Molecular Genetics of Cancer Cells (part II)

Dr. Walaa Bayoumie El Gazzar

Nebras Melhem

Introduction

Imbalance of cell growth: abnormal cell homeostasis (cell gain vs cell loss via apoptosis)

1. Proto-oncogenes and oncogenes

2. **Tumour suppressor genes**

Tumor Suppressor Genes

- **Physiologic function: regulate normal cell growth.**

- Loss of these genes is a key event in many, possibly all, human tumors → named **tumor suppressor genes or anti-oncogene.**

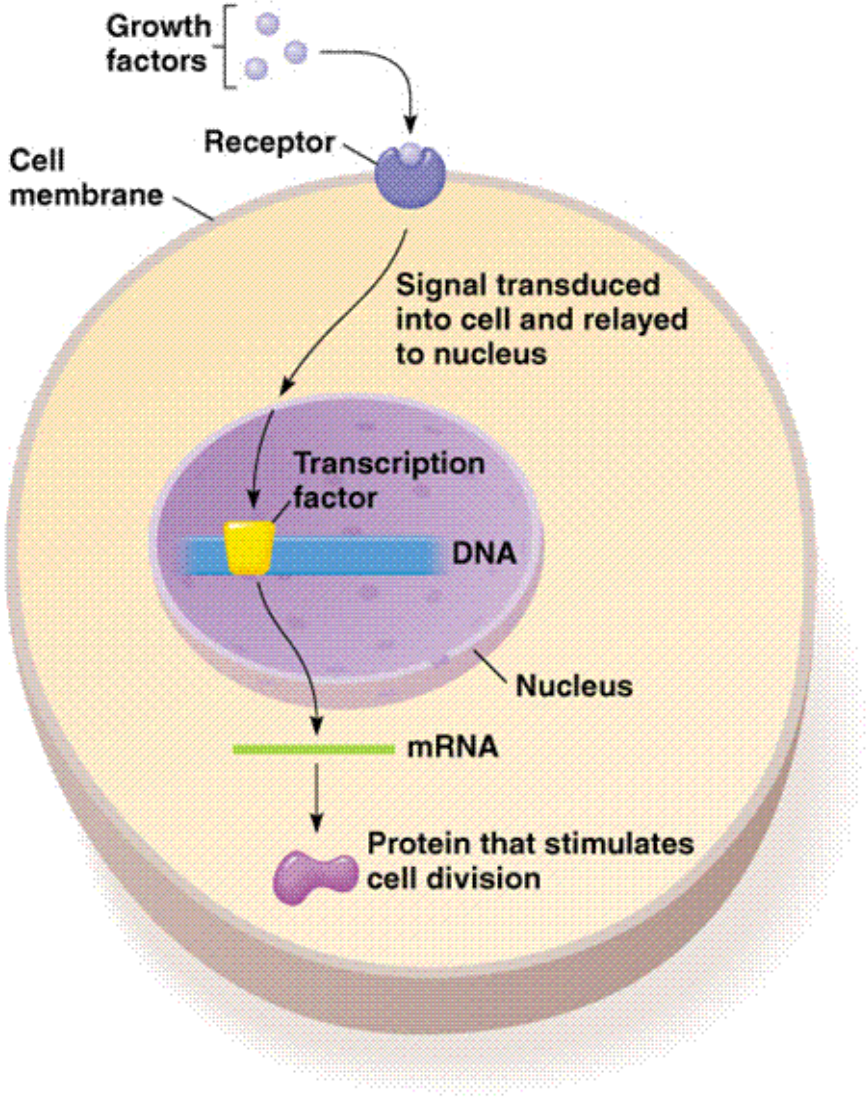
- **They normally participate in the regulation of normal cell growth.**

Tumor Suppressor Genes

- Tumor suppressor genes encode various components of a **growth inhibitory pathway.**

- Similar to growth stimulatory pathway, growth inhibitory signals originate outside the cell and use receptors, signal transducers, cell cycle regulators and nuclear transcription regulators to accomplish their effects.

a) Stimulation of cell division induced by growth factor



Tumor Suppressor Genes

Tumor suppressor genes encode proteins that have at least one of the following functions:

1. Proteins that **slow** **or** **inhibit progression** through a specific stage **of cell cycle** e.g. **p21** & **p16** (CKI)

2. **Check-point control proteins** that **arrest cell cycle** if DNA is damaged or chromosomes are abnormal e.g. **p53**

3. **Receptors** that function to **inhibit cell proliferation**

4. Proteins that **promote apoptosis** e.g. protein Bax

5. **DNA repair** enzymes.

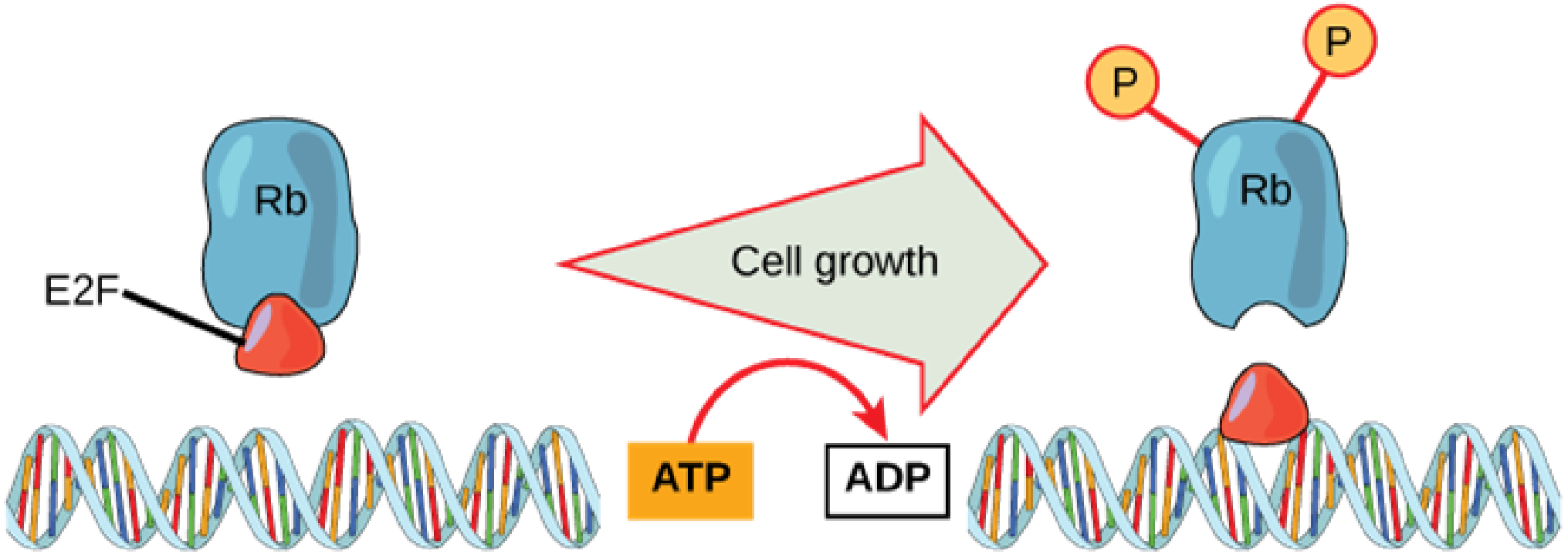
Retinoblastoma (Rb) gene

- This is the first tumor-suppressor gene discovered in human retinoblastoma
  - The **Rb gene** is found on **chromosome 13**

- Exists in:
  - **Active → hypo/unphosphorylated form**
  - **Inactive → phosphorylated**

Retinoblastoma (Rb) gene

- In its active state, Rb serves **as a brake** on the advancement of cells from the G1 to the S phase of the cell cycle.
    - In this state Rb **prevents cell cycle advancement by binding to a transcriptional factor known as E2F** and cells are kept quiescent.



Unphosphorylated Rb binds transcription factor E2F. E2F cannot bind the DNA, and transcription is blocked.

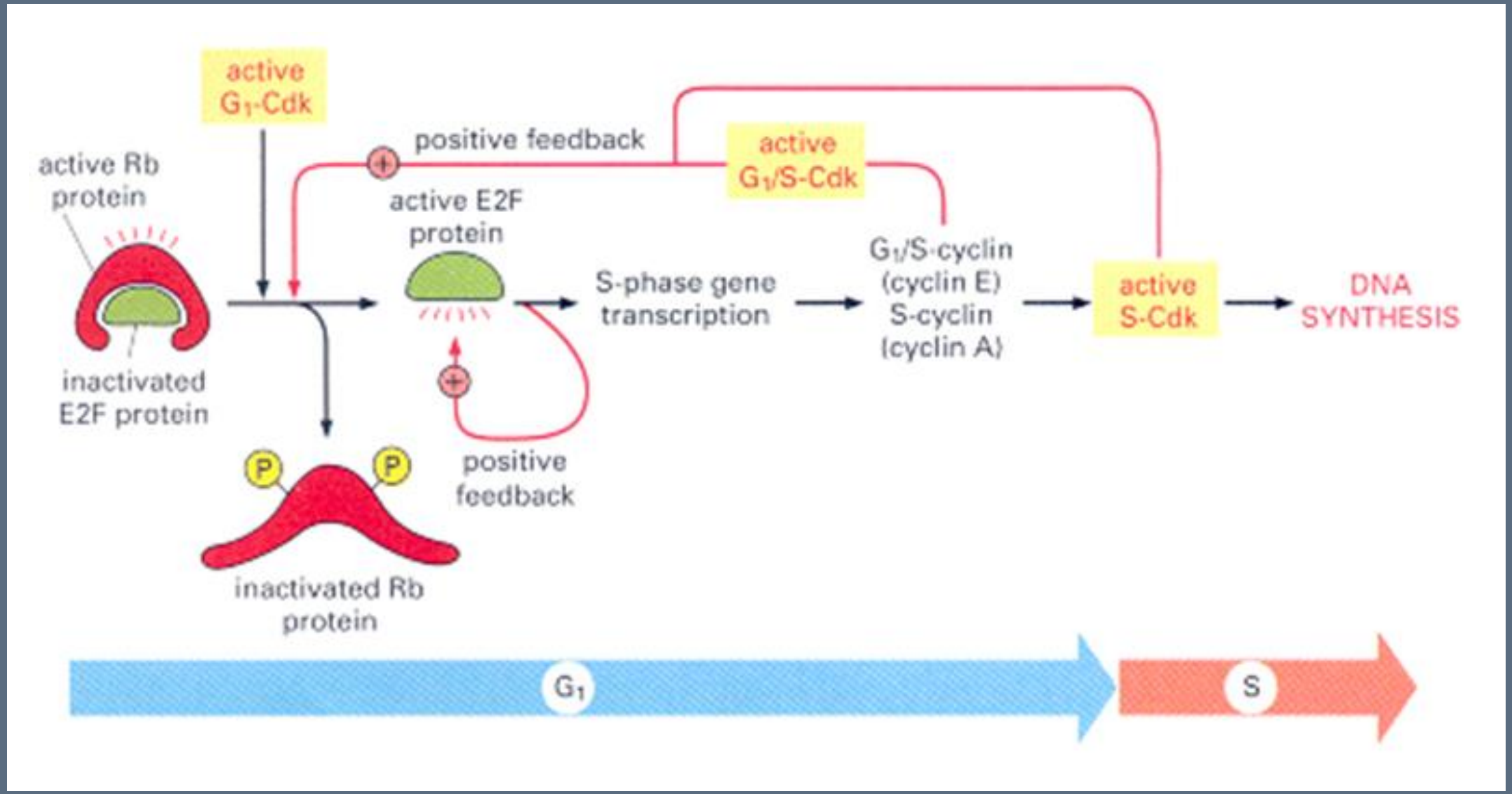
Cell growth triggers the phosphoryation of Rb. Phosphorylated Rb releases E2F, which binds the DNA and turns on gene expression, thus advancing the cell cycle.

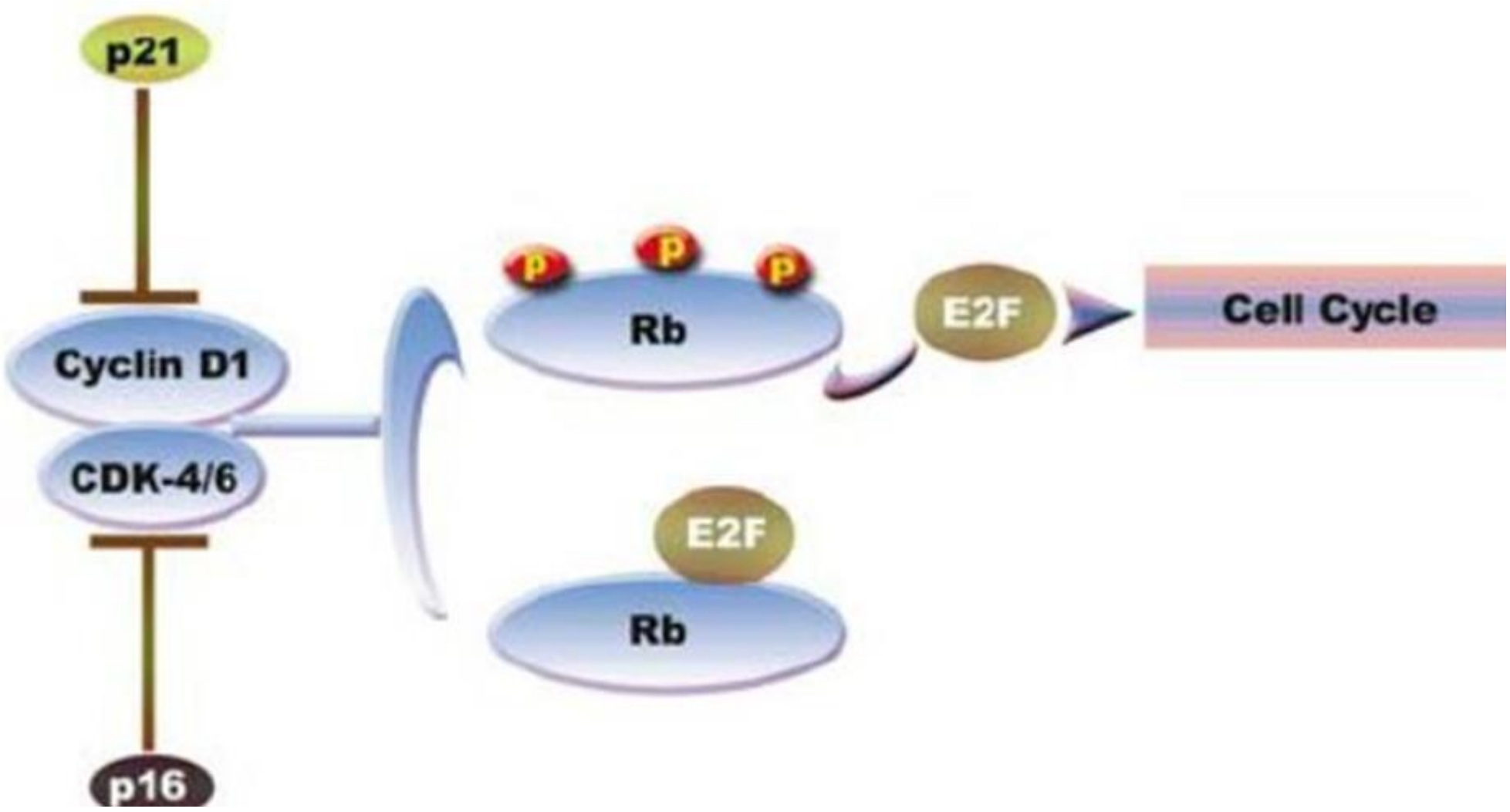
Retinoblastoma (Rb) gene

When the quiescent cells are stimulated by growth factors

→ the concentrations of certain cellular proteins known as **cyclins** will be increased →

- activation of enzymes known as **cyclin dependent kinases (CDK)** → phosphorylation of Rb
- Phosphorylated form of Rb (inactive) **releases** the E2F transcription factors
- **Released E2F proteins then activates the transcription of several other genes, that will stimulate cell replication.**


active
G1-Cdk
positive feedback
active
G1/S-Cdk
active Rb
protein
active E2F
protein
G1/S-cyclin
(cyclin E)
S-cyclin
(cyclin A)
S-phase gene
transcription
active
S-Cdk
DNA
SYNTHESIS
inactivated
E2F protein
P
P
positive
feedback
inactivated Rb
protein
G1
S


p21
Cyclin D1
CDK-4/6
p16
P
P
P
Rb
E2F
Cell Cycle
E2F
Rb

Retinoblastoma (Rb) gene

- Under normal conditions the retinoblastoma protein oscillates between the hypo/unphosphorylated (active) and phosphorylated (inactive) forms.
    - i.e. there will be a brake followed by an acceleration of the cell cycle.

- If the Rb gene is deleted or mutated → the molecular brakes on the cell cycle are released
    - **the cell proliferation is uncontrolled,** and cancer will result (Rb gene is a protective gene against cancer development).

- The main tumors in which inactivation of pRb is an important cause include **tumors of the retina** (**retinoblastoma**)-a rare childhood tumor of the retina- , **lung cancers**, adenocarcinoma of prostate and tumors of bone and connective tissues.

P53 Gene

- It is the most common target for genetic alteration in human tumors.
- Located on chromosome 17.
- Has been described as the **guardian of the genome** (the **policeman** of the cell) → **prevents** the **propagation of genetically damaged cells.**
- To lose functions as a tumor suppressor gene, the two alleles of p53 gene on the two loci of chromosome 17 must be affected by mutation or deletion.

P53 Gene and Normal Development

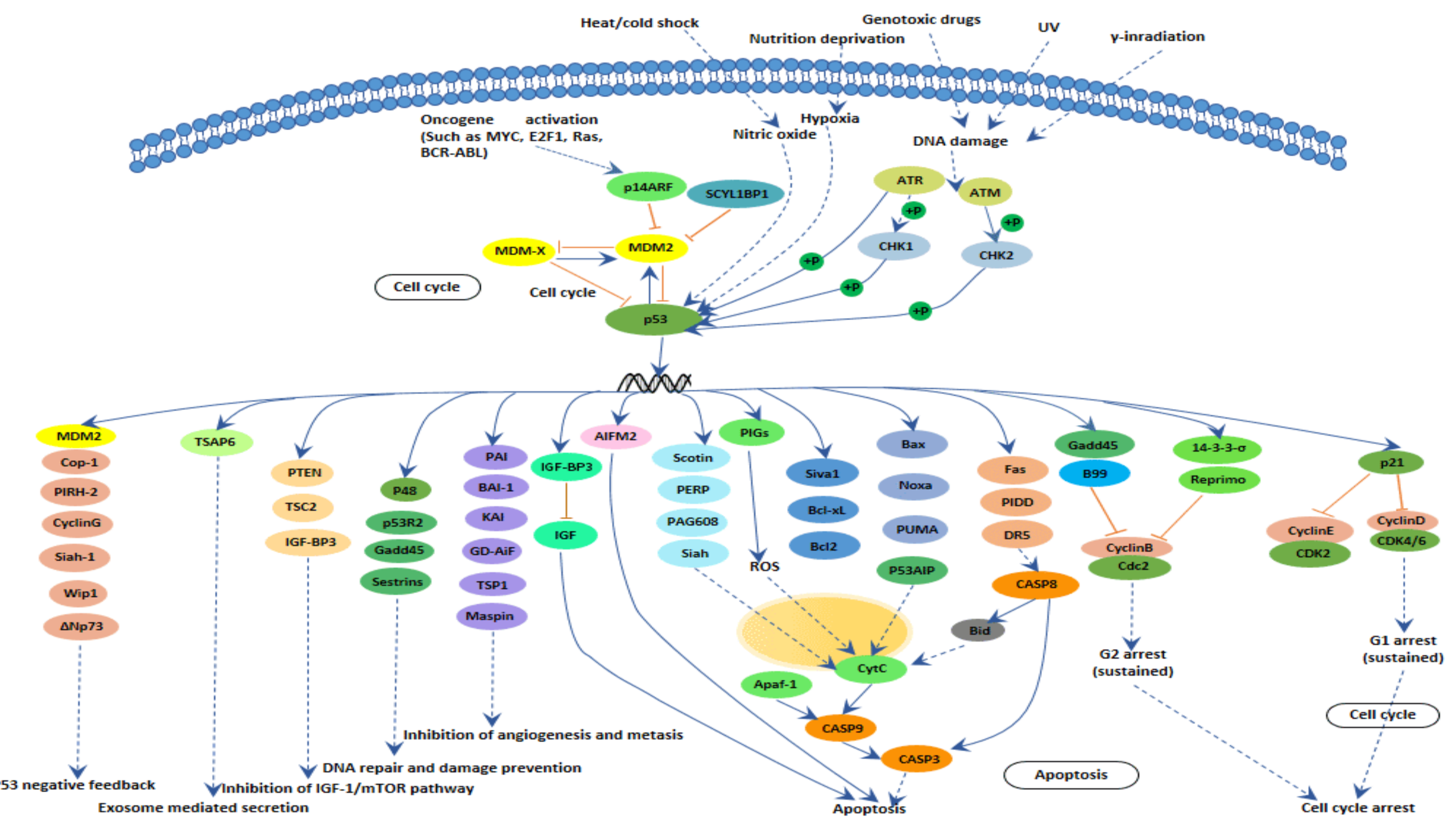
- Under physiological conditions, P53 has a **very short half life**, measured in minutes.
- There is no evidence that this protein is required for normal cell division.
- Transgenic mice (**mouse** models that have had their genomes altered for the purpose of studying gene functions) in which both copies of the gene have been knocked-out (i.e. are made inoperative) appear normal in all respects except one- they usually develop cancer by the age of 3 months & these observations suggest that p53 may serve a function that is required only occasionally or in special circumstances.

Function of p53

- **p53 protein is a transcription factor** that **binds to DNA** and stimulates the transcription of several genes that mediate:
    - **Cell cycle arrest:** by holding cells at the G1/S transition point in case of recognition of DNA damage
    - **DNA repair**
    - **Apoptosis:** if DNA damage cannot be repaired

p53 signalling pathway

- In normal cells, p53 is **inactivated** by its negative regulator **mdm2.**
- Upon DNA damage (UV, radiation, chemicals) → dissociation of p53 from mdm2 → activation of p53.
- DNA DSBs activate ATM (or ATR) → phosphorylation of p53, thereby preventing it's degradation by MDM2.
- Active p53 will either induce cell cycle arrest to allow repair and survival or apoptosis.
- Activated p53 binds DNA and activates expression of genes encoding for p21 (CDKI).
    - P21 is complexed with CDK → **cell cannot pass through G1/S checkpoint.**


Heat/cold shock
Nutrition deprivation
Genotoxic drugs
UV
γ-inradiation
Oncogene activation
(Such as MYC, E2F1, Ras,
BCR-ABL)
Nitric oxide
Hypoxia
DNA damage
p14ARF
SCYL1BP1
MDM-X
MDM2
Cell cycle
Cell cycle
ATR
ATM
CHK1
CHK2
+P
p53
MDM2
Cop-1
PIRH-2
CyclinG
Siah-1
Wip1
ΔNp73
TSAP6
PTEN
TSC2
IGF-BP3
P48
p53R2
Gadd45
Sestrins
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
IGF-BP3
IGF
AIFM2
Scotin
PERP
PAG608
Siah
PIGs
ROS
Siva1
Bcl-xL
Bcl2
Bax
Noxa
PUMA
P53AIP
Fas
PIDD
DR5
CASP8
Bid
Gadd45
B99
CyclinB
Cdc2
14-3-3-σ
Reprimo
p21
CyclinE
CDK2
CyclinD
CDK4/6
CytC
Apaf-1
CASP9
CASP3
Apoptosis
G2 arrest
(sustained)
G1 arrest
(sustained)
Cell cycle
Inhibition of angiogenesis and metasis
DNA repair and damage prevention
Inhibition of IGF-1/mTOR pathway
Exosome mediated secretion
53 negative feedback
Apoptosis
Cell cycle arrest

p53 signalling pathway

- **Mutant** p53 can no longer bind to DNA in an effective way
    - p21 is not made available to act as stop signal for cell division → cells divide in an uncontrolled way → cancer

- If DNA damage is irreparable → p53 can initiate apoptosis by activation of transcription of BAX.

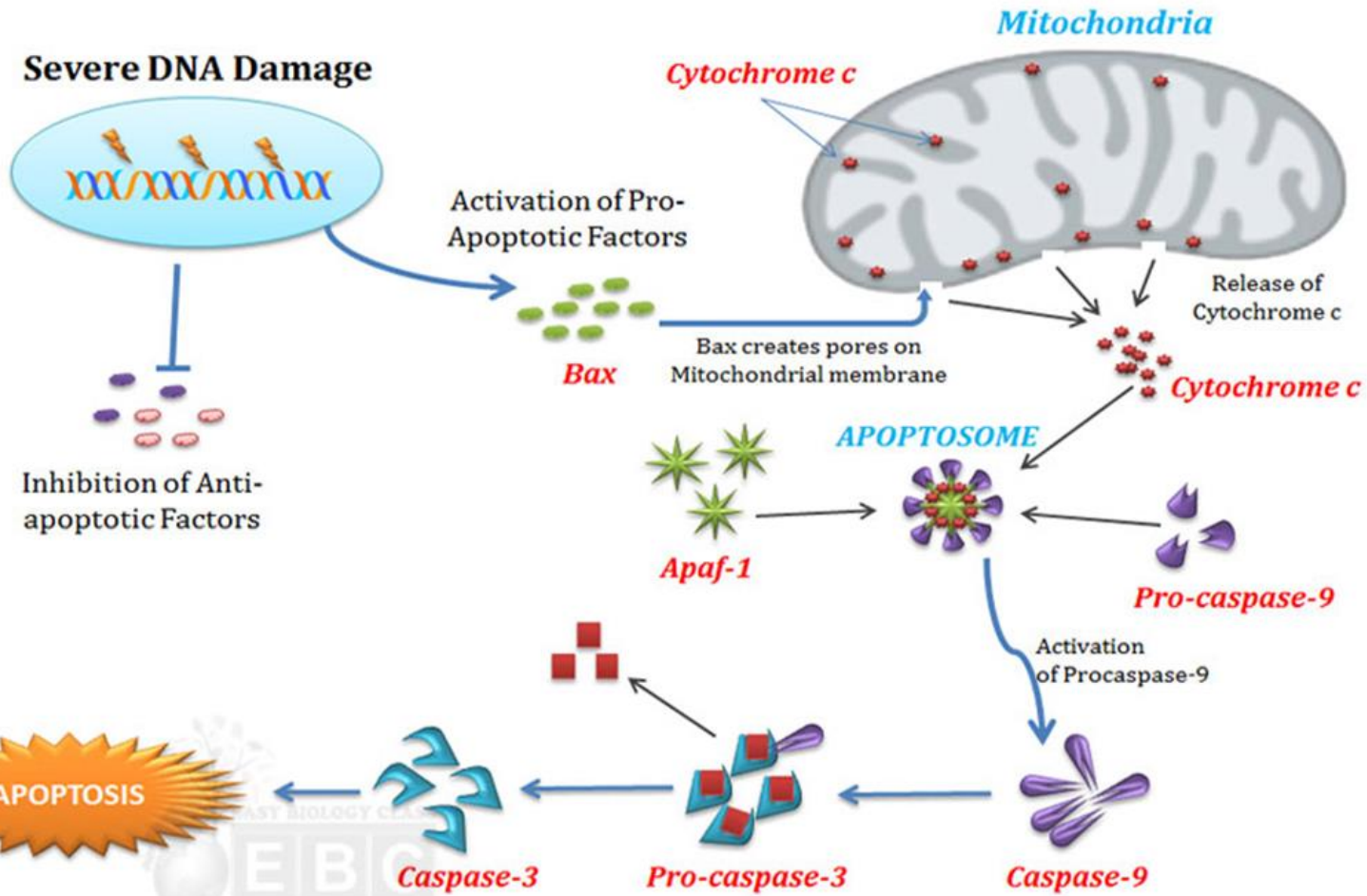
p53 signalling pathway

Intrinsic pathway

- Initiated from within the cell due to severe stress.
- Involves **activation of p53** → transcription of **bcl-2 pro-apoptotic proteins** (e.g. Bax (bcl-2 antagonist x) → mitochondrial release of cytochrome C → activate caspase enzymes

INTRINSIC PATHWAY OF APOPTOSIS

(Mitochondria Mediated Programmed Cell Death Pathway)



Activation of Caspase-3 by Caspase-9

P53 & DNA repair

- P53 may directly & indirectly stimulate the DNA repair machinery.
- The link of p53 to the DNA repair machinery: via another gene called **GADD45** "growth arrest & DNA-damage inducible gene".
- The GADD45 gene is turned on in cells exposed to stresses that stop cell growth & cause DNA damage.

P53 & DNA repair

- The cells' ability to turn GADD45 on depends on the presence of normal p53 gene whose protein product stimulates production of the GADD45 protein (as for p21).

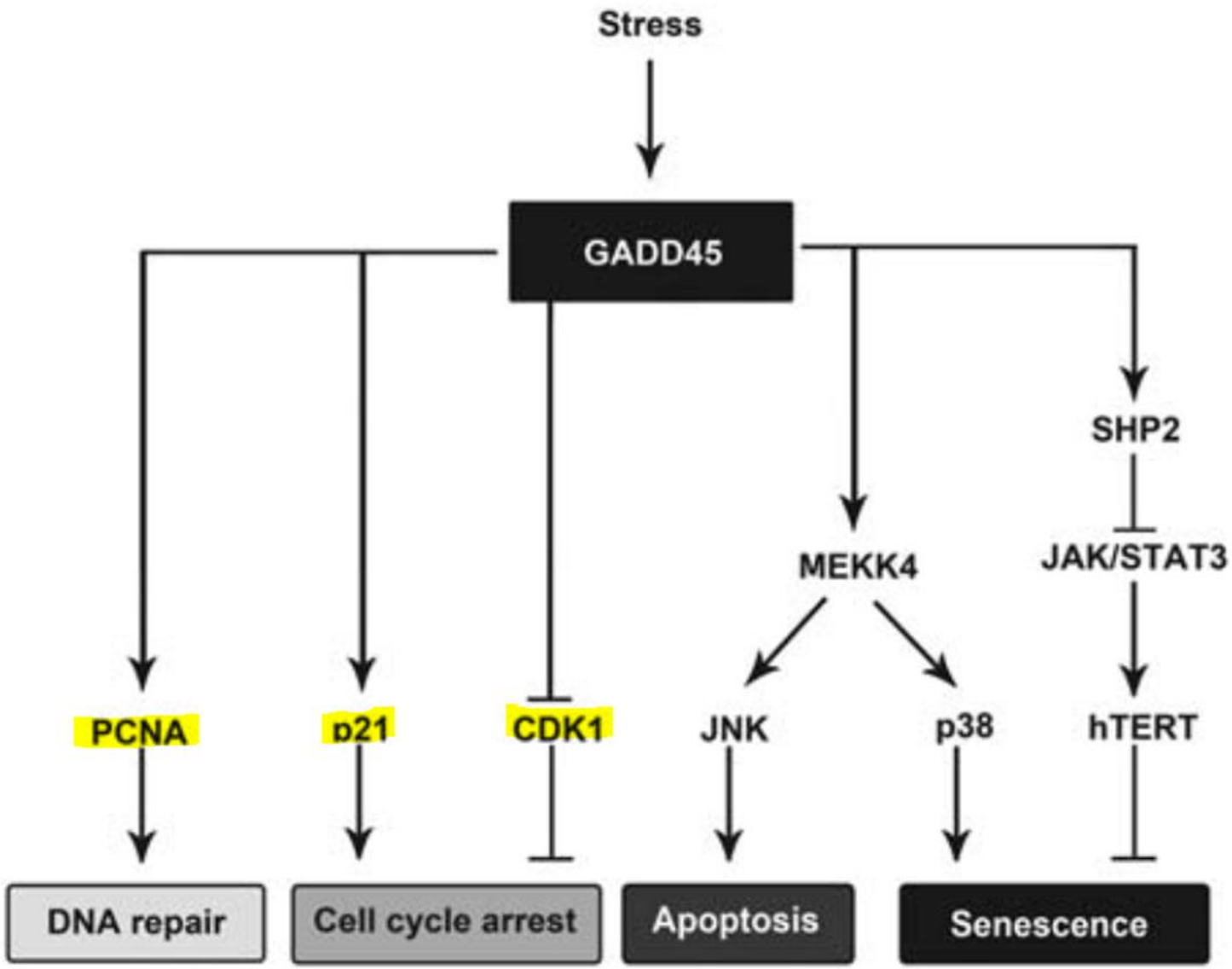
- GADD45 complexes with **proliferating cell nuclear antigen** (PCNA) which is a cofactor of **DNA polymerase delta.**
    - DNA polymerase delta is an enzyme complex found in eukaryotes that is involved in DNA replication and repair.

P53 & DNA repair

- **PCNA** protein is known to have 2 functions

1. It is a necessary component of the machinery that copies (replicate) DNA so that cell division can take place.

2. It is also needed for the resynthesis of DNA after damaged portions are removed by the cells nucleotide excision repair system which remedies damage caused by environmental insults


Stress
GADD45
SHP2
MEKK4
JAK/STAT3
PCNA
p21
CDK1
JNK
p38
hTERT
DNA repair
Cell cycle arrest
Apoptosis
Senescence

BRCA-1 and BRCA-2 genes

- Breast cancer gene-1 (BRCA-1), located on chromosome 17, and Breast cancer gene-2 (BRCA-2) located on chromosome 13, are two **tumor suppressor genes** that are associated with the occurrence of breast and several other cancers.

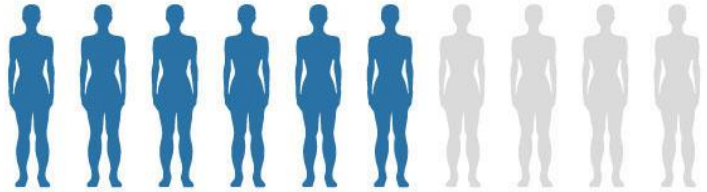
- BRCA2 and BRCA1 are normally expressed in the cells of breast and other tissue, where they **help repair damaged DNA** **or** **destroy cells** if DNA cannot be repaired**.**

- Mutations in BRCA genes → **predispose to errors in DNA replications**, thus leading to mutations in other genes that directly affect cell cycle and cell growth.

NATIONAL CANCER INSTITUTE

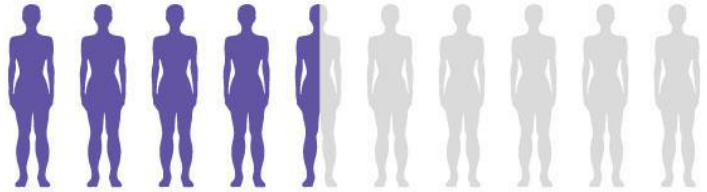
CHANCES OF DEVELOPING BREAST CANCER BY AGE 70

Specific inherited mutations in the BRCA1 and BRCA2 genes increase the risk of breast and ovarian cancers. Testing for these mutations is usually recommended in women without breast cancer only when the person's individual or family history suggests the possible presence of a harmful mutation in BRCA1 or BRCA2. Testing is often recommended in younger women newly diagnosed with breast cancer because it can influence treatment decisions and have implications for their family members.



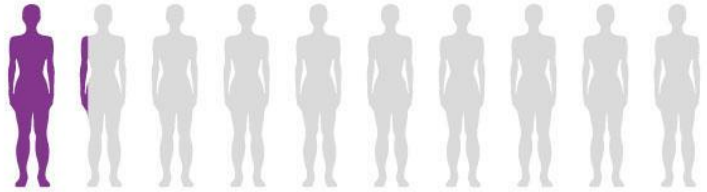
MUTATED BRCA1

55-65%



MUTATED BRCA2

45%



NORMAL BRCA

12%

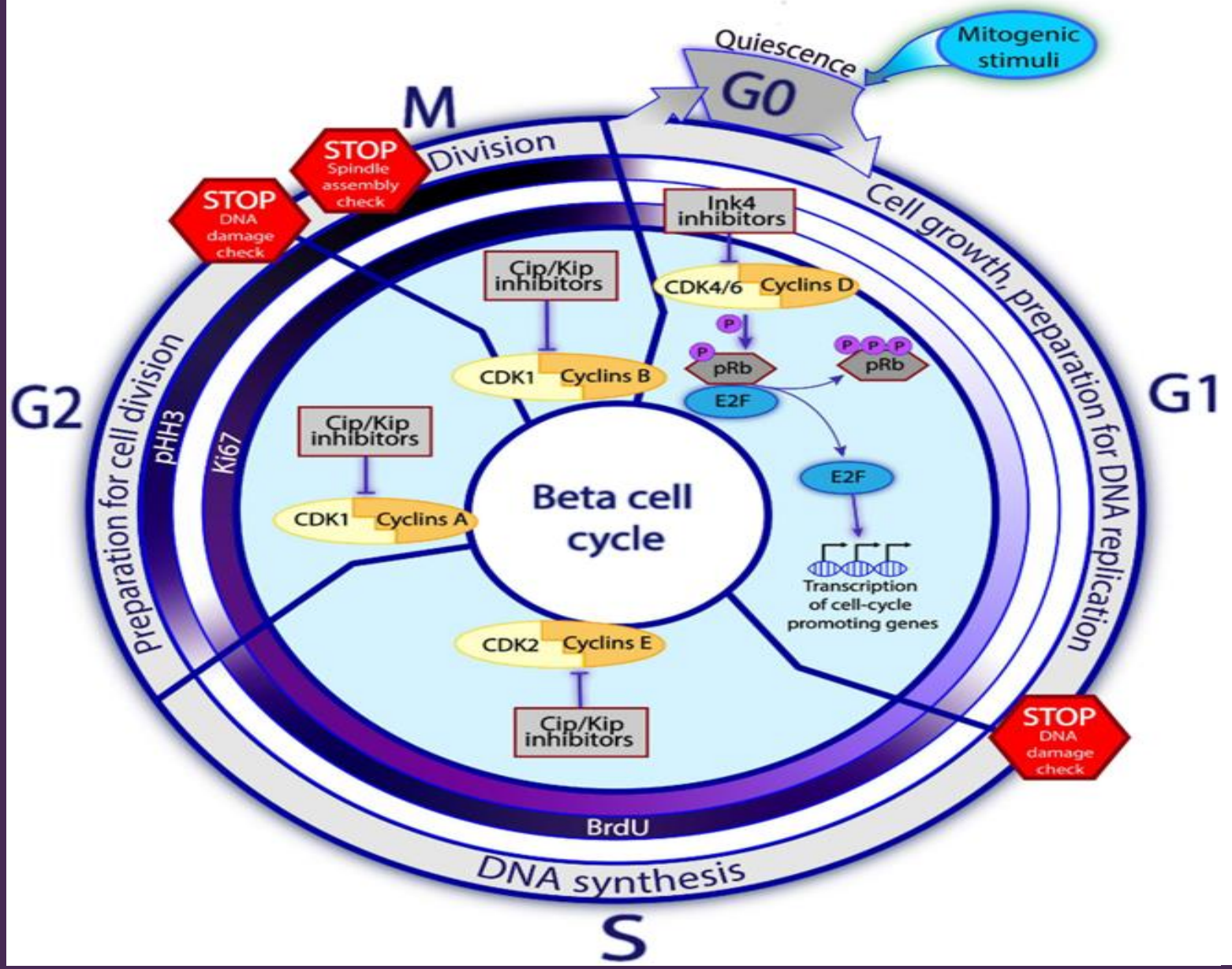
Li–Fraumeni syndrome

- Is a rare, autosomal dominant, hereditary disorder that predisposes carriers to cancer development
- The syndrome is linked to mutations of the p53 tumor suppressor gene
- Li–Fraumeni syndrome is characterized by **early onset of cancer**, a wide variety of types of cancers
- The classical LFS malignancies: sarcoma, cancers of the breast, brain, and adrenal glands—comprise about 80% of all cancers that occur in this syndrome

Genes that regulate DNA repair

- DNA susceptible to alterations resulting from errors that occur spontaneously during DNA replication
- Such mistakes, if not repaired promptly, can also push the cells to cancer formation.
- HNPCC (hereditary nonpolyposis colon cancer) syndrome results from **defects in genes involved in DNA mismatch repair.**
- **DNA mismatch repair (MMR)** is a system for recognizing and repairing errors of bases that can arise during DNA replication and recombination

  → errors slowly accumulate in several genes, including proto-oncogenes and tumor suppressor genes with ultimate production of cancer.



- **INK4** Inhibitors are p15, p16, p18, and p19, specifically inhibits CDK4 and CDK6 activity
- **CIP/KIP** Inhibitors are consisting of p21, p27, and p57, inhibits other cyclin-CDK complexes

Questions

- What is the significance of mutations in the TP53 gene in cancer, and how does it impact cell cycle regulation?

1. TP53 mutations promote cell cycle progression.
2. TP53 mutations inhibit cell cycle progression.
3. TP53 mutations have no impact on cell cycle regulation.
4. TP53 mutations lead to apoptosis.

Questions

- How do chromosomal abnormalities, such as translocations, contribute to the development of cancer?

1. They have no impact on cancer development.
2. They disrupt normal gene function and regulation.
3. Chromosomal abnormalities prevent cell division.
4. Chromosomal abnormalities only occur in non-cancerous cells.

Questions

- Can you describe the relationship between cell cycle checkpoints and the control of cell proliferation in normal and cancer cells?

1. Cell cycle checkpoints are irrelevant to cell proliferation.
2. Cell cycle checkpoints promote uncontrolled cell proliferation in cancer cells.
3. Cell cycle checkpoints prevent uncontrolled cell proliferation.
4. Cell cycle checkpoints have no impact on normal cells.