The Mystery of E7 protein

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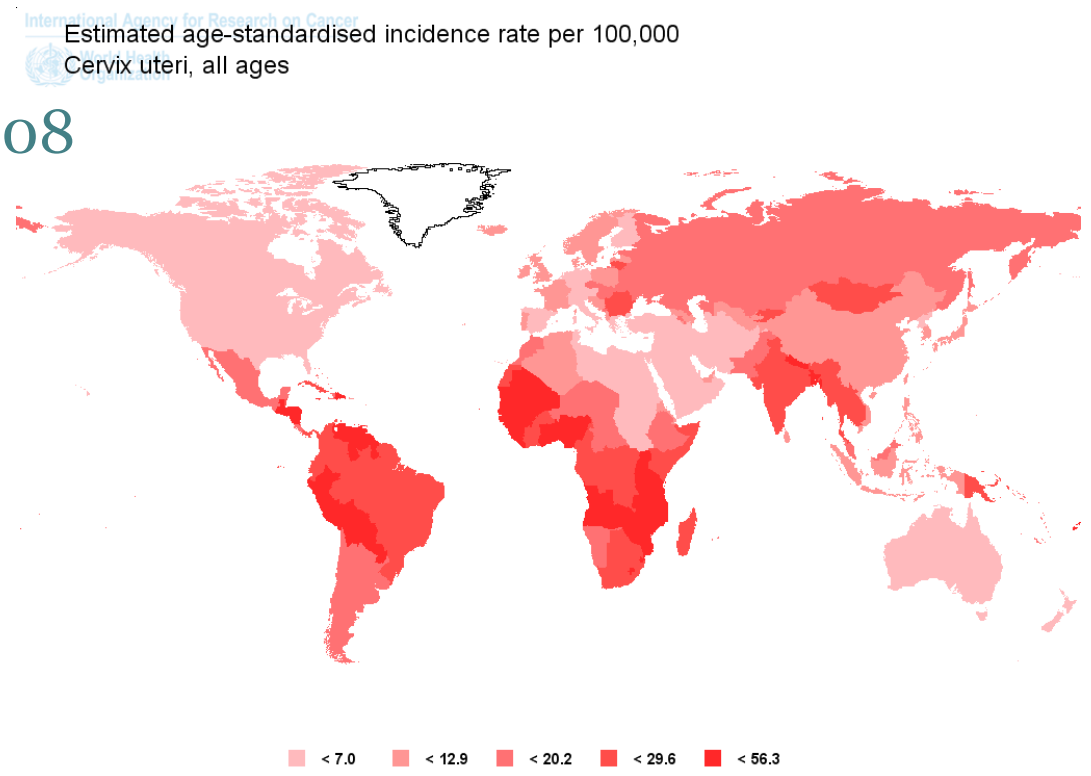
Content

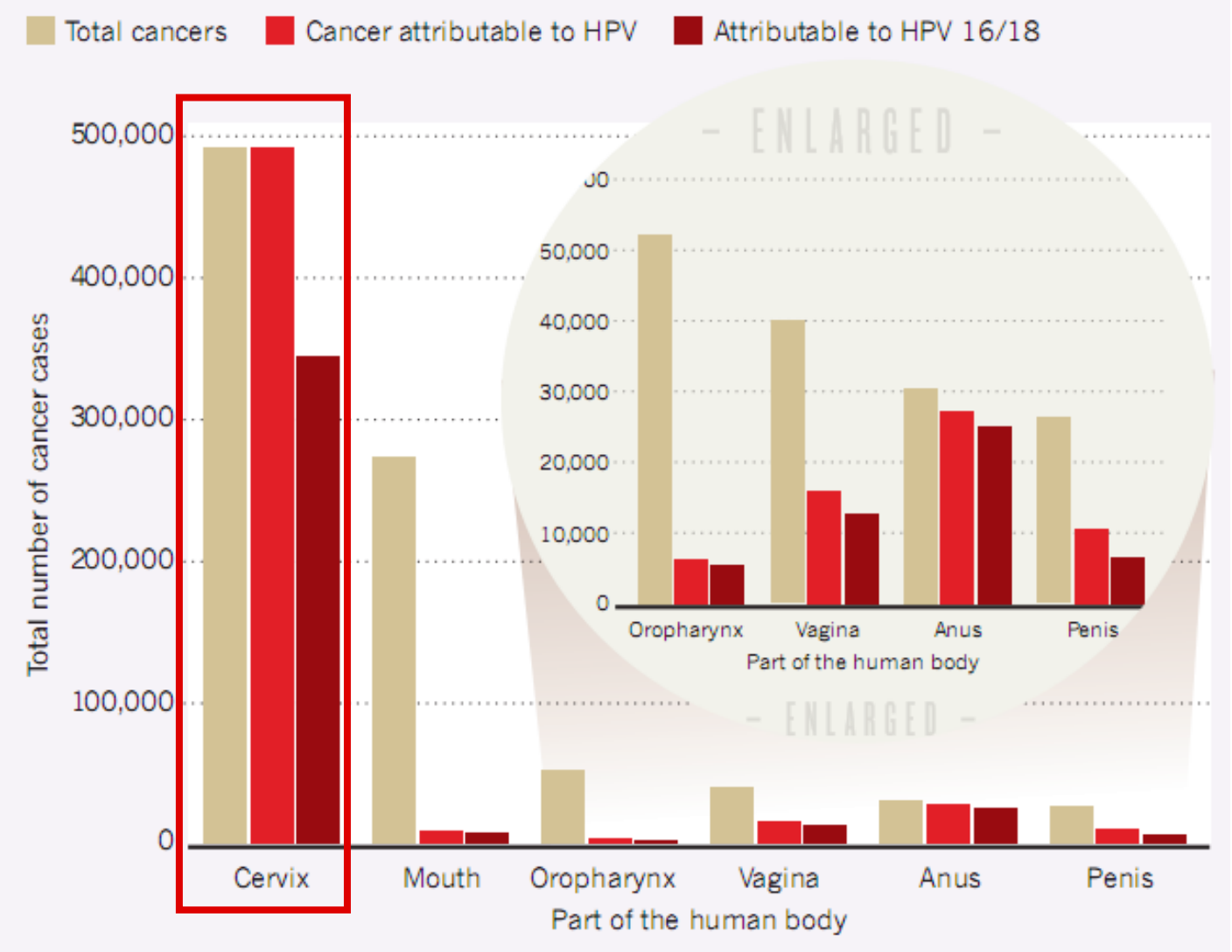
- Cervical cancer
- Cervical cancer and HPV
- HPV and E7
- Structure of E7
- Functions of different domains in E7

Cervical Cancer

- Third most common cancer in Women
- Incidence: 530,000 women in 2008
- Mortality:
  - 275, 000 death in 2008
  - 52%

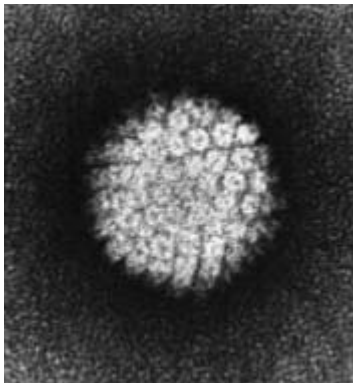


Association of cervical cancer and HPV



(Nature, 2012)

HPV and E7



- Human Papillomavirus (HPV)
- Genome

  Early control region

  Late control region

  Long control region (LCR)
- Protein encoded:

  Early expressed proteins

  Late expressed proteins

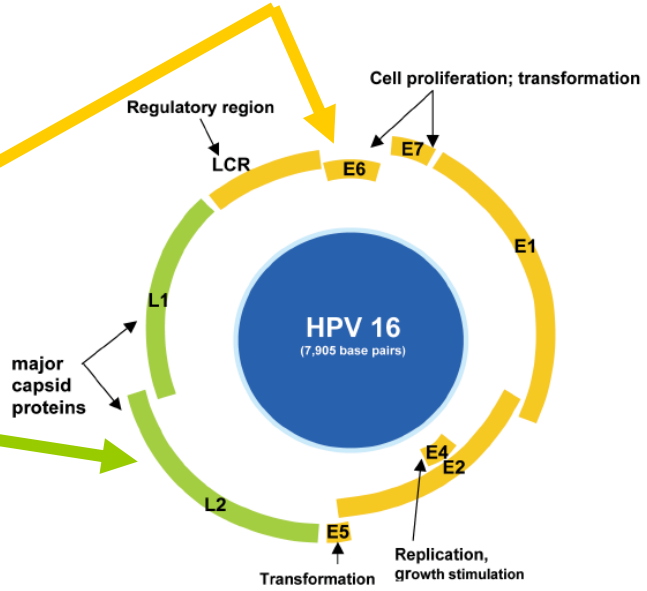


Figure 1 General organization of the HPV genome.

HPV encoded proteins

Early expressed protein

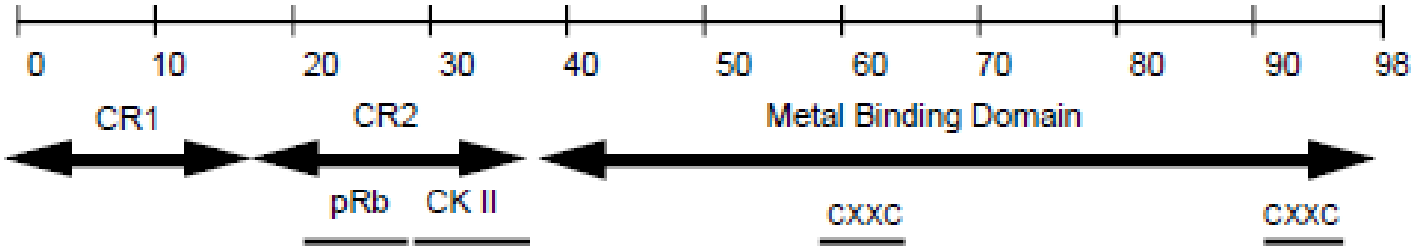
produced
after entry into host cell
prior to DNA replication

First oncogene of
high-risk HPVs
to be discovered

Table 3. Functions of papillomavirus proteins[a]

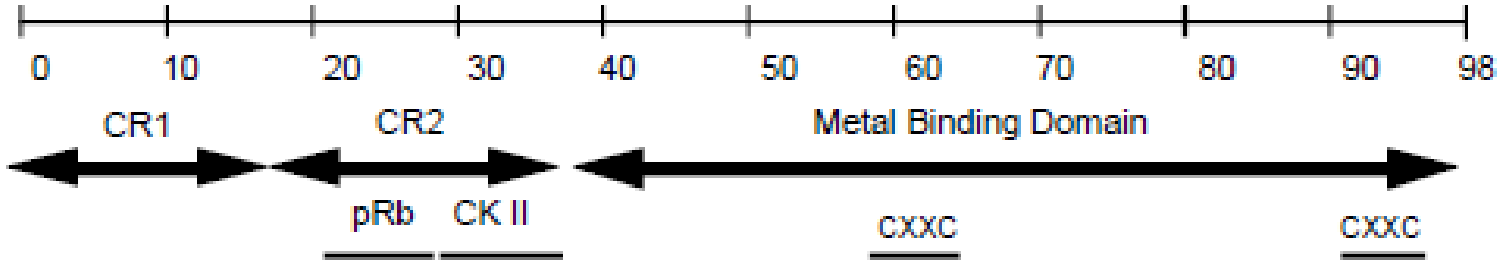
E1	Adenosine triphosphatase (ATPase) and DNA helicase; recognizes and binds to the viral origin of DNA replication as a hexameric complex; necessary for viral DNA replication.
E2	Main regulator of viral gene transcription; binds the viral transcriptional promoter as a dimer; involved in viral DNA replication; interacts with and recruits E1 to the origin.
E4	Acts late in the viral life cycle; interacts with the keratin cytoskeleton and intermediate filaments; localizes to nuclear domain 10; induces G2 arrest; believed to facilitate virus assembly and release.
E5	Induces unscheduled cell proliferation; interacts with 16k subunit c of vacuolar ATPase; may activate growth factor receptors and other protein kinases; inhibits apoptosis; inhibits traffic of major histocompatibility complexes to the cell surface.
E6	Induces DNA synthesis; induces telomerase; prevents cell differentiation; interacts with four classes of cellular proteins: transcriptional co-activators, proteins involved in cell polarity and motility, tumour suppressors and inducers of apoptosis, primarily p53, and DNA replication and repair factors.
E7	Induces unscheduled cell proliferation; interacts with histone acetyl transferases; interacts with negative regulators of the cell cycle and tumour suppressors, primarily p105Rb.
L1	Major viral structural protein; assembles in capsomeres and capsids; interacts with L2; interacts with cell receptor(s); encodes neutralizing epitopes.
L2	Minor viral structural protein; interacts with DNA; interacts with nuclear domain 10s; believed to facilitate virion assembly; may interact with cell receptor(s); encodes linear virus neutralizing epitopes.

Structure of E7



aa 1-15	aa 16-37	aa 38-98
CR1 Conserved region 1	**CR2** Conserved region 2	**Metal Binding Domain**
	• pRB binding site • CK II phosphorylation site	Two CXXC motifs

Functions involved in each domain



CR1	CR2	Metal Binding Domain
	CK II phosphorylation	Metal Binding (Zinc)
	pRB binding	
	Disrupt pRB/E2F complex	
	Cdk inhibitor inactivation	
Transformation and Immortalization		

Role of E7 to HPV itself

- HPV infect undifferentiated basal cells.
  - Proliferation: use host cell's DNA synthesis machinery for viral genome replication
- Differentiating cells:
  - Late viral capsid protein expression
  - Virus assembly
- Shed epithelial squames:
  - Virus particles release

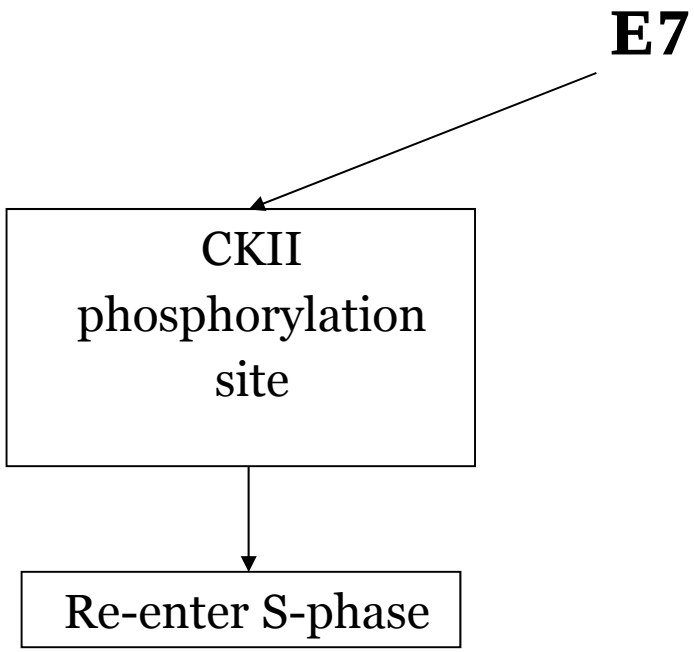
Role of E7 to HPV itself

- E7:
  - Subvert the tight link between proliferation and differentiation
  - Retain differentiating keratinocyte in a DNA-replication competent state
  - Give a high copy number viral genome amplification during differentiation

CR2: CKII Phosphorylation site

- Caesin kinase 2 (CK II)
  - Serine/threonine-selective protein kinase
- When E7 phosphorylated by CKII,
  - S32 & S34
  - Activation of certain S-phase genes
  - Promote S-phase re-entry in differentiated keratinocyte
- Also, induce PCNA (Proliferating Cell Nuclear Antigen), which is a factor holding DNA polymerase ε to DNA

Short Summary



CR2: pRB binding site

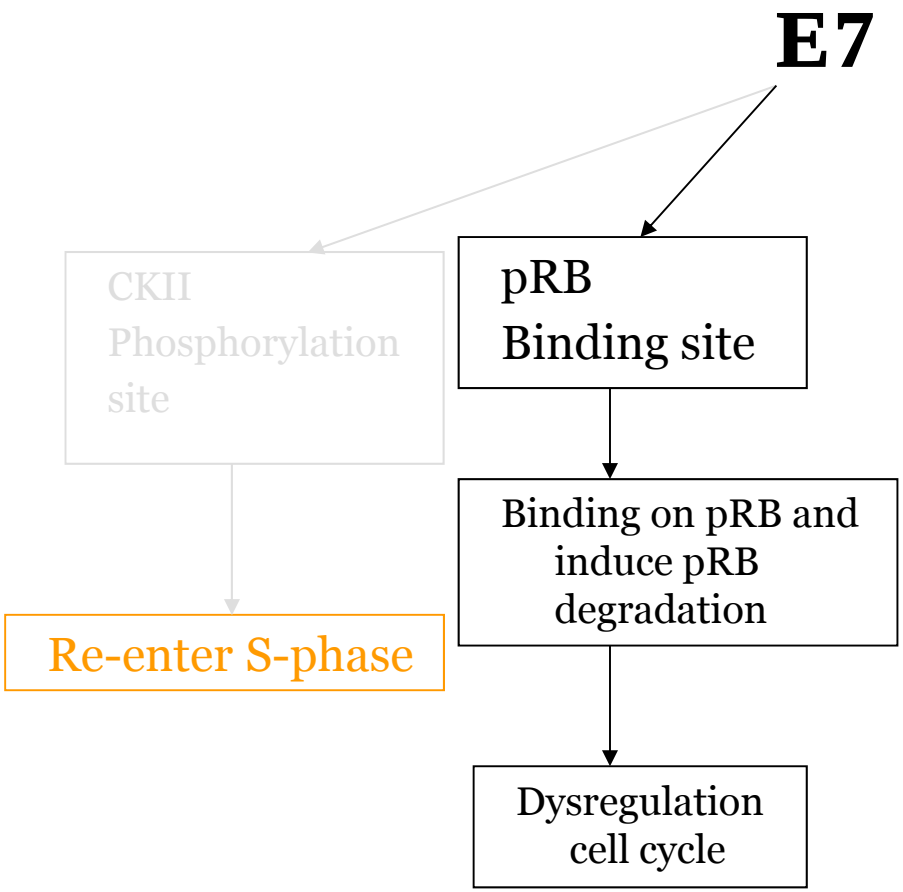
- Retinoblastoma protein (pRB)
  - Tumor suppressor protein
  - Regulating cell cycle progression
  - Preventing damaged DNA replication

CR2: pRB binding site

- Core motif **XLXCXE** for pRB binding
- High-risk E7 VS Low-risk E7
  - Difference in binding affinity
    - Type 16-E7 [**D**LYCYE]
    - Type 6-E7 [**G**LHCYE]

- 16E7 bind to pRB
  - Target on S4 subunit of 26S proteasome
  - pRB degradation

Short Summary



E2F/pRB complex dissociation

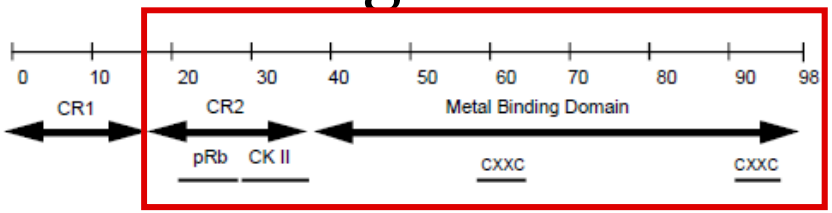
- What is E2F?
  - Family of transcriptional activator
  - Target genes: DNA synthesis, cell cycle progression
  - Important role in control of cell proliferation: Drive cell cycle (G1/S) progression
- Regulation of E2F
  - Association of pRB and pocket protein family (p107, p130) → inhibit E2F-dependent transcription

E2F/pRB complex dissociation

- In quiescent or differentiating cells
  - Majority of E2F bind to pRB-family proteins
- When cells entered S-phase
  - pRB phosphorylated
  - E2F/pRB dissociated
  - E2F is in free form
  - Activate E2f-dependent transcription
- In presence of E7 in differentiating cells…

E2F/pRB complex dissociation

- In presence of E7 in differentiating cells…
  - From experiments

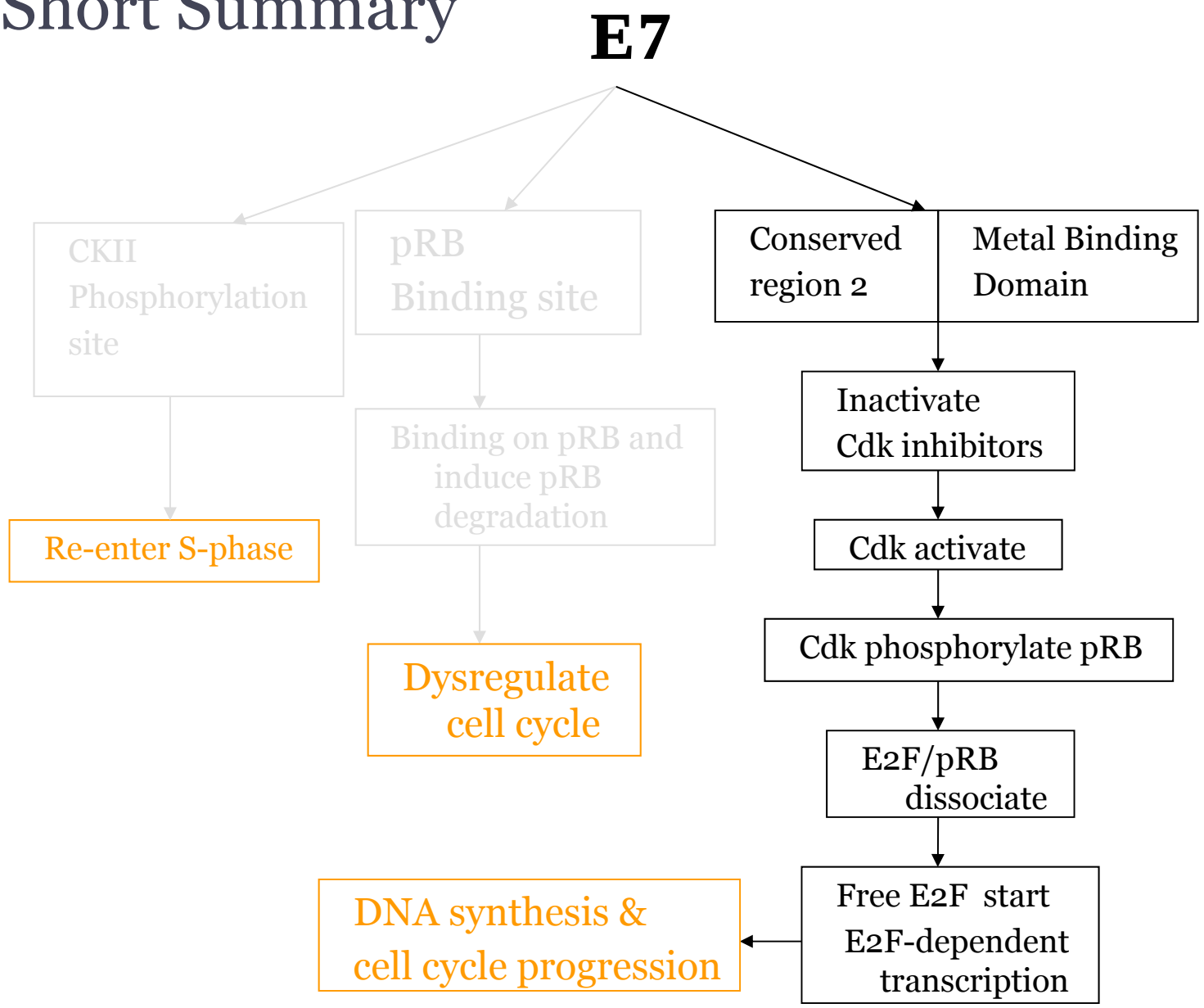


  - Both CR2 & metal binding domain are required for dissociation of E2F/pRB complex through inactivation of cdk inhibitors ?

Inactivate Cdk inhibitors

- Cdk: cyclin-dependent kinase
  - Serine/threonine-selective protein kinase
  - Inhibitors (e.g. p21cip1) regulate cdk activities and mediate inhibitory signals of cellular growth
- E7 interact with p21cip1 and abrogate the inhibition of cdk → cdk activate → phosphorylate pRB → E2F/pRB complex dissociate → free E2F to start E2F-dependent transcription

Short Summary

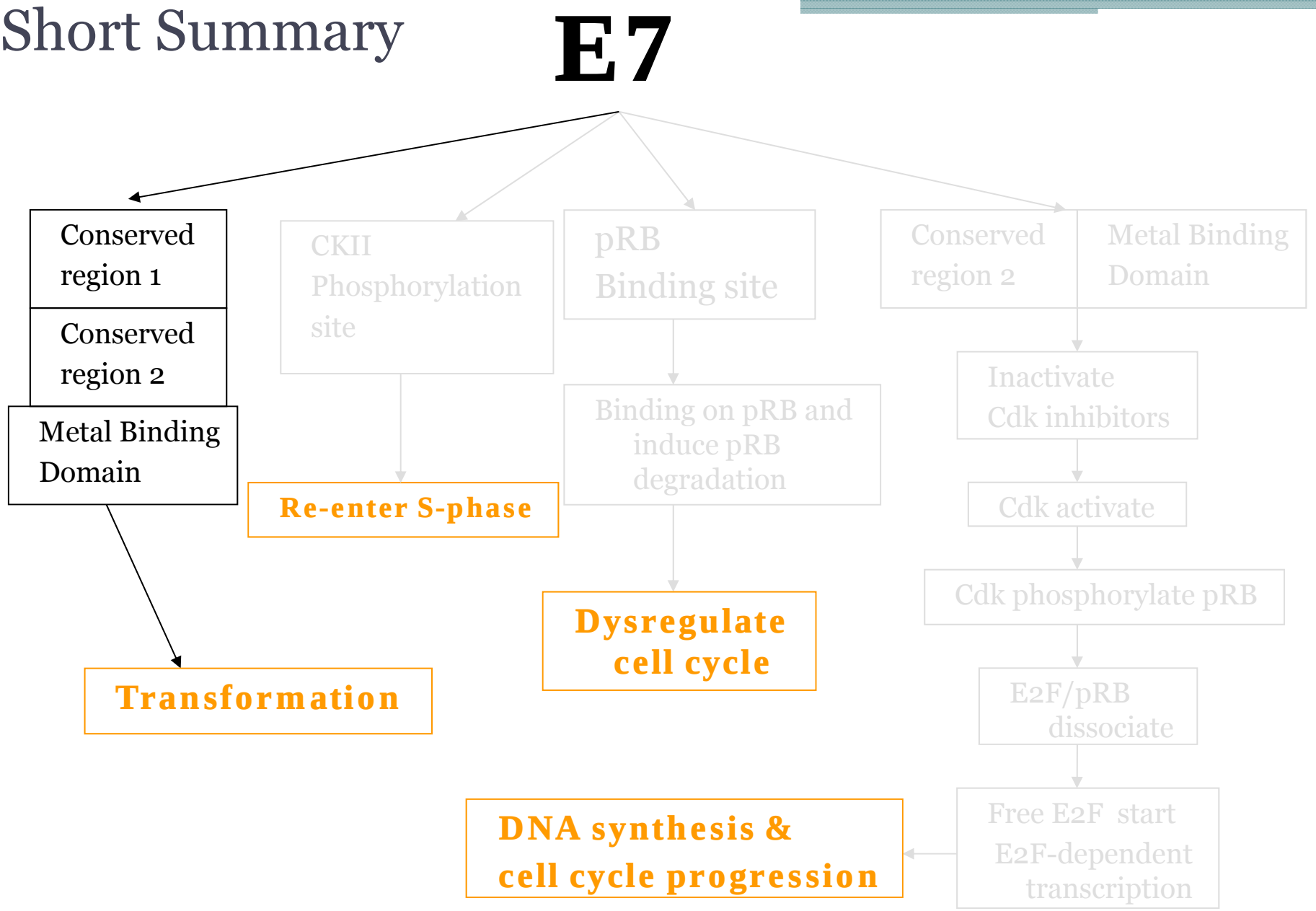


Transformation

- Both CR1, CR2, Metal Binding Domain
- aa 6-10
  - Deletion cause substantial loss of transformation
- CKII phosphorylation site
  - Mutations reduce transformation
- Integrity of CXXC motifs
  - important for transformation

are important for **Tranformation**.

Short Summary



Conclusion

- E7 control the cellular environment in a favorable way for viral replication in differentiating cells
  - pRB binding and degradation
  - Dissociate E2F/pRB complex, free E2F for gene transcription
  - Inactivate Cdk inhibitor
  - CKII phosphorylation
- Leading to Cell Transformation

The End

Thank you