Persistent Infections

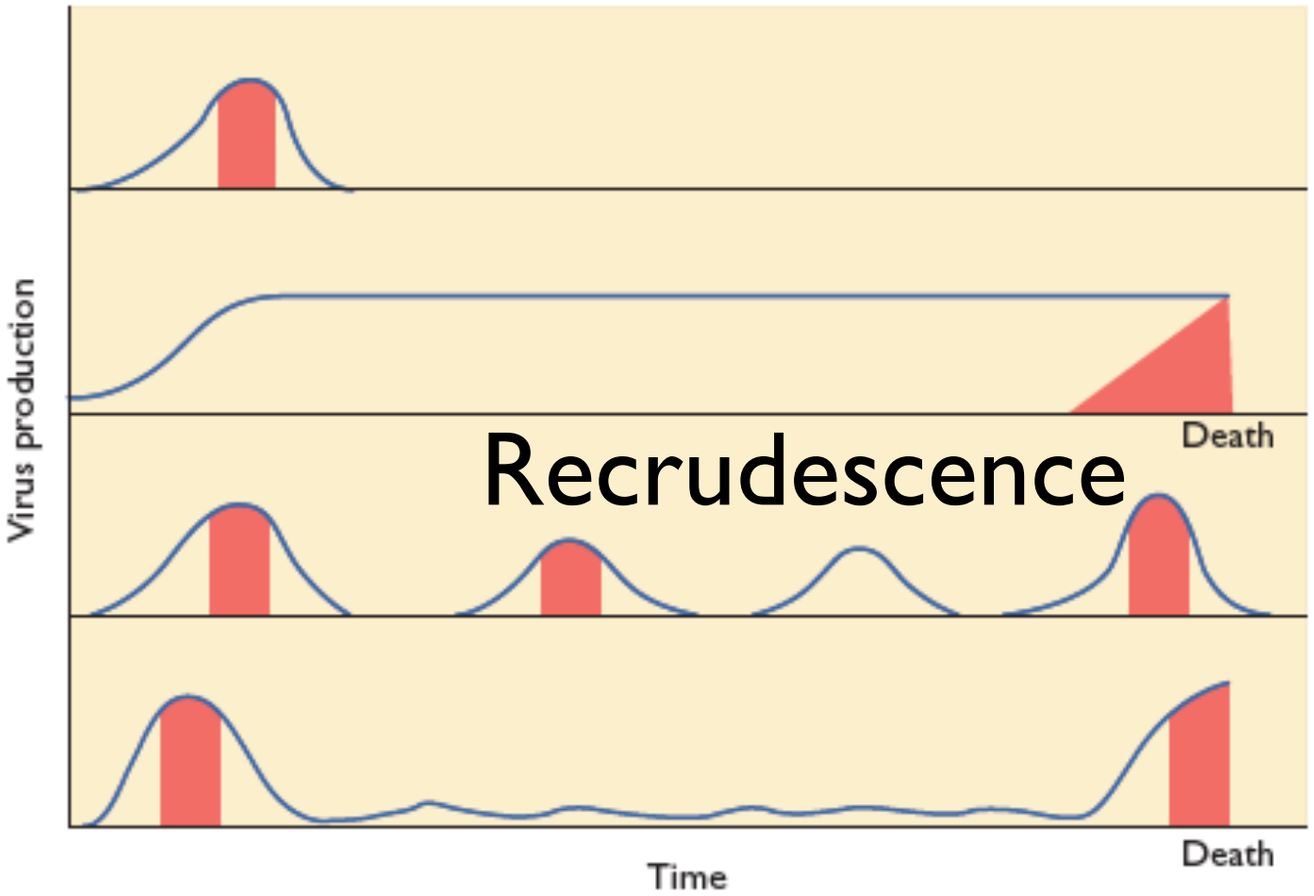
Lecture 16
Virology W3310/4310
Spring 2012

"Breaking Up Is Hard To Do"
Neil Sedaka 1962

Acute vs. Persistent Infections

- Acute - a natural infection that usually is rapid and self limiting
- Persistent - a natural infection that can be long term
    - slow
    - abortive
        - latent
    - transforming

Patterns of Infection



Acute infection
- Rhinovirus
- Rotavirus
- Influenza virus

Persistent infection, smoldering
- Lymphocytic choriomeningitis virus

Persistent infection, latent
- Herpes simplex virus

Persistent infection, slow
- Measles virus SSPE
- Human immunodeficiency virus
- Human T-lymphotropic virus

Antigenic Variation

- Rhino, Influenza & HIV
  -selective pressure can lead to shedding of virions that are resistant to clearing
  - antigenic drift
  - selection

Persistent Infections

- Occur when primary infection is not cleared by the adaptive immune response
  - virus, genomes and/or proteins continue to be produced for years
- Chronic vs. Latent
  - chronic infections are eventually cleared
  - latent infections persist for a lifetime

General Properties of Latent Infections

- Gene products promoting replication are
  - not made
  - found in low concentrations
  - aberrantly localized
- Cells with latent genomes are masked from immune surveillance
- Viral genomes persist intact to reactivate and spread to a new host
  - except for measles and SSPE

Examples of Latent Infections

- Epstein Barr Virus (EBV)
  - novel transcription and replication pattern
  - no new virus
  - but genome replicates

- Adenoviruses
  - isolated from lymphoid tissue, adenoids and tonsils
  - cultured lymphocytes don't support efficient virus replication

Other Examples of Persistent Infections

Virus	Site(s) of persistence	Consequence(s)
Adenovirus	Adenoids, tonsils, lymphocytes	None known
Epstein-Barr virus	B cells, nasopharyngeal epithelia	Lymphoma, carcinoma
Human cytomegalovirus	Kidneys, salivary gland, lymphocytes,[a] macrophages,[a] stem cells,[a] stromal cells[a]	Pneumonia, retinitis
Hepatitis B virus	Liver, lymphocytes	Cirrhosis, hepatocellular carcinoma
Hepatitis C virus	Liver	Cirrhosis, hepatocellular carcinoma
Human immunodeficiency virus	$CD4^+$ T cells, macrophages, microglia	AIDS
Herpes simplex virus types 1 and 2	Sensory and autonomic ganglia	Cold sore, genital herpes
Human T-lymphotropic virus types 1 and 2	T cells	Leukemia, brain infections
Papillomavirus	Skin, epithelial cells	Papillomas, carcinomas
Polyomavirus BK	Kidneys	Hemorrhagic cystitis
Polyomavirus JC	Kidneys, central nervous system	Progressive multifocal leukoencephalopathy
Measles virus	Central nervous system	Subacute sclerosing panencephalitis, measles inclusion body encephalitis
Rubella virus	Central nervous system	Progressive rubella panencephalitis
Varicella-zoster virus	Sensory ganglia	Zoster (shingles), postherpetic neuralgia

How to Promote Persistence

- Failure of innate immune system to clear an acute infection
- Blocking apoptosis can lead to persistence

Host Contributions to Persistence

- Eyes and neurons are devoid of initiators and effectors of the immune system
  - a vigorous immune response would be detrimental to the host
- Persistent infection of these organs is therefore common

State of the Genome

- Nonreplicating genome in a nondividing cell
  - HSV and VZV in neurons
- Autonomous self replicating chromosome in a dividing cell
  - HPV, HCV, HBV and EBV, KSHV
- Integrated in host chromosome, replicates with host
  - Parvoviruses
  - HHV6

Sindbis Virus

- Injection into adult mouse brain results in persistent, noncytopathic infection
- Injection into neonatal mouse brain results in lethal infection
- Why? It's all about the milieu

  - neonatal neurons lack proteins that block virus-induced apoptosis

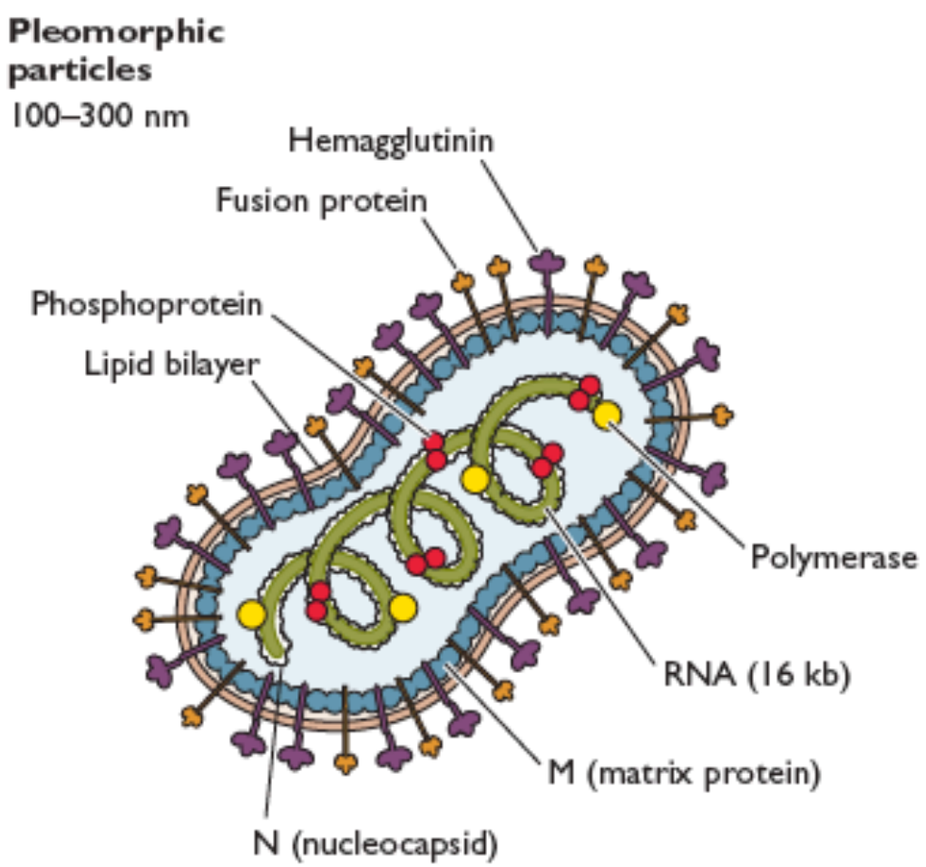
BDV a Pestivirus

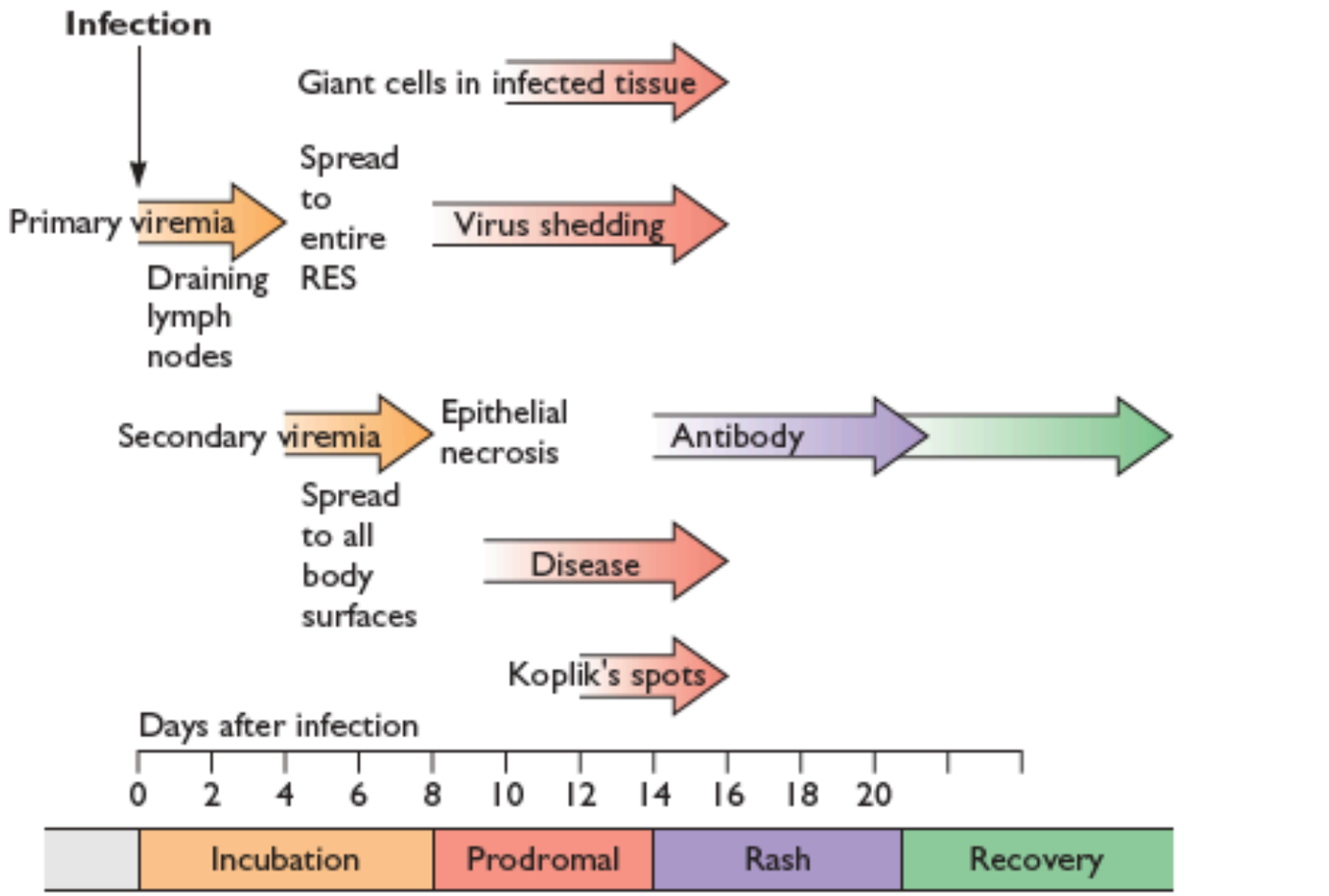
- Two strains of virus
  - cytopathic (C) and noncytopathic (NC)
- Following *in utero* infection NC establishes a lifelong infection of cattle
  - these animals have NO detectable antibody or T-cell response to virus antigens
  - host is tolerized
- When infected with C, IFN response is activated and virus is readily cleared

Measles a Paramyxovirus and SSPE

- No animal reservoir
  - highly contagious
  - 4 X 10^7 infections/yr
  - systemic immunosuppresion
  - lifelong immunity

Infection Pattern





Rare complications

- Encephalitis
- Subacute sclerosing panencephalitis

SSPE - Hypothesis

- Measles enters brain in infected lymphocytes
- Antibody blocks cell - cell fusion
  - removal of fusion protein from surface allows persistence of portions of virus
    - a slow infection, not persistent
- Low levels of envelope, no virions but nucleoprotein complexes spread from cell to cell
- SSPE develops after 6 - 8 years

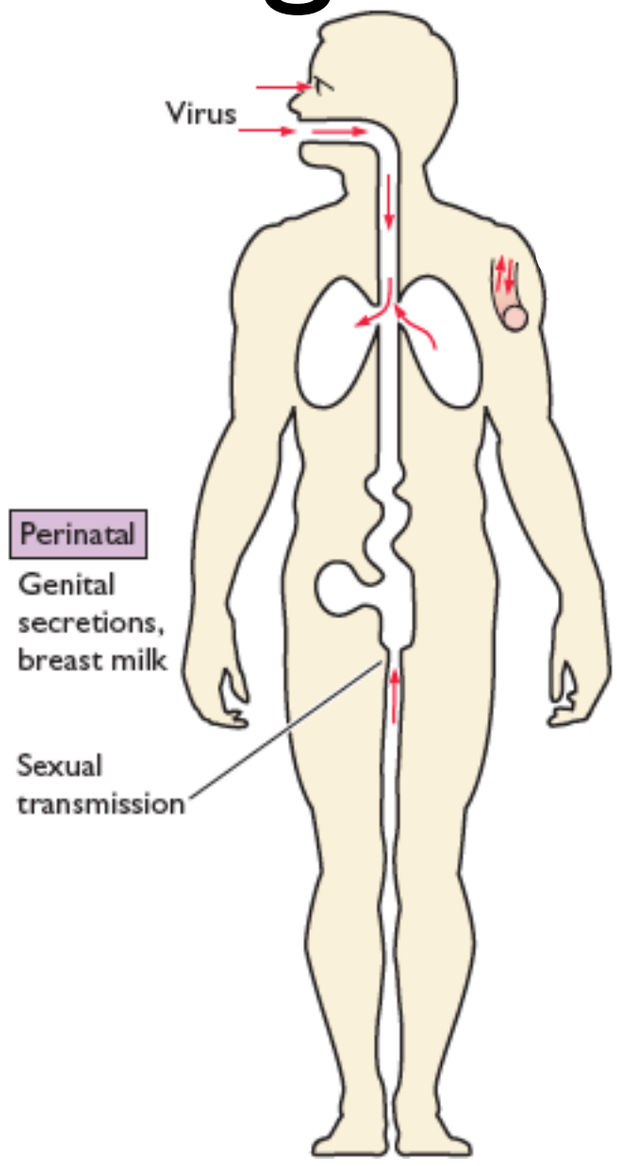
Herpesvirus Latency Primer

- α HSV, VZV are neurotropic
  - default pathway lytic
- β CMV, HHV6 variable but prefer cells of lymphoid origin
  - default pathway lytic
- γ EBV, KSHV markedly lymphotropic
  - default pathway latency

Control of Latent Herpesvirus Genomes

- HSV - LAT transcripts derived from a single region of the chromosome accumulate
- VZV - small subset of aberrantly localized proteins may accumulate
- EBV - virus proteins and small viral RNAs are synthesized
  - required to maintain the latent state
  - modulate host response
- HCMV & KSHV - micro RNAs are thought to play a role in establishment of latency

Acquisition of CytoMegaloVirus



HCMV

- Infects epithelial and other cell types
- Most infections are subclinical
- Cell-mediated immunity required for resolution of infection
- Establishes latency in bone marrow progenitors and macrophages
- Repression of CMI leads to recurrence

HCMV Infections

- Infection *in utero* can be devastating
- Early childhood, less so
  - virus persists
  - found in salivary and mammary glands and semen
- Reactivation can be with dire consequences
  - blood transfusion
  - organ donations
- miRNAs expressed by CMV *in vitro* and *in vivo*
  - are tissue specific
  - associated with a specific stage of viral infection

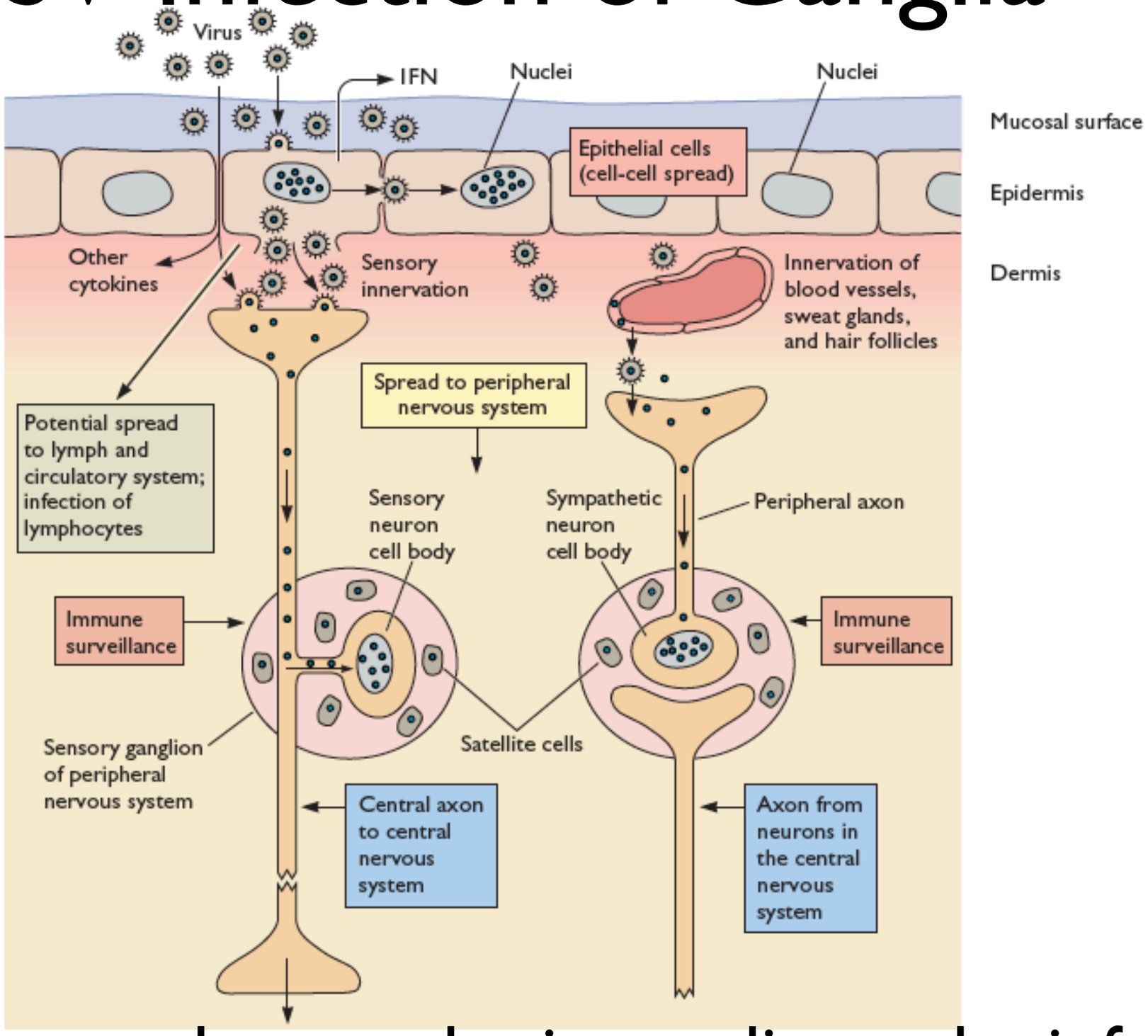
The First Rule of Latency

- Without reactivation there is no latency
- Without reactivation there is no advantage as the virus can no longer spread.

HSV Infections

- Population is >80% seropositive
- ~2.5 X 10^8 have latent virus
- 4 X 10^7 will experience recurrence
  - some asymptomatic shedding

HSV Infection of Ganglia

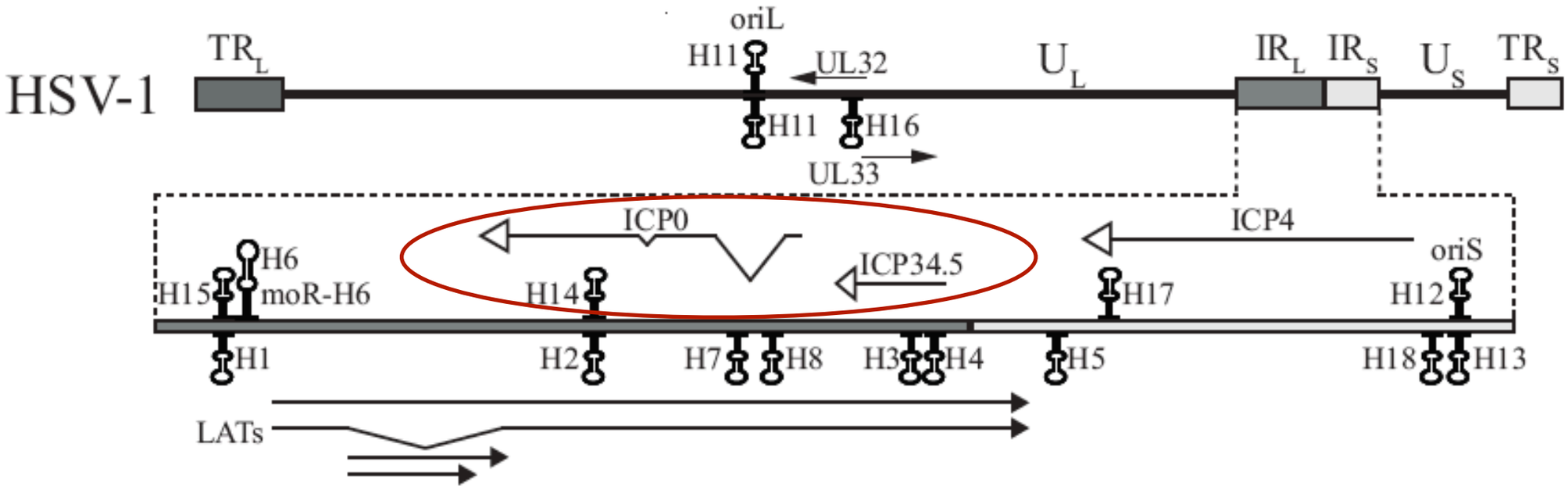


Both sensory and sympathetic ganglia can be infected

Postinfection Events in Neurons

- Nucleocapsid travels up the axon
  - VP16 is separated from nucleocapsid
- Limited productive infection
  - local inflammation leads to resolution
- Genome is silenced and coated by nucleosomes
- Multiple copies of virus DNA
- Nuclear accumulation of LATs

What Do LATs Do?



- LAT$^-$ virus reactivates poorly
- 2 ORFs are contained in the LAT sequence but no know protein has been associated with them
- Encode MIRs that could inhibit expression of
  - ICP0, a potent transcriptional activator
  - γ34.5 a neurovirulence gene, it activates PPla

Why Neurons?

- Neurons don't replicate or divide, genome is established and readily persists
- Insensitive to antivirals and immune surveillance
  - blood brain barrier
- But.....how do they survive the 1° infection?
- Why are there multiple copies of virus DNA?

Reactivation

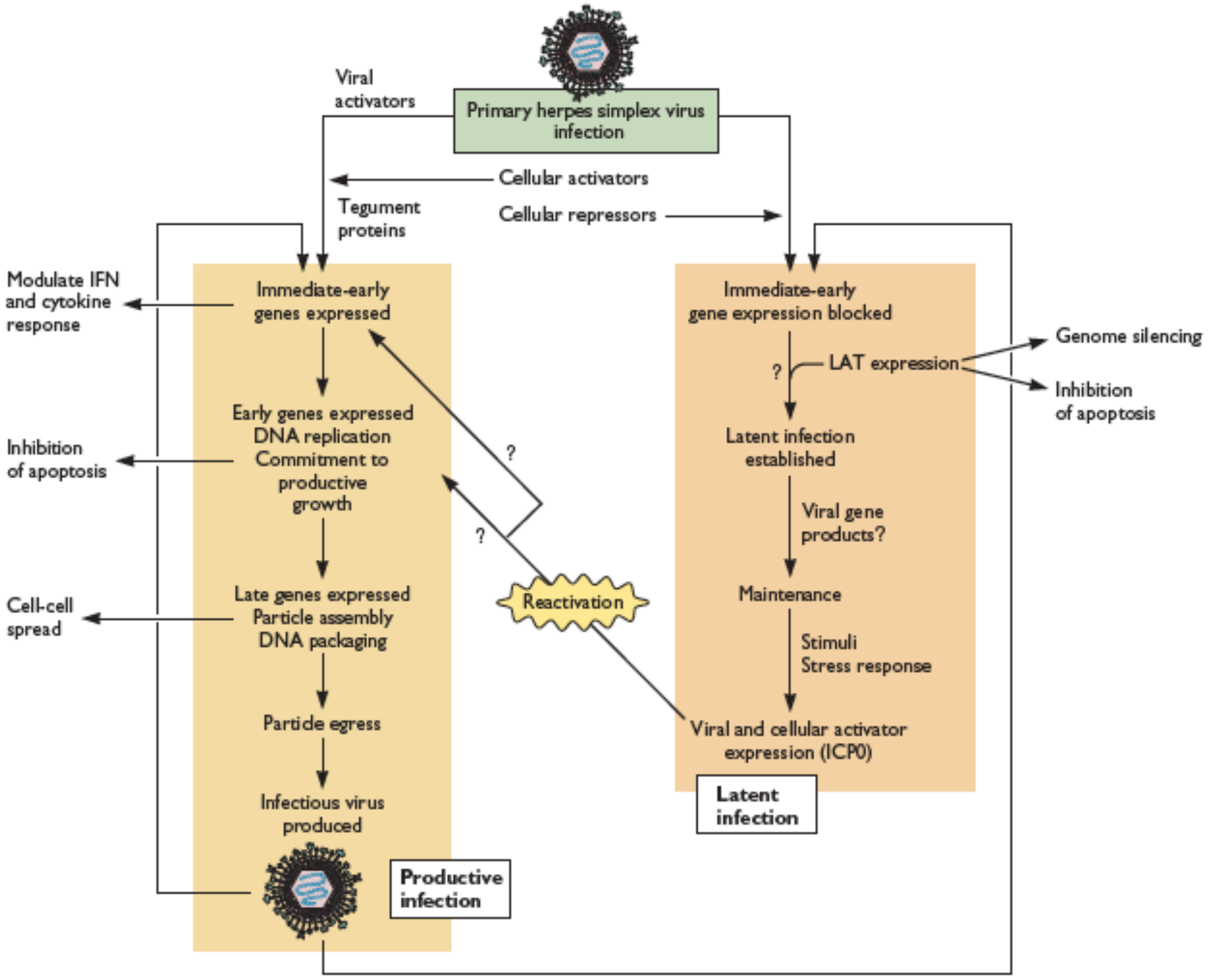
- Only a small number of neurons in a ganglion reactivate
- Virions appear in mucosal tissue innervated by latently infected ganglia, blisters ensue

- What happens to surrounding neurons post reactivation?
- Many times reactivation is silent, virus is shed
- How is virus infection masked from host immune response?

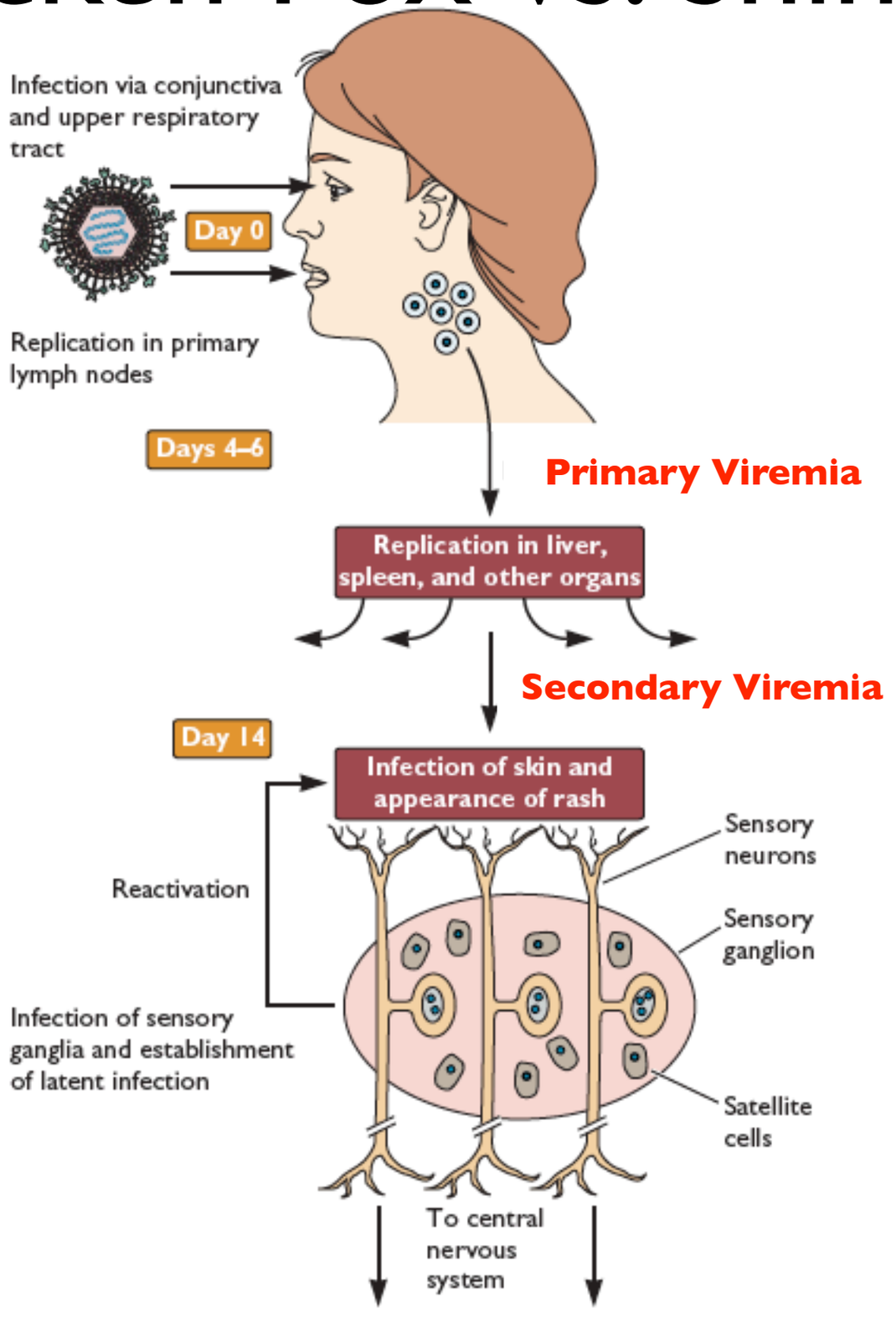
Reactivation Triggers

- What flips the switch?
- Stress
- Glucocorticoids
- In a model system exogenous ICP0 can reactivate
- The VP16 conundrum

Establishment, Maintenance & Reactivation



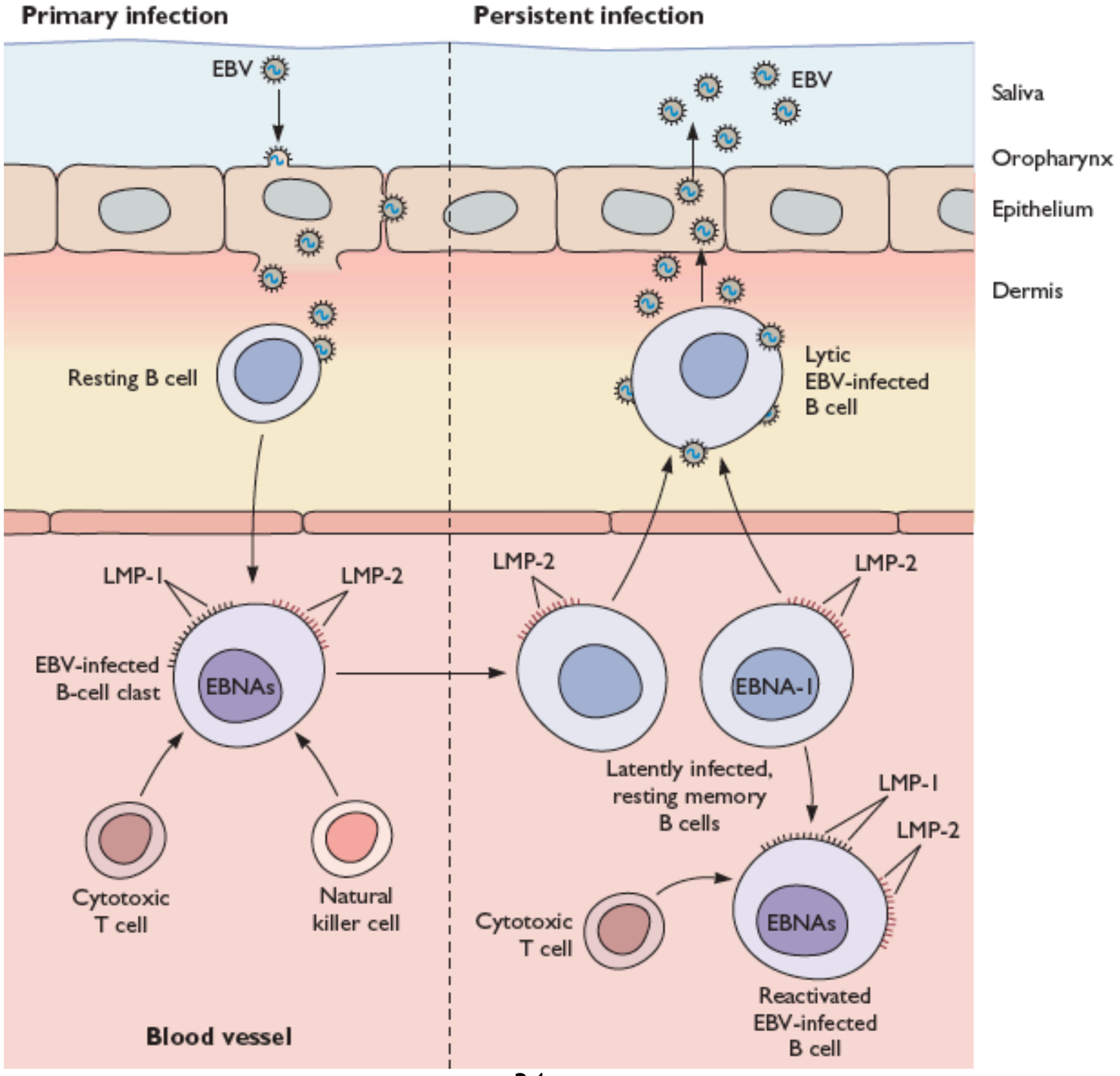
Chicken Pox vs. Shingles



EBV a γ Herpesvirus

- 95% of adults are seropositive and carry the genome
- Virus resides in persistently infected non-proliferating memory B lymphocytes
- Causal agent of:
  - Hodgkins lymphoma
  - Infectious mononucleosis
  - Nasopharyngeal carcinoma
  - Burkitt's lymphoma

EBV Lifecycles



Epigenetic Marking and EBV Replication

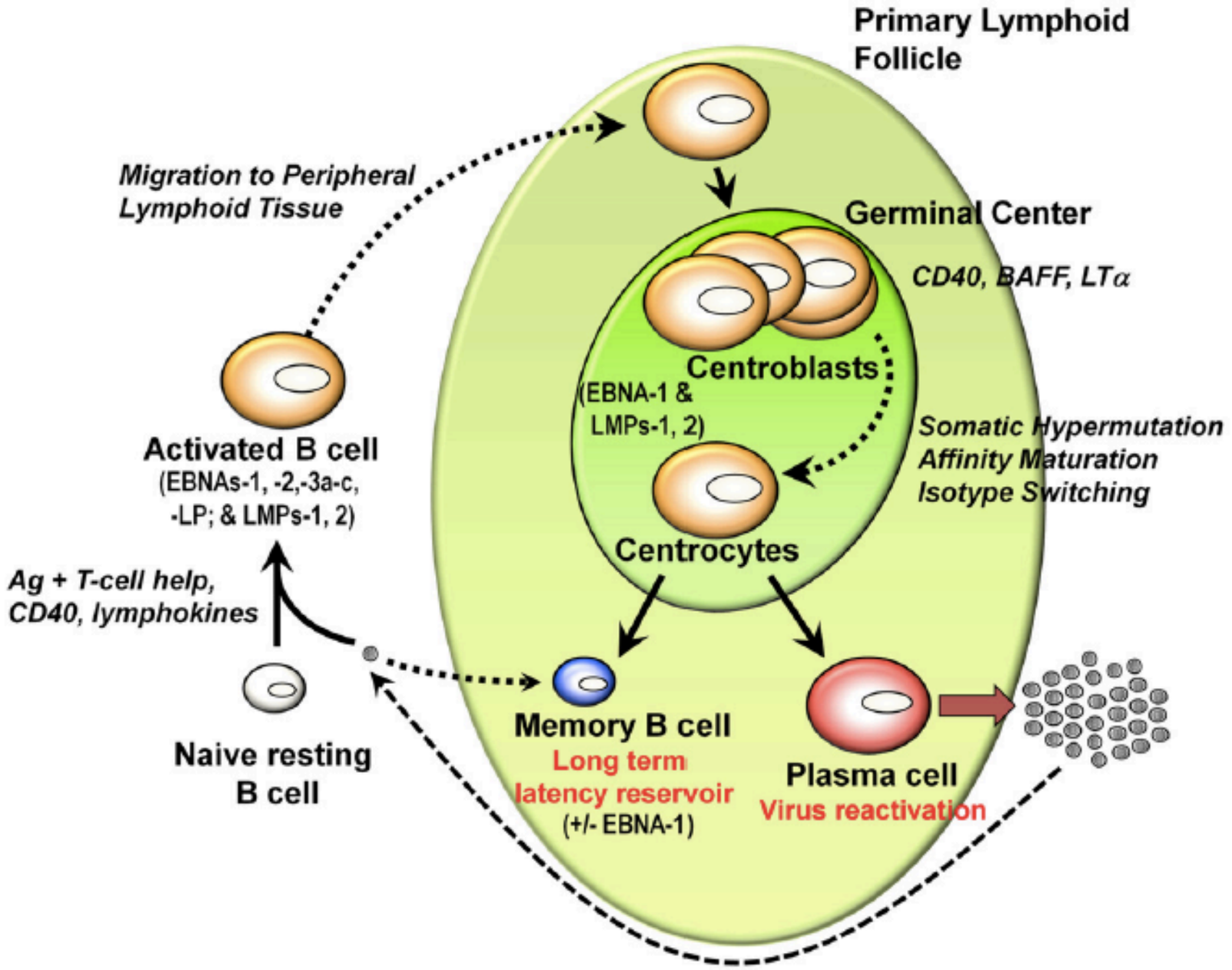
- DNA unmethylated
- Immediate early gene expression (Zta)
  - mode of action
- Susequently methylated but Zta $t_{1/2}$ is short

Latently Infected B Cells & EBV

- Virus chromosome is a self-replicating episome
- Associates with nucleosomes
- Is methylated at CpG residues

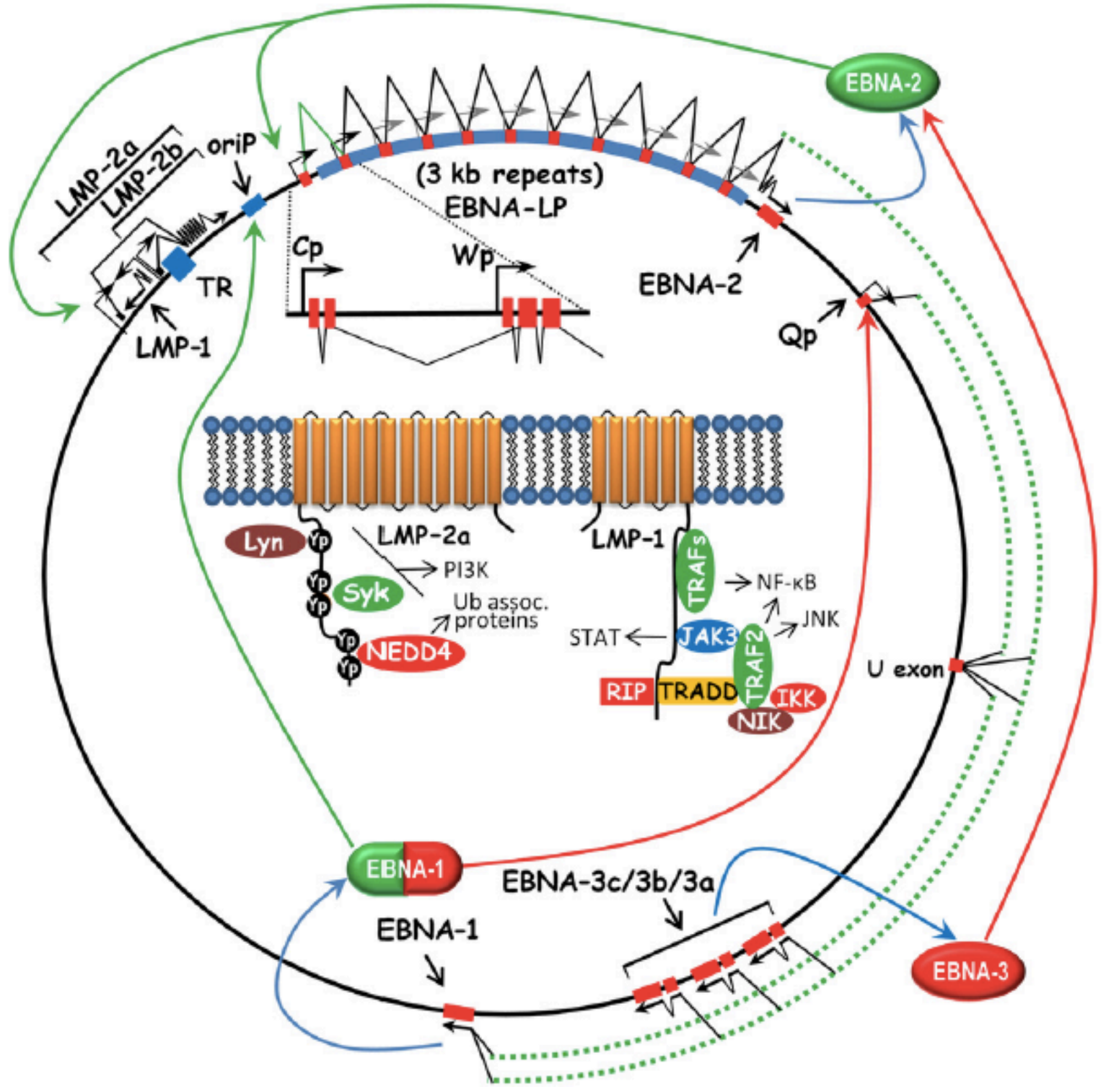
- Expresses limited repertoire of virus genes
- Cells home to bone marrow and lymphoid organs
- Are not seen by CTLs or virus-specific antibody
- Virions produced in a very small fraction of cells

EBV Latency Programs



Progression of Naive B cell through germinal center to become Memory B Cell

EBV Latency Program



What Happens When B Cells Divide?

- Episomal virus genome has to replicate to be distributed to daughter cells
- EBV has two Origins for DNA replication
- Ori Lyt is used for lytic replication
  - high copy #
- Ori P is used for episomal replication in latently infected cells
  - low copy #

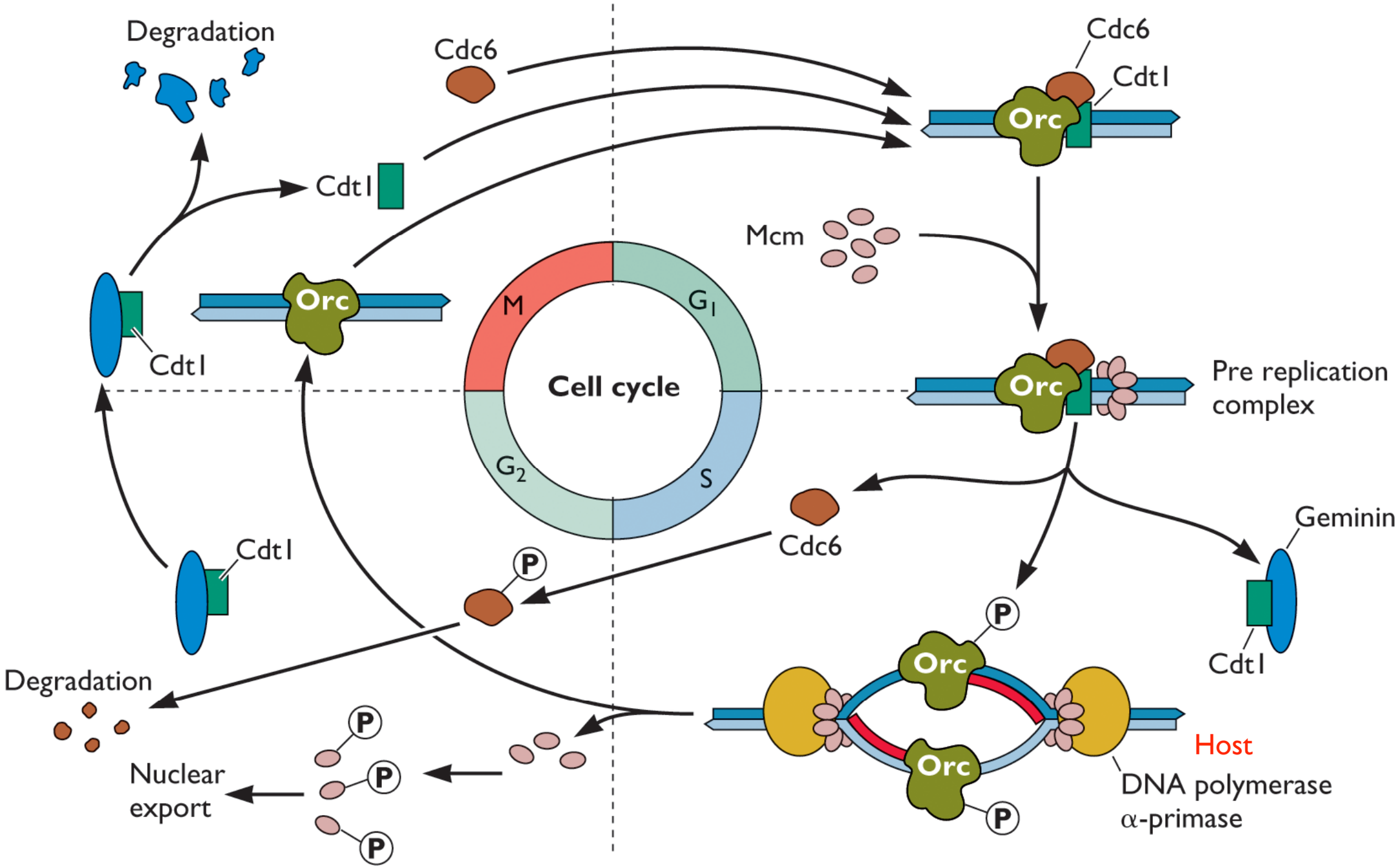
Cell-cycle Regulation of EBV DNA Replication During Latency

- Replication of episomal, nucleosome coated, virus genome is synchronized with the host
    - Why?

- oriP is normally quiescent
    - bound by host regulatory proteins (cdc6, cdt1)

- EBNA-1 interacts with host proteins to form a stable complex Origin Recognition Complex

Replication Licensing



EBV Latent Infection

- EBV replicates in synchrony with the cell
- Replication is licensed by formation of ORC
  -recruits other proteins (mcm)
  -release regulators, initiate DNA replication
- Late in S geminin is produced and it sequesters Cdt1, geminin is subsequently degraded in G2 freeing Cdt to reassociate with ORC
- No second round of replication because during S and G2 mcm and Cdc6 are destroyed

HHV6 a β Herpesvirus

- Causal agent of a mild childhood disease
  - Exanthum subitum
  - 90% of population is seropositive
- Persistently infects the host for life
  - No circular episomal forms
  - Integrates into telomeres
  - Reactivates in the immunosupressed
- Makes integration a plausible molecular strategy for viral latency

Human Papillomaviridae

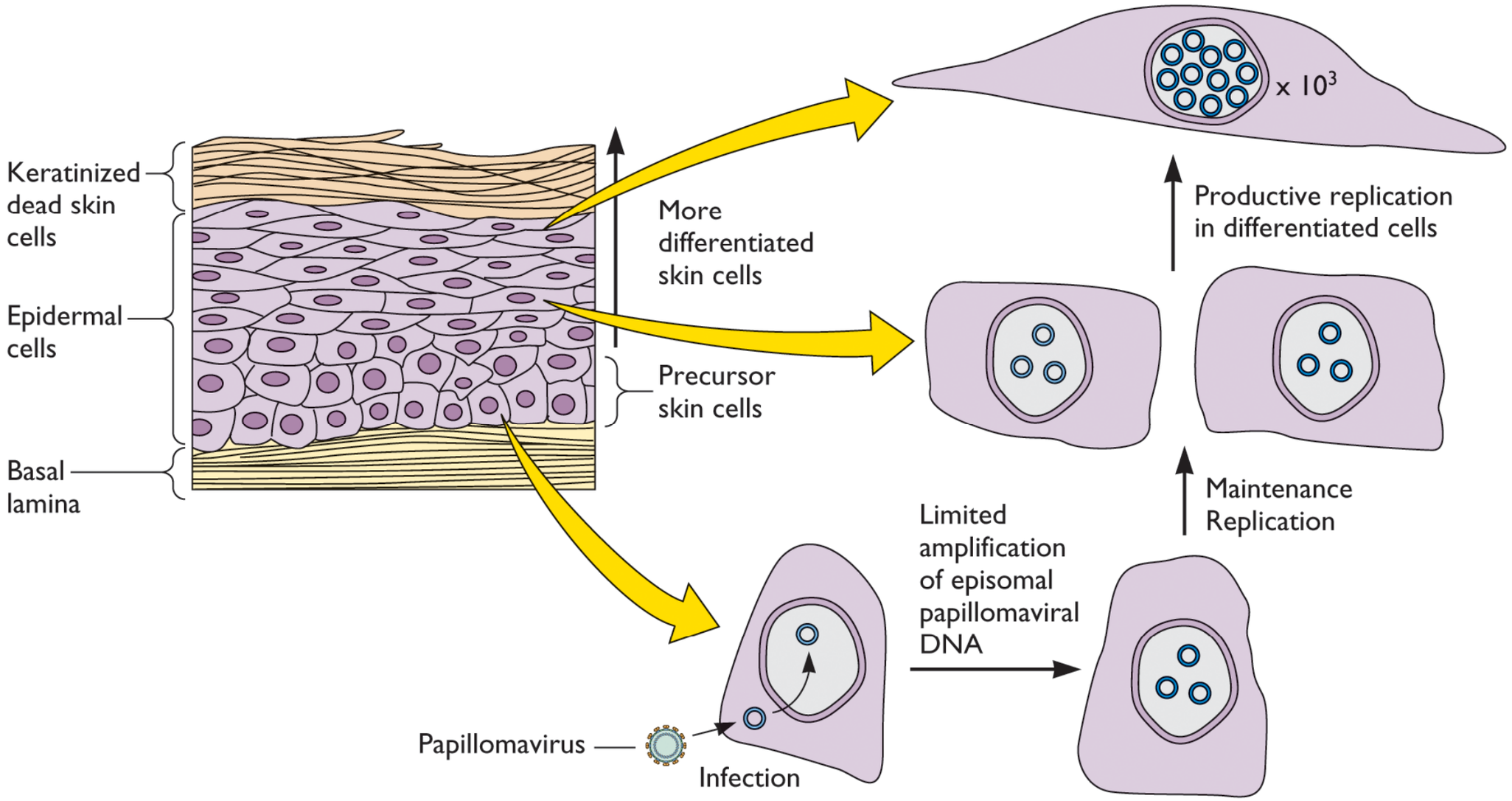
- There are over 100 distinct types of HPVs
  - Genomes that vary by >10%
- Segregate in mucocutaneous and cutaneous types
  - high and low risk types

Papillomavirus DNA Replication

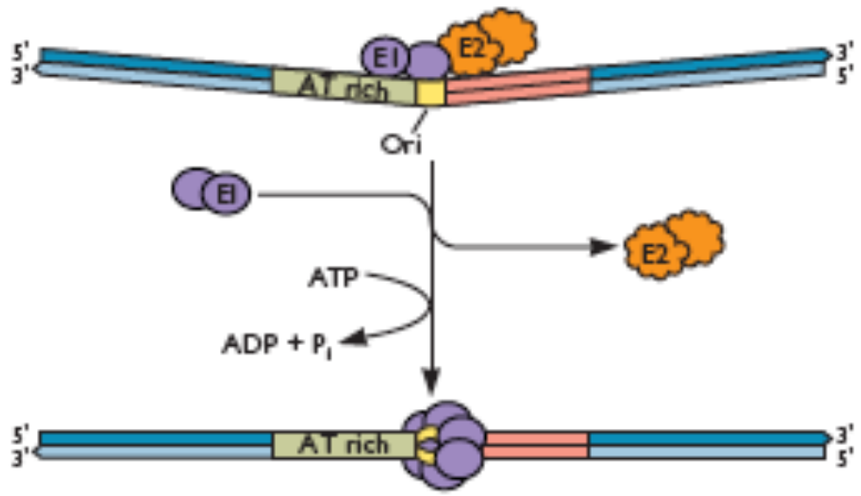
- Infect basal layer of differentiating epithelium
  - first replicate as episomes as cells divide
  - replication as theta forms "ϴ"

- Replicate virus genomes in terminally differentiated epithelial cells
  - interrupt program of terminal differentiation, express HPV E6 and E7

Papillomavirus DNA Replication



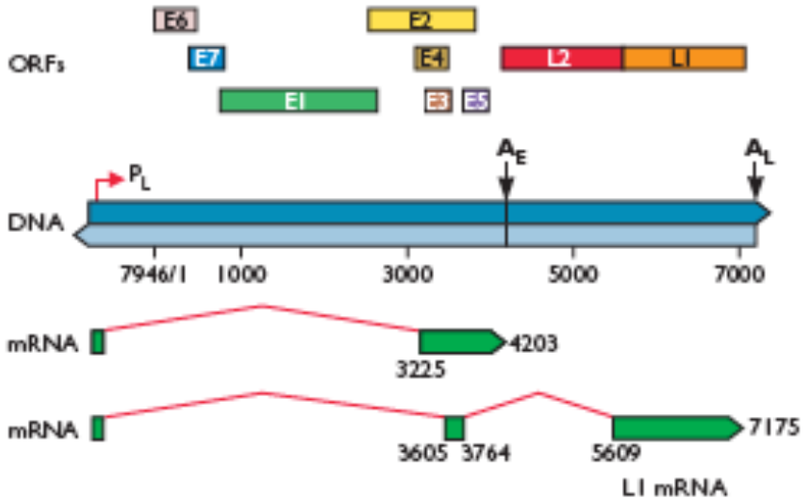
Papillomavirus Replication

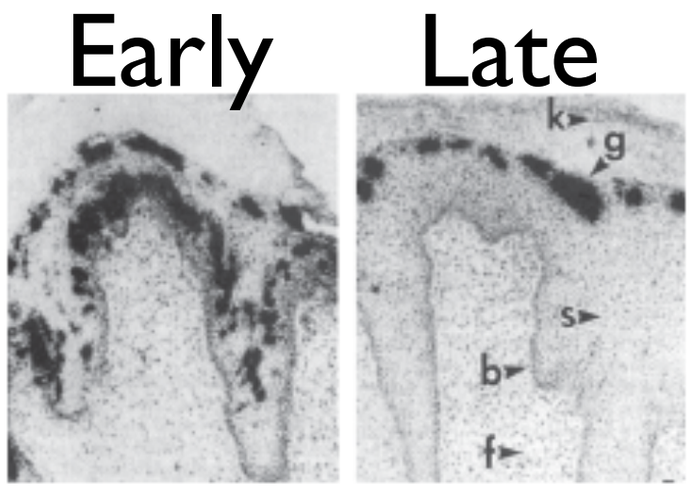


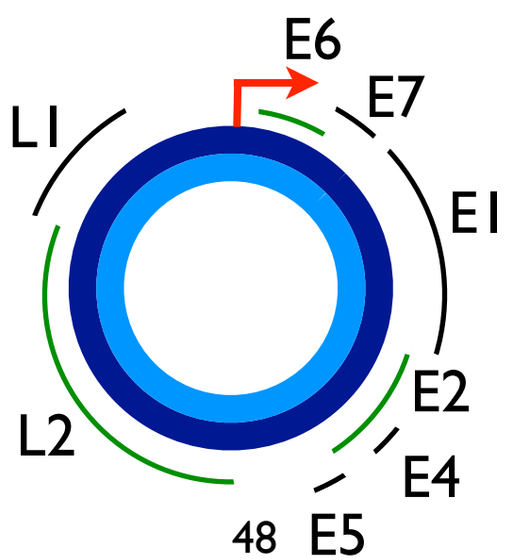
- E1 and E2 are homodimers
- E1 and E2 interact and bind cooperatively to ori
- E2 recruits E1
- Interaction elicits a bend in the DNA at the ori
- E2 dissociates - more E1 is recruited

Papillomavirus Persistence

- Intact virus genomes persist in basal cells of developing epithelium
  - genomes divide as episomes with host
  - infectious virus not present







Papillomavirus Persistence

- In developing cancers virus genome is integrated

  -replicates only when host cell divides

Human Polyomaviridae

- Six known members of the group
  - WUV, BKV, JCV, LPV, KIV and MCV
- Polyomaviruses can cause tumors in animal models
  - only MCV is associated with a human tumor
  - other human PVs appear to latently infect humans

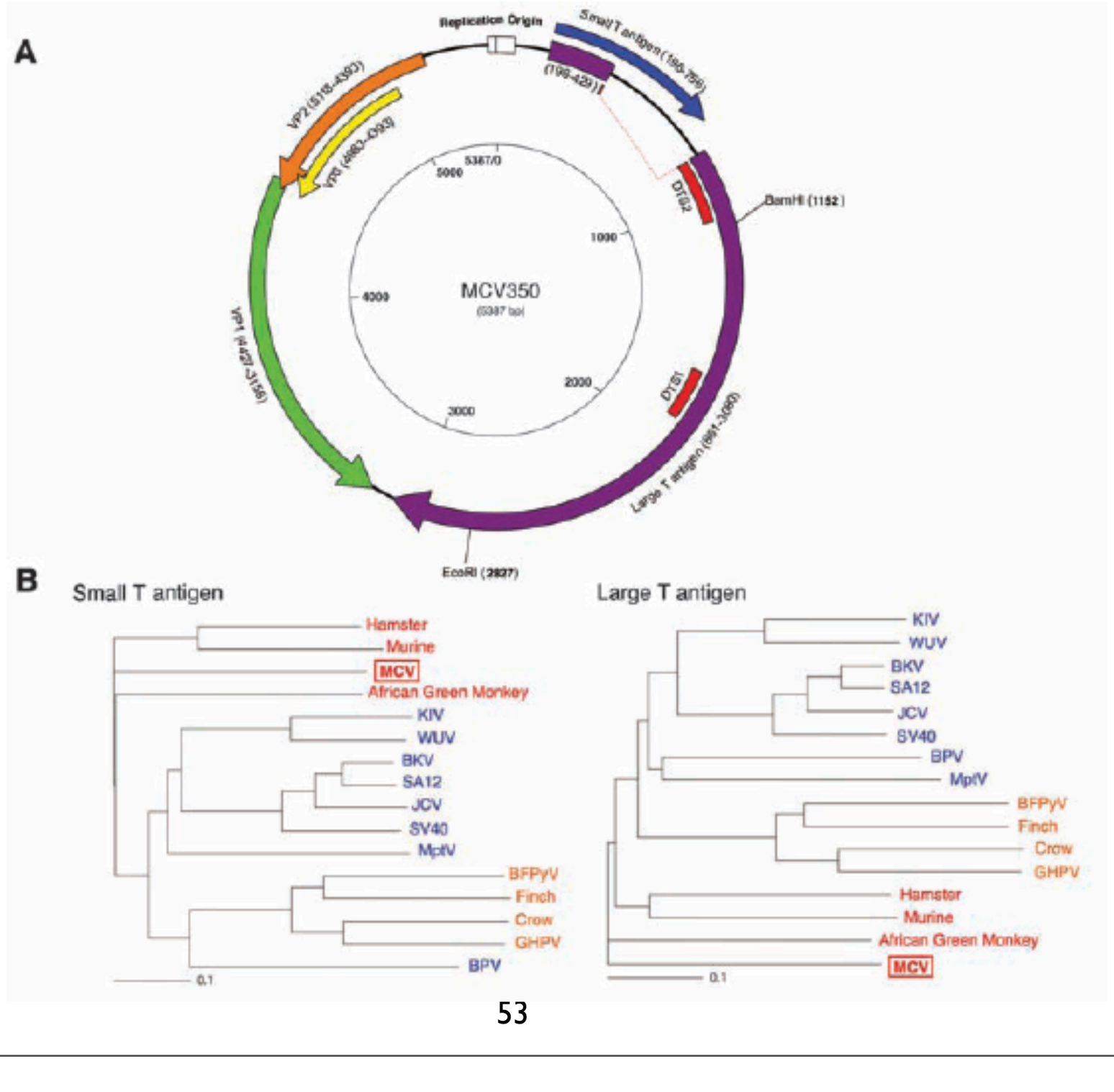
Human Polyomaviridae

- Infection with JC or BK can lead to development of Progressive Multifocal Leukoencephalopathy (PML)
    - myelin is lost and not replaced by oligodendrocytes
    - nerves become damaged and over time stop working properly
- MS patients treated with Tysabri have a much higher than normal occurrence of PML

Human Polyomaviridae

- "Given the high seroprevalence of polyomaviruses in humans, it is not surprising that they are significant pathogens in immunosuppressed populations. An important question is why these viruses can peacefully co-exist in many humans without causing disease. Are human polyomaviruses simply passengers, or do they benefit us in some unknown way?"
  - VRR 2009 Blog

Merkle Cell Carcinoma Polyomavirus



Clonal Integration

- Analysis of MCV DNA in MCC (a neuroectodermal tumor) shows it is integrated in a clonal pattern
  - therefore infection and integration preceded clonal expansion of the tumor cells

- MCV positive tumors have mutations in T
  - thus they are replication deficient

- integrated virus genomes are not excised
  - cells survive

Persistence

- Viruses preferentially target slowly dividing or nondividing cells to host their latent genomes
- They adopt a variety of survival strategies that coordinate replication of their genomes and expression from these genomes to allow them to persist
- In response to a variety of stimuli these latent genomes can on occasion reactivate