Teaching unit 06

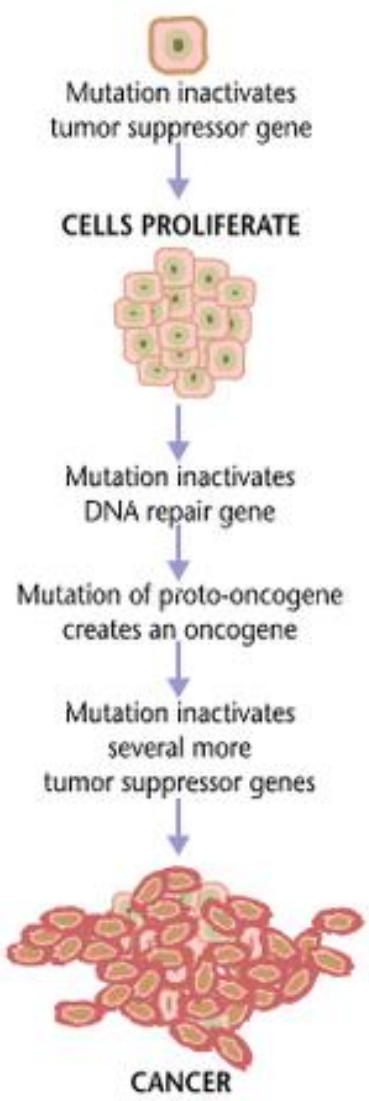
TUMOR SUPPRESSOR GENES 2

Non-specific mechanisms of preventing accumulation of genetic changes

- The tissues in the human body are organized in such a way as to prevent the accumulation of cells with damaged genetic material:
  - Cells that are in constant contact with factors of the external environment (cells of the skin, GIT and bronchial epithelium) have a short life cycle with rapid elimination of differentiated cells that are directly exposed to potentially harmful noxes.
  - in tissues such as the colon, stem cells are protected in crypts.
- However, this system is not perfect.

Specific mechanisms of preventing accumulation of genetic changes

- In addition to numerous protection mechanisms against damage to genetic material, genetic changes occur in cells that escape detection.
- One of the ways in which genetic changes occur is the inactivation of the signaling network for the detection and correction of DNA damage.
- Continuity of cell cycles and unresponsiveness to genetic changes will allow the accumulation of DNA damage that can induce oncogenesis.



- Oncogenesis is a complex process which, in essence, implies inadequate activation of oncogenes and inactivation (loss of function) of other types of genes, i.e. tumor-suppressor genes.

- The formation of tumors is the result of the accumulation of genetic mutations that cause "uncontrolled" proliferation of cells that become **immortalized** and consequently able to invade and metastasize to other tissues.

Continuous proliferation - immortalization of cells

- Boveri 1902. Tumors arise due to changes in the chromosomes in the cell.
- The next half century: changes in one or more genes may be associated with malignancies.

- Some genetic changes → cessation of differentiation → formation of undifferentiated cells with self-renewal capacity.

- Others, on the other hand, block apoptosis, enabling a longer cell life.

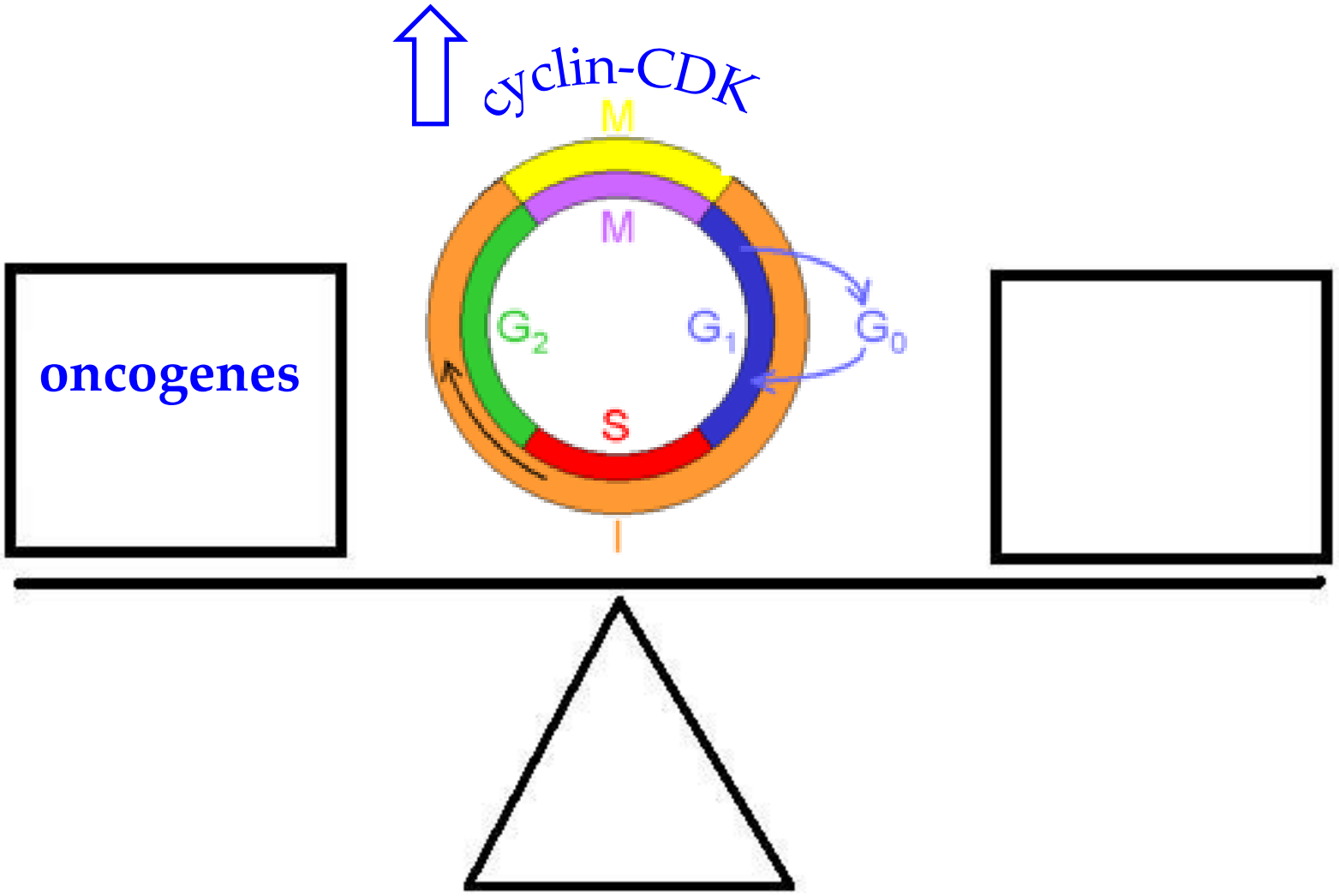
- Such genetic changes induce:

  - Permanent activation of signaling pathways for cell growth (proliferation)
  - Suppression of cell death (apoptosis)
  - Changes in DNA damage control mechanisms

- The aforementioned processes result in "uncontrolled" cell growth and extended cell life → immortalization.

Continuous proliferation - immortalization of cells

- Tumor is partly a disease of "**uncontrolled proliferation**".
- Cell proliferation is a tightly regulated process
- Tumorogenesis is characterized by mutations and changes in the regulation of gene activity:
  - DNA damage control
  - Regulation of the cell cycle
- Genes that after mutation show enhanced function that accelerates cell growth and malignant transformation are called oncogenes (proto-oncogenes). Inadequate expression of these genes can trigger tumor formation.


cyclin-CDK
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G2
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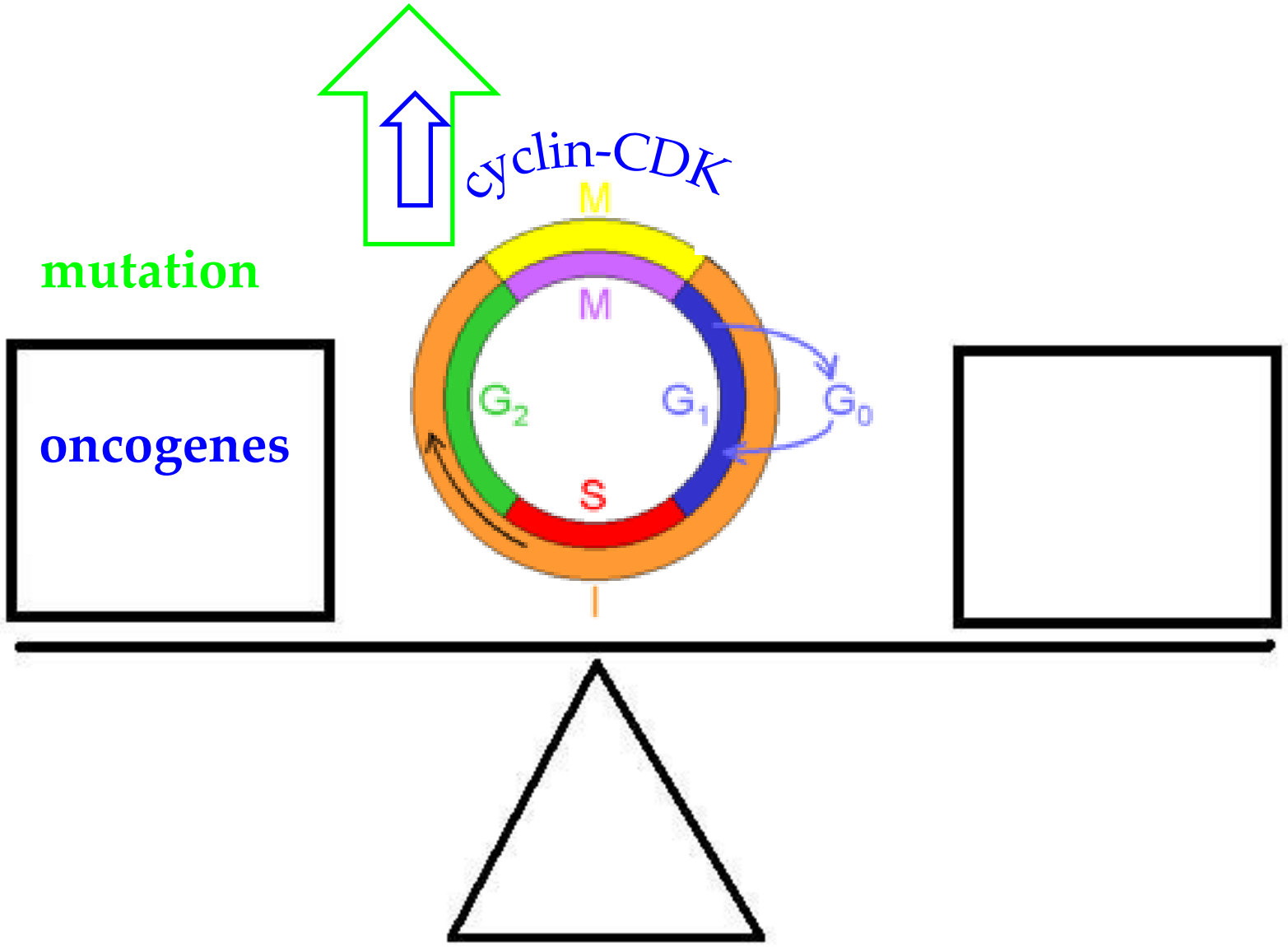
oncogenes


cyclin-CDK
mutation
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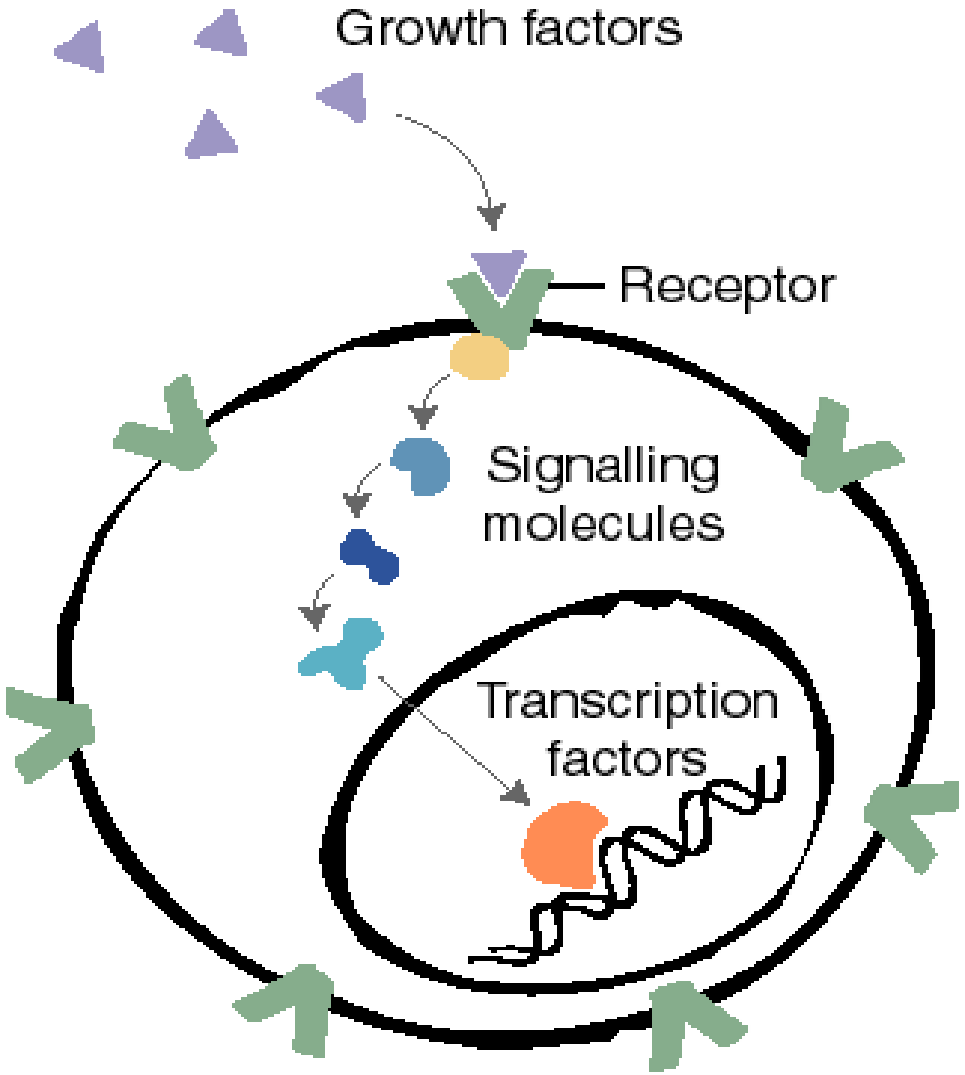
let us remind ourselves...



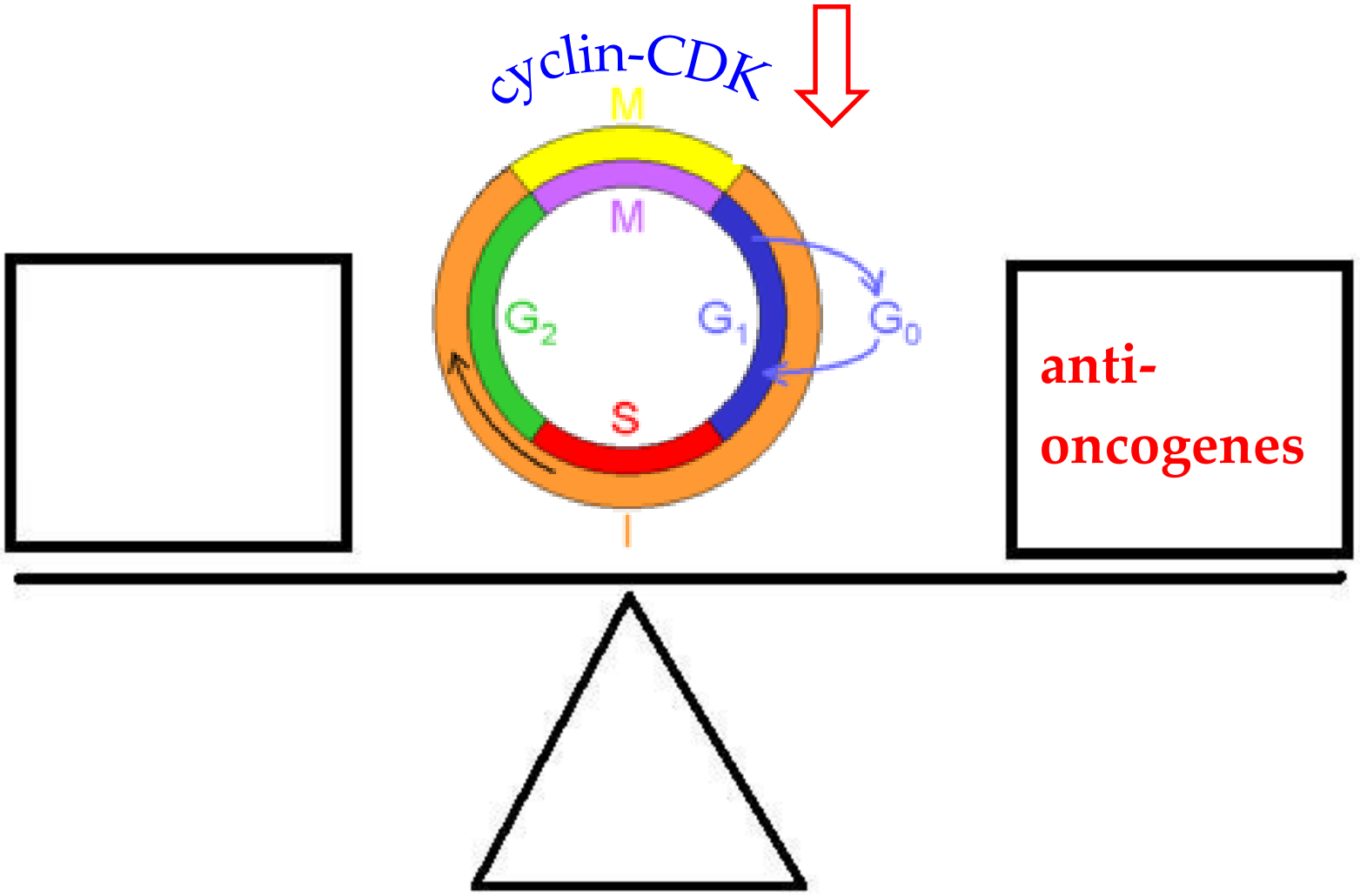
oncogenes encode:

growth factors
receptors for growth factors
signal transducers
transcription factors

One of the causes of disturbances in the regulation of cell proliferation is hyperactivity of proto-oncogenes

Tumor suppressor genes

- Tumor suppressor genes are very sensitive to critical DNA damage.
- They represent a significant physiological barrier to clonal expansion or genetic mutations.
- They are capable of preventing the growth and metastasis of cells that are triggered by uncontrolled proliferation mediated by oncogenes.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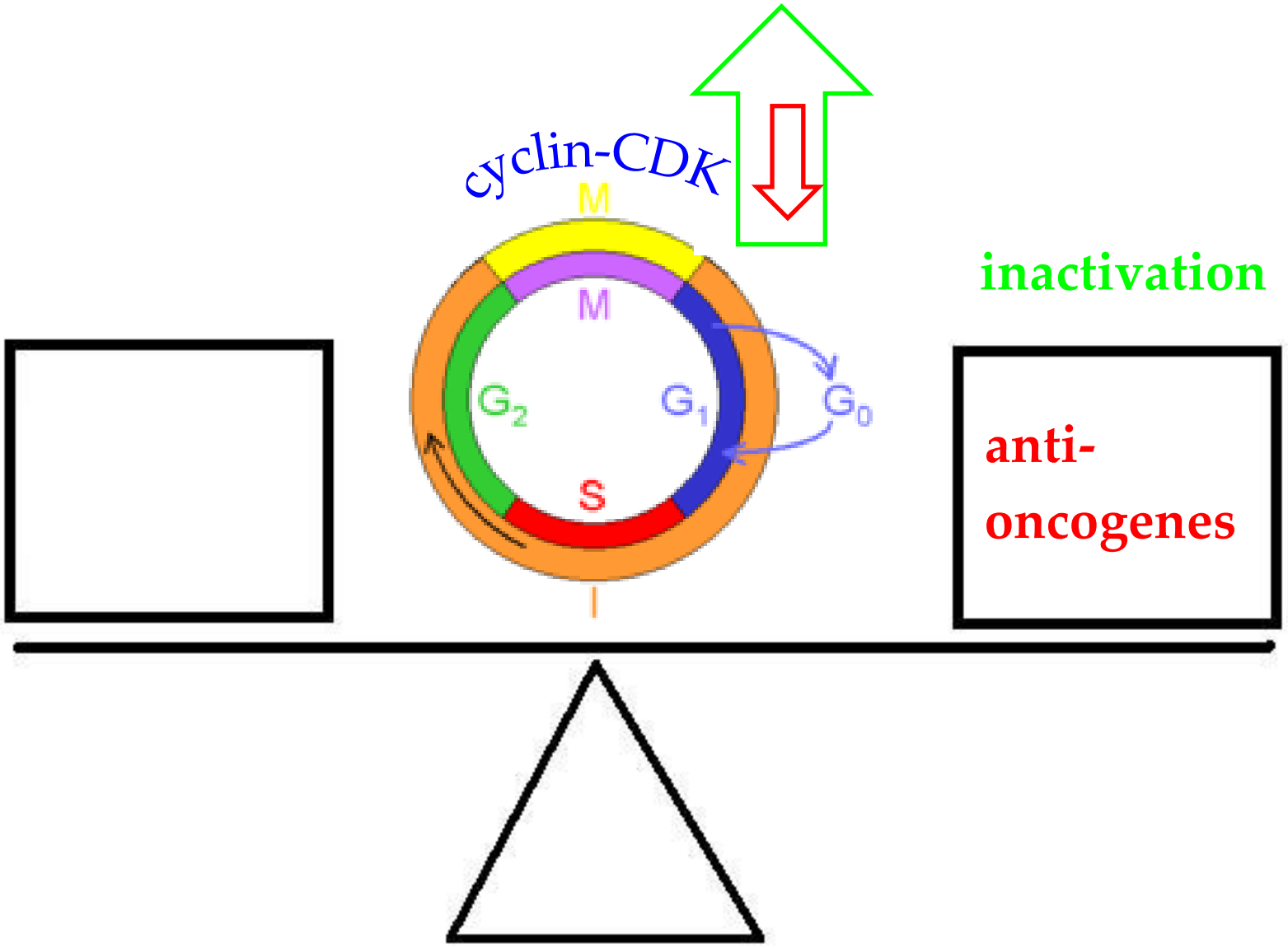
cyclin-CDK
M
M
G2
G1
G0
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inactivation
anti-
oncogenes

Tumor suppressor genes

- They contribute to oncogenesis by losing their function.
- These genes, which include the retinoblastoma gene (Rb-1) and p53, stop cell proliferation.
- The Rb-1 gene inhibits the action of the important transcription factor E2F, and deletion of the Rb gene (seen in hereditary retinoblastoma) relieves E2F suppression.
- On the other hand, p53 enhances the expression of p21/Cip1, which as a suppressor of cyclin-dependent kinases stops further proliferation.

Tumor suppressor genes

- The importance of Rb-1 and p53 genes in the genesis of tumors has been proven by the identification of mutations of the same genes in people with tumor predisposition syndromes, such as congenital retinoblastoma (Rb-1) and Li Fraumeni multicancer syndrome (p53).

- Inactivation or mutation of one allele of the tumor suppressor gene is not sufficient for tumor development, a change in both loci is required - loss of heterozygosity.

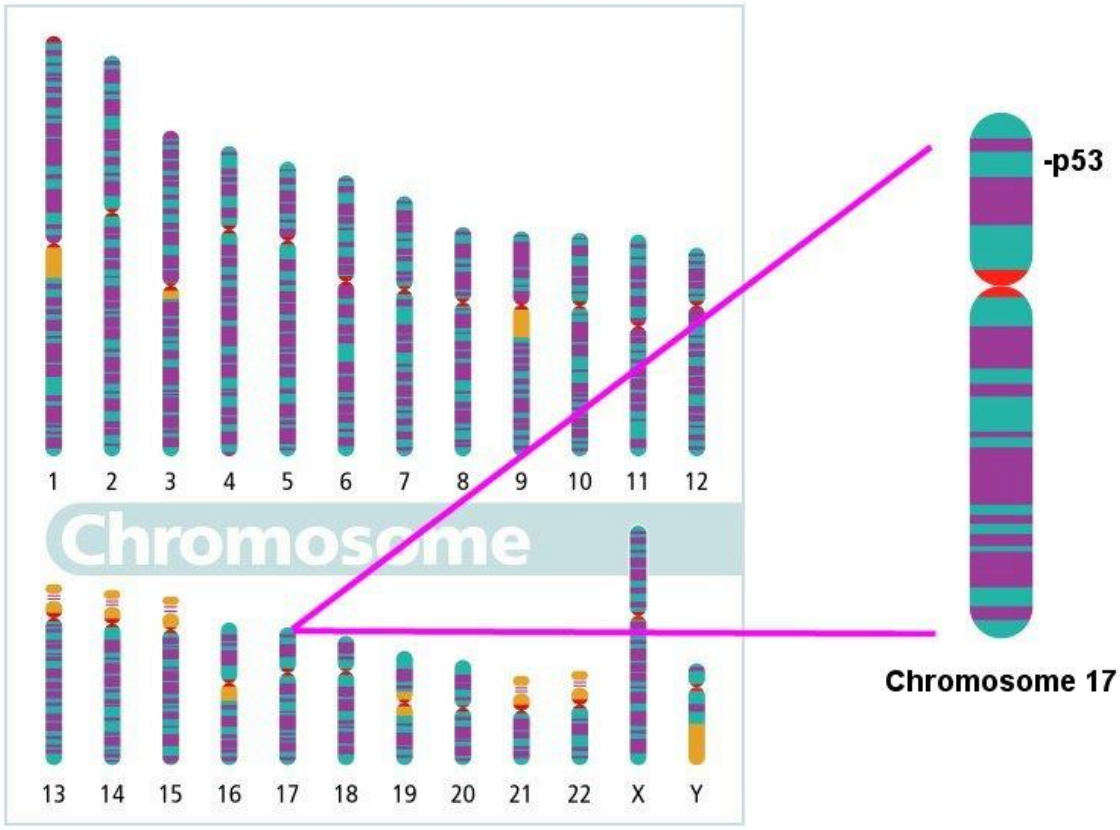
Tumor suppressor genes

- The activity of tumor-suppressor genes can be neutralized by interaction with other cellular proteins or with viral oncoproteins.
- Mutations of the p53 gene directly change the function of the p53 protein, but its function can also be altered under the influence of some oncogenes.
- Increased oncogene expression sometimes causes sequestration or destabilization of the normal form of the p53 protein, inactivating its tumor-suppressor activity.
- Mutations of the p53 gene are present in approximately 50% of human malignancies.

p53 - guardian of the genome

- **p53 is a prototype anti-oncogene with a role in oncogenesis**
- **The product of the normal p53 gene (wild-type p53) is a transcription factor:**
  - **regulation of gene transcription**
  - **interprotein interactions**
- **In normal cells, p53 is generated and degraded continuously, and the half-life of the protein is about 30 minutes.**
- **When the genome is damaged, p53 becomes stabilized and the concentration in the cell increases up to ten times as well as the half-life of 24 hours.**

Tumor suppressor gene p53 is located on chromosome 17p13 and is one of the most frequently mutated genes in human cancers.



p53 - guardian of the genome

- Monitoring of checkpoints in S and M-phase
- Sensitive to DNA damage
- temporarily stopping cell division allowing enzymes of the DNA repair system to correct the error
- In case of severe damage, it induces:

apoptosis or irreversible stopping of cell division, the so-called "replicative age" (senescence).

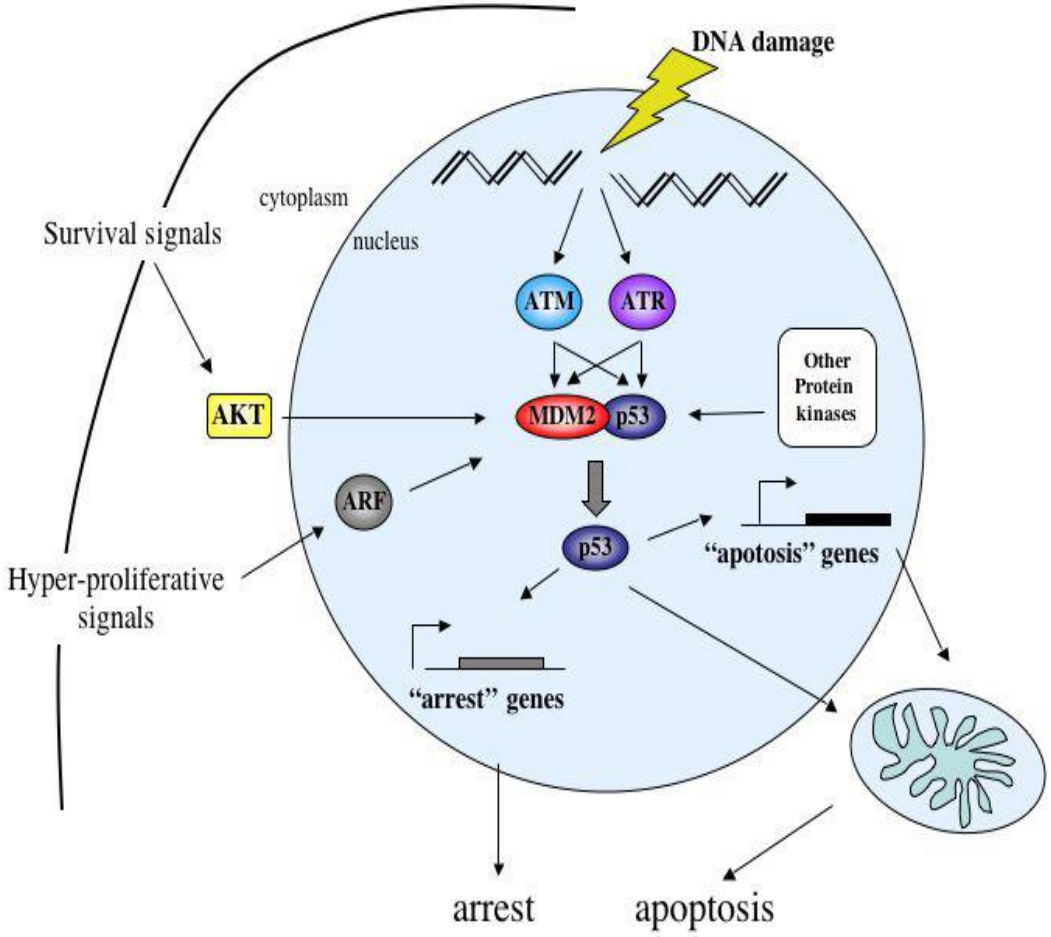
p53 - guardian of the genome

- Eliminates or prevents further division of genetically altered cells (with mutations of oncogenes or tumor-suppressor genes)

- Prevents the accumulation of changes that pose a risk for tumor development.

- UV radiation and DNA double-strand breaks with the help of ATM protein and other DNA-dependent kinases induce p53 expression.

p53 - guardian of the genome

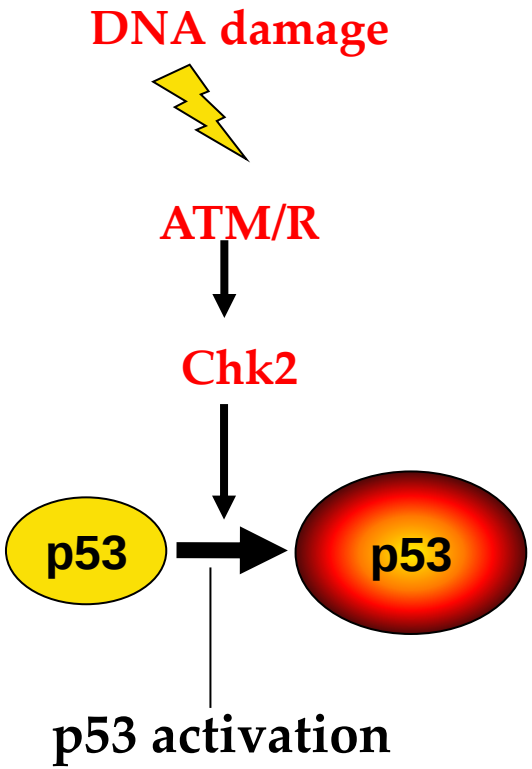


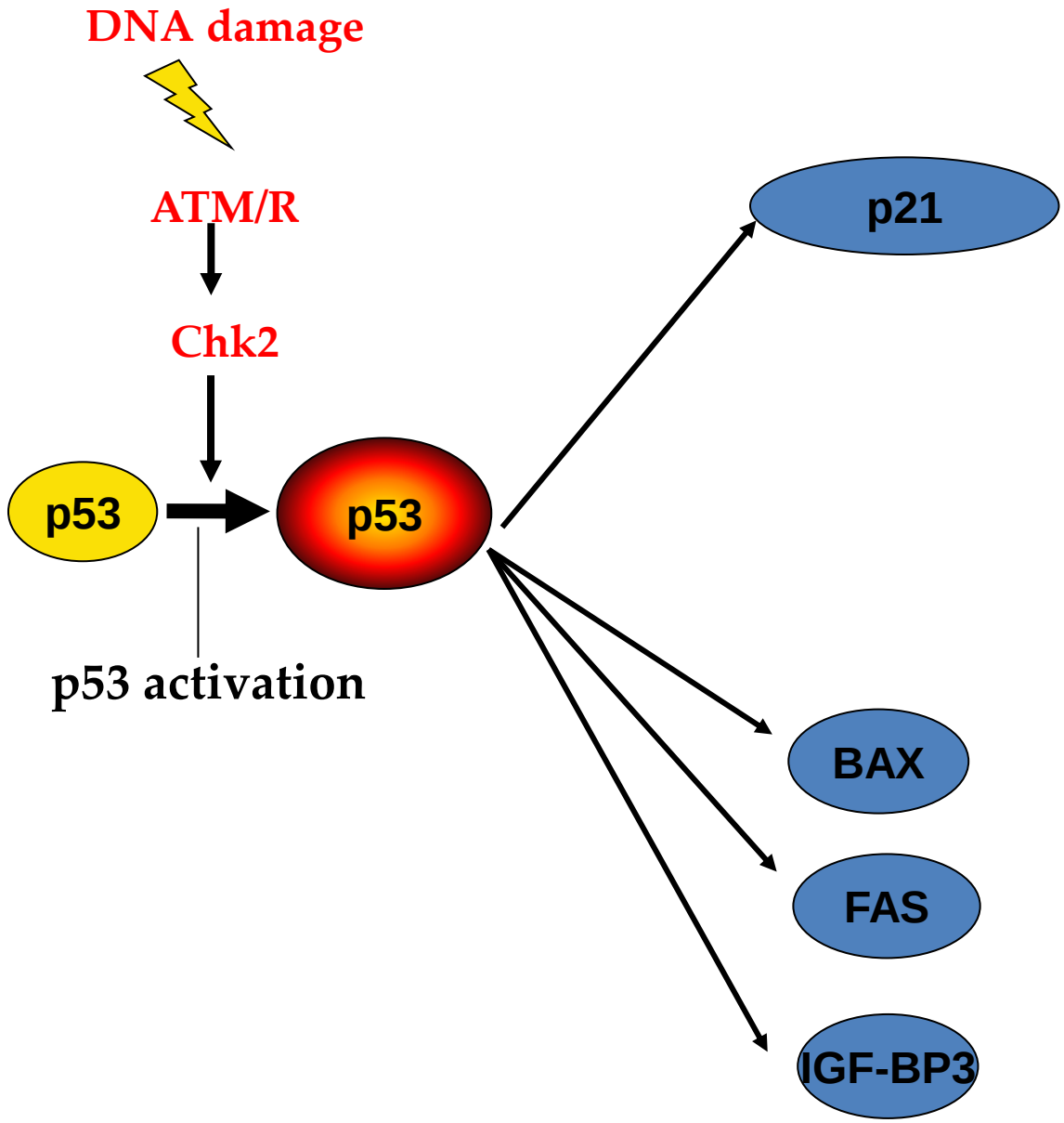
- p53 ↑ Cip/Kip-p21
- Cip/Kip-p21 ↓ cdk2; 4, 6
- G1 arrest
- p53 ↑ BAX, FAS, ☠

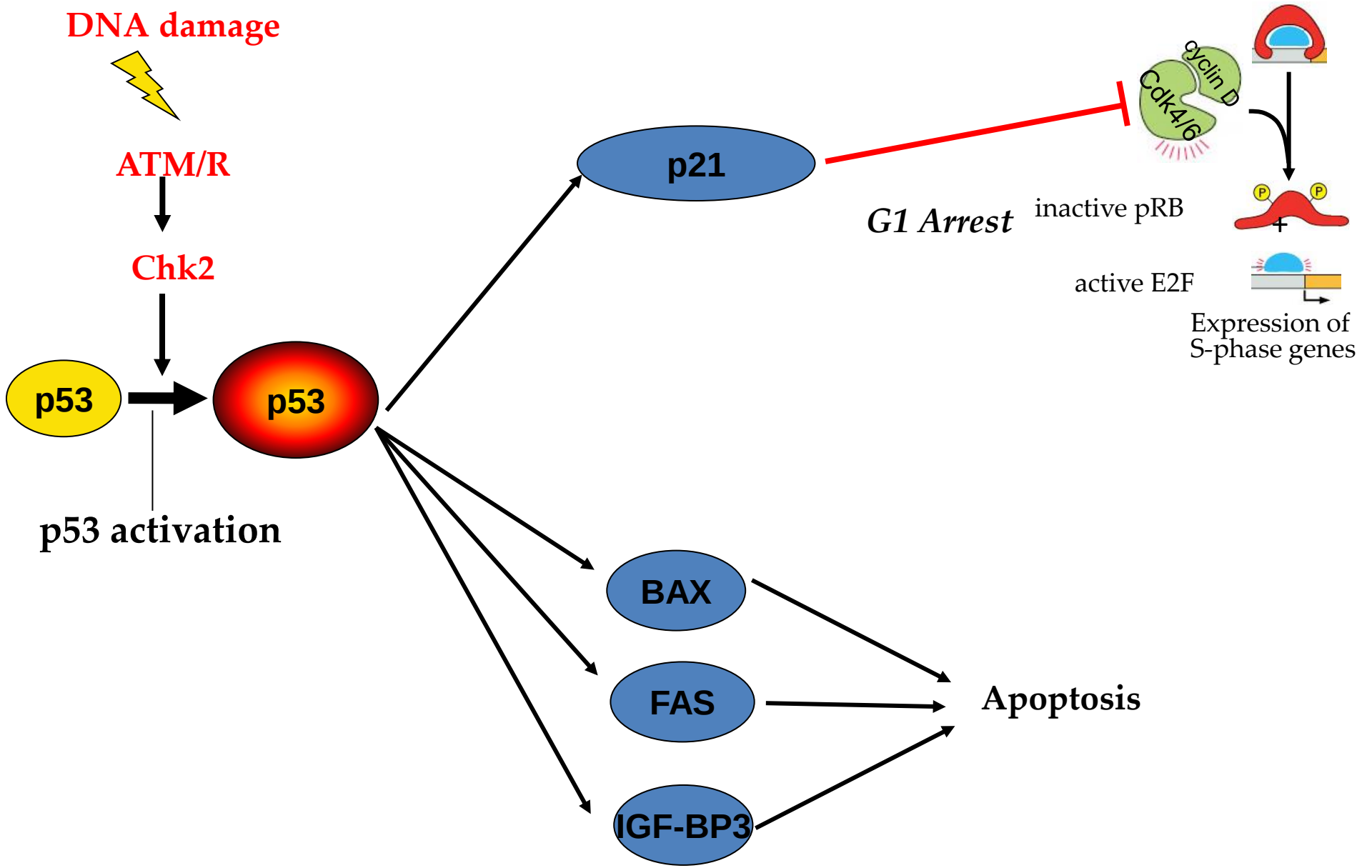
p53 - guardian of the genome

Pro-apoptotic effect of p53 is twofold:

- On the one hand, it inhibits the transcription of Bcl-2 (anti-apoptotic gene)
- On the other hand, it induces the production of the pro-apoptotic Bax protein as well as FAS and other death receptors from the tumor necrosis factor family.
- Ultimate outcome of the pro-apoptotic action of p53 is the elimination of cells with genetically altered material.


DNA damage
ATM/R
Chk2
p53
p53
p53 activation


DNA damage
ATM/R
Chk2
p53
p53
p53 activation
p21
BAX
FAS
IGF-BP3


DNA damage
ATM/R
Chk2
p53
p53
p53 activation
p21
G1 Arrest
cyclin D
Cdk4/6
inactive pRB
active E2F
Expression of
S-phase genes
BAX
FAS
IGF-BP3
Apoptosis

p53

- Missense mutations
- Nonsense mutations
- Tumor cells benefit more from the presence of a slightly altered p53 protein than from its complete absence
- "dominant-interfering" or "dominant-negative" allele
- p53 - homo-tetramer
- The mutant p53 allele actively interferes with the continuously functional "wild type p53" allele, expressed in the same cell.

p53

- Tetramer contains a mixture of mutated and wild type proteins in different proportions.

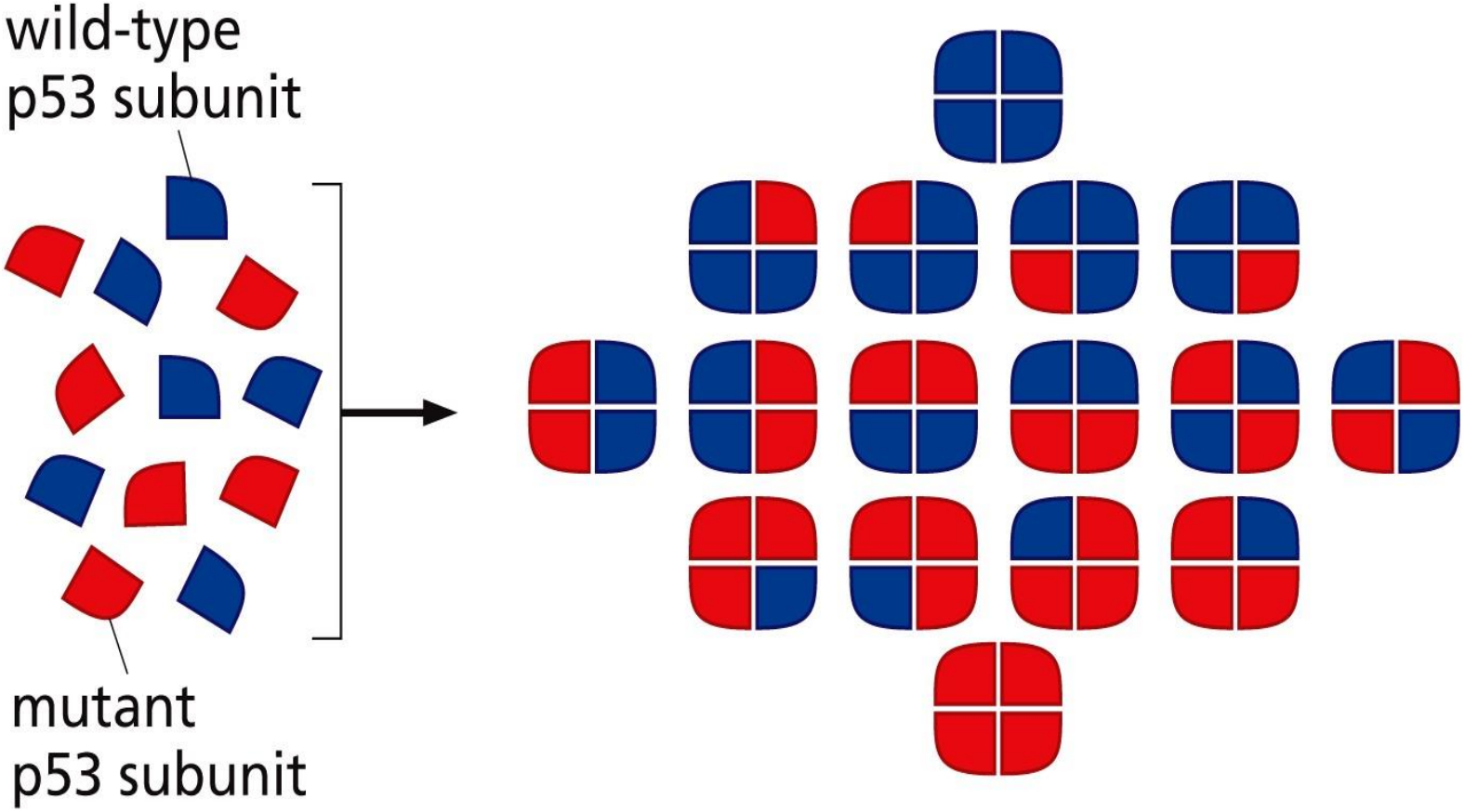


Figure 9.7a The Biology of Cancer (© Garland Science 2014)

p53

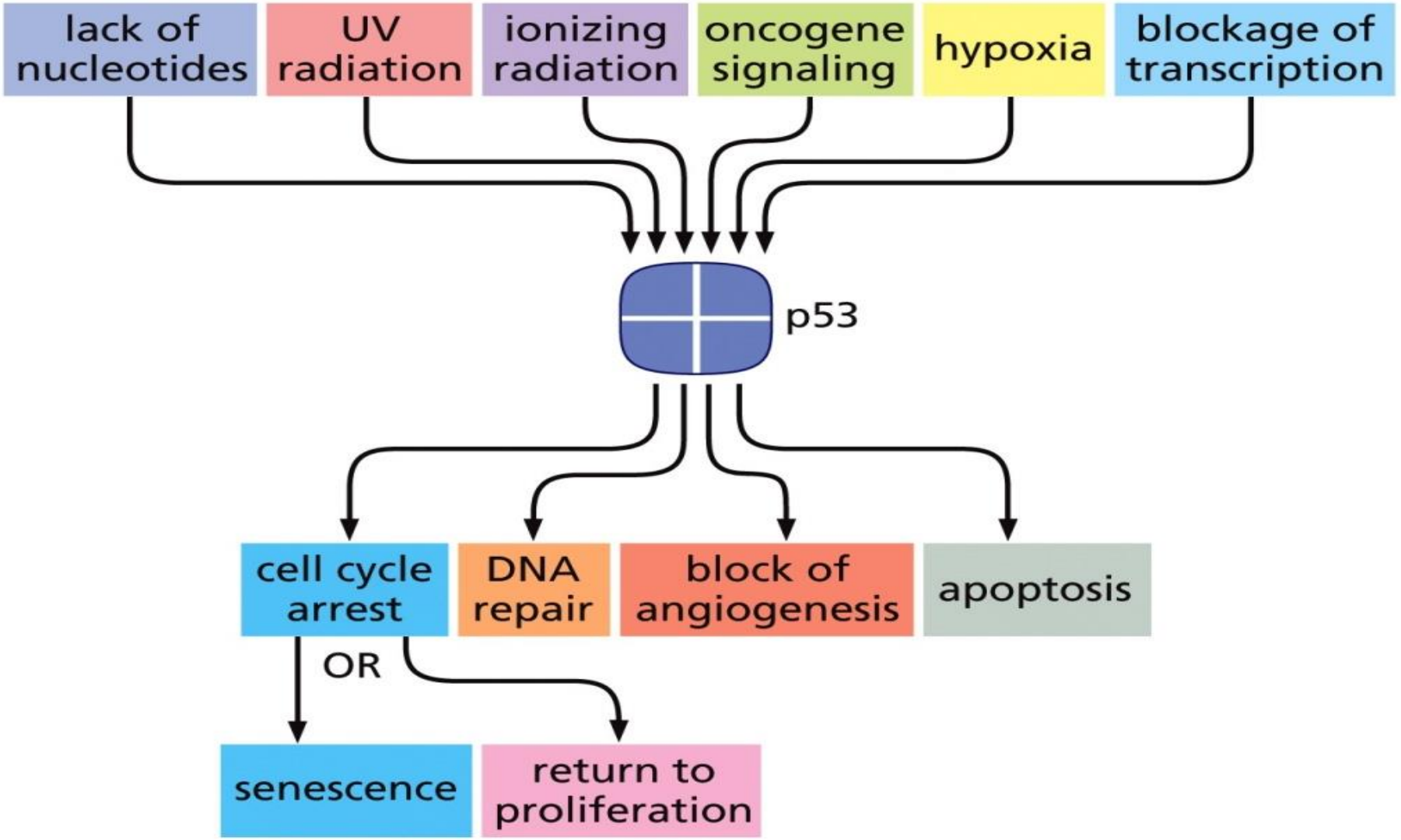


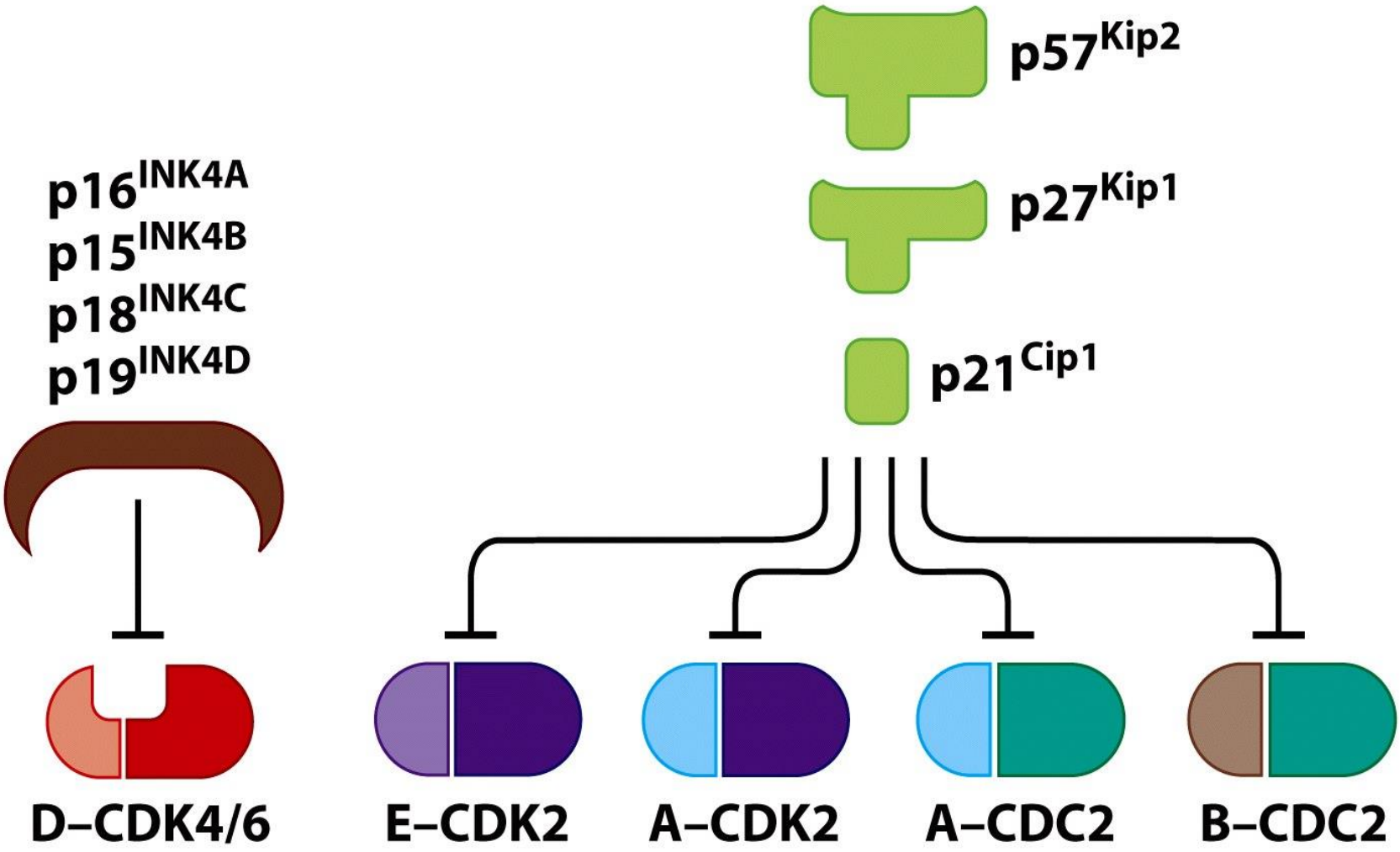
Figure 9.8 The Biology of Cancer (© Garland Science 2014)

Inhibitors of cyclin-dependent kinases (CDK inhibitors)

- 7 proteins that can antagonize the activity of the cyclin-CDK complex:
- 4 proteins are specific inhibitors of cyclin-dependent kinase 4 (CDK4), also known as INK4 proteins (inhibitors of CDK4). Their targets are CDK4 and CDK6 complexes and have no effect on CDK2. These inhibitors include $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$ and $p19^{INK4D}$

- The three remaining inhibitors are non-specific. These include p21, p27 and p57 and inhibit several different cyclin-CDK complexes that are formed during subsequent phases of the cell cycle.

CDK inhibitor activity



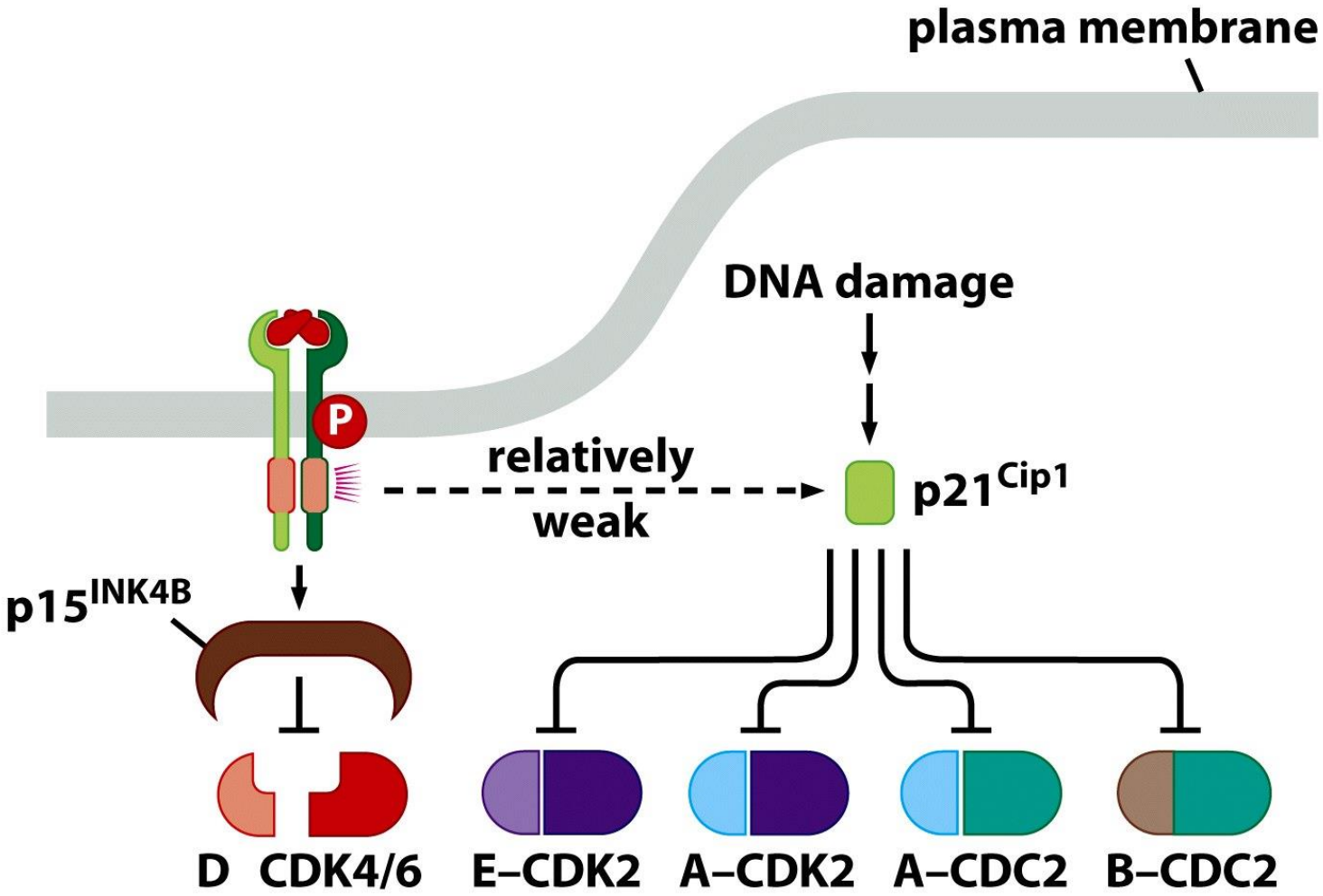
The Biology of Cancer (© Garland Science 2007)

- TGF-β → receptor on epithelial cells → numerous signaling pathways that antagonize cell proliferation:

- Level increase $p15^{INK4B}$ → blocks the formation of cyclin D-CDK4/6 and inhibits already existing complexes.

- Without active cyclin D-CDK4/6 complexes, the cell is unable to "progress" from the G1 phase of the cell cycle.

Mechanisms of TGF-β cell cycle control



The Biology of Cancer (© Garland Science 2007)

Mechanisms of TGF-β cell cycle control

- When the cell "exits" from the G1 phase, the activity of the cyclin D-CDK4/6 complex becomes unnecessary.

- TGF-β inhibits cell growth in the early G1 phase and loses most (almost all) inhibitory characteristics when the cell passes through the G1 phase.

- TGF-β → $p21^{Cip1}$, a broad-acting CDK inhibitor

- $p21^{Cip1}$ arises as a response of the cell to various damages (e.g. DNA damage)
- When present in a significant amount in the cell, $p21^{Cip1}$ it acts in all phases of the cell cycle and stops further "progress" of the cell.

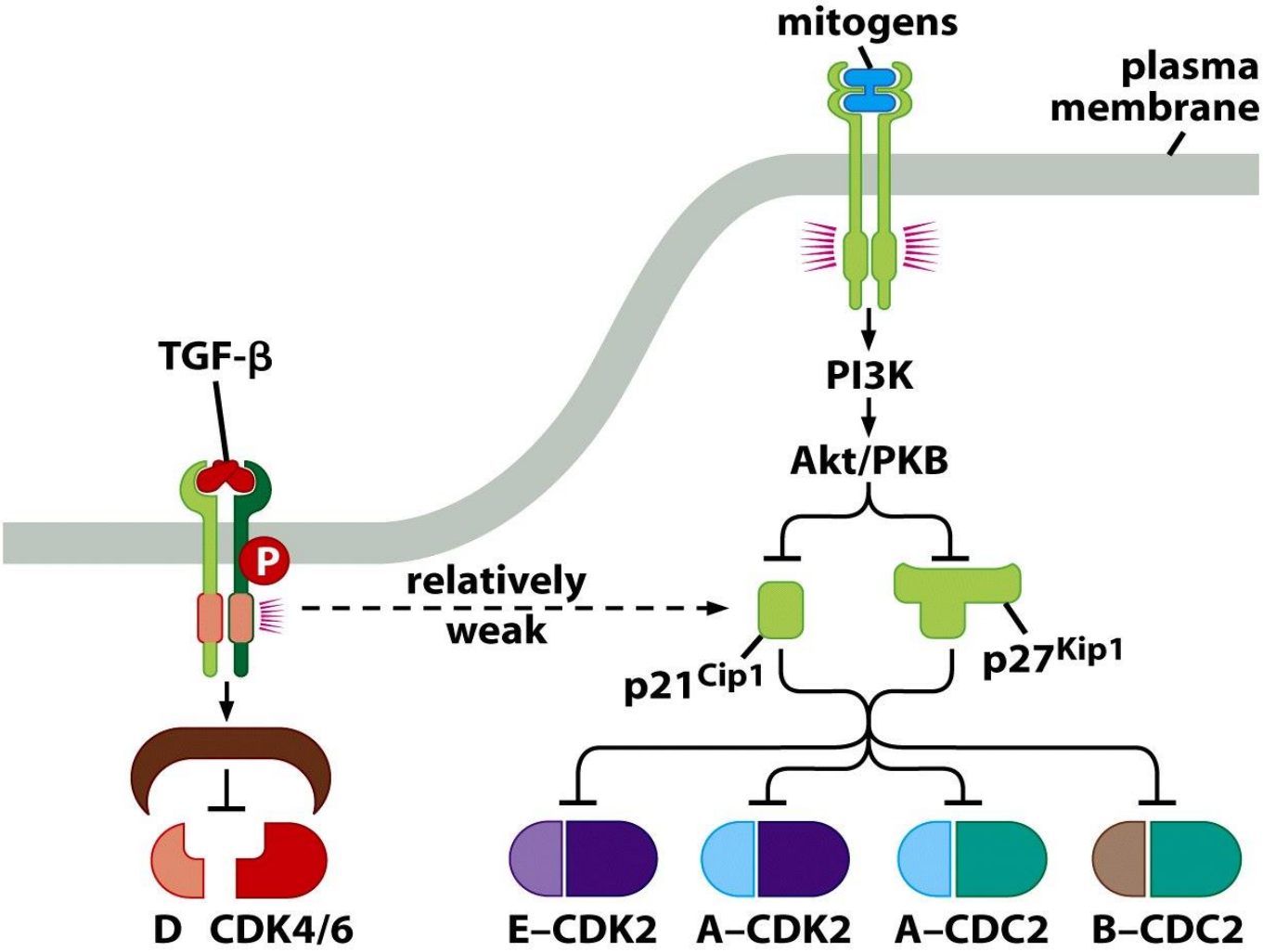
Mitogens

- DNA damage and, to a much lesser extent, TGF-β they can increase the level of $p21^{Cip1}$ in the cell and thus stop the cell cycle

Mitogens have the opposite effect:

- They block the activity of CDK inhibitors and accelerate the cell cycle
- One of the mechanisms of mitogen action is through the phosphatidyl-inositol 3-kinase pathway (PI3K).
- Akt/PKB, a kinase that is activated downstream in the PI3K signaling pathway is phosphorylated $p21^{Cip1}$ molecules localized in the nucleus and causes their exit from the nucleus into the cytoplasm, where they cannot inhibit cyclin-CDK complexes.
- Similarly, Akt/PKB inhibits the function of $p27^{Kip1}$ molecule.
- In aggressive tumors, Akt/PKB kinase is very active, and inhibitors of the cyclin-CDK complex are not found in the nucleus, but in the cytoplasm.

Cell cycle control extracellular signals



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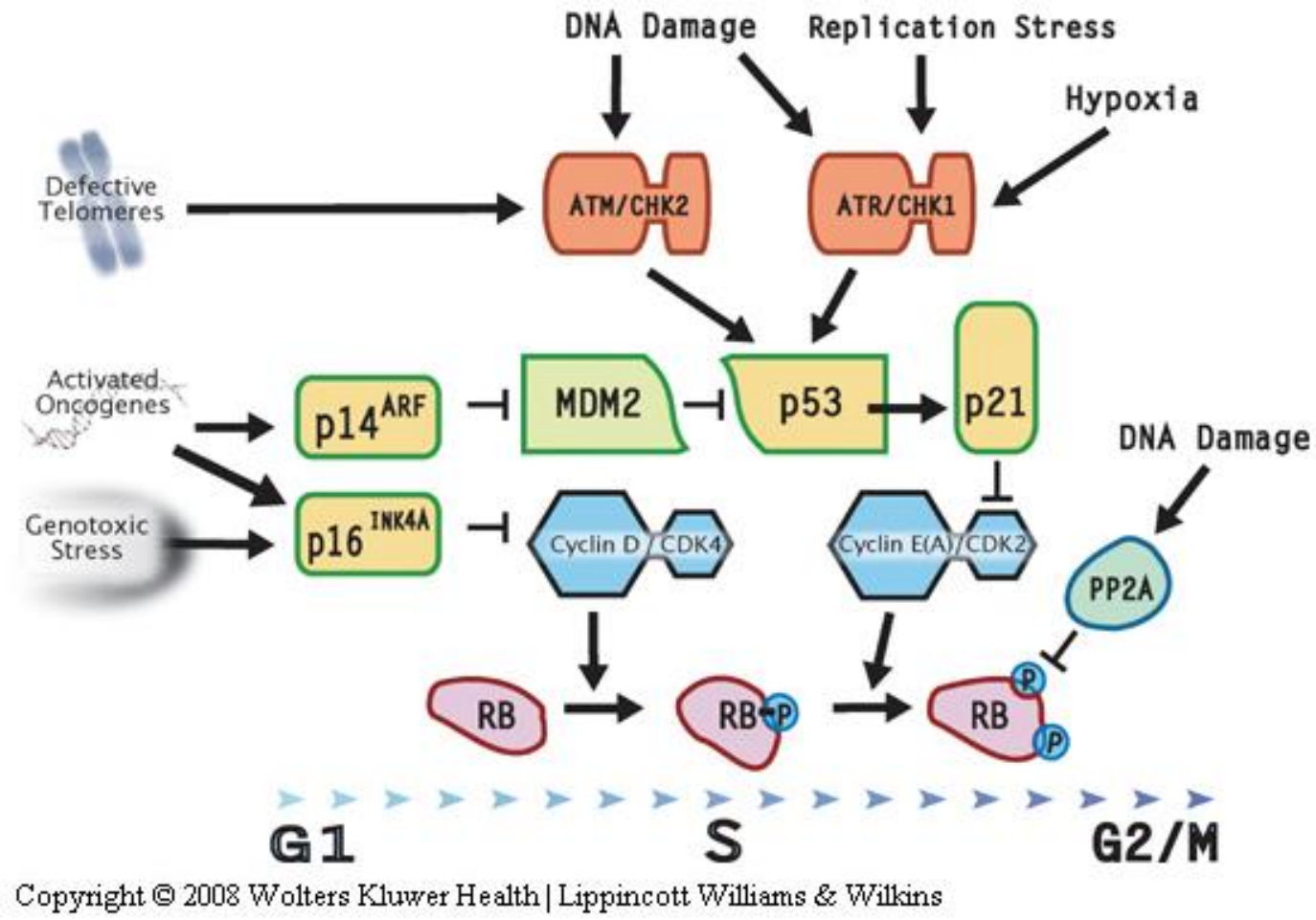
The role of anti-oncogenes in cell cycle regulation



http://www.youtube.com/watch?v=0GlOb76xPW4

Immortalization and oncogenesis

"Death occurs because exhausted tissue cannot be endlessly renewed and because the number of cell divisions of an individual cell is finite.“

August Weissmann, biologist 1881.

Immortalization and oncogenesis

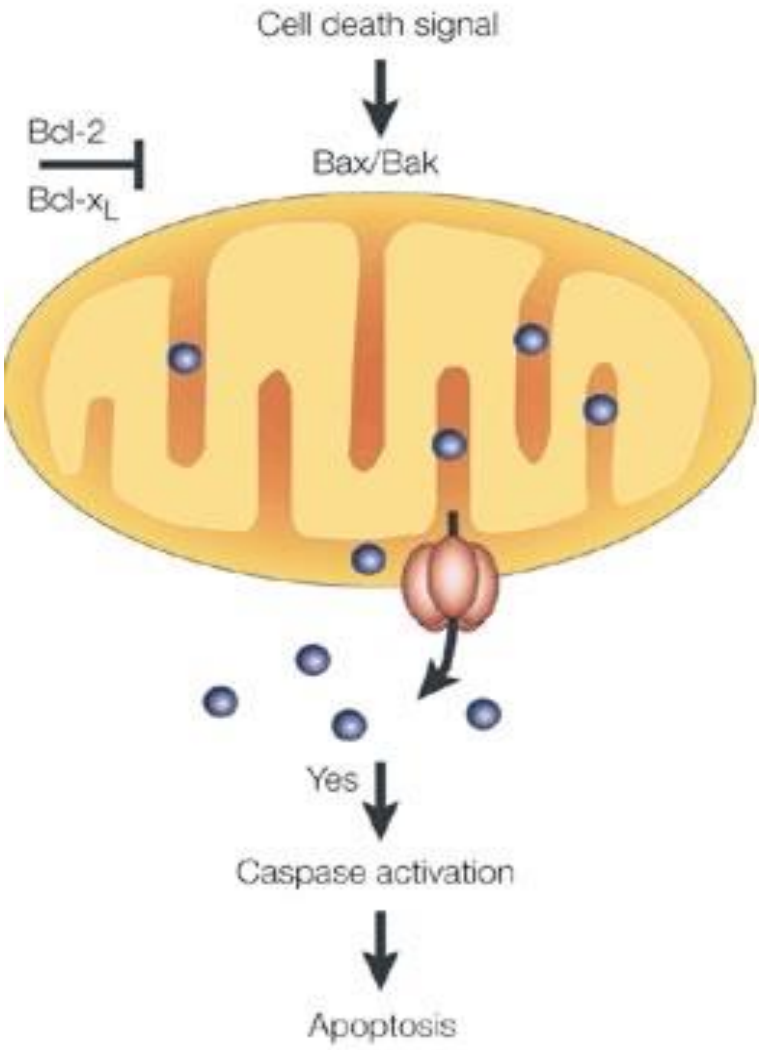
- 1960. Leonard Hayflick- limited replicative potential (50-100 divisions)
- Replicative age - mechanism of protection against malignancy - shortening of telomeres
- Specialized nucleoprotein complexes - RNA primer for the start of replication (25-200bp)
- 1932 Hermann Myollar, Nobel prize winner "Telomere"

Immortalization and oncogenesis

- Accumulation of cdk inhibitors
- Cell cycle arrest

- Mitotic clock (number of divisions and cell lifespan)
- Increased telomerase activity in most primary tumors
- Potential targets in anti-tumor therapy

Inhibition of apoptosis



Induction of apoptosis:

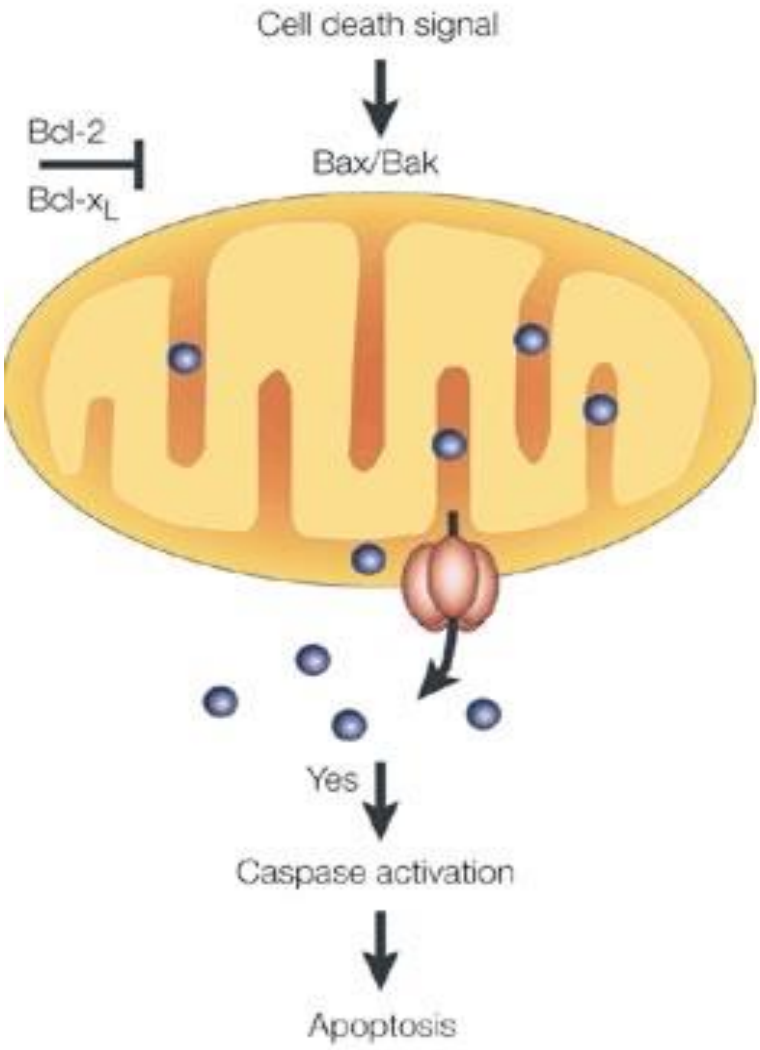
- When the number of mutations reaches a critical level
- Under the influence of cytokines (TNF)
- After DNA damage

Inhibition of apoptosis

- The malignant cell "keeps the mechanisms of apoptosis under control", that is, it blocks apoptosis through the anti-apoptotic proteins of the Bcl family.

- The first identified member of this family is the Bcl-2 gene (B Cell Lymphoma gene-2).

Inhibition of apoptosis



Anti-apoptotic proteins of the Bcl family (Bcl-2, Bcl-x).

- Inhibits apoptosis by blocking the function of pro-apoptotic proteins and preserving the integrity of the mitochondrial membrane.
- Lymphoma: ↑ Bcl-2
  - Prolongs the life of cells
  - Enables accumulation of mutations and rearrangement of the oncogene (c-myc)
  - Cooperative action Bcl-2/c-myc

Inhibition of apoptosis

- For the development of malignancy, more than one oncogene must be active

- Co-transfection of two plasmids (one for induction of proliferation, the other for immortalization) results in transformation.

Autophagy

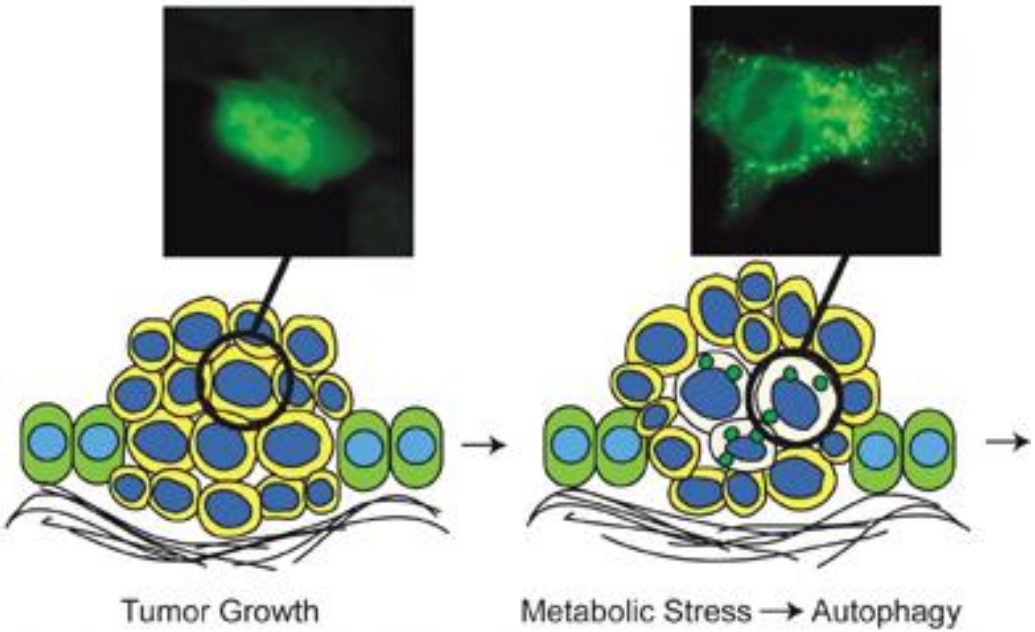
Early stages of oncogenesis, autophagy ⟹ tumor suppressor

Higher level of protein synthesis for tumor growth

Inhibition of autophagy ⟹ continuous tumor growth

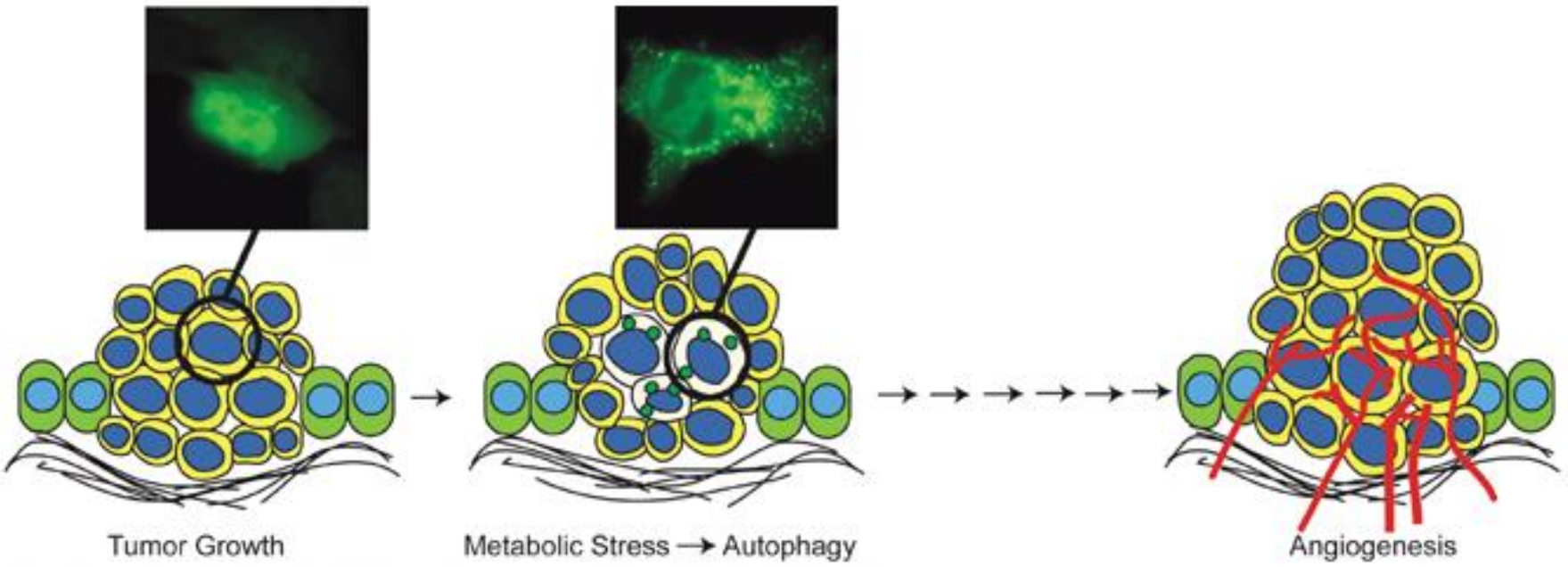
Autophagy reduces mutation rates and suppresses oncogenesis by eliminating damaged organelles that produce genotoxic factors such as free radicals

Autophagy



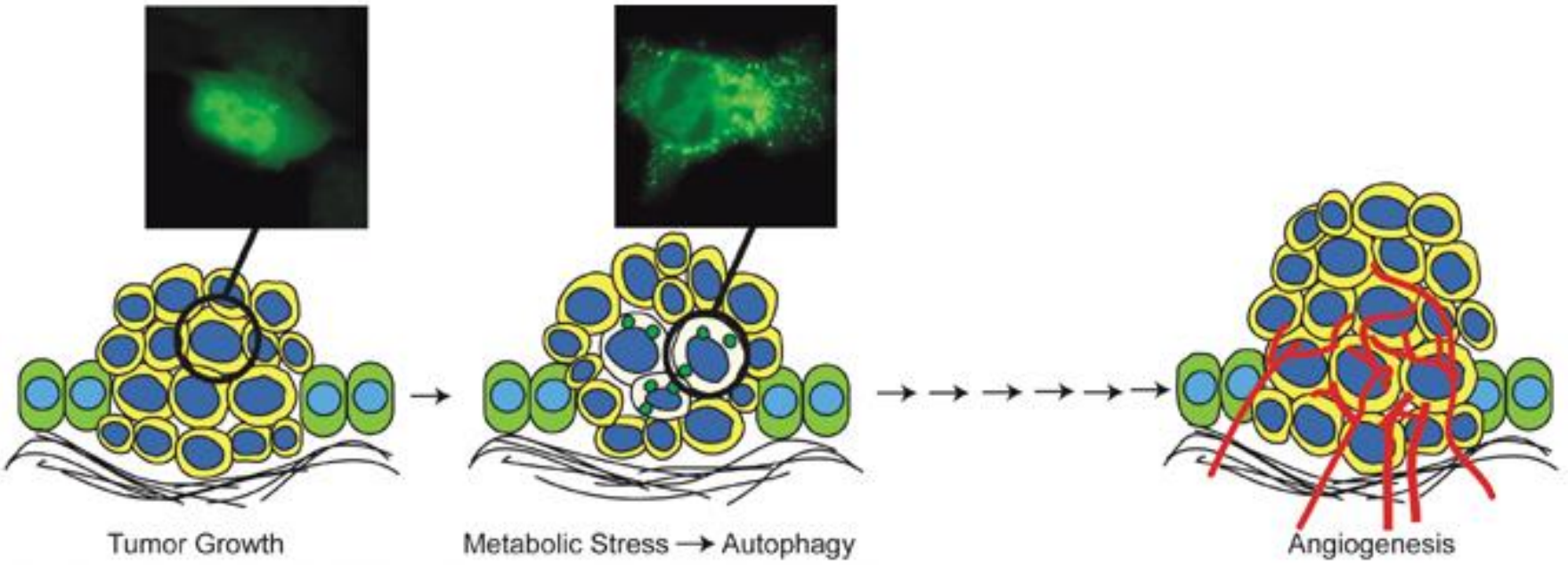
Epithelial tumor cells rapidly proliferate multilayered. Insufficient blood supply induces metabolic stress in the parts of the tumor furthest from nutrients and oxygen, inside the tumor.
Tumor cells with defective apoptosis (inhibition of apoptosis), in regions of metabolic stress, can survive by autophagy.

Autophagy



Also, autophagy induces local neoangiogenesis, through intensifying the proliferation and migration of endothelial cells, which facilitates tumor growth and development.

Autophagy



However, autophagy, as programmed cell death type II, also plays a role in the elimination of tumor cells. The gene encoding Beclin-1, a major autophagy-triggering protein, is often down-expressed in many tumor types, and deletion of this gene in mice significantly increases tumor incidence.