Rb gene

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INSENSITIVITY TO GROWTH INHIBITION AND ESCAPE FROM SENESCENCE: TUMOR SUPPRESSOR GENES

- Failure of growth inhibition is one of the fundamental alterations

- Products of ***tumor suppressor genes apply brakes to cell proliferation***

- Tumor suppressor proteins form a network of checkpoints that prevent uncontrolled growth..

RB & p53,(part of a regulatory network)

- Recognizes genotoxic stress
- Responds by shutting down proliferation.

- Expression of an oncogene leads to quiescence, or

- to permanent cell cycle arrest (oncogene-induced senescence), rather than uncontrolled proliferation

RB, the first, and prototypic, tumor suppressor gene discovered

In molecular terms, Knudson's hypothesis

- Two mutations (hits), involving both alleles of *RB* at chromosome locus 13q14
- required to produce retinoblastoma.

- In some cases, the genetic damage is large enough to be visible in the form of a deletion of 13q14

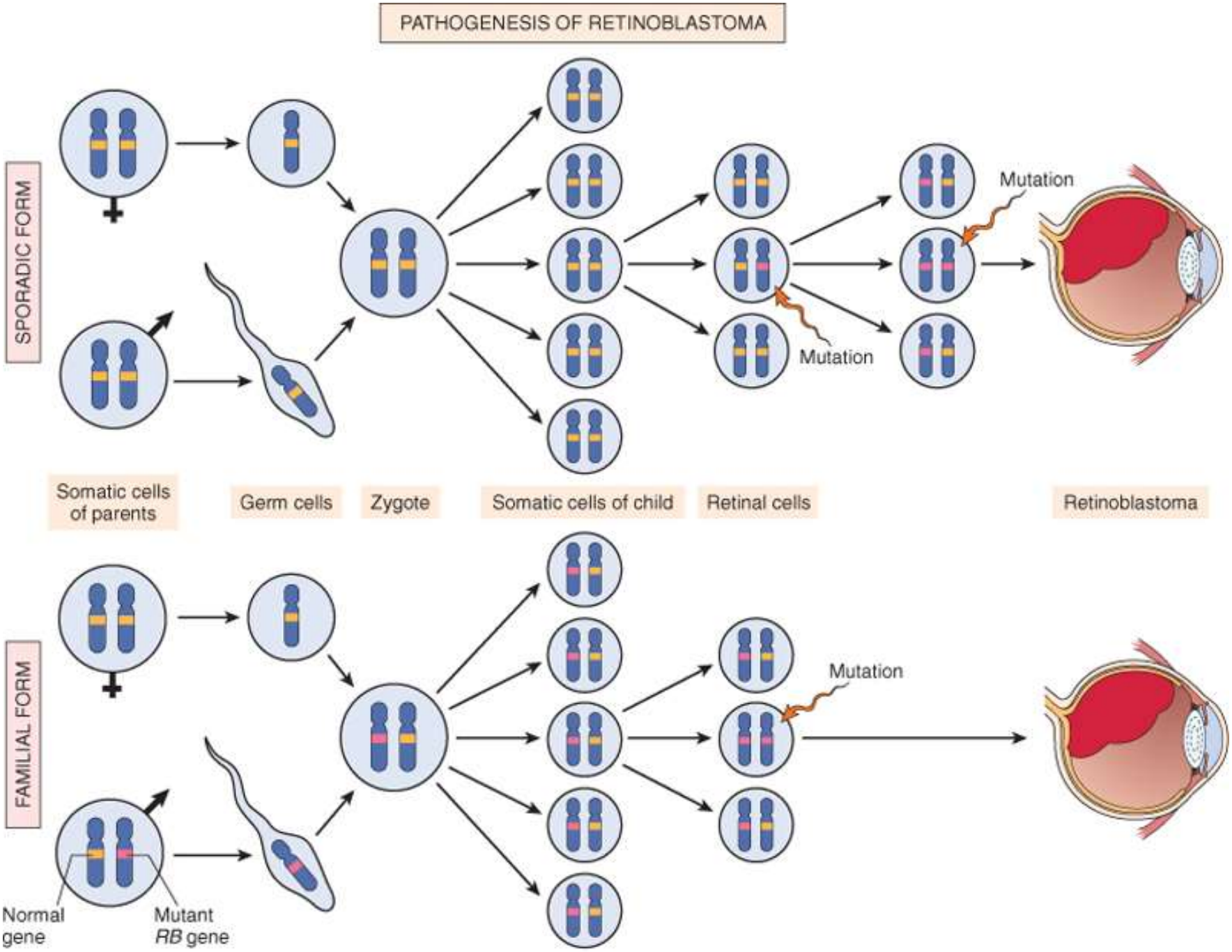
- In familial cases, children inherit one defective copy of the *RB* gene in the germ line (one hit); the other copy is normal

- Retinoblastoma develops when the normal *RB* allele is mutated in retinoblasts as a result of spontaneous somatic mutation (second hit).

- Because only a single somatic mutation is required for loss of RB function in retinoblastoma families, familial retinoblastoma is inherited as an autosomal dominant trait

- In sporadic cases both normal *RB* alleles must undergo somatic mutation in the same retinoblast (two hits).

- The end result is the same: a retinal cell that has completely lost *RB* function becomes cancerous.



LOH, (loss of heterozygosity)

- A child carrying an inherited mutant *RB* allele
- Because such a child is heterozygous at the *RB* locus, this implies that heterozygosity for the *RB* gene does not affect cell behavior.

- *Cancer develops when the cell becomes homozygous for the mutant allele or, put another way, when the cell loses heterozygosity for the normal RB gene LOH,*

RB

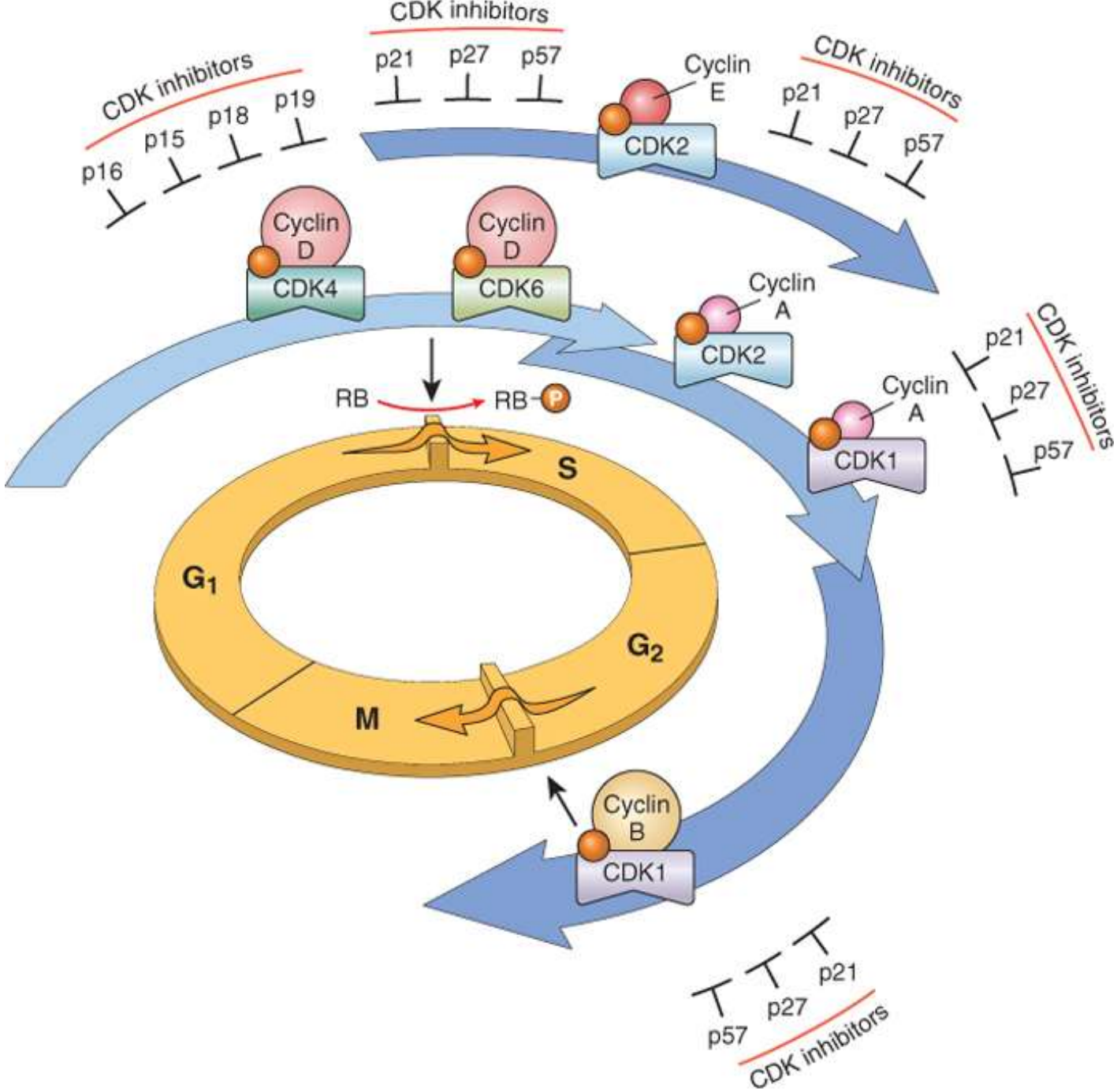
- RB protein, the product of the *RB* gene,
- Key role in regulating the cell cycle.
- Rb exists in an active hypophosphorylated state in quiescent cells and an
- Inactive hyperphosphorylated state in the G_1/S cell cycle transition
- The importance of Rb lies in its enforcement of G_1, or the gap between mitosis (m) and DNA replication (s).
- Two gaps are incorporated into the cell cycle:

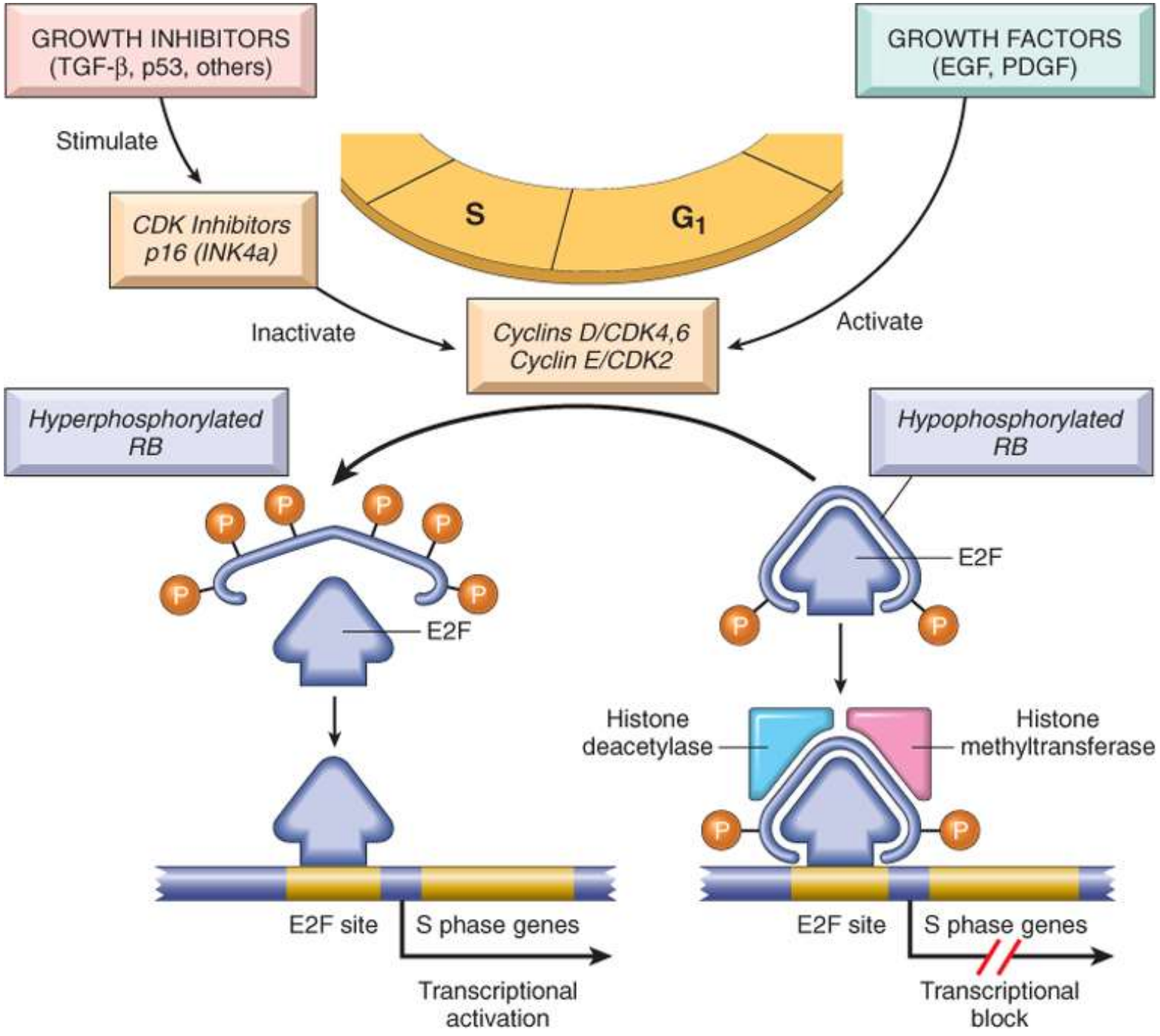
G_1 to S extremely important checkpoint in the cell cycle clock

- In G_1, however, cells can exit the cell cycle, either temporarily, called quiescence, or permanently, called senescence.

- In G_1, therefore, diverse signals are integrated to determine whether the cell should enter the cell cycle, exit the cell cycle and differentiate, or die.

- RB is a key node in this decision process.





The initiation of DNA replication requires the activity of cyclin E–CDK2 complexes,

- Expression of cyclin E is dependent on the E2F family of transcription factors.
- Early in G_1, RB is in its hypophosphorylated active form, and it binds to and inhibits the E2F family of transcription factors, preventing transcription of cyclin E.
- Hypophosphorylated RB blocks E2F-mediated transcription

Hypophosphorylated RB blocks E2F-mediated transcription in at least two ways

- First, it sequesters E2F, preventing it from interacting with other transcriptional activators.

- Second, RB recruits chromatin remodeling proteins, such as histone deacetylases and histone methyltransferases,
- which bind to the promoters of E2F-responsive genes such as cyclin E.

- These enzymes modify chromatin at the promoters to make DNA insensitive to transcription factors.

This situation is changed upon mitogenic signaling

- Growth factor signaling leads to cyclin D expression and activation of cyclin D-CDK4/6 complexes.

- These complexes phosphorylate RB, inactivating the protein and releasing E2F to induce target genes such as cyclin E.

Expression of cyclin E then stimulates DNA replication and progression through the cell cycle.

- When the cells enter S phase, they are committed to divide without additional growth factor stimulation.

- During the ensuing M phase, the phosphate groups are removed from RB by cellular phosphatases, regenerating the hypophosphorylated form of RB.

E2F is not the sole target of RB

- The versatile RB protein has been shown to bind to a variety of other transcription factors that regulate cell differentiation.
- RB stimulates myocyte-, adipocyte-, melanocyte-, and macrophage-specific transcription factors.
- Thus, the RB pathway couples control of cell cycle progression at G_1 with differentiation,

RB is not mutated in every cancer.

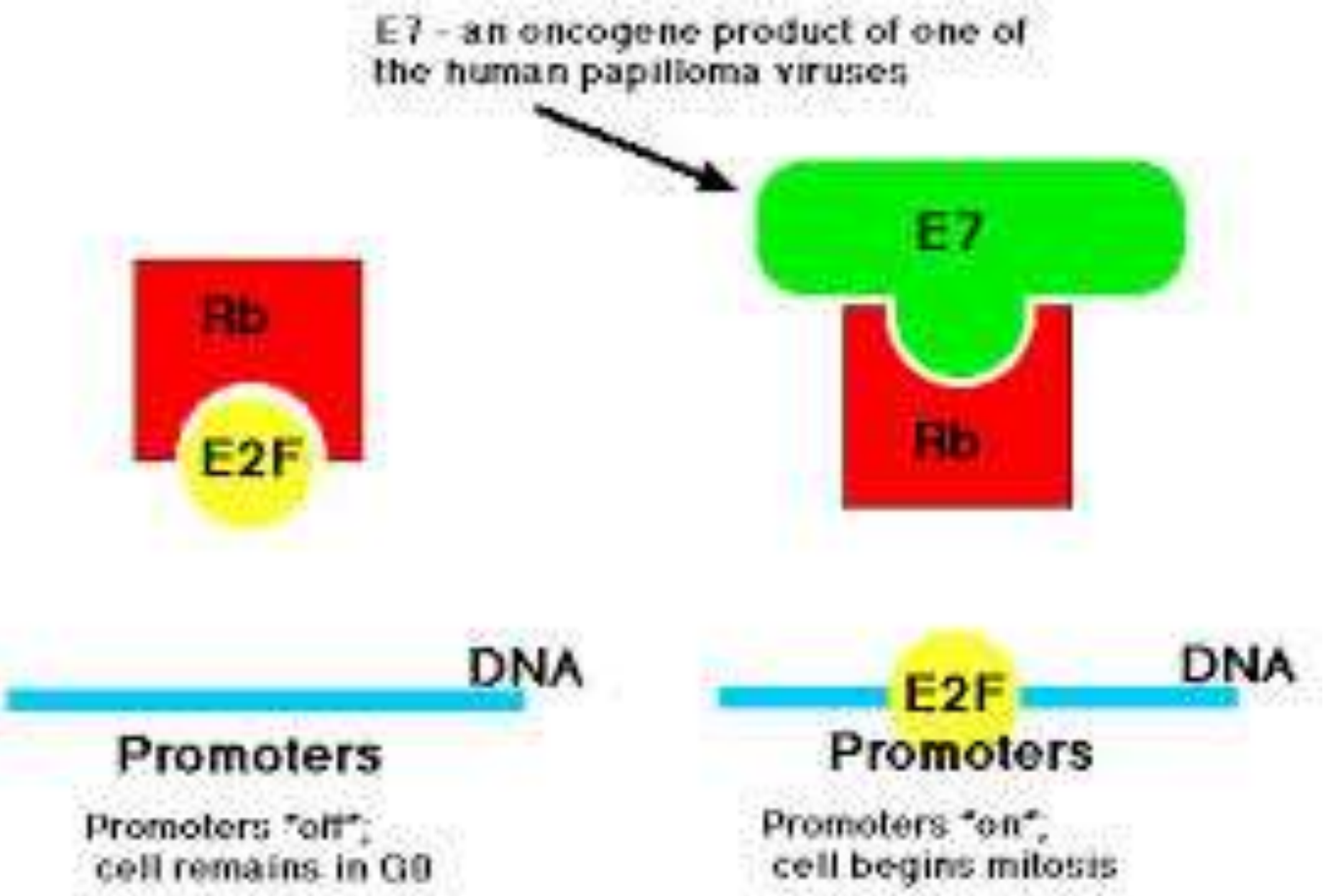
- Mutations in other genes that control RB phosphorylation can mimic the effect of *RB* loss;
- Mutational activation of CDK4 or overexpression of cyclin D
- Cyclin D is overexpressed in many tumors because of gene amplification or translocation.
- Mutational inactivation of cdkis

The emerging paradigm

- *Loss of normal cell cycle control is central to malignant transformation and that*

- *At least one of the four key regulators of the cell cycle (CDKN2A, cyclin D, CDK4, RB) is mutated in most human cancers.*

- Transforming proteins of several oncogenic animal and human DNA viruses seem to act, in part, by neutralizing the growth-inhibitory activities of RB.
- (HPV) E7 protein all bind to the hypophosphorylated form of RB.
- The RB protein, unable to bind to the E2F transcription factors, is functionally deleted, and
- the cells lose the ability to be inhibited by antigrowth signals that funnel through the RB nexus.


E7 - an oncogene product of one of the human papilloma viruses
E7
Rb
E2F
Rb
DNA
Promoters
Promoters "off"; cell remains in G0
E2F
DNA
Promoters
Promoters "on"; cell begins mitosis