NEOPLASIA 4

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- **ANATOMICAL PATHOLOGY**

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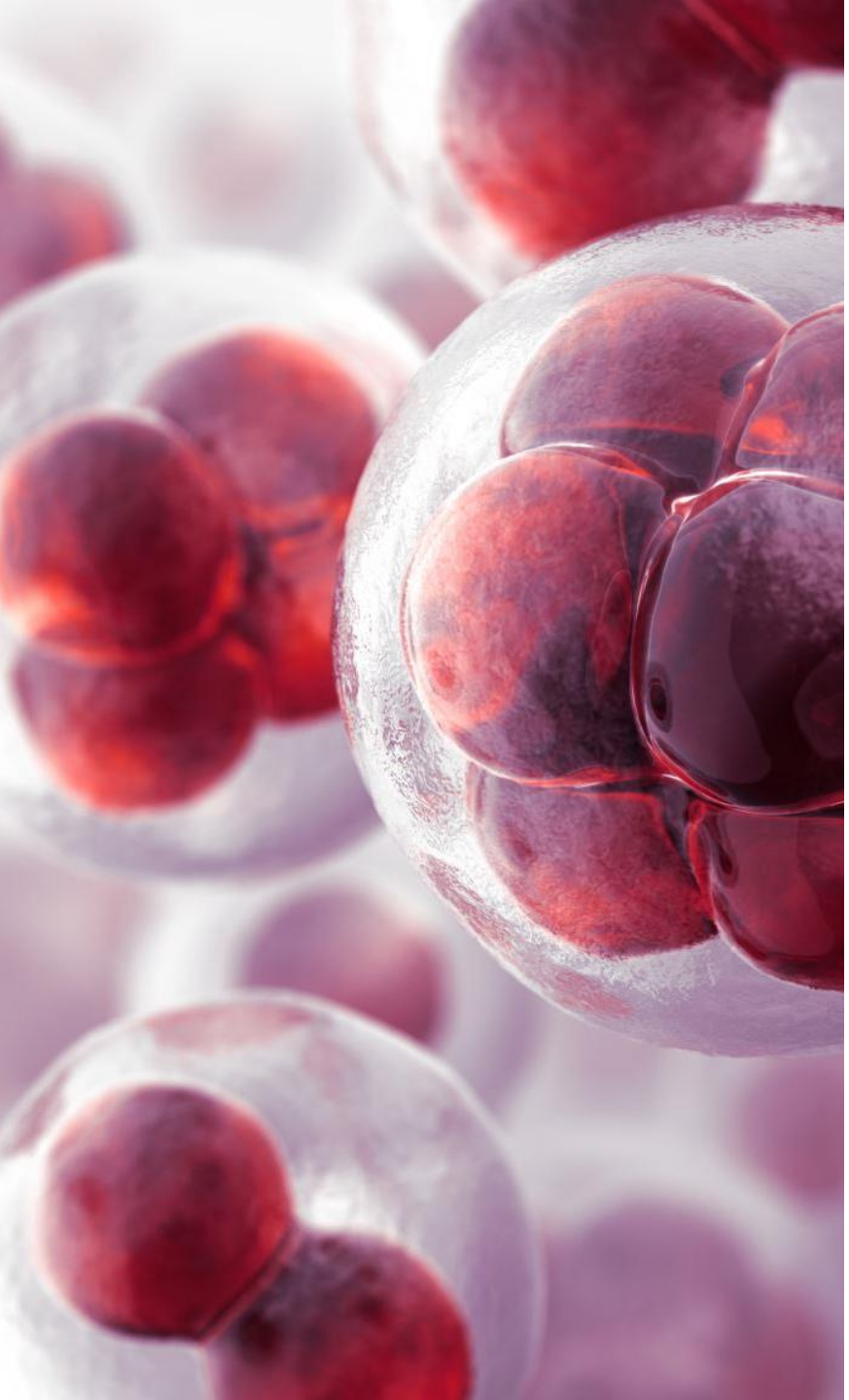
SCHOOL OF MEDICINE- DEPARTMENT OF HISTOPATHOLOGY

GENERAL PATHOLOGY LECTURES 2023



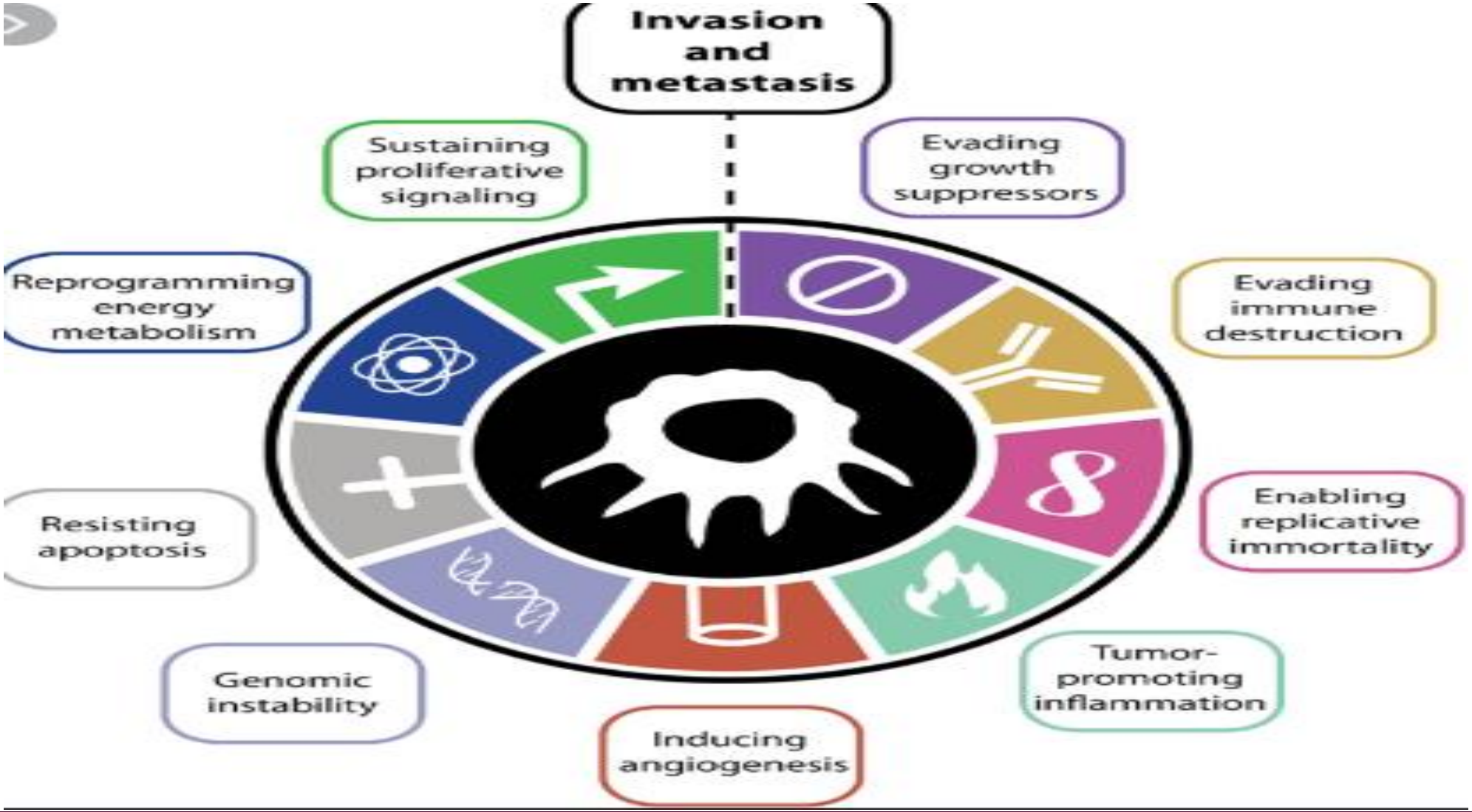
HALLMARKS OF CANCER

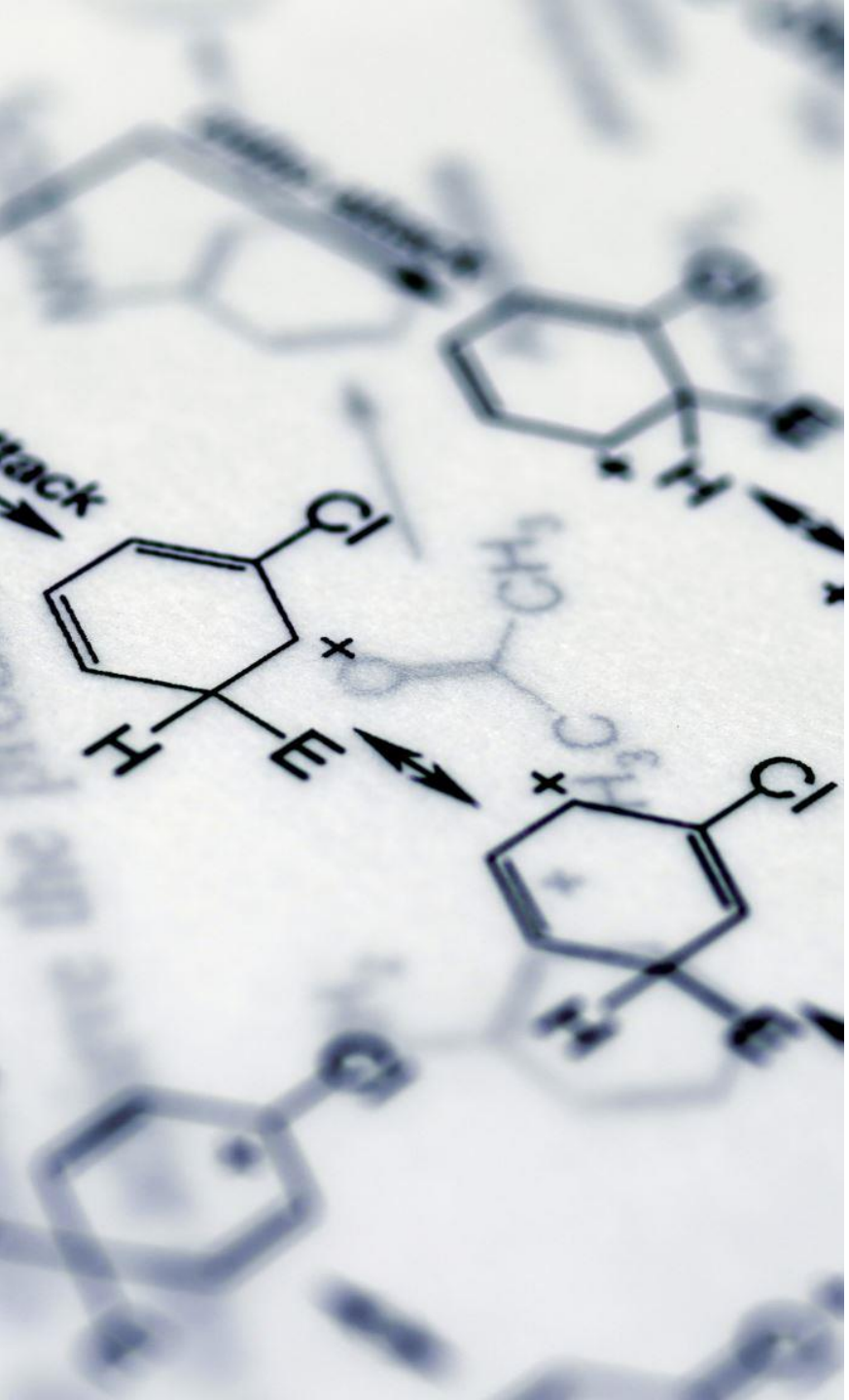
- Hallmarks of cancer: changes in cell physiology resulting from genetic changes that result in cancer.

SO:

- FOR CANCER TO DEVELOP: we need the 8 characteristics (hallmarks) plus the 2 enabling characteristics and the correct microenvironment.
- All the above are acquired as mutations or epigenetic alterations accumulating overtime.
- So: no single mutation is enough to cause cancer.

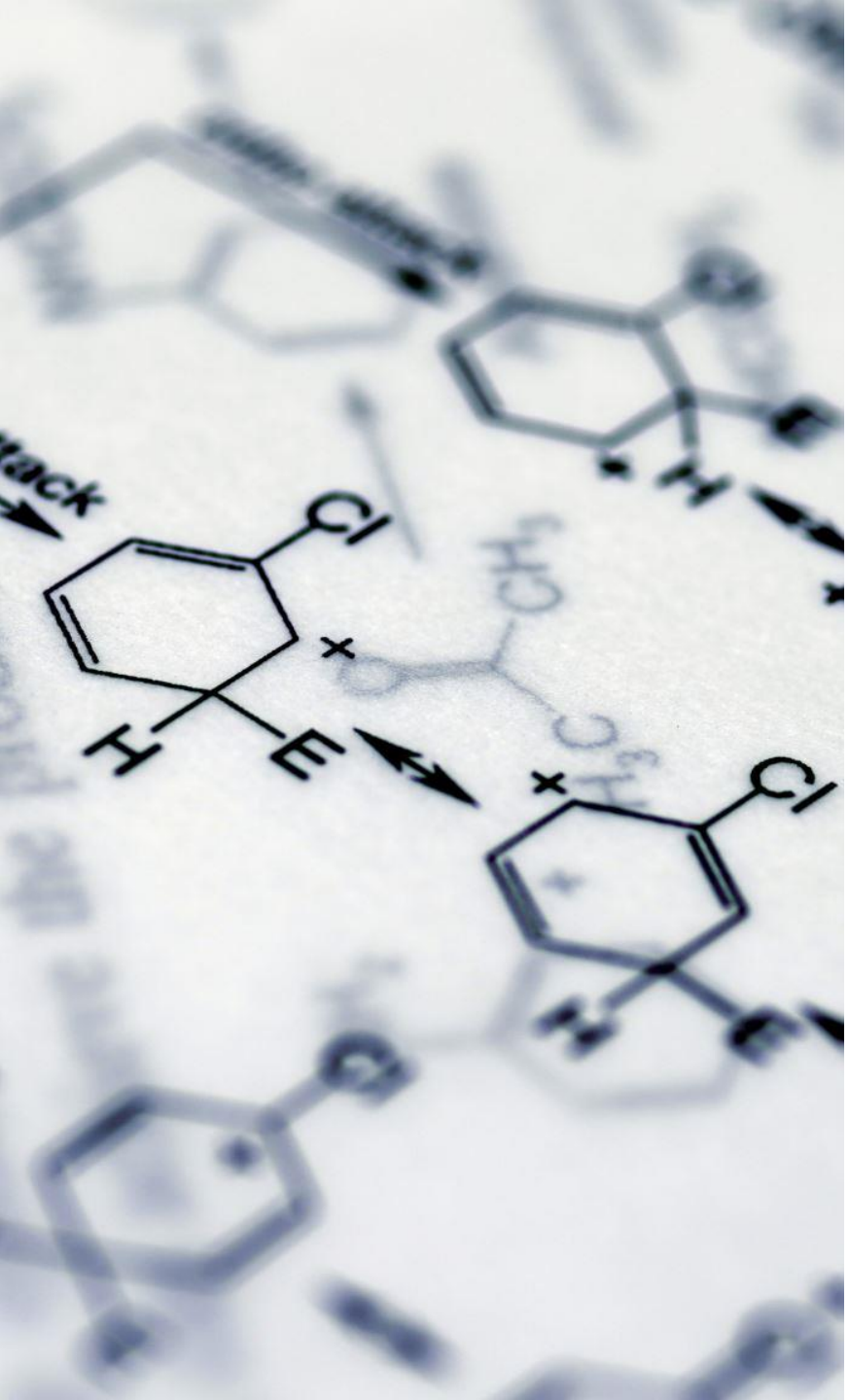
- Carcinogenesis is a multistep process.
- Each step results from a genetic change.
- Each genetic change results in acquiring a certain phenotype (one of the hallmarks).
- There is no specific sequence of acquiring these phenotypes… the main issue is to acquire them all.


Invasion and metastasis
Sustaining proliferative signaling
Evading growth suppressors
Reprogramming energy metabolism
Evading immune destruction
Resisting apoptosis
Enabling replicative immortality
Genomic instability
Tumor-promoting inflammation
Inducing angiogenesis



1. SELF SUFFICIENCY IN GROWTH SIGNALS

- Tumors proliferate regardless of the normal regulatory mechanisms.
- This is achieved by mutations in proto-oncogenes.
- Oncogenes cause increased growth by increasing oncoproteins which act as growth factors, growth factor receptors, transcription factors..etc

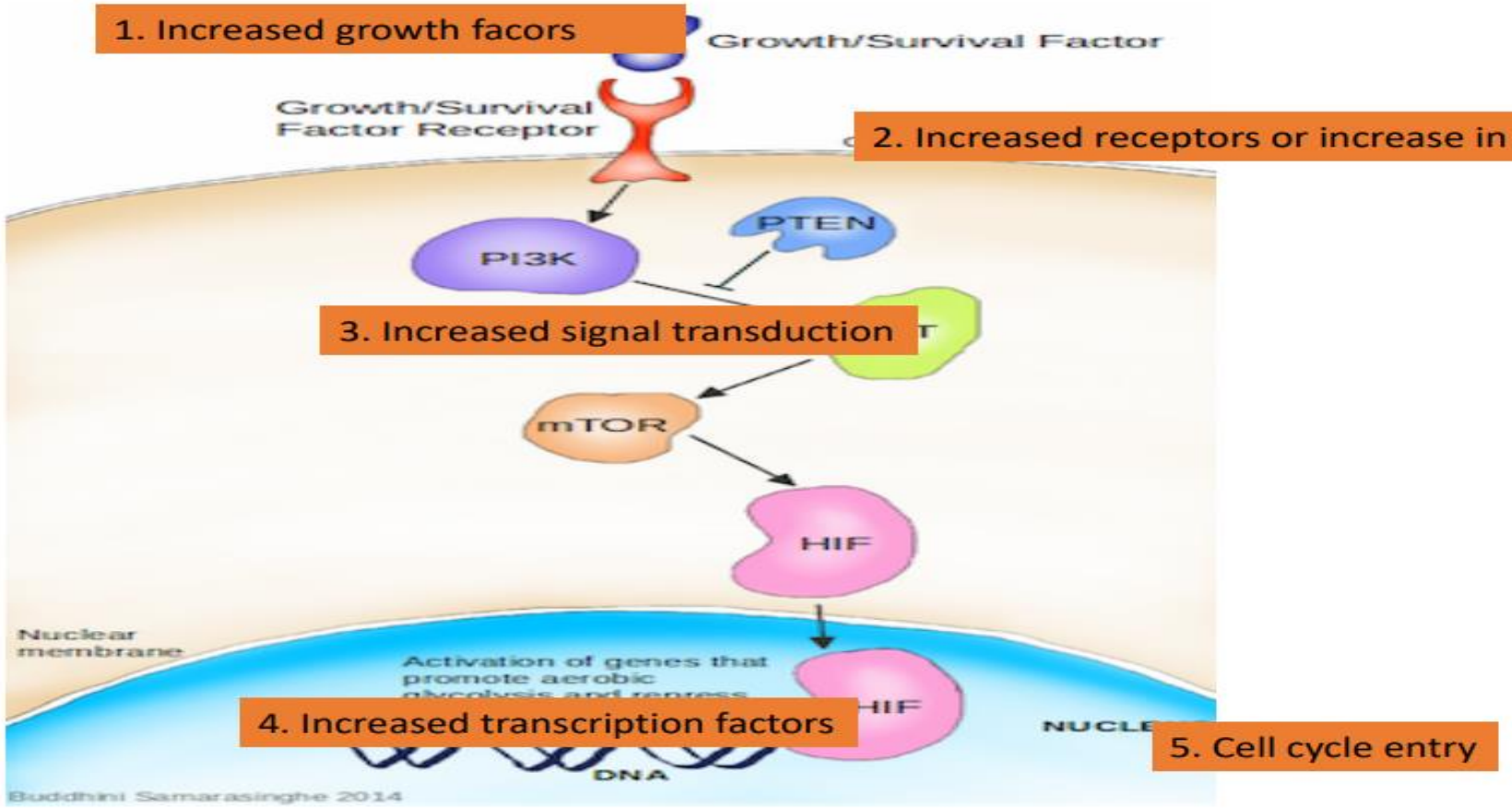


SELF-SUFFICIENCY IN GROWTH SIGNALS

Normal cell proliferation involves the following steps:

- Growth factor binding to cell surface receptor
- Transient and limited activation of the receptor and associated membrane or cytoplasmic signal-transduction proteins.
- Nuclear transmission via second messengers.
- Induction and activation of nuclear regulatory factors that initiate DNA transcription.
- Entry into and progression through the cell cycle.

INCREASED GROWTH CAN HAPPEN IF THERE IS INTERFERENCE WITH ANY OF THE 5 STEPS IN THE PREVIOUS SLIDE.



GROWTH FACTORS

Some tumors produce their own growth factors.

This creates an autocrine loop

Eg: glioblastomas produce PDGF and express its receptor

GROWTH FACTORS

Tumor cells can interact with their stroma to produce growth factors.

This is an example of how microenvironment (interaction with host cells including stromal cells) can help tumors to grow.

GF RECEPTORS

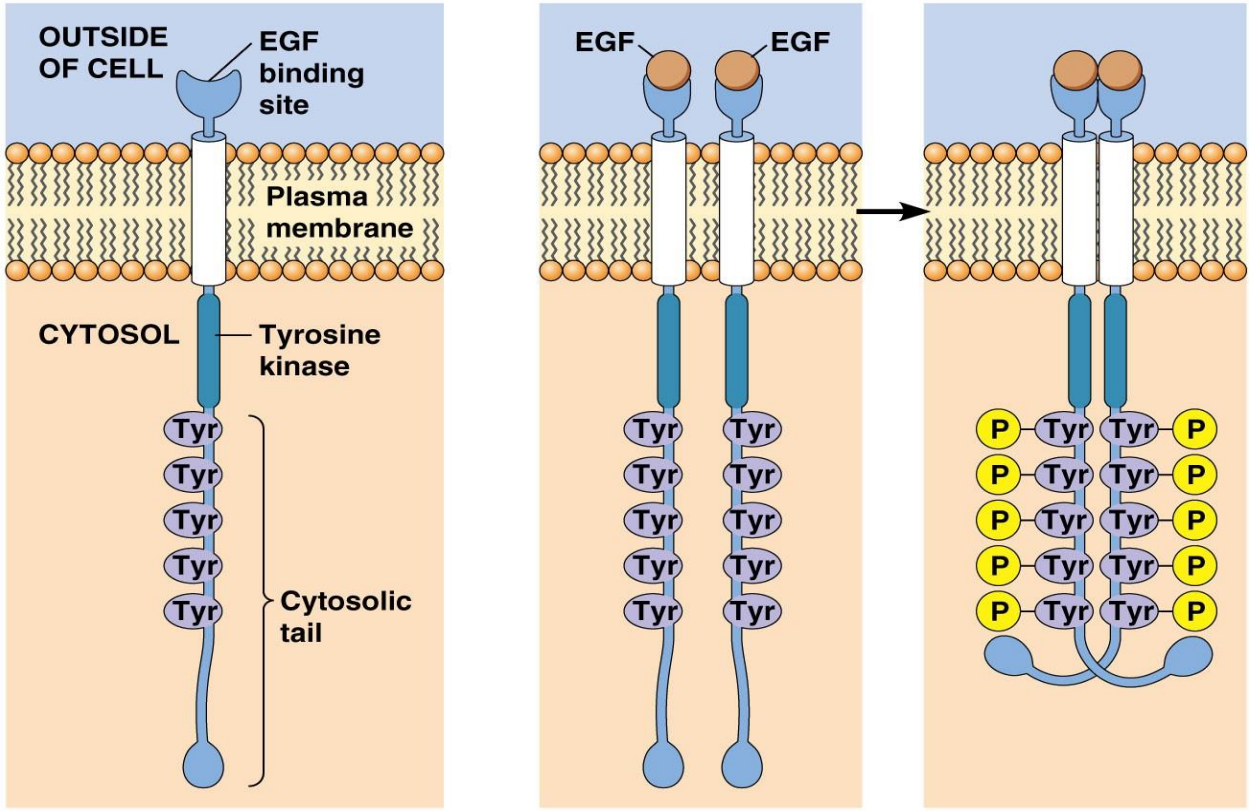
GF receptors can increase cell proliferation in two situations:

1. Overexpression of the receptor .. So, Receptor is hyper responsive to GF even in levels that don't normally trigger proliferation.

2. Mutant receptor proteins. So the receptor itself is mutated and acquires a configuration that is always stimulated

This causes continuous mitogenic signal even in the absence of GF.

NOTICE THE SHAPE CHANGE AND DIMERIZATION OF THIS RECEPTOR WHEN ATTACHED TO ITS LIGAND.



(a) Structure of the epidermal growth factor (EGF) receptor

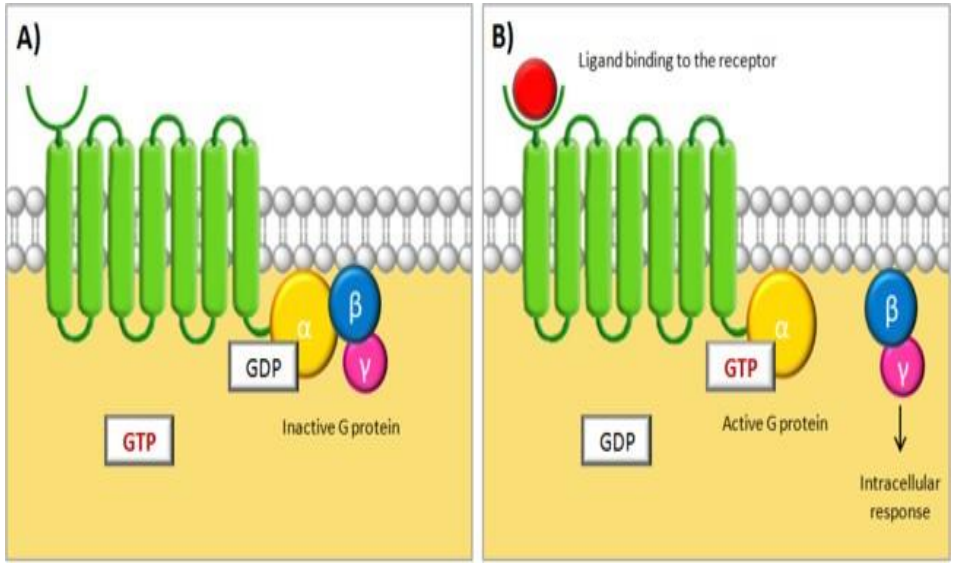
(b) Activation of the EGF receptor

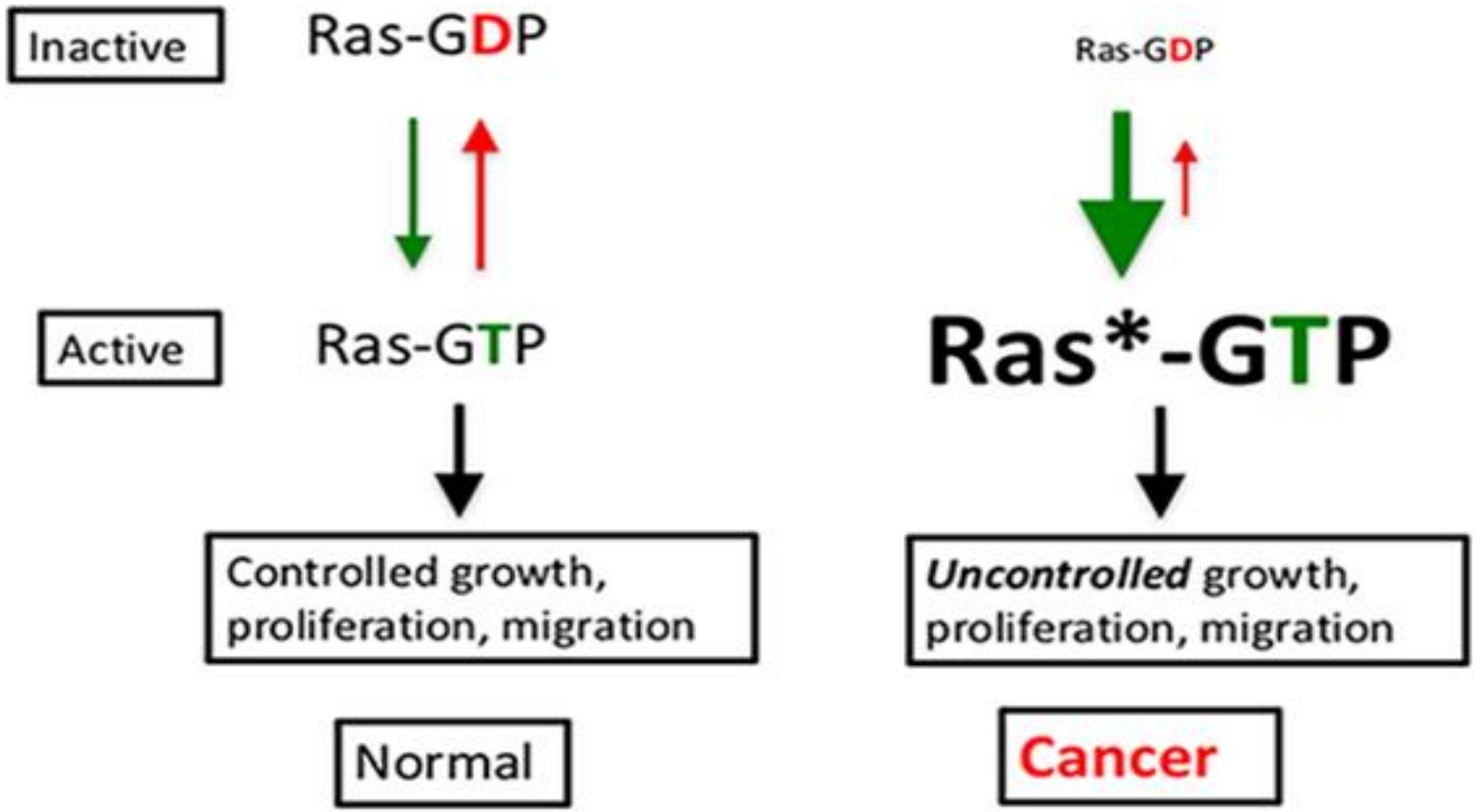
SIGNAL TRANSDUCING PROTEINS

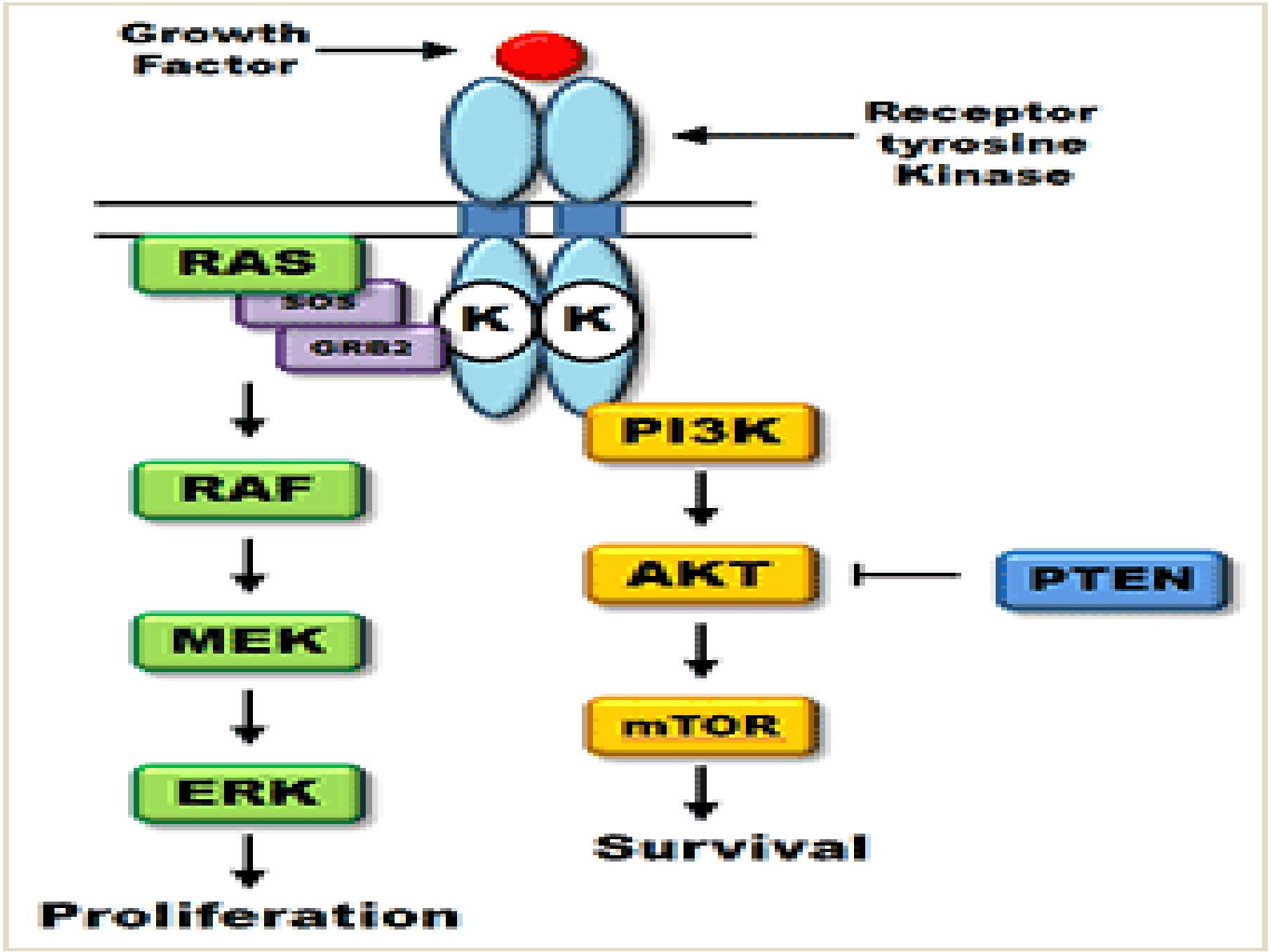
- Main signal transducing proteins involved: RAS and ABL

G COUPLED PROTEINS, WHICH RAS IS ONE OF THEM.

- Seven transmembrane alpha helices, coupled with G protein (GTP binding protein).
- Ligand binding: GDP in the G protein changes to GTP… receptor activated.




Inactive
Ras-GDP
Active
Ras-GTP
Controlled growth, proliferation, migration
Normal
Ras-GDP
Ras*-GTP
Uncontrolled growth, proliferation, migration
Cancer


Growth Factor
Receptor tyrosine Kinase
RAS
SOS
GRB2
K
K
PI3K
RAF
AKT
PTEN
MEK
mTOR
ERK
Survival
Proliferation

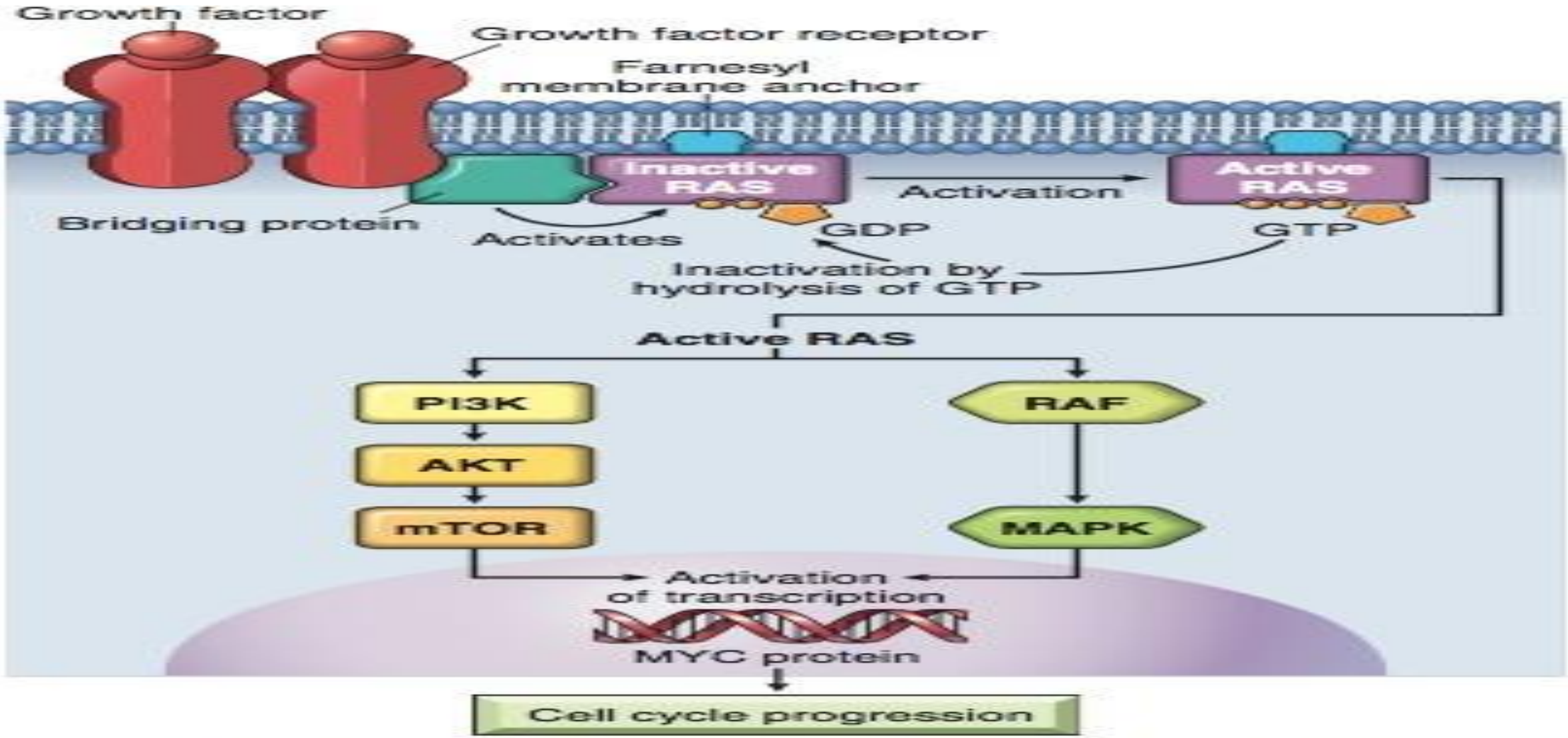
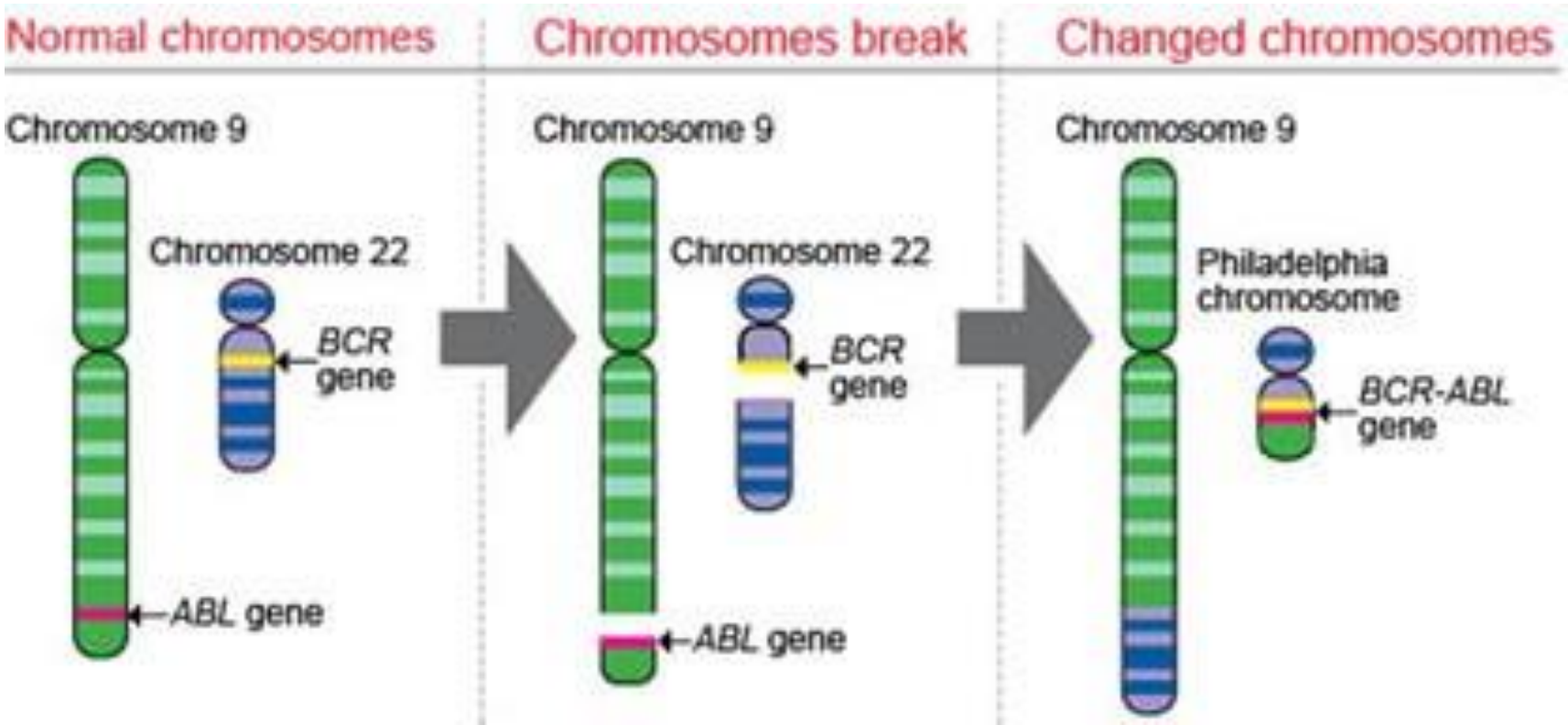


Fig. 6.18 Model for action of RAS. When a normal cell is stimulated through a growth factor receptor, inactive (GDP-bound) RAS is activated to a GTP-bound state. Activated RAS transduces proliferative signals to the nucleus along two pathways: the so-called "RAF/ERK/MAP kinase pathway" and the *PI3 kinase/AKT pathway*. *GDP*, Guanosine diphosphate; *GTP*, guanosine triphosphate; *MAP*, mitogen-activated protein; *PI3*, phosphatidylinositol-3.

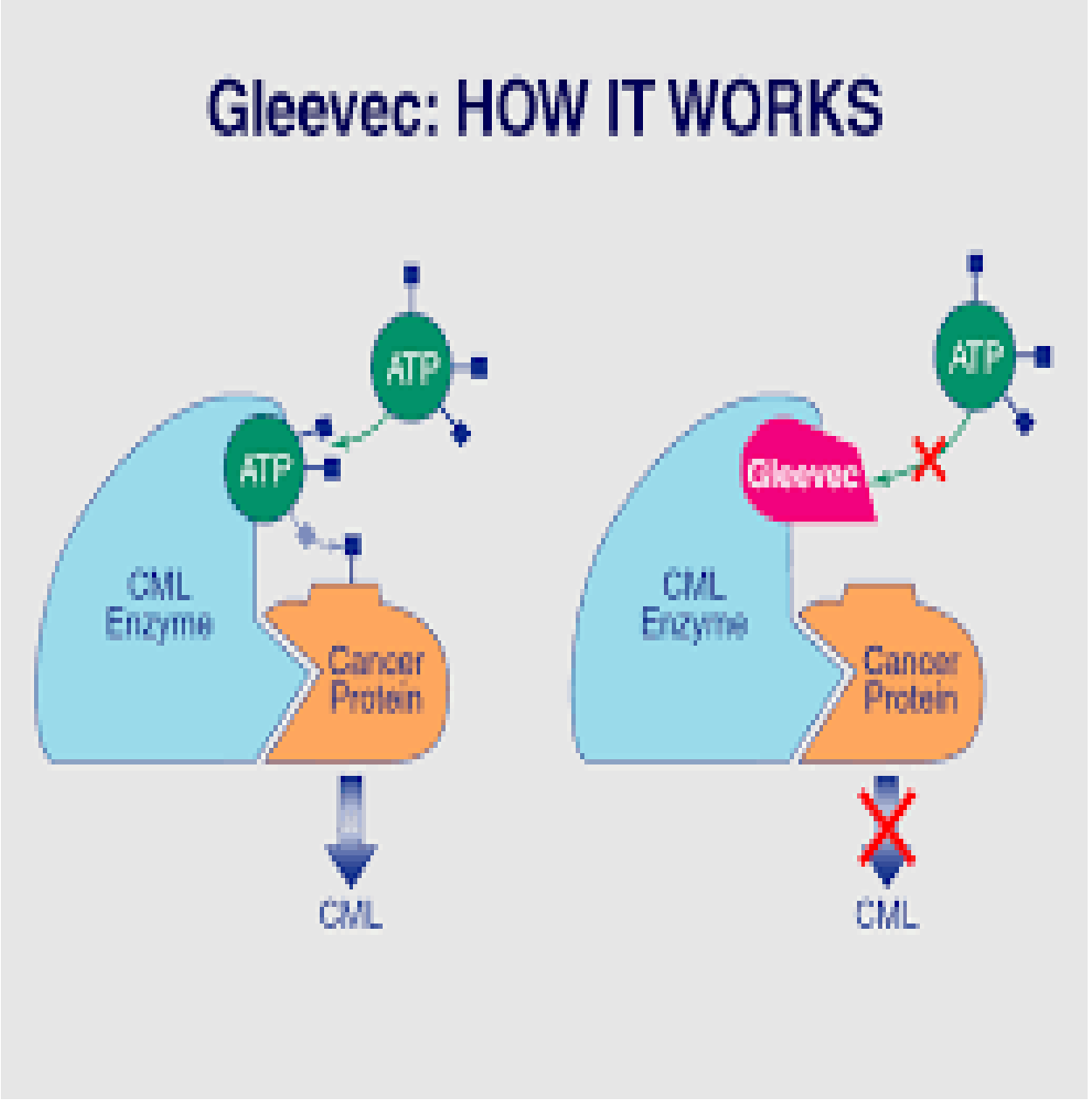
HOW RAS IS ACTIVATED (MUTATED)

- **Point mutation in an amino acid residues within the GTP binding pocket or in the enzymatic region of GTP hydrolysis** both result in defective hydrolysis.. Leading to trapped RAS in active phosphorylated GTP bound RAS

PHILADELPHIA CHROMOSOME




NOVARTIS
Gleevec®
(imatinib mesylate)
Tablets
100 mg
Rx only
Each tablet contains 100 mg
of imatinib free base.
90 Tablets


Gleevec: HOW IT WORKS
ATP
ATP
CML
Enzyme
Cancer
Protein
CML
ATP
Gleevec
CML
Enzyme
Cancer
Protein
CML

NUCLEAR TRANSCRIPTION FACTORS

- Most commonly involved in human cancers: MYC

MYC:

- Can activate or repress expression of other genes
- Activates cyclins and cyclin dependent kinases CDK (so cells enter cell cycle and divide)
- Myc also inhibits cyclin dependent kinase inhibitors (CDKI).
- These cyclins, CDK, CDKI play a role in entry to cell cycle, which we will discuss in detail in this lecture.

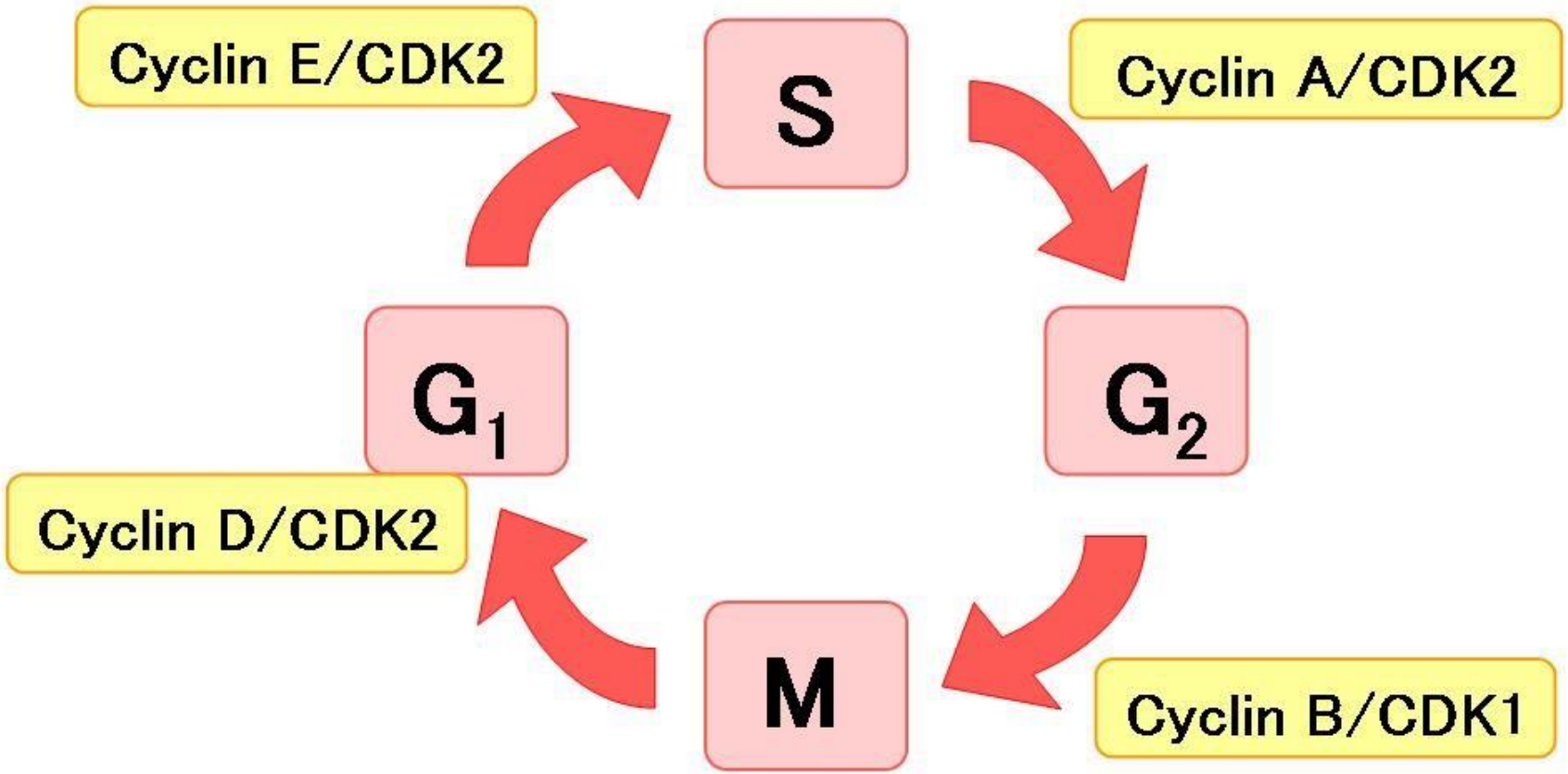
ENTRY INTO CELL CYCLE

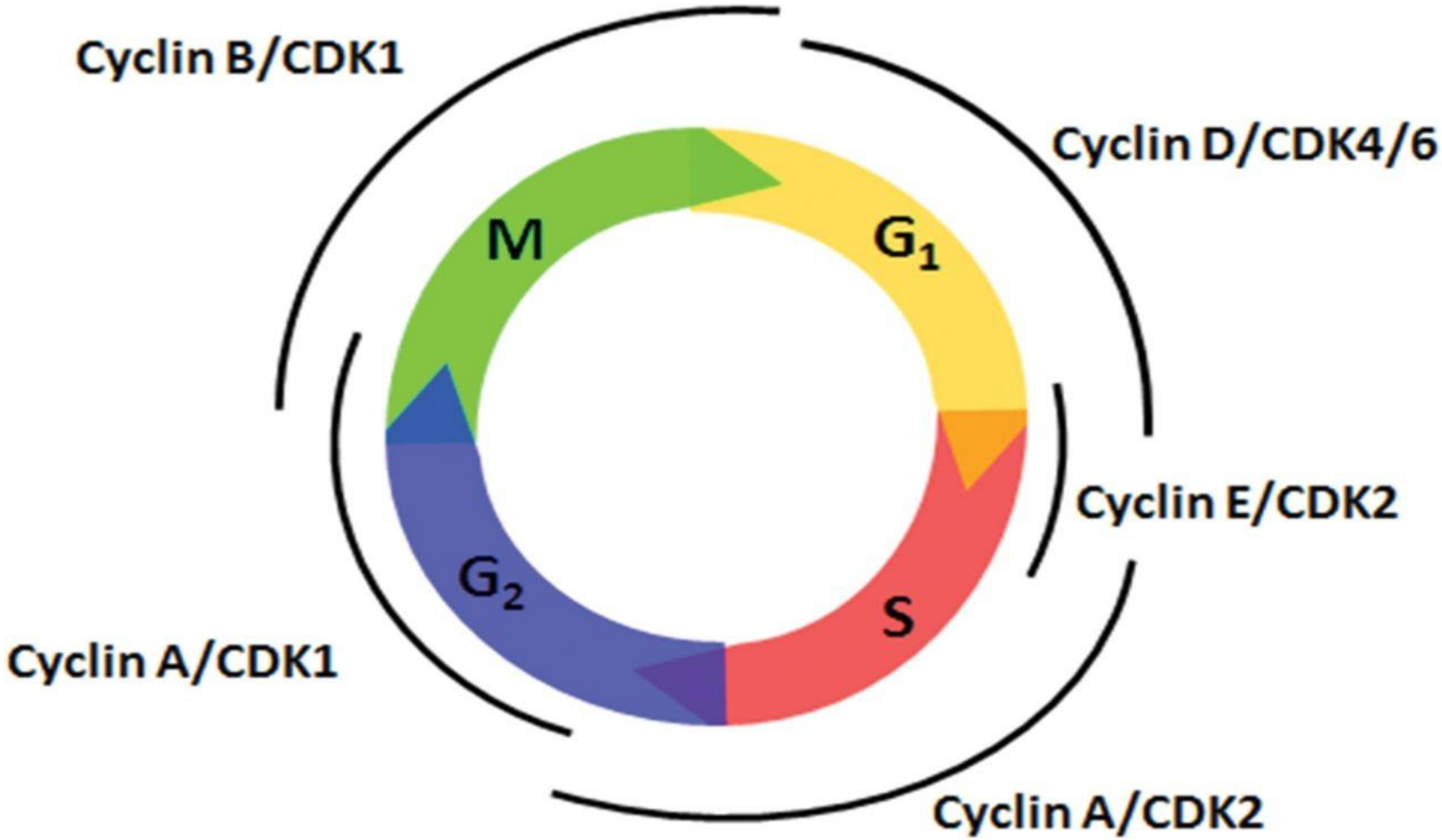
- All the stimuli mentioned till now aim for quiescent cells to enter the cell cycle .
- Each phase in the cell cycle depends on successful completion of the previous one
- Cycle stops when essential gene function is lost

CYCLINS

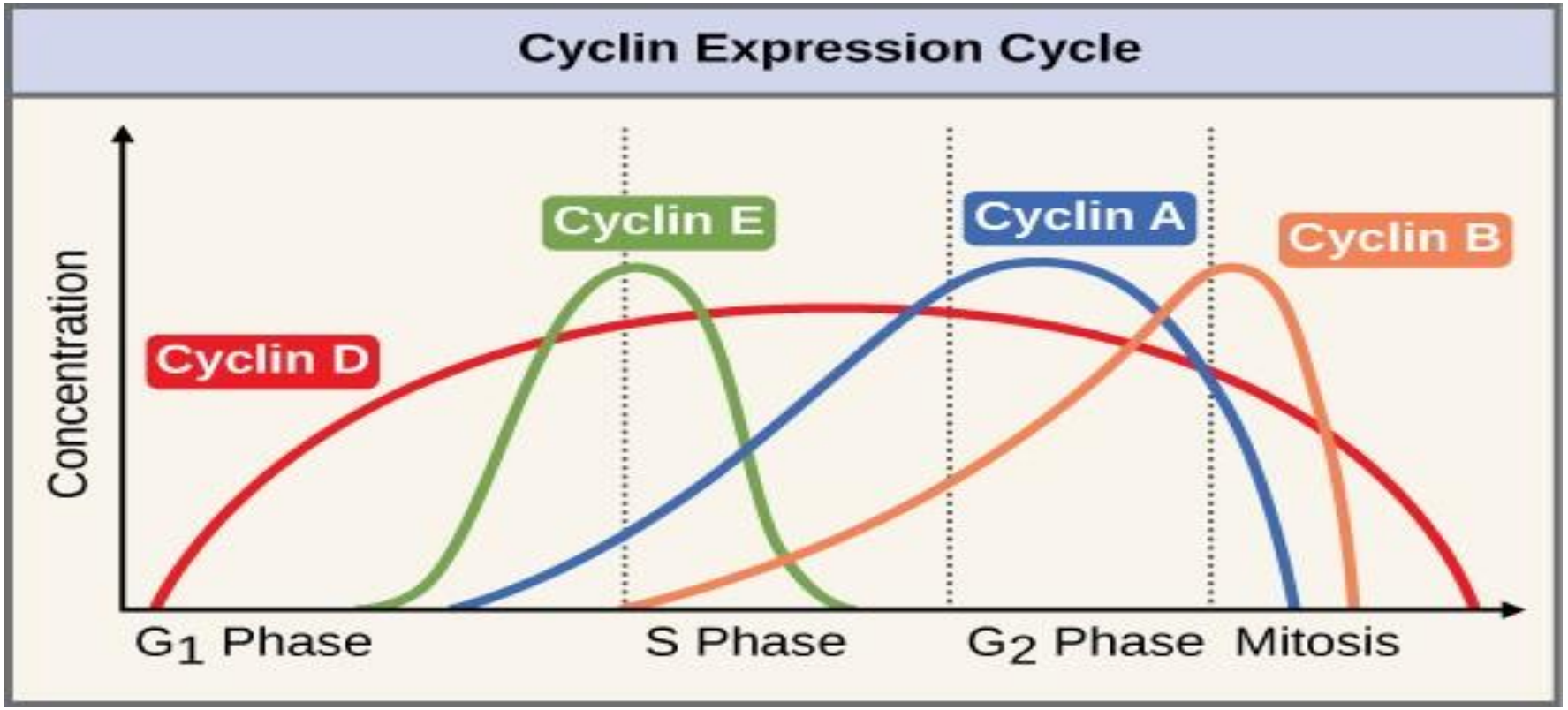
- Progression through cell cycle, especially G1-S is regulated by proteins called **cyclins**
- Cyclins activate kinases CDK (cyclin dependent kinases)
- Cyclin and CDK form complexes that phosphorylate target proteins that drive the cell through the cell cycle.
- Cyclins :D, E, A , B (they appear in this sequence)

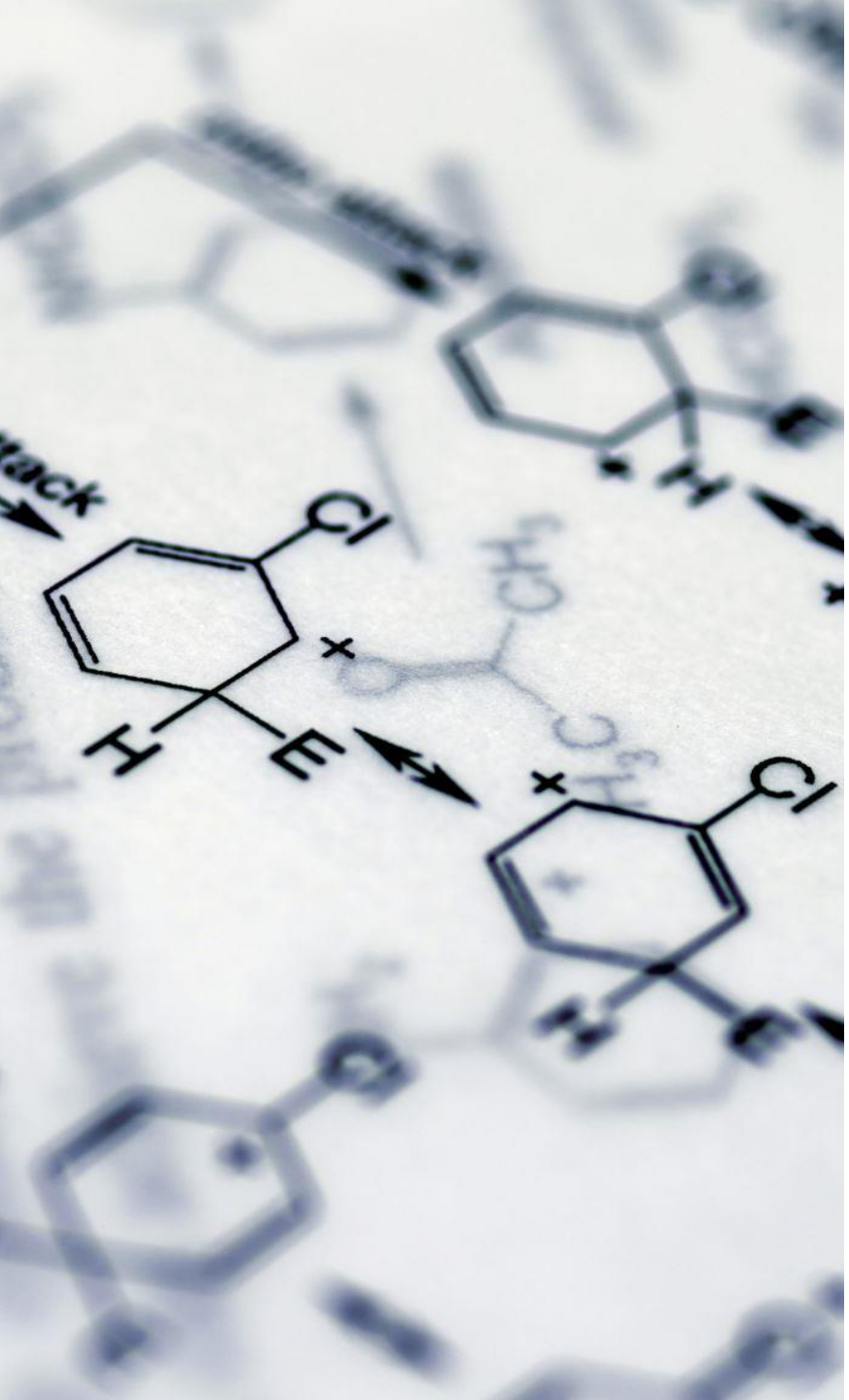
Cell Cycle and Cyclin–CDK complex



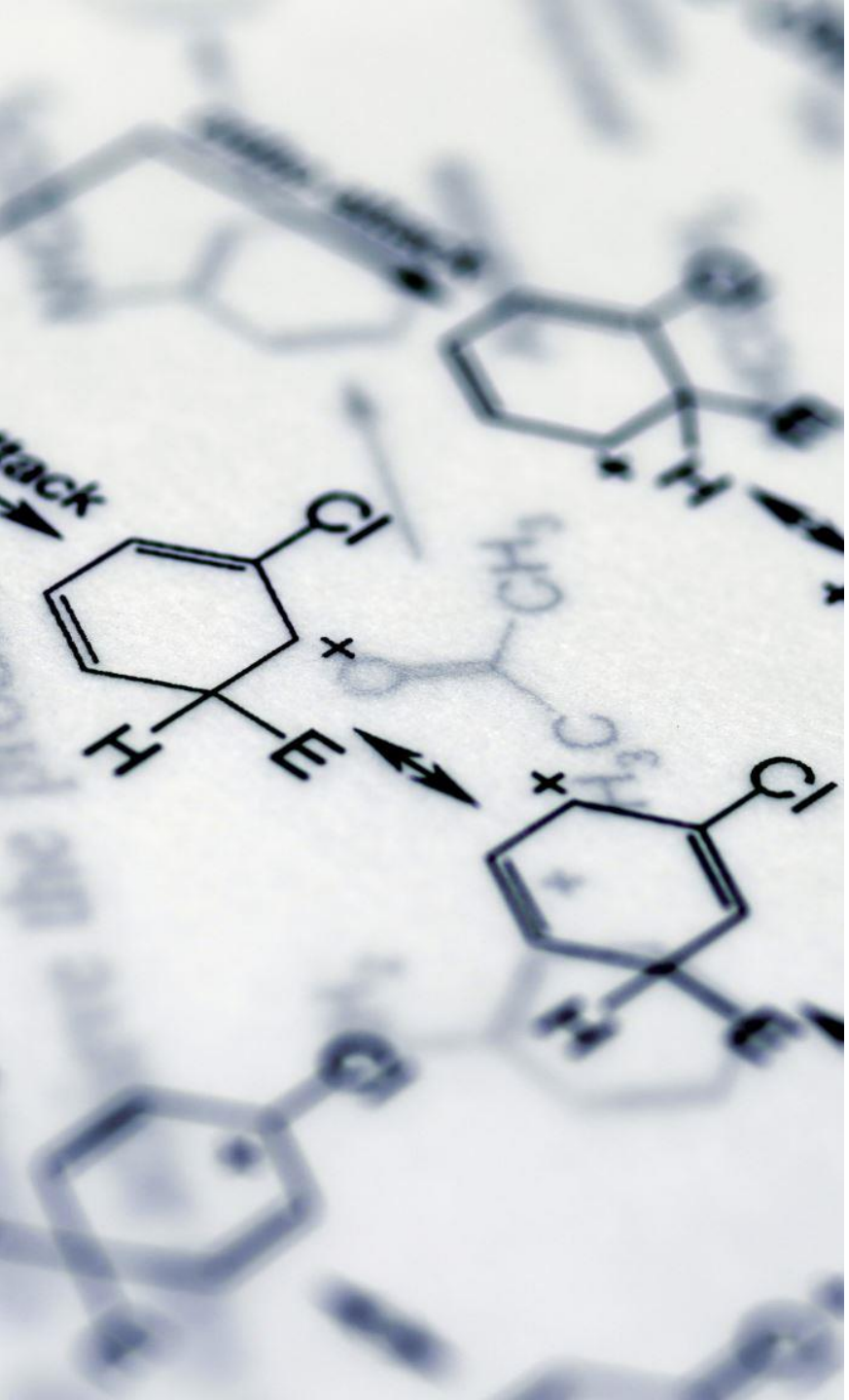

Cyclin B/CDK1
Cyclin D/CDK4/6
M
G1
Cyclin E/CDK2
G2
S
Cyclin A/CDK1
Cyclin A/CDK2

THE DEAB SEQUENCE OF CYCLINS !



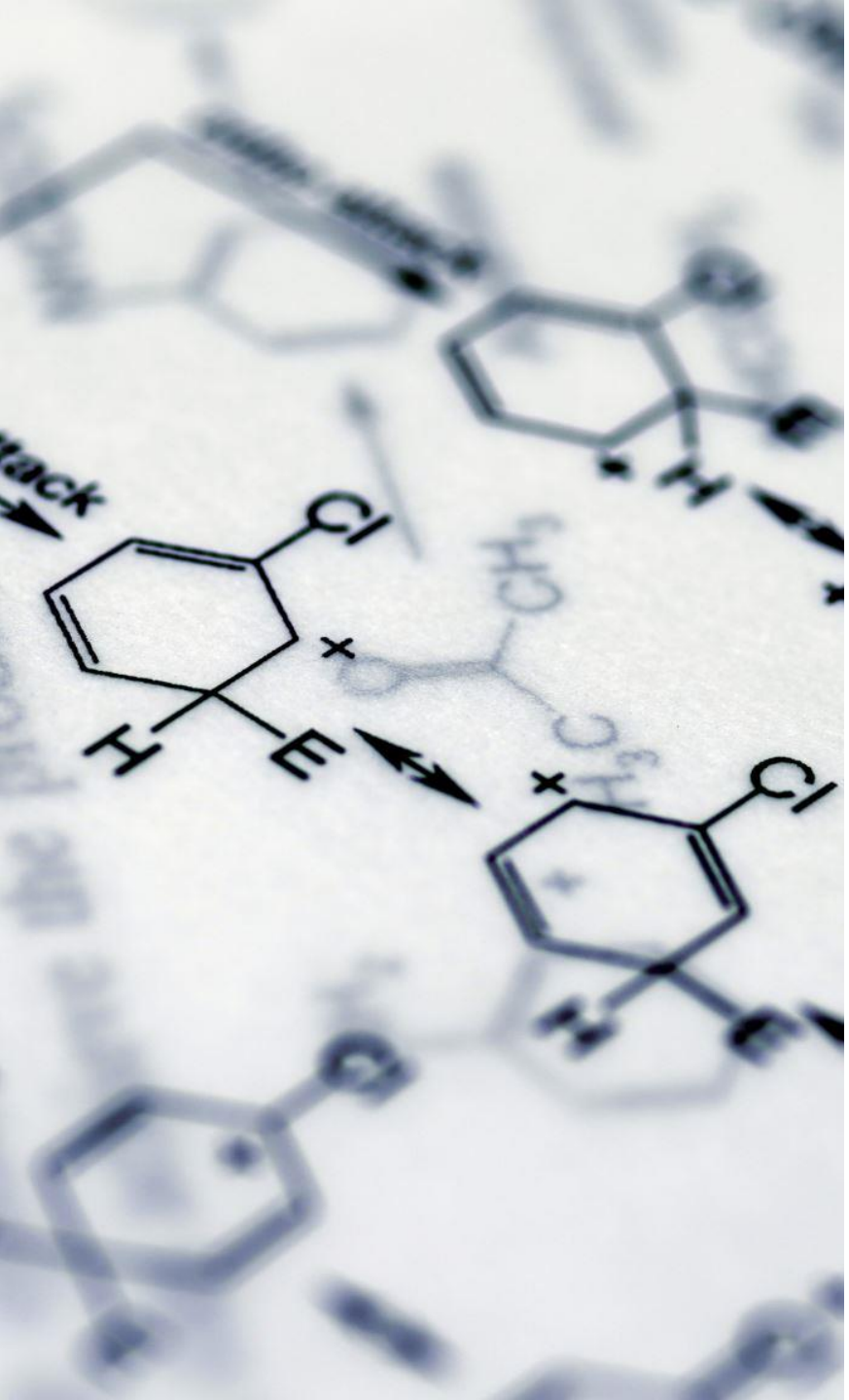


- SO: cyclin/CDK complexes cause proliferation.
- Theses are inhibited by cyclin dependent kinase inhibitors (CDKI)
- CDKI important for enforcing the checkpoints and delaying cell cycle.
- Mutations causing increased cyclins or CDK cause self sufficiency in growth signals.
- Mutations inhibiting CDKI will cause increased growth.
- Examples: cyclin D is activated in several tumors mainly lymphomas.



SECOND HALLMARK OF CANCER: INSENSITIVITY TO GROWTH INHIBITORS

- Main genes/ pathways mutated to cause insensitivity to growth inhibition:
- RB gene
- TP53 gene
- TGF beta pathway
- Contact inhibition
- APC gene



RB GENE

- RB gene= retinoblastoma gene = governor of cell cycle
- **RB is a key negative regulator of the cell cycle, it is directly or indirectly inactivated in most human cancers**
- The function of the RB protein is to **regulate the G1/S checkpoint**, the portal through which cells must pass before DNA replication starts.

HOW DOES RB ACT? ..THE DETAILS!

- RB is active at the beginning of G1 phase.
- This activity of RB depends on its phosphorylation state. Strangely RB is active
  - when it's hypo-phosphorylated. *Note that most of our proteins are activated by gaining phosphate groups. RB is an exception to this rule.*
- So: at the beginning of G1 phase, active, hypo-phosphorylated RB binds to E2F transcription factor family, preventing cyclin E formation.

HOW DOES RB ACT? ..THE DETAILS!

- *Cyclin E/ CDK2 complex is important for initiation of DNA replication (for entering the S phase*).
- **As long as cyclin E/CDK2 complex is not formed , cells are trapped in the G1 phase and cannot move to the S phase.. In other words they cannot cross the G1/S checkpoint**.
- Some cells leave cell cycle to G_0 or go into senescence at this stage.. But if they cross the G1/S they are committed to undergo mitosis without the need of any extra growth signals. That's why this checkpoint is important.

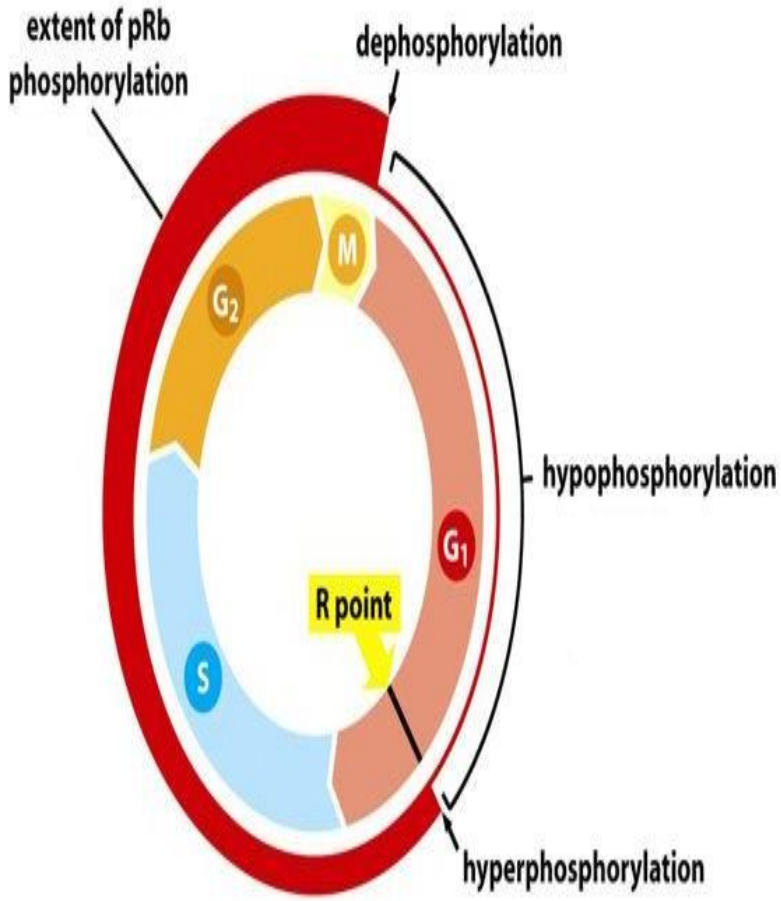
HOW DOES RB INACTIVATE E2F?

- This inactivation occurs via 2 mechanisms
- 1. RB **sequesters:** E2F which prevents E2F from interacting with other transcription factors.
- 2. RB recruits proteins that bind to and **inhibit E2F promotor.** This makes E2F gene insensitive to transcription factors
- The net result is preventing transcription of E2F.

RB regulates progression through G1 phase

- This pause is important because cells that cross the G1/S are committed to DNA replication.. We don't want this to happen unless cells have normal protein content and are really ready to divide.
- However, this inactivation cannot continue forever..We need to deactivate RB at a certain point to allow cells to enter the S phase.

Un- and **Hypo-**phosphorylated **pRb** inhibits the cell from entering a new cell cycle

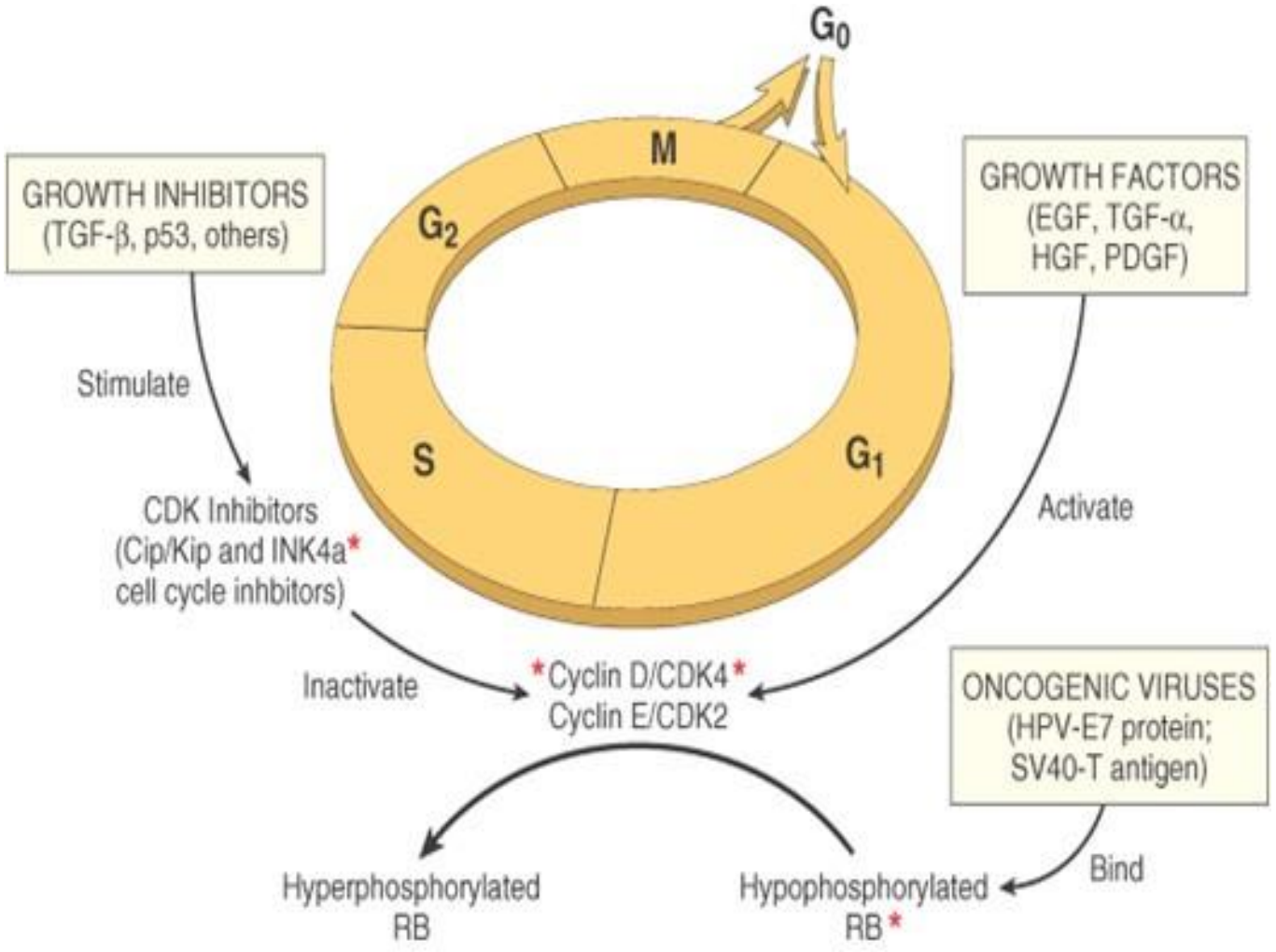


HOW RB IS DEACTIVATED/ HOW CELLS CAN CROSS THE G1/S CHECKPOINT?

- RB is deactivated by phosphorylation.
- This happens when cells are subject to mitogenic (growth) signals.
- Growth signals cause cyclin D expression.
- Cyclin D complexes with CDKs… these complexes phosphorylate and inactivate RB.
- Once RB is deactivated, E2F genes can be transcribed, resulting in cyclin E formation.
- Cyclin E/ CDK complexes can start the S phase.
- Remember the DEAB sequence we talked about last time.. Cyclin D is the first needed, then E.. Makes sense now??

Note that in the presence of growth inhibitory signals, RB is active and cells cannot divide With growth stimulatory signals RB is deactivated via cyclin D/ CDKs complexes

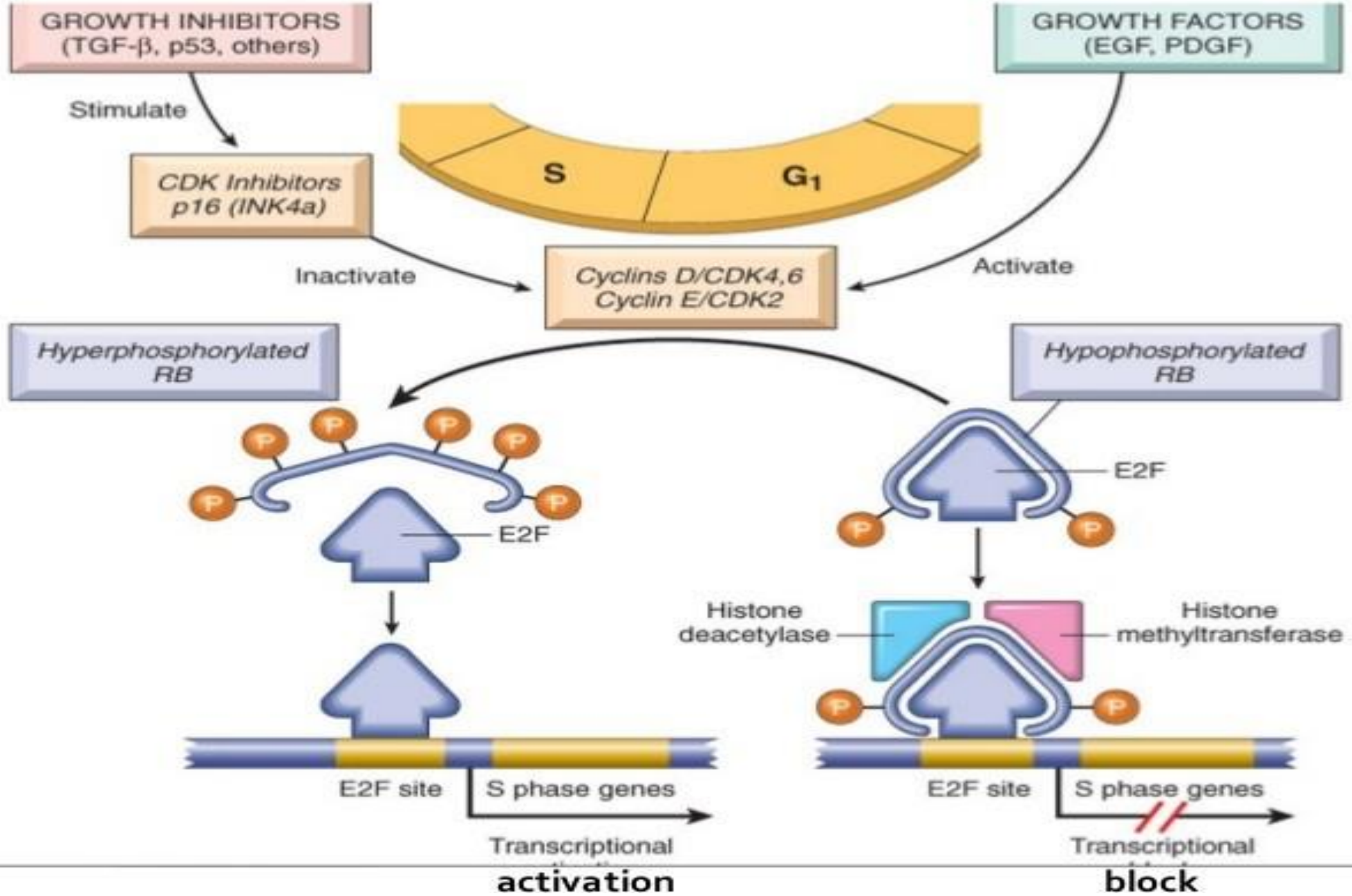
Note that certain viruses can deactivate RB by hyper-phosphorylating it.. This is how these viruses cause cancer (become oncogenic)



Role of RB as a cell cycle regulator

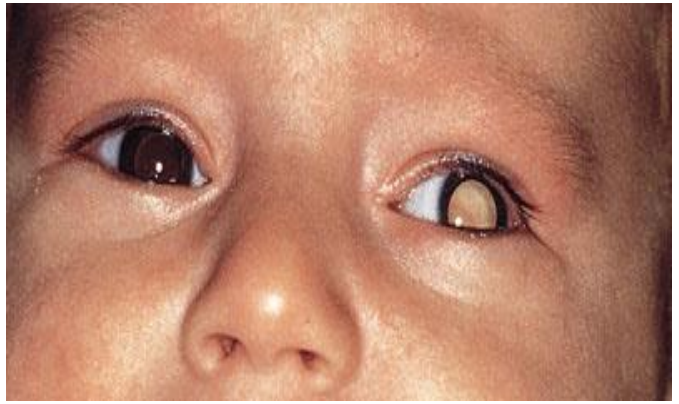
NOTE

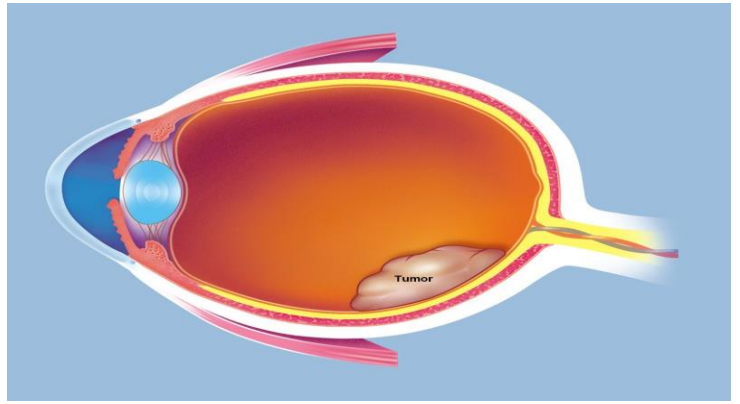
- Once in S phase cells are committed to division.. They don't need additional growth signals
- In M phase phosphate removed from RB ,so it goes back to its inactive state .


GROWTH INHIBITORS
(TGF-β, p53, others)
GROWTH FACTORS
(EGF, PDGF)
Stimulate
S
G1
CDK Inhibitors
p16 (INK4a)
Inactivate
Cyclins D/CDK4,6
Cyclin E/CDK2
Activate
Hyperphosphorylated
RB
Hypophosphorylated
RB
E2F
E2F
Histone
deacetylase
Histone
methyltransferase
E2F site
S phase genes
Transcriptional
E2F site
S phase genes
Transcriptional
activation
block

RB IS NAMED AFTER A TUMOR CALLED: RETINOBLASTOMA

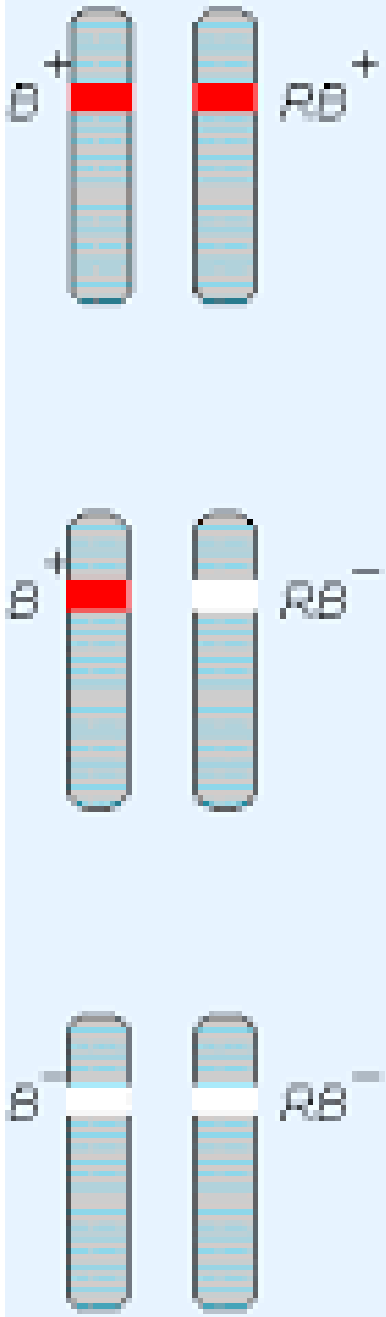
- Retinoblastoma is a rare childhood tumor affecting the eye (retino)
- RB gene was first discovered in this tumor and it's named after it
- However, RB is mutated in most human cancers, not just retinoblastoma tumor. People with inherited RB have increased risk of other cancers.. Mainly osteosarcomas and soft tissue sarcomas.





RETINOBLASTOMA

- 60 % of cases are sporadic, 40% familial
- In familial cases the predisposition to develop the tumor is inherited as an autosomal dominant trait
- However, to develop retinoblastoma: we need both copies of the RB gene to be mutated (remember that tumor suppressor genes are recessive)
- Loss of the two genes is called the two hit hypothesis



TWO HIT HYPOTHESIS

- There are two alleles for RB
- BOTH must be deleted or functionally deactivated before developing a tumor.
- In familial cases there is an inherited, germ line mutation in one of the alleles. The second allele is mutated or deleted later in somatic cells.
- In sporadic cases both alleles need to be mutated and/or deleted to have cancer.

TWO HIT HYPOTHESIS

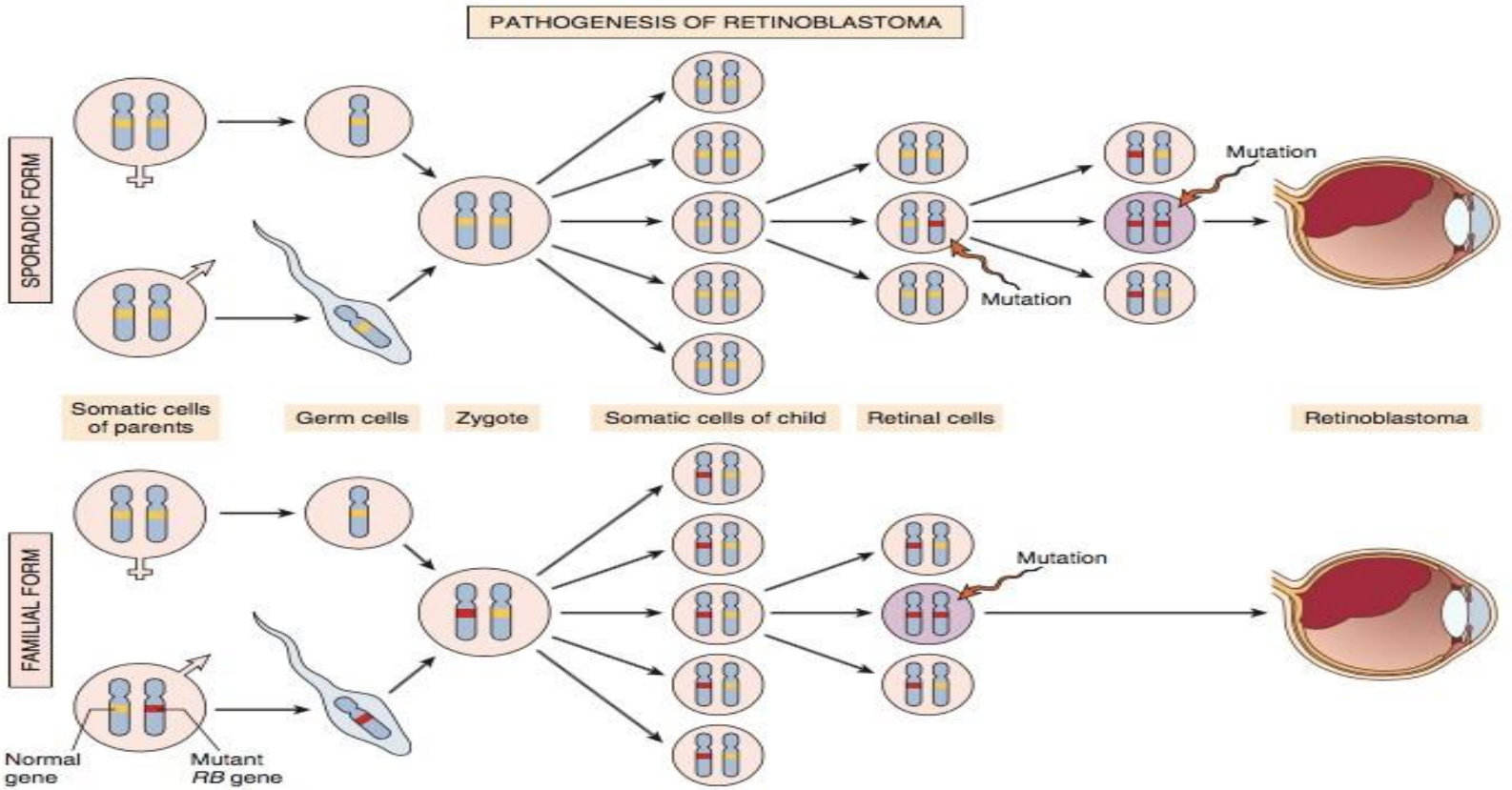
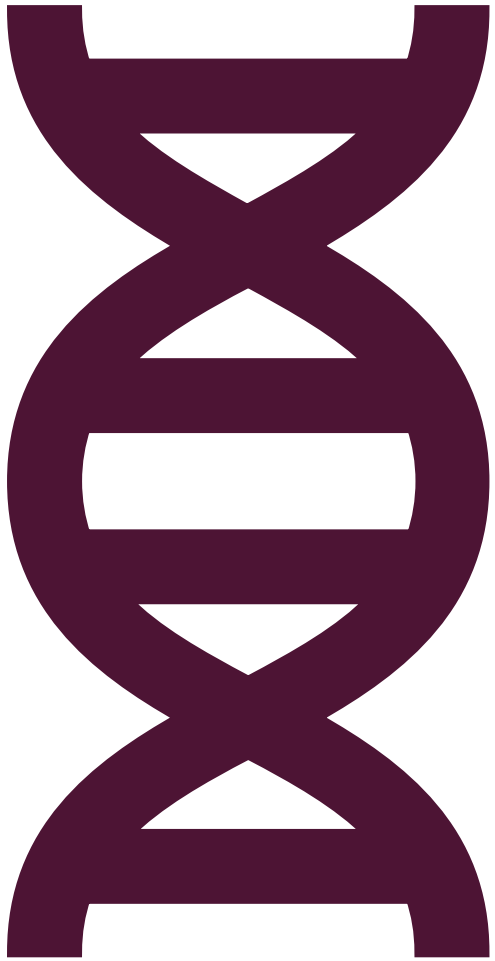


Fig. 6.19 Pathogenesis of retinoblastoma. Two mutations of the *RB* chromosomal locus, on 13q14, lead to neoplastic proliferation of the retinal cells. In the sporadic form, both *RB* mutations in the tumor-founding retinal cell are acquired. In the familial form, all somatic cells inherit one mutant *RB* gene from a carrier parent, and as a result only one additional *RB* mutation in a retinal cell is required for complete loss of *RB* function.

TWO HIT HYPOTHESIS

- Two mutations (hits) required to develop retinoblastoma
- The 2 mutations involve the RB gene on chromosome 13 (13q14)locus
- **Both copies of RB gene need to be deactivated to develop retinoblastoma**

- In familial cases, one hit is inherited (germ line mutation) the other is acquired
- In sporadic cases, both mutations are acquired



DON'T BE LOST IN TERMINOLOGY!!!

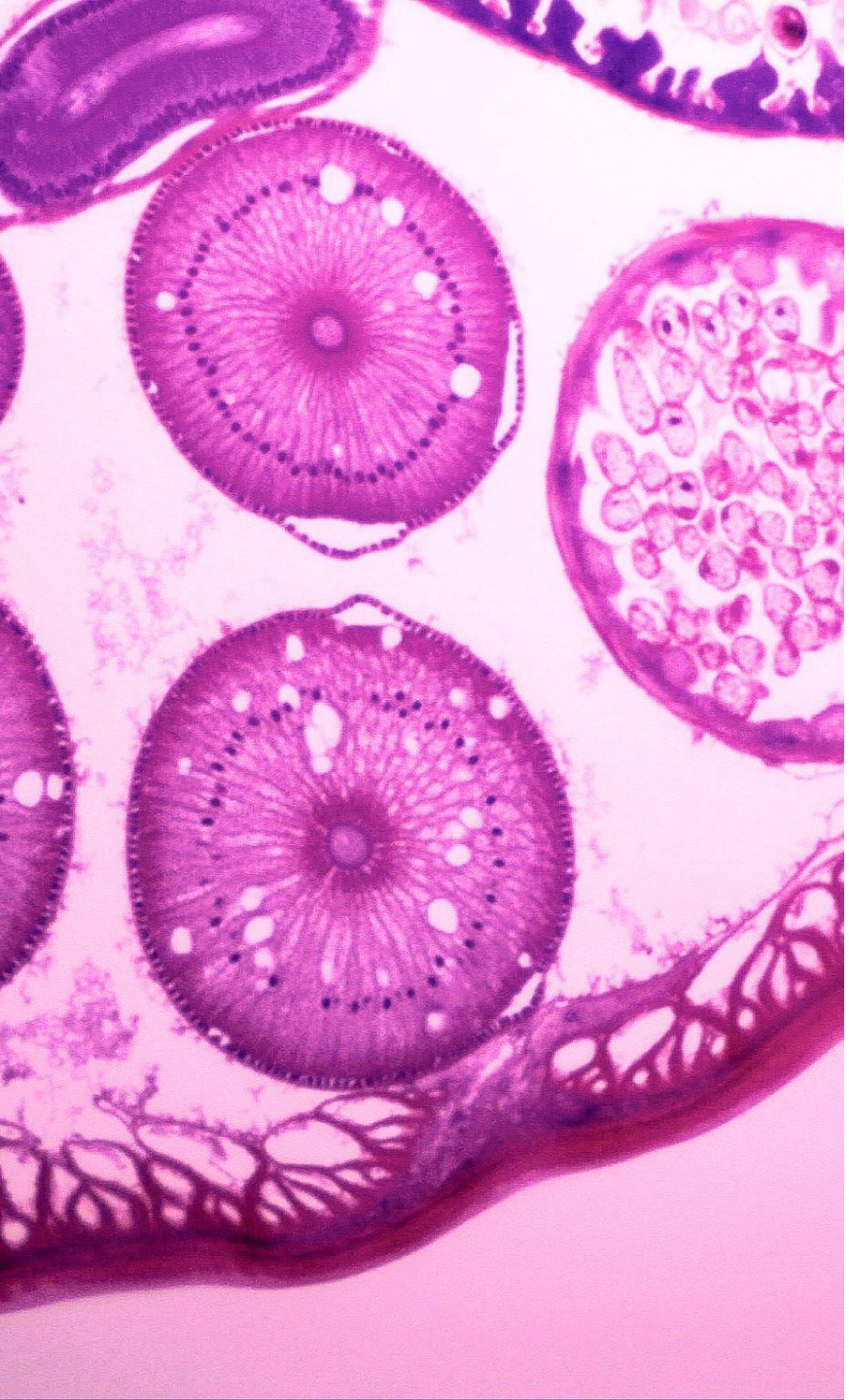
- Homozygous loss (both copies lost) of RB gene can be seen in many cancers like breast, bladder…
- **A cell heterozygous in RB locus is not neoplastic (one normal and one abnormal allele)**
- The two hits are essential for neoplastic transformation.. This makes both copies abnormal, and this is called homozygous loss.
- Note that people who inherit a germline mutation are born with a heterozygous RB (one normal copy and one mutated). When they acquire anther mutation, both copies become abnormal.. They loose the heterozygosity! This is called loss of heterozygosity (LOH)
- So LOH means: homozygous abnormality; both copies abnormal

MODE OF INHERITANCE OF RB

- Let's come back to this point: we said retinoblastoma is inherited as an autosomal dominant trait In familial cases, However, we know that RB gene is recessive?
- In familial cases affected individuals inherit one abnormal allele, this mutation is enough to transmit the risk of malignant transformation because almost all those born with one abnormal gene will acquire a second somatic mutation.
- This means all those born with one abnormal chromosome will have the disease.
- In other words, one abnormal inherited mutation will result in the phenotype (increased risk of developing cancer).. By definition, this is a dominant trait.
- But we know that the phenotype needs a second mutation.. Because the gene is recessive.
- This concept applies to ALL inherited syndromes that cause tumors by tumor suppressor genes.. We will discuss these in the relevant lectures.

NOTE

- **Loss of nomal cell cycle control is found in all tumors through mutations of RB, cyclin D, CDK4 or CDKN2A (which is a CDKI).**
- Some viruses like HPV have protein (E7) which binds to the hypophsphorylated RB and prevents it from inhibiting E2F so RB is functionally deleted.



TP53 GENE: THE GUARDIAN OF THE GENOME

This is also an important regulator of cell cycle.
P53 causes growth inhibition by three mechanisms

1. Temporary cell cycle arrest: quiescence
2. permanent cell cycle arrest: senescence
3. Triggering apoptosis

- TP53 is one of the most commonly mutated genes in cancer
- It encodes p53 protein

- **P53 is triggered by several stresses: anoxia, inappropriate oncogene activity (MYC or RAS) or DNA damage.**

HOW P53 WOKS?

- In non-stressed healthy cells, p53 is short lived: 20 minutes because it binds MDM2 which is a protein that targets it for destruction via ubiquitin proteasome pathway.
- When cells are stressed ..sensors that include protein kinases are activated (ATM is one of these kinases)
- These activated kinases catalyze post translational modifications of p53 and release it from MDM2
- Now p53 has longer life span and can drive transcription of certain genes.. hundreds of them

ONCE ACTIVATED P53 CAUSES TRANSCRIPTION OF MANY GENES INCLUDING:

- 1. genes mediating cell cycle arrest, like p21.
- 2. DNA repair genes.
- 3. genes involved in senescence like CDKI
- 4. pro-apoptotic genes including BAX and PUMA

CELL CYCLE ARREST

- **1. genes mediating cell cycle arrest. This occurs late in G1. caused by p53 dependent transcription of CDKI gene= p21:**
  - P21 protein inhibit cyclin/CDK complexes and prevents phosphorylation of RB…. So cell is arrested in G1 …. Pause to repair any DNA damage

- **2. expression of DNA damage repair genes:**
  - If DNA is repaired successfully ,p53 upregulates transcription of MDM2.. Destruction of p53.. Removal of the block on cell cycle.
  - If DNA not repaired p53 makes cells enter apoptosis or senescence
- **3. Senescence needs activation of p53 and or RB and expression of their mediators like CDKI:**
  - Mechanisms of senescence unclear but seem to involve global chromatin change, with permanent change gene expression

4. P53 INDUCED APOPTOSIS

- Induced by pro-apoptotic genes including BAX and PUMA
- P53 also **represses** proliferative and anti-apoptotic genes (bcl2)
- P53 is a transcriptional activator so how could it repress certain gene expression
- Answer by miRNAs.. P53 stimulates expression of micrRNAs that result in inactivation of certain genes, like the example above: bcl2.

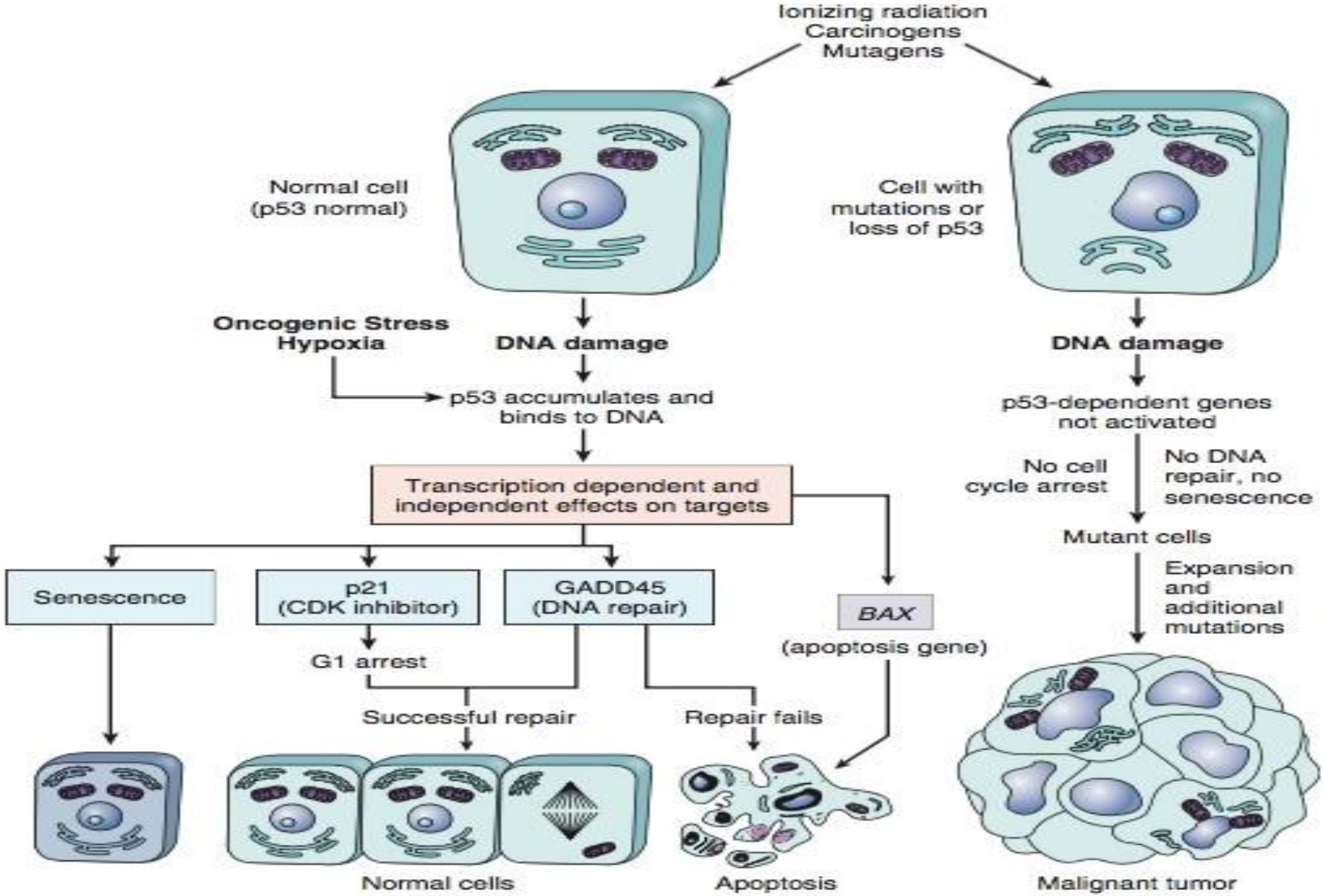


Fig. 6.21 The role of p53 in maintaining the integrity of the genome. Activation of normal p53 by DNA-damaging agents or by hypoxia leads to cell cycle arrest in G1 and induction of DNA repair, by transcriptional upregulation of the cyclin-dependent kinase inhibitor CDKN1A (p21) and the *GADD45* genes. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, p53 triggers either apoptosis or senescence. In cells with loss or mutations of TP53, DNA damage does not induce cell cycle arrest or DNA repair, and genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.

- More than 70% of human cancers have mutated TP53.
- Both copies of the gene need to be lost for cancer to develop.
- Mostly somatic.
- Rare Li Fraumeni syndrome: inherit defect in one allele.. More predisposition to cancer (Sarcoma, breast carcinoma , leukemia and brain tumor).
- P53 can become nonfunctional by some DNA viruses (HPV, Hep B, EBV)Proteins can bind to p53 and deactivate it.

- Note p53 activated by phosphorylation

REFERENCE: ROBIN BASIC PATHOLOGY, 10TH EDITION

THANK YOU