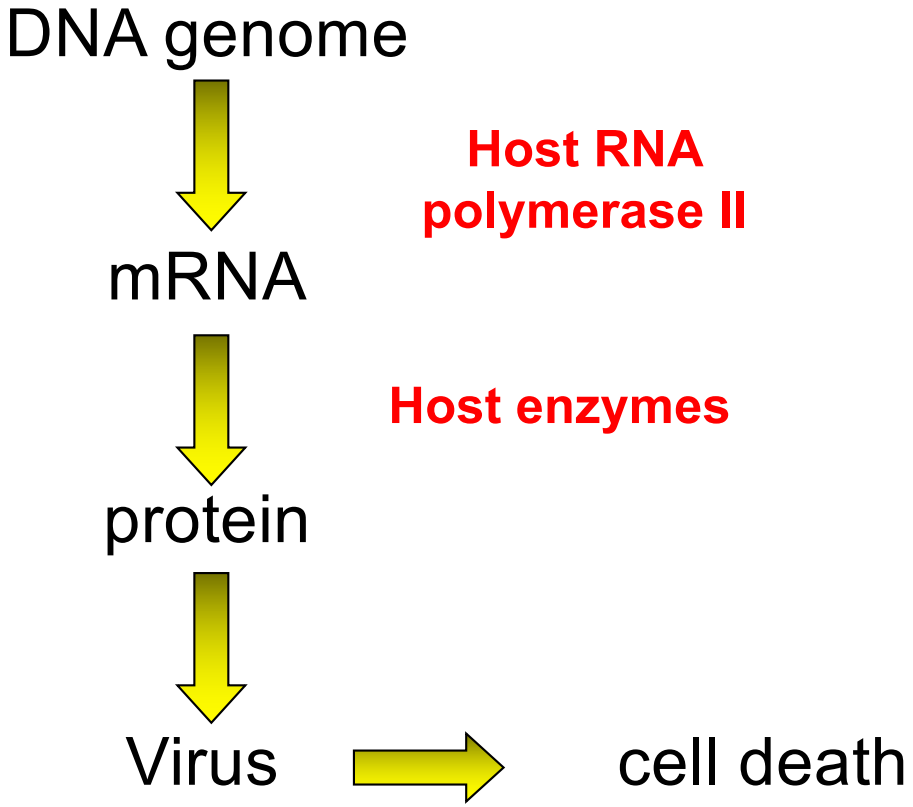
DNA tumour viruses

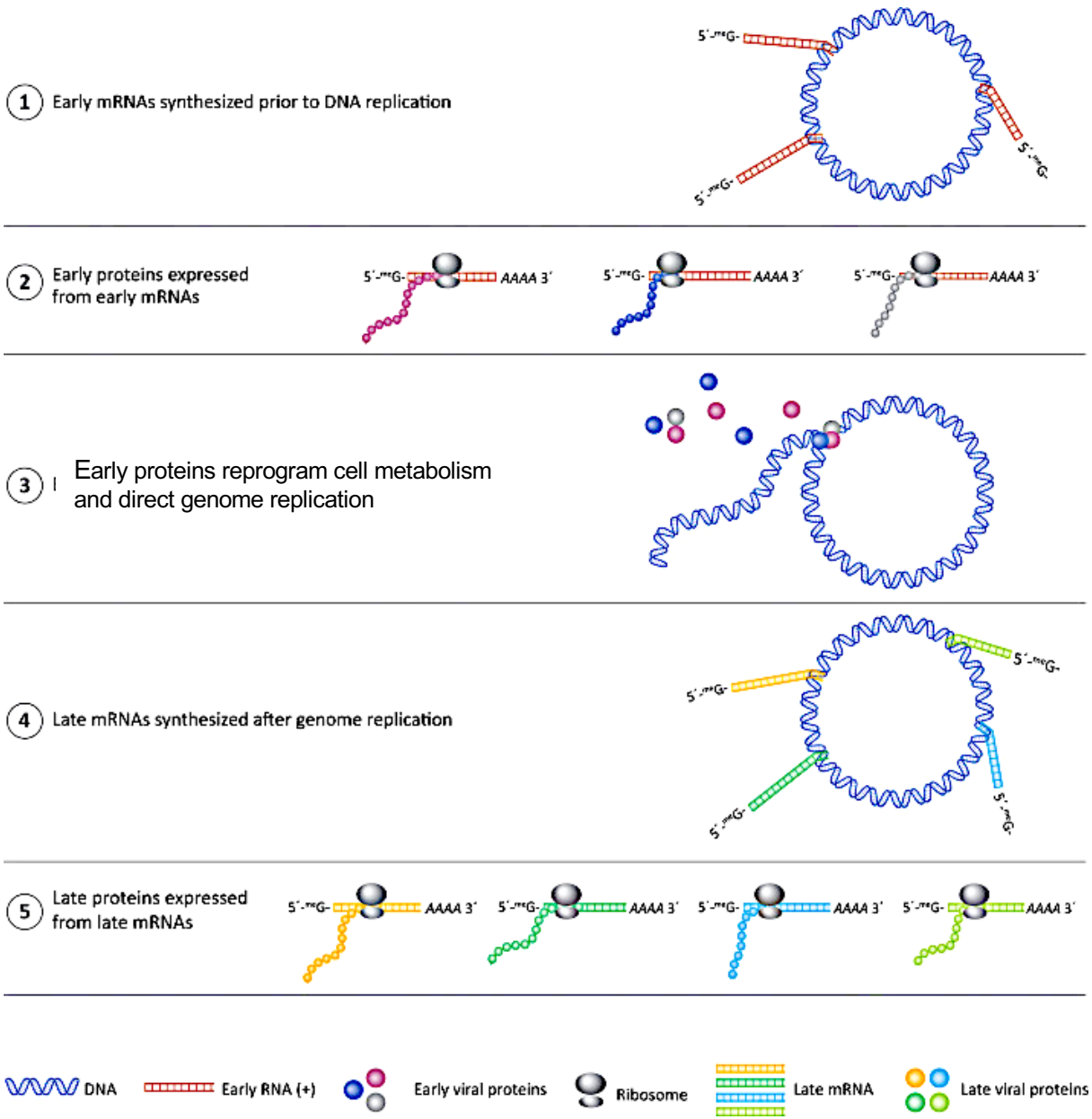
DNA Tumor Viruses
(*Adenoviridae, Papillomaviridae, Polyomaviridae*)



OR TRANSFORMATION

In transformation usually only EARLY functions are expressed

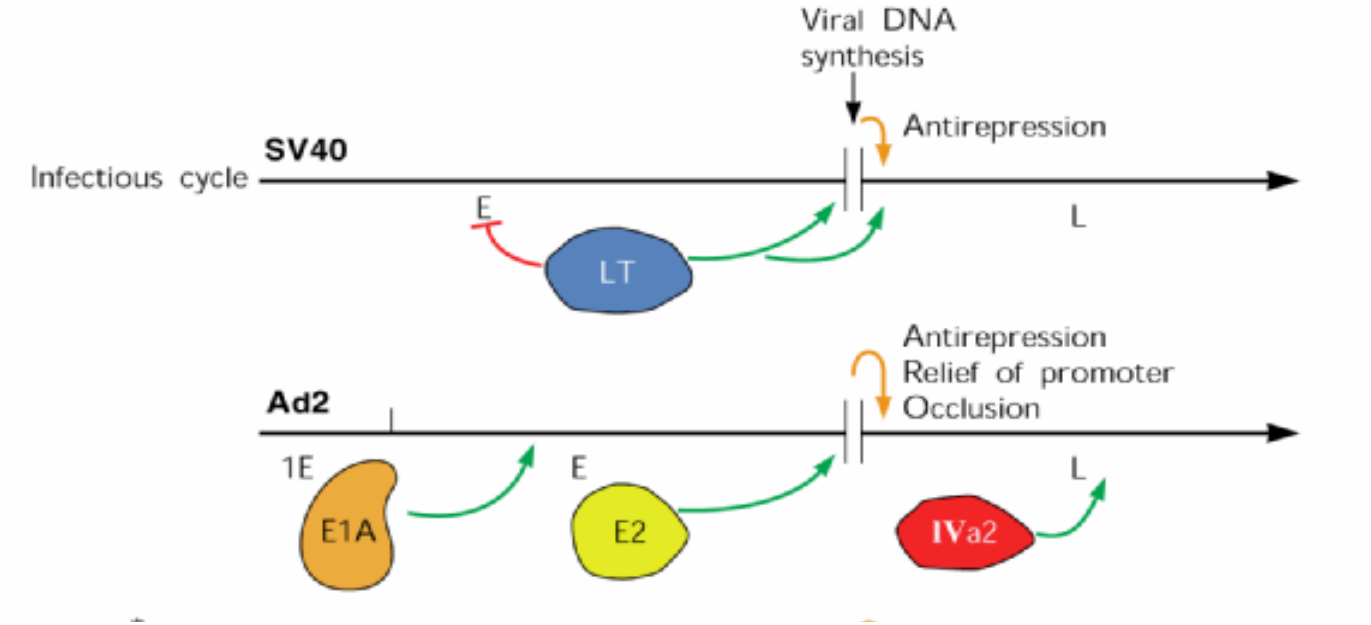
DNA virus expression timing



Upon uncoating, the genomes of DNA viruses are transcribed to produce an “early” set of mRNAs. Early mRNAs typically encode for proteins that modulate the host cell environment and/or are required for viral genome replication. After genome replication another set of mRNAs, the “late” mRNAs are expressed. Late genes encode structural proteins (and other proteins that are packaged within virions).

VIRUSES 2018 Elsevier Inc.

Early gene expression

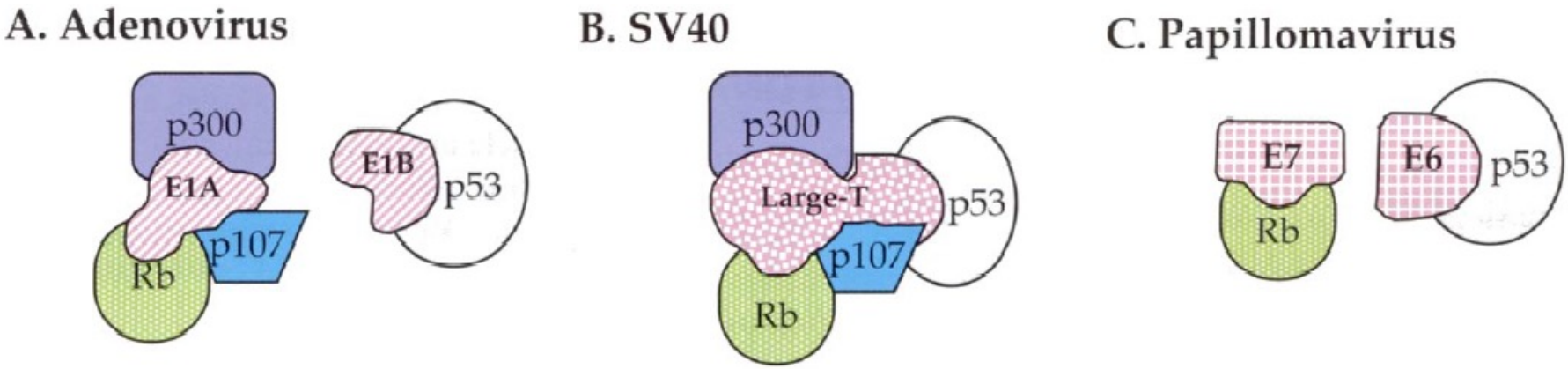


ASM Virology
Fig. 08.15 #815

DNA tumour virus oncoproteins

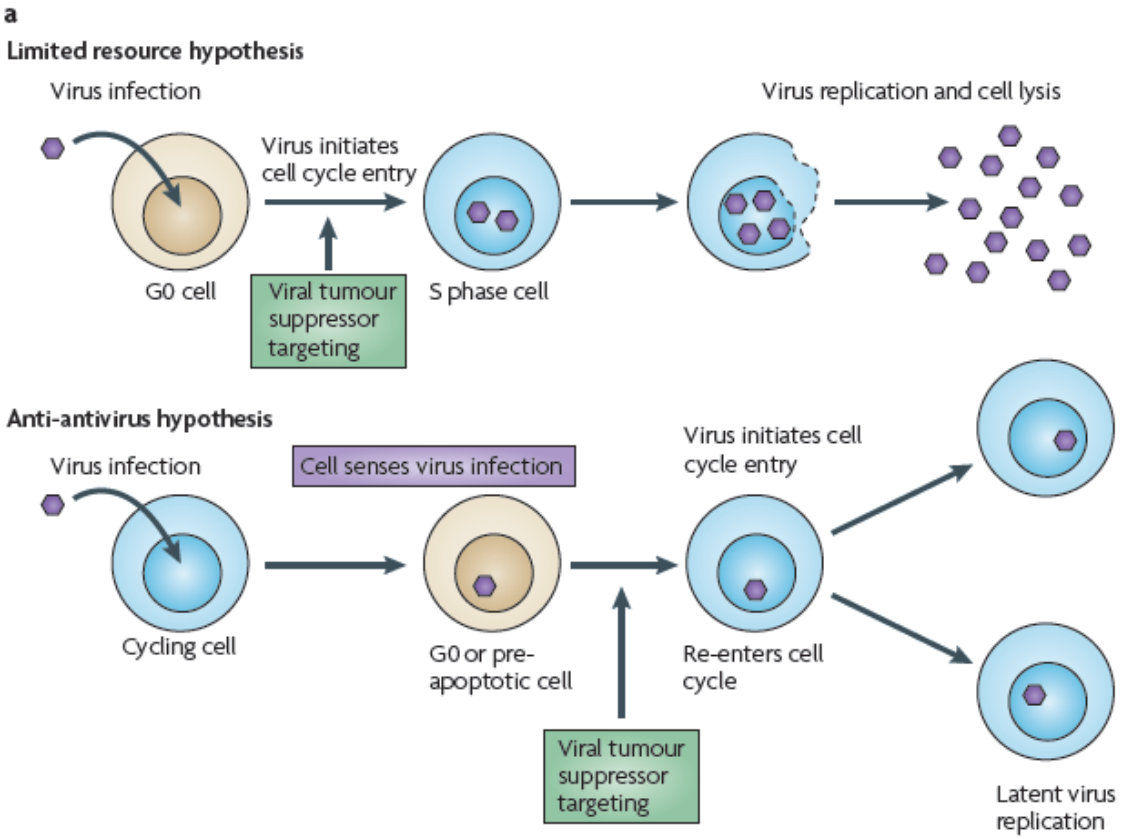
Table 7.5 Transforming proteins of DNA tumour viruses

Virus	Transforming protein(s)	Cellular target
Adenoviruses	E1A + E1B	Rb, p53
Polyomaviruses (SV40)	T antigen	p53, Rb
Papillomaviruses:		
BPV-1	E5	PDGF receptor
HPV-16, 18	E6	p53
	E7	Rb



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

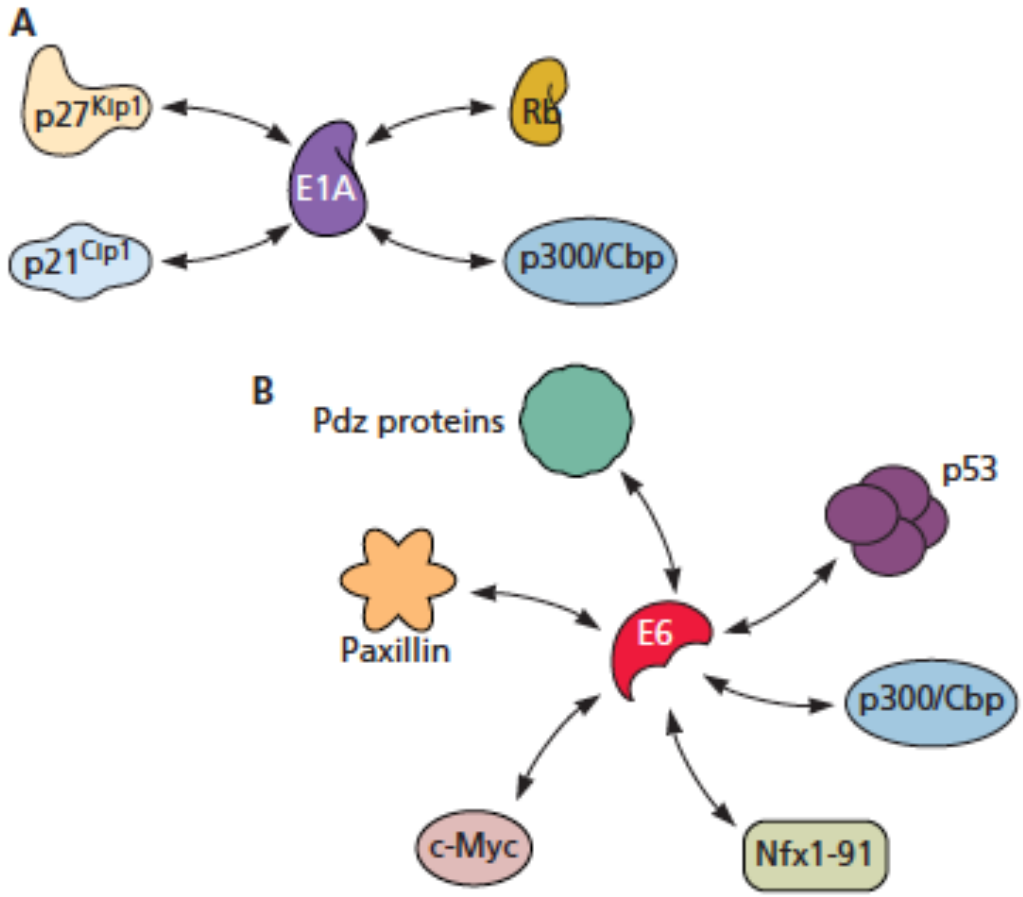
Two views for the origins of viral oncoproteins



The tumour virus proteins target RB1 and p53 to drive a quiescent G0 cell into S phase of the cell cycle, allowing viral access to the nucleotide pools and replication machinery that are needed for replication and transmission. Viral tumourigenesis is a by-product of the molecular parasitism by viruses to promote their own replication. Cells respond to virus infection by activating RB1 and p53 to inhibit virus replication as part of the innate immune response. To survive, tumour viruses have evolved the means for inactivating these and other immune signalling pathways that place the cell at risk for cancerous transformation. This view holds that many tumour suppressor proteins have dual functions in preventing cancer formation and virus infection.

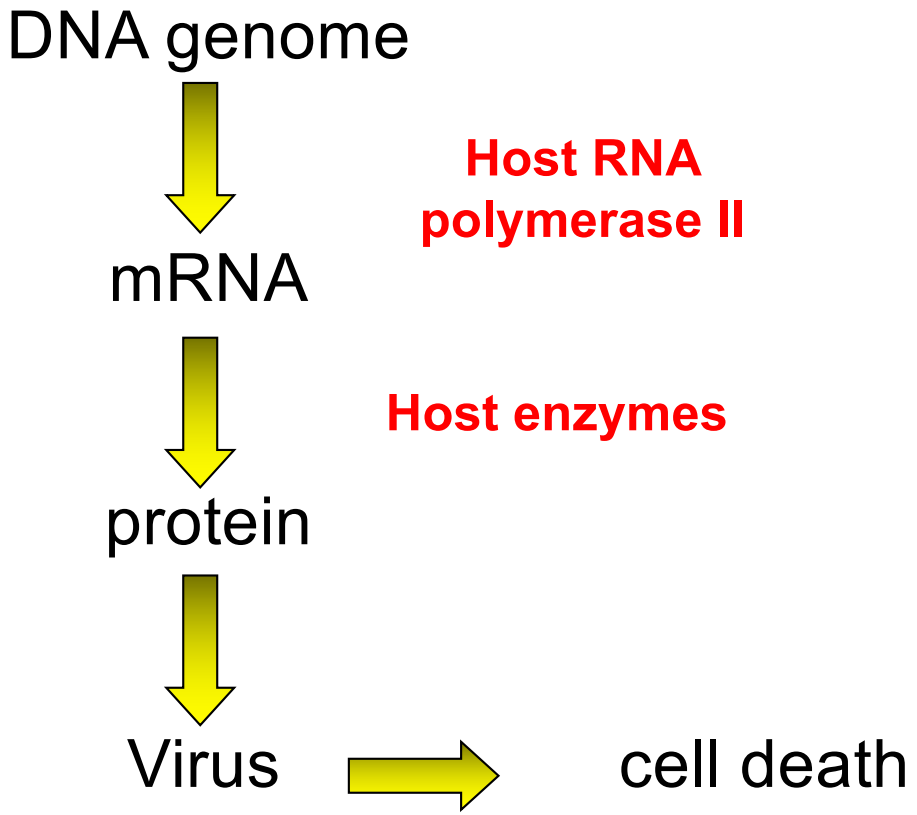
NATURE REVIEWS | CANCER

Interactions of DNA virus transforming proteins with multiple cellular proteins



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

DNA Tumor Viruses



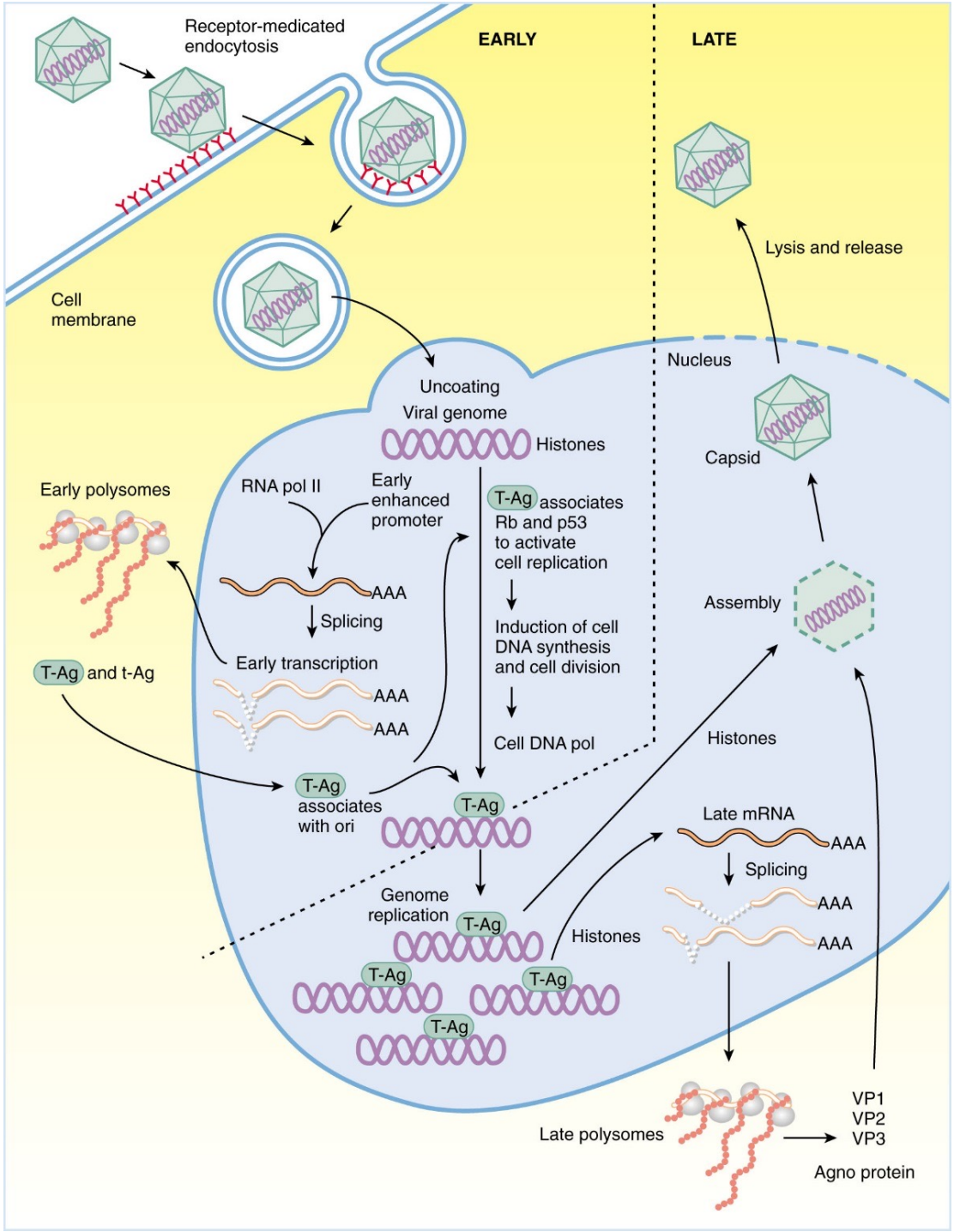
Viral tumourigenesis is a by-product of the molecular parasitism by viruses to promote their own replication

OR TRANSFORMATION
In transformation usually only EARLY functions are expressed

Permissive cells: Replication, lysis and death

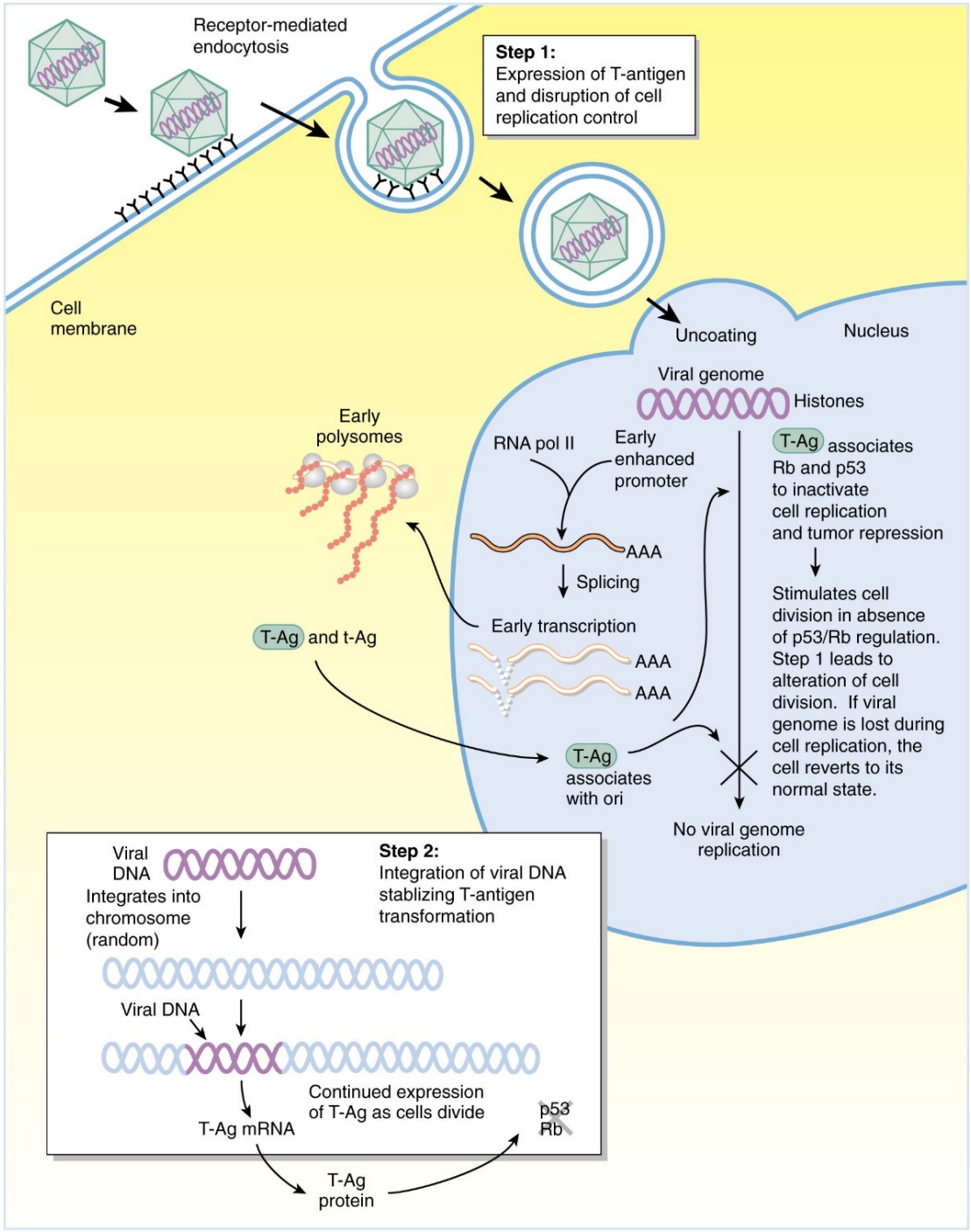
Non-permissive cells: transformation. Usually, DNA is integrated. Early functions only are expressed. Control information, rather than structural proteins

SV40 life cycle, infection of permissive cells



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

SV40 life cycle, infection of non-permissive cells

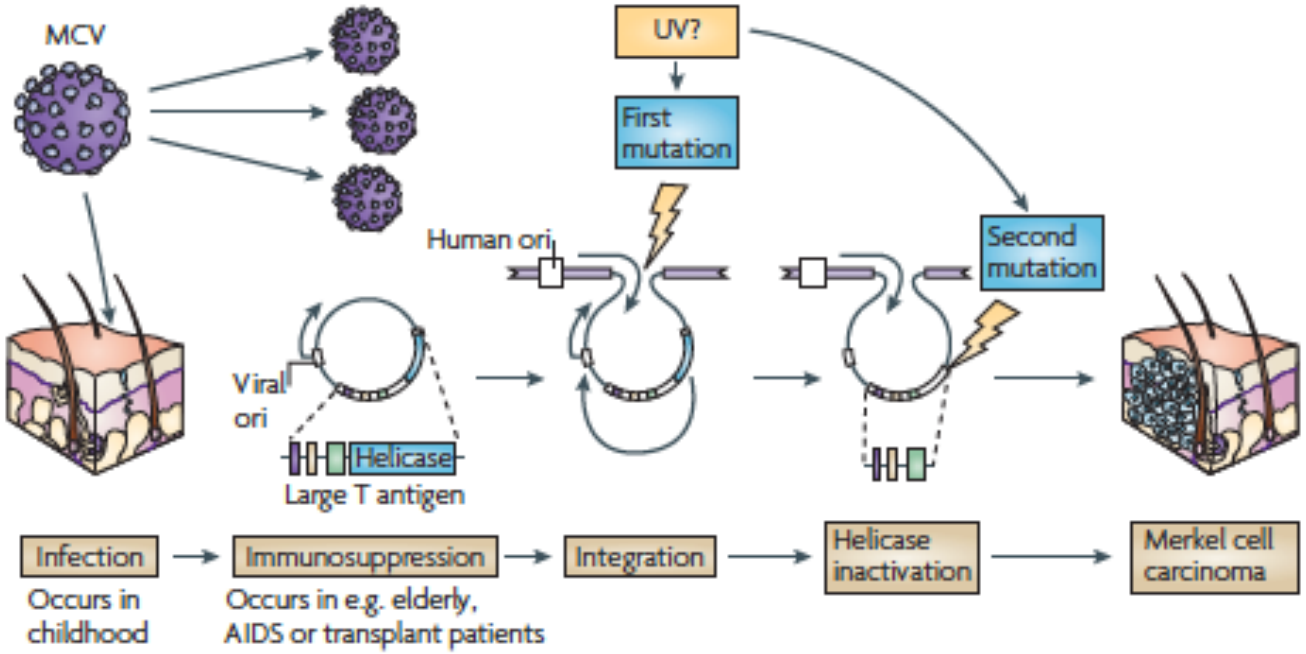


From Wagner and Hewlett *Basic virology* (2003) Blackwell Science Press

The human cancer viruses

Virus	Genome	Notable cancers	Year first described	Refs
Epstein–Barr virus (EBV; also known as human herpesvirus 4 (HHV4))	Double-stranded DNA herpesvirus	Most Burkitt's lymphoma and nasopharyngeal carcinoma, most lymphoproliferative disorders, some Hodgkin's disease, some non-Hodgkin's lymphoma and some gastrointestinal lymphoma	1964	15
Hepatitis B virus (HBV)	Single-stranded and double-stranded DNA hepadenovirus	Some hepatocellular carcinoma	1965	25
Human T-lymphotropic virus-I (HTLV-I)	Positive-strand, single-stranded RNA retrovirus	Adult T cell leukaemia	1980	20
High-risk human papillomaviruses (HPV) 16 and HPV 18 (some other α-HPV types are also carcinogens)	Double-stranded DNA papillomavirus	Most cervical cancer and penile cancers and some other anogenital and head and neck cancers	1983–1984	29, 30
Hepatitis C virus (HCV)	Positive-strand, single-stranded RNA flavivirus	Some hepatocellular carcinoma and some lymphomas	1989	31
Kaposi's sarcoma herpesvirus (KSHV; also known as human herpesvirus 8 (HHV8))	Double-stranded DNA herpesvirus	Kaposi's sarcoma, primary effusion lymphoma and some multicentric Castleman's disease	1994	33
Merkel cell polyomavirus (MCV)	Double-stranded DNA polyomavirus	Most Merkel cell carcinoma	2008	34

The molecular evolution of a human tumour virus

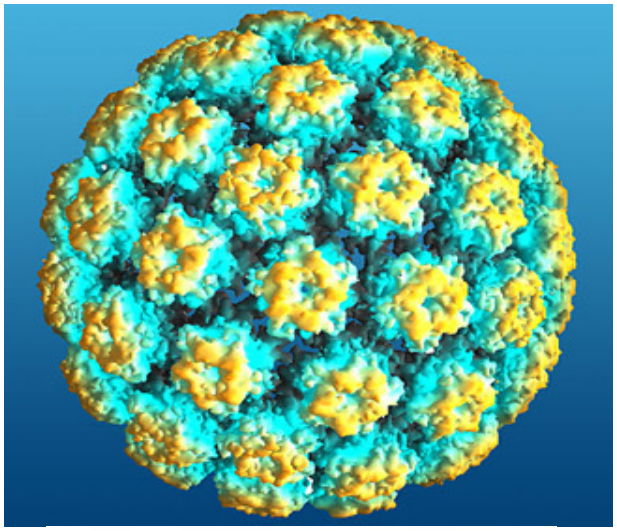


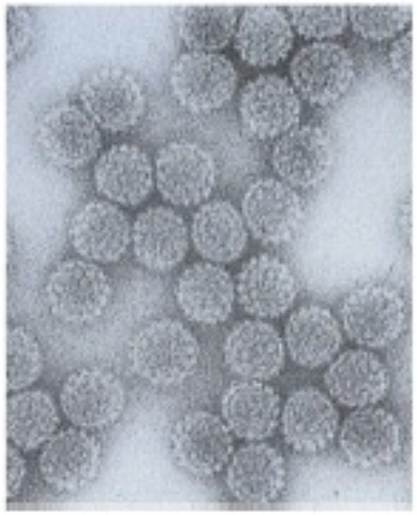
Merkel cell polyomavirus (MCV), which has tumour-specific truncation mutations, illustrates common features among the human tumour viruses involving immunity, virus replication and tumour suppressor targeting. Although MCV is a common infection, loss of immune surveillance through ageing, AIDS or transplantation and subsequent treatment with immunosuppressive drugs may lead to resurgent MCV replication in skin cells. If a rare integration mutation into the host cell genome occurs, the MCV T antigen can activate independent DNA replication from the integrated viral origin that will cause DNA strand breaks in the proto-tumour cell. A second mutation that truncates the T antigen, eliminating its viral replication functions but sparing its RB1 tumour suppressor targeting domains, is required for the survival of the nascent Merkel tumour cell. Exposure to sunlight (possibly ultraviolet (UV) irradiation) and other environmental mutagens may enhance the sequential mutation events that turn this asymptomatic viral infection into a cancer virus.

NATURE REVIEWS | CANCER 2010

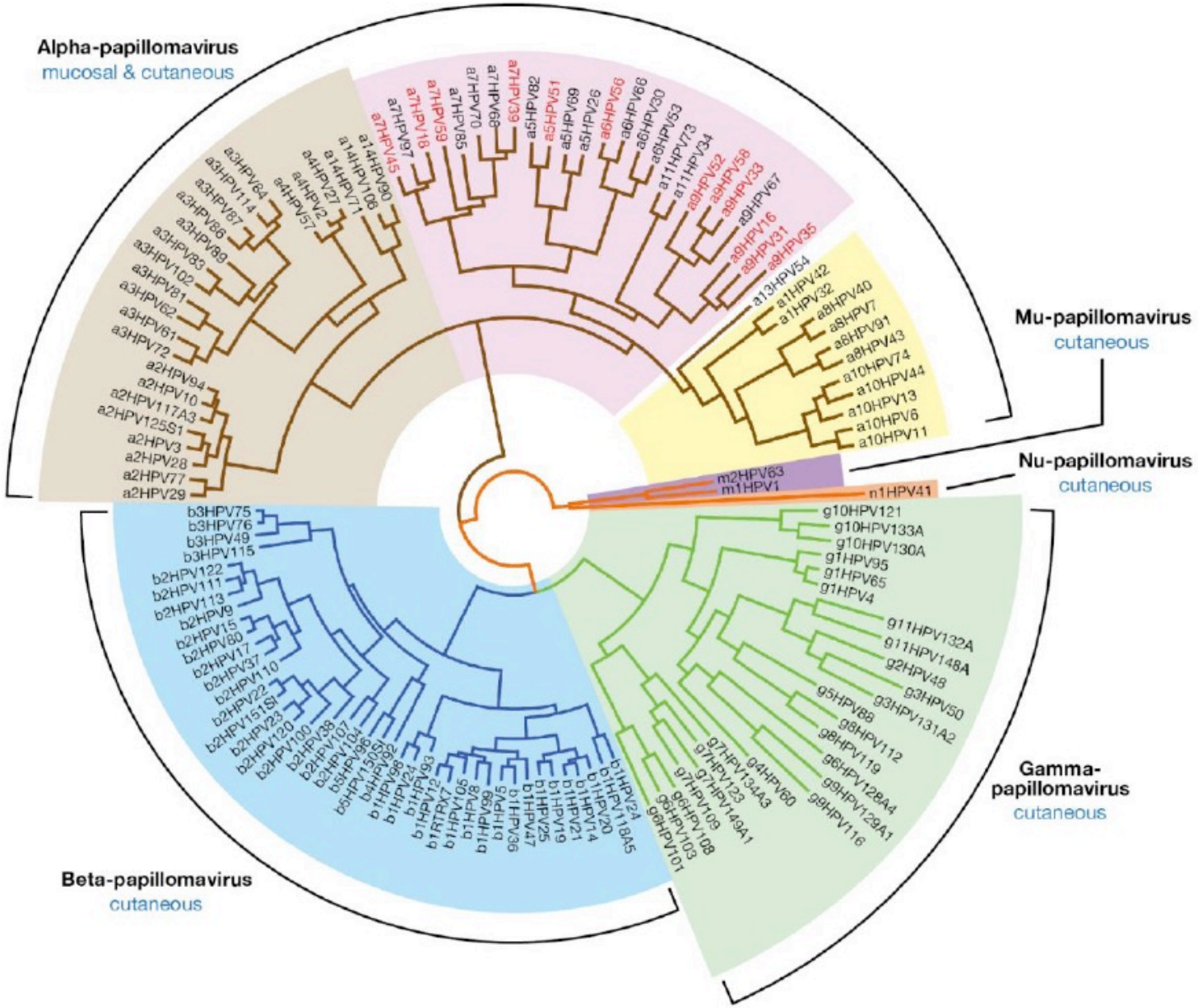
The Human Papillomavirus

- Family: Papillomaviridae
- Genome: circular dsDNA, 7900bps
- Capsid: icosahedra, 52-55 nm
- 2 structural proteins: L1 major capsid protein, L2 minor capsid protein
- Specie-specific
- Tropism: epithelial cells (skin, mucosa)





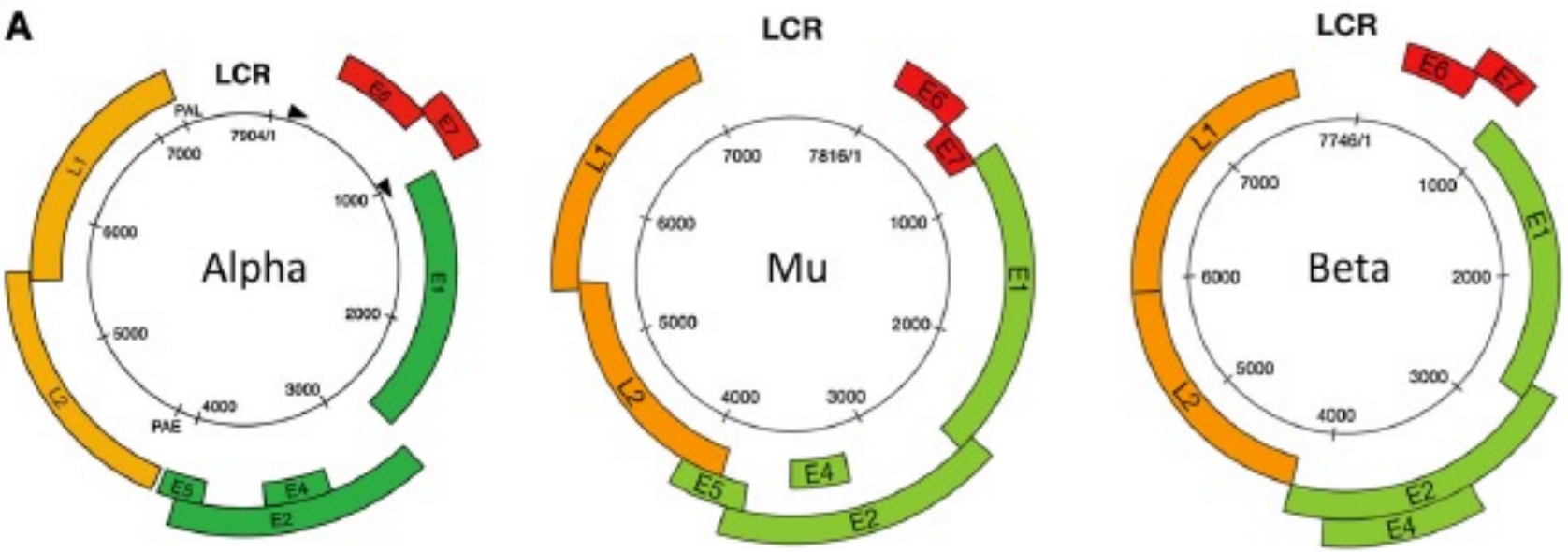
Classification



Human papillomavirus (HPV) grouping according to their risk to produce cancerous or precancerous lesions

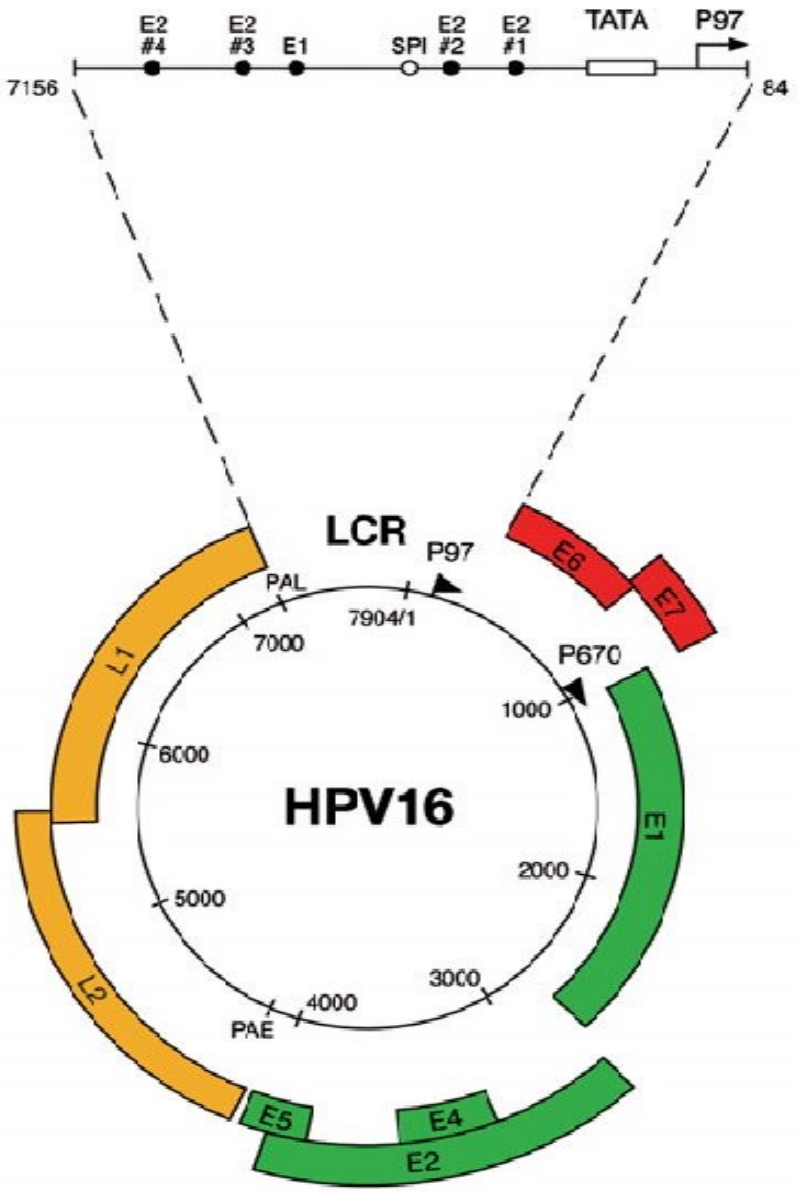
More than 150 different HPV types are known, and about 40 of these are sexually transmitted

HPV group	HPV types
High risk	HPV-16, HPV-18, HPV-45, HPV-56
Intermediate risk	HPV-31, HPV-33, HPV-35, HPV-51, HPV-52, HPV-58
Low risk	HPV-6, HPV-11, HPV-42, HPV-43, HPV-44



Typical genome organization of the high-risk Alpha, Mu, and Beta HPV genomes. Although all share a common genetic organization, the size and position of the major ORFs can vary, with Beta HPV types lacking an E5 ORF. The positions of the major promoters are marked with arrows on the high-risk Alpha HPV genome map, with early and late polyadenylation sites marked as polyadenylation late and polyadenylation early,

Papillomavirus genome

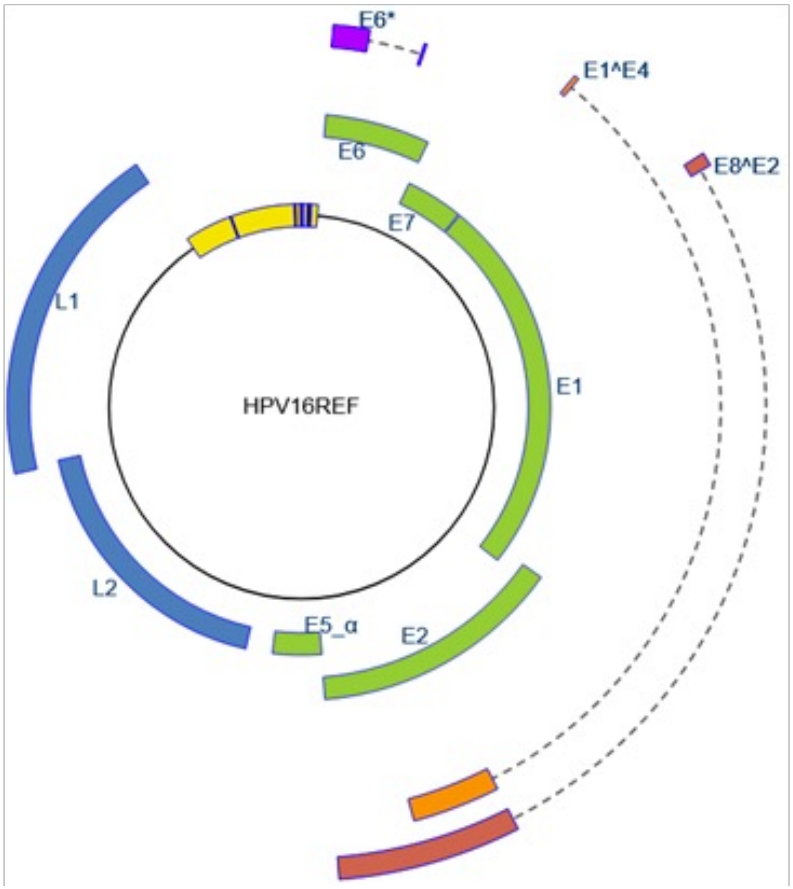


The human papillomavirus type 16 (HPV-16) genome is nearly 8 kb in size and exists as a circular episome in the nucleus of the infected cell. The viral genome encodes the so-called early (E) genes, designated ***E1***–***E7***, and the late (L) genes ***L1*** and ***L2***. The non-protein-coding sequence between the ***L1*** stop codon and the ***E6*** AUG is termed the long control region (LCR) and contains the origin of DNA replication (***ori***) and the early viral promoter, p97

Clinical Science (2006)

E1	DNA helicase activity, DNA-dependent ATP-binding, ATPase activity. Role in replication and replication repression.
E2	Regulator of viral transcription and replication, control of early region viral gene expression, necessary for efficient viral DNA replication together with E1.
E4	Expressed as a late gene primarily in differentiating epithelium, role in productive infection, associated with the keratin cytoskeleton of cultured epithelial cells, role in viral egress.
E5	Transforming activity in HPV-16 *in vitro* (EGFR binding). Presumably stimulates benign cell proliferation *in vivo* but might have a role in the initiation of carcinogenesis
E6	Role in transformation process together with E7. Transcriptional activation properties. E6 of high-risk HPVs inactivates p53 by inducing its degradation. Together with E7 provides a cellular environment for viral DNA replication
E7	Transactivating properties, induces DNA synthesis in quiescent cells, role in rodent cell transformation in co-operation with an activated ras oncogene. E7 binds to the hypophosphorylated form of the retinoblastoma protein (pRB) resulting in its functional inactivation permitting cell progression to S phase of the cell cycle. E7 proteins from the low-risk HPV types 6 and 11 bind less efficiently than the E7 proteins from high-risk HPVs (types 16 and 18).
L1	Major capsid protein.
L2	Minor capsid protein.

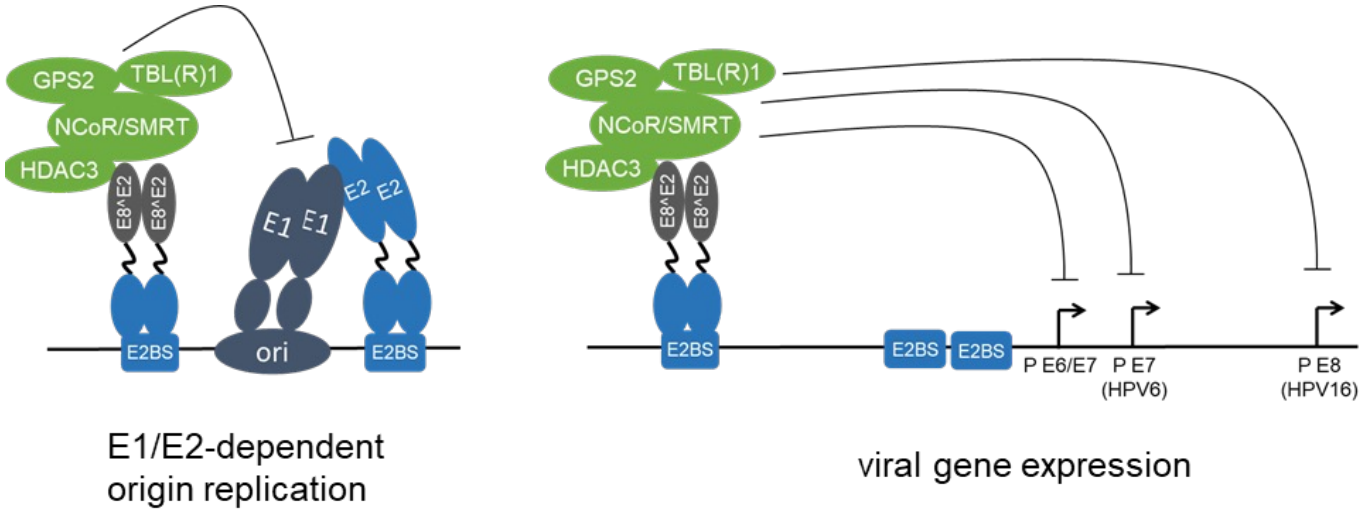
HPV16 genome



E8^E2 protein plays a role in limiting the replication of viral DNA in keratinocytes. Recruits the host NCoR/SMRT complex to viral replication foci to mediate repression of both viral replication and transcription.

The HPV E8^E2 mRNA is generated from a separate promoter within the E1 gene with transcriptional start sites located 70–150 nt upstream of the E8 ATG start codon. Transcript analyses of HPV16 suggest that the main function of this promoter is to drive E8^E2 expression.

HPV E8^E2 proteins repress viral replication and transcription.



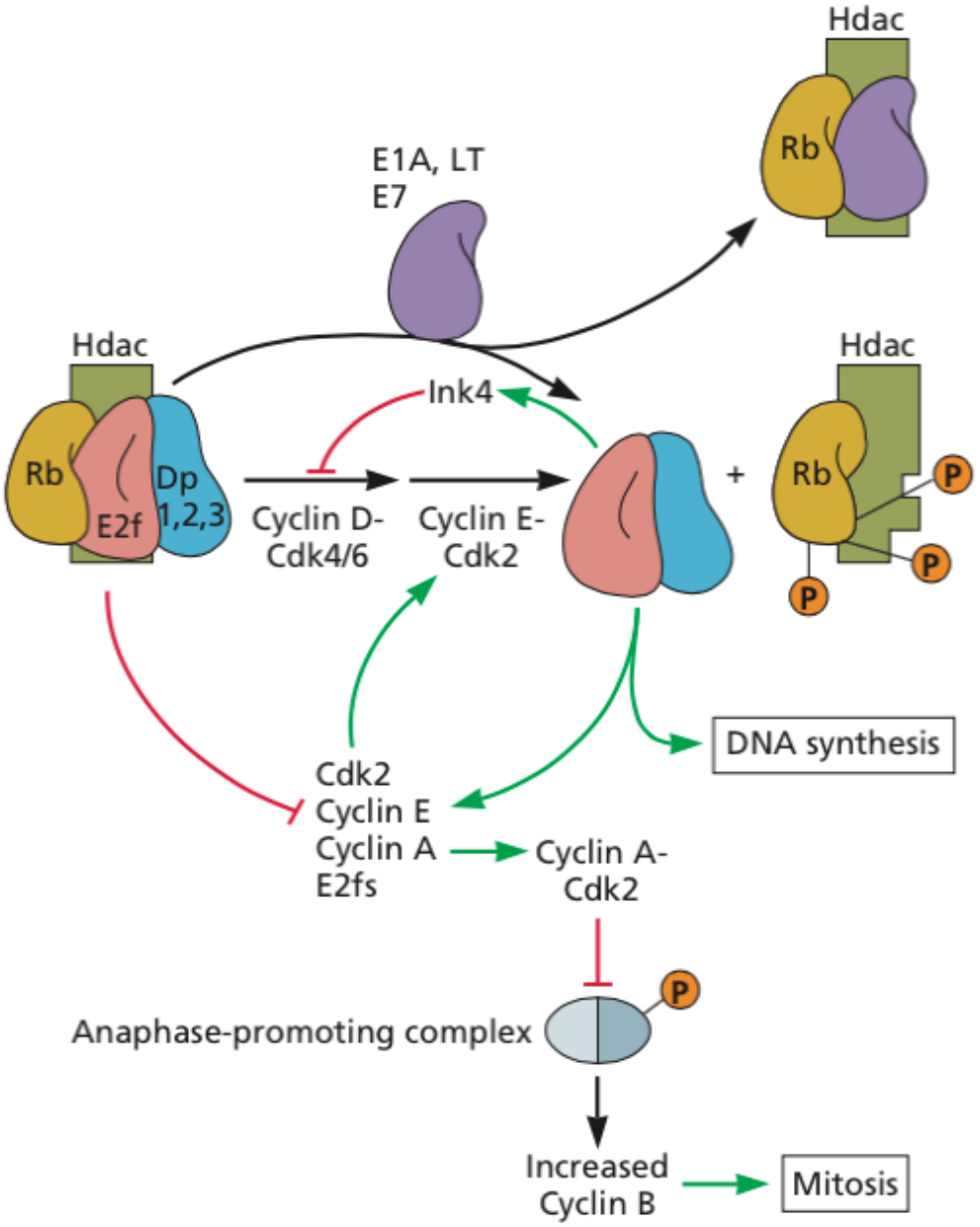
E1/E2-dependent origin replication

viral gene expression

HPV E8^E2 proteins bind to E2BS via the DNA binding domain within the C-terminal part shared by full-length E2 and E8^E2. The E8 domain recruits NCoR/SMRT corepressor complexes composed of GPS2, HDAC3, NCoR, SMRT, TBL1, and TBLR1, and this inhibits both E1/E2-dependent replication of the viral origin and the transcription from different viral promoters.

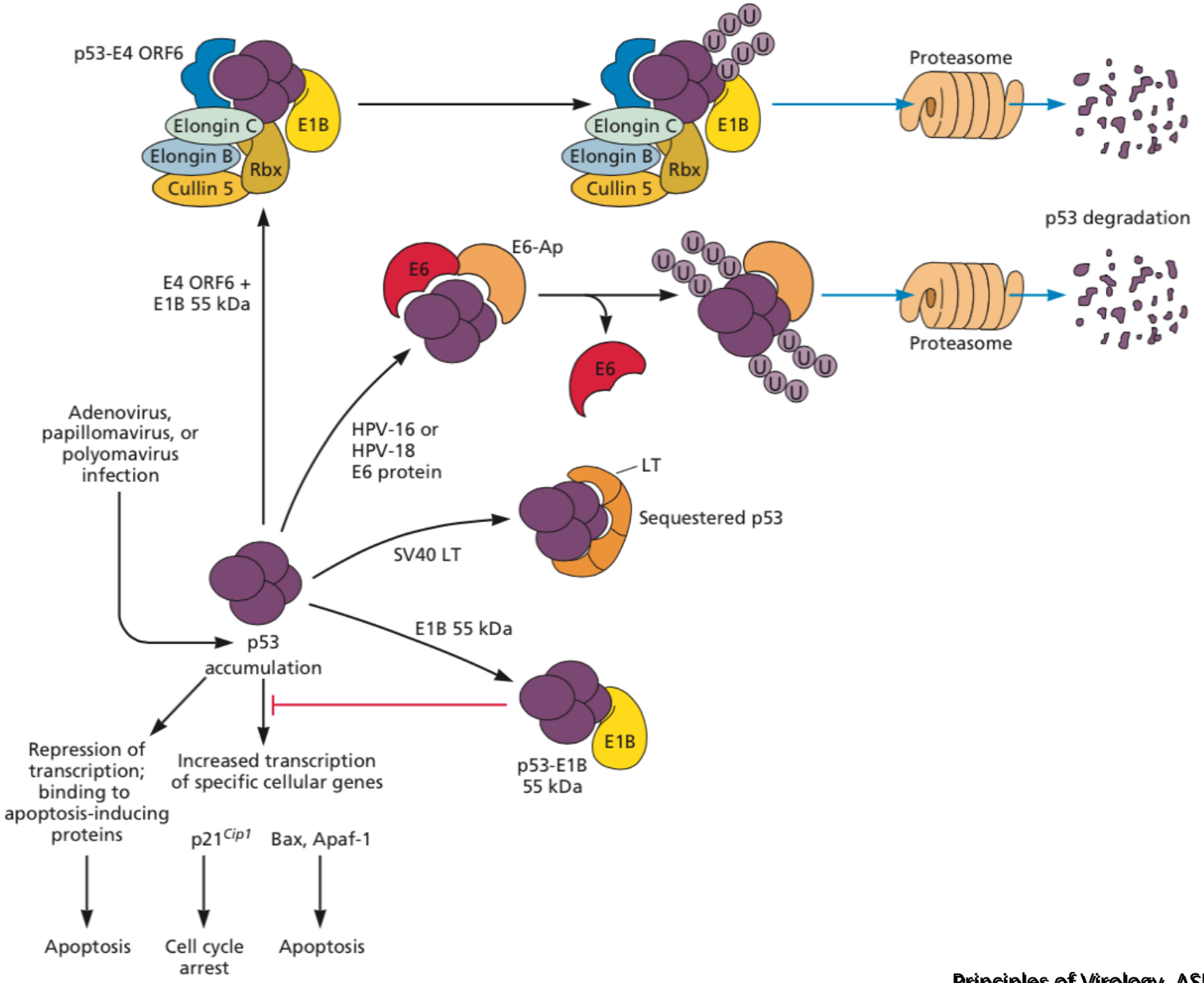
Viruses 2022, 14, 953. https://doi.org/10.3390/v14050953

Interference with Rb protein function



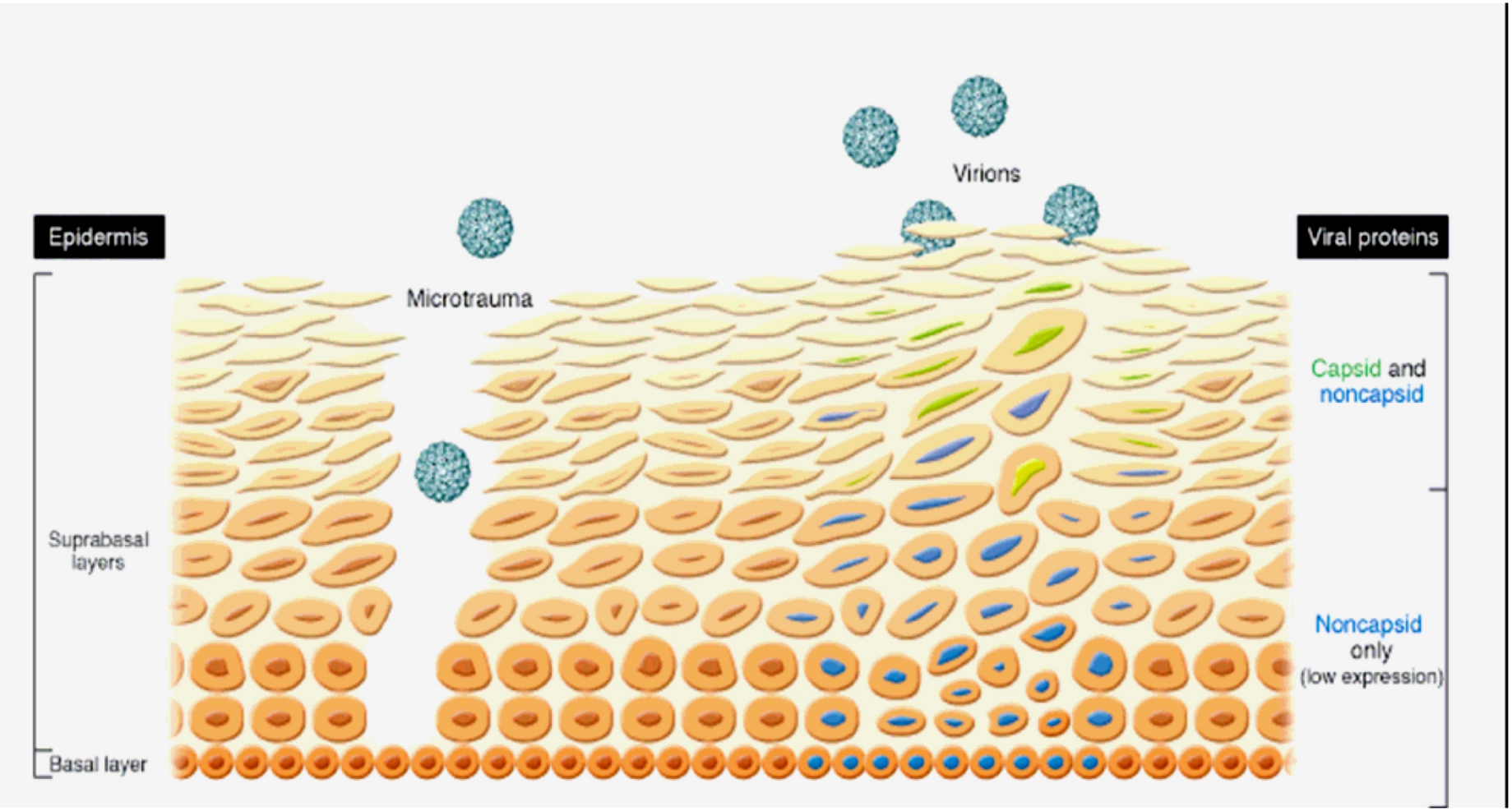
Principles of Virology, ASM Press

Interference with p53 protein function

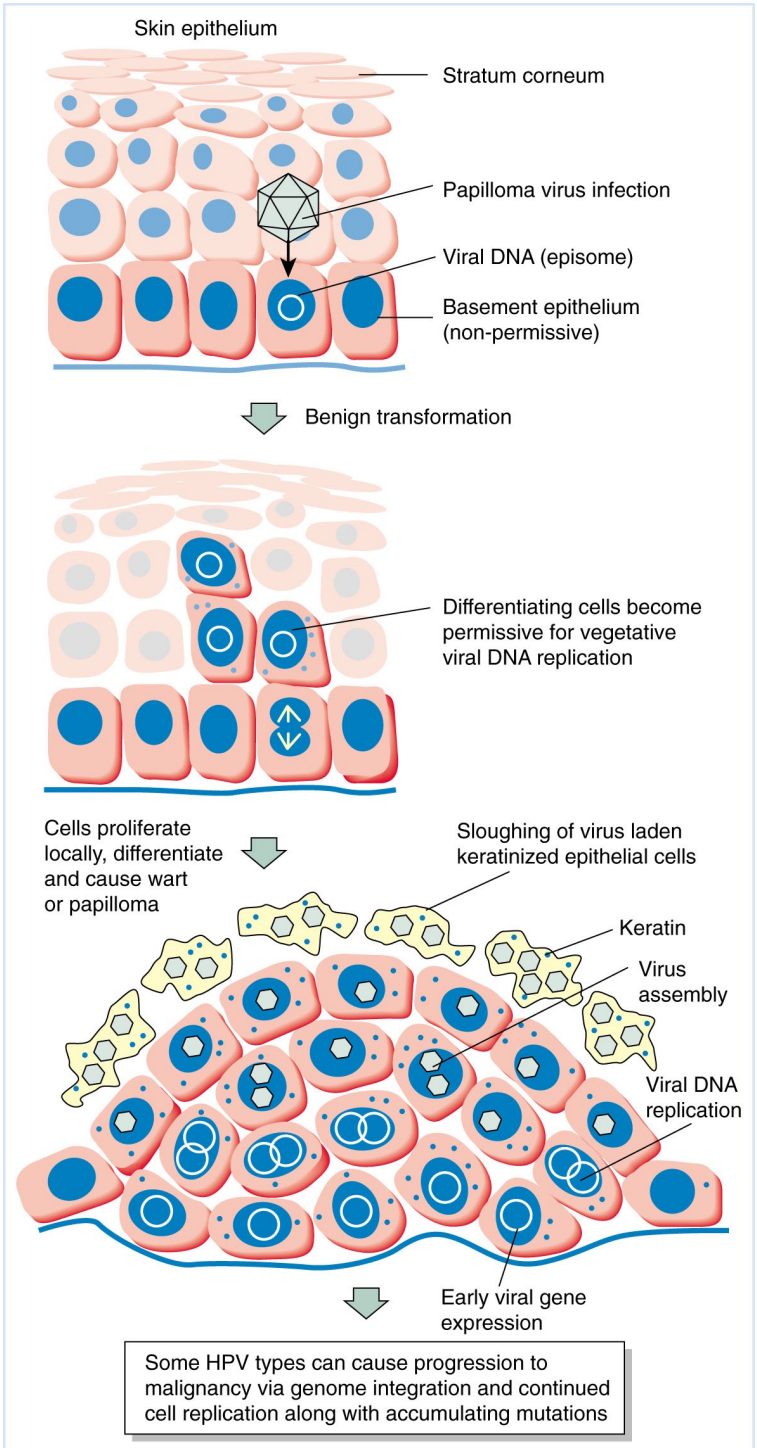


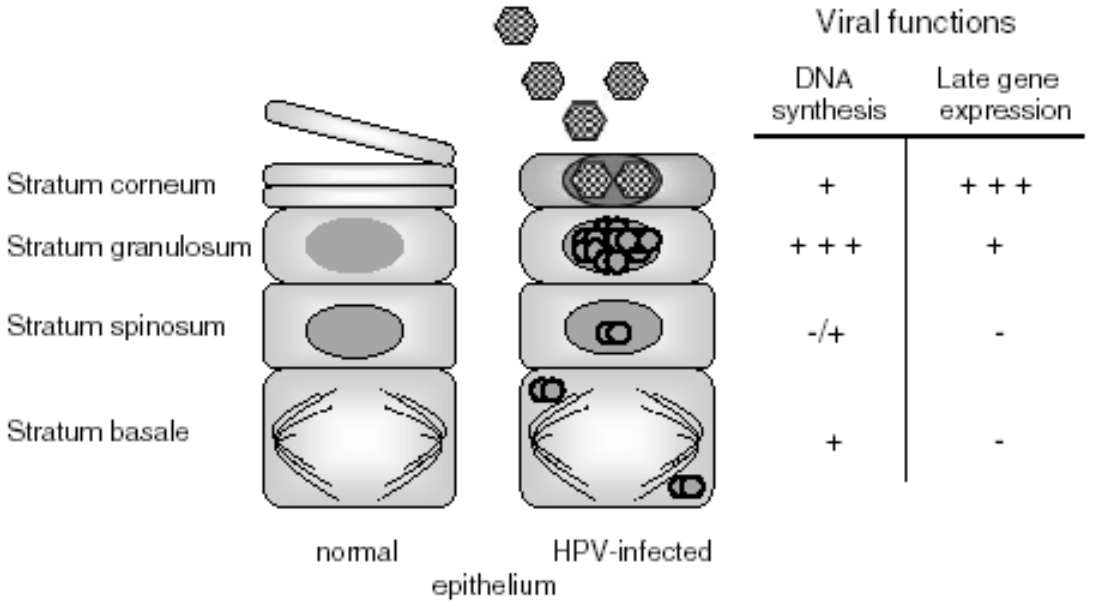
Principles of Virology, ASM Press

HPV life cycle



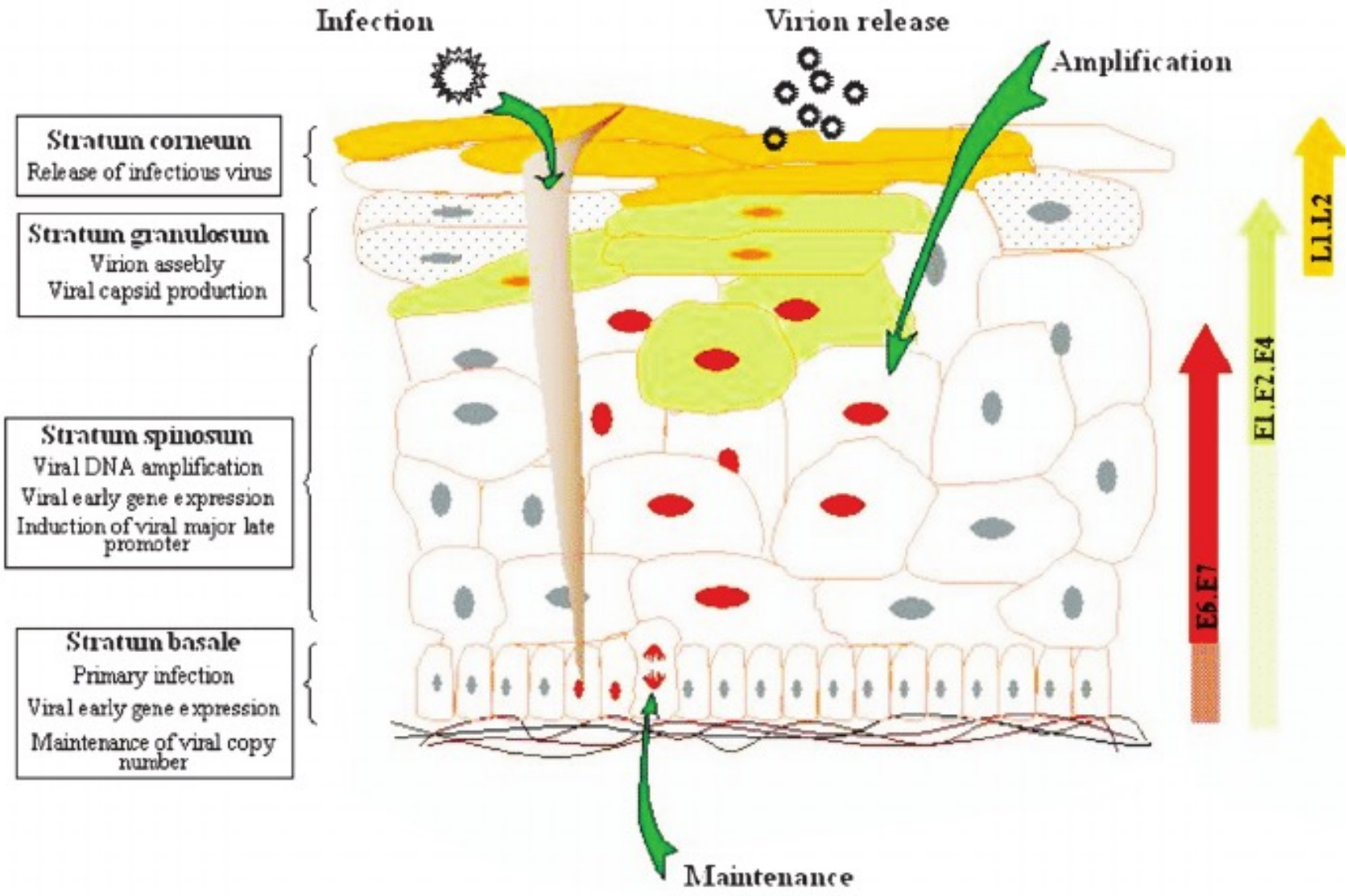
HPV life cycle



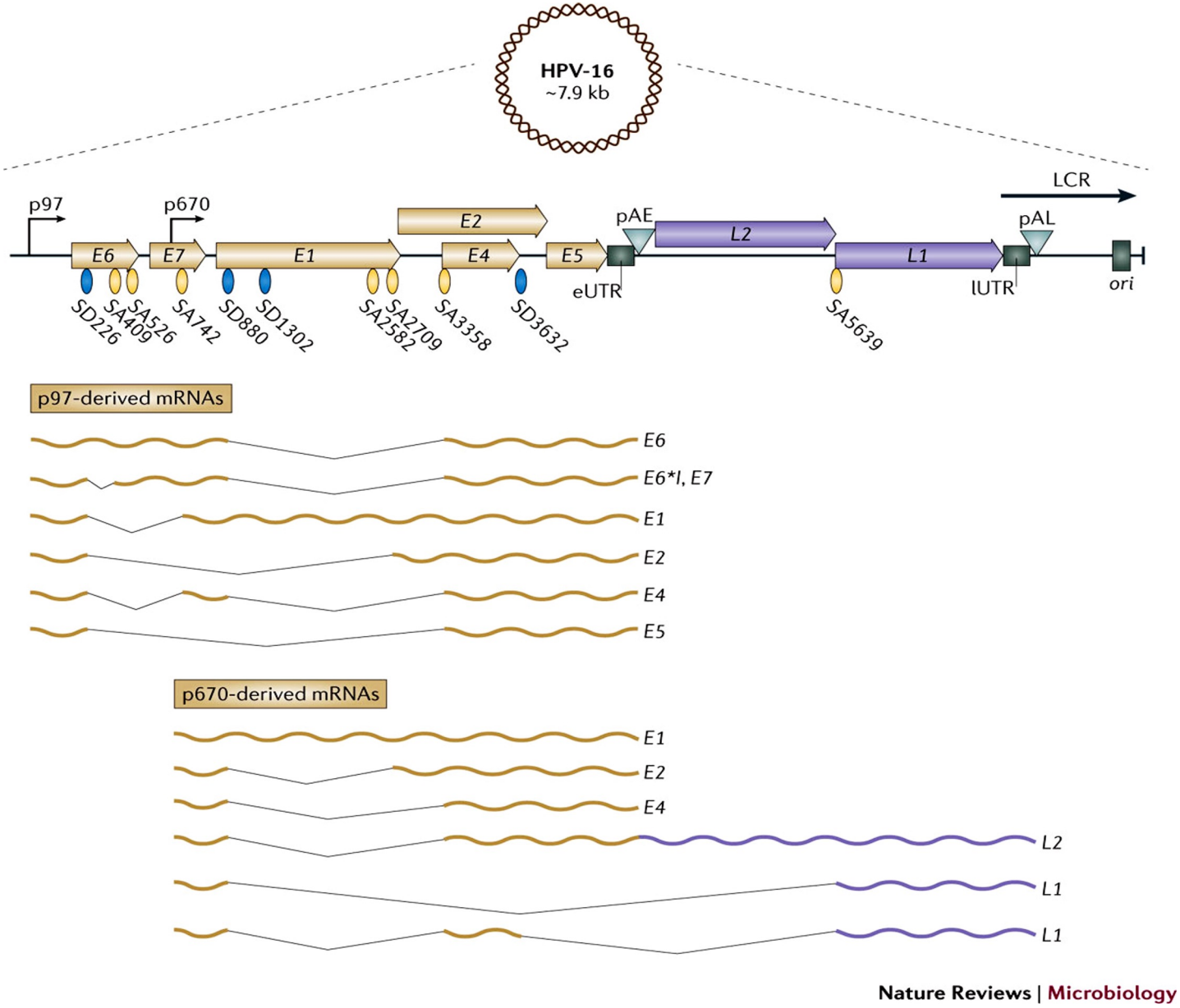


From Wagner and Hewlett *Basic virology* (2003) Blackwell Science Press

HPV life cycle

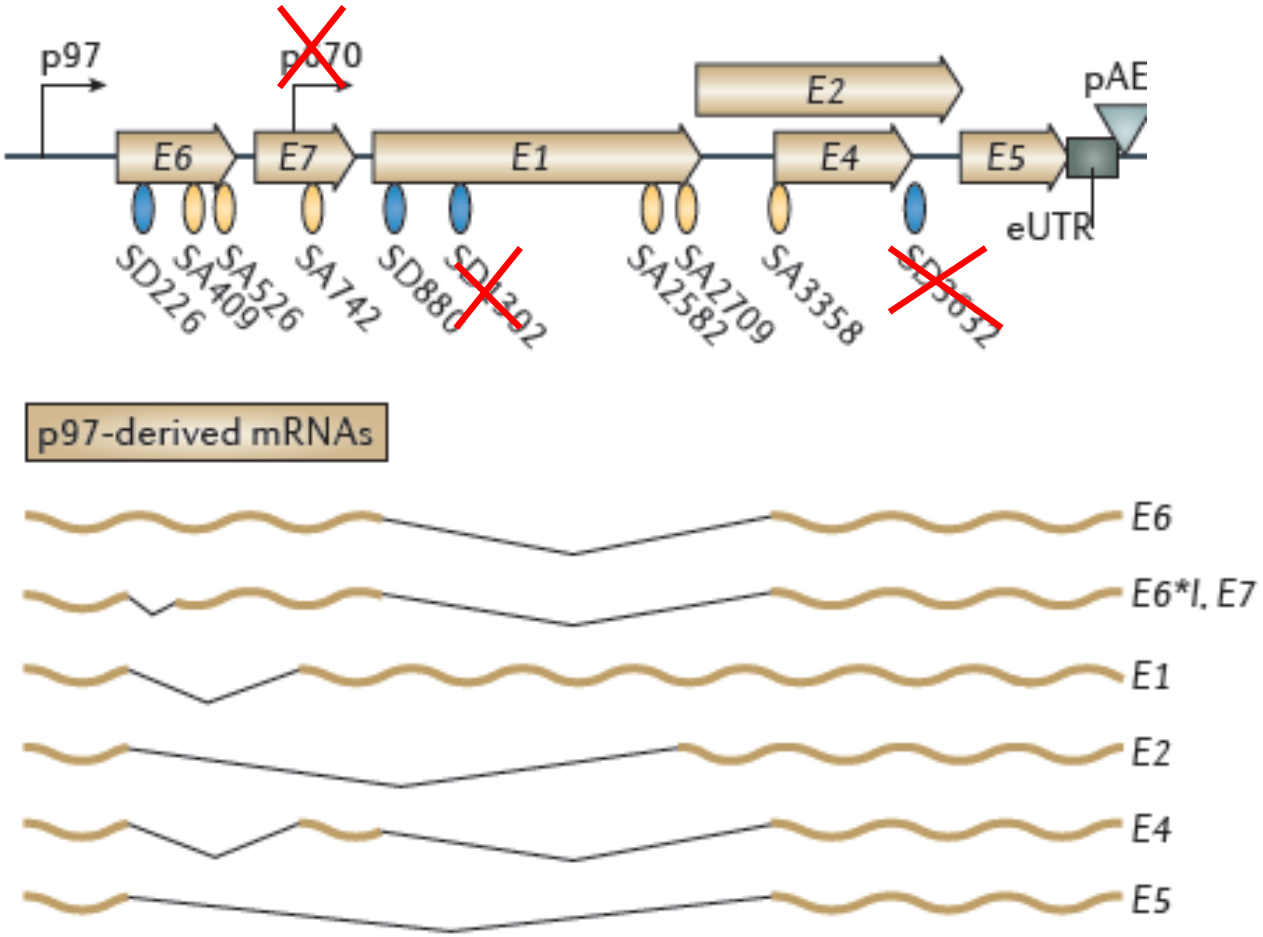


Papillomavirus genome



Nature Reviews | Microbiology

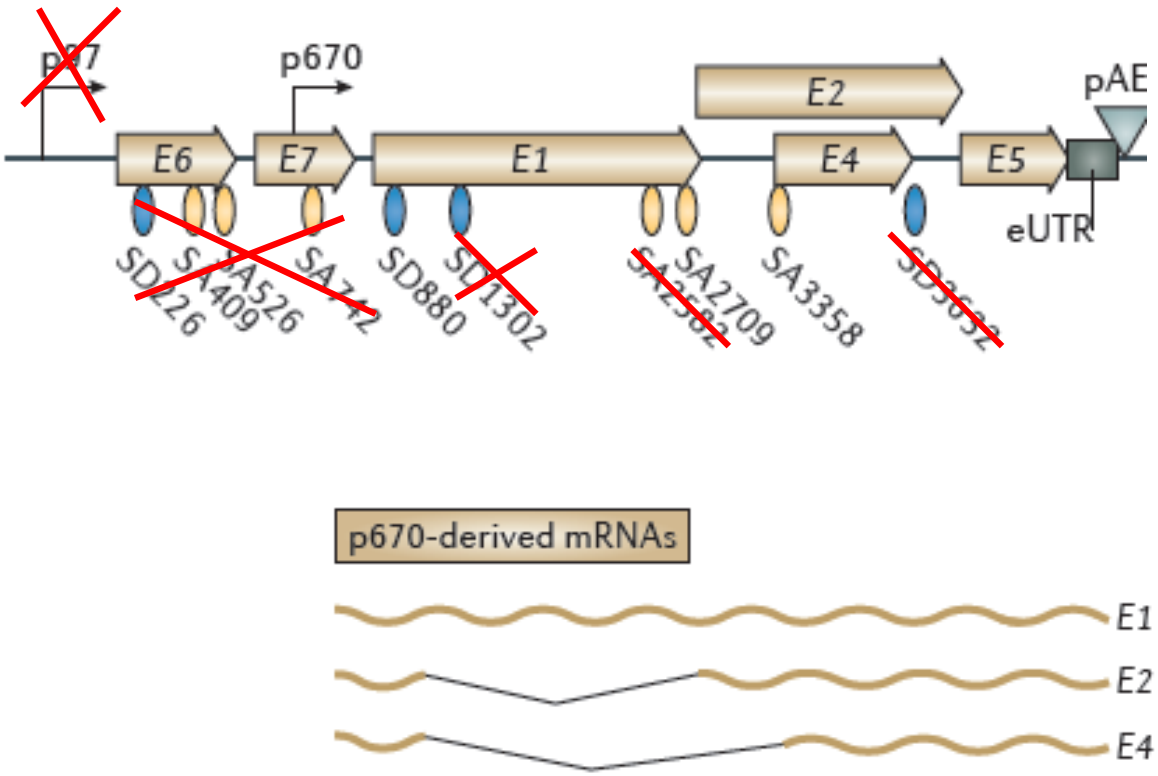
Papillomavirus very early gene expression



In the early stages of the viral life cycle, transcription from the early promoter p97 generates mRNAs encoding all of the early genes, which are polyadenylated at the early polyadenylation signal (pAE). These polycistronic mRNAs are subjected to alternative splicing through the differential use of various early splice sites (splice donor 226 (SD226), splice acceptor 409 (SA409), SA526, SA742, SD880, SA2582, SA2709 and SA3358.

NATURE REVIEWS | MICROBIOLOGY

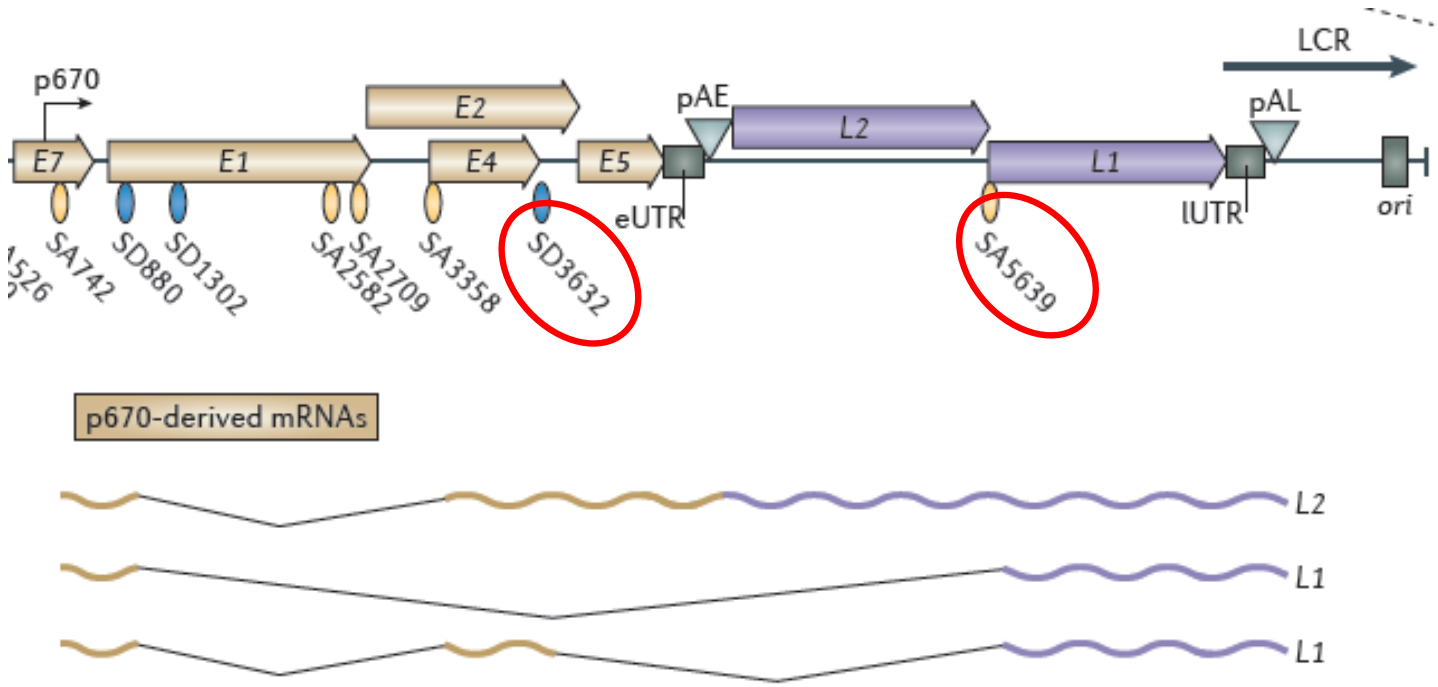
Papillomavirus early-late gene expression



As the infected cells undergo into differentiation program, the late promoter p670 is activated, and expression from this promoter bypasses the *E6* and *E7* genes and induces high expression of *E1*, *E2* and *E4* mRNAs using primarily the early splice sites SD880, SA2709 and SA3358. These mRNAs are also polyadenylated at pAE.

NATURE REVIEWS | MICROBIOLOGY

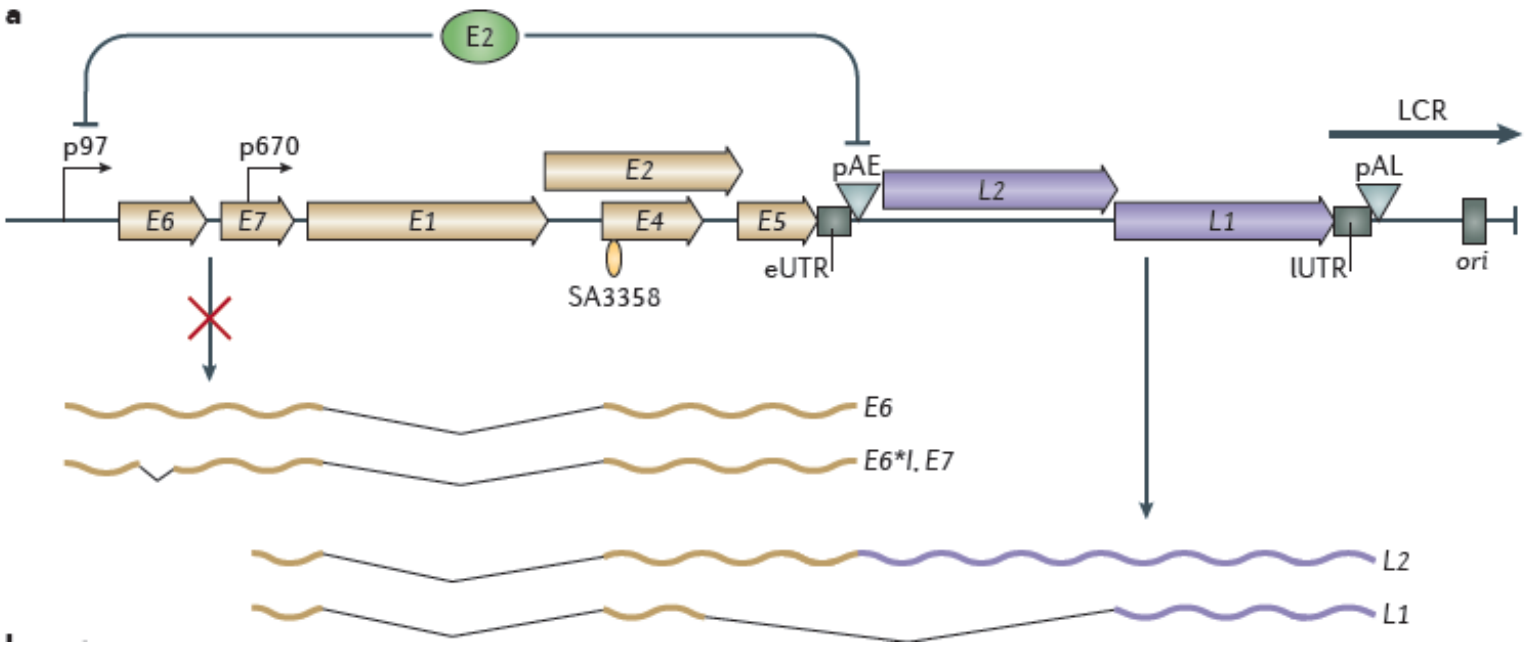
Papillomavirus late gene expression



The E1 and E2 proteins bind to the origin of replication (*ori*), which is located in the LCR, to replicate the viral DNA genome.
Terminal differentiation of the host cell downregulates the activity of pAE, resulting in readthrough into the true late region of the genome (encoding L1 and L2) followed by polyadenylation at the late polyadenylation signal (pAL) to generate *L2* mRNAs.
Derepression of the exclusively late splice sites SD3632 and SA5639 generates *L1* mRNAs in addition to the *L2* mRNA.

NATURE REVIEWS | MICROBIOLOGY

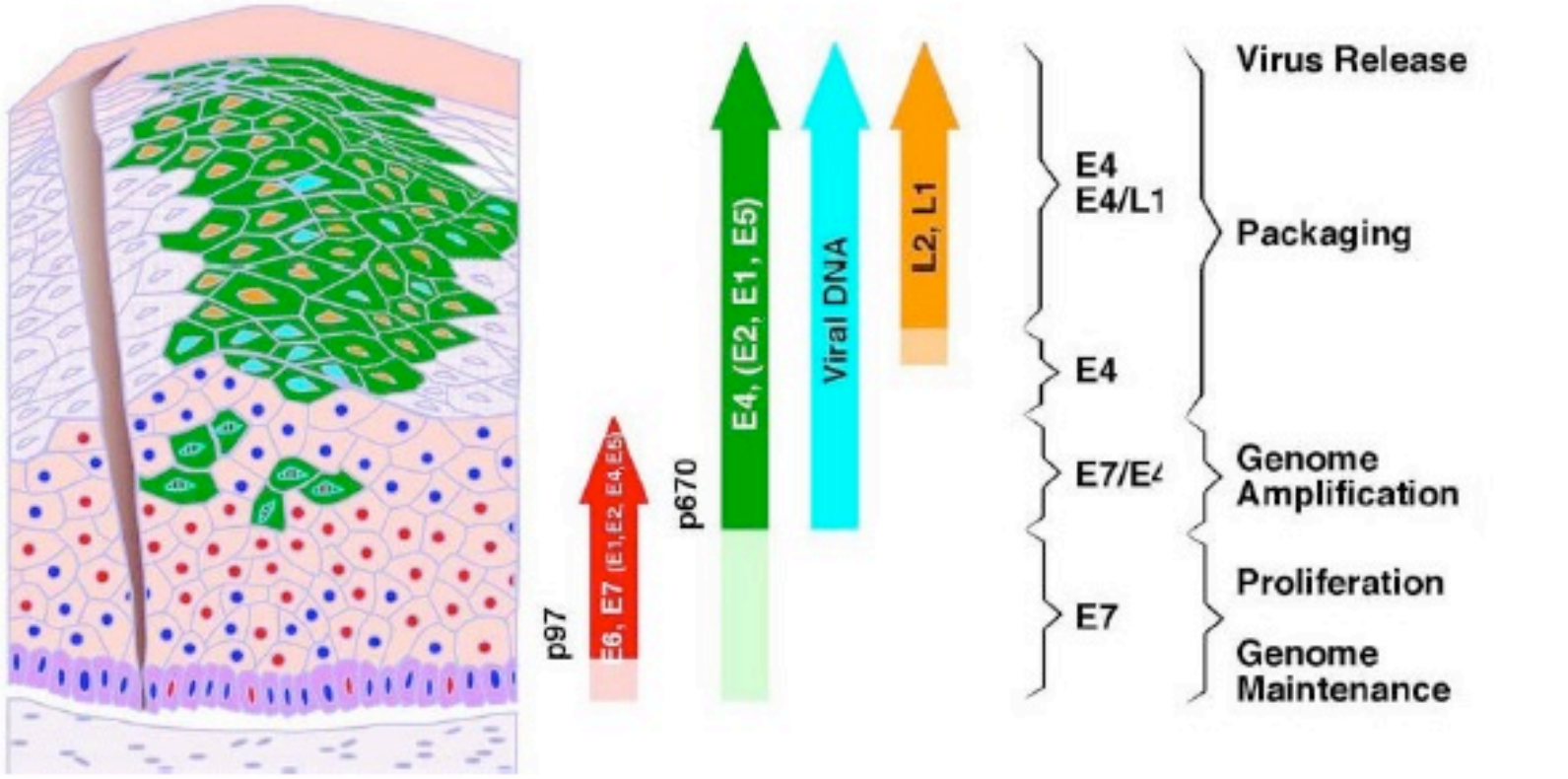
HPV 16 E2 protein inhibits the early polyadenylation signal to induce late gene expression



HPV-16 produces two early (E) gene *E2* mRNAs, one produced from the early promoter, p97, and one produced from the late promoter, p670. Activation of the differentiation-dependent promoter, p670, drives the expression of high levels of *E2* mRNAs. An increase in the level of the E2 protein inhibits p97 and thereby shuts down E6 and E7 expression to pave the way for cell differentiation and entry into the late stage of the viral life cycle. High levels of E2 also inhibit use of the early polyadenylation signal (pAE), allowing readthrough into the late region of the HPV-16 genome, followed by production of the late (L) mRNAs encoding L1 and L2.

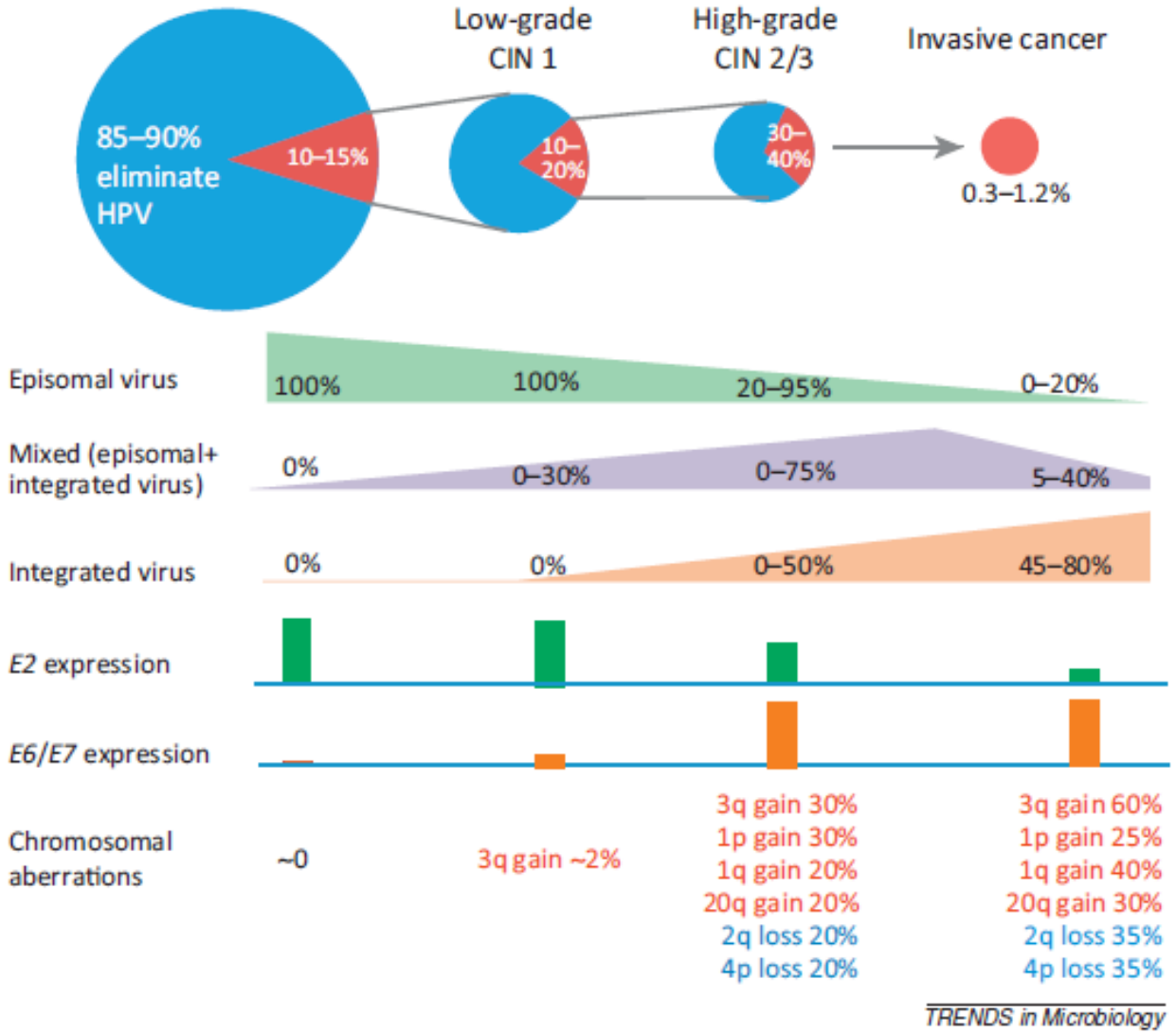
NATURE REVIEWS | MICROBIOLOGY

HPV life cycle

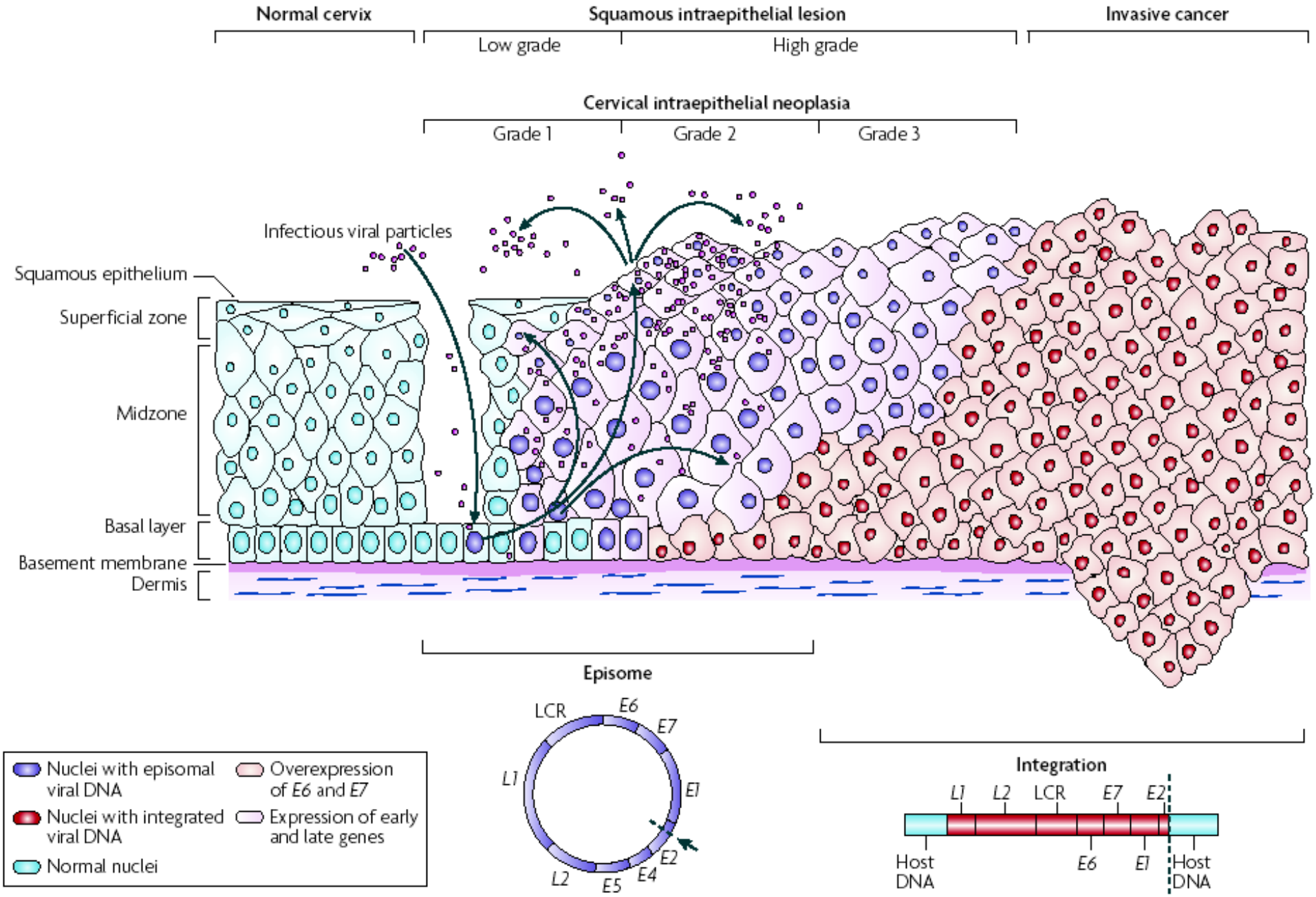


Front Biosci, 2006

Progression of human papillomavirus (HPV) cervical infection to cancer



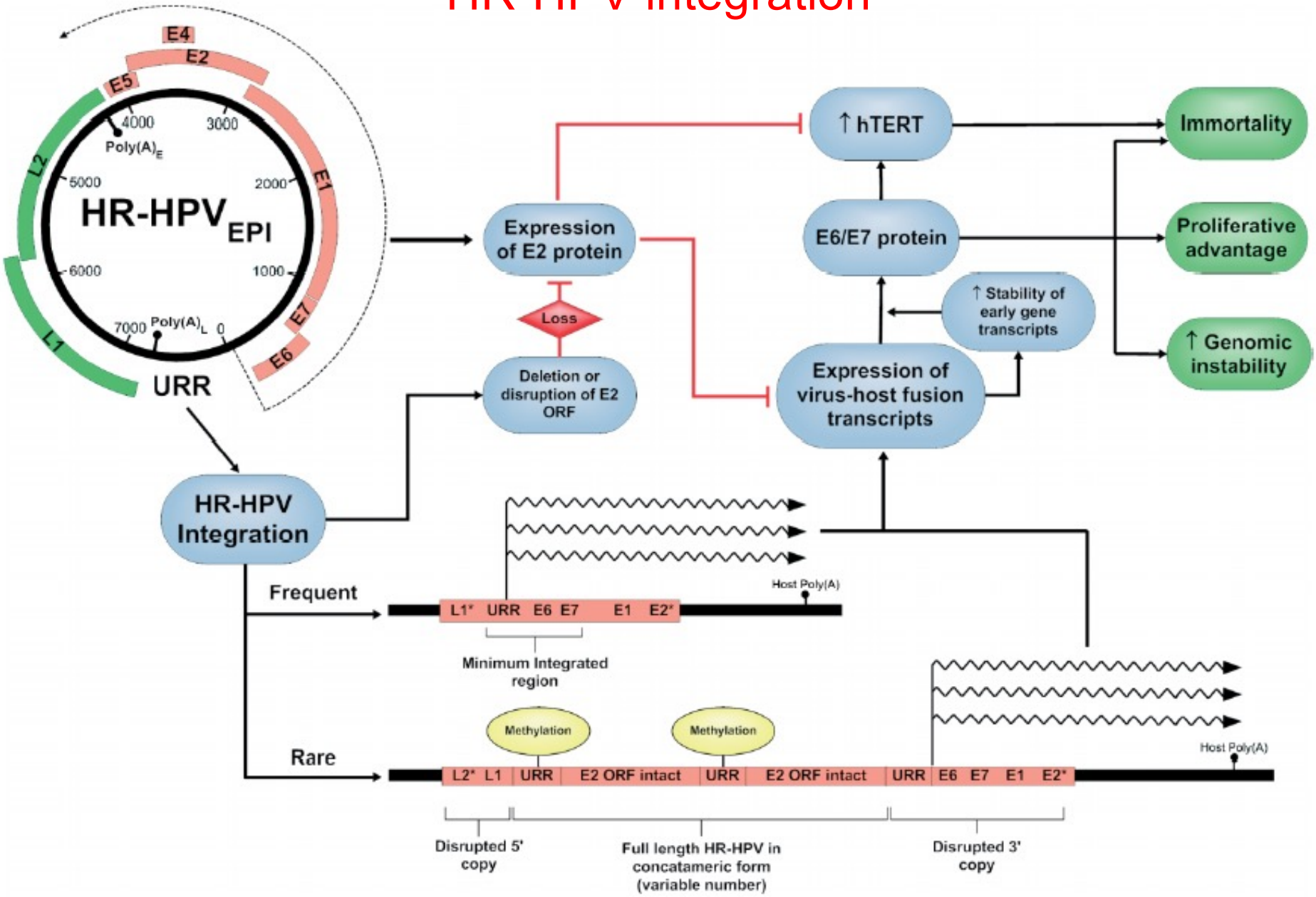
Trends in Microbiology June 2014



CIN: Cervical intraepithelial neoplasia

NATURE REVIEWS | CANCER

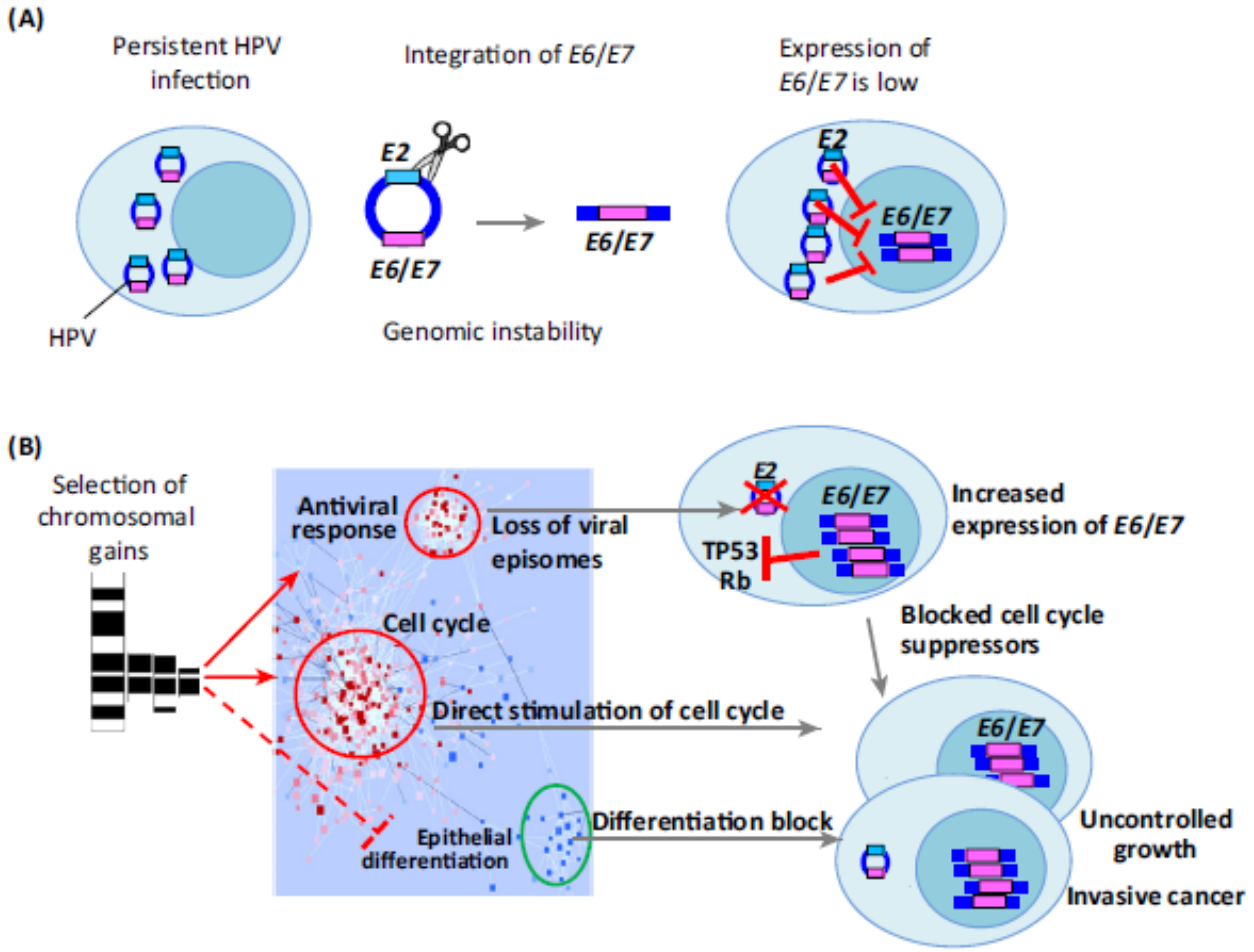
HR-HPV integration



J Pathol 2007; **212**: 356–367

Significance of HR-HPV integration events detected in cervical carcinomas. The majority of integrants that derive from insertion of HR-HPV episomes (HR-HPVEPI) into the host genome are detected at low copy number and retain at least the E6 and E7 oncogenes together with the viral upstream regulatory region (URR). Integrant copy number is often increased through amplification of viral and flanking host DNA. Typical integrants also have complete or partial disruption of the open reading frame (ORF) for E2, the viral gene that regulates viral replication and which, by binding sites in the URR, can inhibit expression from integrated virus. Disruption of the viral genome also dissociates viral early (E) gene transcription from the viral early polyadenylation signal, leading to use of host poly(A) signals and transcription of virus–host fusion transcripts with a longer half life. These events lead to increased levels of E6 and E7 proteins, which, together with loss of additional inhibitory effects of E2, result in cellular immortalization, deregulated proliferation, and increased genomic instability.
More rarely, concatameric integrants are observed, where viral copies (including intact E2) are arranged in a head-to-tail fashion with partially deleted copies at the 5 and 3 ends.
The dashed line in the figure represents transcription from the early promoter of HR-HPVEPI (P97 in HPV16)

A model of cervical carcinogenesis



(A) Persistent high-risk human papillomavirus (HPV) infection may result in the integration of virus into the host genome upon which E2 is disrupted. The integration leads to the increased genomic instability, however, the expression of E6/E7 oncogenes is still controlled by episomal E2. (B) Frequent chromosomal aberrations (gains) occur in the regions containing antiviral genes, which will induce the elimination of inhibitory episomal E2 and the release of E6/E7 that will block suppressors of the cell cycle [tumor protein 53 (TP53) and retinoblastoma (Rb)]. The same chromosomal gains contain drivers of the cell cycle that directly induce cell proliferation and miRNAs that may inhibit cell differentiation. All three processes act synergistically allowing the dysplastic cell to become a malignant tumor.

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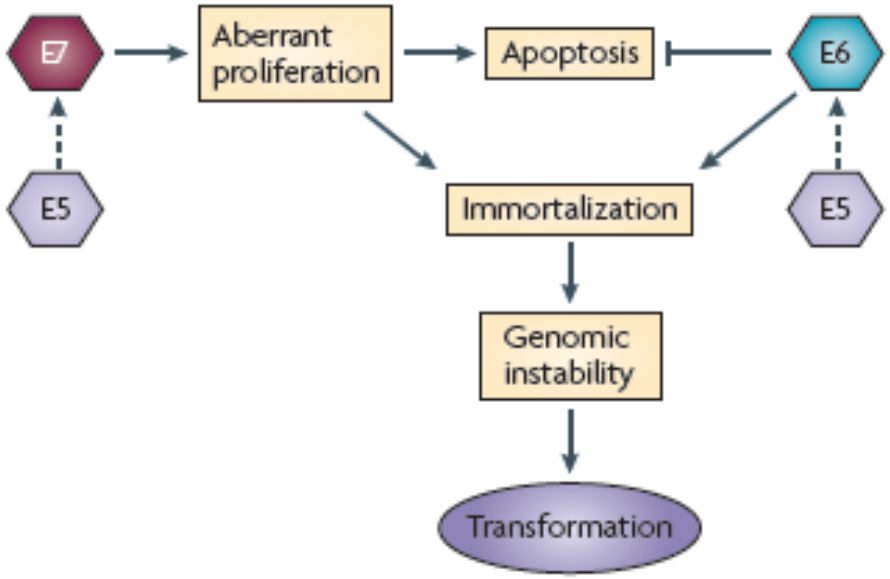
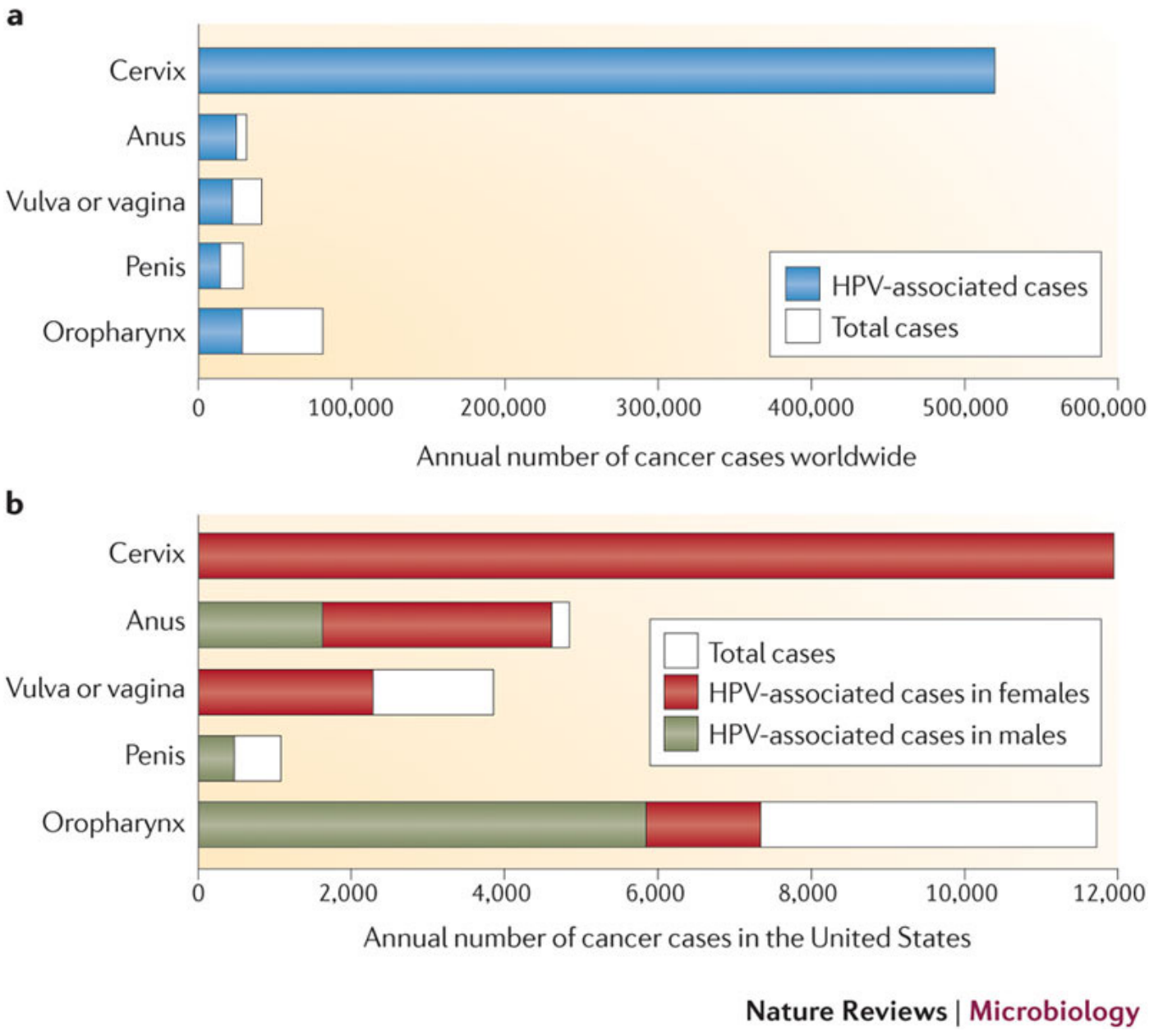
Molecular mechanisms by which the human papillomavirus oncoproteins cooperate to induce cervical carcinogenesis.



The induction of hyperproliferation by the E7 protein triggers apoptosis, which is blocked by the actions of the E6 protein. The cooperative actions of E6 and E7 efficiently immortalize cells and this process is augmented by the actions of the E5 protein. The ability of E6 and E7 to target crucial regulators of proliferation, apoptosis, immortalization and genomic stability collectively promotes the emergence of a clonal population of cells with a growth advantage and an increased propensity for transformation and malignant progression

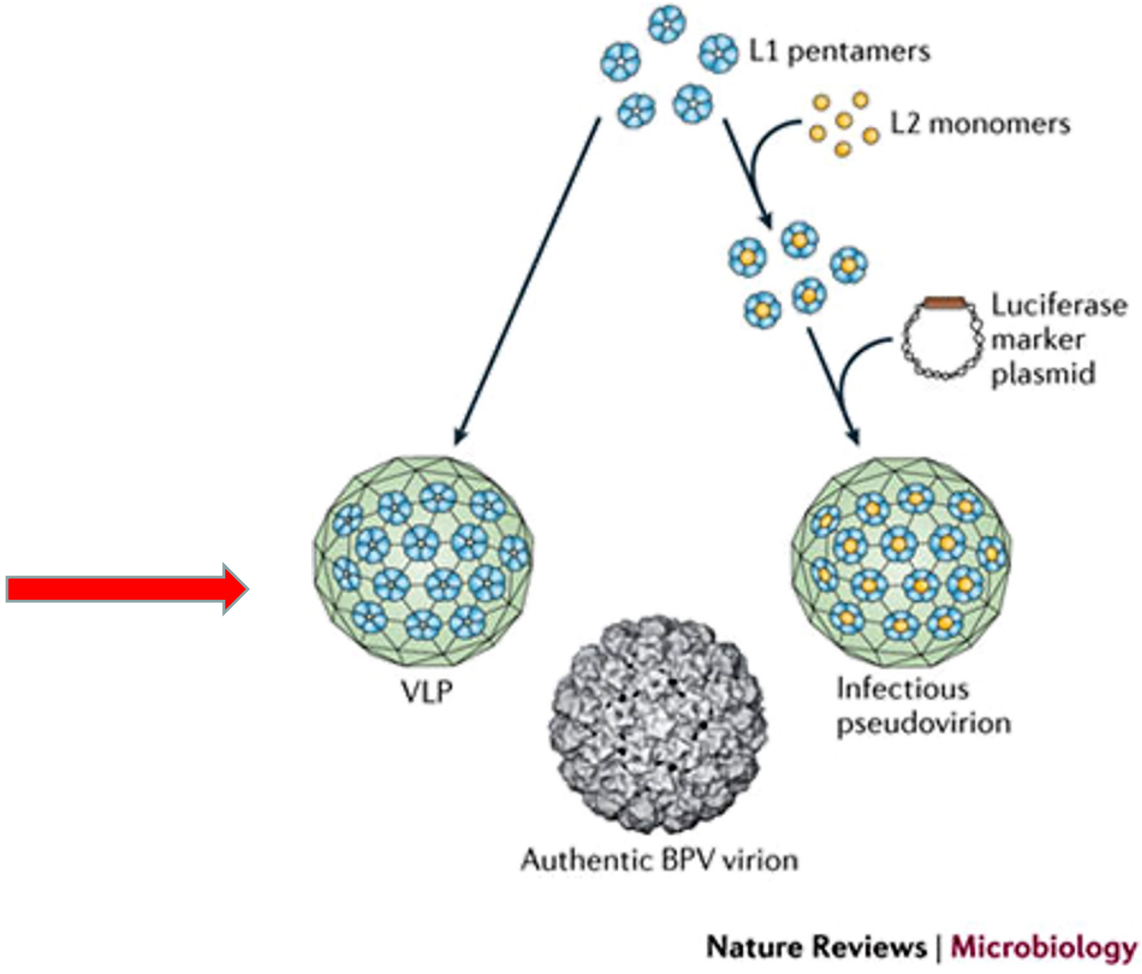
NATURE REVIEWS | CANCER

Prevalence of human papillomavirus-associated cancers



Nature Reviews | Microbiology

Human papillomavirus (HPV) vaccines



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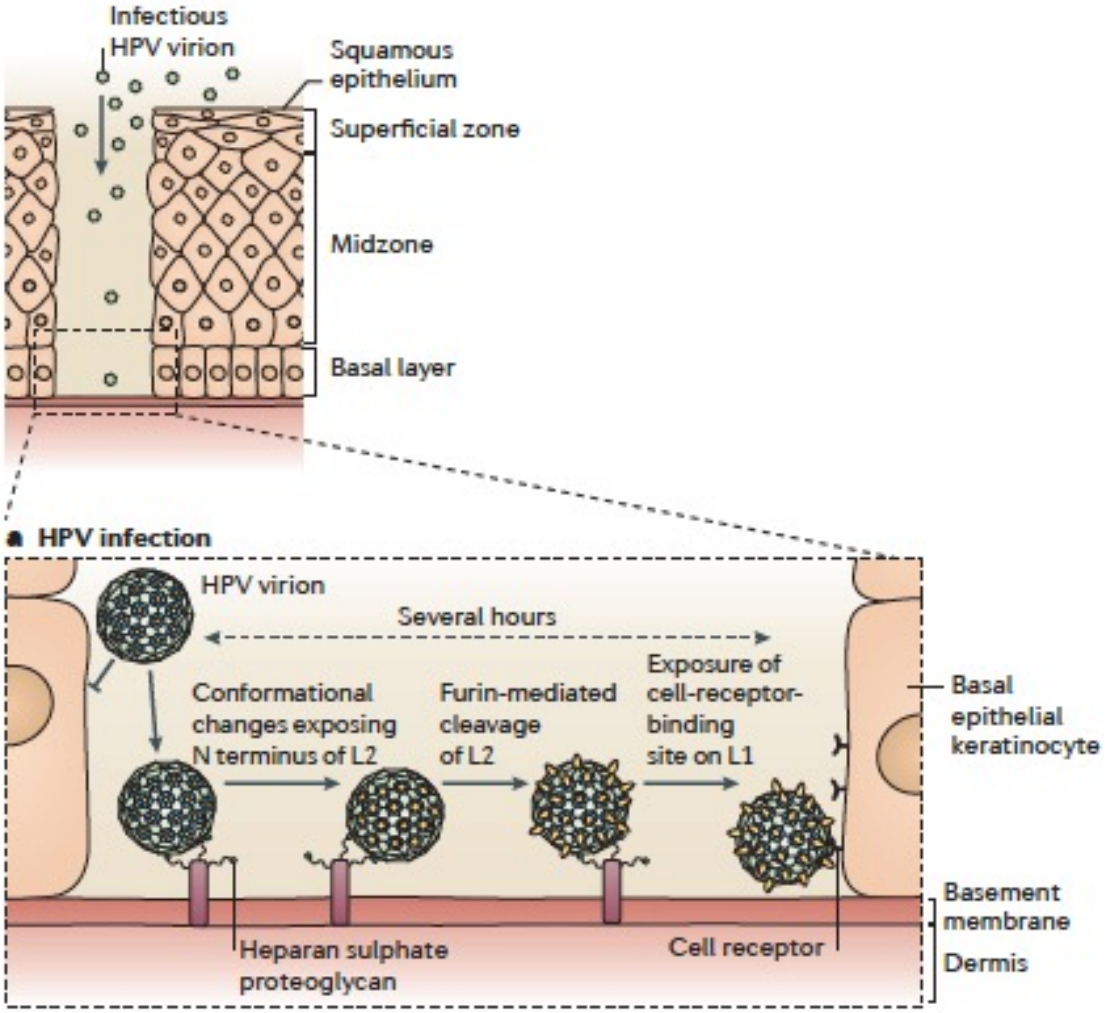
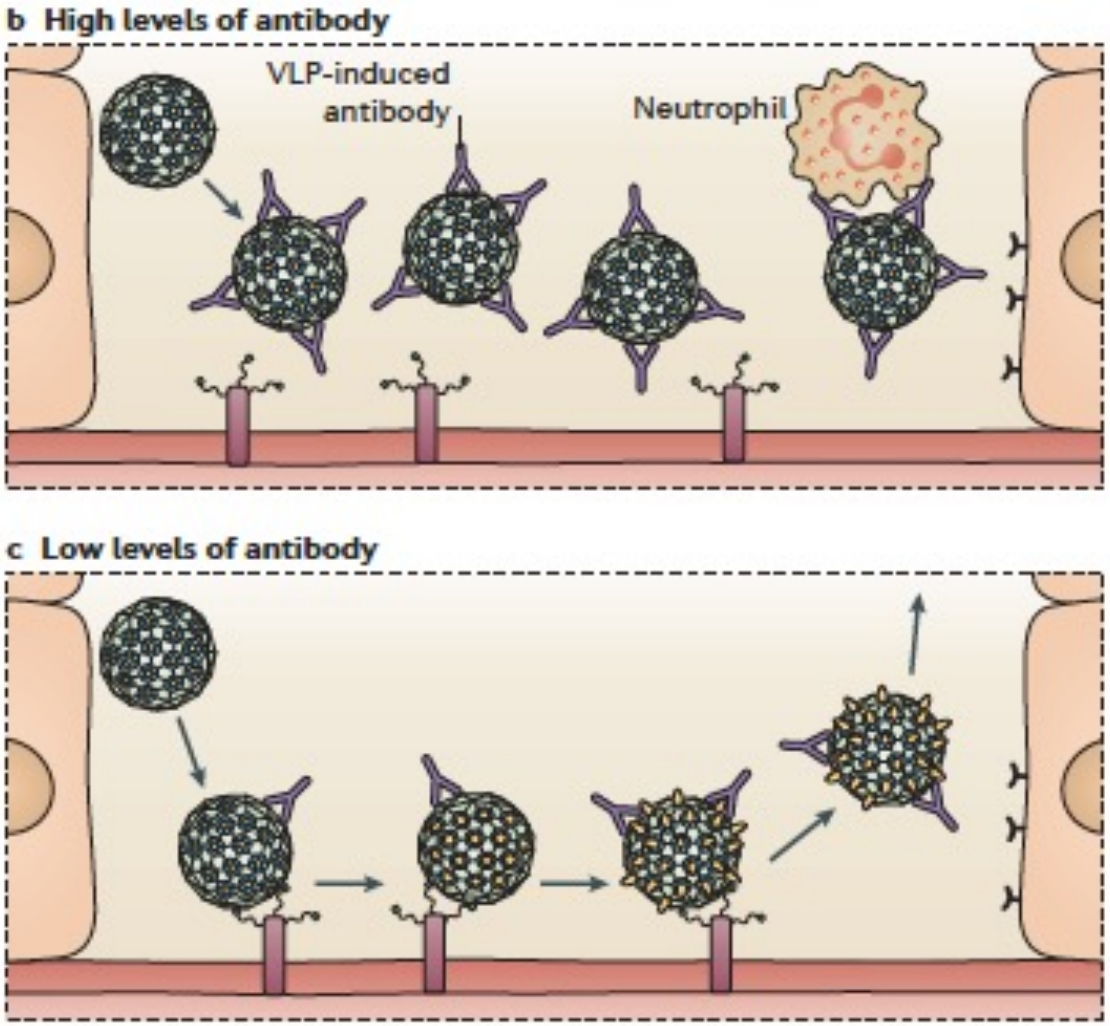


a | Human papillomavirus (HPV) virions cannot bind or infect intact squamous epithelium.
They must first bind the basement membrane via heparan sulphate proteoglycans. Then, in a process that takes several hours, they must undergo a series of conformational changes, beginning with furin-mediated cleavage of the minor capsid protein, L2 (yellow), to expose their receptor-binding site on the major capsid protein, L1 (blue), followed by binding to the cell surface receptor and infection of basal epithelial keratinocytes.

Human papillomavirus (HPV) vaccines

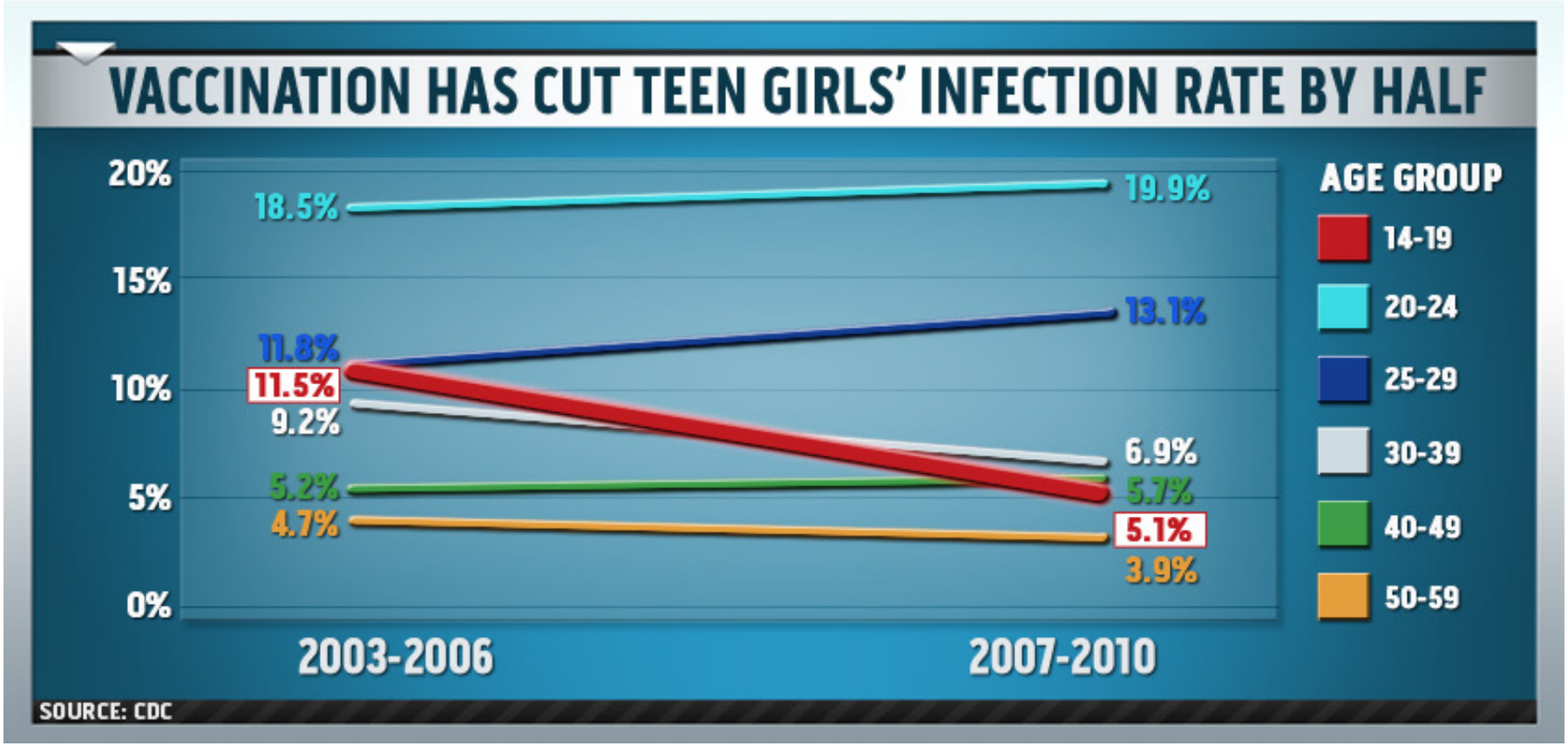


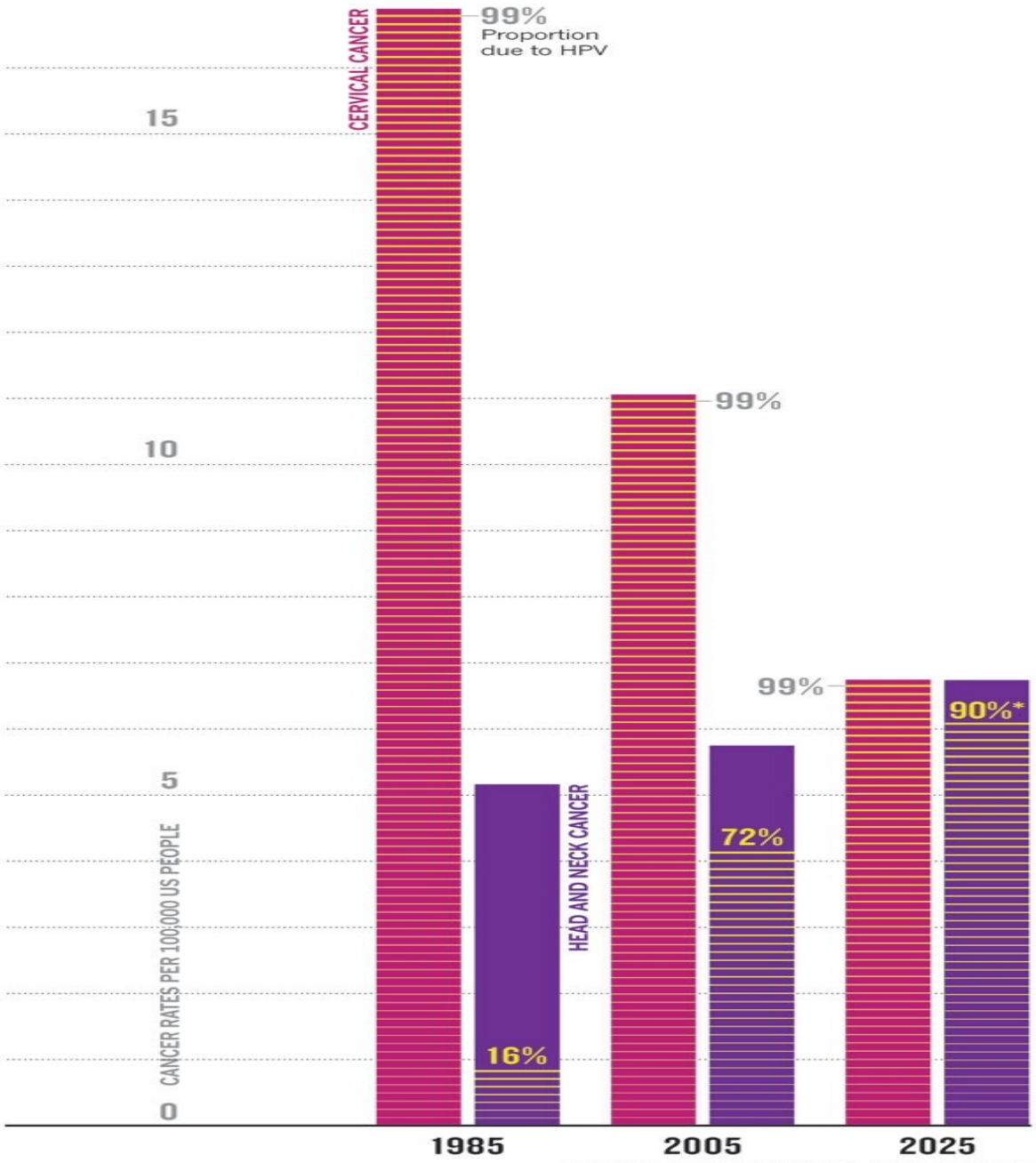
b | High levels of virus-like particle (VLP)-induced antibodies prevent attachment of the virus to the basement membrane, and this in turn prevents the conformational changes required for cell surface binding. Virus–antibody complexes associate with neutrophils in the cervicovaginal mucus.

c | Low levels of VLP-induced antibodies permit basement membrane attachment and the conformational changes leading to furin-mediated L2 cleavage, but they prevent a stable association of the virion with the cell surface.

Gardasil HPV types 6, 11, 16, and 18

Cervix HPV types 16, and 18





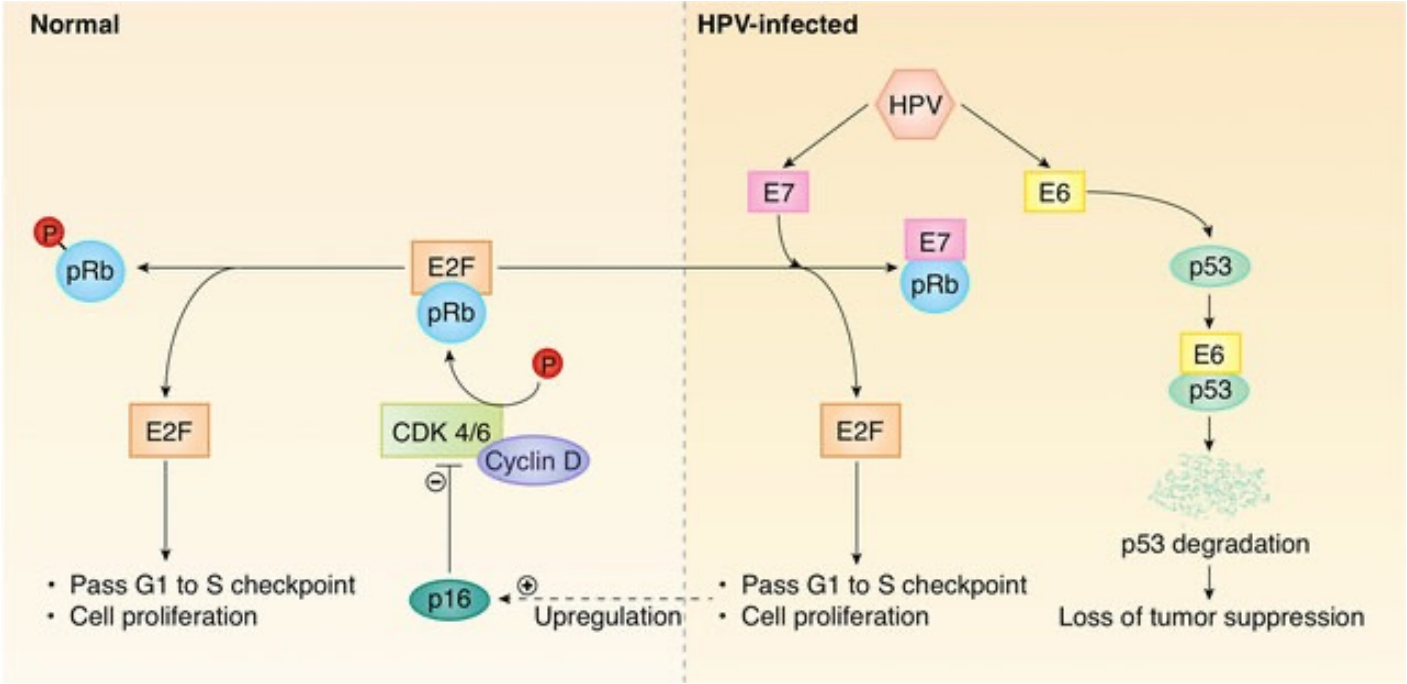
EMERGING THREAT

Rates of head and neck cancer (purple) have risen — and they are set to grow further. An increasing proportion of cases is caused by human papillomavirus (HPV, yellow). At the same time, rates of cervical cancer (red; nearly all caused by HPV) have declined, owing to increased screening.

*Estimate based on clinical observations

	HPV-positive HNSCC	HPV negative SCC
Epidemiology		
Incidence	Increasing	Decreasing
Demographic background		
Age	Younger	Older
Socioeconomic status	Higher	Lower
Risk factors	High-risk sexual practices, marijuana exposure	Tobacco and alcohol exposure
Molecular etiology		
P53 pathway	E6-mediated degradation of cellular p53	TP53 genetic mutation
RB pathway	E7-mediated degradation of Rb	17p LOH, hypermethylation of p16INK4A promoter
P16 expression	Overexpression	Decreased expression
Clinical characteristics		
Location of primary tumor	Oropharynx (palatal and lingual tonsils)	All head and neck sites
Survival	Better	Worse
Response to chemoradiation	Better	Worse
Tumor recurrence	Lower risk	Higher risk

Human papillomavirus (HPV)+ cancer increases expression of p16



Left panel: Normal, uninfected cell. Cyclin D-cyclin dependent kinase (CDK) 4/6 complex initiates phosphorylation of the tumor suppressor protein, pRb. The hyperphosphorylation of pRb leads to release of the transcription factor E2F into its active state, which drives the expression of downstream gene products allowing the cell to transition from the G1 to S phase. As a cyclin kinase inhibitor, p16 is a tumor suppressor and negative regulator of the cyclin D-CDK 4/6 complex. Right panel: HPV infected cell. When the transcription factor E2F is bound to pRb, it remains inactive. The overexpression of the E7 oncoprotein by high-risk HPV subtypes disrupts the E2F-pRb complex by displacing E2F and binding to pRb. The subsequent release of E2F into its active state drives the expression of downstream gene products, allowing the cell to transition from the G1 to S phase. In a regulatory feedback attempt to inhibit further cell proliferation, p16 is upregulated, and thus can be a surrogate for HPV+ tumors. The overexpression E6 oncoprotein acts via a separate mechanism. E6 binds to the tumor suppressor protein, p53, and ultimately leads to degradation of p53. Loss of the regulatory function of p53 causes aberrant propagation of the cell cycle and prevents apoptosis.

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