Cell cycle, signaling to cell cycle, and molecular basis of oncogenesis

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CELL CYCLE - SUMMARY

Basic terminology:

Cyclins – conserved proteins with homologous regions; their cellular level profoundly oscilate during the cell cycle due to transcriptional regulation and different degradation of the protein.

Cyclins are catalytic subunits of active cyclin-cdk complexes.

CYCLINS A, B, D(1,2,3), E

Cyclin-dependent kinases (cdks) – kinases which require a catalytic subunit (cyclin) and their activity is regulated by phosphorylation/dephosphorylation and by cdk-inhibitors.

CDK 1,2,3,4,6,7

Substrates of cyclin-cdk complexes – the most important is the retinoblastoma protein (Rb).
Rb gene family: Rb, p107, p130.

Cdk inhibitors – bind and inactivate cyclin-cdk complexes

E2F transcription factors – heterodimers of E2Fs (1-5) and DPs (1,2) activate transcription of several genes important for the S-phase.

Transcription by E2F is repressed by Rb protein. Only hypophosphorylated Rb protein is capable of repressing transcription. Upon phosphorylation, Rb protein becomes inactive.

E2F targets are promoters of: DNA polymerase α, dihydrofolate-reductase, thimidine kinase,
Cyclin E, cyclin A, c-myc, E2F-1 (positive loop)
cdc6

Summary of the regulation of cyclin/cdk complexes during cell cycle

Cell cycle phase	**Cyclin-cdk complex**	**inhibitor**	**activation**	**Substrate(s)**
G1	**Cyclin D/cdk 4,6**	p16 family, p21 family	CAK, Cdc25A	Rb protein
G1/S	**Cyclin E/cdk 2**	p21 family	CAK, Cdc25A	Rb protein, NPAT, cdc6
S	**Cyclin A/cdk 2** (CyclinA/cdk 1)	p21 family	CAK, Cdc25	Rb protein, pre-RC, E2F
G2/M	**Cyclin B/cdk 1** (Cyclin A/cdk 1)	p21 family	CAK, Cdc25C	Several substrates required for mitosis (APC, lamins, condensins,..)

Cell cycle checkpoints

- restriction point
  a regulatory checkpoint, operates under physiologic conditions (in the absence of DNA damage), regulates S-phase entry. After this point, the cell is committed to enter the S-phase.

Other checkpoints enable cells to halt the cell cycle and repair damaged DNA or complete spindle assembly at mitosis:

- DNA replication checkpoint (in G2/M)
  this checkpoint ensures that mitosis occurs only after DNA has replicated completely and faithfully. DNA replicates only once during a single cell cycle (exception: endoreduplication)

- spindle assembly checkpoint
  ensures proper segregation of chromosomes during mitosis (at the metaphase to anaphase transition)

- DNA damage checkpoint(s)
  cell cycle can be arrested in G1, S , or G2

Other cell processes that result also in effects on cell cycle

Differentiation.
Differentiated cells are in the G1(G0) phase of the cell cycle.
Terminal differentiation (nerves, muscles) normally does not allow
the re-entry into the cell cycle. Differentiated cells have specific differentiated phenotype which includes morphology and expression of cell type-specific markers.

Senescence.
Replicative senescence results in exit from the cell cycle into G0.
Again, normally, senescent cells are unable to re-enter the cell cycle.
Senescent cells have also specific morphology and express senescent specific markers.

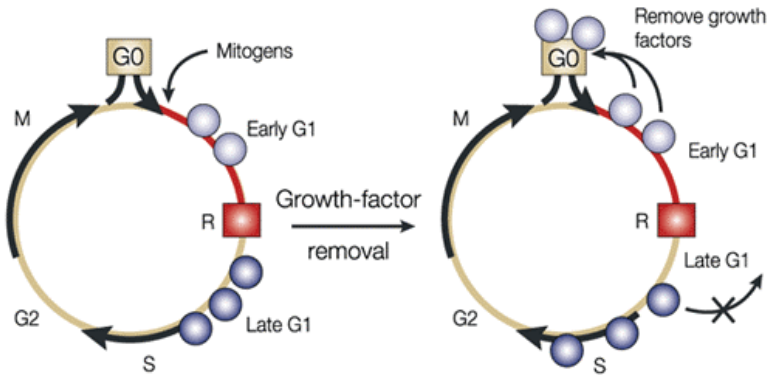
Apoptosis.
Apoptosis (programmmed cell death) occurs after the activation of pro-apoptotic genes or inhibition of anti-apoptotic genes and in most instances it is initiated in G1.

Signaling after DNA damage

(DNA damage checkpoints)

Crucial players:
ATM (ataxia-teleangiectasia mutated), ATR

Chk1, Chk2



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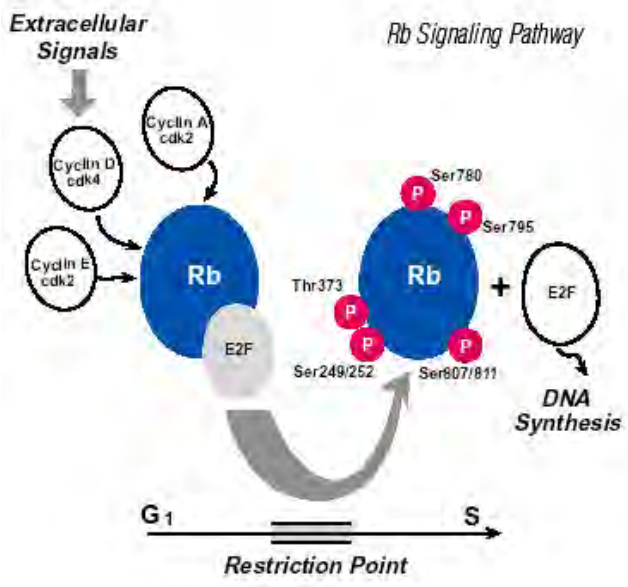
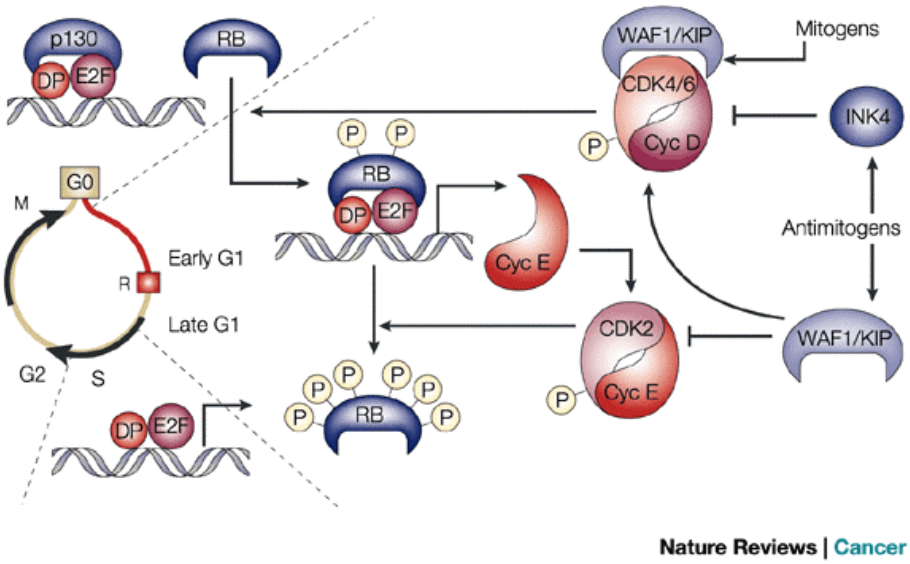




Cdk inhibitors

INK4 family:
p16 (INK4a), p15 (INK4b) p18 (INK4c) p16(INK4d)

Inhibit only cyclin D/cdk 4,6 complexes

p21 (Cip1) family

p21 (Cip1, WAF1), p27 (kip1), p57 (Kip2)

Inhibit both cyclin D/cdk 4,6 complexes nad cyclin E/cdk2 and cyclin A/cdk2 complexes

p14ARF (p19ARF in mouse)
stabilizes the p53 protein

APC = Anaphase promoting complex

APC is mainly required to induce progression and exit from mitosis by inducing proteolysis of different cell cycle regulators including securin and cyclin B.
APC has ubiquitin-ligase activity and is a large complex that contains at least 11 subunits.

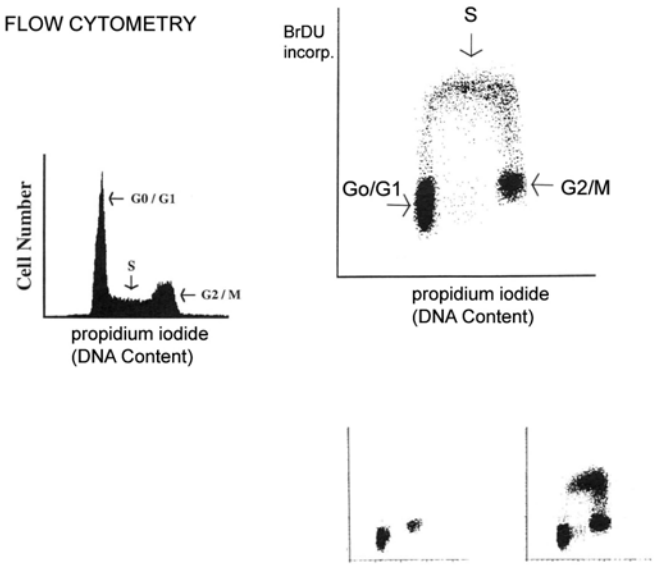
APC^{Cdc20} enables activation of the protease separase by mediating the degradation of securin.

Active separase separates sister chromatids from each other by cleaving cohesin complexes.

APC^{Cdc20} also helps to activate separase by initiating the degradation of cyclin B and other mitotic cyclins.

Anaphase promoting complex has two different adaptors

Cdc20 and Cdh1 are adaptors helping APC to couple with substrates .
APC^{Cdc20} is activated at the onset of prometaphase (PM) when it initiates the degradation of cyclin A (CycA). During anaphase (A) and telophase (T), APC^{Cdh1} is activated and mediates the destruction of additional substrates. APC^{Cdc20} is inactivated during mitotic exit, whereas APC^{Cdh1} remains active until the onset of the next G1 phase.



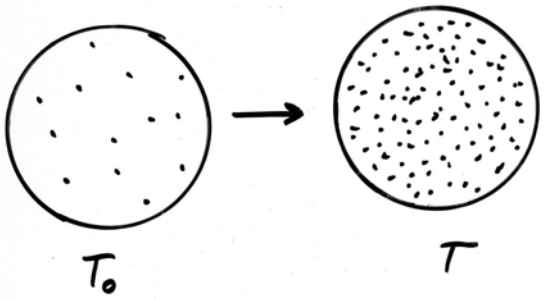
Cells in culture

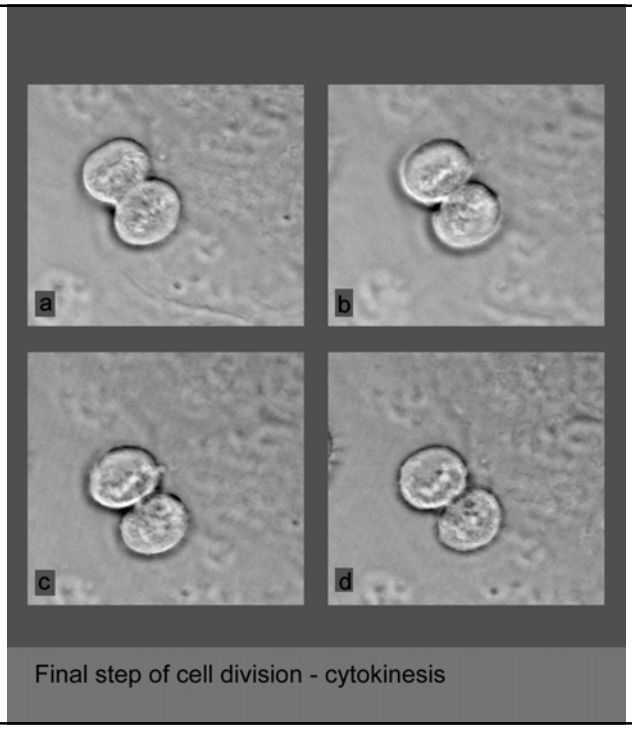
doubling time ... the time interval in which the cell number doubles

number of doublings = log N/No x 3.33

No...number of cells at time To

N....number of cells at time T





Final step of cell division - cytokinesis

Ras signalling

RAS proteins can be activated constitutively by oncogenic mutations (typically codon 12 in K-ras, codon 13 in K-ras, codon 61 in H-ras), as in cancer cells, or physiologically through growth factors and their receptors (e.g. EGFR = EGF receptor).

Ras proteins activate a checkpoint resulting in G1 cell cycle arrest upon forced ras stimulation, provided all other cell cycle proteins that brake the cycle work normally (= are not mutated or inactivated).

If ras is stimulated in the cell where p16 or p19ARF is inactivated (e.g. by mutation), deregulation of cell cycle occurs, resulting in tumor formation (in mice).

Ras is physiologically required for normal cell cycle progression through G1 phase.

The MAPK pathway:

encompasses a cascade of phosphorylation events involving three key kinases, namely Raf, MEK and ERK:

RAS signaling (ras: H-ras, K-ras, N-ras – GTP-binding membrane proteins, with GTPase activity), active form: GTP-ras (constitutively active when mutated, such as in tumors)

↓

RAF = MAP kinase kinase kinase (MAPKKK)

↓

MEK = MAP kinase kinase (MAPKK)

↓

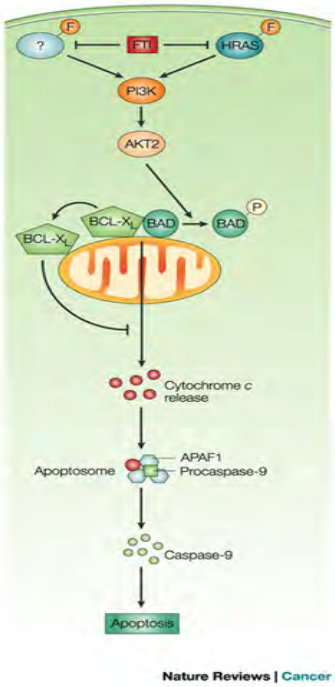
ERK = MAP kinase (MAPK)

↓

protein substrates

Activation of AKT kinase by PI 3-kinase

Activation of growth factor receptor protein tyrosine kinases results in autophosphorylation on tyrosine
residues > **PI3K** is recruited to the membrane > this leads to allosteric activation of the
catalytic subunit > activation results in production of the second messenger **phosphatidylinositol-3,4,5-trisphosphate (PIP3).**
The lipid product of PI3K, PIP3, recruits a subset of signaling proteins, including **AKT (=PKB)**. (PTEN, is a PI-3,4,5-P3 phosphatase, which negatively regulates the PI3K/Akt pathway).
Once activated, **AKT mediates the activation and inhibition of several targets (by phosphorylating them)**,
resulting in cellular survival, growth and proliferation through various mechanisms.



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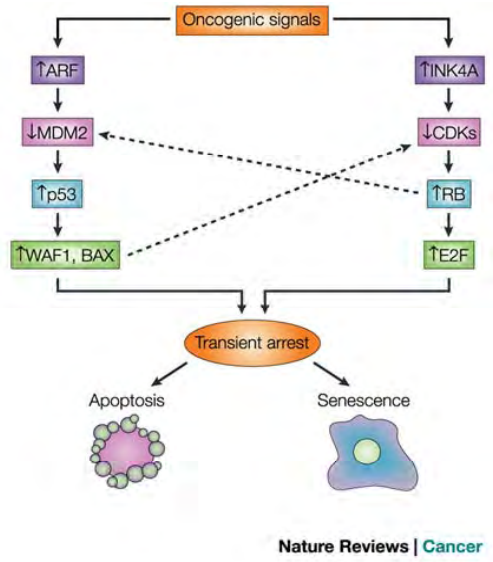
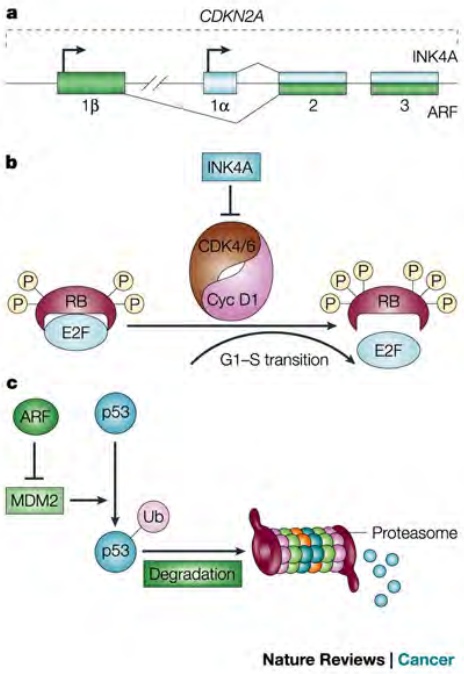
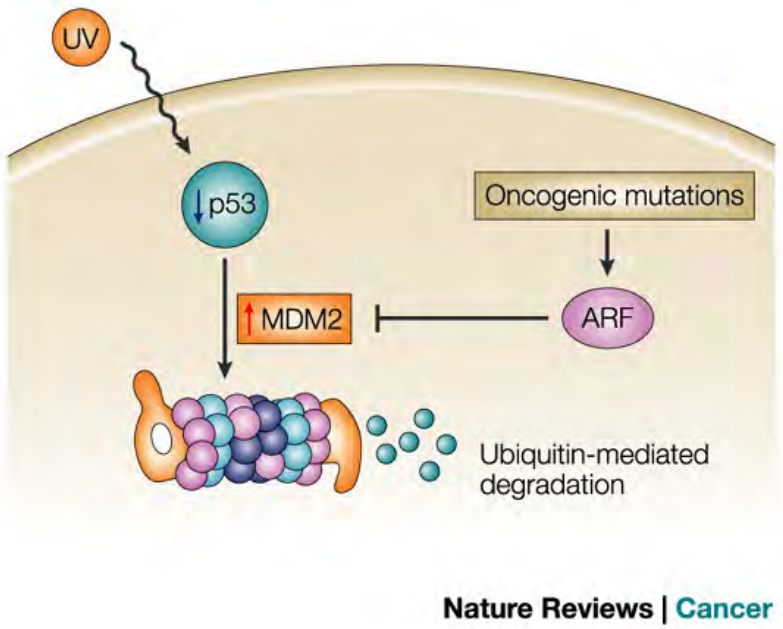
The PI3K–AKT cascade is an anti-apoptotic signalling pathway.

An important downstream effector of PI3K function is the AKT serine/threonine kinase. One of the best-characterized substrates of AKT that is important in promoting cell survival is the pro-apoptotic BAD protein. BAD interacts with the anti-apoptotic protein BCL-X_L and prevents BCL-X_L function, thereby leading to cytochrome *c* release from the mitochondria.

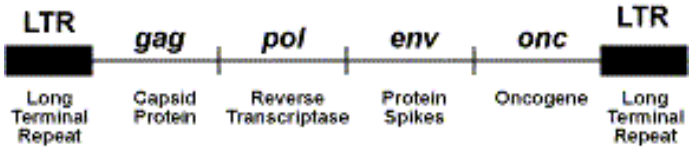
Rb- and p53- pathways are deregulated in cancer cells







RETROVIRAL ONCOGENESIS



Inserted oncogenes:

•**Growth factor receptors** - One example is epidermal growth factor receptor which promotes wound healing by stimulating cell growth. Some factors function as transmembrane protein kinases that are activated by an extracellular signal. An example is ***v-erbB*** found in the Avian erythroblastosis virus that infects chicken.

•**Protein kinases** - These proteins alter the function of other proteins by phosphorylating specific amino acid residues. The ***v-src*** from the Rous Sarcoma virus which infects chickens is an example.

•**G-proteins** - These proteins bind the nucleotide GTP, and also exhibit GTPase activity. The ***v-H-ras*** oncogene of the Harvey murine sarcoma virus which infects rats is an example.

•**Transcription factors** - These proteins function by binding to DNA and activating transcription. An example is the ***v-jun*** oncogene of the Avian sarcoma virus that infects chickens.

CANCER GENES - 2004:

•So far, 291 cancer genes have been reported (more than 1% of all the genes in the human genome).

•90% of cancer genes show somatic mutations in cancer, 20% show germline mutations and 10% show both.

•The most common mutation class among the known cancer genes is a chromosomal translocation that creates a chimeric gene or apposes a gene to the regulatory elements of another gene.

•Many more cancer genes have been found in leukemias, lymphomas and sarcomas than in other types of cancer, despite the fact that they represent only 10% of human cancer. These genes are usually altered by chromosomal translocation.

•The most common domain that is encoded by cancer genes is the protein kinase. Several domains that are involved in DNA binding and transcriptional regulation are common in proteins that are encoded by cancer genes.

•*Futreal et al.: Nature Reviews Cancer* **4**, 177 -183 (2004)

Oncogenic DNA viruses.

Virus:	Oncoprotein:	Genome size (kb):
SV-40 virus	large T Ag	5
Polyomavirus	middle T (large T)	5
Adenoviruses (Ad12)	E1a (E1b)	35
Papilomaviruses (HPV16)	E7, E6	8

Hepatitis B viruses
Herpesviruses, Epstein-Barr virus
Poxviruses

Cellular processes disrupted in cancer cells:

(Cellular processes that result also in effects on cell cycle)

Differentiation:
Differentiated cells are usually in the G0 phase of the cell cycle.
Terminal differentiation (nerves, muscles) normally does not allow
the re-entry into the cell cycle. Differentiated cells have specific differentiated phenotype which includes morphology and expression of cell type-specific markers. **Differentiation program is often impaired in cancer cells.**

Senescence:
Replicative senescence results in exit from the cell cycle into G0.
Again, normally, senescent cells are unable to re-enter the cell cycle.
Senescent cells have also specific morphology and express senescent specific markers. **Cancer cells are unable to senesce (cancer cells are always immortal).**

Apoptosis:
Apoptosis (programmmed cell death) occurs after the activation of pro-apoptotic genes or inhibition of anti-apoptotic genes and in most instances it is initiated in G1. **The execution of apoptosis is impaired in cancer cells.**

Cell cycle checkpoints:
Cell cycle control, mostly in **G1**, is **deregulated in cancer cells**.
Also, checkpoints are deregulated in cancer cells.

Tumor suppressor genes

- In addition to mutational "gain", cancer can be caused by genetic loss
- genes whose loss contributes to cancer are classified as tumor suppressor genes because when they function normally they help to suppress tumors
- normal functions of tumor suppressor genes is to
  - suppress cellular growth
  - promote cellular death
- Also known as anti-oncogenes

October 1998
Oliver Bogler, Ph.D.

Oncogenes and Protooncogenes

- An "oncogene" is a gene that contributes to cancer formation when it is mutated to become active
  - it is mutated for example, by:
    - point mutation
    - overexpression/amplification
    - deletion of parts of it
    - fusion of two proteins
  - it can be a cellular oncogene **c-onc**, meaning that is in the genome of the cancer cell, or
  - it can be a viral oncogene **v-onc**, meaning that it is brought into the cell by an infecting virus
- a protooncogene is the normal gene, before it was mutated
- an oncogene is dominant over a protooncogene

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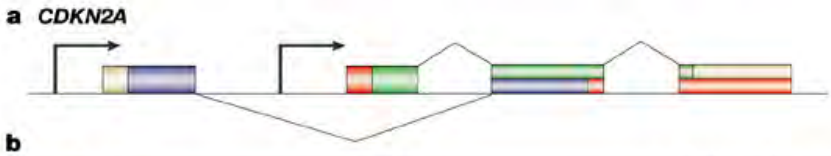
The *INK4A* locus and proliferation control in human and mouse cells



Gene product		Pathway			Enforces growth arrest secondary to: Proliferation	RAS
Human						
	INK4A	⊣	D-type cyclins CDK4/6	⊣ RB	+	+
	ARF	⊣	MDM2	⊣ p53	–	–
Mouse						
	Ink4a	⊣	D-type cyclins Cdk4/6	⊣ Rb	+/–	–
	Arf	⊣	Mdm2	⊣ p53	+	+

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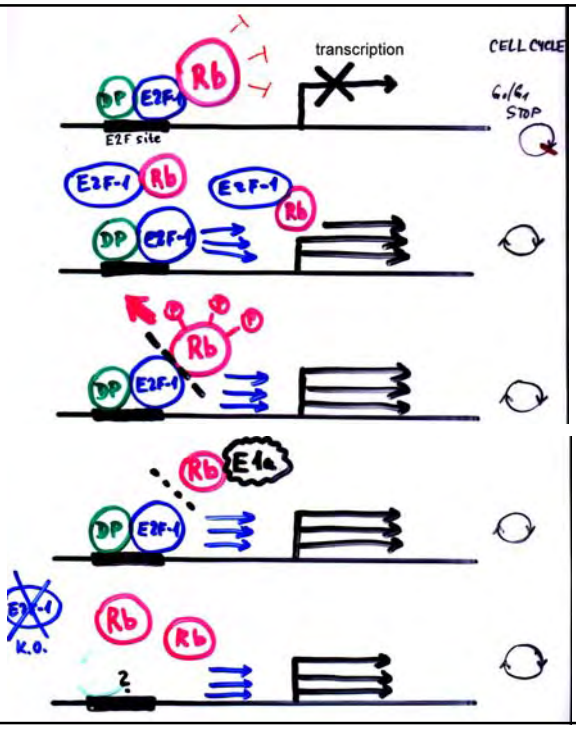

Unphosphorylated Rb blocks transcription

High level of E2F sequesters Rb from promoter

Phosphorylation with cdk-cyclin complexes inactivates Rb

Oncogenic proteins of DNA viruses inactivate Rb

Absence of E2F-1 prevents formation of repressor complex Rb-E2F