Human Herpesviruses: Features

Alphaherpesvirinae (Neurotropic):

Human herpes simplex virus 1 e 2 (HSV1, HSV2). Primary agents of recurrent faciaal and genital herpetic letions, respectively.

Varicella-zoster virus (VZV). Causative agent of cicken pox ad shingles.

Betaherpesvirinae (Lymphotropic):

Cytomegalovirus (HCMV). Congenital infections; pneumonia.

Human herpesvirus 6, 7 (HHV6, HHV7). Some roseola

Gammaherpesvirinae (Lymphotr., tumor-assoc.):

Epstein-Barr virus (EBV). Tumors (B cell, epithelial).

Human herpesvirus 8/Kaposi's sarcoma herpesvirus (HHV8/KSHV).

Biology: Large dsDNA genome (125-236 kb; ~70-200 genes about half of which are non-essential in cell culture). Regulated gene expression. Nuclear replication

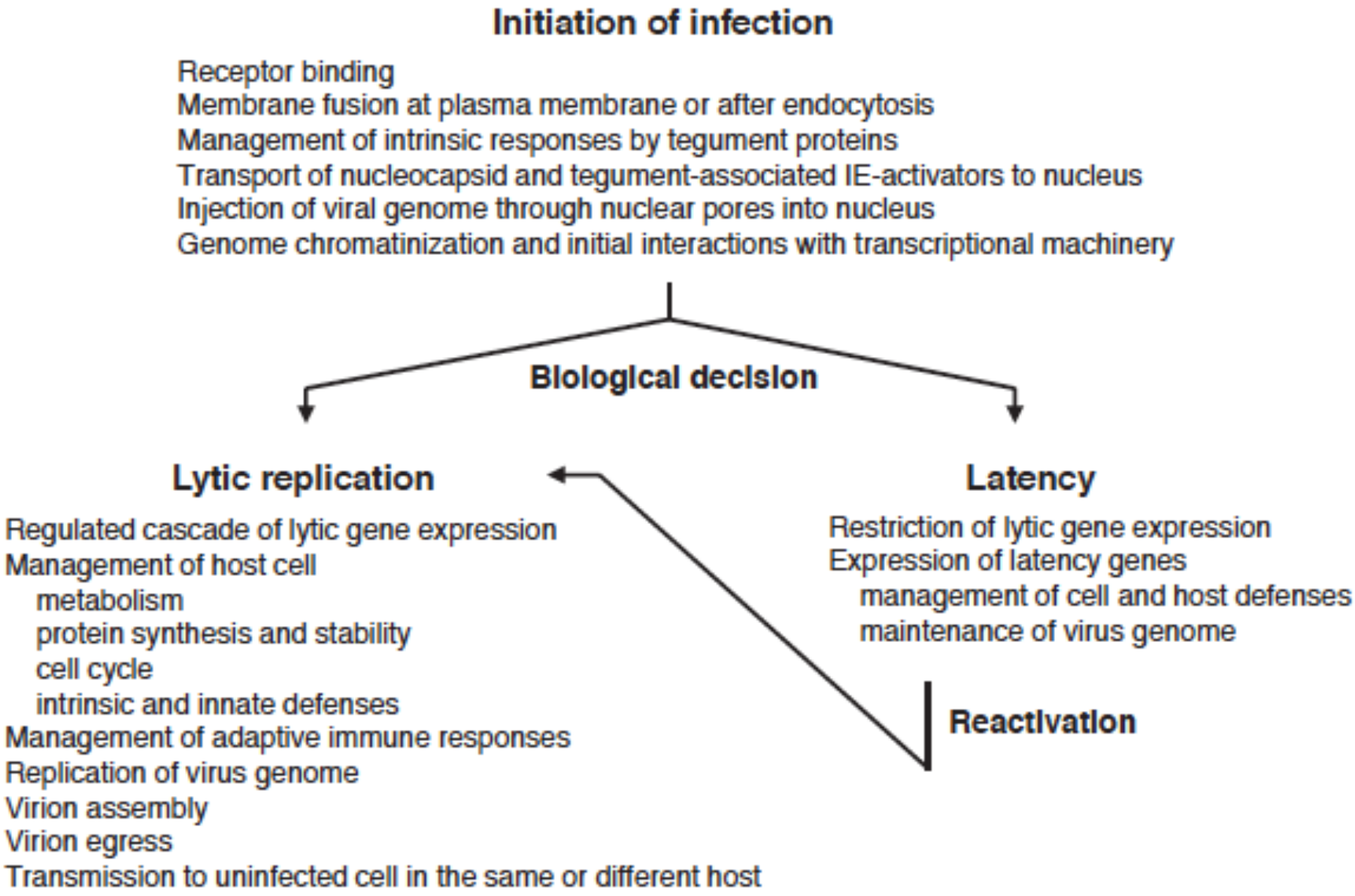
Herpesviruses: Common Biological Properties

Enzymology: Encode enzymes for DNA metabolism, DNA synthesis, protein processing/modification

Nuclear replication: Synthesis of DNA and capsid assembly occur in the nucleus

Cytolytic: Production of infectious progeny results in host cell death

Latency: All herpesviruses have the capacity for latency


Initiation of infection
Receptor binding
Membrane fusion at plasma membrane or after endocytosis
Management of intrinsic responses by tegument proteins
Transport of nucleocapsid and tegument-associated IE-activators to nucleus
Injection of viral genome through nuclear pores into nucleus
Genome chromatinization and initial interactions with transcriptional machinery
Biological decision
Lytic replication
Regulated cascade of lytic gene expression
Management of host cell
metabolism
protein synthesis and stability
cell cycle
intrinsic and innate defenses
Management of adaptive immune responses
Replication of virus genome
Virion assembly
Virion egress
Transmission to uninfected cell in the same or different host
Latency
Restriction of lytic gene expression
Expression of latency genes
management of cell and host defenses
maintenance of virus genome
Reactivation

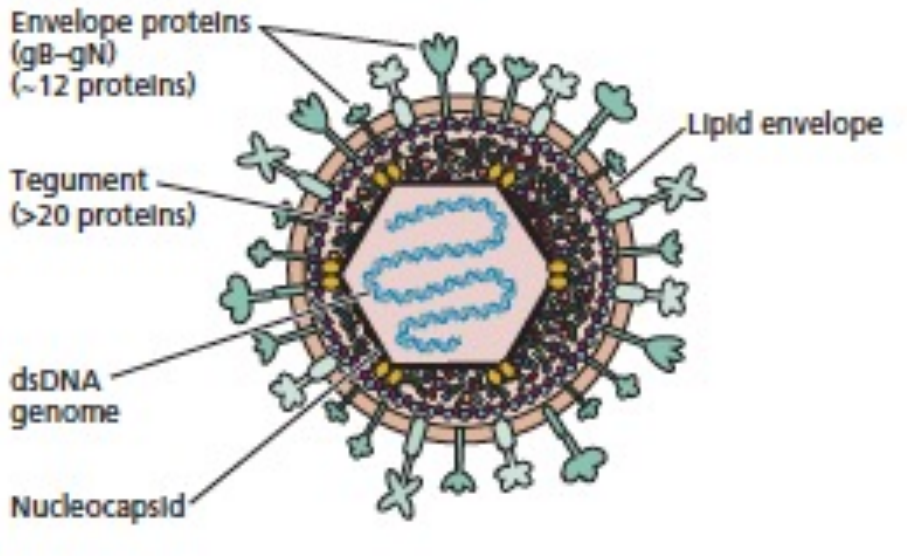
Latency

"Reversible non-productive infection of a cell by a replication-competent virus"

Requirements for latency:

1. **Evasion of host immunity - e.g., by minimizing gene expression (HSV-1 in neurons) or specific genes designed to suppress immunity (various)**
2. **Genome persistence. Relatively easy in non-dividing cells (neurotropic herpesviruses). More complex in dividing cells (lymphotropic herpesviruses) - e.g., EBV *oriP* permits genome replication, retention and segregation.**

Herpesvirus Structure



Nucleocapsid icosaedron made up of six different proteins

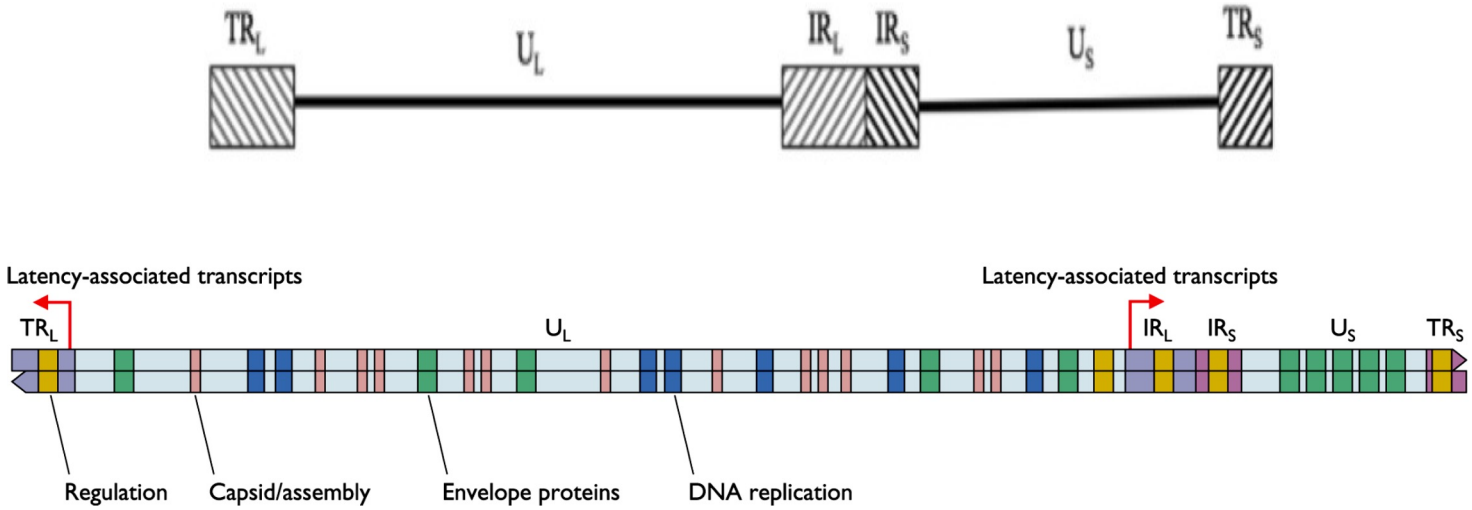
Matrix called *tegument* lies between the capsid and the envelope and contains at least 15 or 20 proteins (and virion RNAs).

Envelope contains 10 or more glycoproteins

Note: Herpesvirus particles also contain virion RNAs. This may allow for immediate synthesis of new gene products following virus entry, setting up the cell for efficient viral replication. RNA packaging may depend on specific viral RNA-binding proteins (e.g., HSV-1 VP22) and may reflect the concentration of RNAs within producer cells.

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

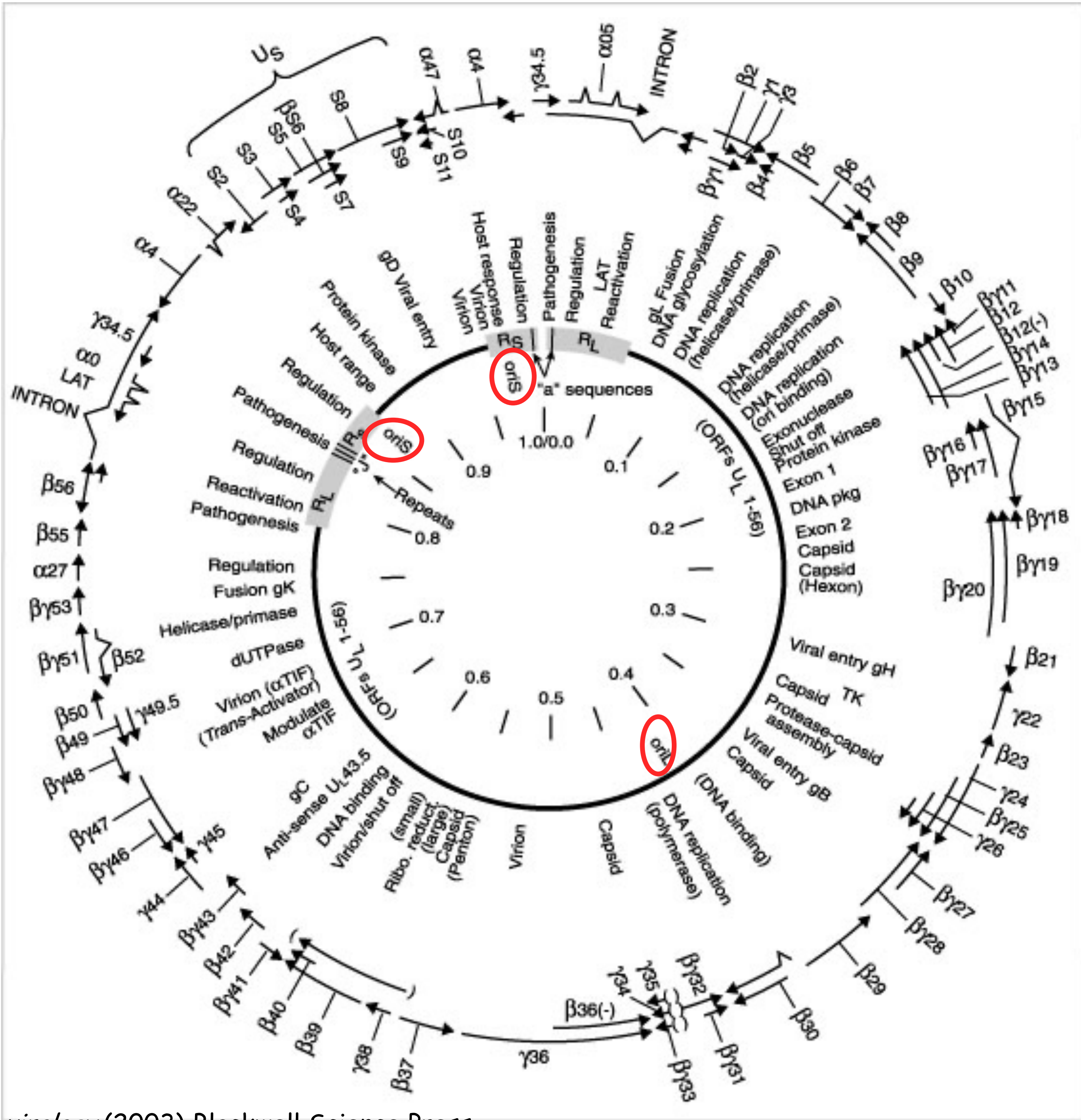
HSV 1 Genome (152 kbp)



HSV 1-around 84 ORF. The position of genes and transcription units associated with the latency is indicated . Genes encoding similar functions are marked with the same color. Genes are dispersed rather than clustered

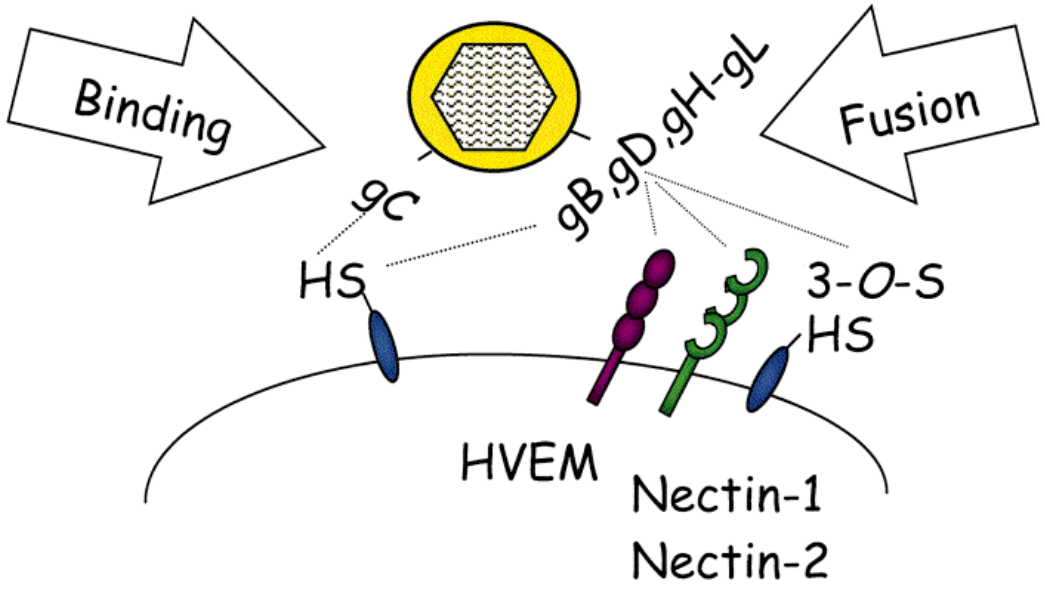
From Flint et al. *Principles of Virology* (2000), ASM Press

HSV 1 Genome ca 152kbp



From Wagner and Hewlett *Basic virology* (2003) Blackwell Science Press

HSV-1 Attachment and Entry



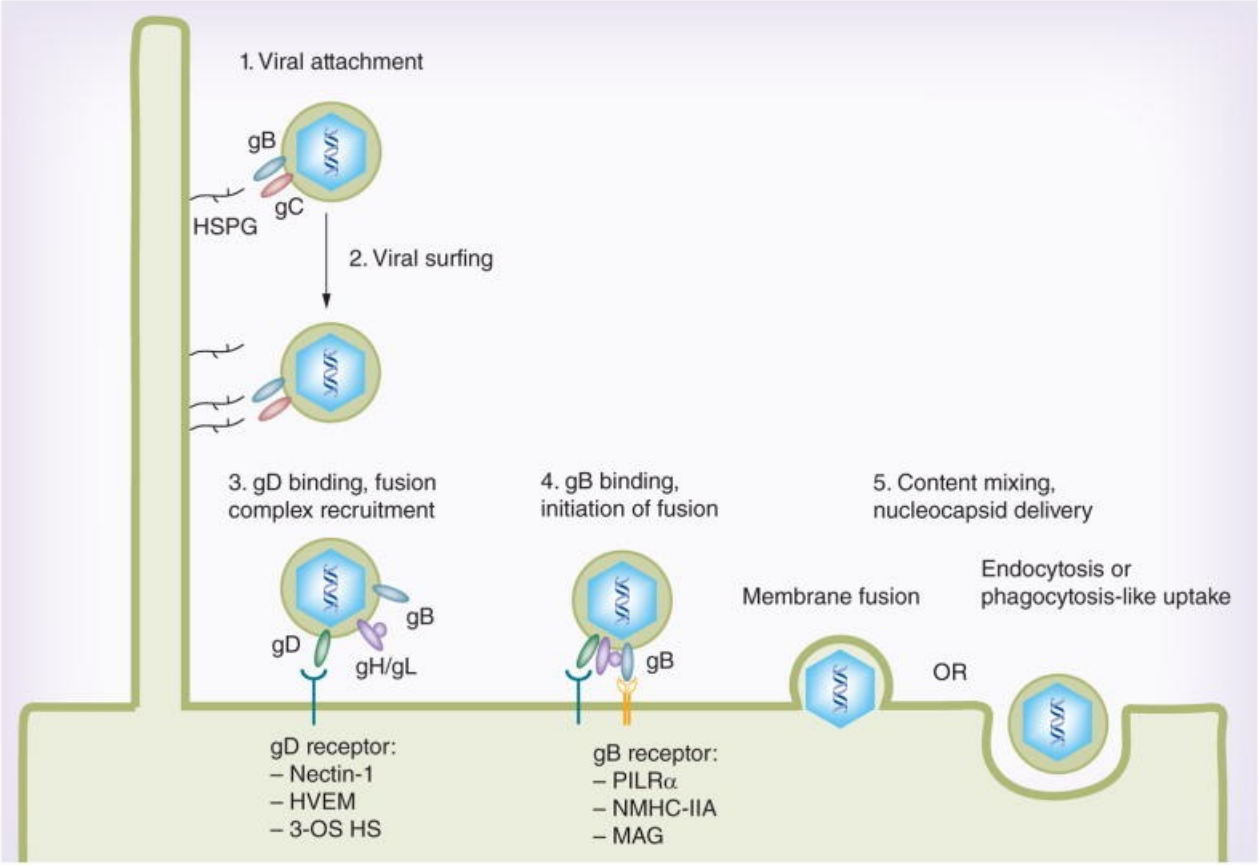
Spear, P.G. Cell. Microbiol. 6:401, 2004.

Binding: gC binds Heparan Sulfate (HS) on cell surface proteoglycans.

Entry Receptors: 3 receptors are bound by **gD - (1) HVEM**, a TNF receptor superfamily member; **(2) Nectin 1 & 2**, Ig-gene superfamily members & (3) modified HS sites generated by **3-O-sulfotransferases.**

Binding causes a **conformation change** in gD, facilitating fusion. *gB/gH & gL are needed also for fusion as they allows the lipids in both the viral and cell membranes to mix.*

HSV-1 Attachment and Entry



Initial attachment to cells is facilitated by binding of HSPGs by HSV glycoproteins gC and/or gB. Virions are then transported along cellular filopodia toward the cell body in a process termed viral surfing. Recruitment of a fusion complex comprising gB, gD and gH/gL is initiated by gD binding to one of its receptors, nectin-1, herpes virus entry mediator or 3-O-sulfated heparan sulfate. Binding of gB to one of its receptors, PILRα, NMHC-IIA or MAG, is then required for delivery of the viral nucleocapsid to the cytoplasm, accomplished either by membrane fusion or endocytosis/phagocytosis-like uptake. 3-OS HS: 3-O-sulfated heparan sulfate; HVEM: Herpes virus entry mediator.

Future Virol. 2015 Oct 1; 10(10): 1145–1154.

HSV Gene Expression

Often infect post-mitotic cells & consequently encode DNA synthetic machinery of their own.

Viral gene expression is tightly regulated:

1. **All viral proteins fall into groups whose synthesis is coordinately regulated (i.e., all genes within a group are turned on at the same time)**
2. **All viral proteins are expressed in a cascade fashion - from immediate early (IE or α) to early (E or β) to late (L or γ)**

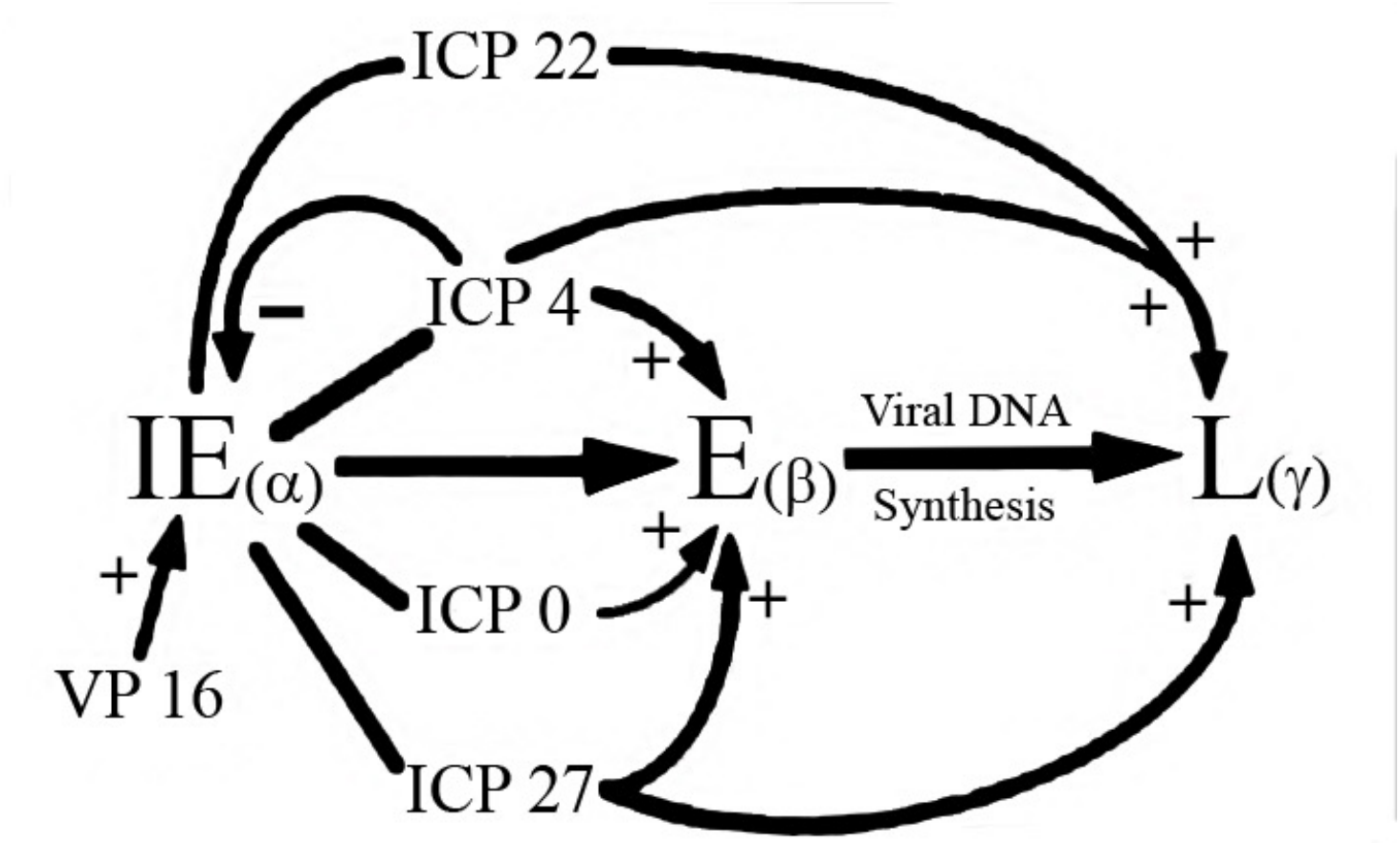
HSV Gene Groups

Immediate-early (IE): 6 genes, which are designated as ICP (infected cell proteins) 0, 4, 22, 27 and 47, plus Us1.5. The RNAs are made even in the presence of protein synthesis inhibitors. All are transactivators of other viral genes. ICP4, ICP0 and ICP27 are essential for virus replication *in vitro*; other proteins are not essential *in vitro.*

Early (E): Made before viral DNA replication. Include enzymes involved in DNA metabolism, including thymidine kinase, ribonucleotide reductase, DNA polymerase.

Late (L): Made after viral DNA replication. Typically, structural proteins, as well as VP16 and vhs.

Herpesvirus Gene Expression



Key HSV-1 Virion Proteins

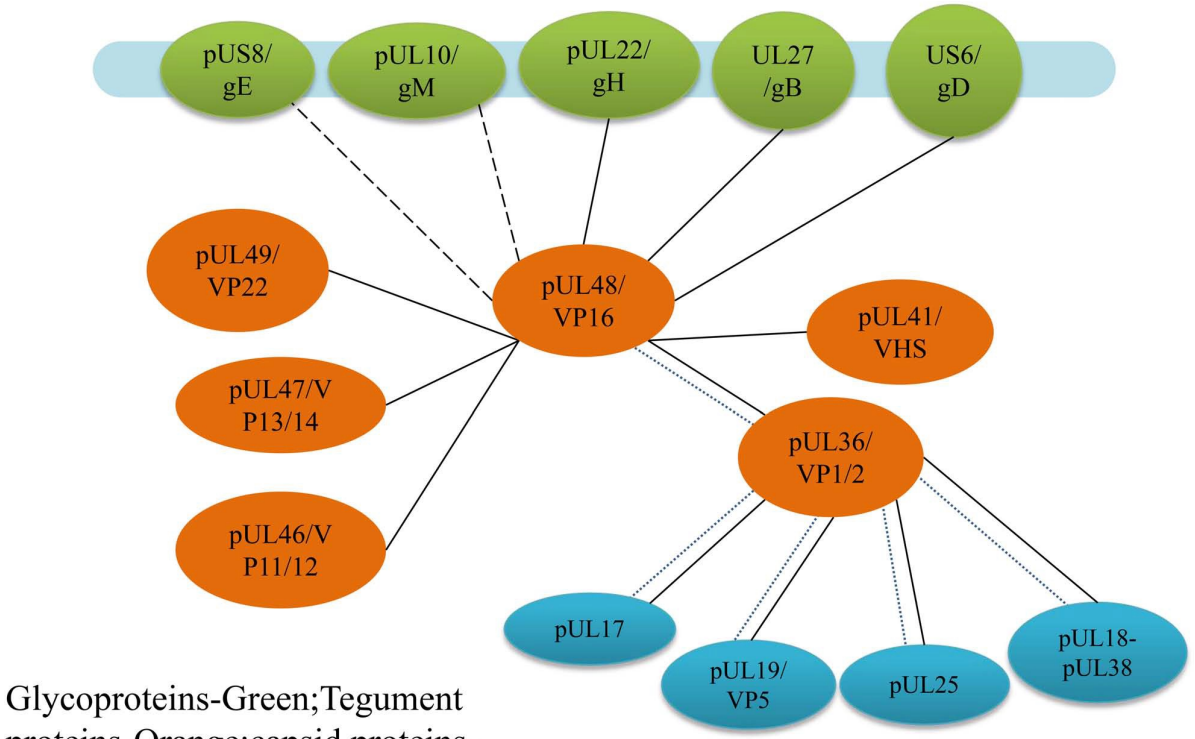
VP16 (α–TIF or UL48): An essential transactivator of viral immediate-early genes. Acts in concert with cellular transcription factors (including Oct-1 & the endogenous regulator of cell proliferation, HCF)

Vhs (virion host shutoff or UL41): Not essential, but enhances replication efficiency. Causes non-specific mRNA degradation. Role of ICP27 in degradation specificity. Late in infection, it is complexed to VP16 and inactivated.

Action of VP16 in activating HSV α genes

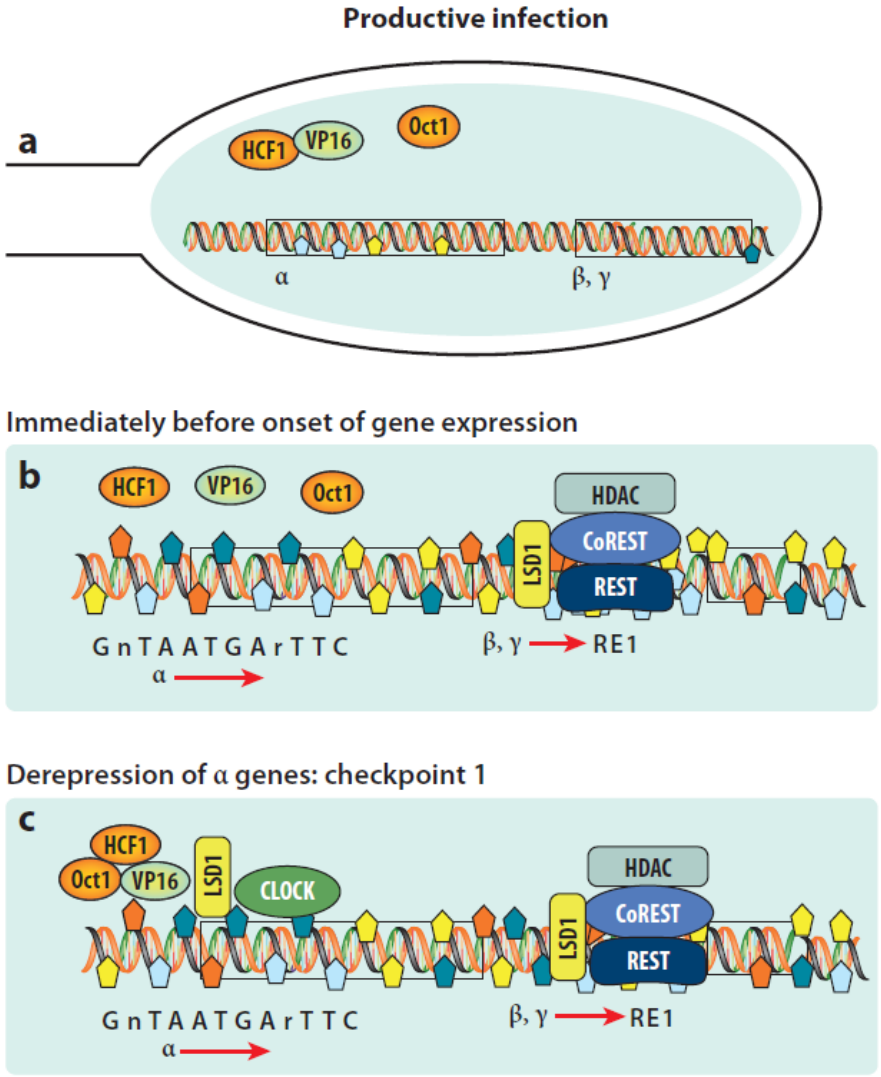
- VP16 has a potent transcriptional activation function when recruited to a promoter.
- Two host proteins, Oct1 and HCF, are required for VP16 to up-regulate α gene expression
- HCF associates with VP16 and mediates its transit into the nucleus
- Oct1 is a DNA binding transcription factor that binds to α gene promoters adjacent to a VP16 binding site. VP16/HCF complex can only bind DNA in the presence of Oct1
- Why the virus has evolved this indirect mechanism for activation by VP16 is uncertain; it may relate to the switch into and out of latency.

VP16 HAS ALSO A ROLE IN VIRAL ASSEMBLY



Glycoproteins-Green;Tegument proteins-Orange;capsid proteins-Blue

Model of regulation of viral gene expression in productively infected cells.

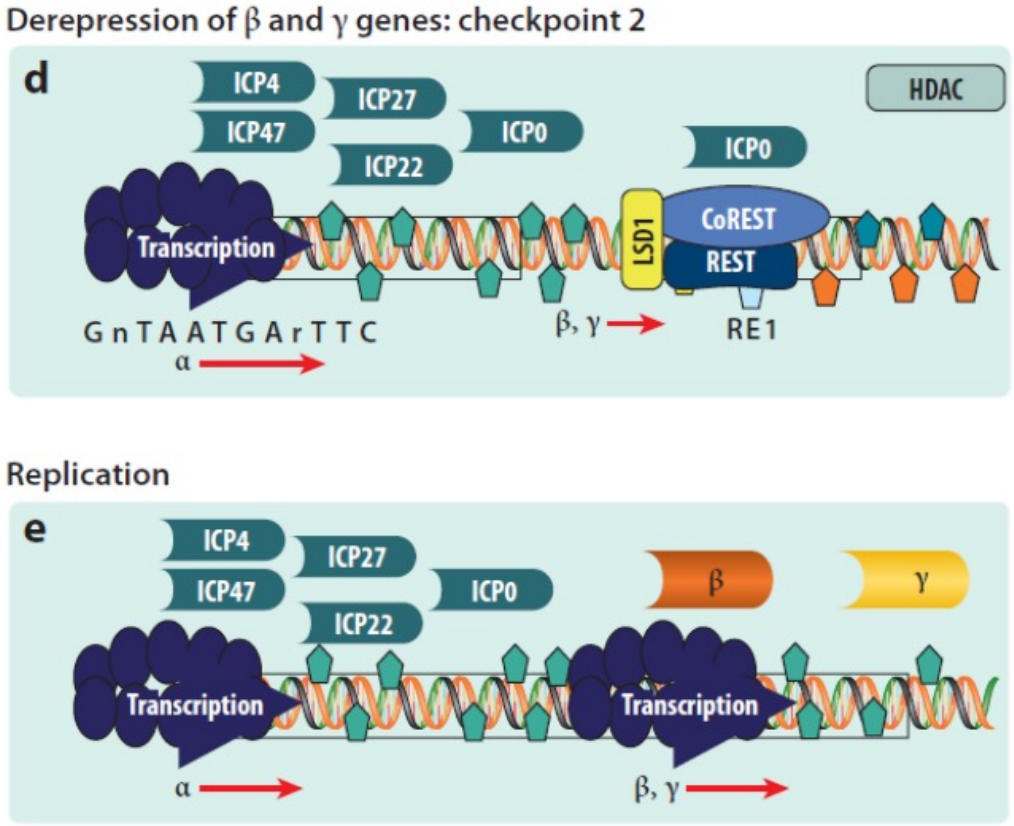


(a, b) Upon entry into cells, viral DNA is immediately bound by histone, histone- and DNA-modifying enzymes, and repressors.

(c) VP16 assembles with HCF1, Oct1, to derepress gene promoters. The complex recruits transcriptional factors to transcribe α genes and overcome checkpoint 1.

Annu. Rev. Microbiol. 2013. 67:355–74

Model of regulation of viral gene expression in productively infected cells.



(*d, e*) ICP0, an α protein, binds to CoREST and dislodges HDAC1 or HDAC2. The complex dissociates from DNA and is translocated to the cytoplasm. Checkpoint 2 is overcome and β and γ genes are expressed. Viral replication ensues

Annu. Rev. Microbiol. 2013. 67:355–74

Among the β gene products: TK and RR

Function: Thymidine kinase and ribonucleotide reductase are involved in the generation of DNA precursors.

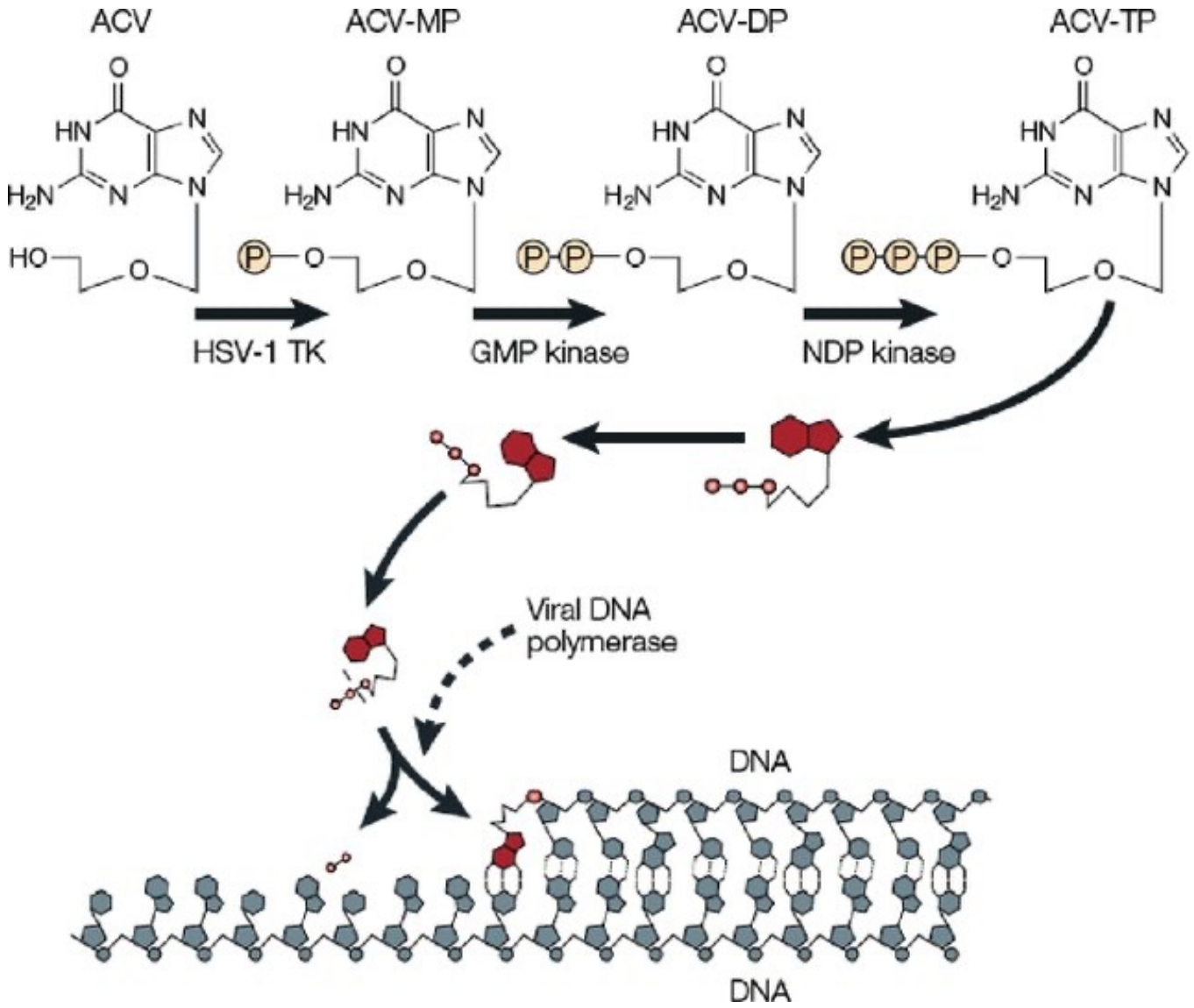
- Thymidine kinase (phosphorylates thymidine and other nucleosides)
- Ribonucleotide reductase (converts ribonucleotides to deoxyribonucleotide)

Phenotype of TK- or RR- HSV-1: Such mutants replicate fine in dividing cells, but fail to replicate in post-mitotic cells like neurons (where the available pool of DNA precursors is small).

Use of TK and RR deleted viruses: Being used for therapy of brain tumors: CNS cells (including neurons) are all essentially post-mitotic, while tumor cells are not. So these viruses can replicate only in the tumor cells (killing them).

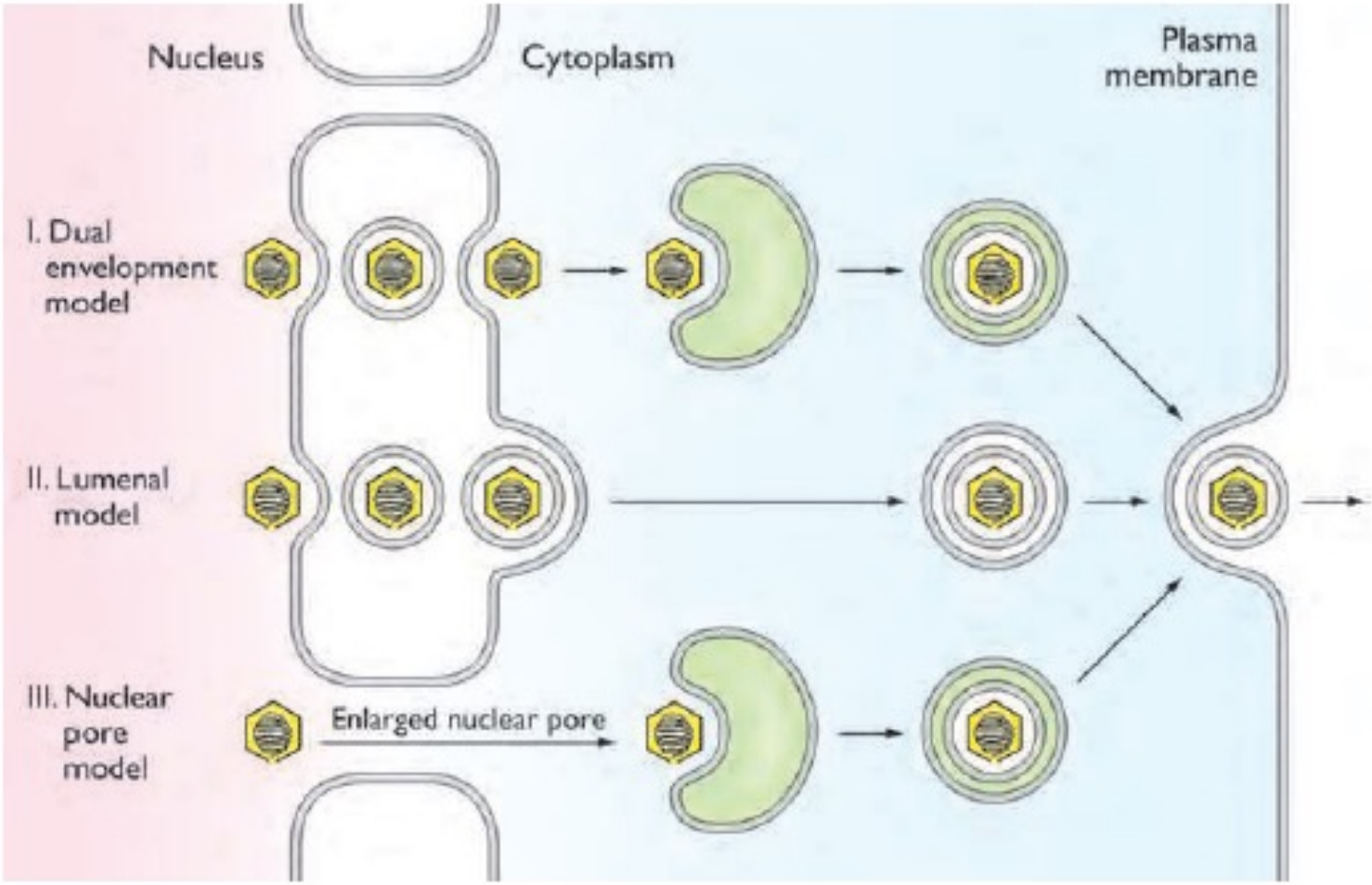
Moreover…TK activity has been exploited to develop the first antiviral specific drug

Mechanism of antiviral action of acyclovir



Antiviral Strategies pp 53-84

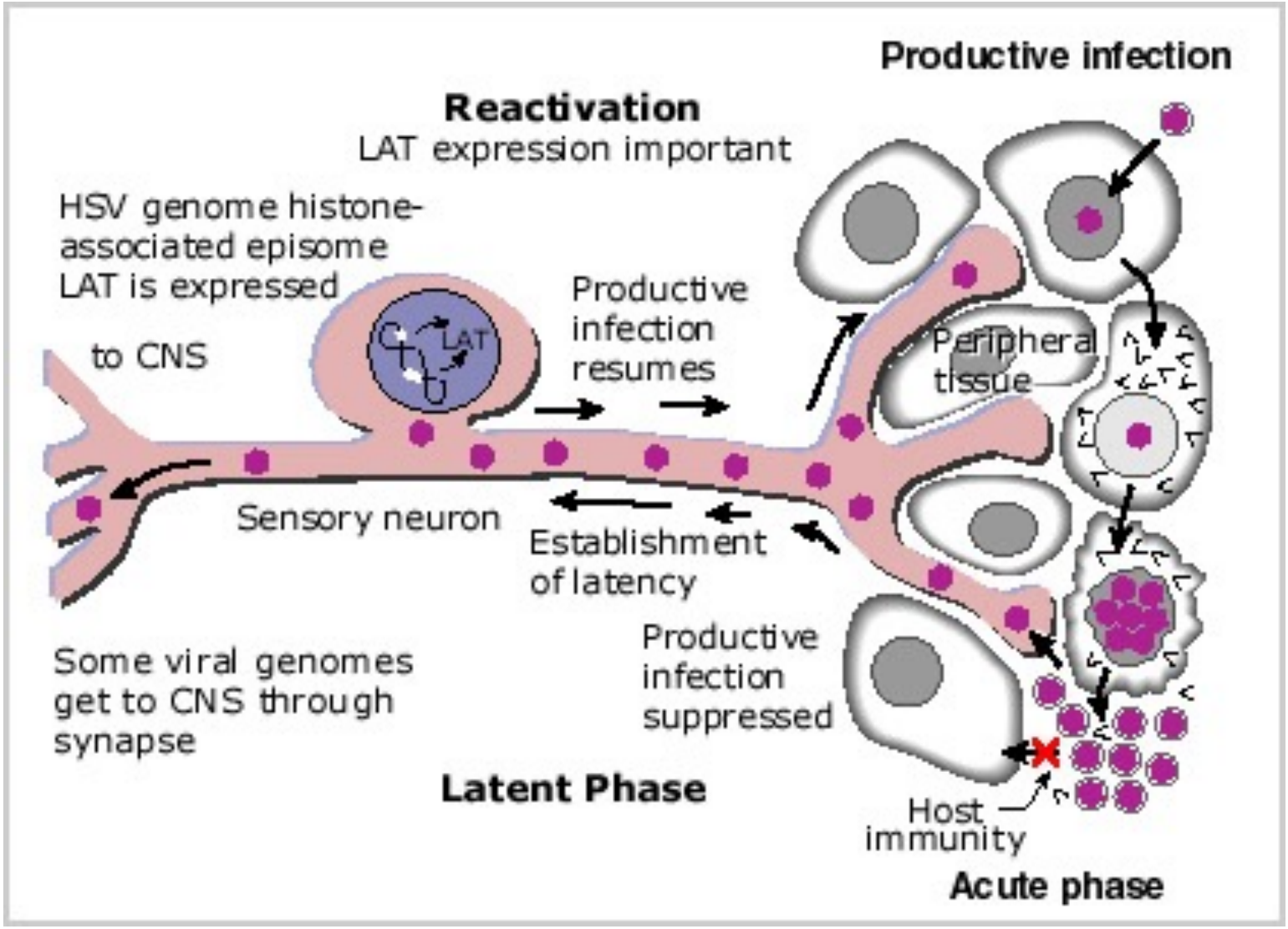
Models for egress of herpes simplex virus (HSV) from the host cell



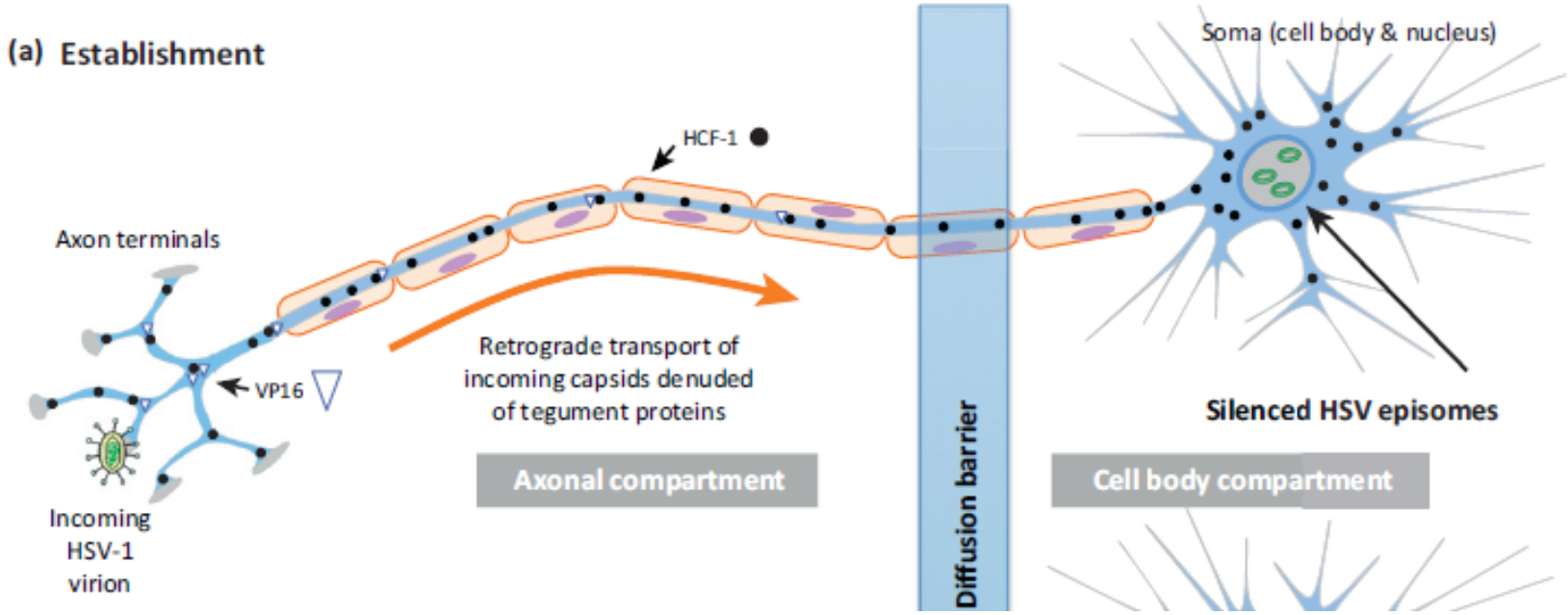
Although this has been a controversial area, the first model—envelopment–de-envelopment–re-envelopment—is supported by substantial data and is considered to be the “most prominent model of virion egress”.

Models for egress of herpes simplex virus (HSV) from the host cell. Shown are the proposed pathways for maturation of filled capsids from the nucleus to the exterior of the infected cell. I: In the dual envelopment pathway, nucleocapsids undergo primary envelopment at the inner nuclear membrane, become de-enveloped at the outer nuclear membrane, undergo secondary envelopment at cytoplasmic membranes, and then are transported in vesicles to the plasma membrane where fusion of the vesicle with the plasma membrane releases the virion to the extracellular space. II: In the lumenal model, the nucleocapsid is enveloped at the inner nuclear membrane, enters a vesicle at the outer nuclear membrane, and is then transported to the plasma membrane where the virion is released. III: In the nuclear pore model, filled capsids exit the nucleus through enlarged nuclear pores and become enveloped by budding into cytoplasmic vesicles, and the virion is transported to the plasma membrane where the virion is released. Wild et al have proposed a dual pathway for egress, in which HSV uses the luminal pathway at early times of infection but the bulk of extracellular virus is formed by the nuclear pore pathway at late times of infection. (Copyright Lynne Chang and David Knipe.)

Latent Infections by HSV 1



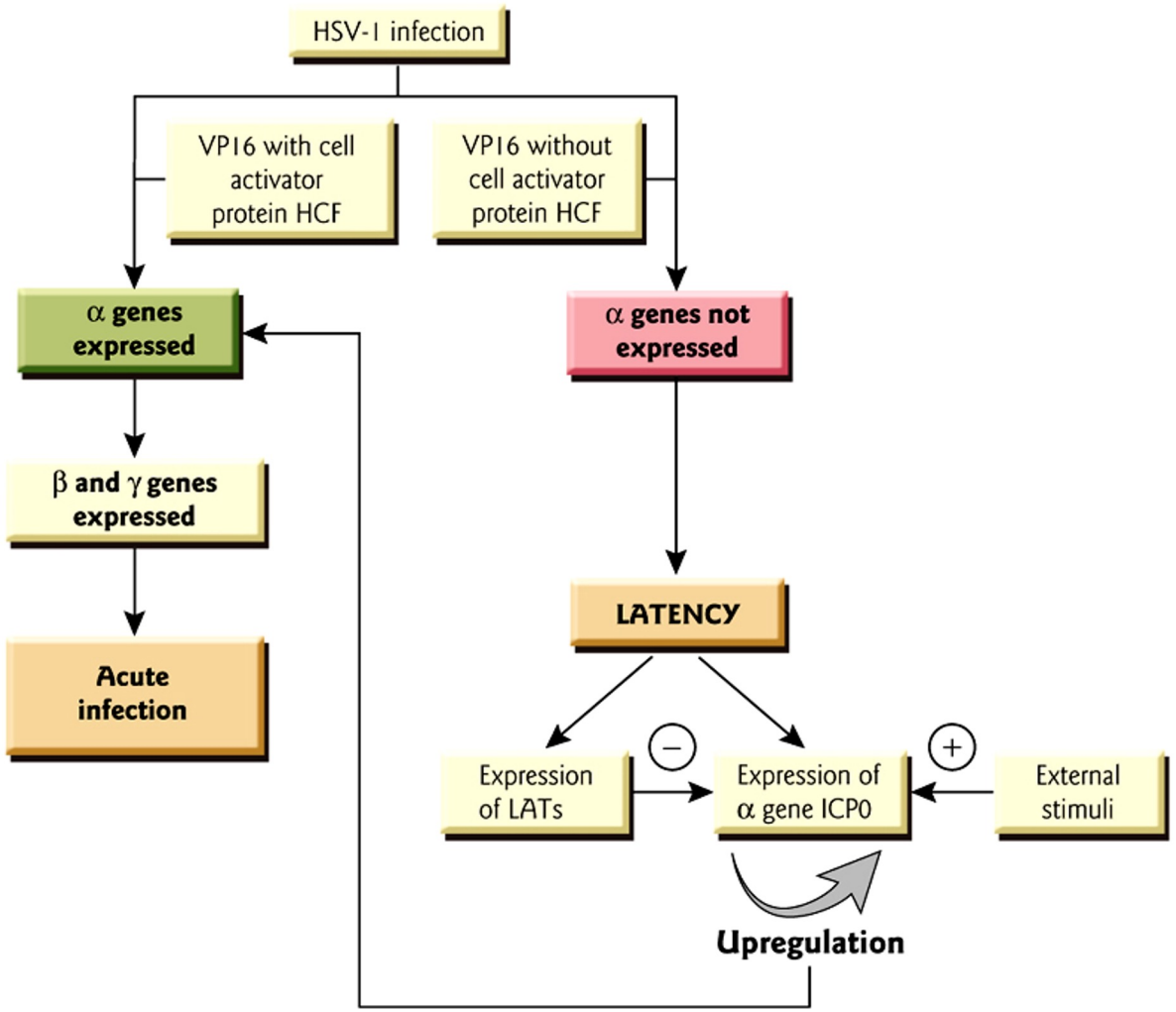
Latent Infections by HSV 1



The unique polarized morphology of neurons contributes to the establishment and control of HSV-1 latency. (a) During natural infections, HSV-1 enters the nervous system via axon terminals of peripheral neurons innervating the mucosal or corneal epithelial layers, and capsids undergo retrograde axonal transport to the cell body where the genome (green circles) is delivered into the nucleus. It is proposed that tegument protein VP16 (blue triangles) dissociates from the capsid almost immediately after release into the cytoplasm, and translocates to the nucleus with very low efficiency, due perhaps to the presence of host transcription factor HCF-1 (black dots) in the cytoplasm of both the axons and cell body. VP16 is required for productive replication in neurons, and thus the absence of tegument-derived VP16 facilitates establishment of latency

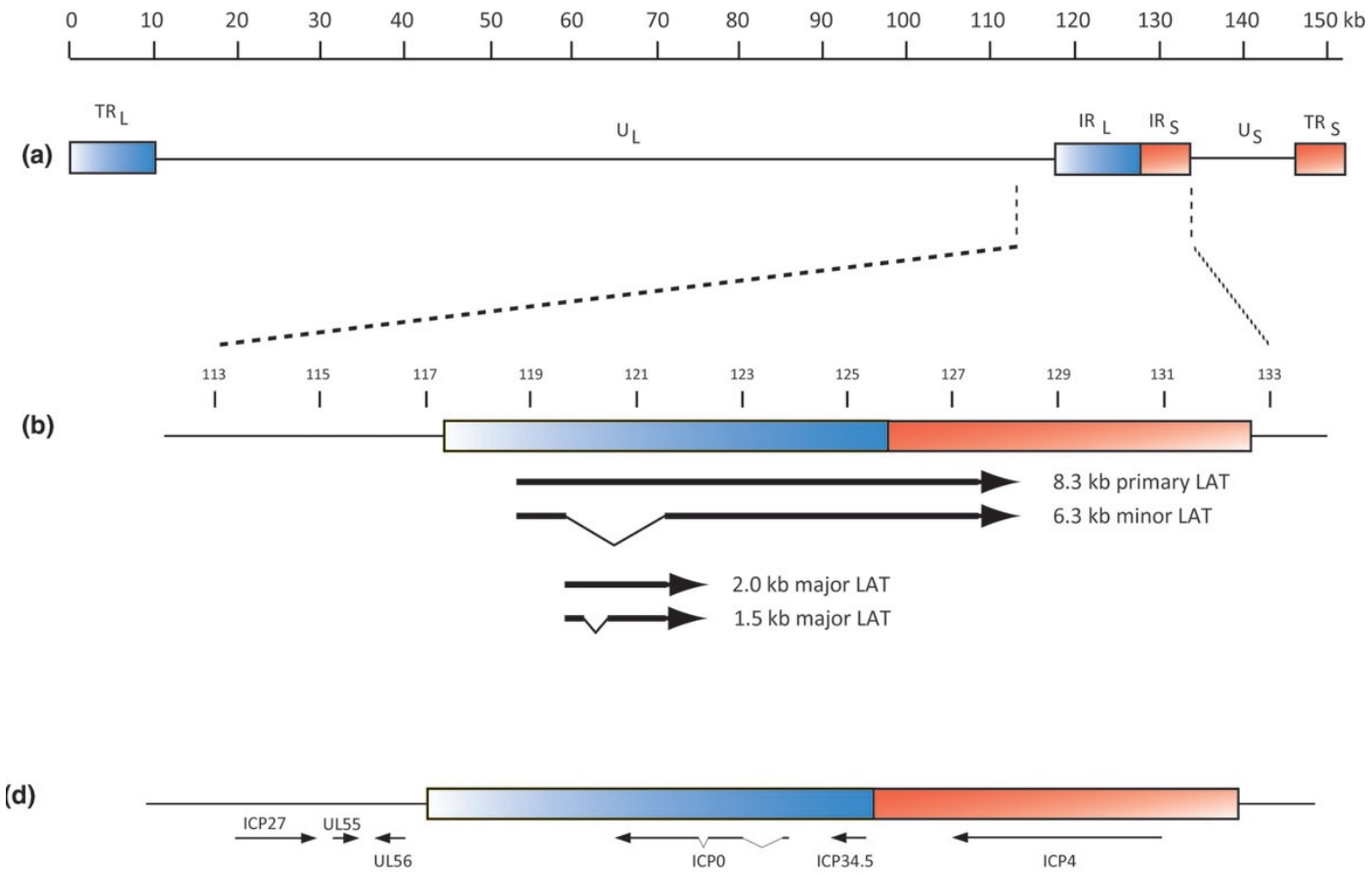
A.C. Wilson et al. Trends in Microbiology 2012, 20: 604-611

Latent Infections by HSV 1



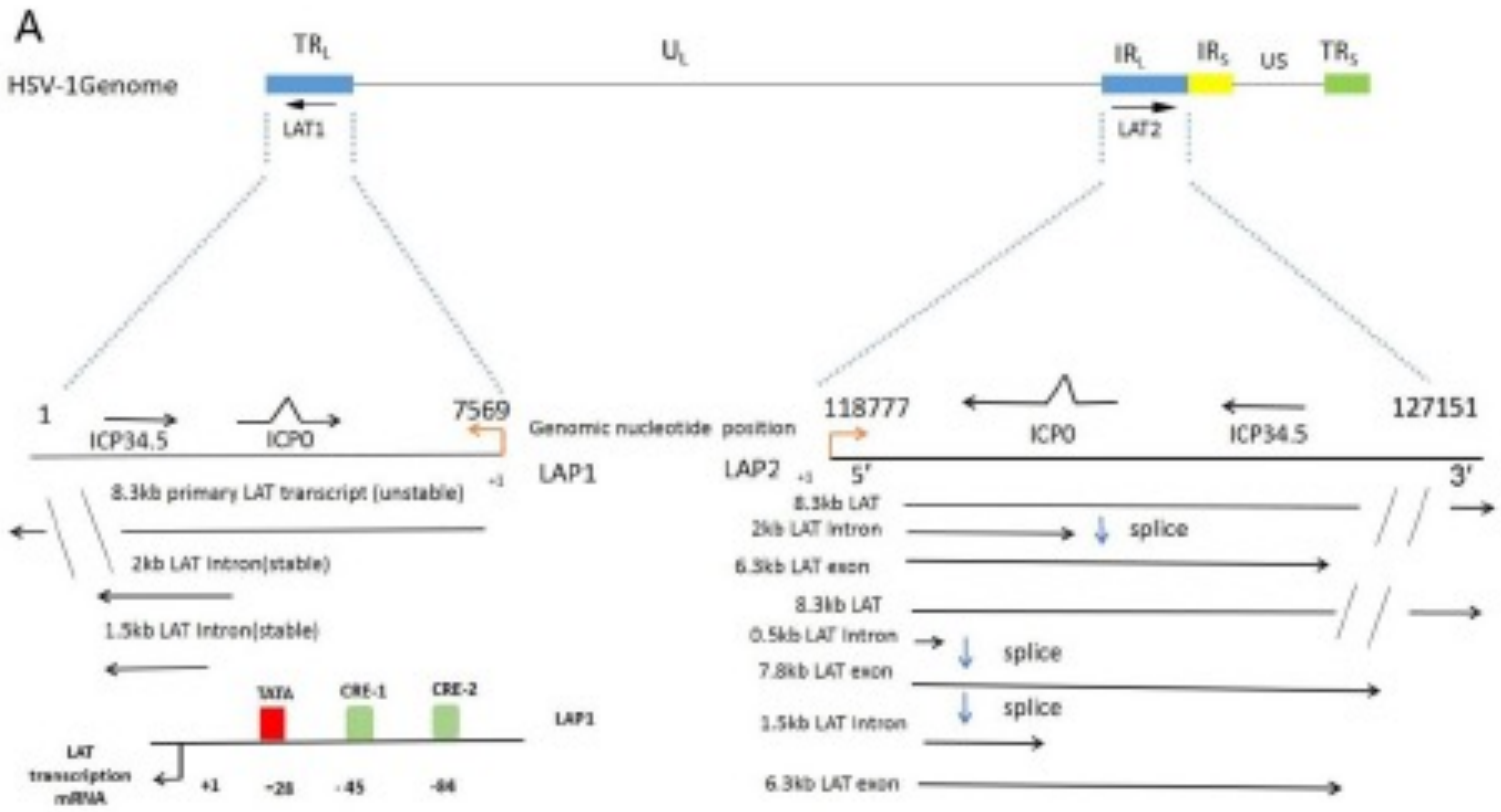
From Dimmock, Easton and Leppard, "Introduction to Modern *Virology*" (2007). Blackwell Publishing

LAT transcripts



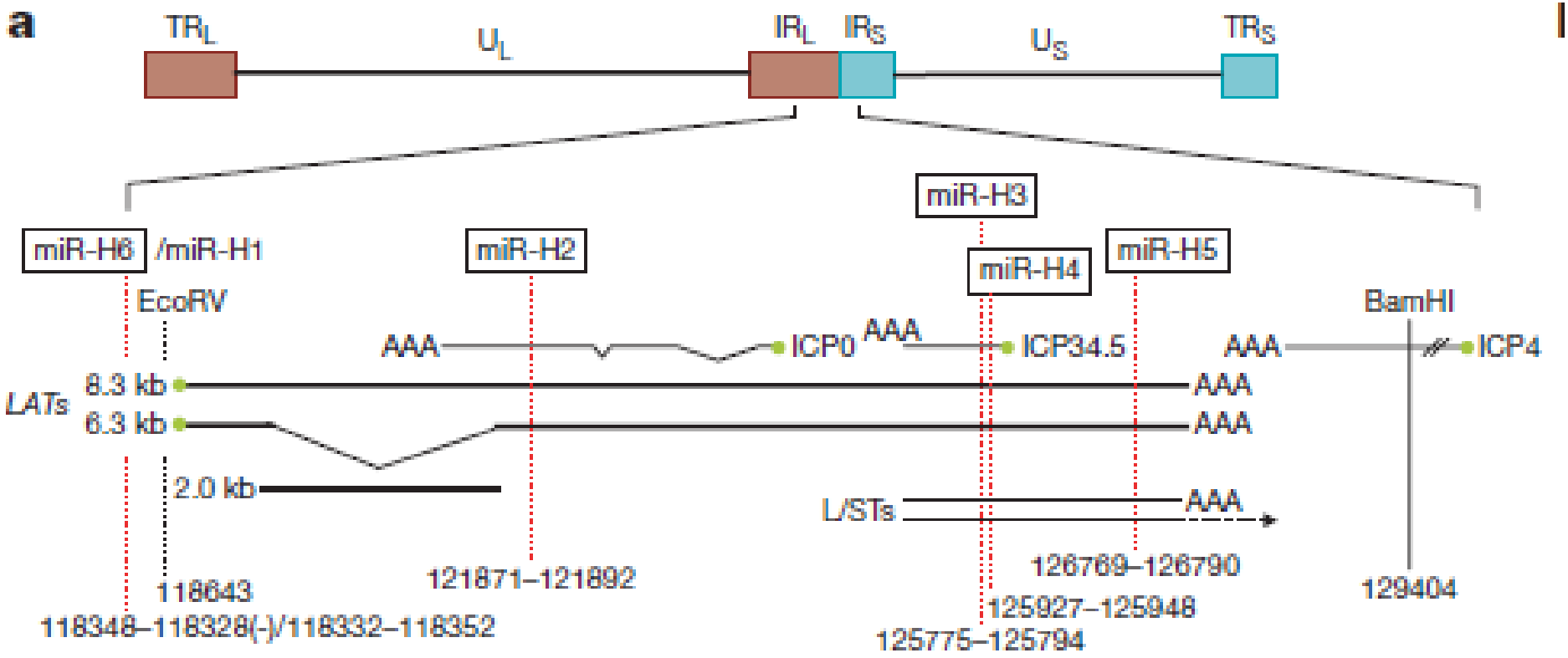
M.P. Nicoll et al., FEMS Microbiol Rev 36 (2012) 684–705

LAT transcripts



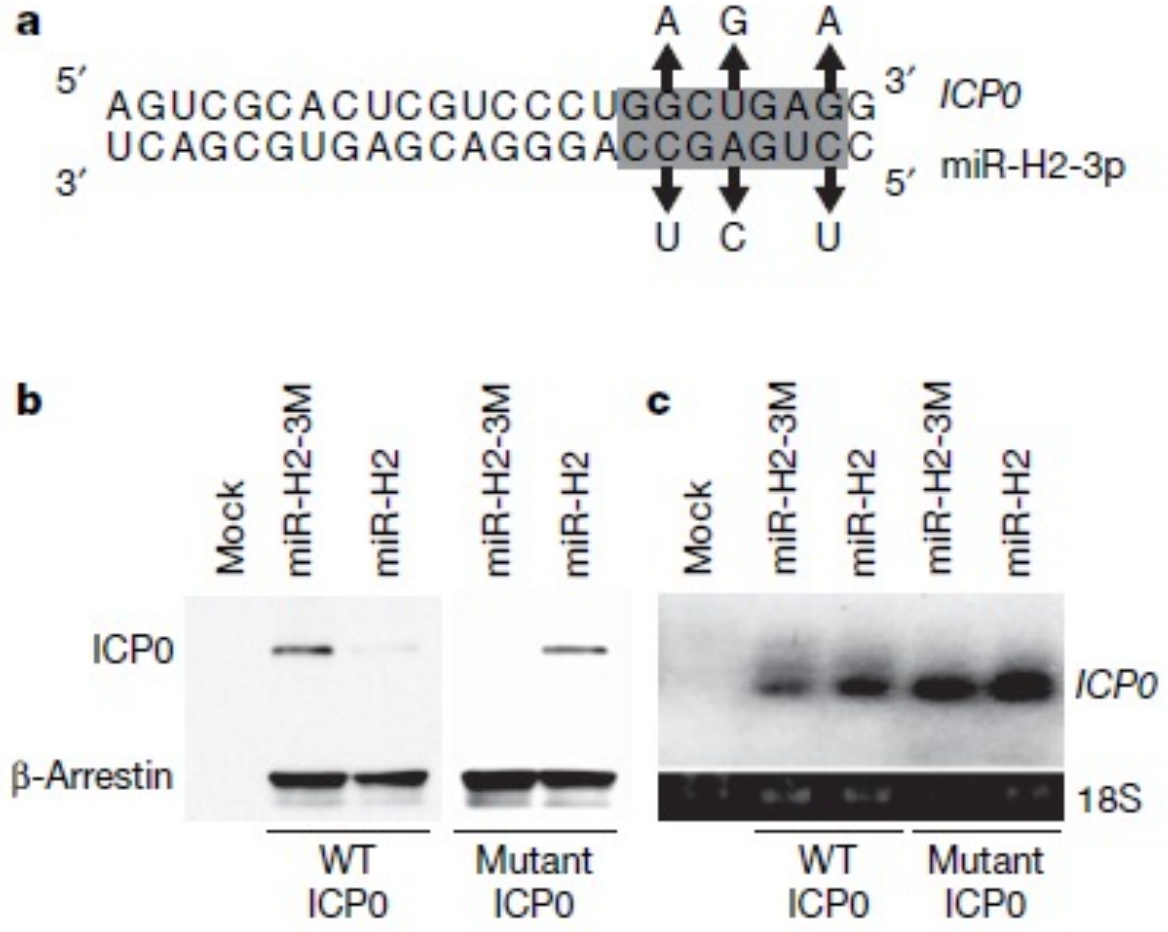
Journal of Cancer 2020, 11(11): 3387-3399

The exonic regions of LAT might function as a primary miRNA precursor



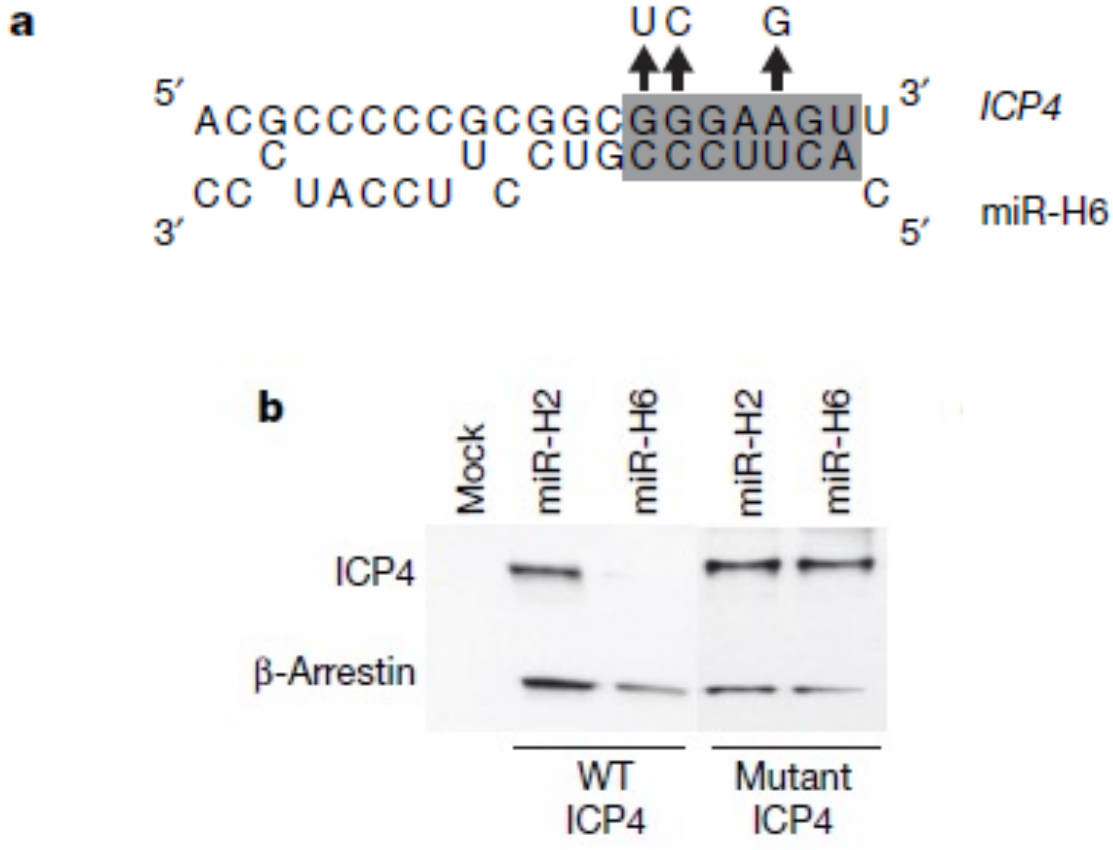
J.L. Umbach et al., Nature 2008, 454: 780-783

Downregulation of ICP0 protein expression by HSV-1 miR-H2



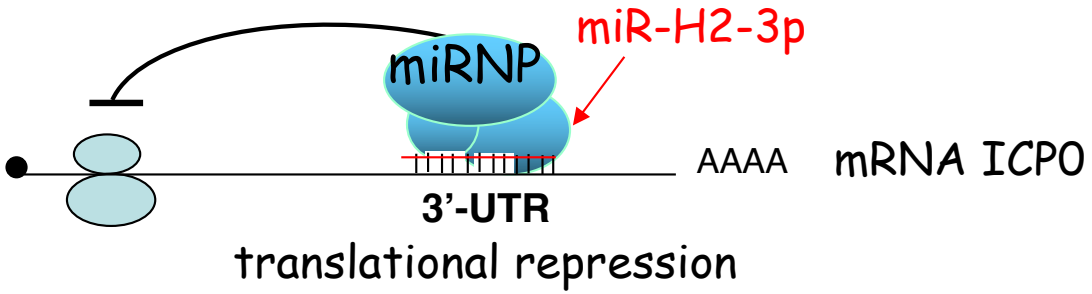
J.L. Umbach et al., Nature 2008, 454: 780-783

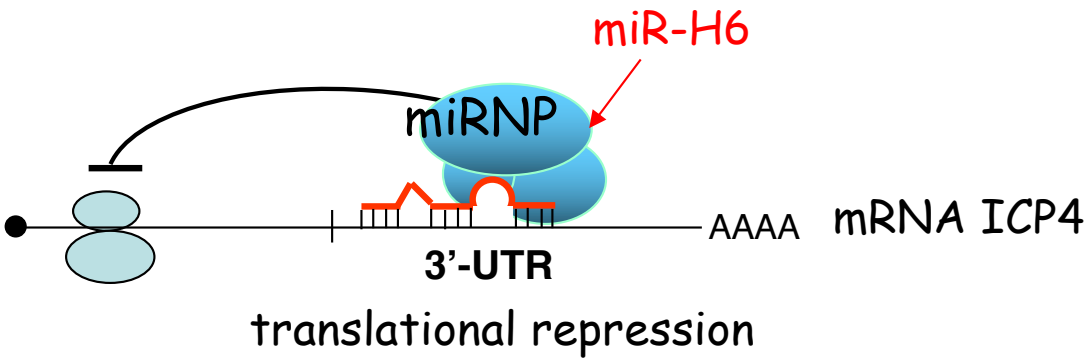
Downregulation of ICP4 protein expression by HSV-1 miR-H6



J.L. Umbach et al., Nature 2008, 454: 780-783

miR-H2-3p: derived from the antisense transcript of the viral mRNA encoding the immediate early protein ICP0



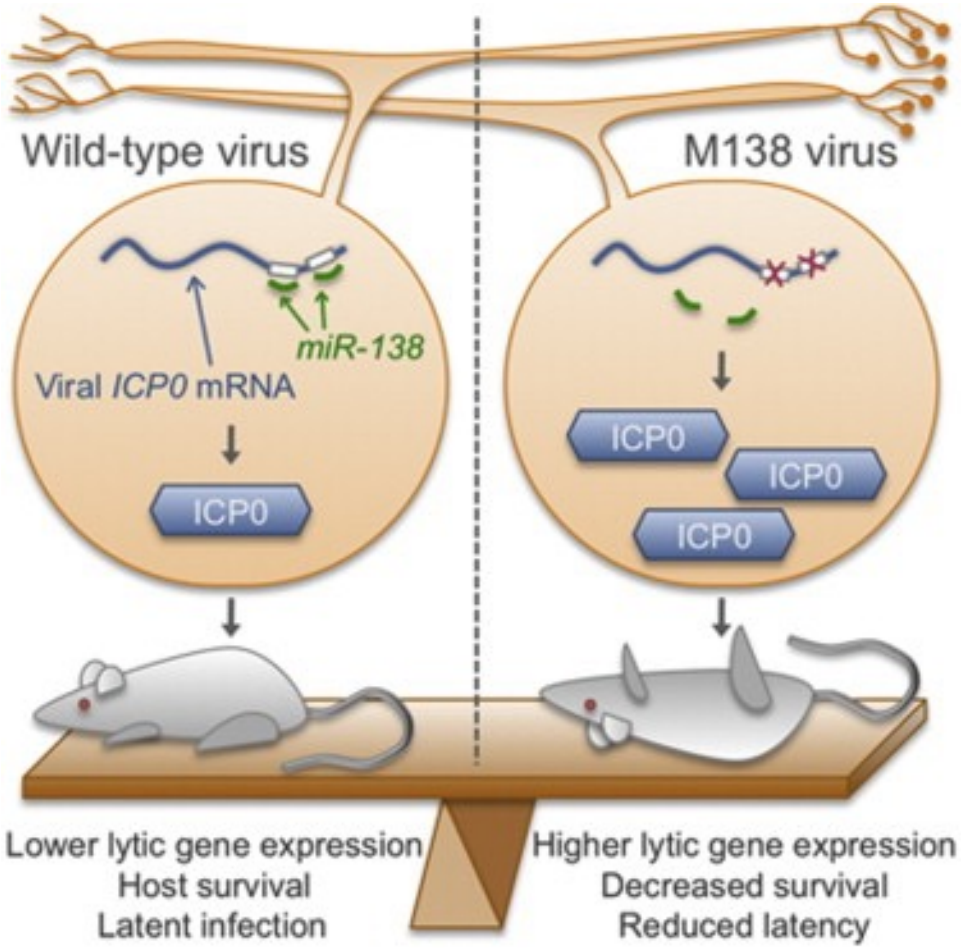


miR-H2-3p and miR-H6 negatively regulate 2 immediate early proteins

Important

- for entry into latency
- to inhibit the transition from latency to lytic cycle.

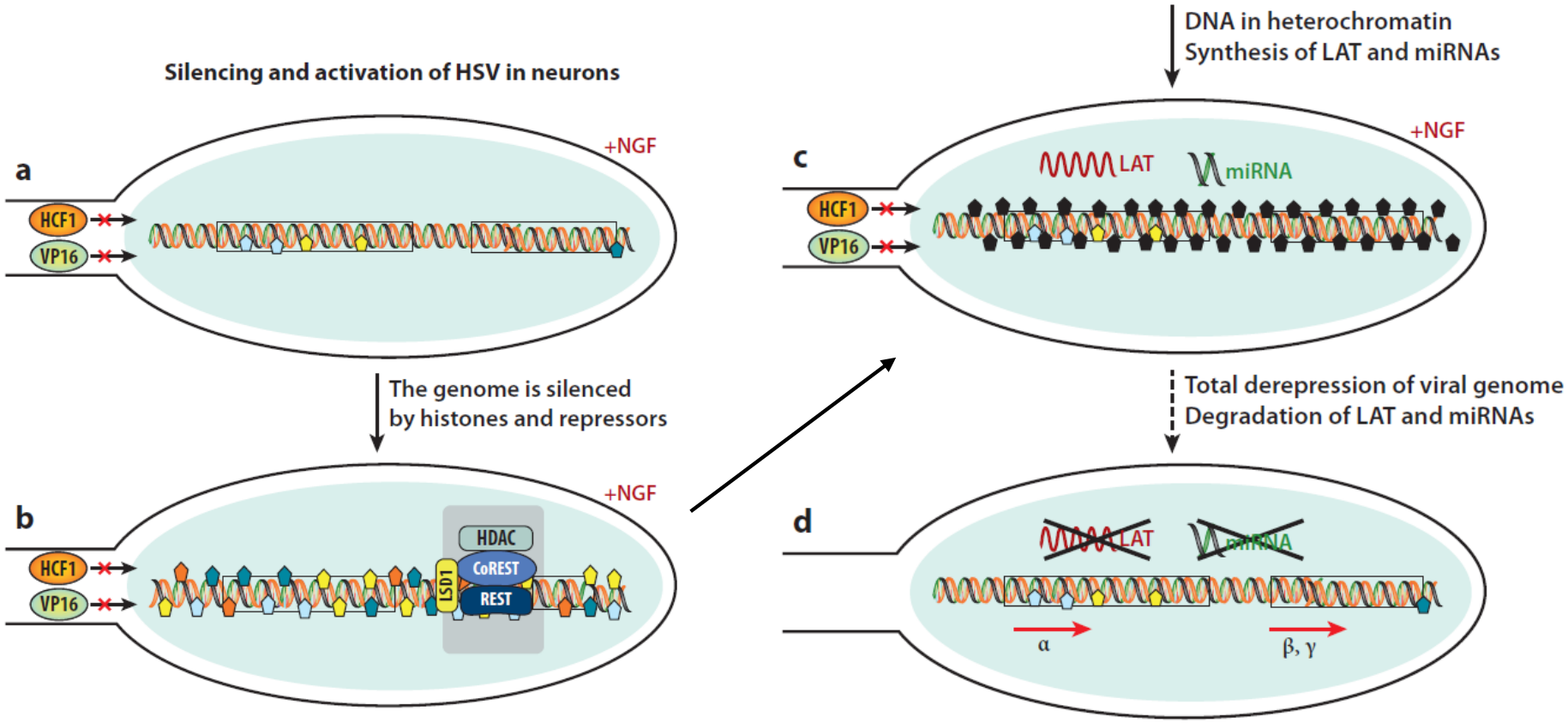
A Neuron-Specific Host MicroRNA Targets Herpes Simplex Virus-1 ICP0 Expression and Promotes Latency



After infecting peripheral sites, herpes simplex virus (HSV) invades the nervous system and initiates latent infection in sensory neurons. Establishment and maintenance of HSV latency require host survival, and entail repression of productive cycle ("lytic") viral gene expression. We find that a neuron-specific microRNA, miR-138, represses expression of ICP0, a viral transactivator of lytic gene expression. A mutant HSV-1 (M138) with disrupted miR-138 target sites in *ICP0* mRNA exhibits enhanced expression of ICP0 and other lytic proteins in infected neuronal cells in culture. Following corneal inoculation, M138-infected mice have higher levels of*ICP0* and lytic transcripts in trigeminal ganglia during establishment of latency, and exhibit increased mortality and encephalitis symptoms. After full establishment of latency, the fraction of trigeminal ganglia harboring detectable lytic transcripts is greater in M138-infected mice. Thus, miR-138 is a neuronal factor that represses HSV-1 lytic gene expression, promoting host survival and viral latency.

Cell Host and Microbe, Volume 15, Issue 4, 2014, 446 - 456

A model of silencing and activation of HSV in neurons



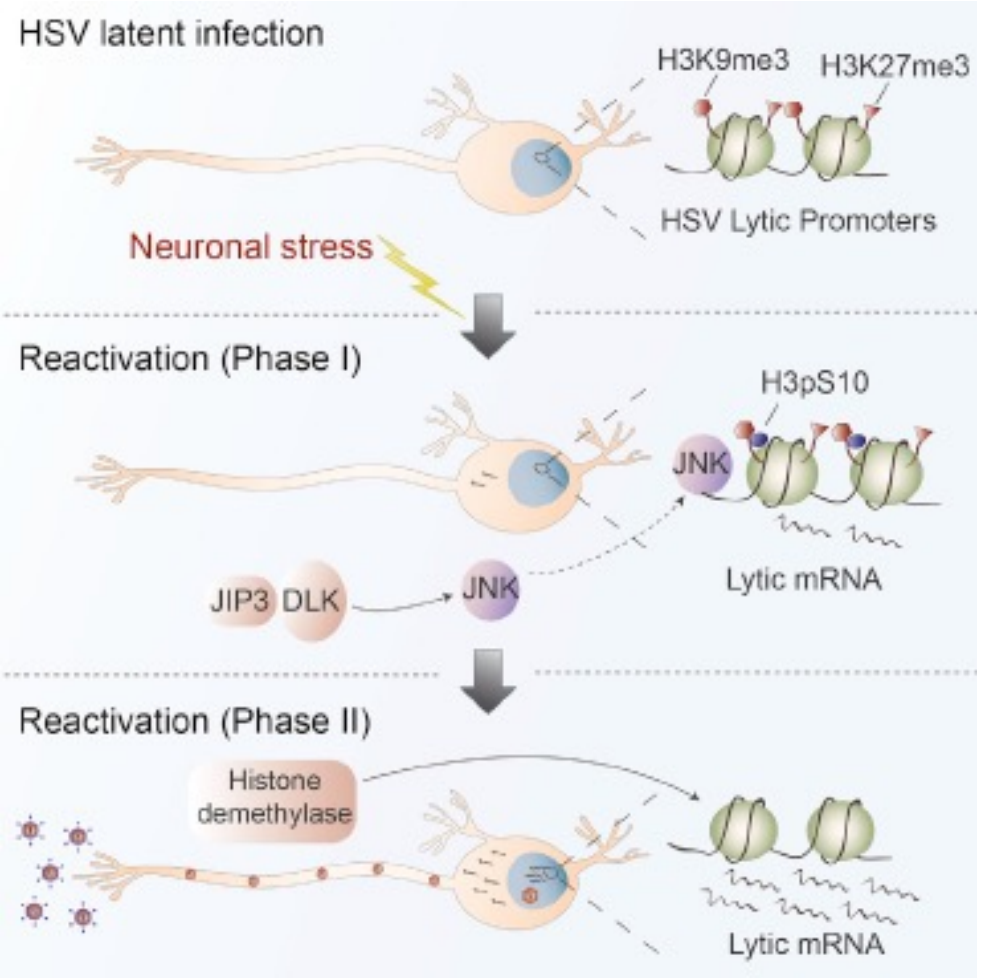
Possible involvment of JNK in reactivation

Annu. Rev. Microbiol. 2013. 67:355–74

A model of silencing and activation of HSV in neurons

(*a*) The model is based on the proposed evidence that HCF1, VP16, or both are retained in the axons or cytoplasm and are not translocated to the neuronal nucleus. (*b, c*) In the absence of HCF1 and VP16 viral DNA is progressively silenced by histones and histone-modifying enzymes and becomes encased in heterochromatin. The HDAC1/CoREST/LSD1/REST complex initiates the repression of the viral genome in peripheral neurons. Over time, LATs and viral miRNAs abound. Viral DNA equilibrates between total silencing and sporadic gene expression. The function of HDACs, LATs, and miRNAs is to maintain the DNA silence and to degrade small amounts of transcripts, whose expression may shift the dynamic state to activation of the viral genome. (*d*) The stress of a specific type or of a high magnitude causes the entire genome to become derepressed and all viral genes are expressed at once. LATs and miRNAs are degraded.

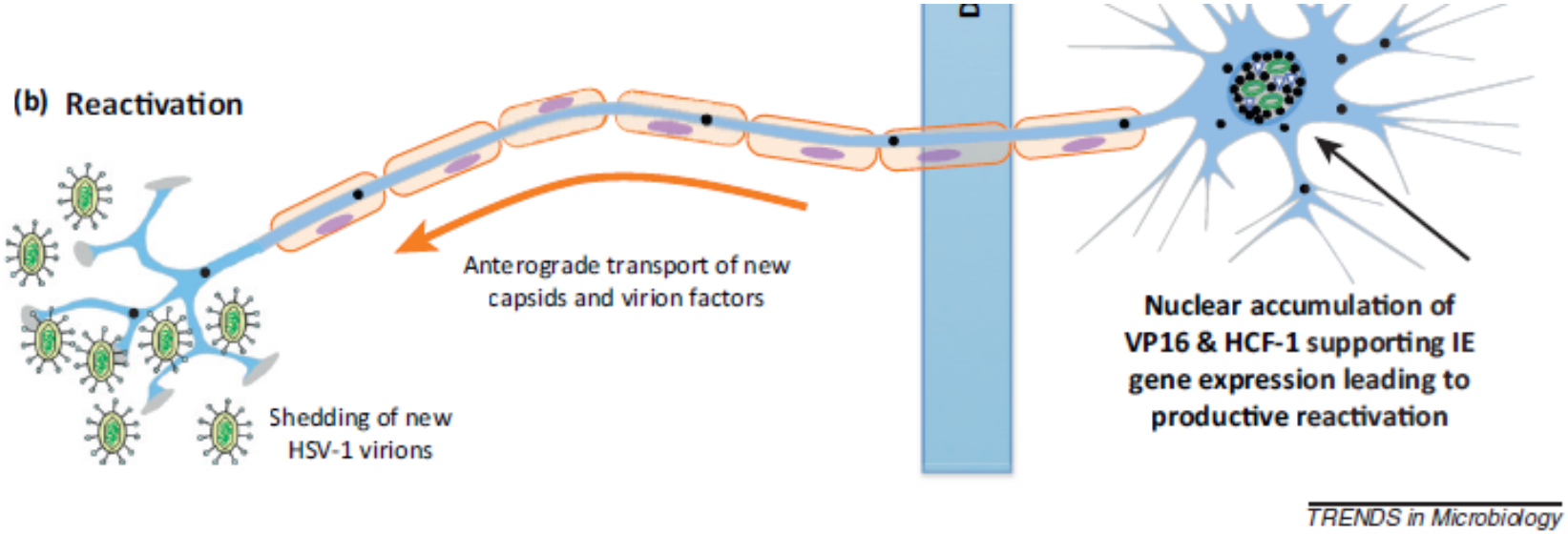
Stress Flips a Chromatin Switch to Wake Up Latent Virus



Initially, the episomal viral genome is coated with repressive chromatin marks such as H3K9me3 at promoters. As stress increases, the JNK signaling pathway is activated and phosphorylation on H3S10 allows transcription of viral genes without the removal of H3K9me3, passing the threshold for reactivation. With an increase in transcription of viral genes such as **VP16**, the reactivation enters Phase II with the re-writing of active marks, such as H3K9ac, on the viral genomes, viral replication, and progeny production.

Cliffe et al., 2015, Cell Host & Microbe 18, 649–658 December 9, 2015

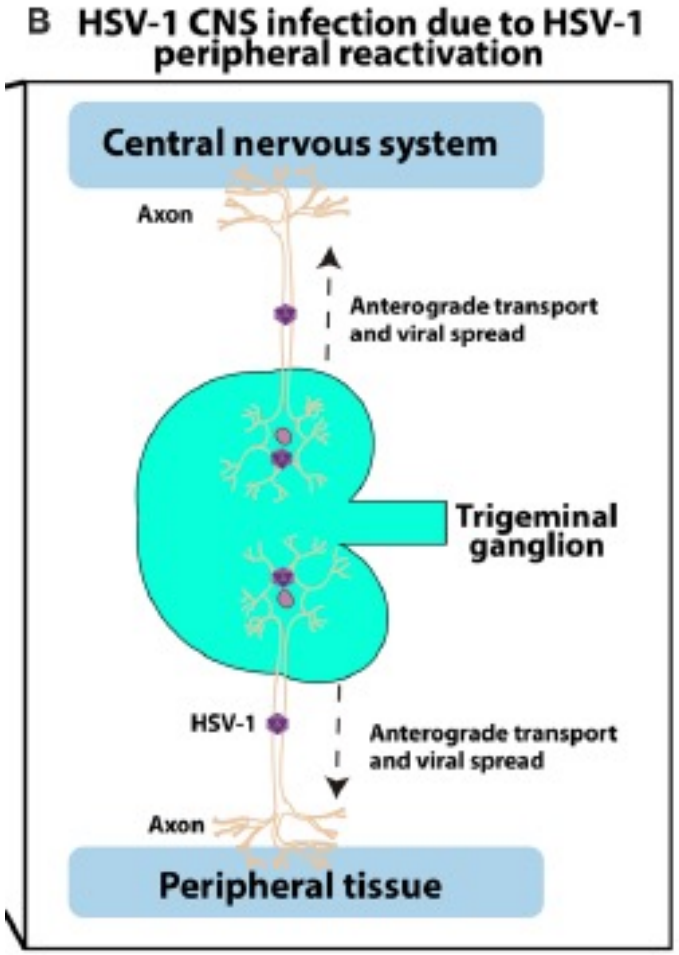
Latent Infections by HSV 1



Reactivation stimuli can elicit many changes in the neuron, including nuclear accumulation of HCF-1 and VP16, which is synthesized de novo along with other viral regulatory proteins. Stimulation of viral lytic transcription by VP16 leads to viral DNA amplification and synthesis of virion proteins. Capsids are transported in an anterograde fashion to the axonal termini where they mature and are then released, bringing the HSV-1 life cycle full circle.

A.C. Wilson et al. Trends in Microbiology 2012, 20: 604-611

HSV-1 and neurodegenerative disorders



70% of herpetic simplex encephalitis (HSE) is due to HSV-1 reactivation in peripheral trigeminal nerve.
It has been also shown that HSV-1 can establish latency in neurons of the CNS.
Herpes simplex virus 1 (HSV-1) has long been suspected to be one of the factors involved in the pathogenesis of the Alzheimer's disease.

Duarte et al., Frontiers in Cellular Neuroscience, 2019