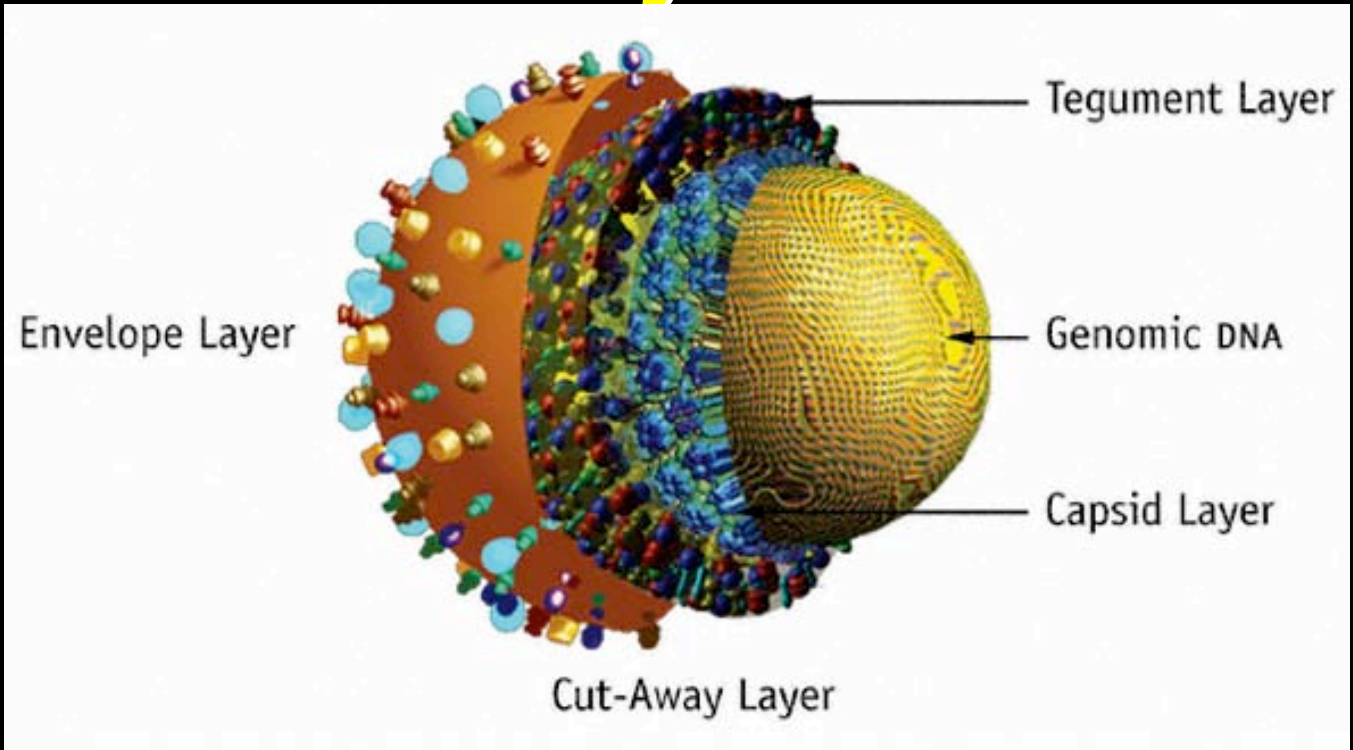
Herpesviruses:
Latent and Lytic Infection



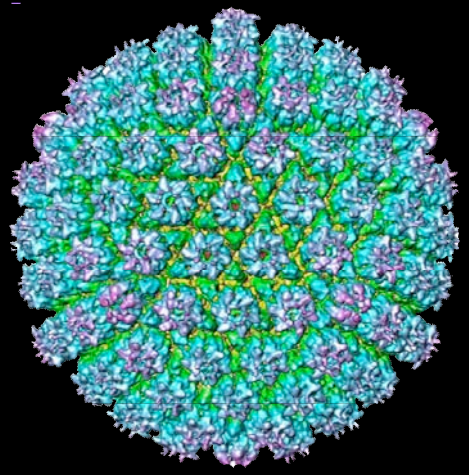
Published in The PRN Notebook, Volume 7, Number 1, March 2002 and The PRN Notebook Online at www.prn.org.
Three-dimensional model of KSHV created by Louis E. Henderson, Ph.D., Frederick Cancer Research Center.

Viruses, Cells and Disease
November 6, 2008

Diversity within DNA viruses

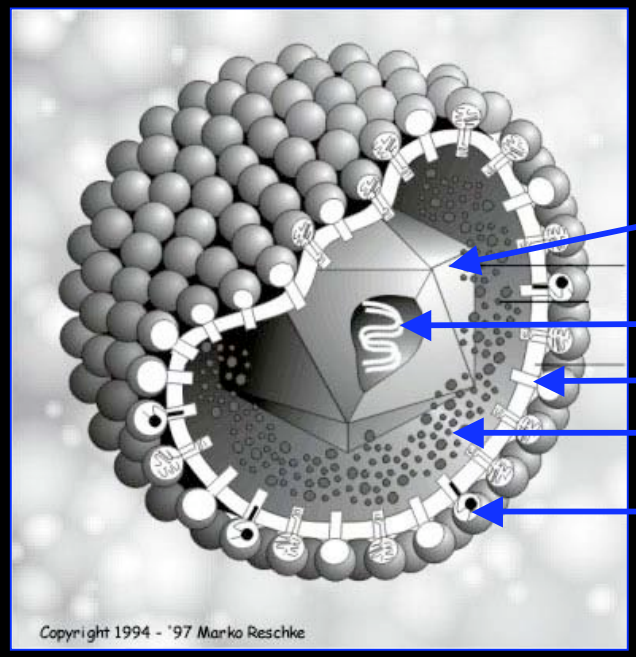
Classification	Example	Genome size	# genes	envelope?	Unique features
Polyomavirus	SV40	5 kb. ds	7	NO	Transformation
Papillomavirus	HPV	8 kb. ds	10	NO	>100 strains; Cervical cancer agent; Transformation following integration
Adenovirus	Ad2, Ad5	36 kb.ds	14 tx. Units	NO	Leader RNAs; Common respiratory pathogen; Transformation; Gene therapy vector
Parvovirus	AAV	5 kb. ss	2	NO	Require helper virus; Gene therapy vector
Herpesvirus	HSV (HHV-1)	152 kb. ds	86	YES	Tegument
	HCMV (HHV-5)	235 kb. ds	222		Latency
	EBV (HHV-4)	172 kb. ds	82		Transformation
	KSHV (HHV-8)	170 kb. ds	86		Neural gene therapy vector
Poxvirus	Vaccinia	200 kb. ds	150	YES	Smallpox agent (Variola); Cytoplasmic replication (RNA pol, capping enz, poly(A) pol)
Hepadnavirus	HBV	3.2 kb ds*	8	YES	RNA repli. Intermediate; Hepatitis agent; liver cancer; 1st recomb. vaccine

Herpesviruses



- Extremely common, highly disseminated in nature
- >200 herpesviruses identified to date
- Every mammal is infected by at least one:
  Eight have been isolated from humans
- Spread through direct physical contact
- Alternating Lytic (Productive) infection and
  Latent (non-productive) infection

Virion Structure



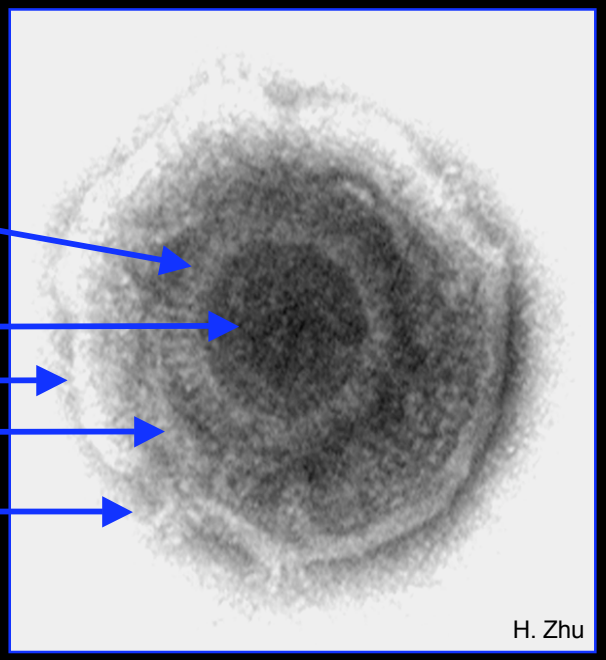
icosahedral nucleocapsid

dsDNA genome

envelope

tegument

glycoprotein



<u>Human Herpesviruses genomes:</u>
150-235 kb. DNA, encode 80-225 proteins

<u>Tegument:</u> >20 proteins

Classification: Three subfamilies -

α herpesviruses:

Human herpesvirus 1,2 **Herpes simplex virus 1, 2 (HSV-1, HSV-2)**
Human herpesvirus 3 **Varicella-Zoster virus (VZV)**

- a relatively rapid, cytocidal growth cycle
- establish latent infections primarily in sensory ganglia

β herpesviruses:

Human herpesvirus 5 **Cytomegalovirus (CMV)**
Human herpesvirus 6 **HHV-6**
Human herpesvirus 7 **HHV-7**

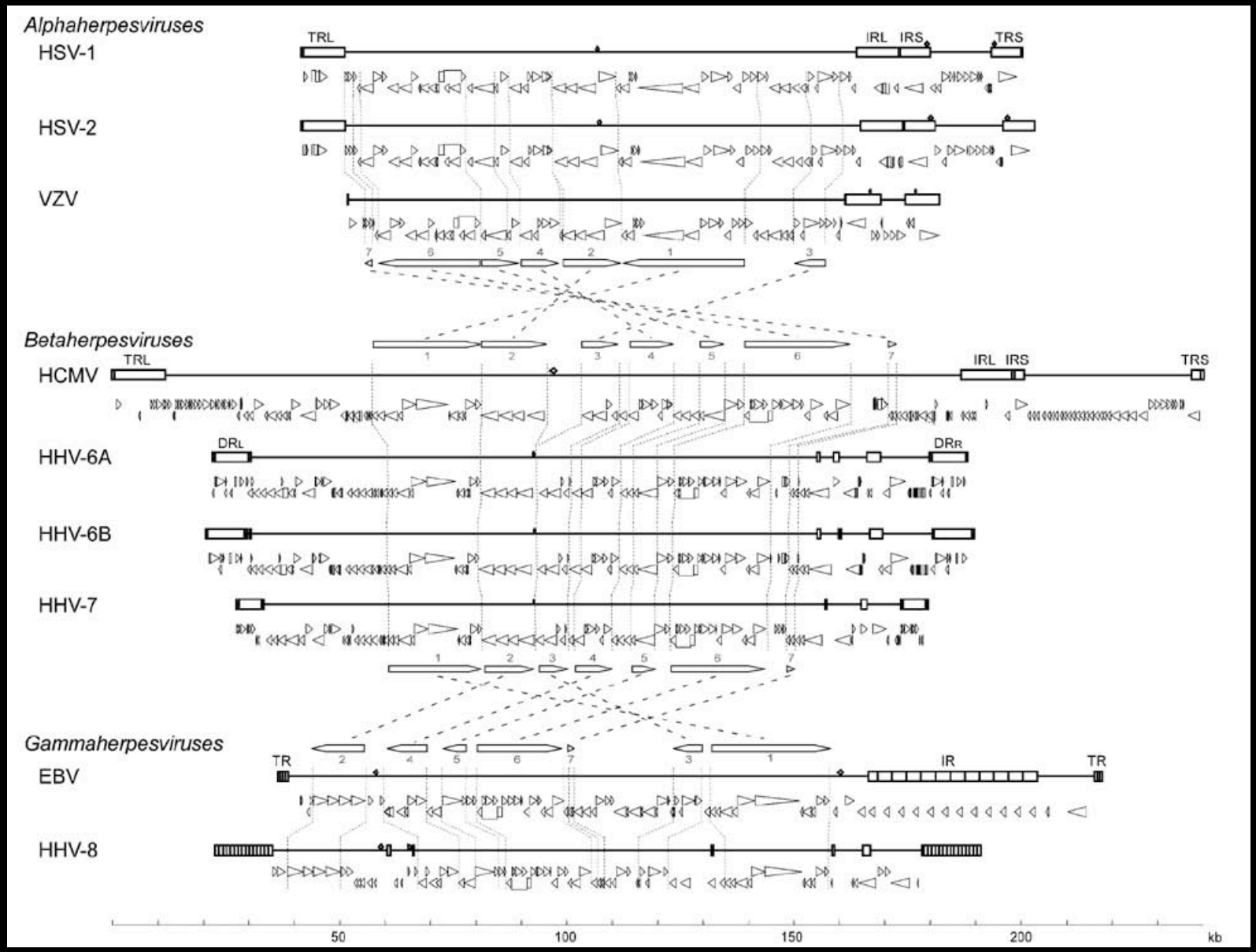
- the reproductive cycle is long and grow slowly in culture
- establish latency in monocytes (CMV) or T cells (HHV-6, 7)

γ herpesviruses:

Human herpesvirus 4 **Epstein Barr virus (EBV)**
Human herpesvirus 8 **Kaposi's sarcoma associated herpesvirus (KSHV/HHV-8)**

- establish latency in B cells
- can transform cells; they are oncogenic herpesviruses

Although divergent in size and genomic architecture, herpesviruses share 7 conserved blocks of ancient genes



Approximately 40 core genes are conserved among all herpesviruses

Gene regulation

Transcriptional/post-transcriptional transactivator

Nucleotide Metabolism

Ribonucleotide reductase, large subunit
Uracil DNA glycosylase
dUTPase

DNA Replication

Helicase/primase (3 subunits)
DNA polymerase**
Polymerase processivity factor
ssDNA binding

Other

Cell-to-cell fusion

Virion

Maturation

- Alkaline exonuclease
- Terminase/packaging
- Capsid nuclear egress (2)
- Genome cleavage/packaging (3)
- Transport
- Scaffold protease
- Virion protein
- Scaffold

Capsid

- Major capsid protein (MCP)
- Minor capsid protein
- Capsid triplex (2)
- Hexon tips

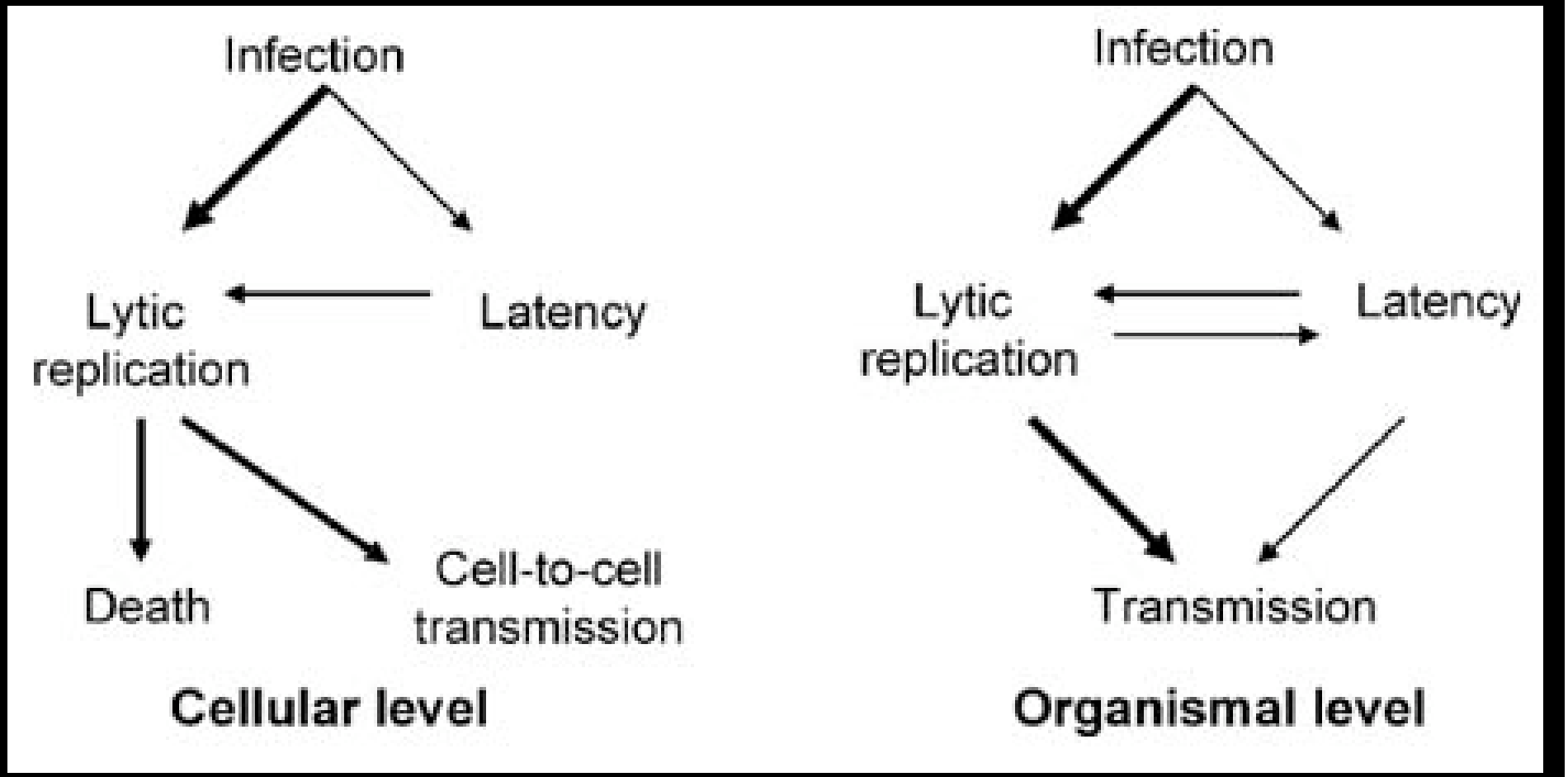
Tegument

- Large tegument protein
- 6 with unknown function
- Protein kinase

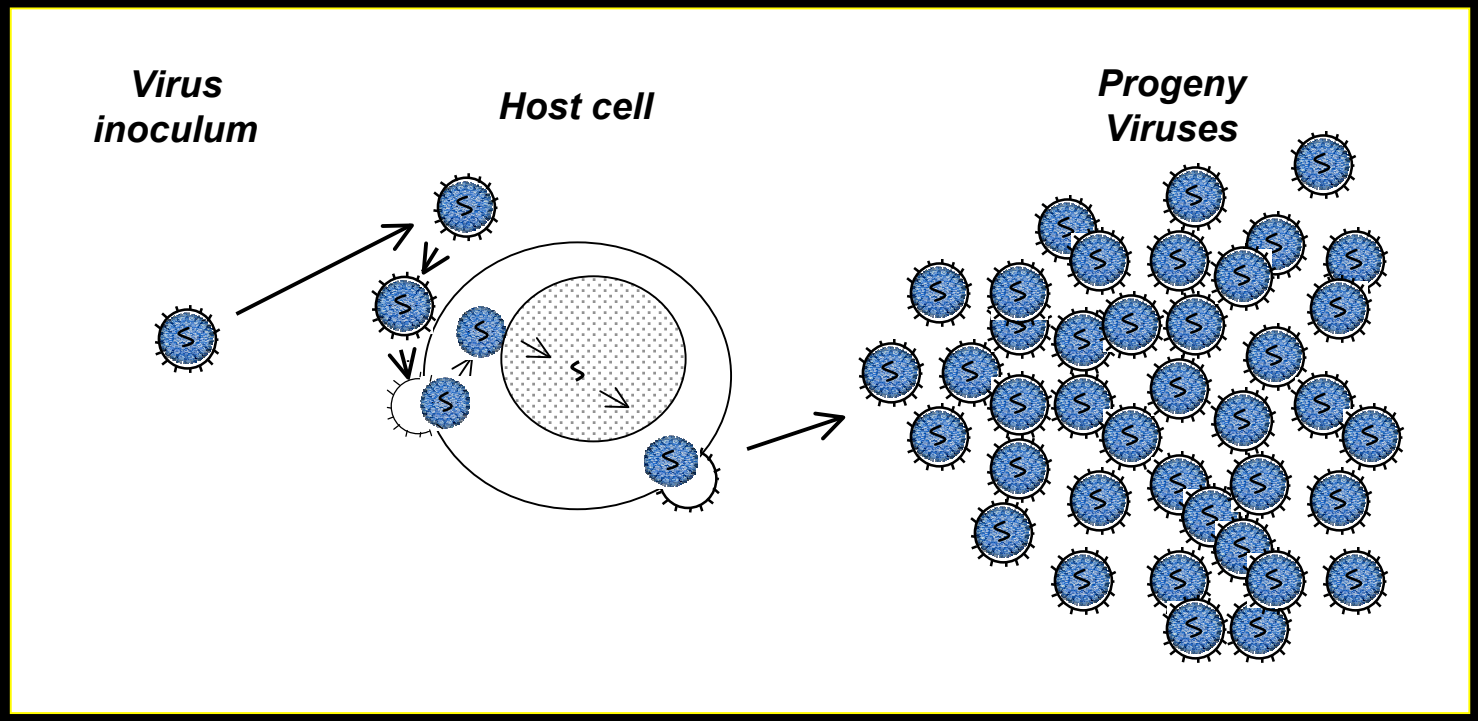
Envelope

- Glycoproteins B (gB), gH, gL, gM, gN

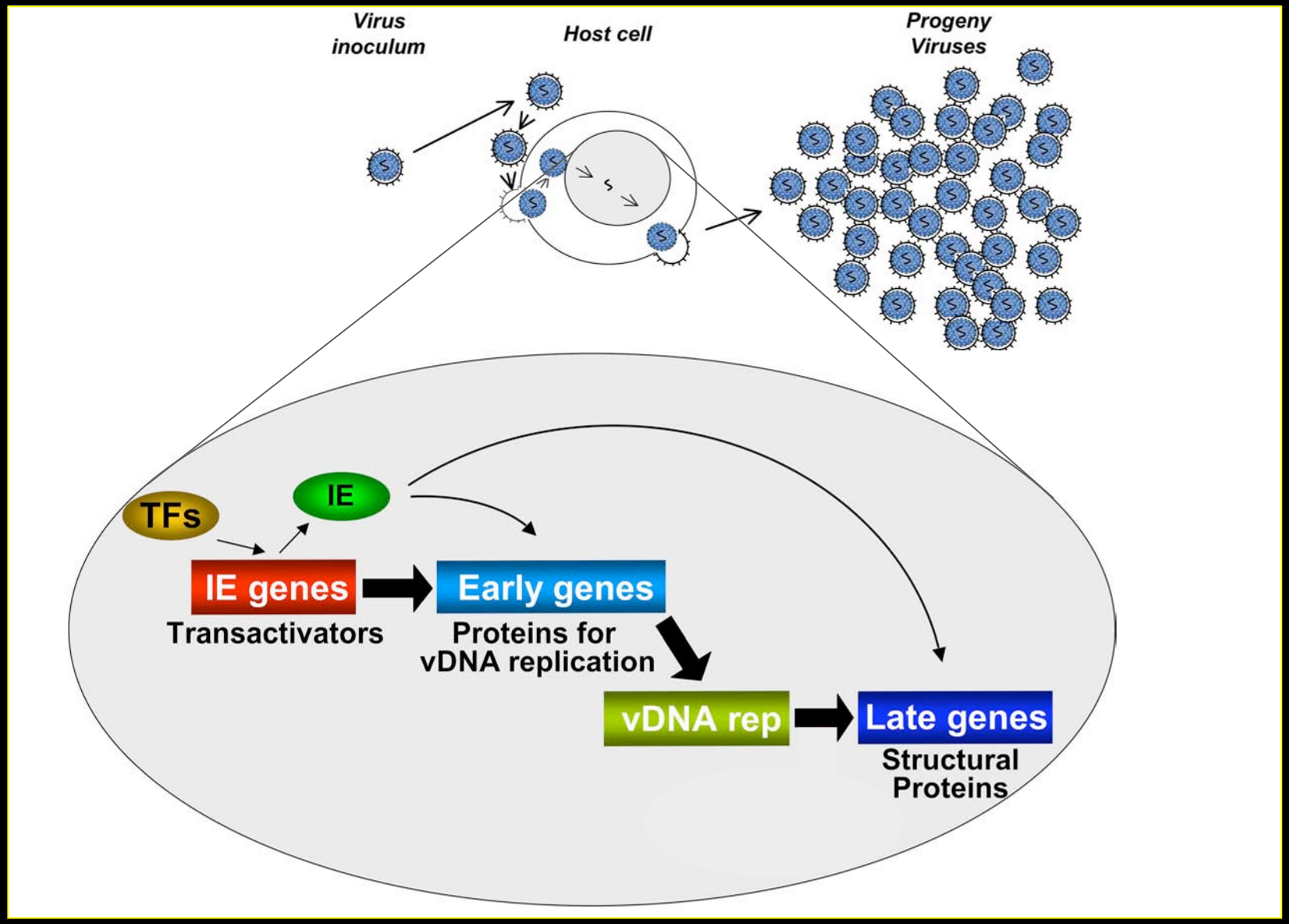
Patterns of herpesviral replication must be considered at the cellular and organismal levels



Reproduction and transmission determine the biological success of a virus



Viral genes are expressed in a cascade fashion during replication of a DNA virus



Productive gene expression cascade of herpesviruses

Immediate Early (IE; α) genes:

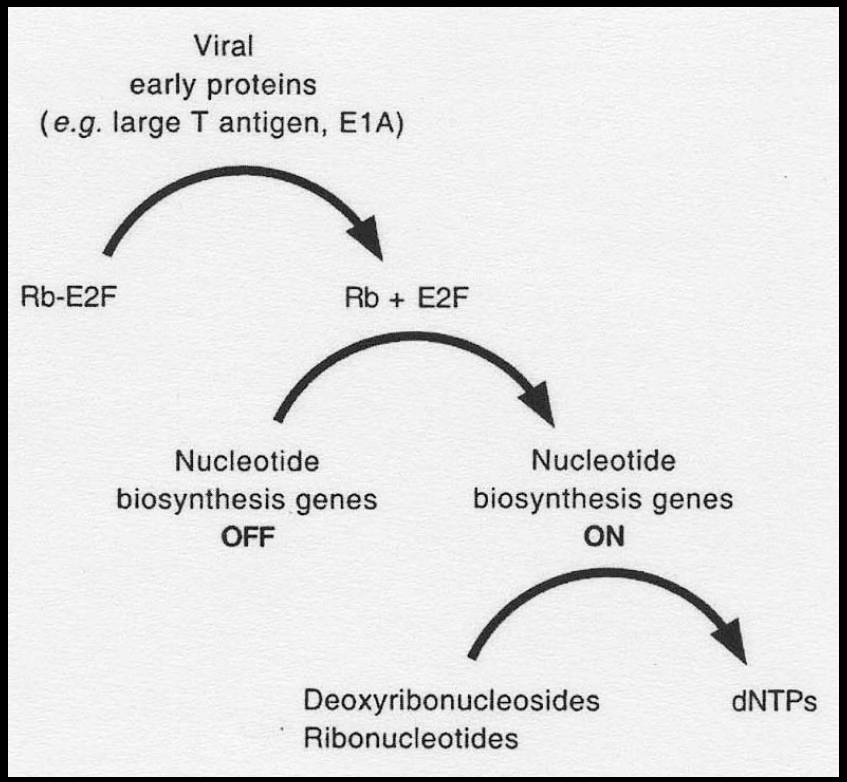
CHX resistant (no prior protein synthesis req).
Transcriptional and post-transcriptional activation and de-repression. Inhibit IFN response
Pro and anti-apoptotic Block antigen presentation
Reduce host gene expression E3 ubiquitin ligase

Delayed Early (DE; β) genes: all require prior expression of combinations of IE transactivators
Do not require prior viral DNA replication
Can function as DE genes when present on a plasmid

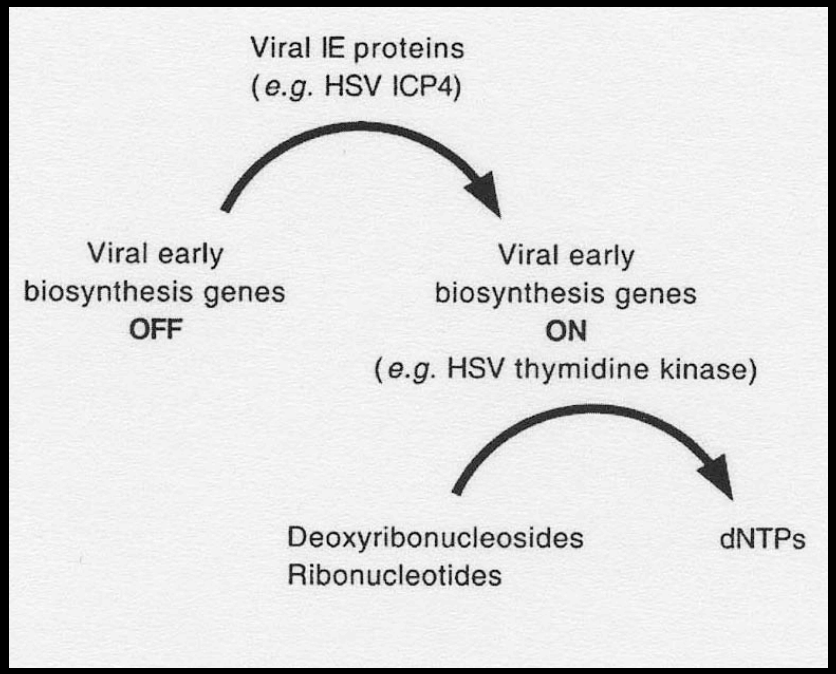
Late (L; γ) genes: expression enhanced by viral DNA synthesis
require IE transactivators + ssDNA binding protein (DE)
γ1-expression does not require viral DNA synthesis
γ2-expression strictly dependent on viral DNA synthesis
Not understood--cis acting regulation

Small DNA viruses, but not herpesviruses, target central growth control proteins to replicate productively

Small DNA Viruses (Papova and Adenoviruses)



Herpesviruses



Nuclear events--the goal is to replicate viral DNA

Viral coding capacity generally predicts autonomy vs. reliance on host functions

Function	SV40	HSV-1
DNA pol	*cellular DNA pol δ*	**UL30**
Pol processivity	*cellular PCNA*	**UL42**
ssDNA binding protein	*cellular RF-A*	**UL29**
helicase/primase	**Large T antigen**	**UL5,8,52**
Origin binding protein	**Large T antigen**	**UL9**
RNAse H/5'-3' exonuclease	*cellular RNAse H, MF-1*	**UL30,42**
Nucleoside phosphotransferase	*cellular thymidine kinase*	**UL23**
Ribonucleotide reductase	*cellular*	**UL39,40**
Deoxyuridine triphosphatase	*cellular*	**UL50**
Deoxyribonuclease	*cellular*	**UL12**
Uracil-DNA-glycosylase	*cellular*	**UL2**
Topoisomerase I, II	*cellular*	*cellular*
RF-C	*cellular*	*cellular*
Ligase I	*cellular*	*cellular*

Lytic cycle (productive) herpesviral DNA replication

Requires: Lytic origin of replication ("ori $_{Lyt}$")

+

7 viral proteins that participate directly at replication fork.

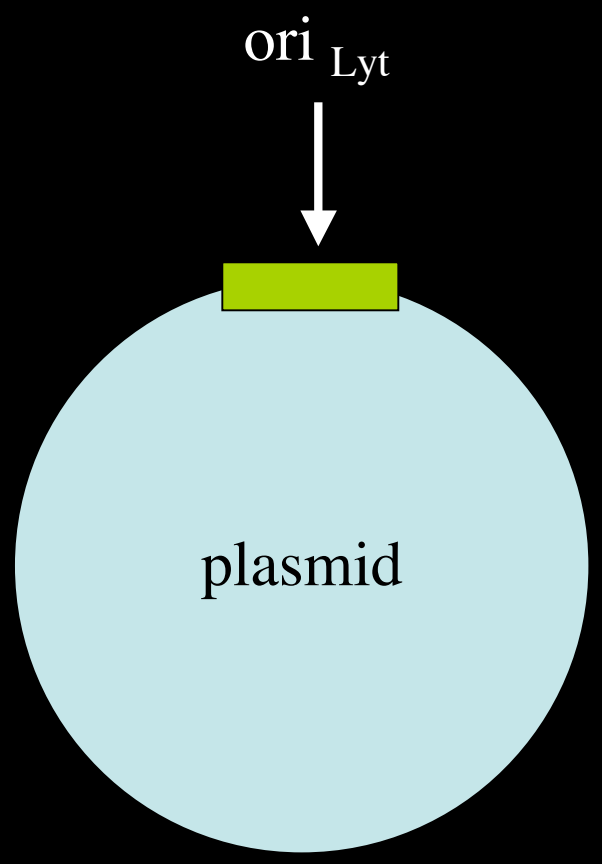
Rolling circle replication

Cleavage of genome concatemers into single units

Produces linear genomes that are packaged into capsids

DE gene products replicate viral DNA

Challberg. 1986. PNAS. 83: 9094-8.



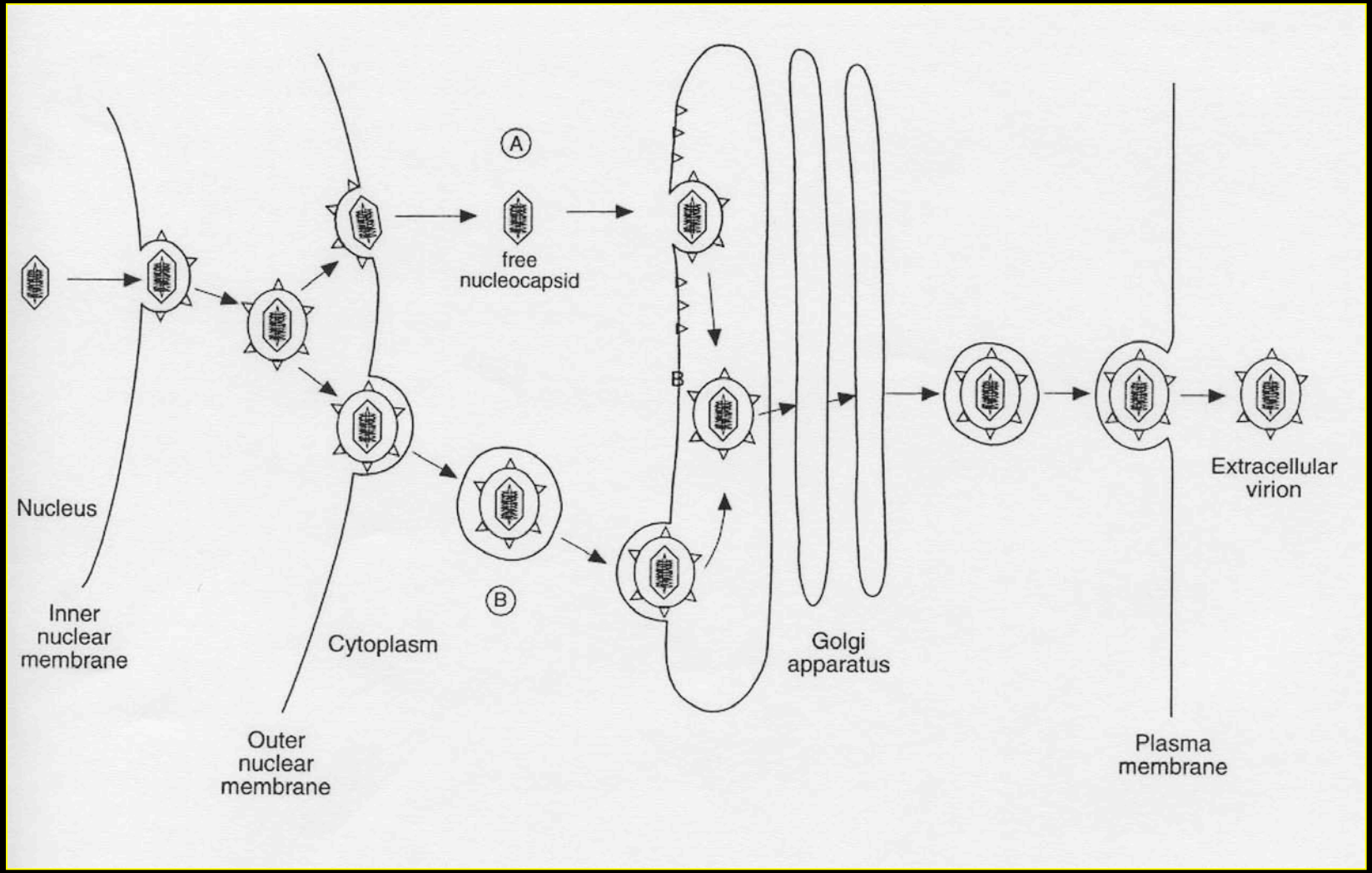
1) Co-transfect mammalian cells with plasmids encoding 7 viral proteins:
DNA polymerase
Polymerase processivity protein
ssDNA binding protein
Origin binding protein
3 helicase/primase proteins

2) Harvest viral DNA

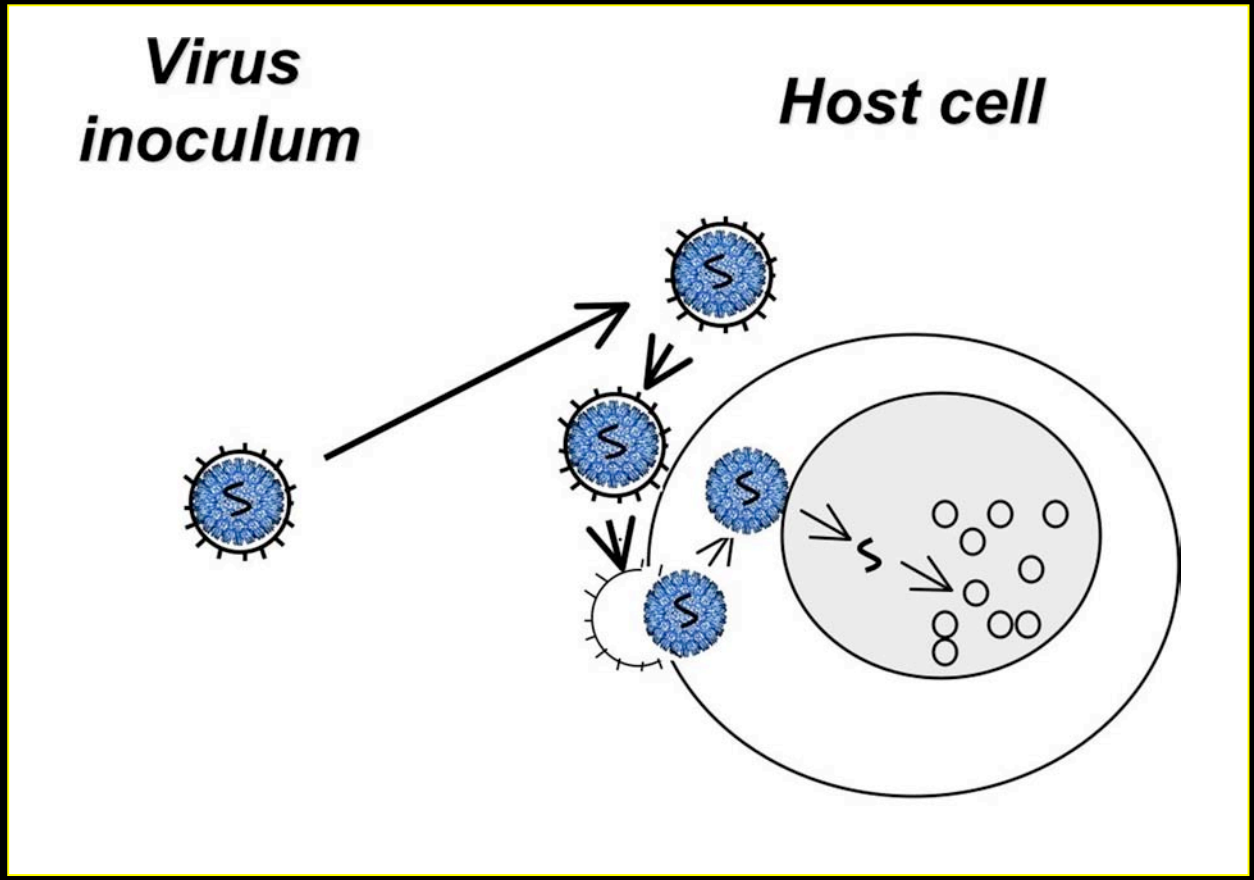
3) Digest with Dpn I (digests transfected DNA ONLY) + specific REs

4) Southern blot for plasmid

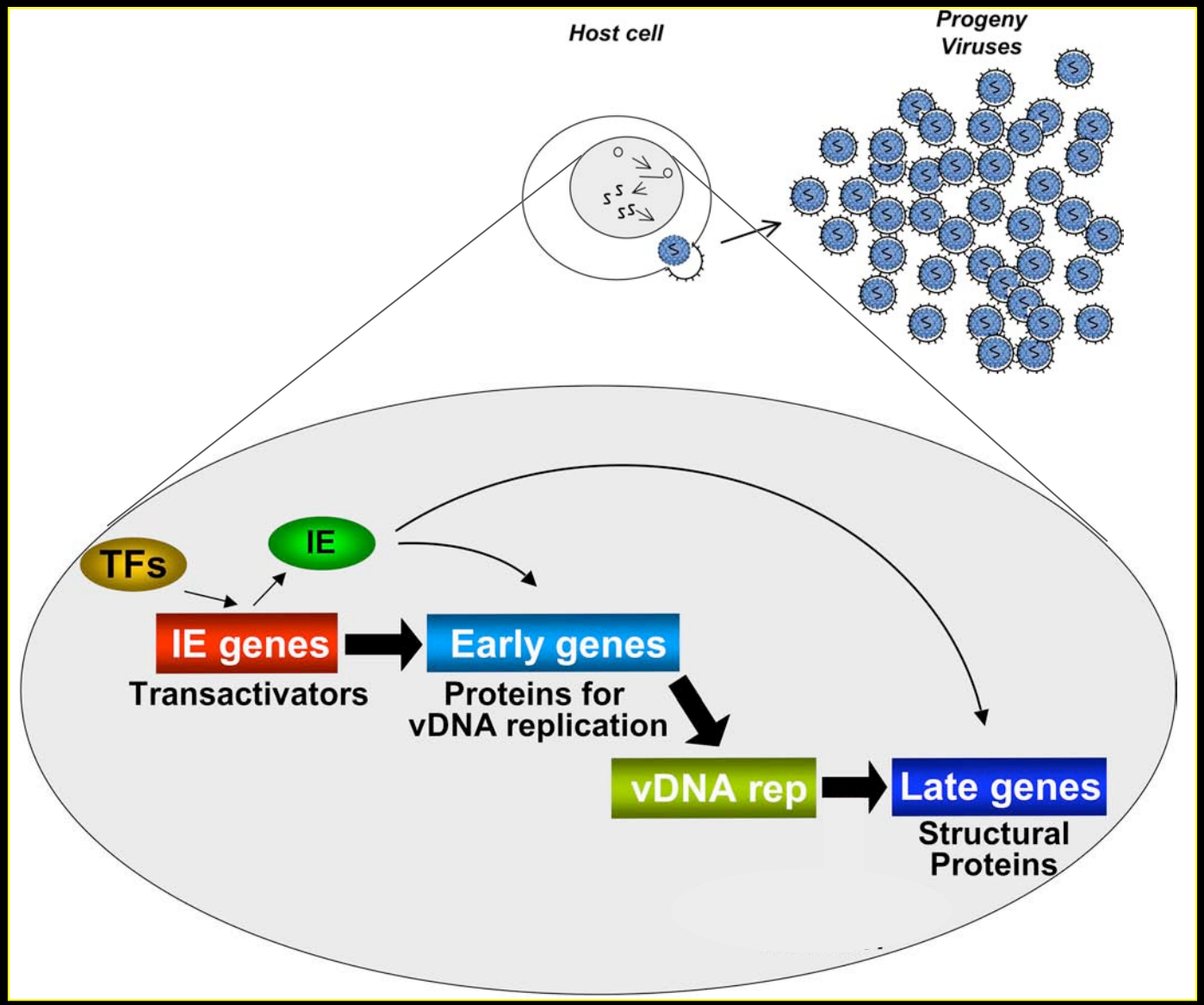
Successful replication leads to viral budding and cell lysis



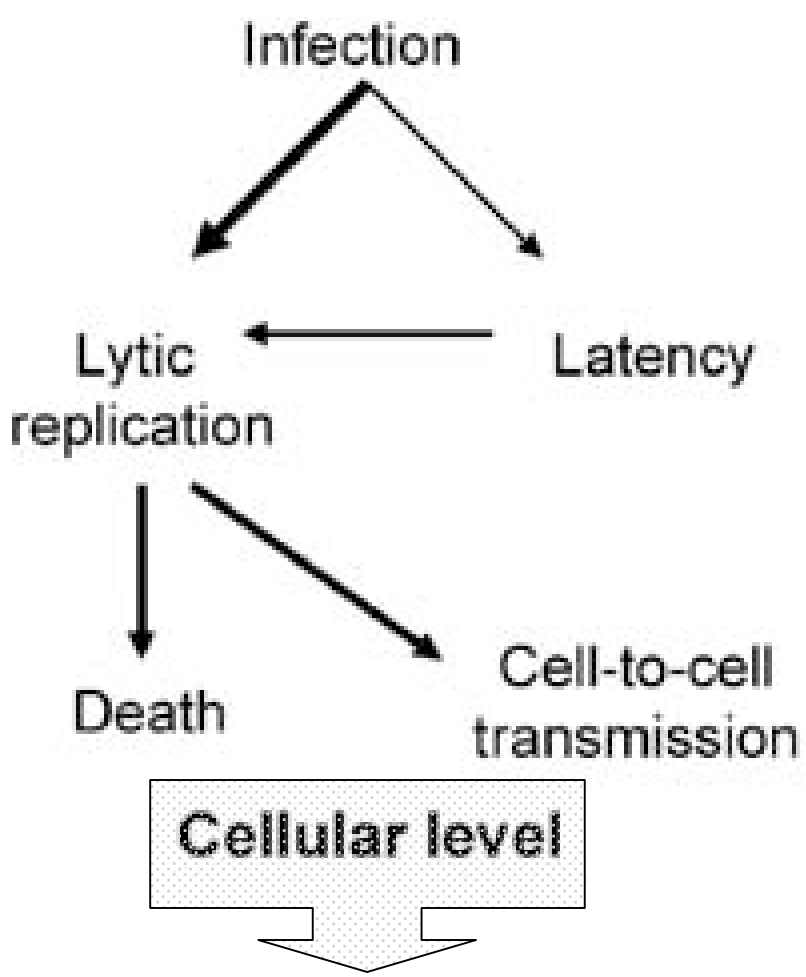
Herpesviruses establish latent, non-productive infections

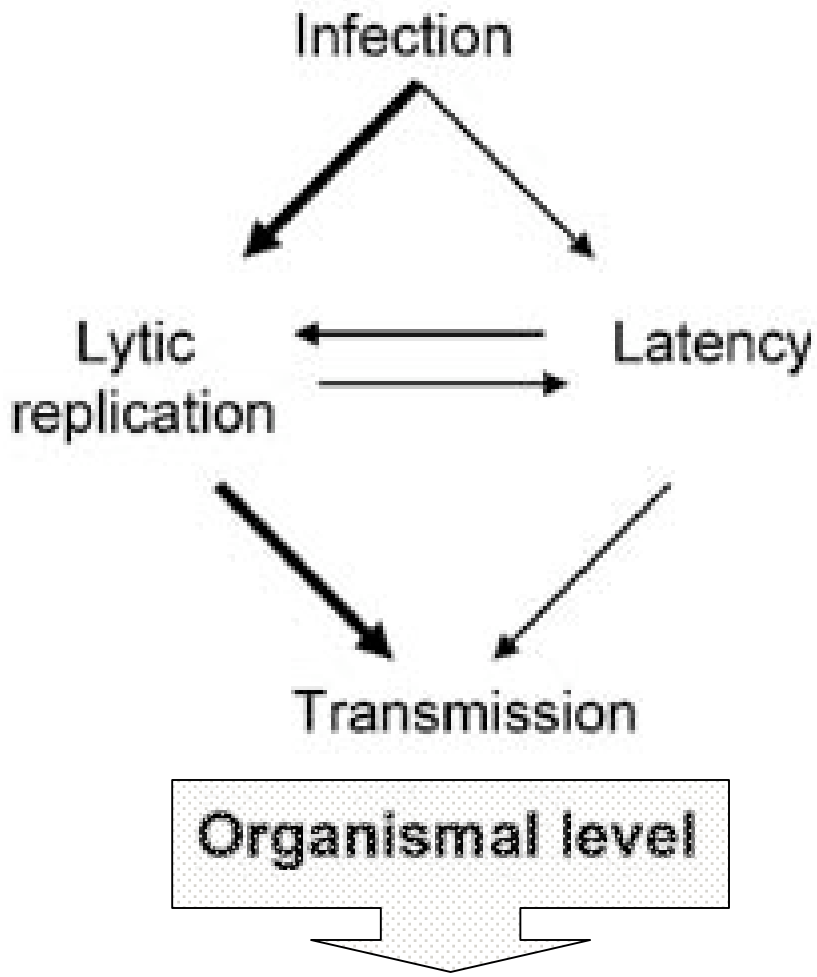


In herpesviral reactivation, the gene expression cascade is probably identical as in primary, productive infection



Experimental Models for Latency and Reactivation of Human herpesviruses are few in number.



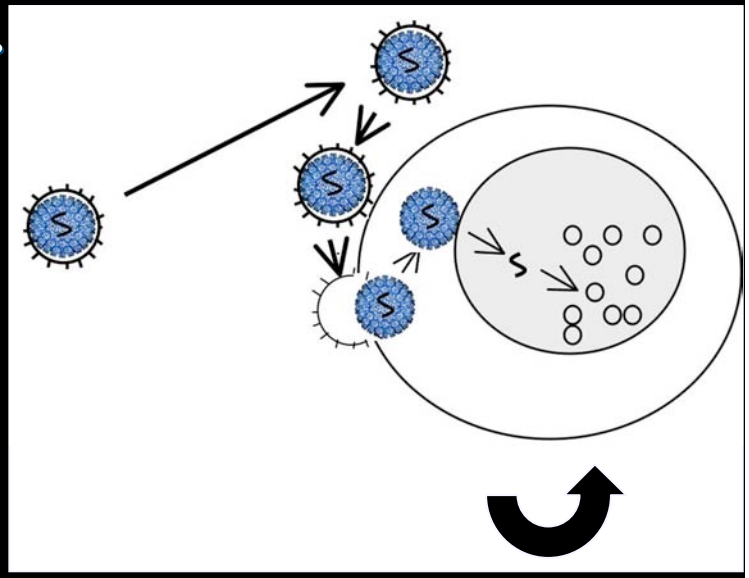


Gamma-herpesviruses **:Model:** **Herpes Simplex 1 and 2**

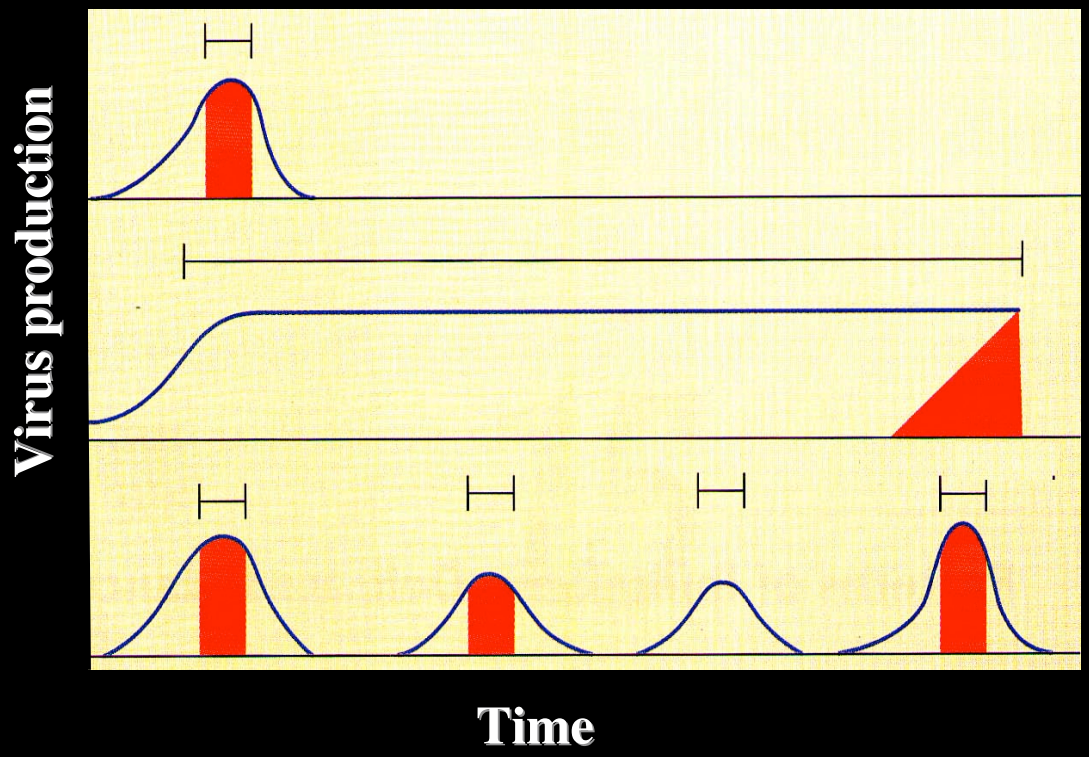
Non-human herpesviruses provide animal models for others

Herpesvirus Latency--Characteristics (cellular level)

1. Viral genomes persist as nuclear episomes:
   some herpesviruses express protein that tethers viral chromatin to host chromatin.
2. Viral DNA is nucleosomal.
3. Viral gene expression is extremely limited.
4. Viral DNA replicates along with host.
   Requires ori_{lat} + cellular replication machinery. Proceeds via a theta form. Yields circular progeny genomes.
5. Immune detection of the virus is reduced or eliminated.
6. Mature virions are not produced.
7. Establishment and maintenance of latency can quantitated separately. Establishment is not well-understood in herpesviruses.
8. Virus can be reactivated into productive cycle at a later time.



Herpesviral infection alternates between productive and non-productive replication for the life of the host (organismal level)



Acute infection
- Rhinovirus
- Rotavirus
- Influenza virus

Persistent infection
- Lymphocytic Choriomeningitis virus

Latent, reactivating infection
- Herpesviruses

Mechanisms that control productive vs. latent infection

Lack of expression of immediate early (IE) genes.

Expression is repressed by host cell factors
(transcription factors or repressive chromatin).

Expression is repressed by viral factors

Absence of host cell factors
- Lack of expression
- Lack of proper modifications
- Absence from nucleus

Inhibition of viral replication by the host immune response.

Evidence supports a combination of all of the above.

Balance between above mechanisms and those promoting productive infection probably determines outcome.

Specific players differ for different herpesviruses.

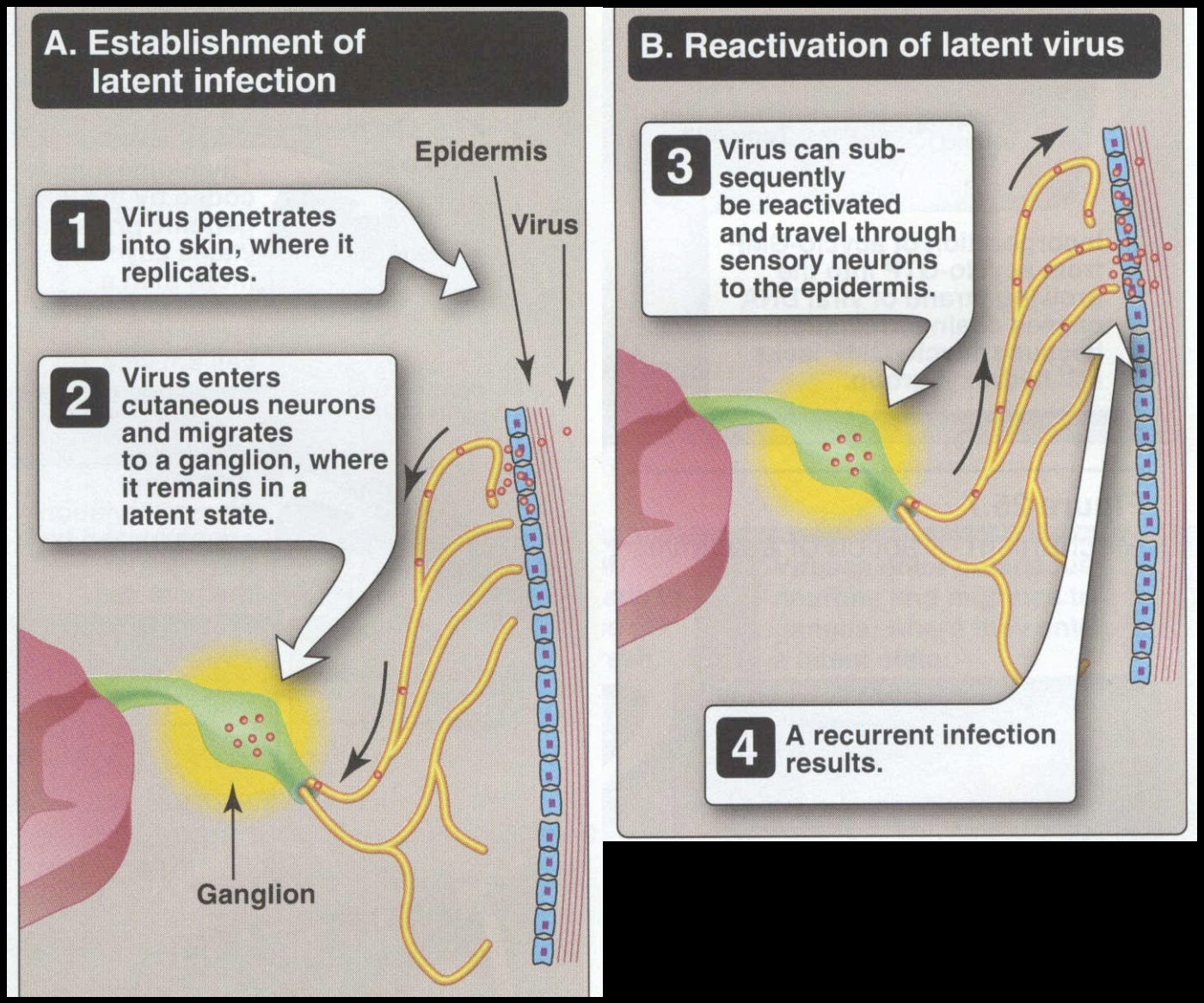
Latent and Lytic Herpesviral replication is Cell and Disease-specific

Classif.	Common name	LatentSite-Disease	Reactivation-Disease
HHV-1 HHV-2	Herpes Simplex Virus-1 Herpes Simplex Virus-2	Neurons-none	Cutaneous Epithelium lesions-Face or Genital
HHV-3	Varicella/Zoster Virus	Neurons-none	Cutaneous Epithelium lesions-shingles, pain
HHV-4	NEXT SLIDE		
HHV-5	Human cytomegalovirus	Monocytes/ Macrophages -none	Widespread Epithelium, and Endothelium-Fever, Retinitis, Cardiovascular disease
HHV-6A HHV-6B		Monocytes/Macro-none CD4+ T cells-none	Same-Bone Marrow Suppression, URI, AIDS dementia?
HHV-7		CD4+ T cells-none	Salivary Epithelium
HHV-8	NEXT SLIDE		

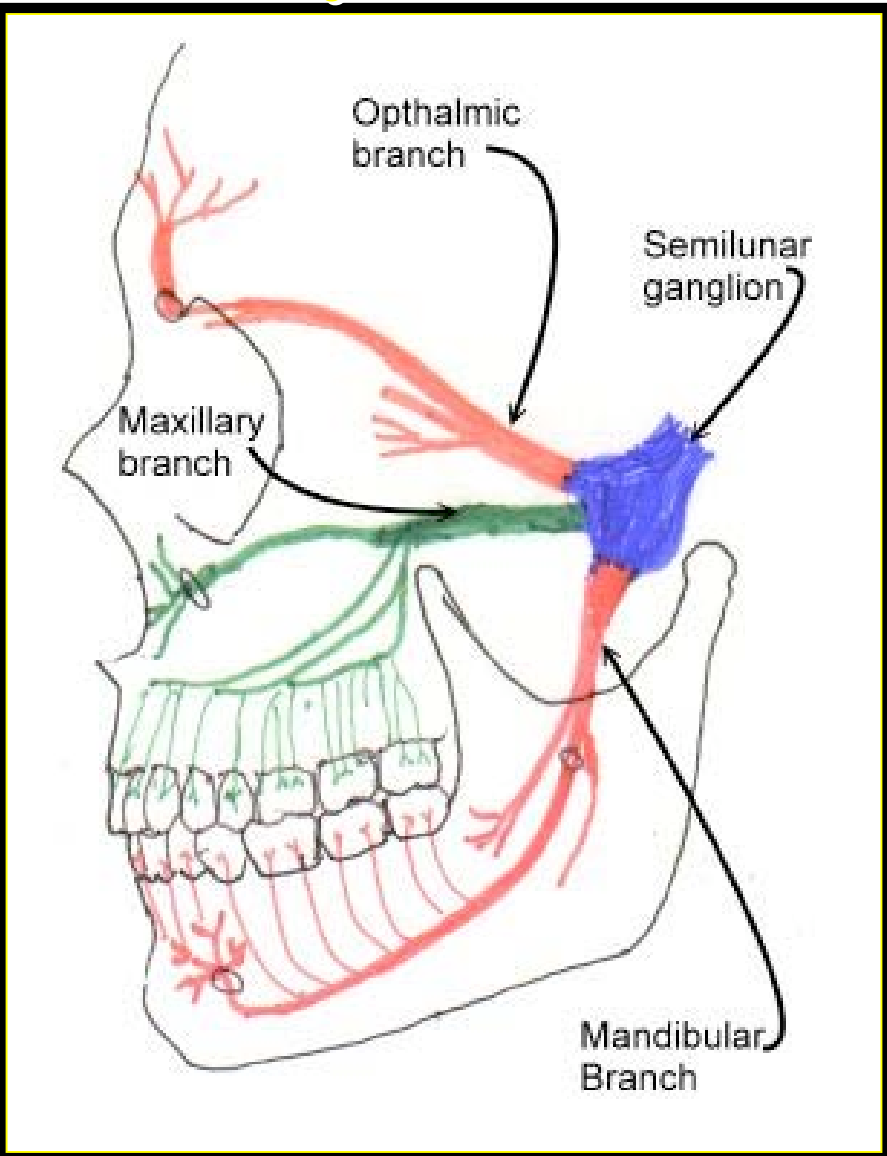
Only Epstein-Barr Virus (EBV) and Kaposi's sarcoma-associated Herpesvirus (KSHV) are conclusively associated with Human cancers.

Classif.	Common name	LatentSite-Disease	Reactivation-Disease
HHV-4	Epstein-Barr Virus	Resting, memory B cells-Lymphomas, incl. Burkitt's, AIDS-associated, Hodgkin's disease, others Nasal epithelium-Nasopharyngeal carcinoma	Oral epithelium-Oral Hairy Leukoplakia
HHV-8	Kaposi's sarcoma-associated Herpesvirus	B cells- Primary Effusion Lymphoma	Lymphatic Endothelium - Kaposi's sarcoma B cells-Multi-Centric Castleman's Disease

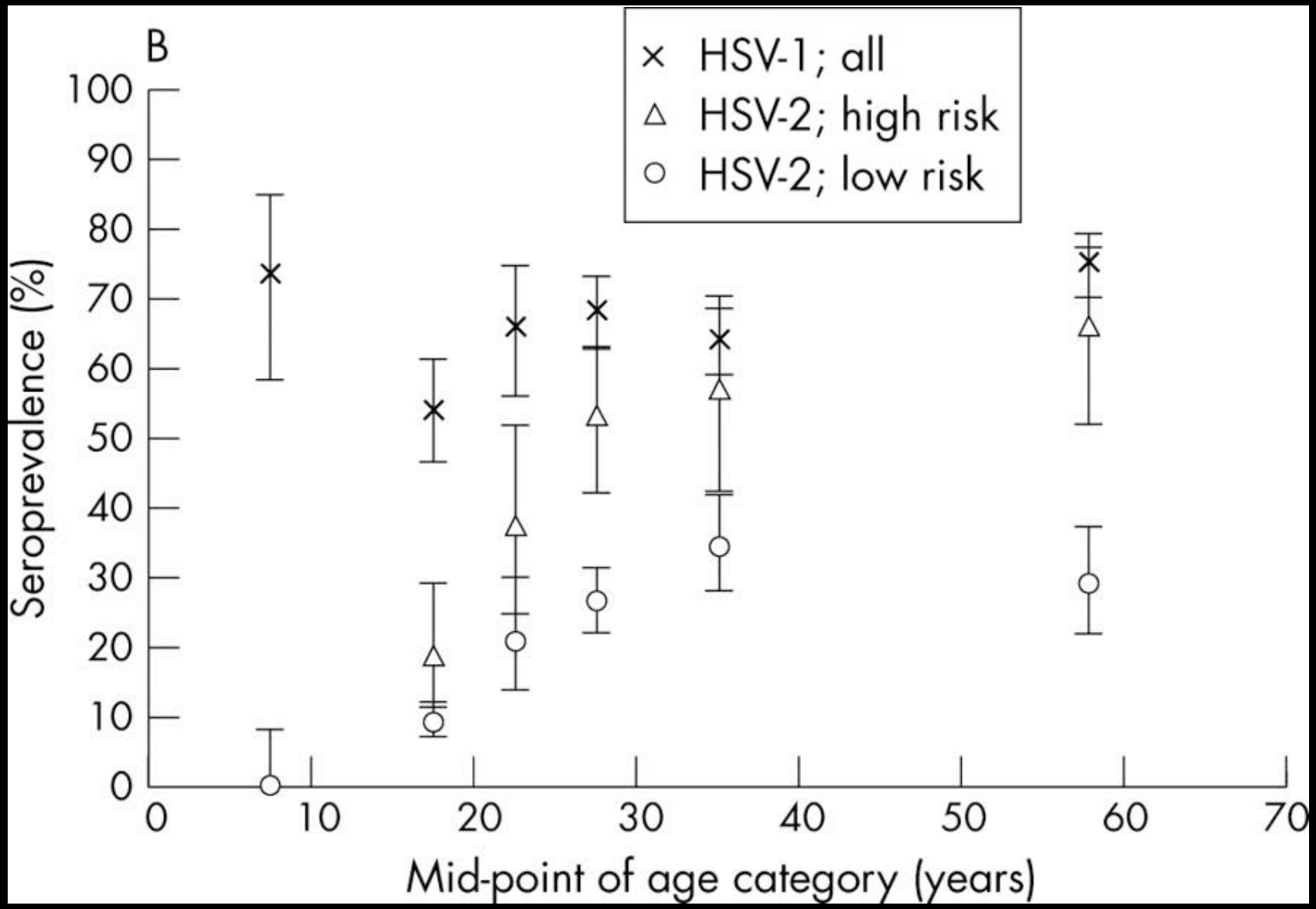
HSV Lifesytles



The trigeminal nerve is the major site of HSV-1 latency in mice and humans

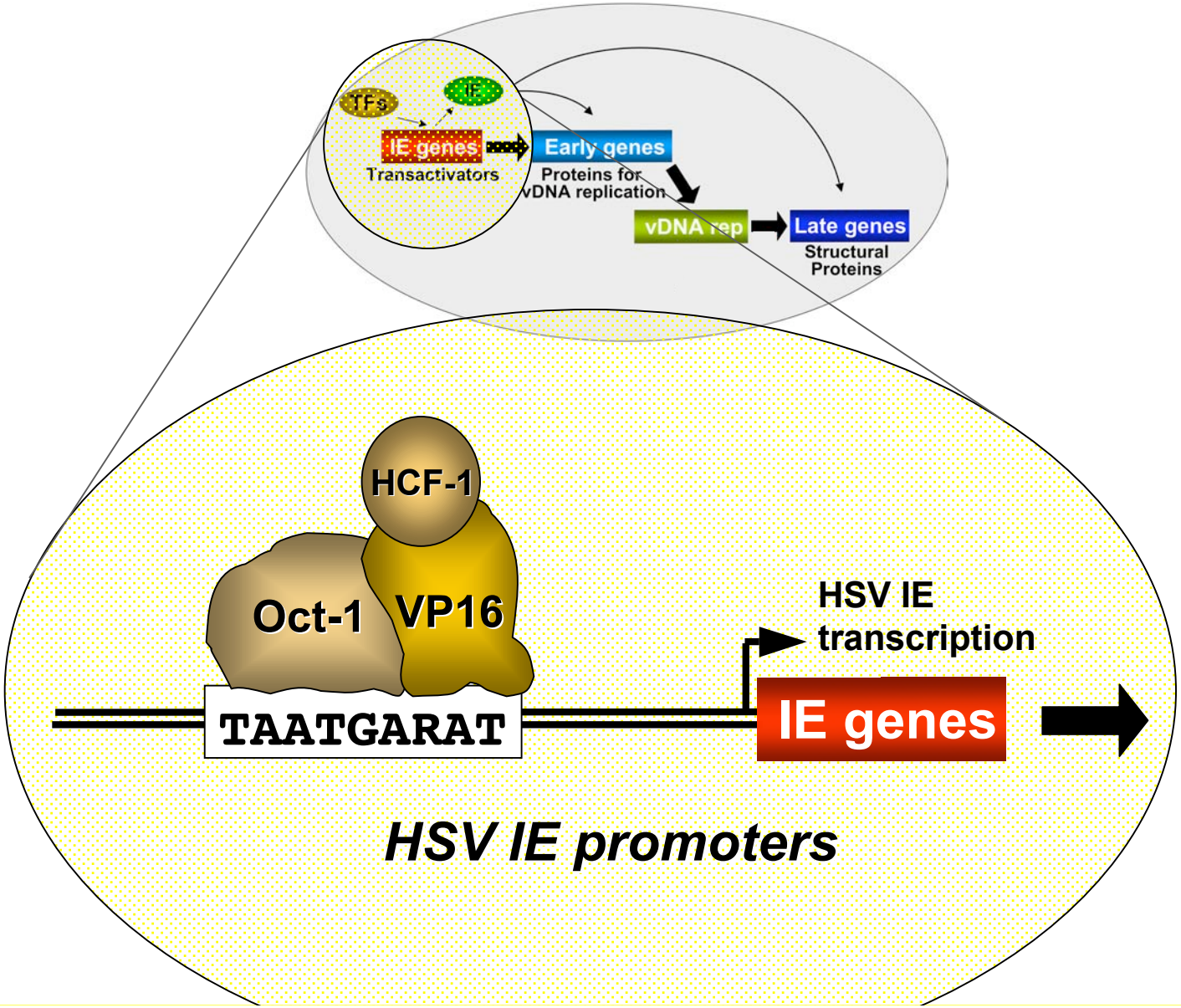


Comparative seroprevalence of HSV-1 and 2 in the US

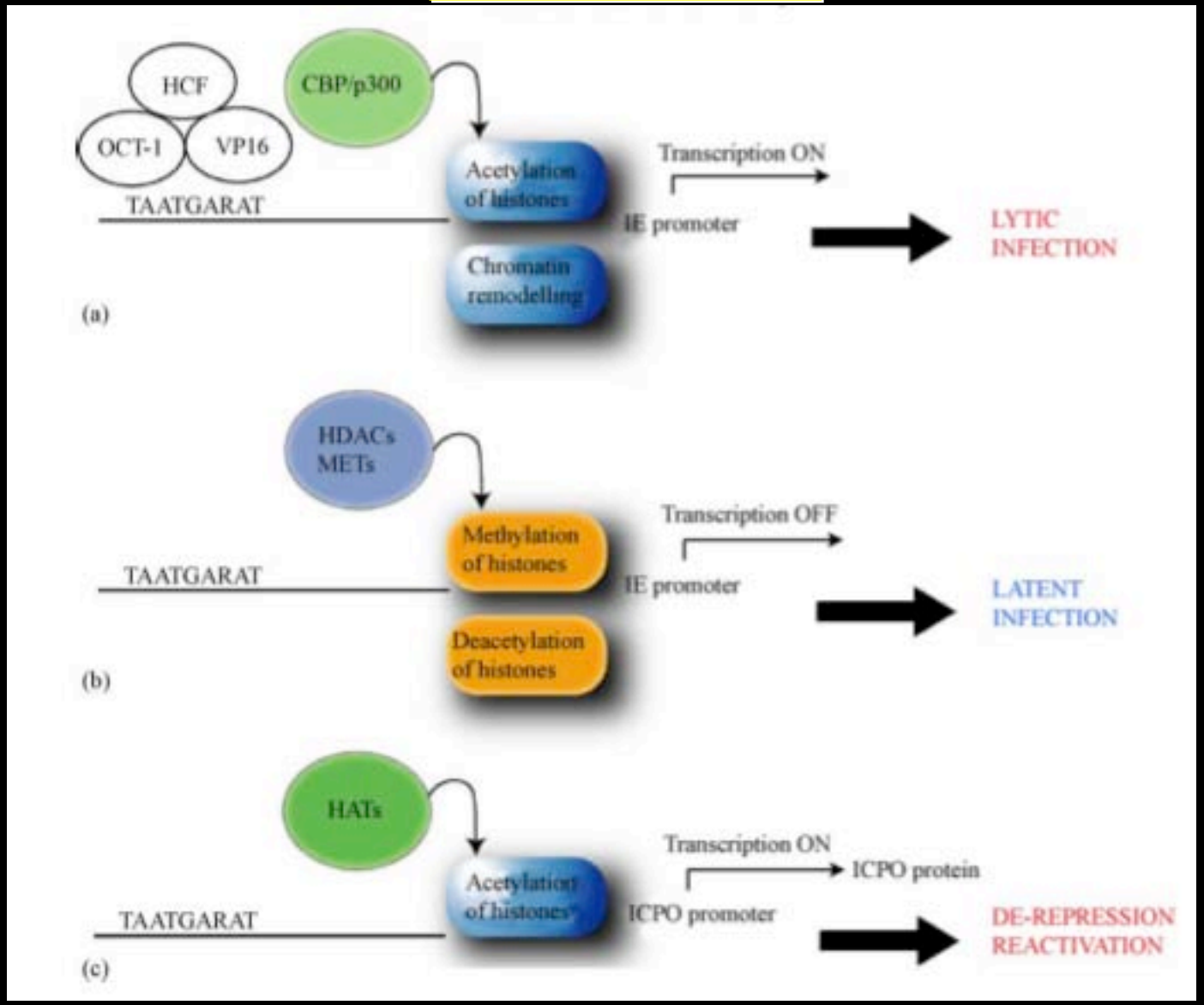


Looker and Garnett *Sex Transm Infect* (2005) 81: 103-7

HSV virion protein (VP)-16: a paradigm for promoter-specific transactivation in viral replication

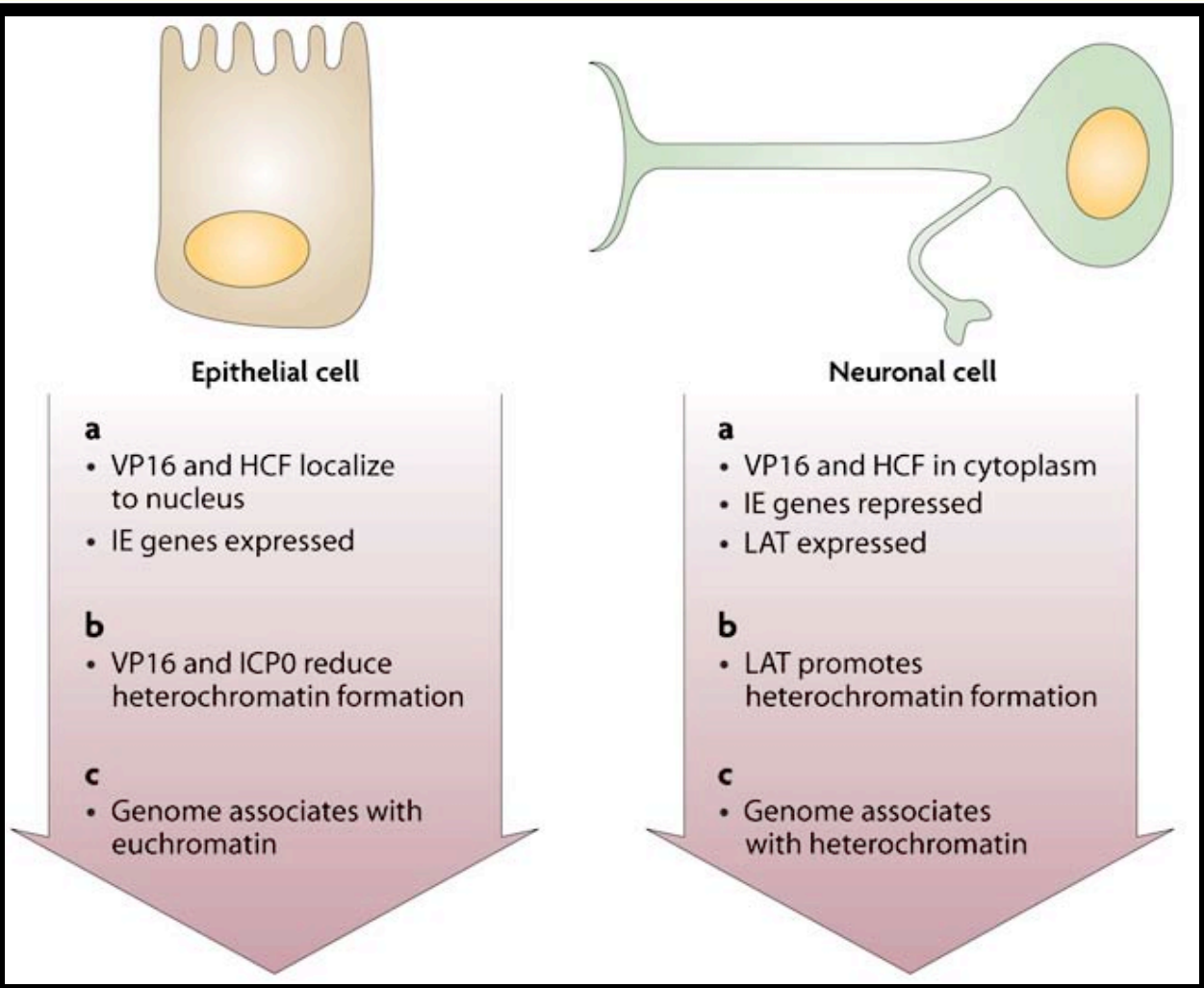


Histone modifications are critical in the latent to lytic switch of HSV



Virus Res. 111: 108-119.

Establishment/maintenance of HSV latency

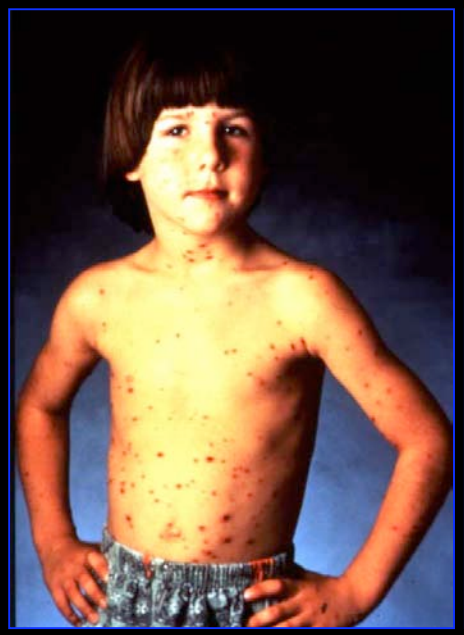


Productive infection **Latent infection**

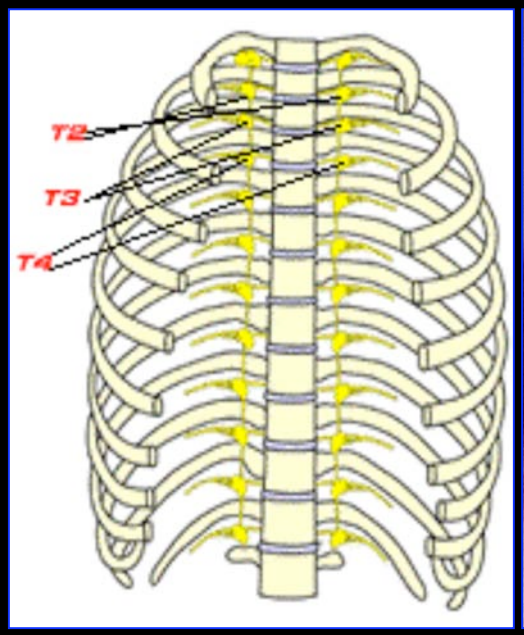
Nat. Rev. Microbiol. 6: 211-221.

Varicella-Zoster Viruses (VZV)

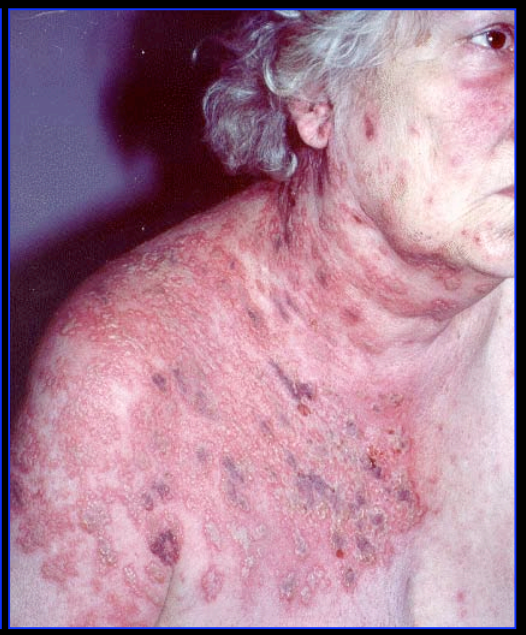
VZV gives rise to two distinct clinical syndromes



Varicella = Chicken pox
Primary infection

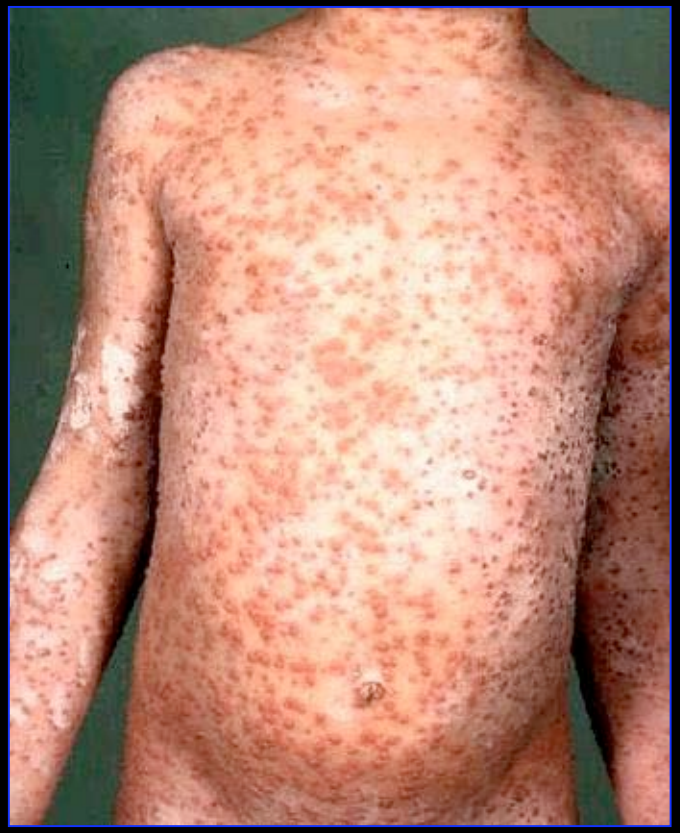


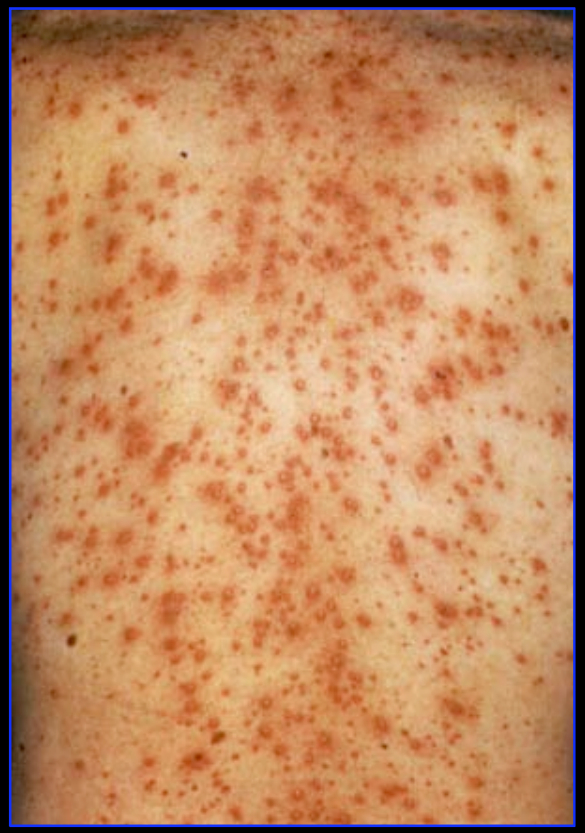
Latency in
ganglia



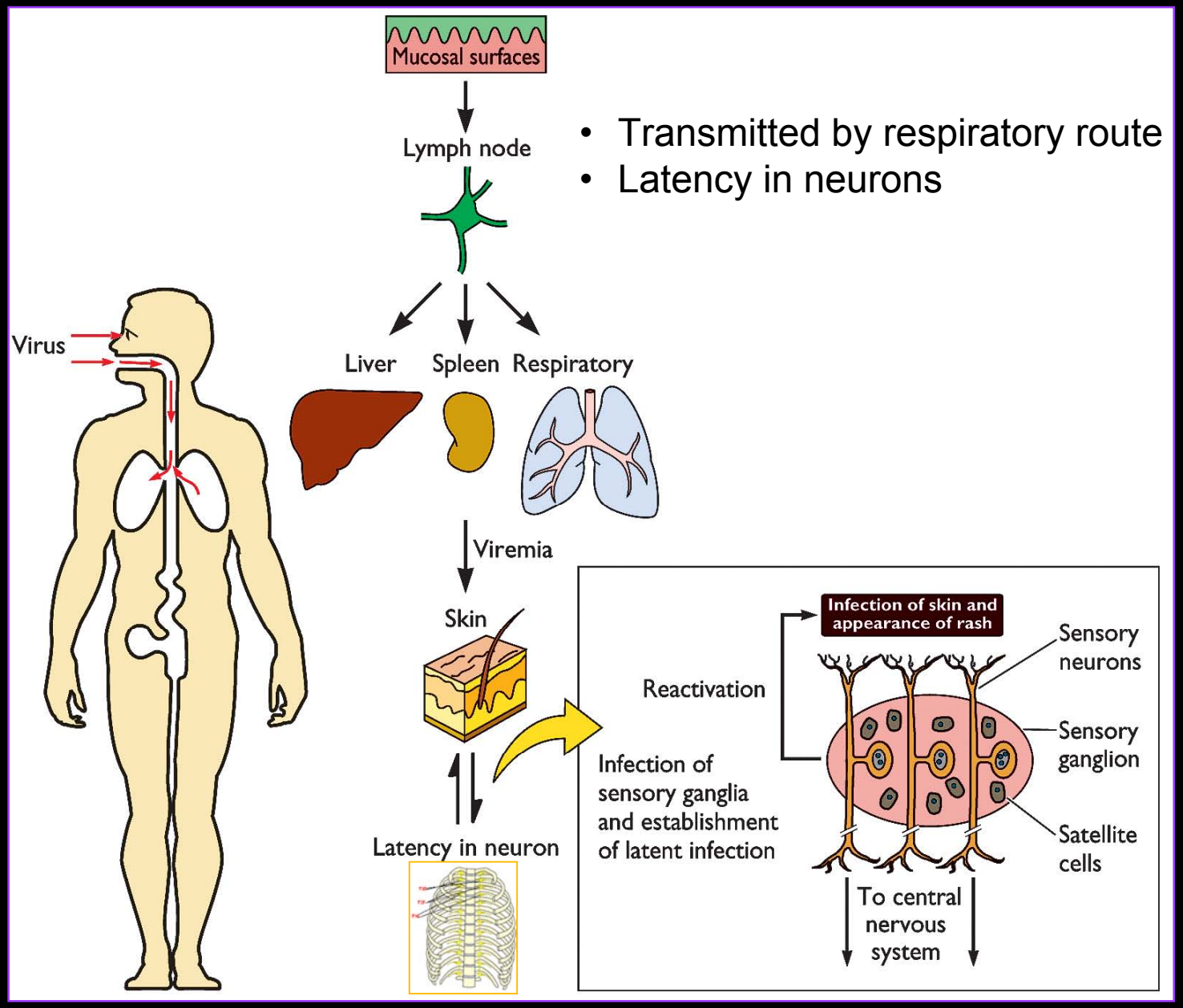
Zoster = Shingles
Reactivation

Chicken pox





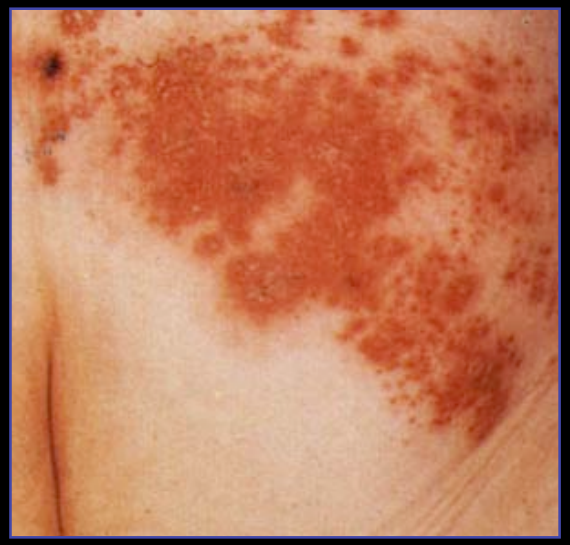
VZV Disease Mechanism



- Transmitted by respiratory route
- Latency in neurons

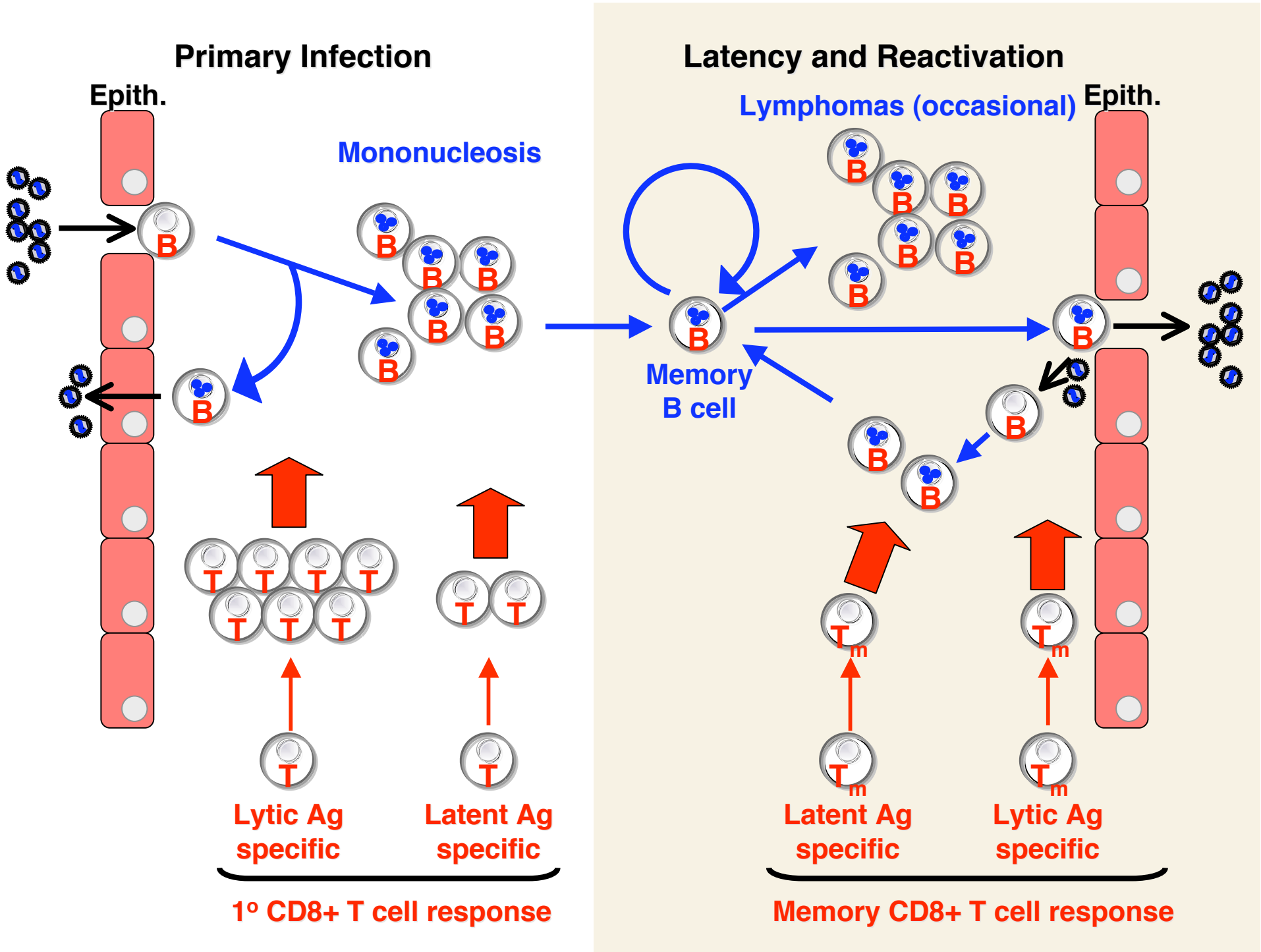
Zoster (Shingles)

- ~ 1 million cases/year in US
- > 65 year old individuals
- Reactivation of VZV from dorsal root ganglia - viruses transport to skin
- Occurs only in persons who have previously had chicken pox
- Symptoms:
  A rash that develops into clear blisters (full of infectious virus) and moderate to severe pain, potentially dangerous in the elderly



Epstein-Barr Virus (EBV)

- 1958- first described in a childhood tumor by Dr. Burkitt
  Burkitt's lymphoma
  1964- identified by Epstein and Barr by EM
- 90-95% of adults show evidence of EBV infection


Primary Infection
Epith.
Mononucleosis
B
T
Lytic Ag specific
Latent Ag specific
1° CD8+ T cell response
Latency and Reactivation
Lymphomas (occasional)
Epith.
Memory B cell
T_m
Latent Ag specific
Lytic Ag specific
Memory CD8+ T cell response

Maintenance of EBV latency and transformation of B cells requires the same proteins.

1. To maintain a latent infection, EBV must stimulate growth and survival of host B cell. This can lead to lymphoma

   Three EBV proteins are essential for latency/transformation:

   EBNA-2 (EBV nuclear antigen-2) - a transcriptional activator that orchestrates latent gene expression

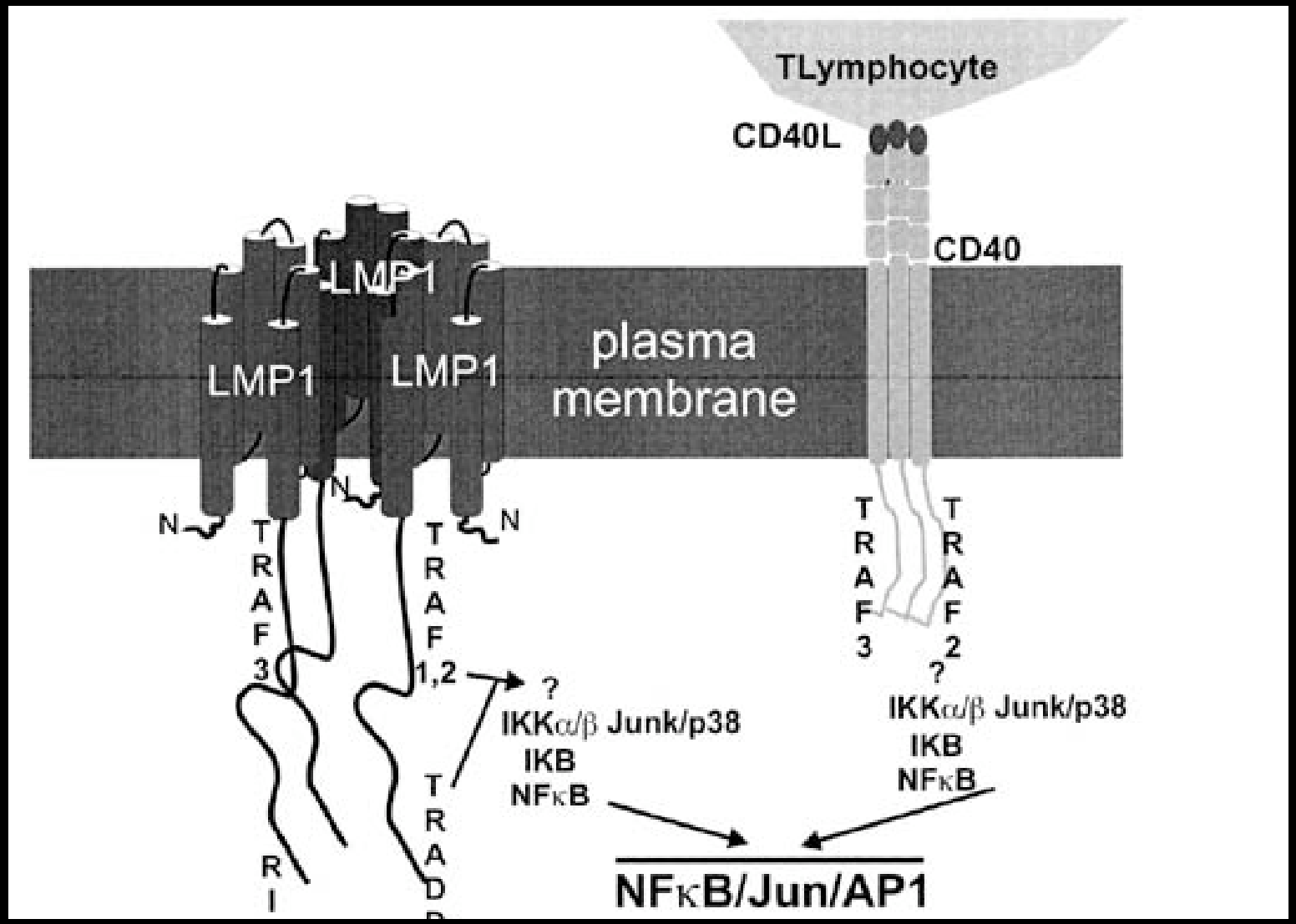
   LMP-1 (latent membrane protein-1) - can transform permanent cell lines and induce B-cell lymphomas in transgenic mice.

   EBNA-1 - required for replicating the latent episomal genome, tethers genome to host chromosome

2. c-myc is overexpressed by one of two mechanisms:
   a. EBNA2 directly transactivates it
   b. C-myc is translocated adjacent to a strong cellular promoter.

3. NF-kB is constitutively activated by LMP-1--inhibits apoptosis.

LMP1, the major EBV oncoprotein, mimics constitutively active CD40 receptor to activate growth, division, of infected B cells



Kaposi's Sarcoma-Associated Herpesvirus
(KSHV or HHV-8)

- KS was first described by Moritz Kaposi in the 1870s
- KS is a cancer that develops in lymphatic endothelial cells
- KS is more common in AIDS patients

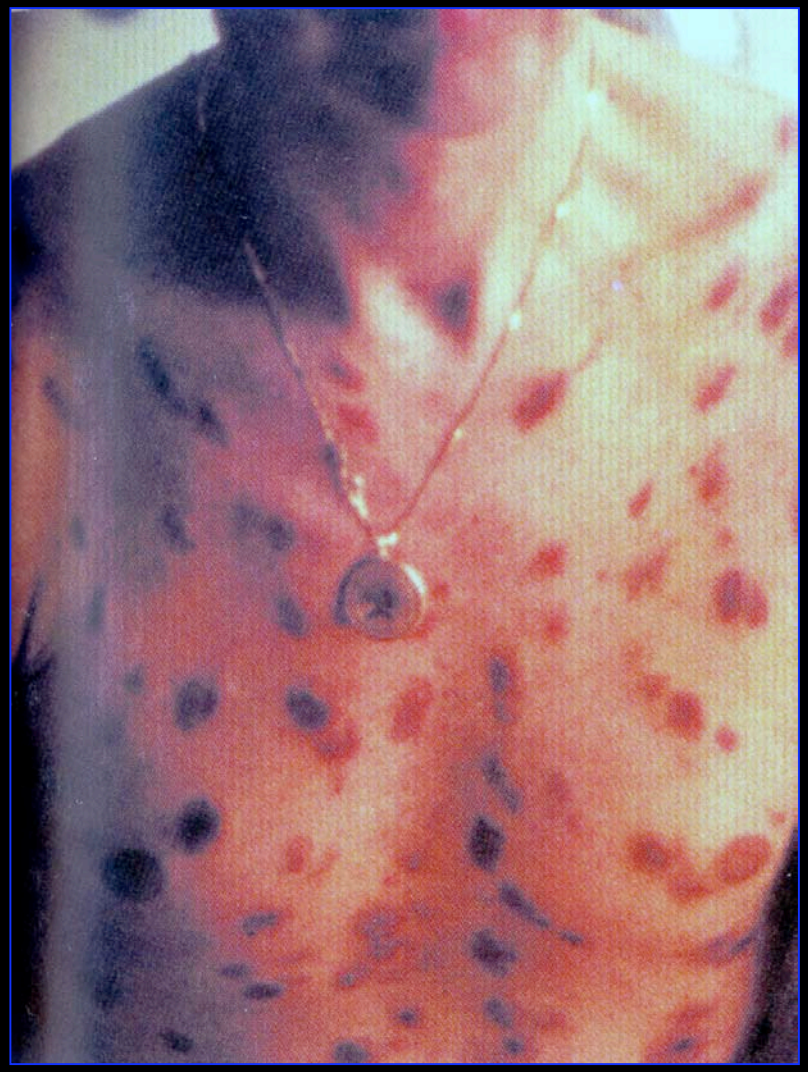
  91,000 persons with AIDS (1989), 15% have KS

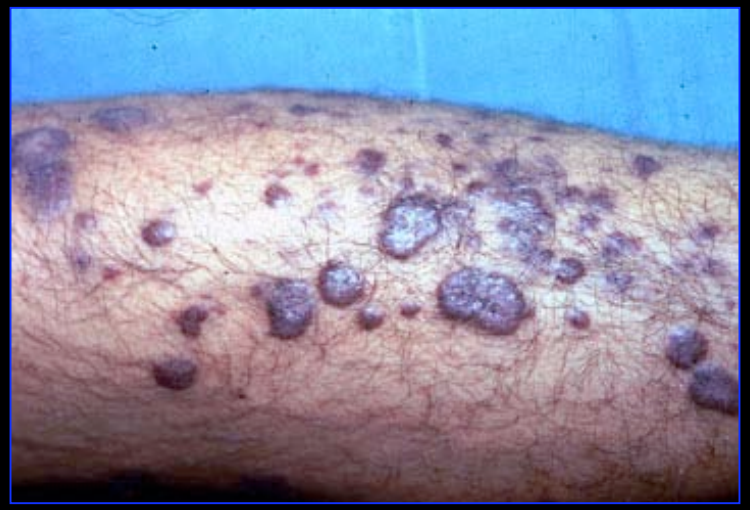
  >20,000X more common in person with AIDS

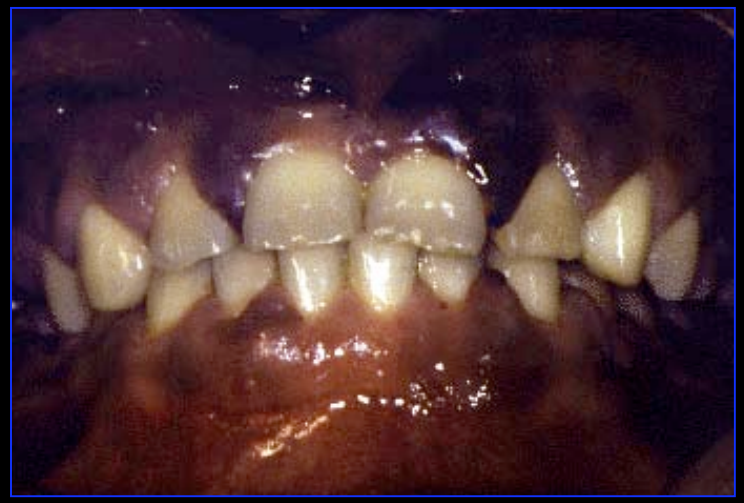
  ~300X more common in other immunosuppressed groups
- A sexually transmitted factor other than HIV plays a role in KS

  KS is 10X more common in homosexual or bisexual men
- 1994, using a PCR-based technique Chang and Moore identified two small DNA fragments present in AIDS-KS samples - homology to EBV
- 4 clinico-epidemiologic forms: KSHV is etiologic agent of all.

Kaposi's sarcoma in patients infected with HIV-1

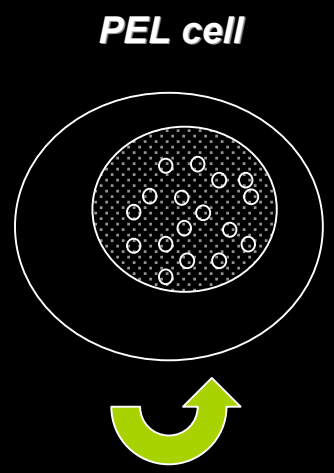






Red, blue or purple flat or raised lesions.

Primary Effusion Lymphoma cells: *The* tissue culture system to study lytic reactivation of KSHV



KSHV is the etiologic agent of

Primary Effusion Lymphoma (PEL)
B cell lymphoma--
B cells are KSHV reservoir *in vivo*

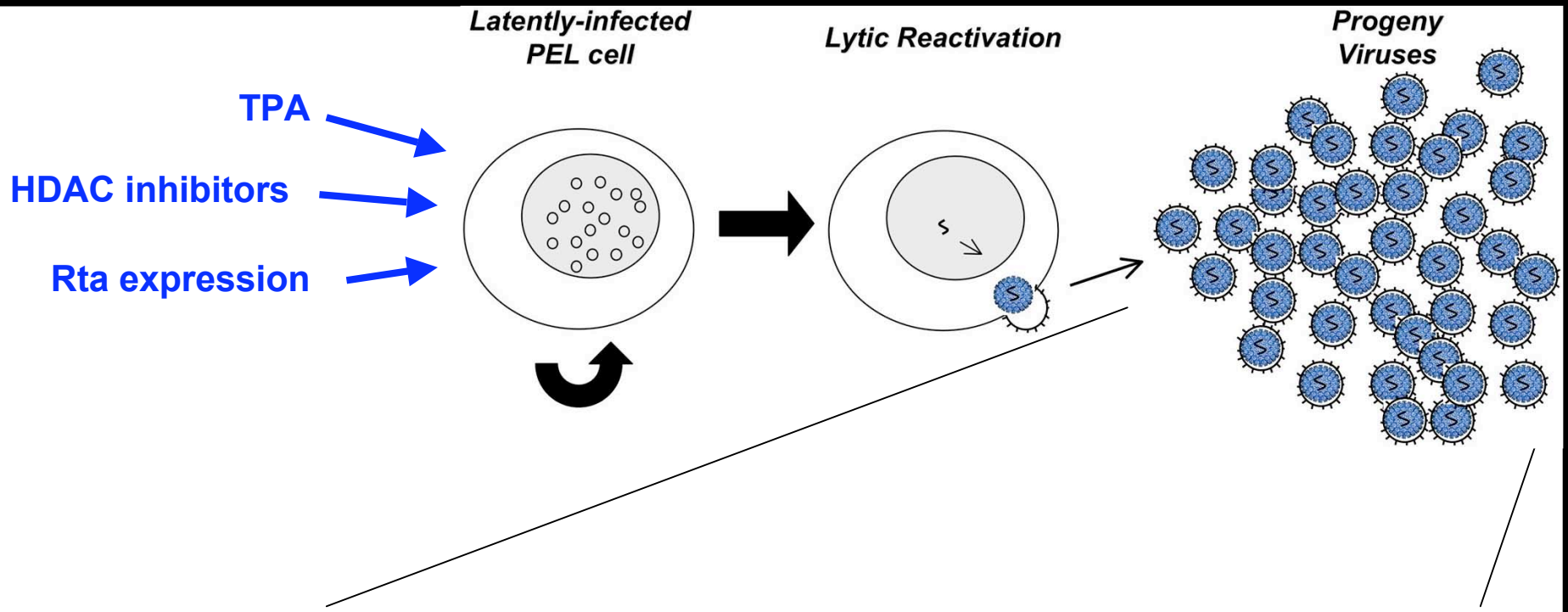
PEL Cell Lines

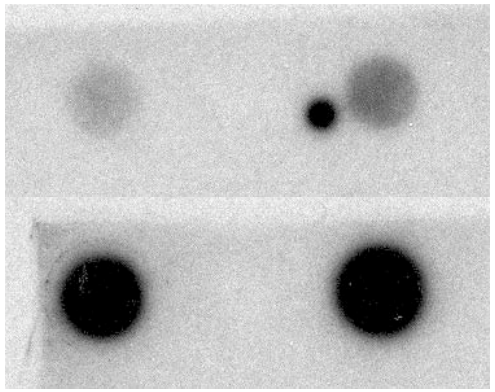
Explanted from PEL patients
50 copies KSHV per cell genome

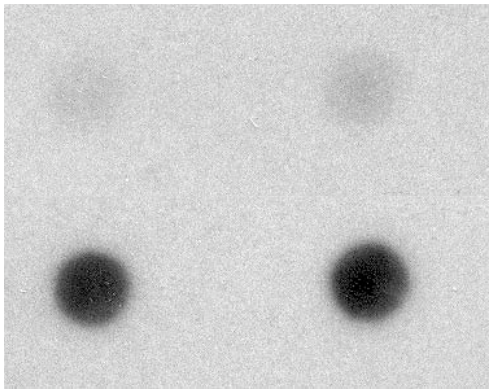
Latency

Virus is latent in >95% of cells
Highly restricted viral gene expression
(ca. 6 genes expressed)
Little spontaneous reactivationB

In PEL cells, KSHV is reactivated by chemicals or by ectopic expression of the KSHV ORF50/Rta protein

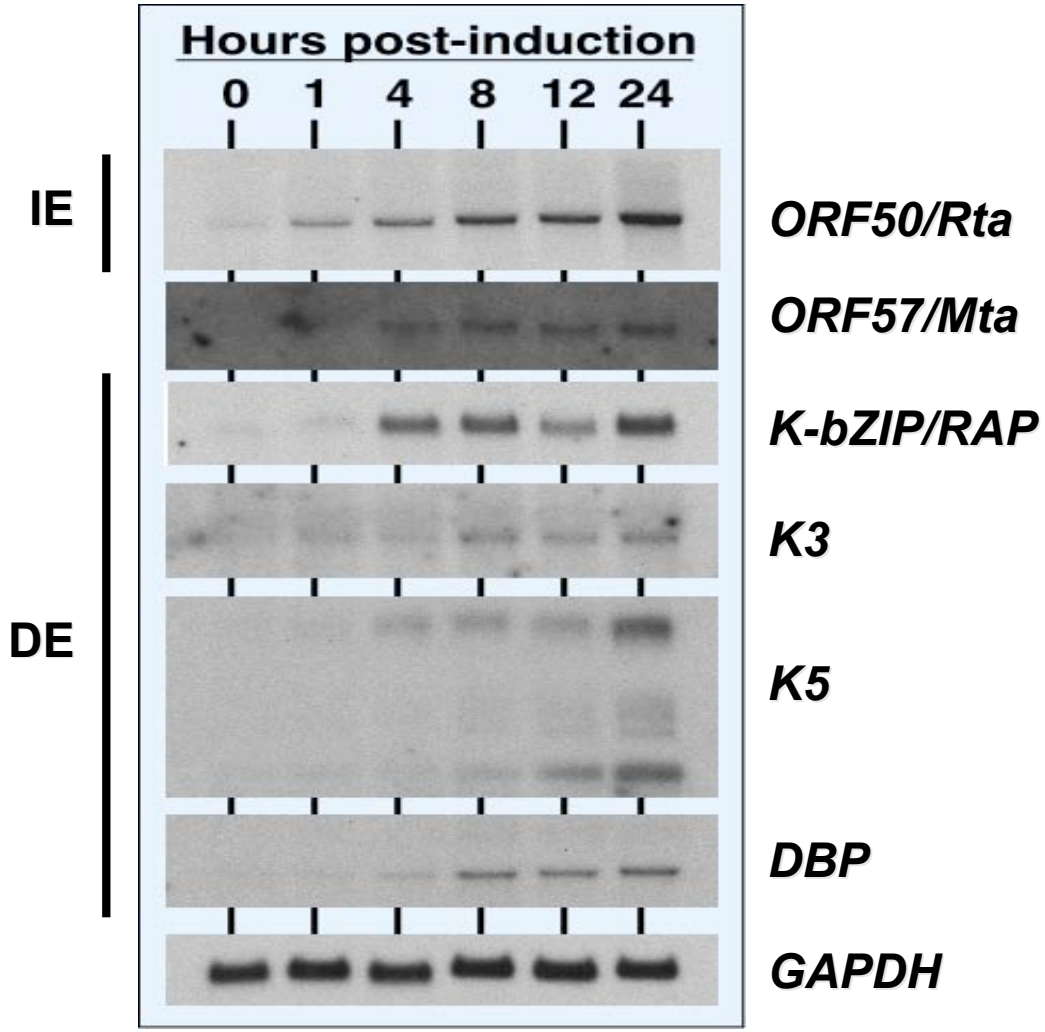






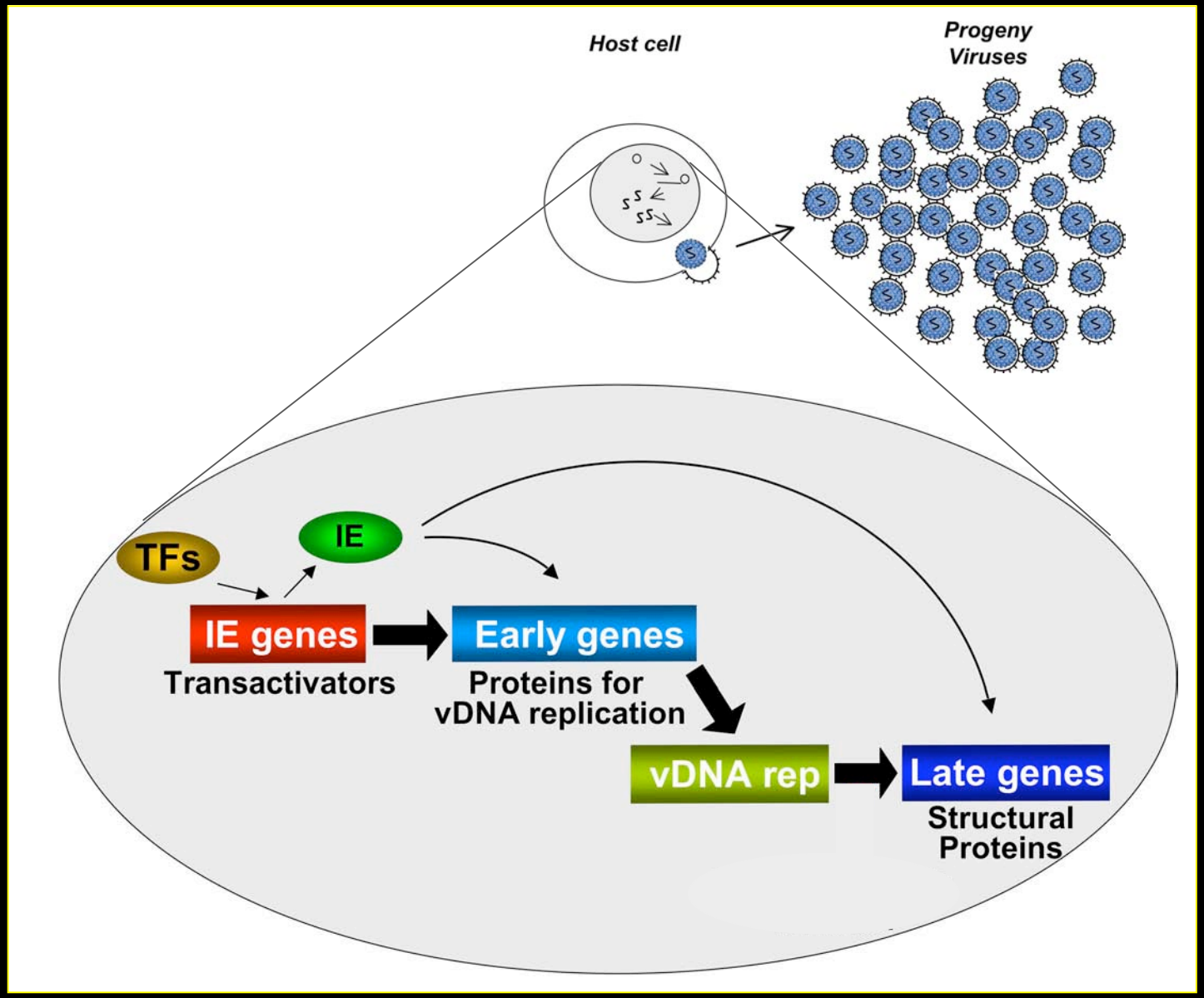
Palmeri, et al. J. Virol. 81: 13299-13314

TPA induces expression of Rta, leading to a cascade of viral gene expression

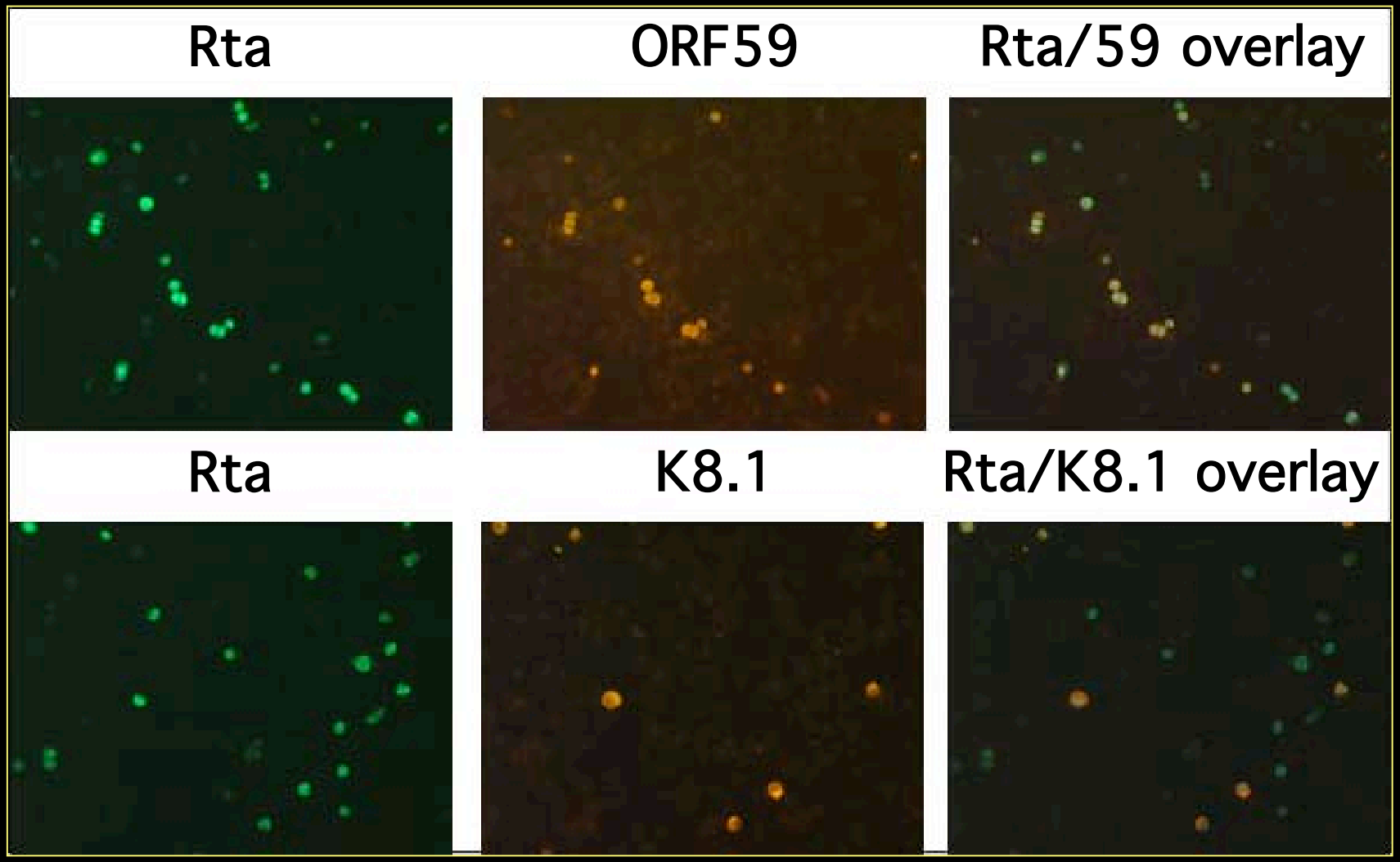


Lukac, et al. J. Virol. 73: 9348-9361.

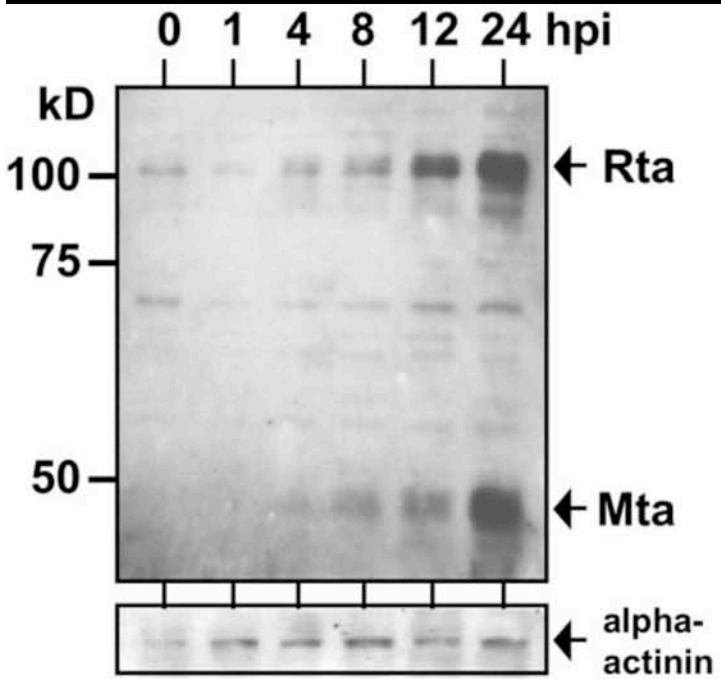
Does Herpesviral reactivation follow a gene expression cascade similar to that of de novo lytic infection?

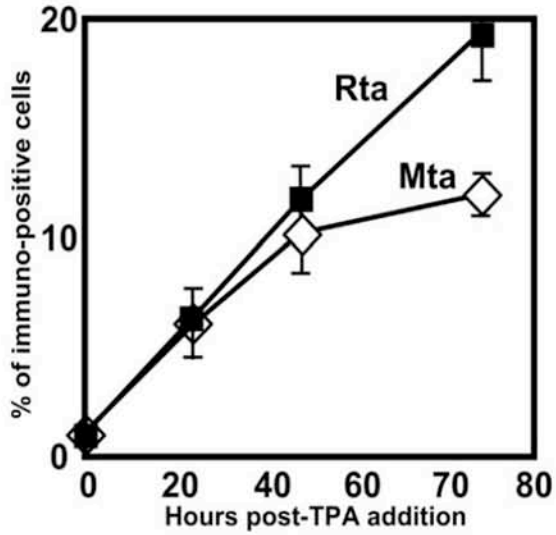


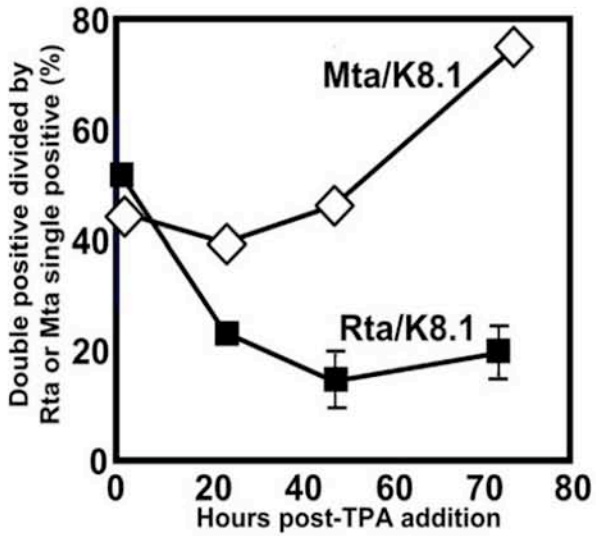
Rta is an inefficient reactivator



Mta is a commitment factor for reactivation





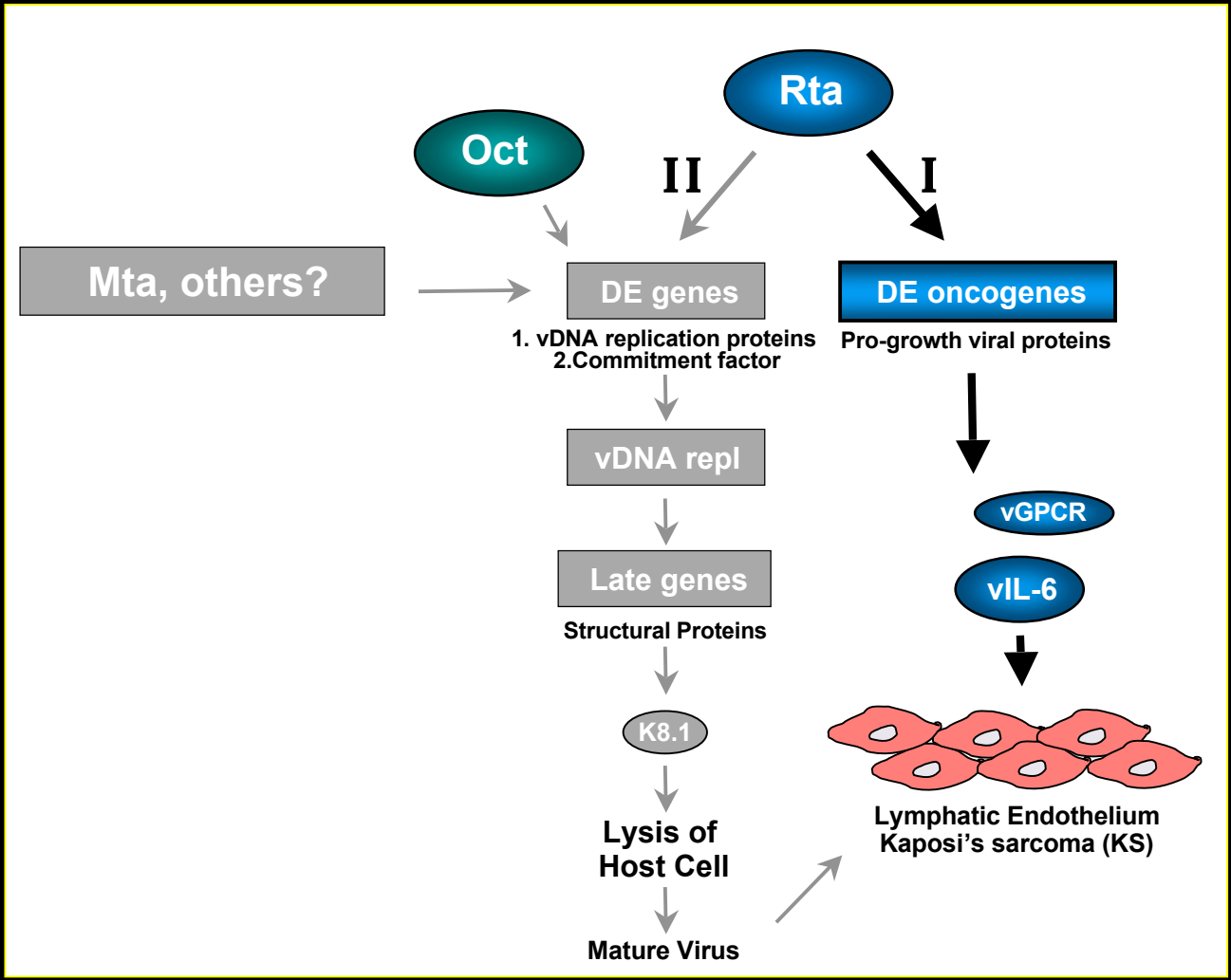


Pathogenesis?:

- KSHV encodes many homologues of cellular growth-control proteins. Most are expressed after reactivation.

vIL-6:	cell growth
vBCL2:	anti-apoptosis
vIRF:	inhibition of IFN signaling
vCYC:	cell cycle control
vGPCR:	(IL-8 receptor homolog) cell growth
vCCLs:	(chemokines) immune modulation

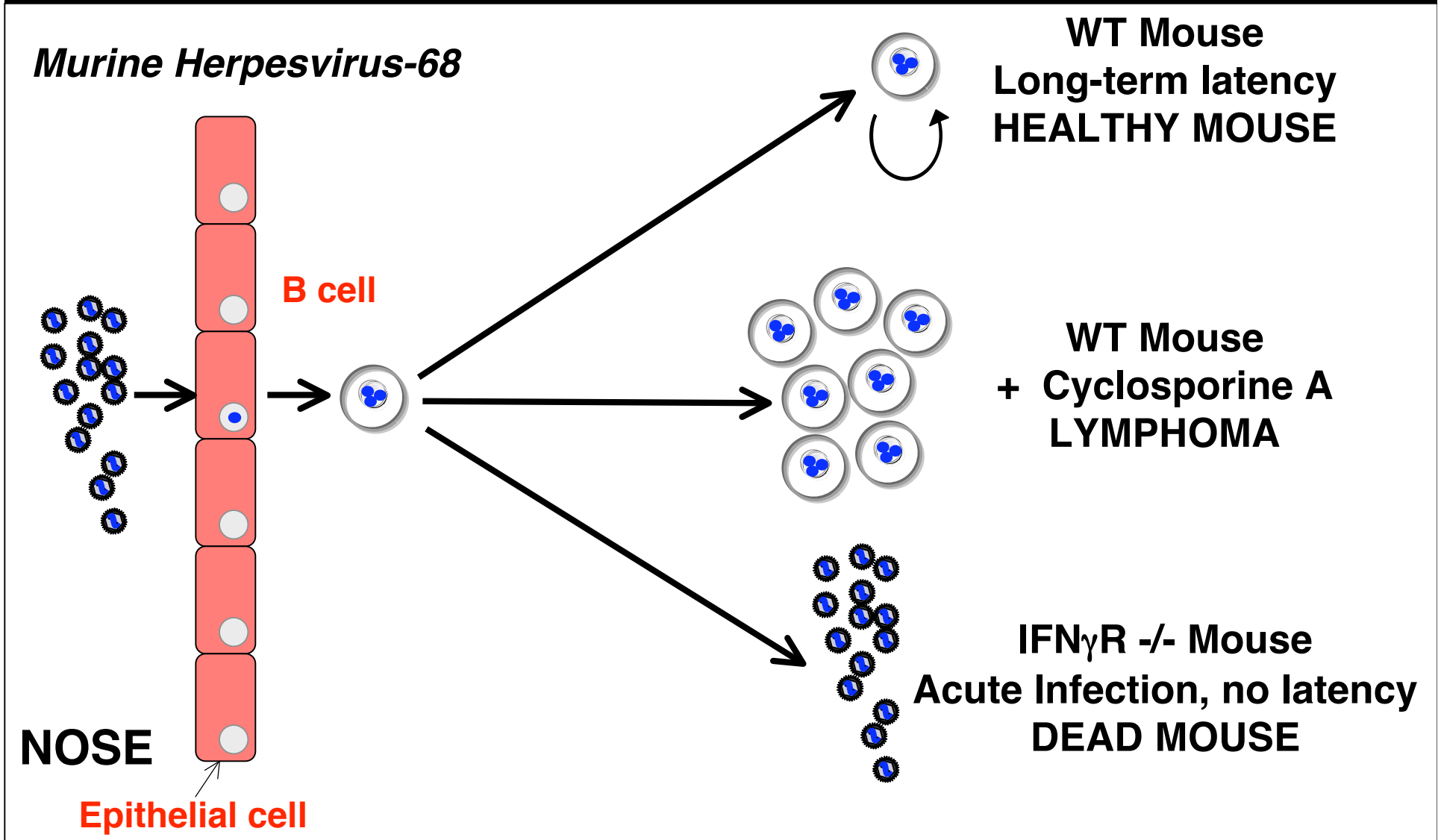
Sub-optimal progression of KSHV reactivation probably contributes to pathogenesis



The host immune response tempers herpesviral reactivation and pathogenesis.

- In immunocompromised hosts (AIDS, transplant, some elderly) herpesvirus reactivation ↑, viral load ↑, and risk and severity of disease ↑.
- Can often be reversed by restoring immunity.

Role of immune system--modeling gamma-herpesvirus infection in the mouse (MHV-68)



Role of immune system--modeling gamma-herpesvirus infection in the mouse (MHV-68)

	ΔCD8+	IFNγ –/–	ΔMHC II	Autologous Primed CD4+	ΔB cells
# of latently-infected cells	↑				
Lytic/Latent Infection			↑		
Lymphoma regression				!	
Reactivation efficiency	↑	↑			↑
Longer period of reactivation					↑

Assignment for Next Week:

Oral presentation of two papers on KSHV infection in humans