Neoplasia
Lecture 3

CARCINOGENESIS

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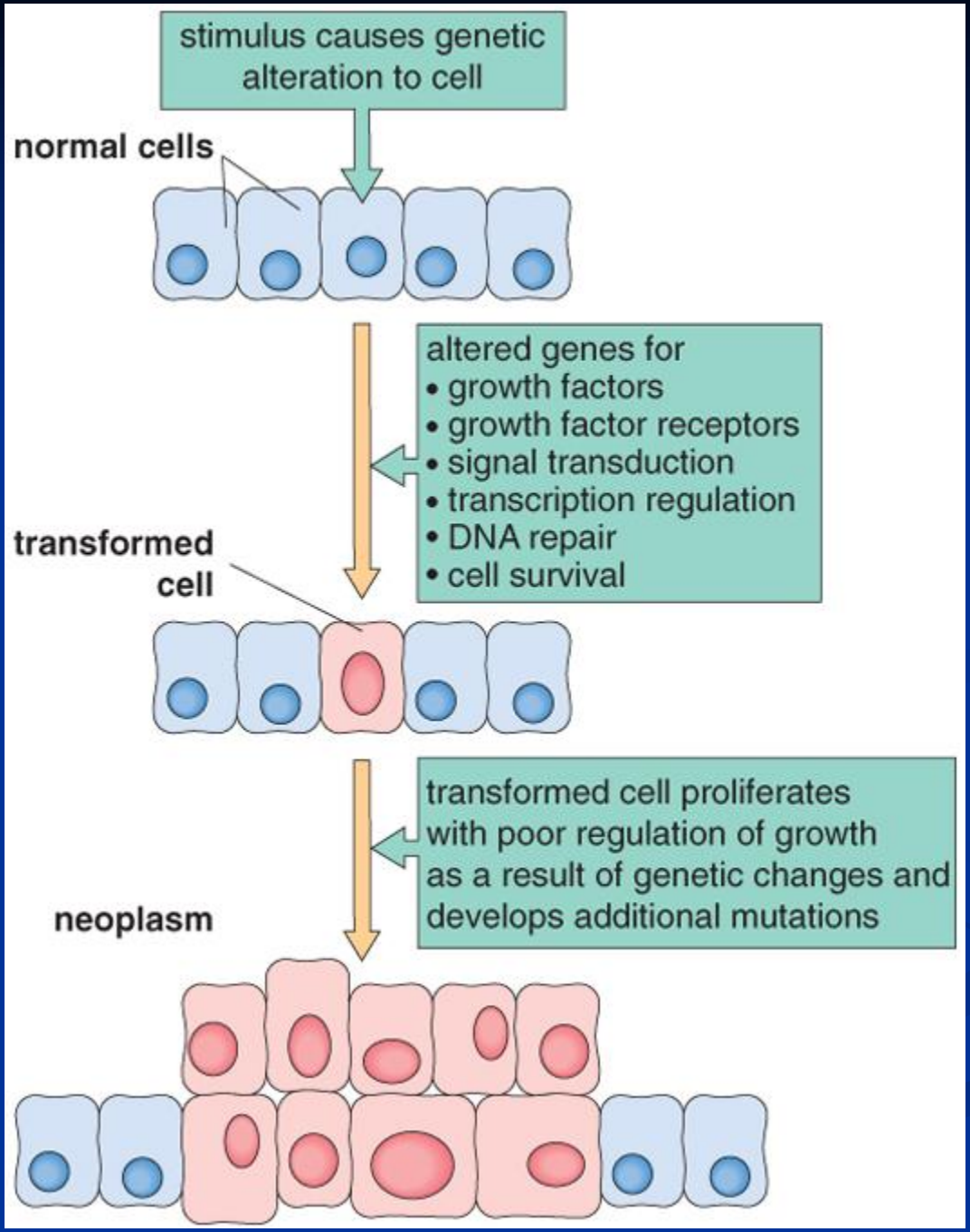
Foundation block 2014
Pathology

CARCINOGENESIS

- Carcinogenesis is a multistep process at both the phenotypic and the genetic levels.
- It starts with a genetic damage:
  - Environmental
    - Chemical
    - Radiation
    - Infectious
  - Inhereted

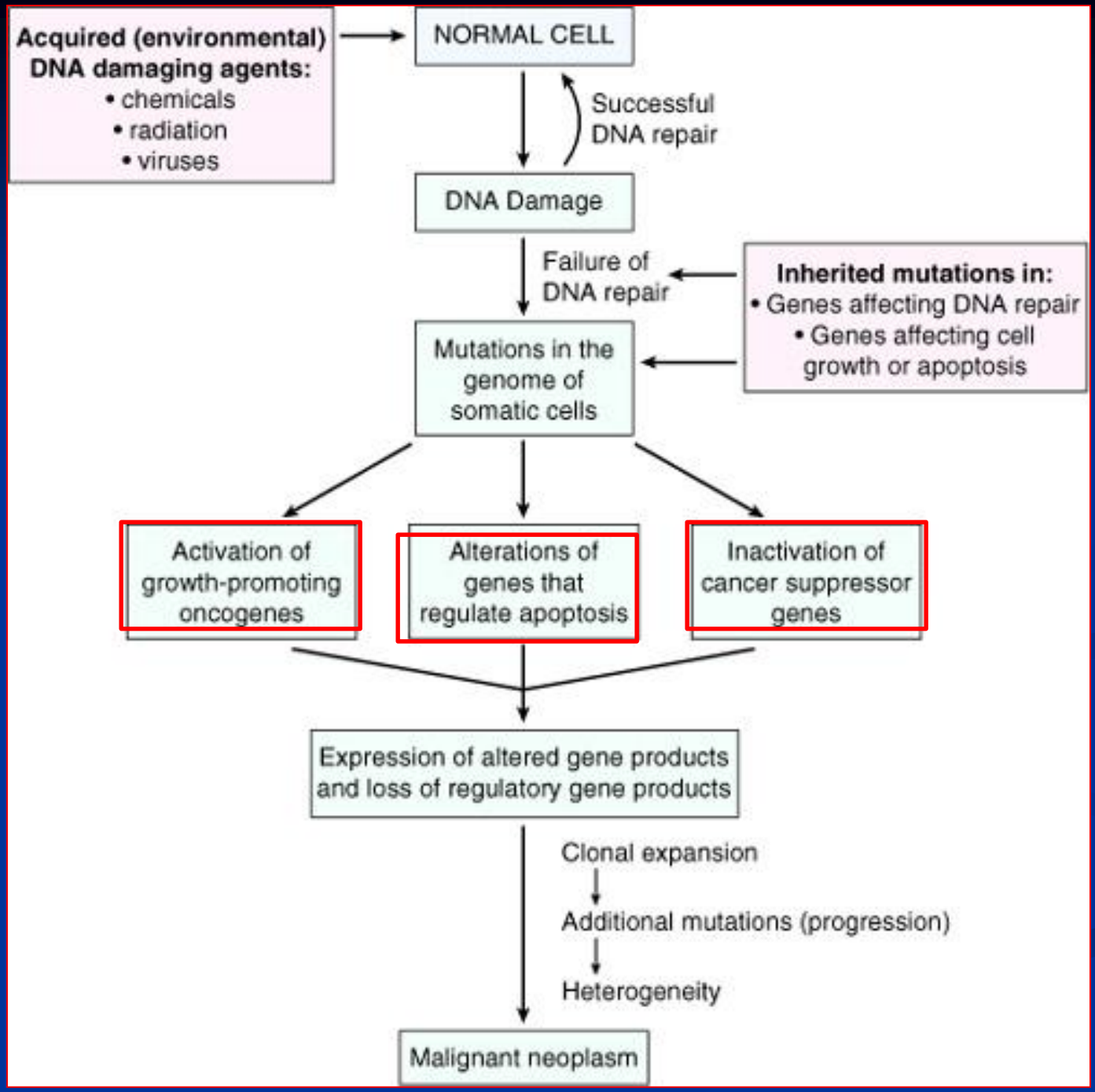
Carcinogenesis

- Genetic damage lead to “ mutation”
- single cell which has the genetic damage undergoes neoplastic proliferation (clonal expansion) forming the tumor mass


stimulus causes genetic alteration to cell
normal cells
altered genes for
• growth factors
• growth factor receptors
• signal transduction
• transcription regulation
• DNA repair
• cell survival
transformed cell
transformed cell proliferates with poor regulation of growth as a result of genetic changes and develops additional mutations
neoplasm

Carcinogenesis

- Where are the targets of the genetic damage??
- Four regulatory genes are the main targets:
  - Growth promoting protooncogenes
    - Protooncogene > mutation > oncogene
  - Growth inhibiting (supressors) genes
  - Genes regulating apoptosis
  - DNA repair genes


Acquired (environmental) DNA damaging agents:
• chemicals
• radiation
• viruses
NORMAL CELL
Successful DNA repair
DNA Damage
Failure of DNA repair
Inherited mutations in:
• Genes affecting DNA repair
• Genes affecting cell growth or apoptosis
Mutations in the genome of somatic cells
Activation of growth-promoting oncogenes
Alterations of genes that regulate apoptosis
Inactivation of cancer suppressor genes
Expression of altered gene products and loss of regulatory gene products
Clonal expansion
Additional mutations (progression)
Heterogeneity
Malignant neoplasm

Carcinogenesis

- Main changes in the cell physiology that lead to formation of the malignant phenotype:
  - Self-sufficiency in growth signals
  - Insensitivity to growth-inhibitory signals
  - Evasion of apoptosis
  - Limitless replicative potential
  - Sustained angiogenesis
  - Ability to invade and metastsize

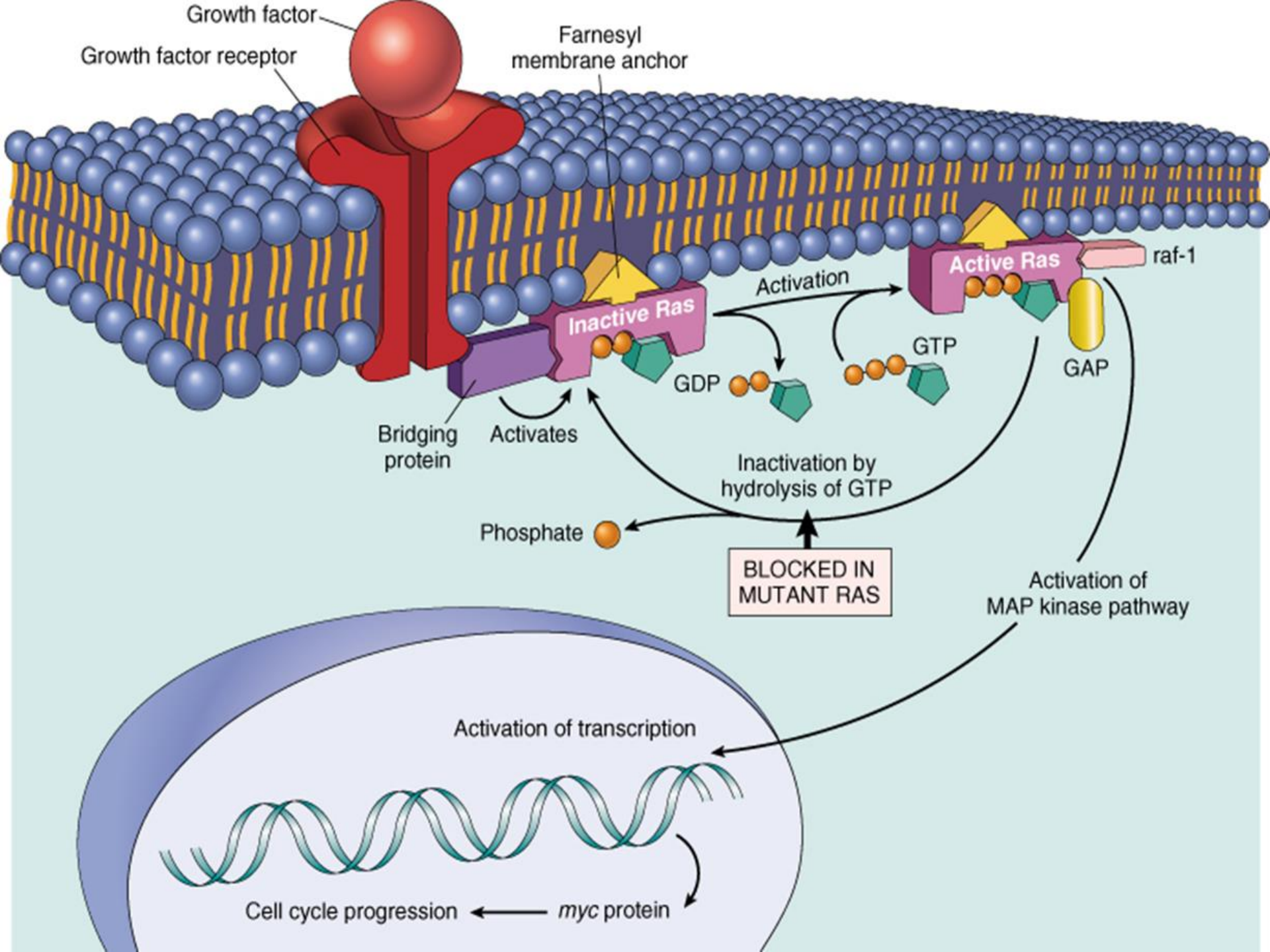
Carcinogenesis

A - Self-sufficiency in Growth signals:

- Oncogene: Gene that promote autonomous cell growth in cancer cells
- They are derived by mutations in protooncogenes
- They are characterized by the ability to promote cell growth in the absence of normal growth-promoting signals
- Oncoproteins : are the products

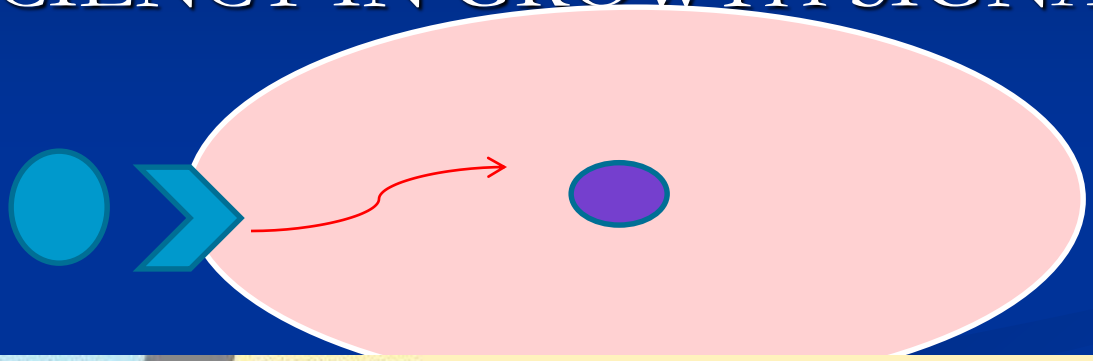
Carcinogenesis

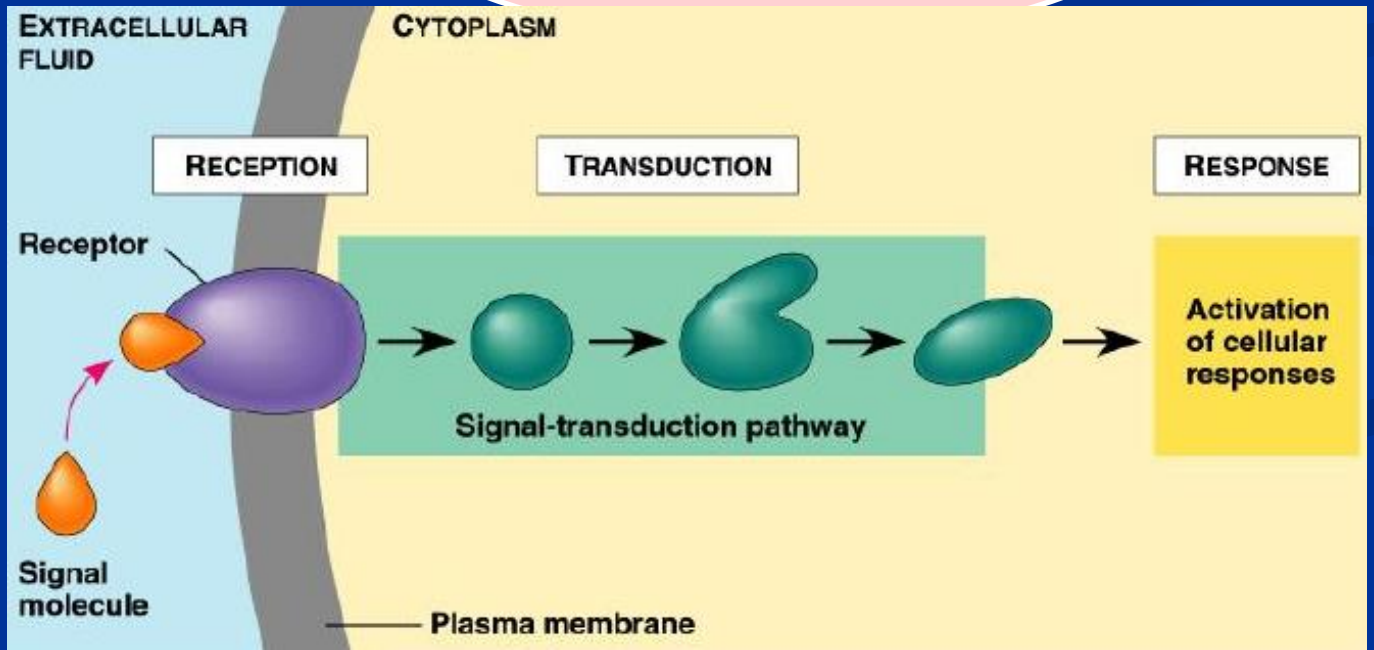
- Remember the cell cycle !!
  - Binding of a growth factor to its receptor on the cell membrane
  - Activation of the growth factor receptor leading to activation of signal-transducing proteins
  - Transmission of the signal to the nucleus
  - Induction of the DNA transcription
  - Entry in the cell cycle and cell division


Growth factor
Growth factor receptor
Farnesyl membrane anchor
Inactive Ras
Activation
Active Ras
raf-1
GTP
GDP
GAP
Bridging protein
Activates
Inactivation by hydrolysis of GTP
Phosphate
BLOCKED IN MUTANT RAS
Activation of MAP kinase pathway
Activation of transcription
Cell cycle progression
myc protein

Carcinogenesis

- HOW CANCER CELLS ACQUIRE SELF-SUFFICIENCY IN GROWTH SIGNALS??



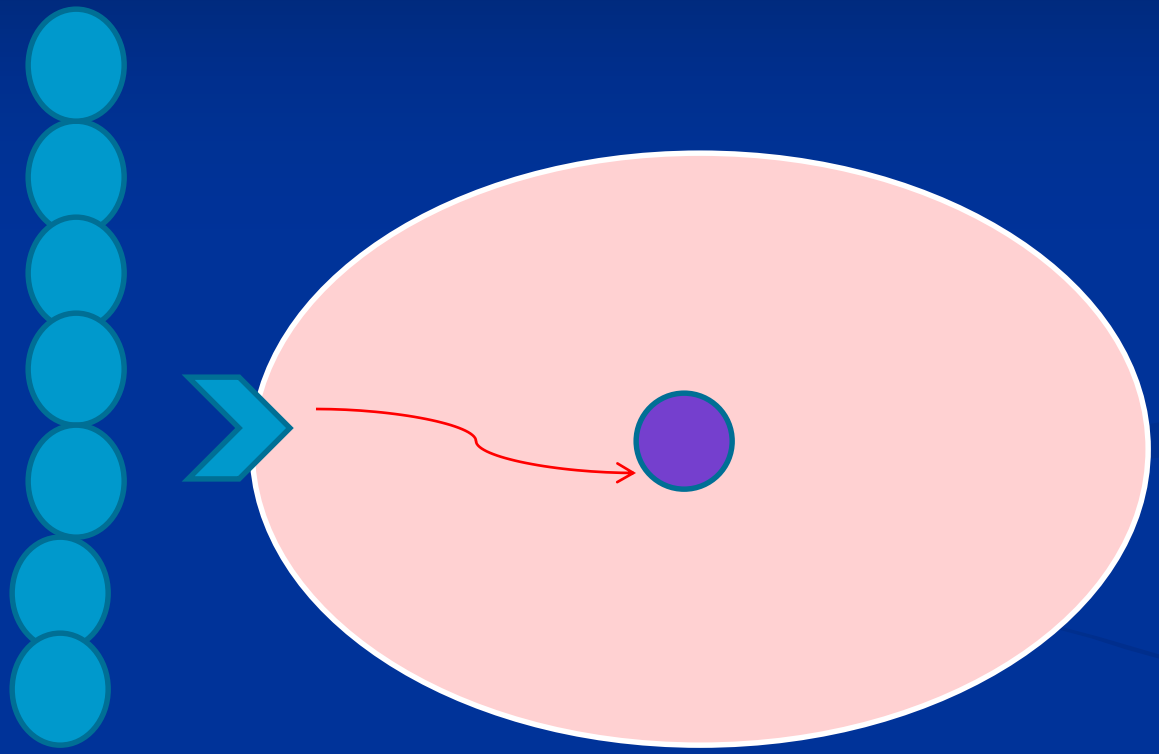


Carcinogenesis

1- Growth factors:

- Cancer cells are capable to synthesize the same growth factors to which they are responsive
  - E.g. Sarcomas ---- > TGF-α

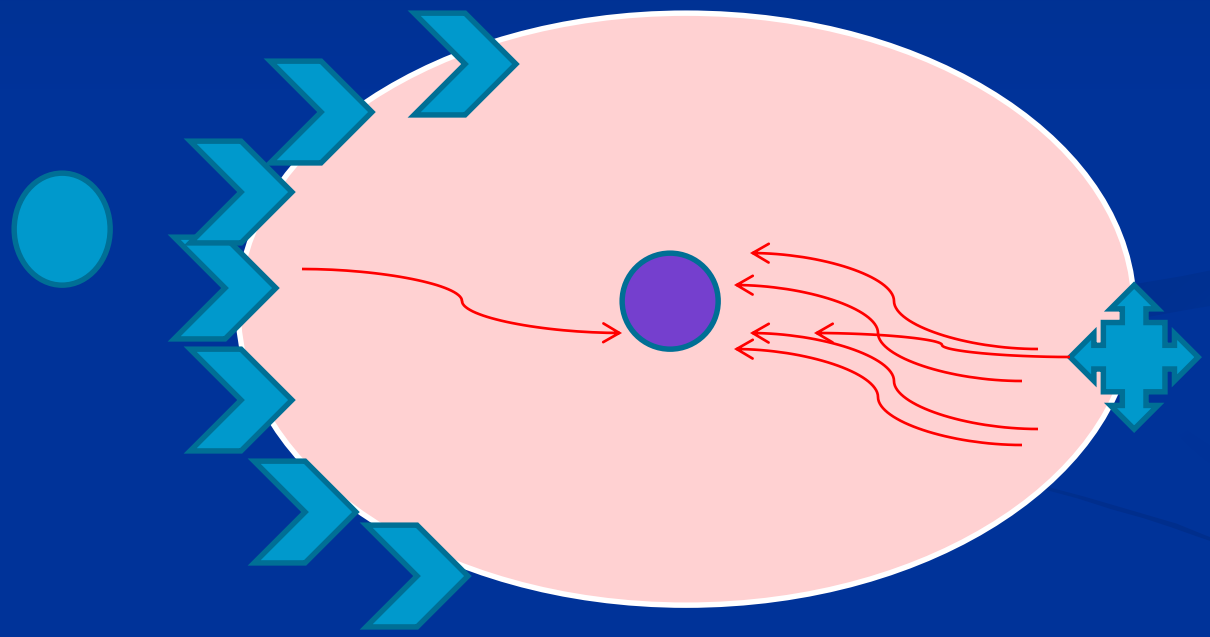
    Glioblastoma-----> PDGF



Carcinogenesis

2-Growth factors receptors:

- Receptors --- mutation ----continous signals to cells and uncontroled growth
- Receptors --- overexpression ---cells become very sensitive ----hyperresponsive to normal levels of growth factors



Carcinogenesis

- Example :
  - Epidermal Growth Factor (EGF) Receptor family
    - HER2
      - Amplified in breast cancers and other tumors
      - High levels of HER2 in breast cancer indicate poor prognosis
      - Anti- HER2 antibodies are used in treatment

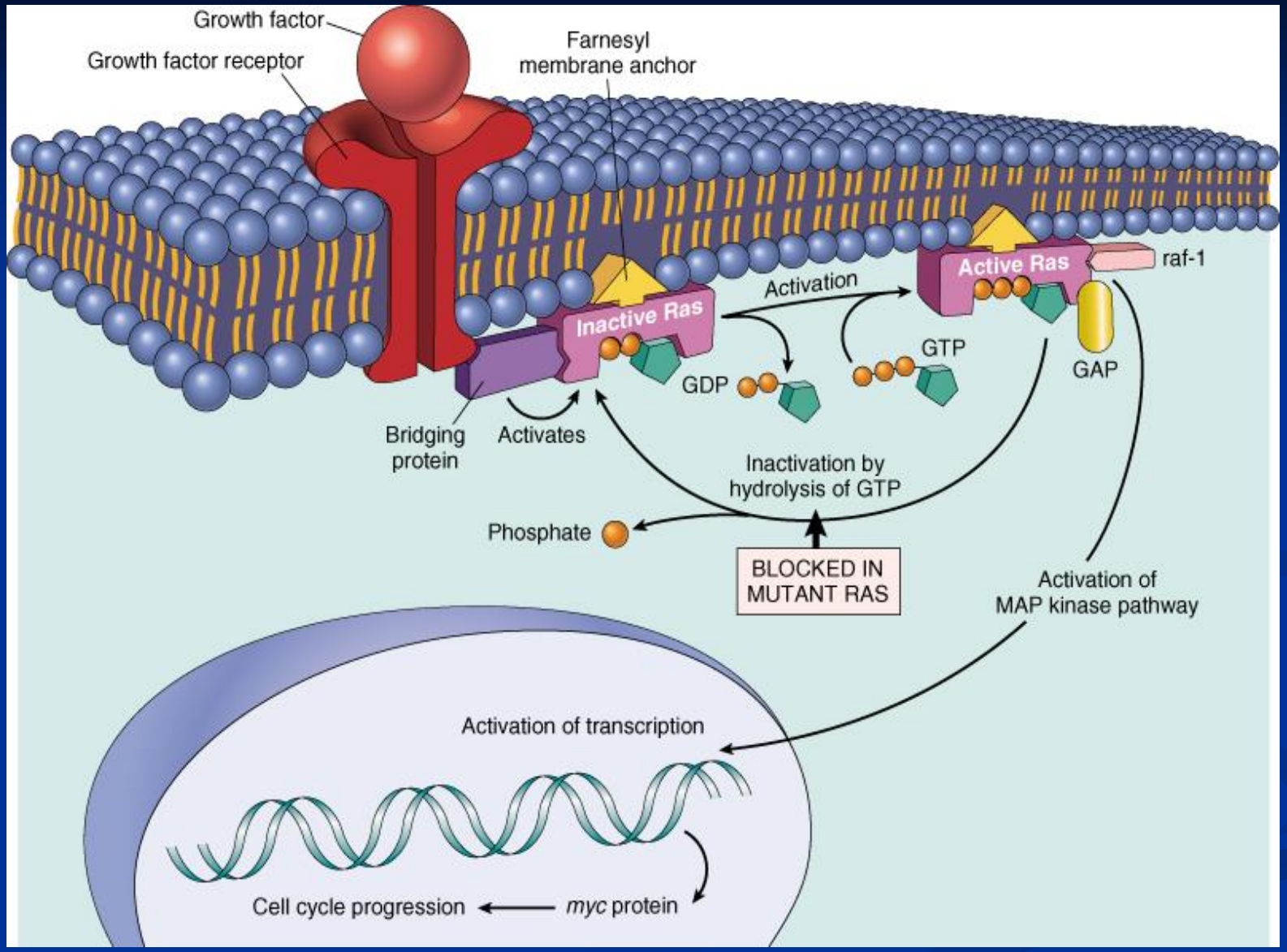
Carcinogenesis

3- Signal-transducing proteins :

- They receive signals from activated growth factors receptors and transmitte them to the nucleus. Examples :
  - RAS
  - ABL

Carcinogenesis

- RAS :
  - 30% of all human tumors contain mutated RAS gene . E.g : colon . Pancreas cancers
  - Mutations of the RAS gene is the most common oncogene abnormality in human tumors
  - Mutations in RAS --- cells continue to proliferate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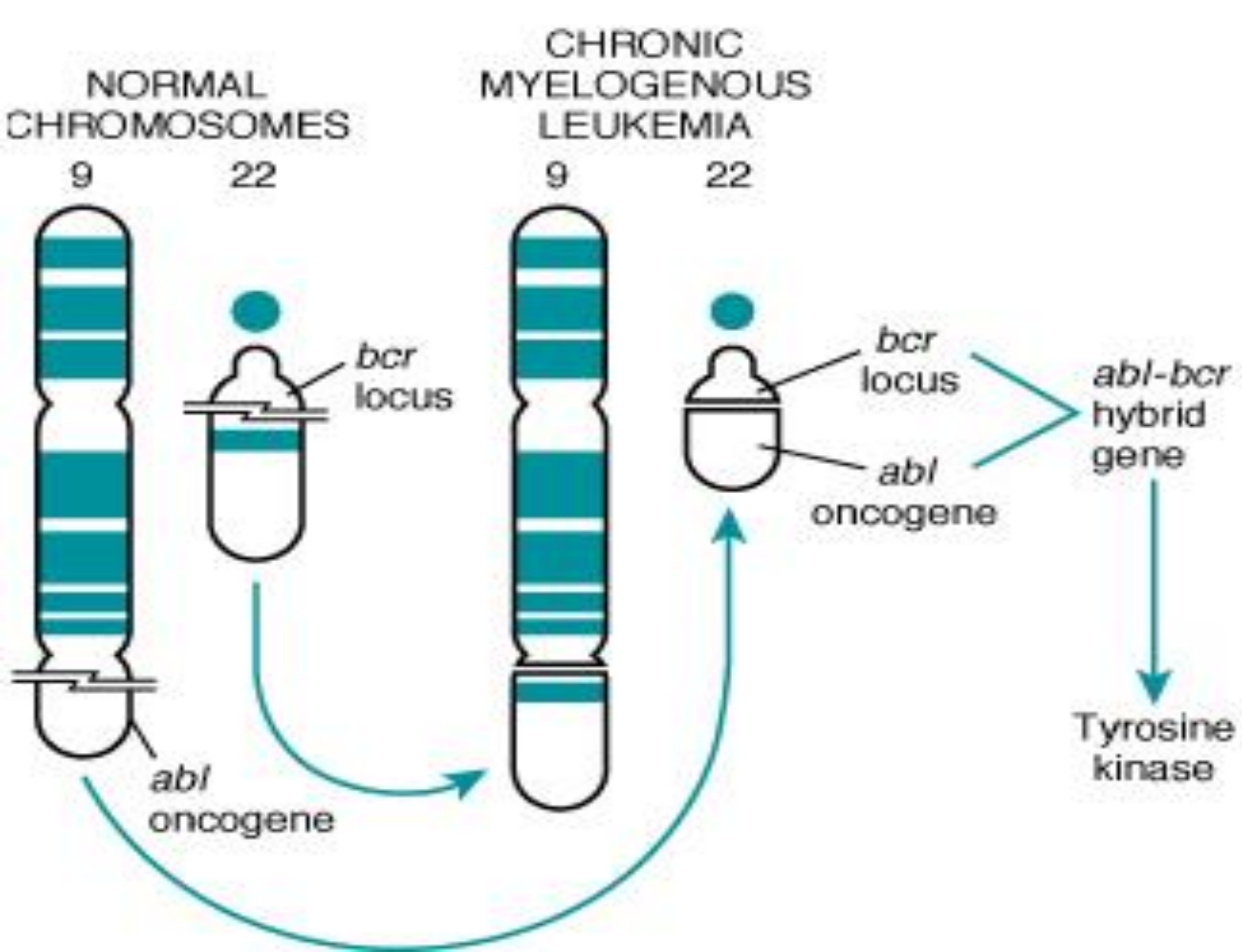
myc protein

Carcinogenesis

- ABL gene
  - ABL protooncogene has a tyrosine kinase activity
  - Its activity is controlled by negative regulatory mechanism
  - E.g. : chronic myeloid leukemia (CML) :
    - t(9,22) ---ABL gene transferred from ch. 9 to ch. 22
    - Fusion with BCR ---> BCR-ABL
    - BCR-ABL has tyrosine kinase acttivity ---(oncogenec)


NORMAL
CHROMOSOMES
9
22
CHRONIC
MYELOGENOUS
LEUKEMIA
9
22
bcr
locus
abl
oncogene
bcr
locus
abl
oncogene
abl-bcr
hybrid
gene
Tyrosine
kinase

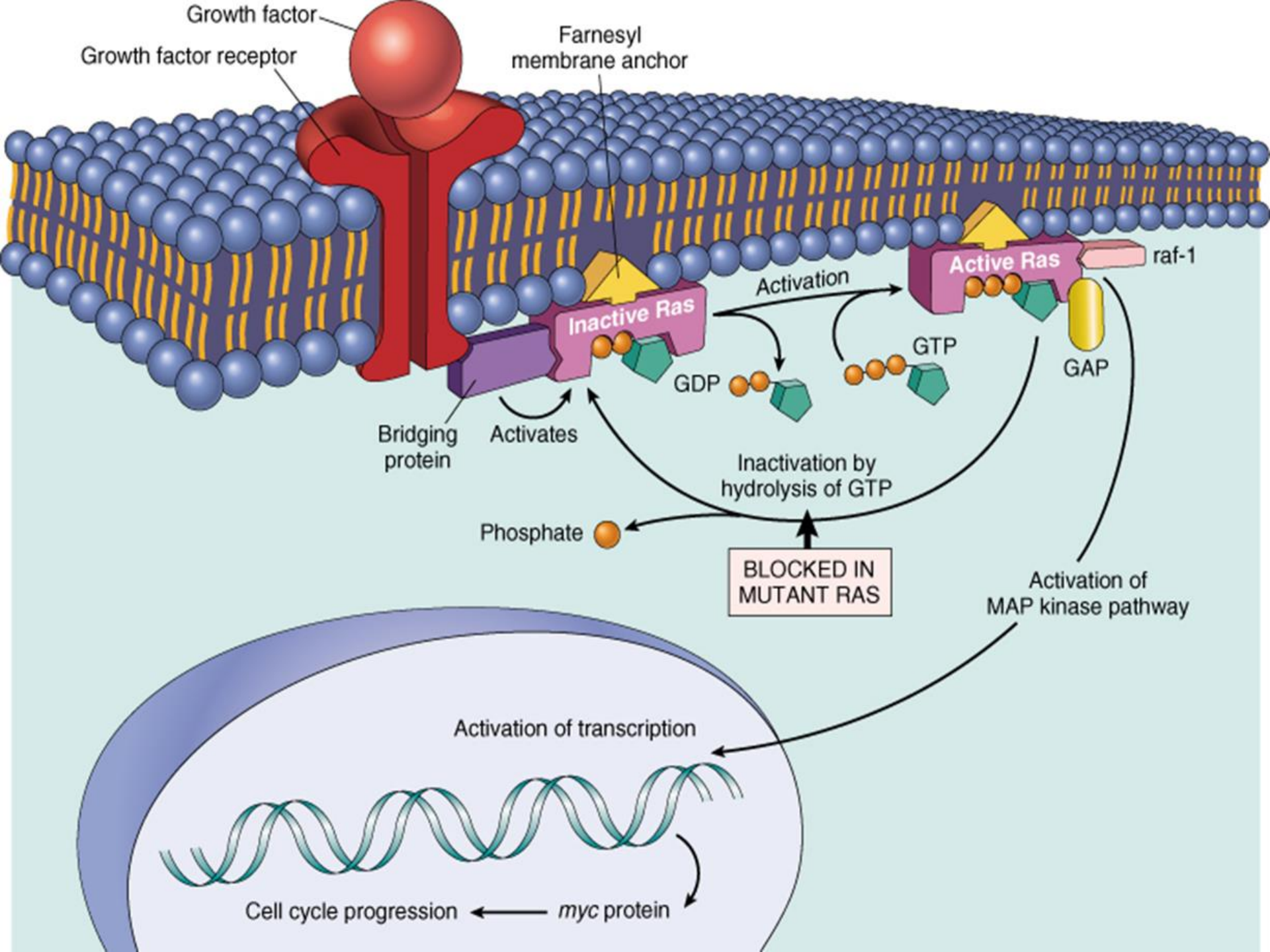
Carcinogenesis

- CML patients are treated with (Gleevec) which is inhibitor of ABL kinase

Carcinogenesis

4- Nuclear transcription factors :

- Mutations may affect genes that regulate transcription of DNA → growth autonomy
- E.g. MYC
  - MYC protooncogene produce MYC protein when cell receives growth signals
  - MYC protein binds to DNA leading to activation of growth-related genes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Activation of transcription
Cell cycle progression
myc protein

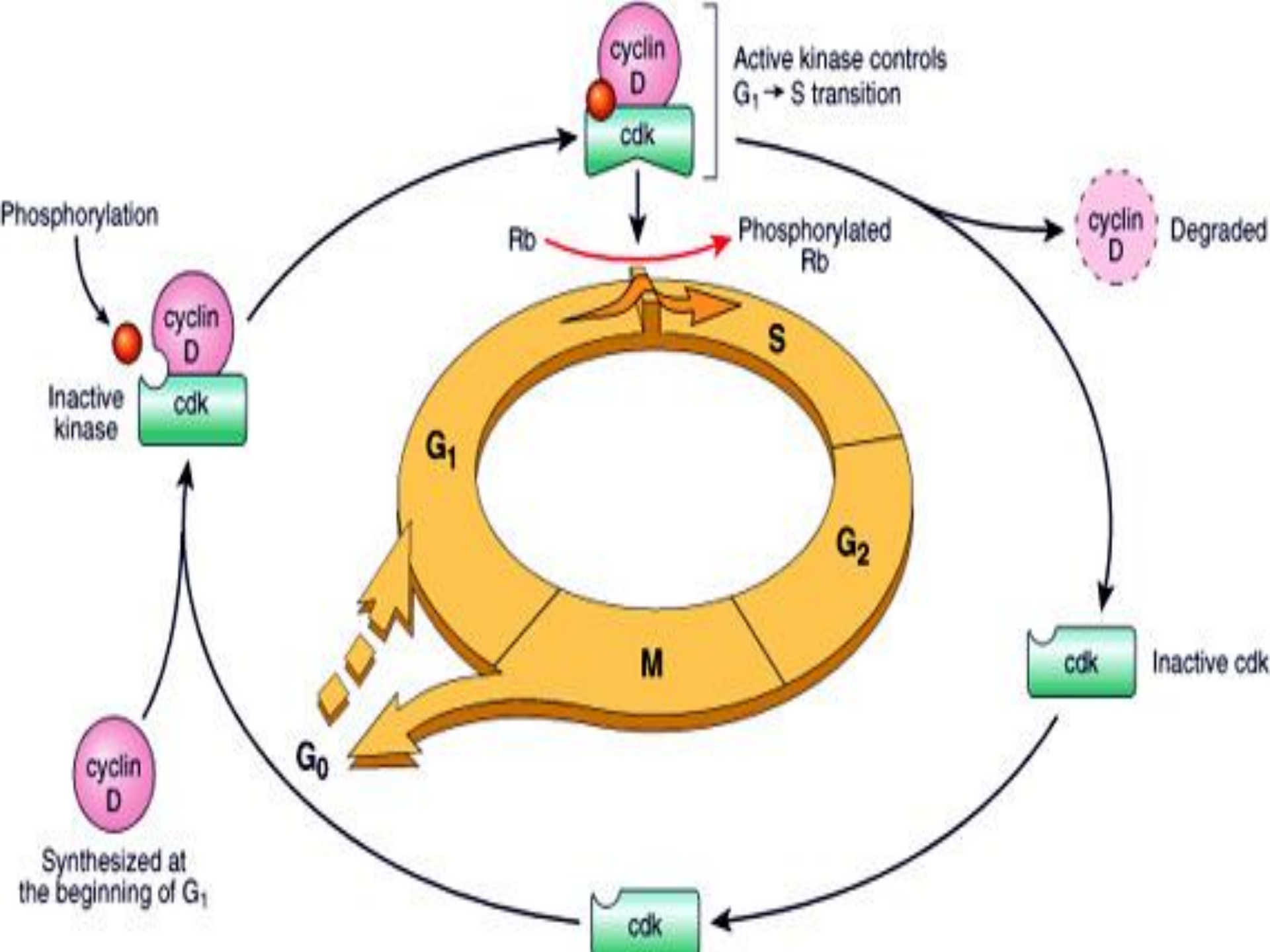
Carcinogenesis

- Normally … MYC decrease when cell cycle begins …but ..in tumors there is sustained expression of MYC → continuous proliferation
- E.g. Burkitt Lymphoma ; MYC is dysregulated due to t(8,14)

Carcinogenesis

5- Cyclins and cyclins- dependent kinases (CDKs)

- Progression of cells through cell cycles is regulated by CDKs after they are activated by binding with cyclins
- Mutations that dysregulate cyclins and CDKs will lead to cell proliferation …e.g.
  - Cyclin D genes are overexpressed in breast, esophagus and liver cancers.
  - CDK4 is amplified in melanoma and sarcomas


cyclin D
cdk
Active kinase controls $G_1 \rightarrow S$ transition
Phosphorylation
Rb
Phosphorylated Rb
cyclin D
Degraded
cyclin D
cdk
Inactive kinase
S
G_1
G_2
M
G_0
cdk
Inactive cdk
cyclin D
Synthesized at the beginning of G_1
cdk

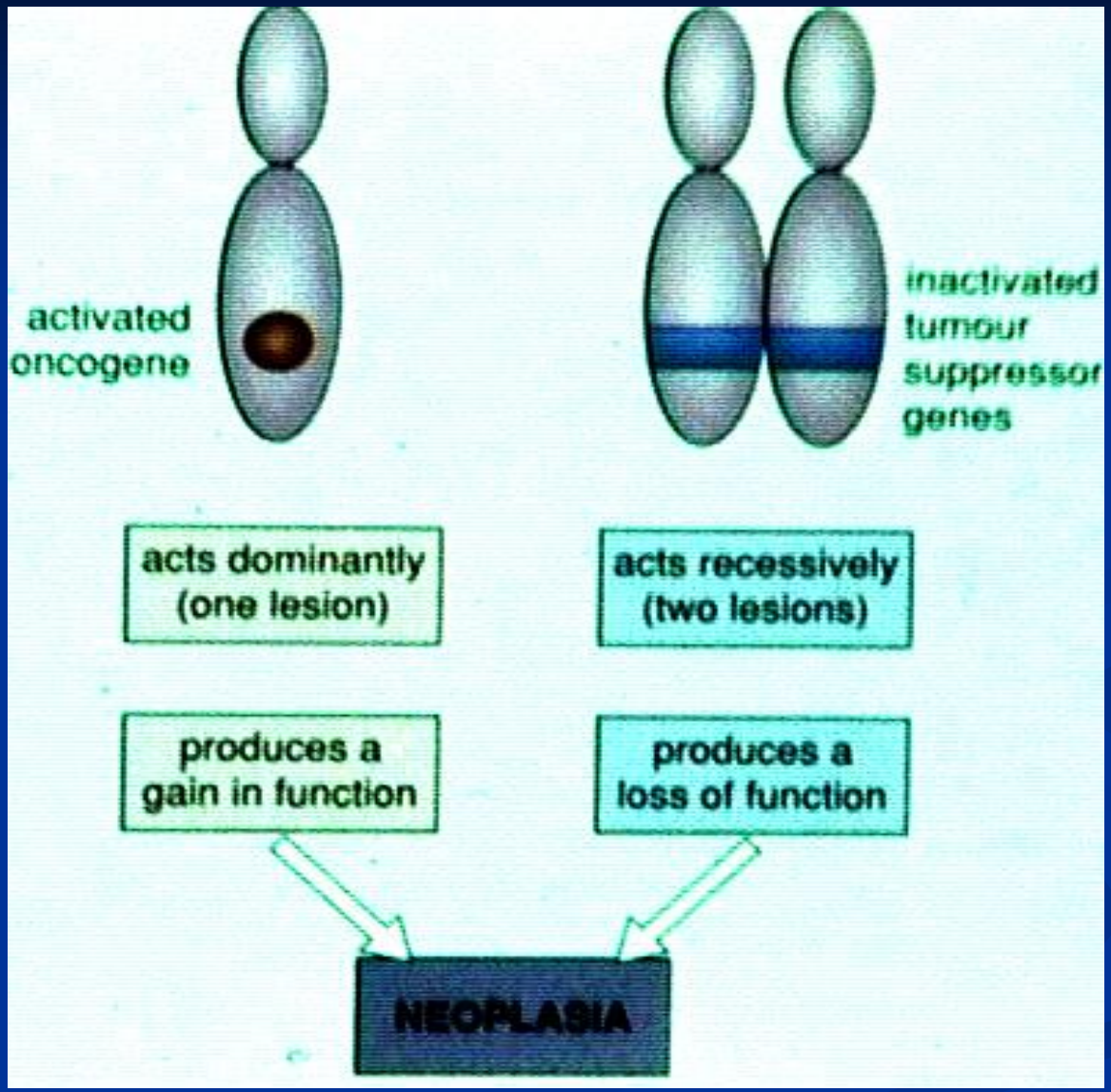
Carcinogenesis

- Main changes in the cell physiology that lead to formation of the malignant phenotype:
  - A- Self-sufficiency in growth signals
  - **B- Insensitivity to growth-inhibitory signals**
  - C- Evasion of apoptosis
  - D- Limitless replicative potential
  - E- Sustained angiogenesis
  - F- Ability to invade and metastsize

Carcinogenesis

2. Insensitivity to growth-inhibitory signals

- Tumor suppressor genes control (apply brakes) cells proliferation
- If mutation caused disruption to them → cell becomes insensitive to growth inhibition→ uncontrolled proliferation
- Examples: RB, TGF-β, APC, P53

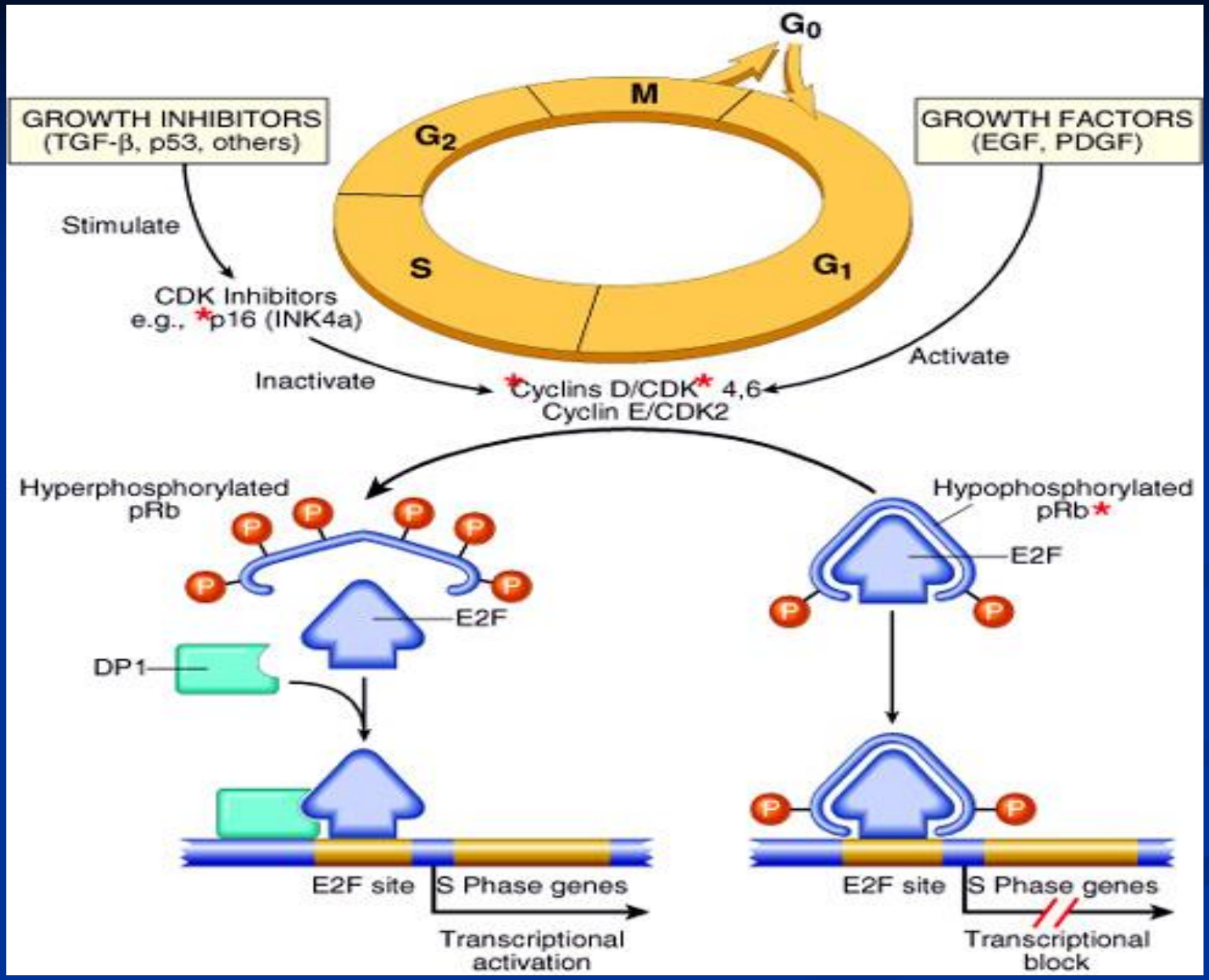

activated oncogene
inactivated tumour suppressor genes
acts dominantly (one lesion)
acts recessively (two lesions)
produces a gain in function
produces a loss of function
NEOPLASIA

Carcinogenesis

- RB (retinoblastoma) gene :
  - First tumor supressor gene discovered
  - It was discovered initially in retinoblastomas
  - Found in other tumors, e.g. breast ca
  - RB gene is a DNA-binding protein
  - RB is located on chromosome 13

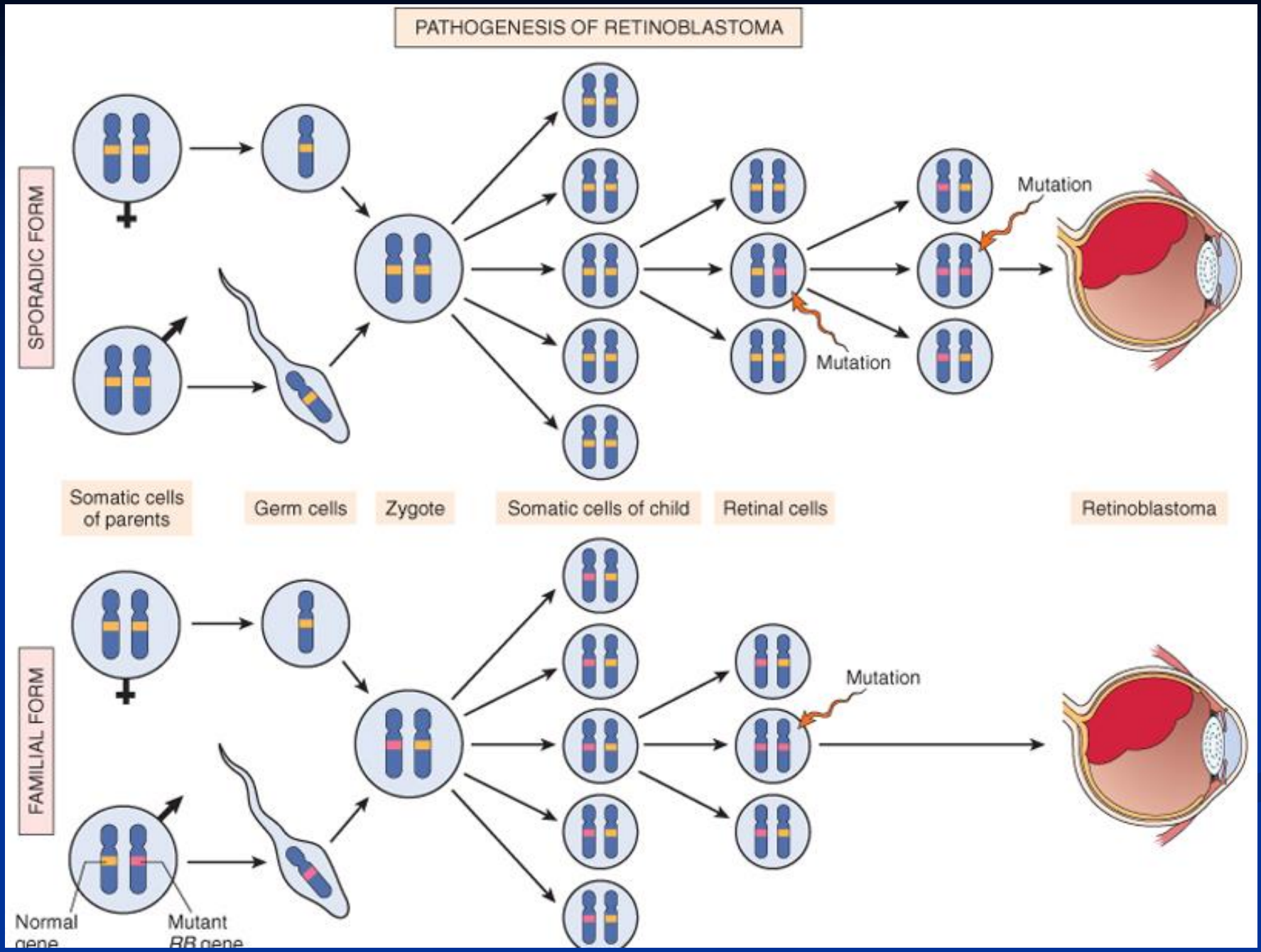
Carcinogenesis

- RB gene exists in “ active “ and “ inactive” forms
- If active→ will stop the advancing from G1 to S phase in cell cycle
- If cell is stimulated by growth factors → inactivation of RB gene →brake is released→ cells start cell cycle …G1 →S→M …then RB gene is activated again


G0
M
G2
S
G1
GROWTH INHIBITORS
(TGF-β, p53, others)
GROWTH FACTORS
(EGF, PDGF)
Stimulate
CDK Inhibitors
e.g., *p16 (INK4a)
Inactivate
Activate
Cyclins D/CDK 4,6
Cyclin E/CDK2
Hyperphosphorylated
pRb
P
E2F
DP1
Hypophosphorylated
pRb*
E2F site
S Phase genes
Transcriptional
activation
E2F site
S Phase genes
Transcriptional
block

Carcinogenesis

- Retinoblastoma is an uncommon childhood tumor
- Retinoblastoma is either sporadic (60%) or familial (40%)
- Two mutations required to produce retinoblastoma
- Both normal copies of the gene should be lost to produce retinoblastoma


PATHOGENESIS OF RETINOBLASTOMA
SPORADIC FORM
Mutation
Mutation
Somatic cells of parents
Germ cells
Zygote
Somatic cells of child
Retinal cells
Retinoblastoma
FAMILIAL FORM
Mutation
Normal gene
Mutant RB gene

Carcinogenesis

- Transforming Growth Factor- β pathway:
  - TGF-β is an inhibitor of proliferation
  - It regulate RB pathway
  - Inactivation of TGF-β lead to cell proliferation

Mutations in TGF-β pathway are present in :

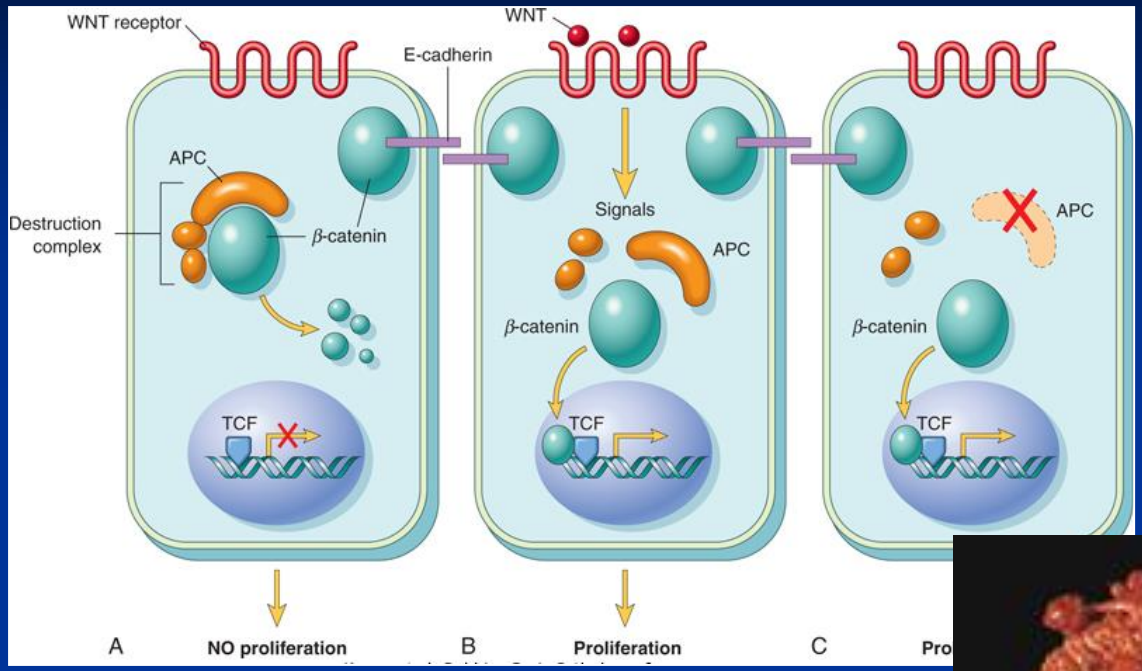
100% of pancreatic cancers

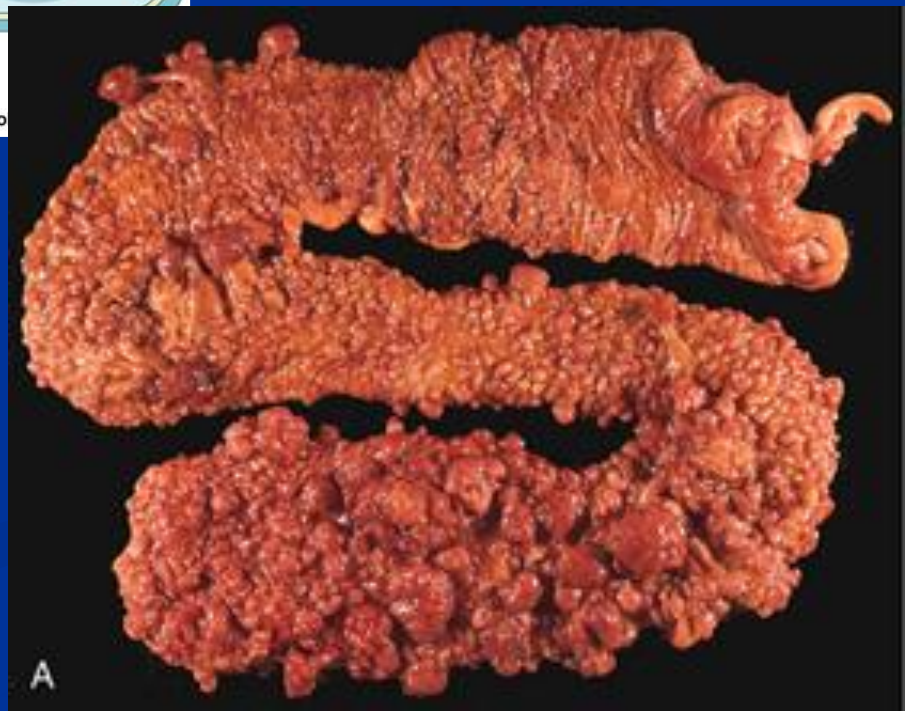
83% of colon cancers

Carcinogenesis

- Adenomatous Polyposis Coli – β Catenin pathway:
  - APC is tumor supressor gene
  - APC gene loss is very common in colon cancers
  - It has anti-proliferative action through inhibition of β–Catenin which activate cell proliferation
  - Individuals with mutant APC develop thousands of colonic polyps

Adenomatous Polyposis Coli





Carcinogenesis

- One or more of the polyps will progress to colonic carcinoma
- APC mutations are seen in 70% to 80% of sporadic colon cancers

Carcinogenesis

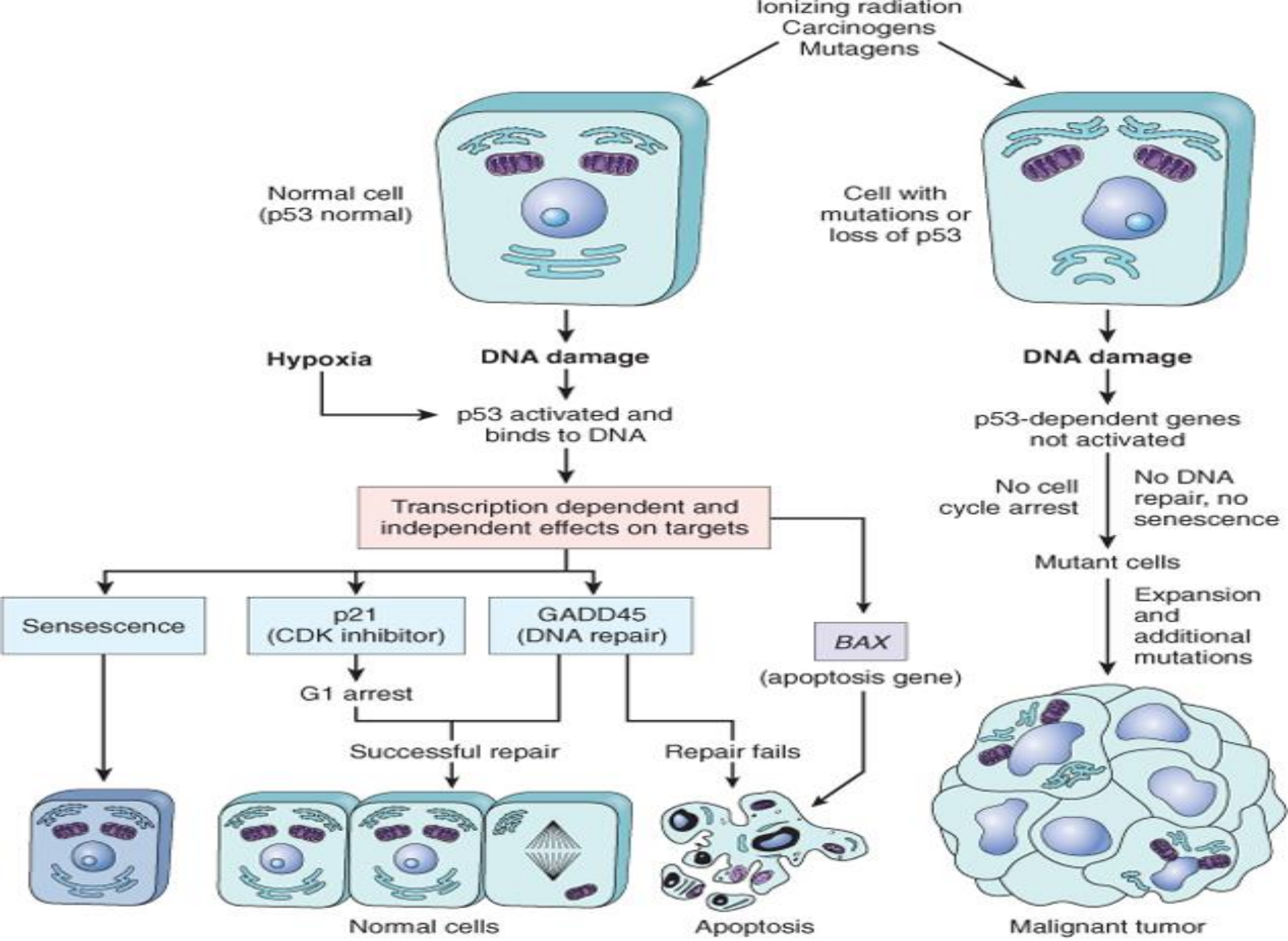
- P53
  - It has multiple functions
  - Mainly :
    - Tumor suppressor gene (anti-proliferative)
    - Regulates apoptosis

Carcinogenesis

- P53 senses DNA damage
- Causes G1 arrest to give chance for DNA repair
- Induce DNA repair genes
- If a cell with damaged DNA cannot be repaired, it will be directed by P53 to undergo apoptosis

Carcinogenesis

- With loss of P53, DNA damage goes unrepaired
- Mutations will be fixed in the dividing cells, leading to malignant transformation



Carcinogenesis

- P53 is called the “ guardian of the genome”
- 70% of human cancers have a defect in P53
- It has been reported with almost all types of cancers : e.g. lung, colon, breast
- In most cases, mutations are acquired, but can be inhereted, e.g : Li-Fraumeni syndrome

Carcinogenesis

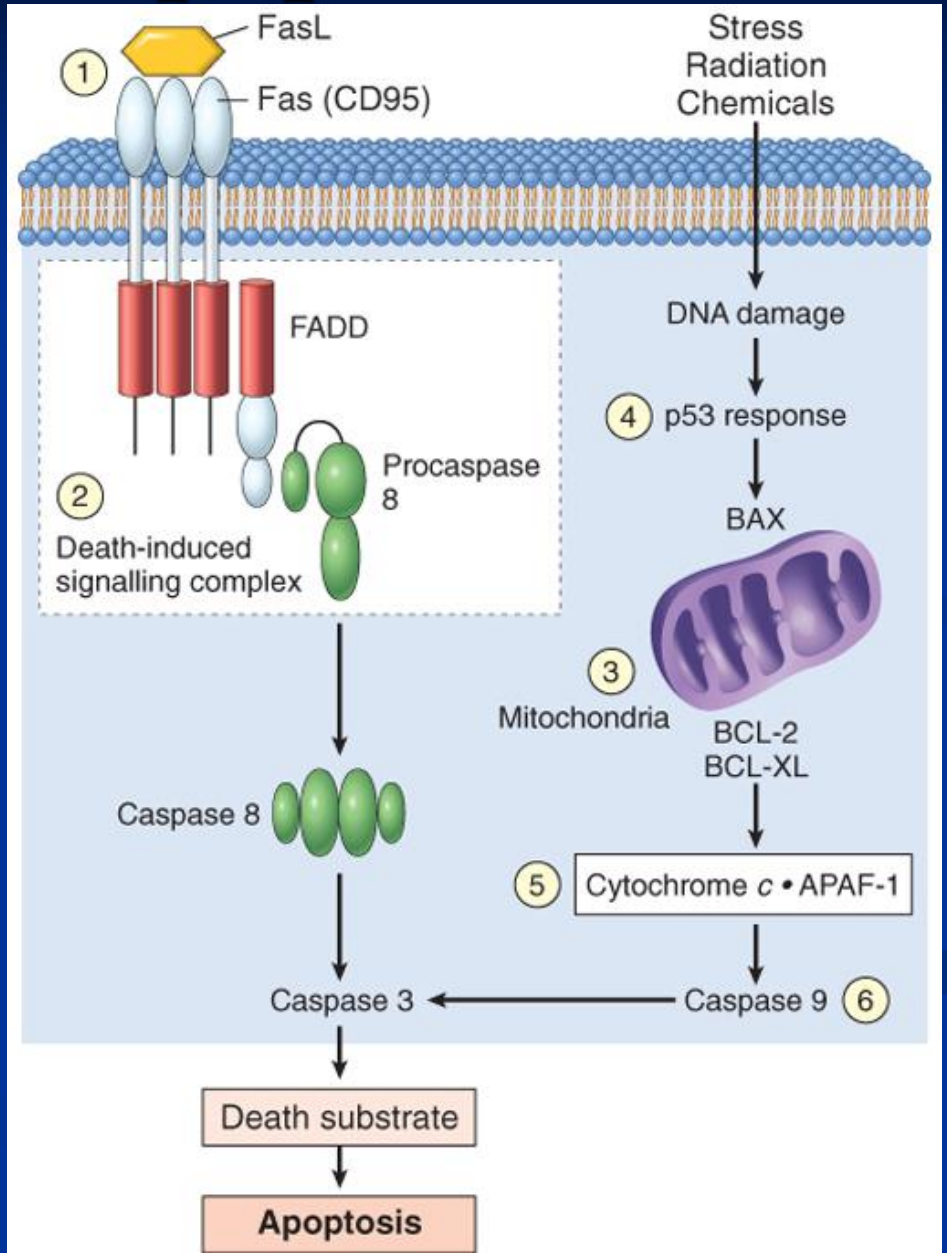
- Main changes in the cell physiology that lead to formation of the malignant phenotype:
  - A- Self-sufficiency in growth signals
  - B- Insensitivity to growth-inhibitory signals
  - **C- Evasion of apoptosis**
  - D- Limitless replicative potential
  - E- Sustained angiogenesis
  - F- Ability to invade and metastsize

Carcinogenesis

- **Evasion of apoptosis:**
  - Mutations in the genes regulating apoptosis are factors in malignant transformation
  - Cell survival is controlled by genes that promote and inhibit apoptosis

Evasion of apoptosis

- Reduced CD95 level inactivate death – induced signaling cascade that cleaves DNA to cause death→ tumor cells are less susceptible to apoptosis
- DNA damage induced apoptosis (with the action of P53) can be blocked in tumors
- loss of P53 and up-regulation of BCL2 prevent apoptosis e.g. follicular lymphoma



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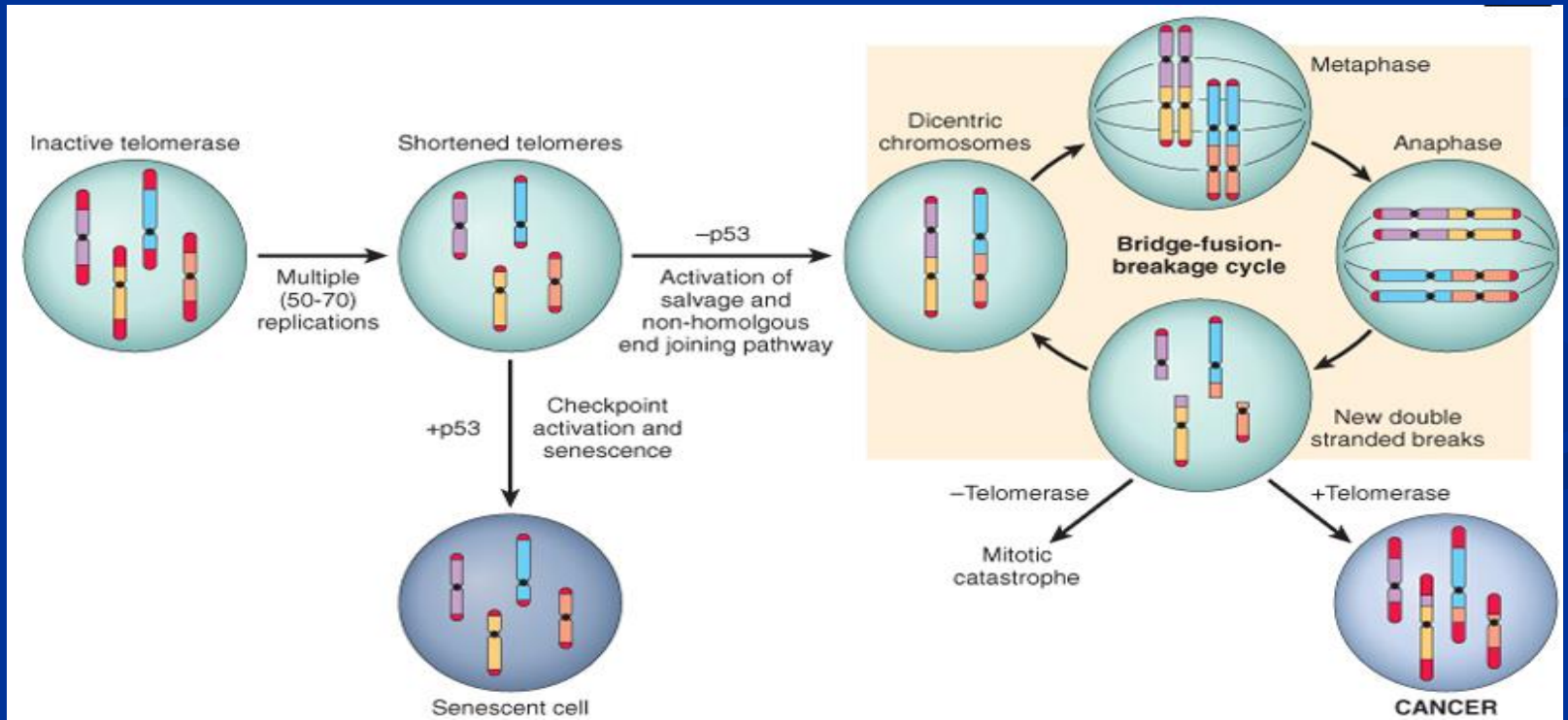
- **Limitless replicative potential:**
  - Normally there is progressive shortening of telomeres at the ends of chromosomes
  - Telomerase is active in normal stem cells but absent in somatic cells
  - In tumor cells : activation of the enzyme telomerase, which can maintain normal telomere length



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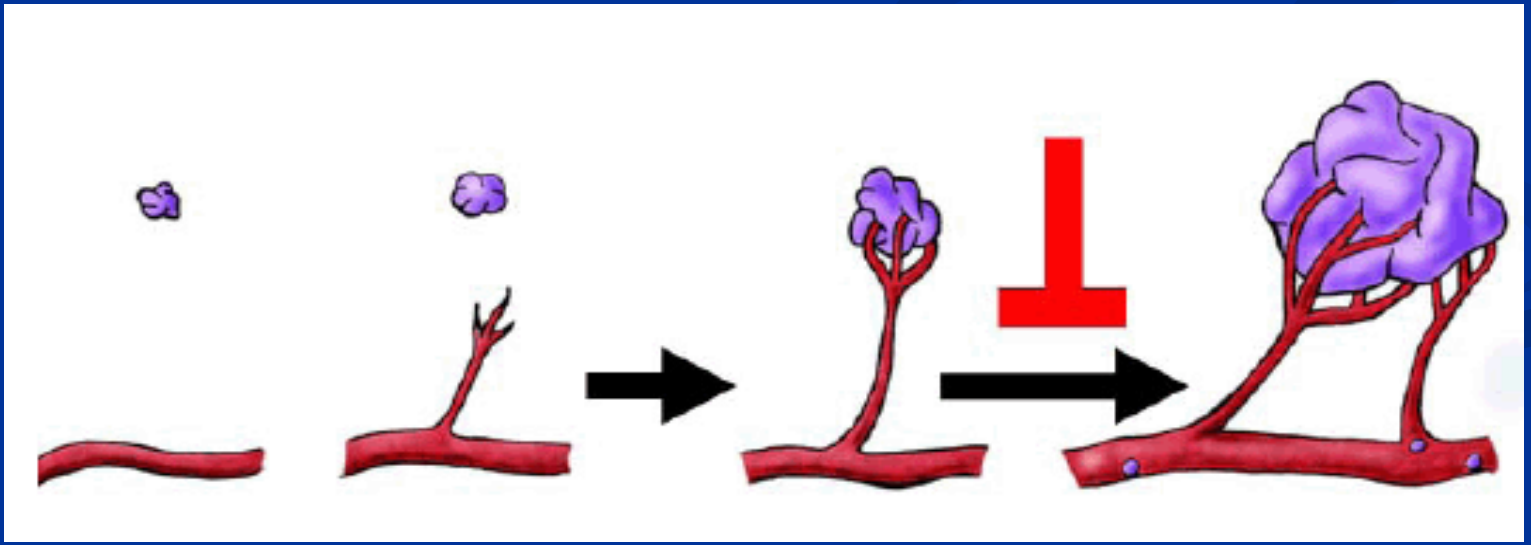
Carcinogenesis

- **Sustained angiogenesis**
  - Neovascularization has two main effects:
    - Perfusion supplies oxygen and nutrients
    - Newly formed endothelial cells stimulate the growth of adjacent tumor cells by secreting growth factors, e.g : PDGF, IL-1
  - Angiogenesis is required for metastasis

- How do tumors develop a blood supply?
  - Tumor-associated angiogenic factors
  - These factors may be produced by tumor cells or by inflammatory cells infiltrating the tumor e.g. macrophages
  - Important factors :
    - Vascular endothelial growth factor(VEGF)
    - Fibroblast growth factor



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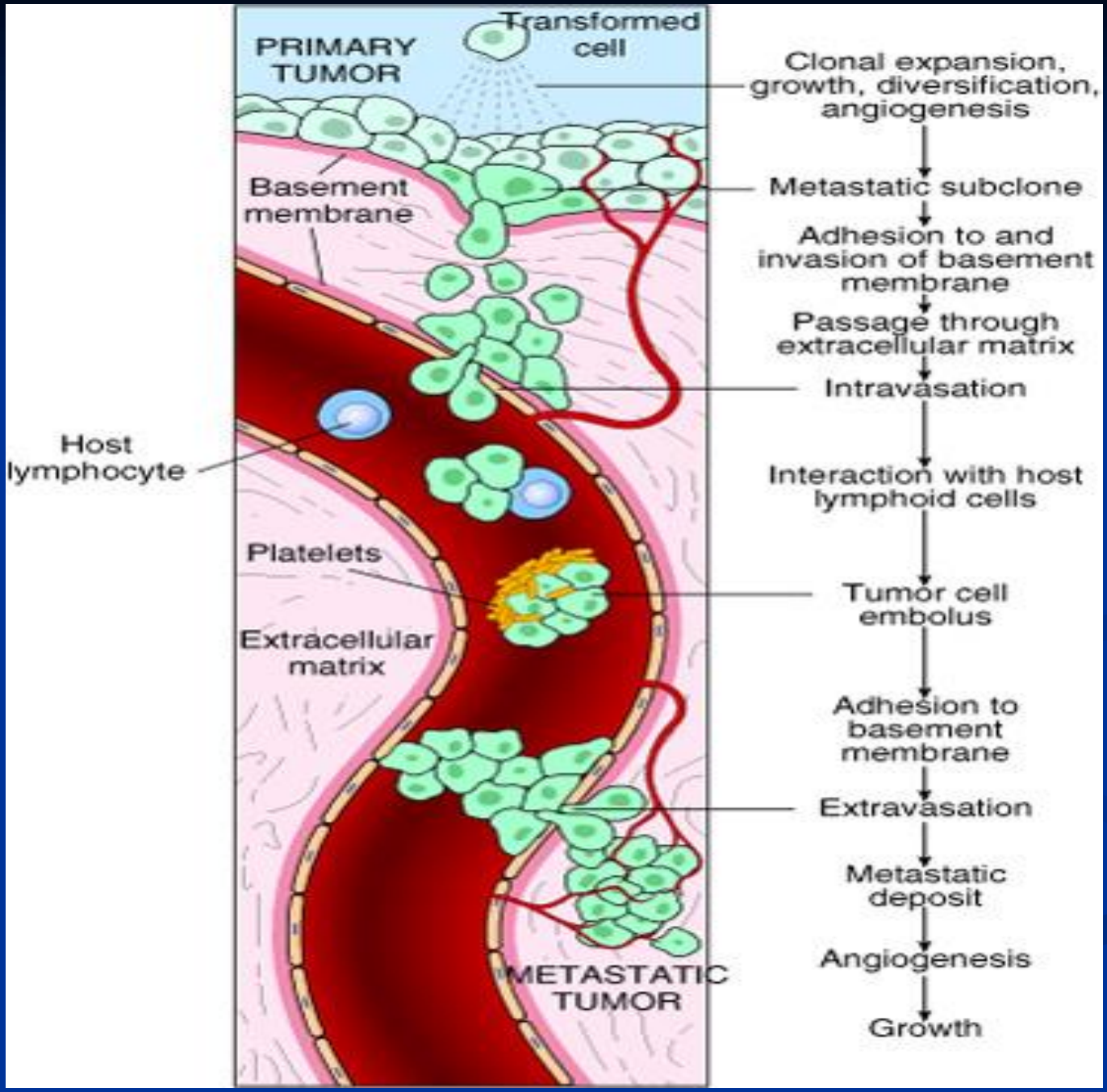

PRIMARY TUMOR
Transformed cell
Clonal expansion, growth, diversification, angiogenesis
Basement membrane
Metastatic subclone
Adhesion to and invasion of basement membrane
Passage through extracellular matrix
Intravasation
Host lymphocyte
Interaction with host lymphoid cells
Platelets
Tumor cell embolus
Extracellular matrix
Adhesion to basement membrane
Extravasation
Metastatic deposit
Angiogenesis
METASTATIC TUMOR
Growth

Carcinogenesis

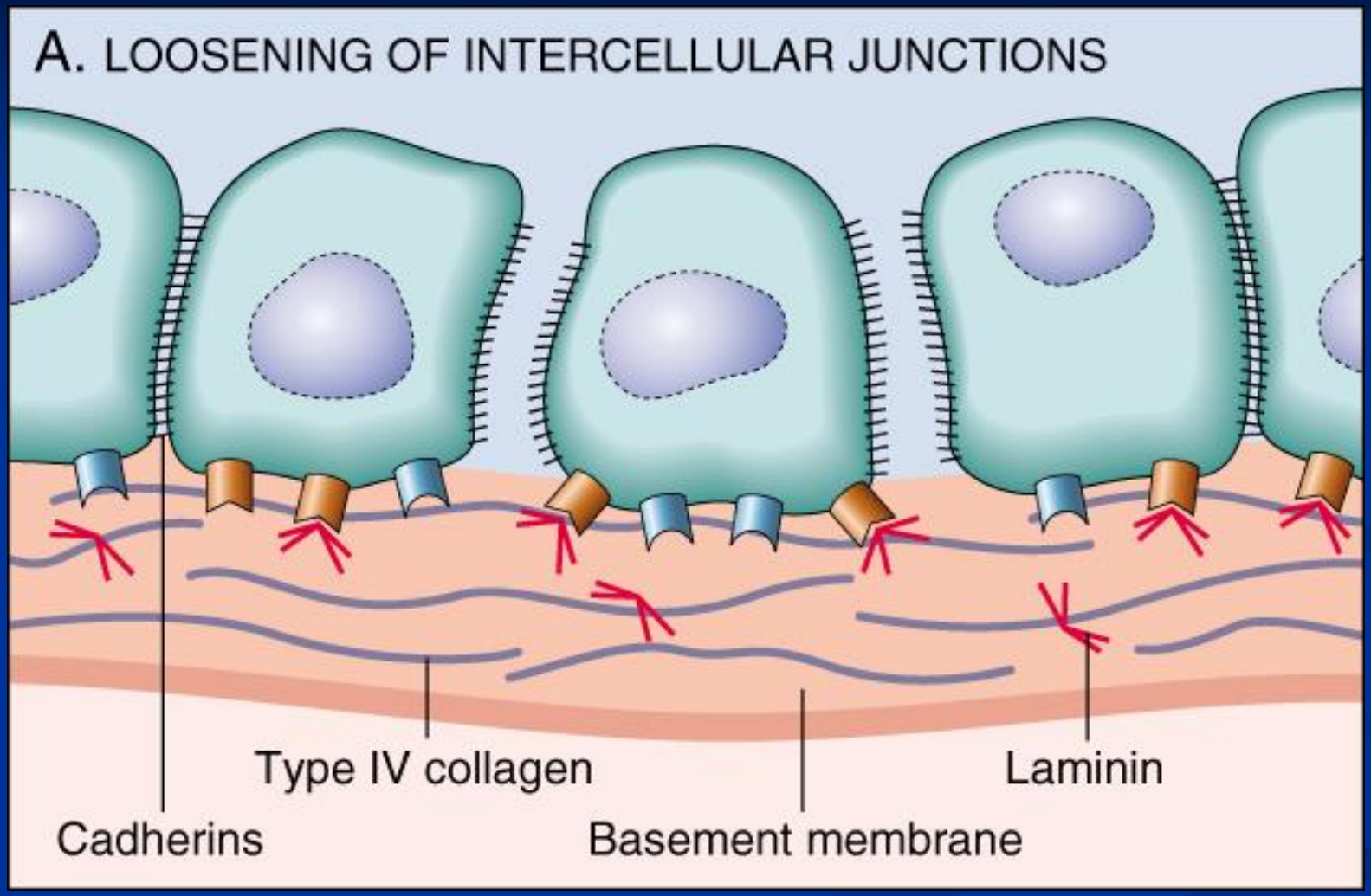
- **Ability to invade and metastsize:**
  - Two phases :
    - Invasion of extracellular matrix
    - Vascular dissimenation and homing of tumor cells

Carcinogenesis

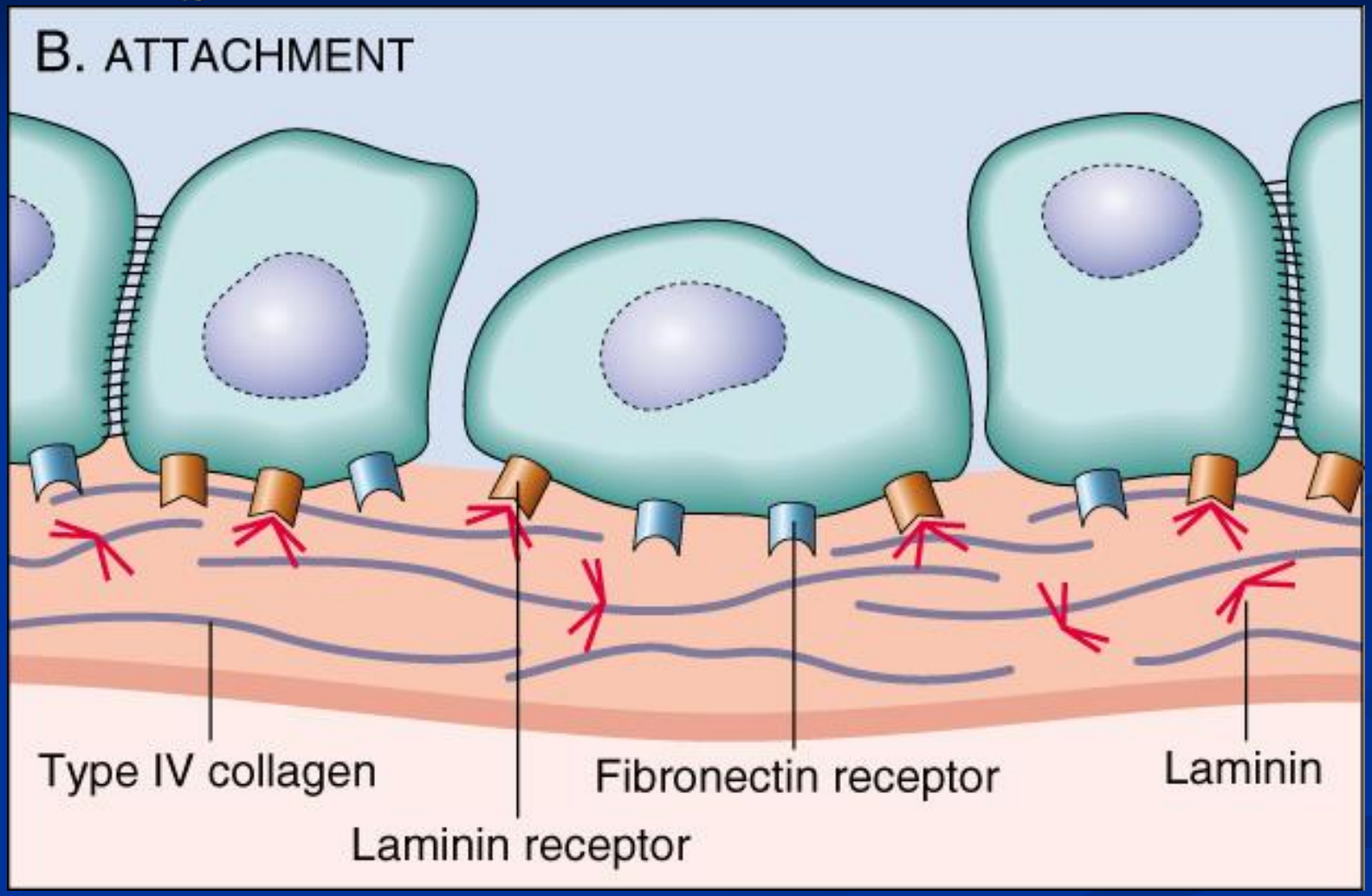
- Invasion of ECM:
  - Malignant cells first breach the underlying basement membrane
  - Traverse the interstitial tissue
  - Penetrate the vascular basement membrane
  - Gain access to the circulation

  - Invasion of the ECM has four steps:

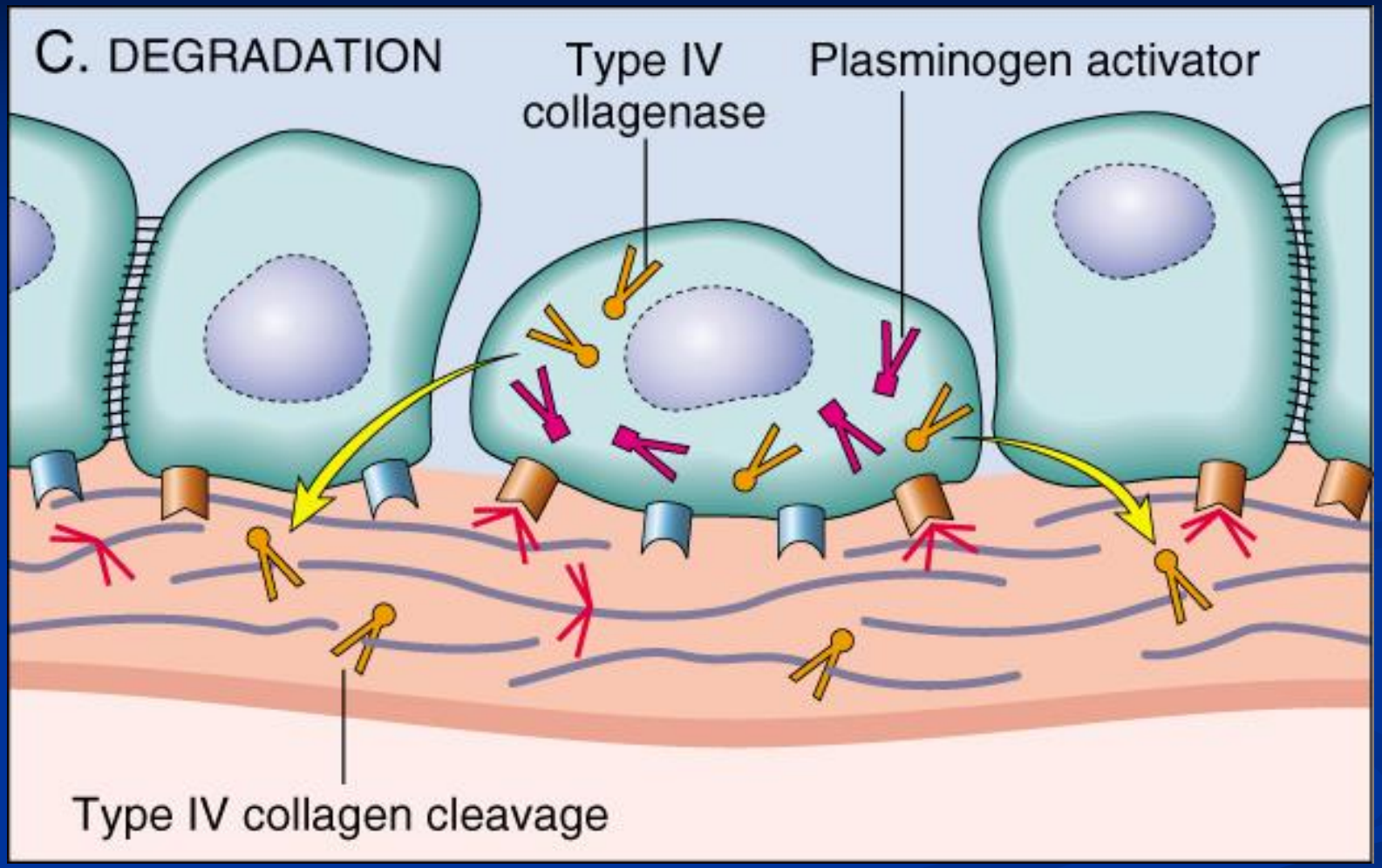
1. Detachment of tumor cells from each other



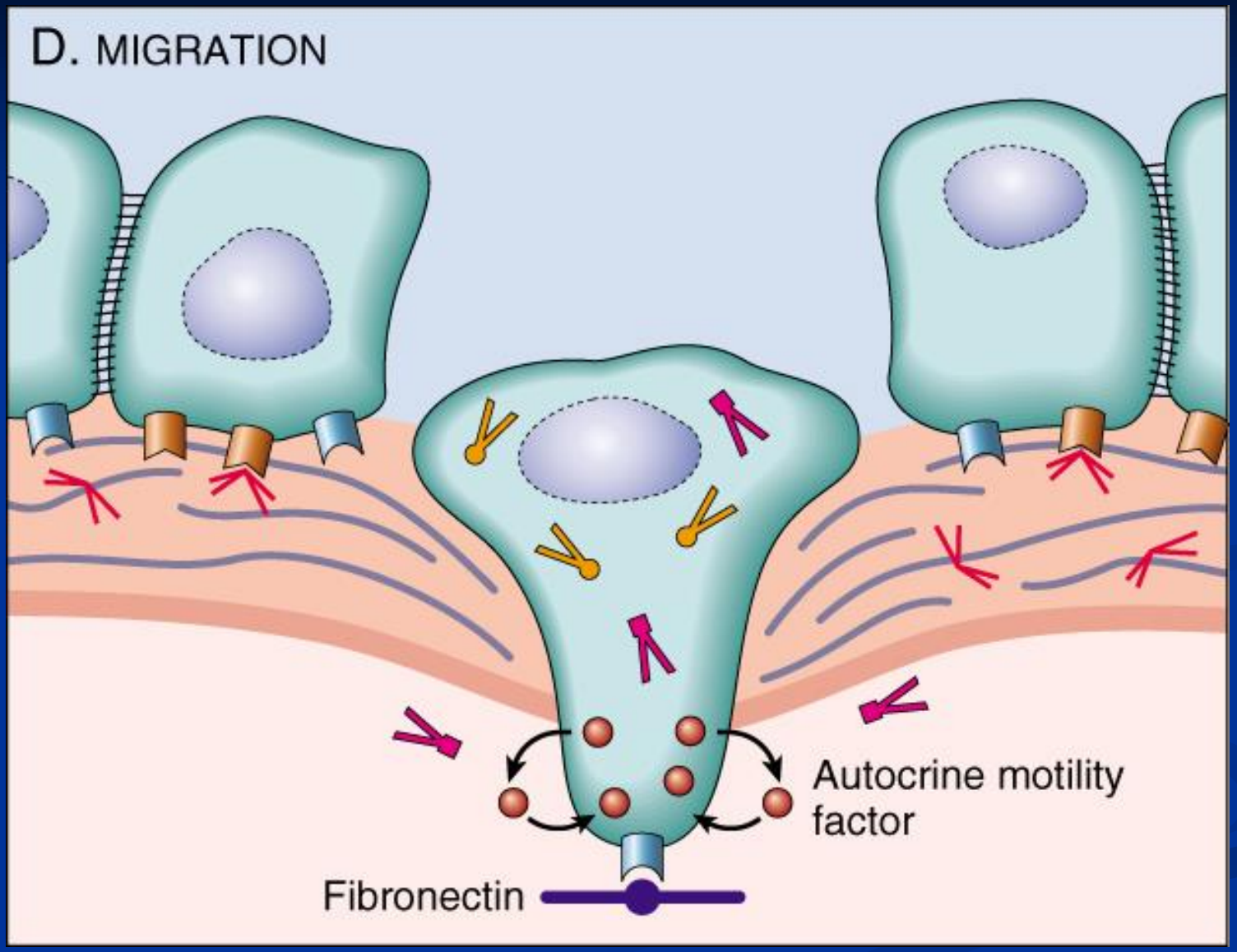
2. Attachments of tumor cells to matrix components



3. Degradation of ECM by collagenase enzyme



4. Migration of tumor cells



Carcinogenesis

- Vascular dissemination and homing of tumor cells:
  - May form emboli
  - Most travel as single cells
  - Adhesion to vascular endothelium
  - extravasation

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Genomic Instability

- Enabler of malignancy
- Due to defect in DNA repair genes
- Examples:
  - Hereditary Nonpolyposis colon carcinoma(HNPCC)
  - Xeroderma pigmentosum
  - Familial breast cancer

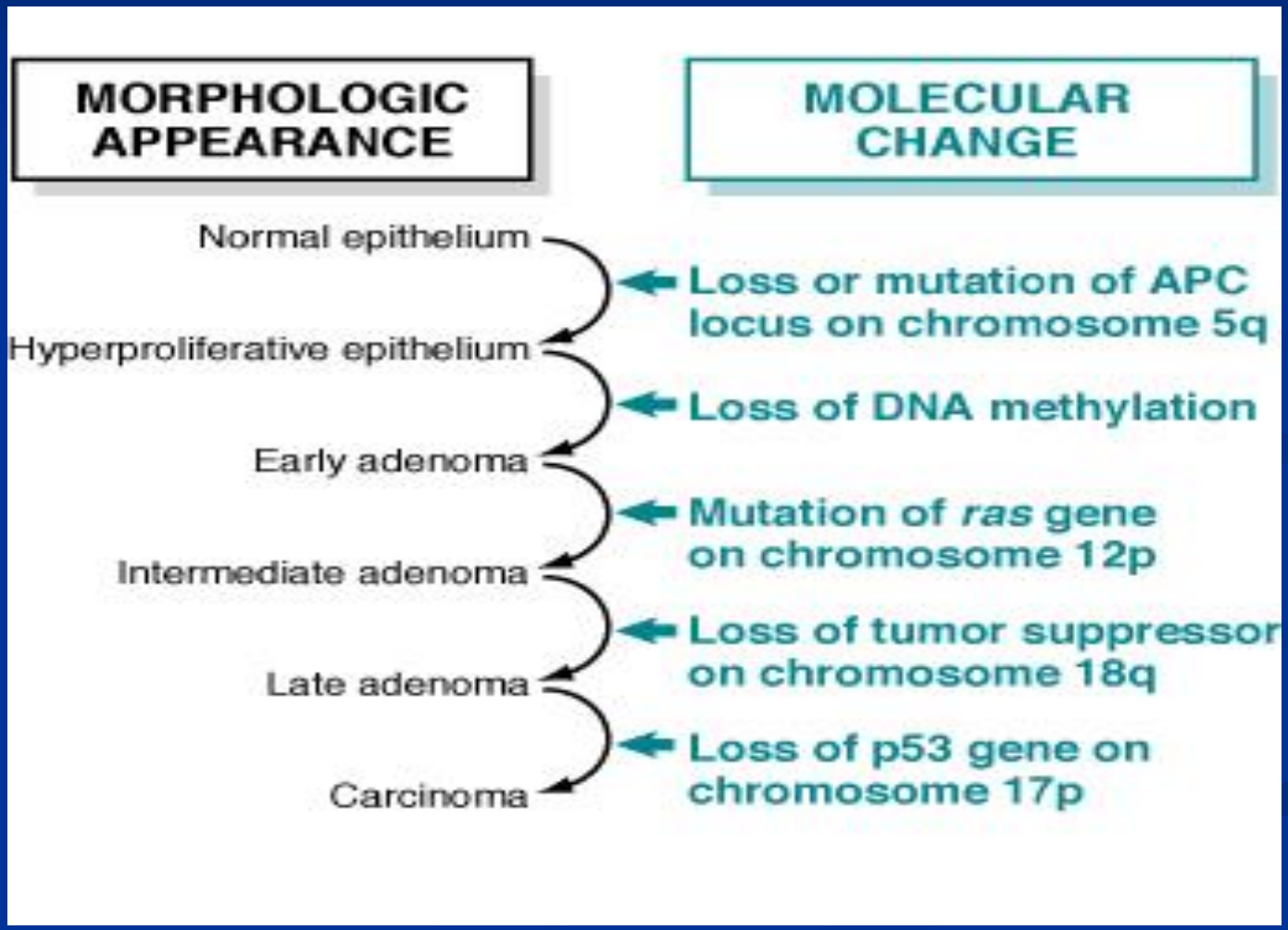
Genomic Instability

- Familial breast cancer:
  - Due to mutations in BRCA1 and BRCA2 genes
  - These genes regulate DNA repair
  - Account for 80% of familial breast cancer
  - They are also involved in other malignancies

Molecular Basis of multistep Carcinogenesis

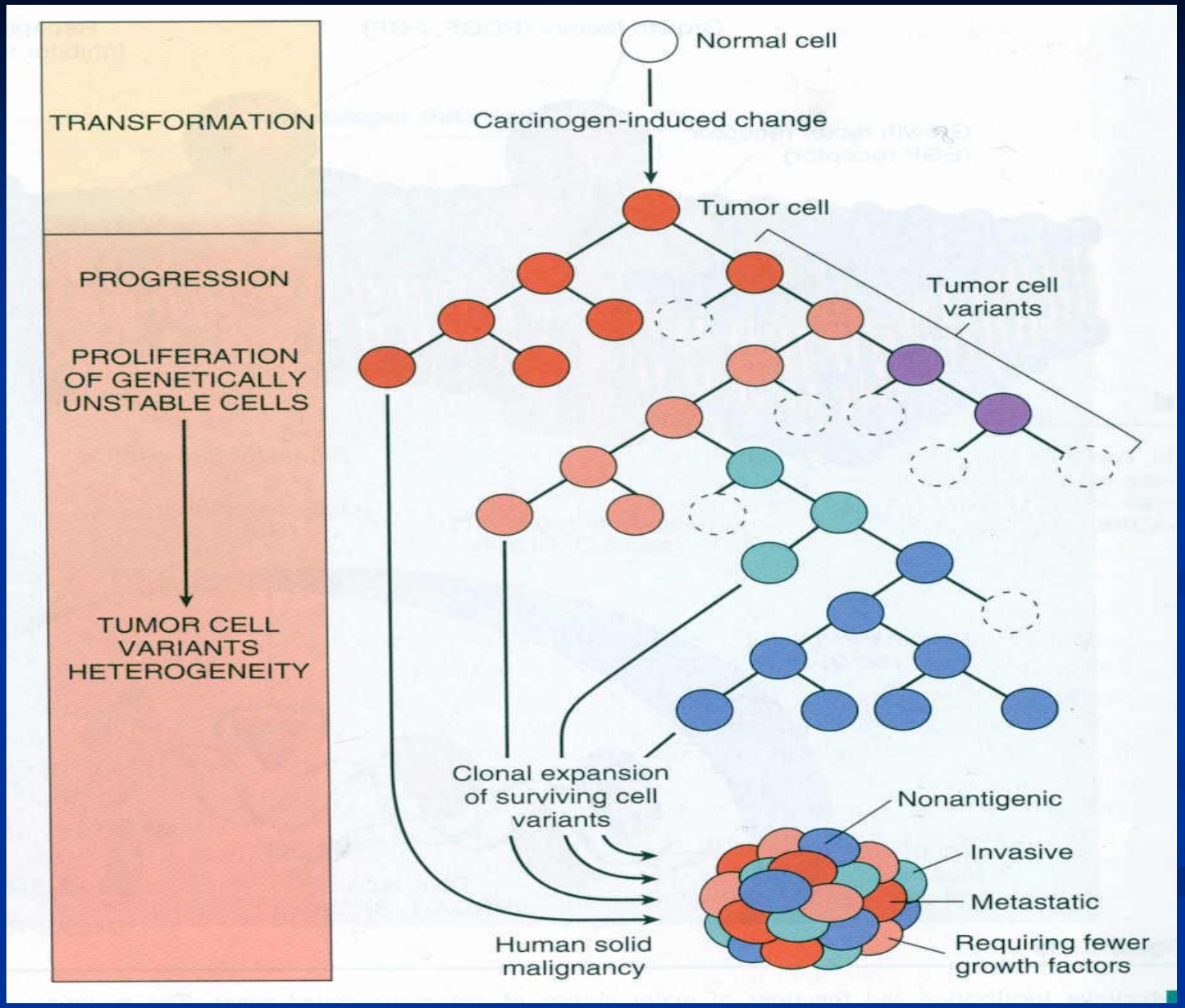
- Cancer results from accumulation of multiple mutations
- All cancers have multiple genetic alterations, involving activation of several oncogenes and loss of two or more tumor suppressor genes

Molecular Basis of multistep Carcinogenesis



Tumor progression

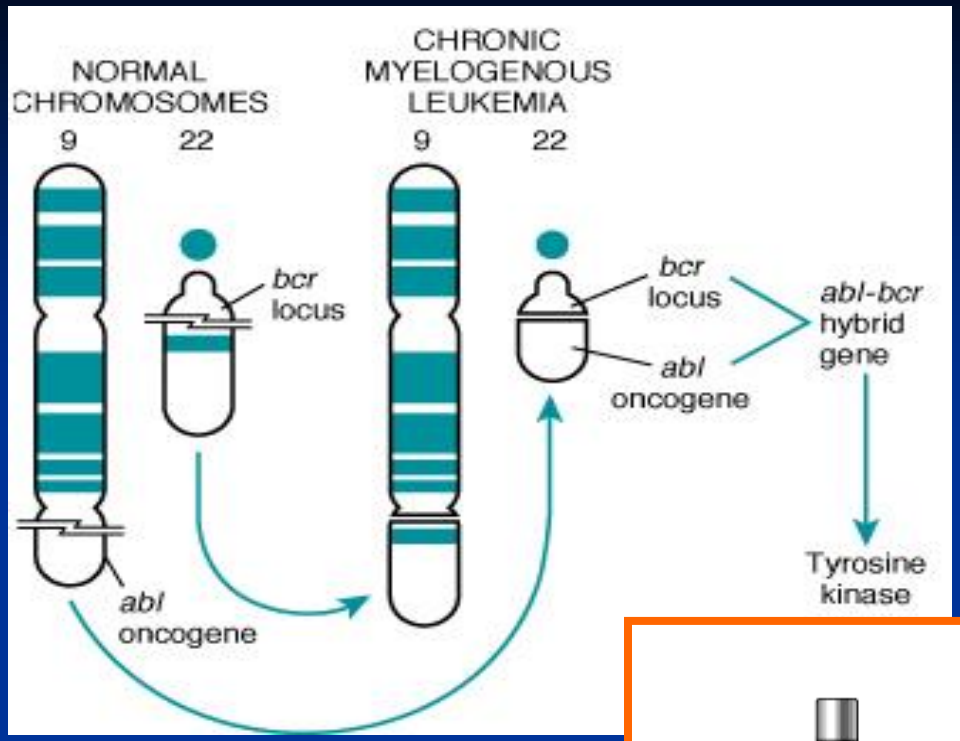
- Many tumors become more aggressive and acquire greater malignant potential…this is called “ tumor progression” …not increase in size!!
- By the time, the tumor become clinically evident, their constituent cells are extremely heterogeneous


TRANSFORMATION
PROGRESSION
PROLIFERATION OF GENETICALLY UNSTABLE CELLS
TUMOR CELL VARIANTS HETEROGENEITY
Normal cell
Carcinogen-induced change
Tumor cell
Tumor cell variants
Clonal expansion of surviving cell variants
Nonantigenic
Invasive
Metastatic
Requiring fewer growth factors
Human solid malignancy

Karyotypic Changes in Tumors

- Translocations:
  - In CML : t(9,22) …" Philadelphia chromosome"
  - In Burkitt Lymphoma : t(8,14)
  - In Follicular Lymphoma : t(14,18)
- Deletions
- Gene amplification:
  - Breast cancer : HER-2

Translocations



Gene amplification