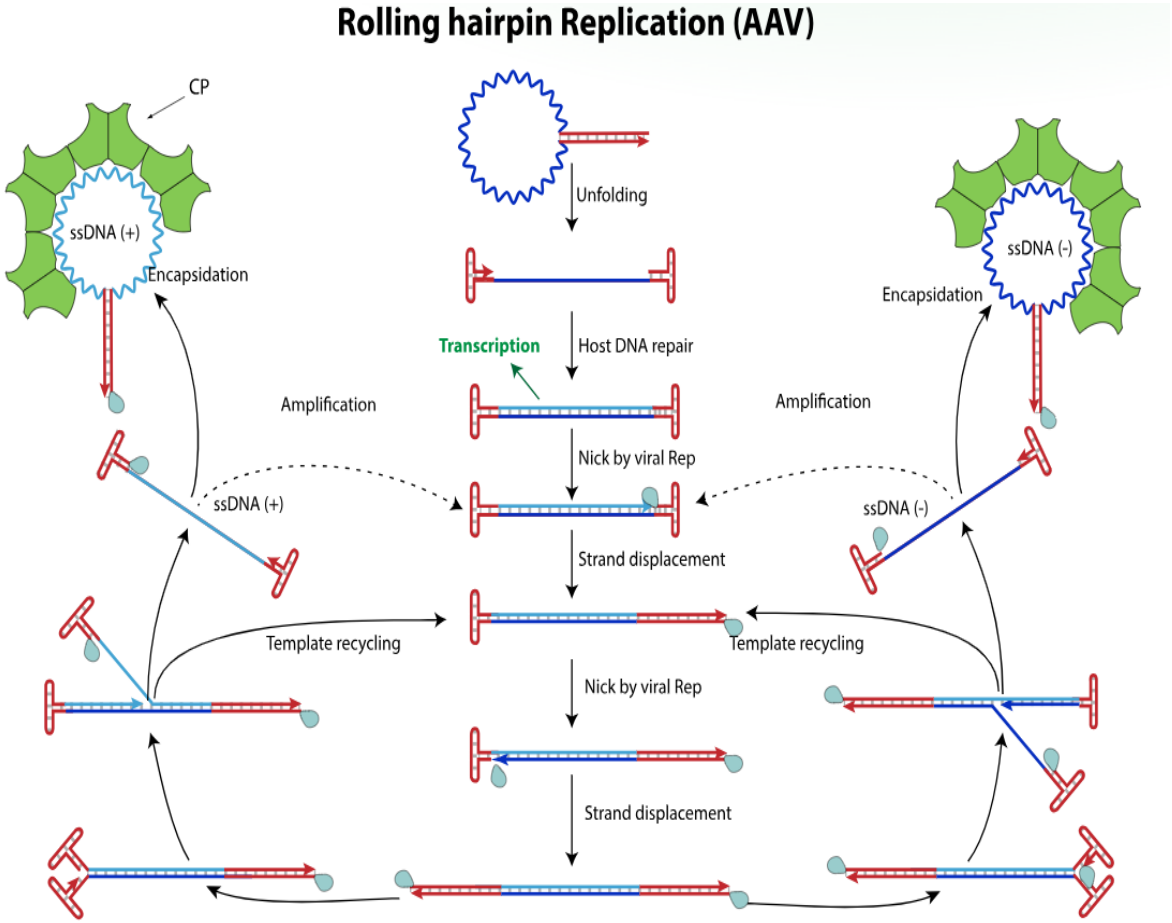
Parvovirus



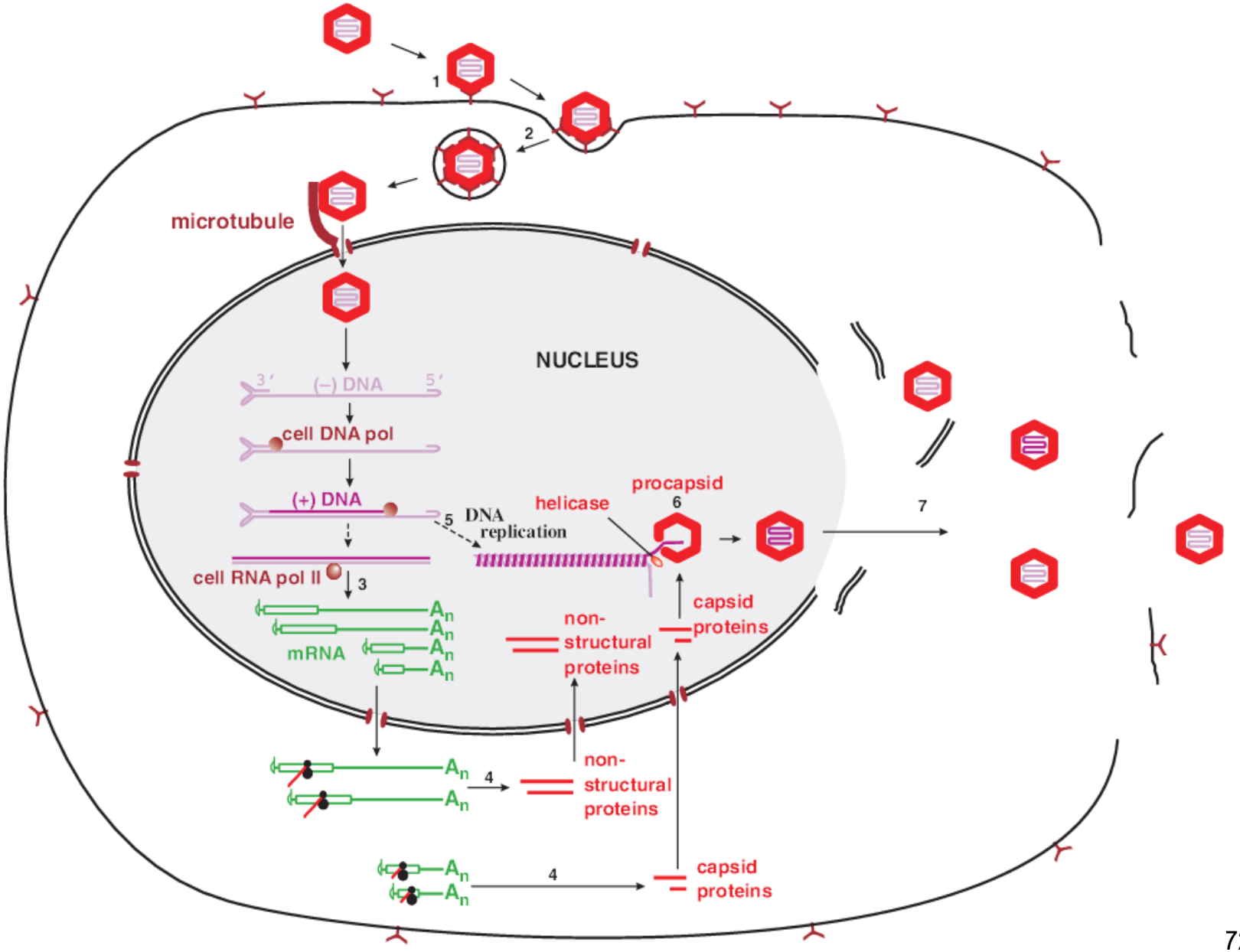
Cellular RNA pol II transcribes genes and cellular transcription factor play key roles
1°transcript –various splicing events to make 2 classes based on size:

1. larger mRNAs –non-structural, phoshorylated and play role in control of gene expression and in DNA replication
2. smaller mRNAs –structural

Parvovirus



- **Inizio della replicazione:** Il DNA a singolo filamento (ssDNA) si dispiega e forma un intermedio a doppia elica tramite la riparazione del DNA dell'ospite o la trascrizione.
- **Amplificazione del genoma:** La proteina virale Rep introduce un taglio (nick) nel DNA, dando inizio allo spostamento del filamento e alla sintesi di nuovi filamenti di ssDNA, sia positivi (+) che negativi (-).
- **Riciclo del templato e incapsidamento:** I filamenti di ssDNA neo-sintetizzati possono essere riciclati come templati per ulteriore amplificazione o incapsidati in nuovi capside virali.


1
2
microtubule
NUCLEUS
3′
(–) DNA
5′
cell DNA pol
(+) DNA
5
DNA
replication
helicase
procapsid
6
7
cell RNA pol II
3
An
An
mRNA
An
An
non-
structural
proteins
capsid
proteins
An
An
4
non-
structural
proteins
An
An
4
capsid
proteins

	FAMILY
Animal viruses	PARVOVIRIDAE, CIRCOVIRIDAE
Plant viruses	GEMINIVIRIDAE
Bacteriophages	MICROVIRIDAE

I *Parvoviridae* sono i più piccoli virus a DNA. Le loro piccole dimensioni e il repertorio genetico limitato li rendono più dipendenti di qualsiasi altro virus a DNA dalla cellula ospite, oppure richiede la presenza di un virus helper per la loro replicazione.
La famiglia Parvoviridae comprende: Parvovirus B19, e i Dependovirus (virus satellite e adeno/herpes-associati)
B19 e *Bocavirus* sono gli unici parvovirus noti per causare malattie umane.
B19 normalmente causa eritema infettivo, o quinta malattia, una lieve malattia esantematica febbrile che si verifica nei bambini.

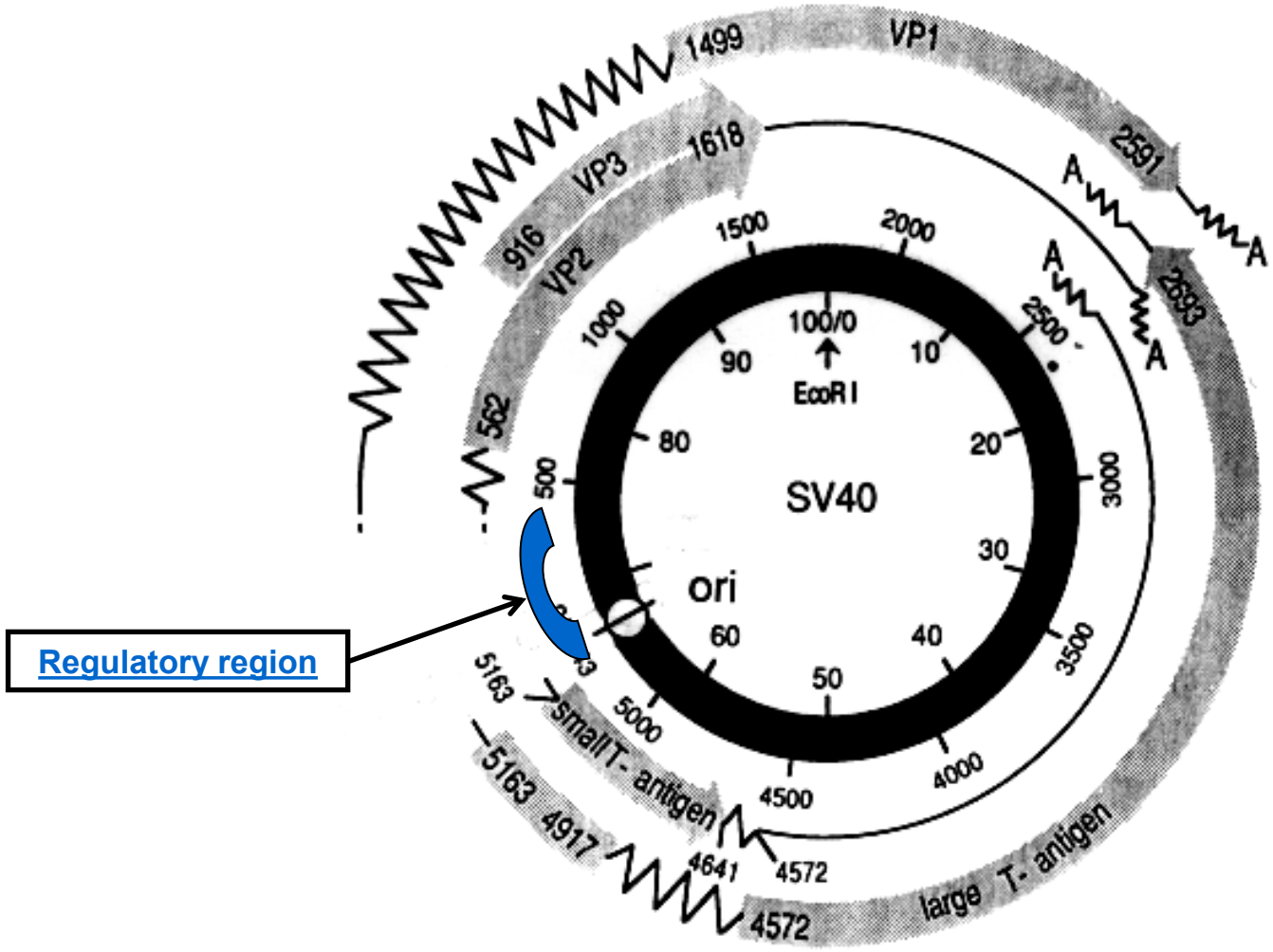
Parvoviridae family

TAXONOMY

Group II: ssDNA viruses
Realm: *Monodnaviria*
Kingdom: *Shotokuvirae*
Phylum: *Cossaviricota*
Class: *Quintoviricetes*
Order: *Piccovirales*
Family: ***Parvoviridae***
Subfamily: *Parvovirinae*
Genus: *Amdoparvovirus*
Aveparvovirus
Bocaparvovirus
Copiparvovirus
Erythroparvovirus
Dependoparvovirus
Protoparvovirus
Tetraparvovirus
Subfamily: *Densovirinae*
Genus: *Ambidensovirus*
Iteravirus
Brevidensovirus
Hepandensovirus
Penstyldensovirus
Unassigned:
Bombyx mori densovirus 3

ETYMOLOGY

Parvo: from Latin, 'small'



Unique regulatory region : controls the expression of all viral genes.
Both upstream of early genes and late genes.
+ unique origin of DNA replication