Fundamentals of oncology

Teaching unit 3

SIGNALING PATHWAYS IN THE CELL

Ras/Raf/MAPK signaling pathway

PI3K/Akt signaling pathway

JAK/STAT signaling pathway

Wnt signaling pathway

Hedgehog signaling pathway

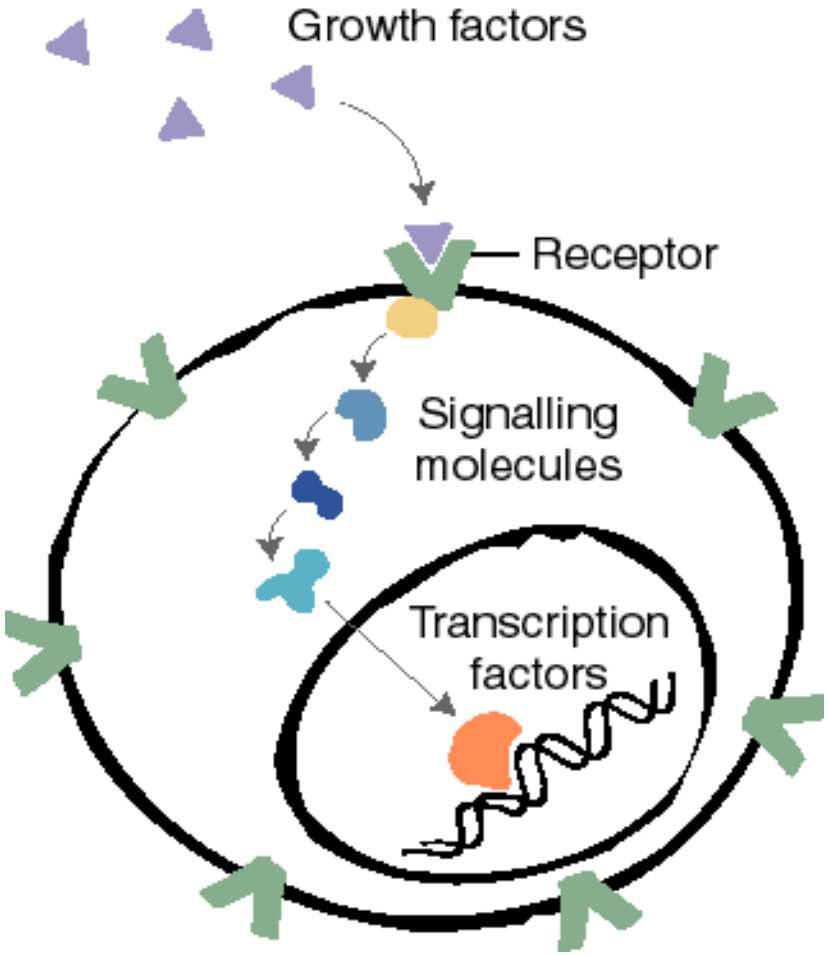
The role of transcriptional factor NF-κB in oncogenesis

SIGNALING PATHWAYS IN THE CELL

Essential processes for all living organisms, such as **growth**, **proliferation**, **differentiation** and **programmed cell death**, are strictly regulated by numerous control mechanisms.

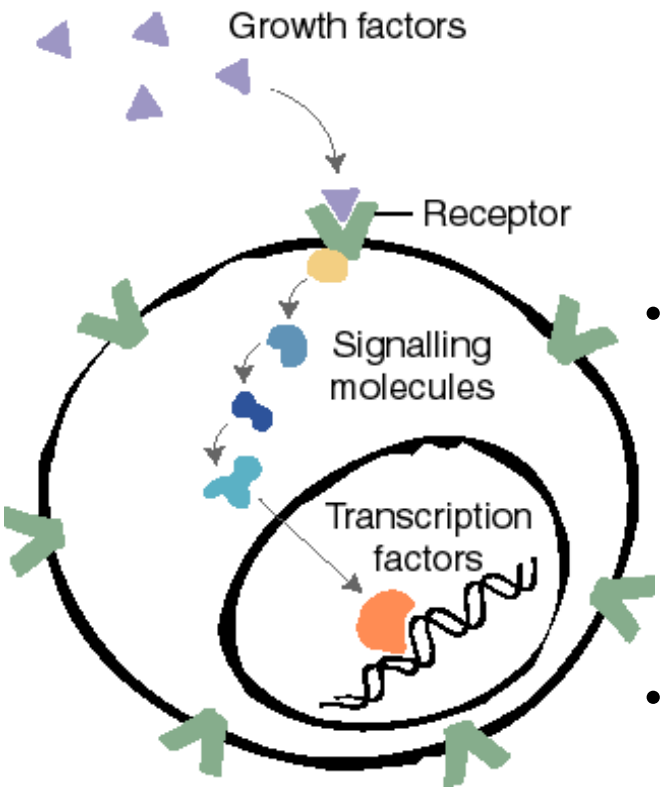
Thanks to the **complex signaling pathway networks**, extracellular signals are integrated and distributed to different places in cell from where begins appropriate biological answer to the stimulus.

SIGNALING PATHWAYS IN THE CELL



Growth, proliferation, differentiation and programmed cell death are under permanent control of extracellular signals which include **interaction with extracellular matrix**, **intercellular contact** and, finally, **effect of soluble molecules known as growth factors.**

TRANSDUCTION OF SIGNAL IN THE CELL

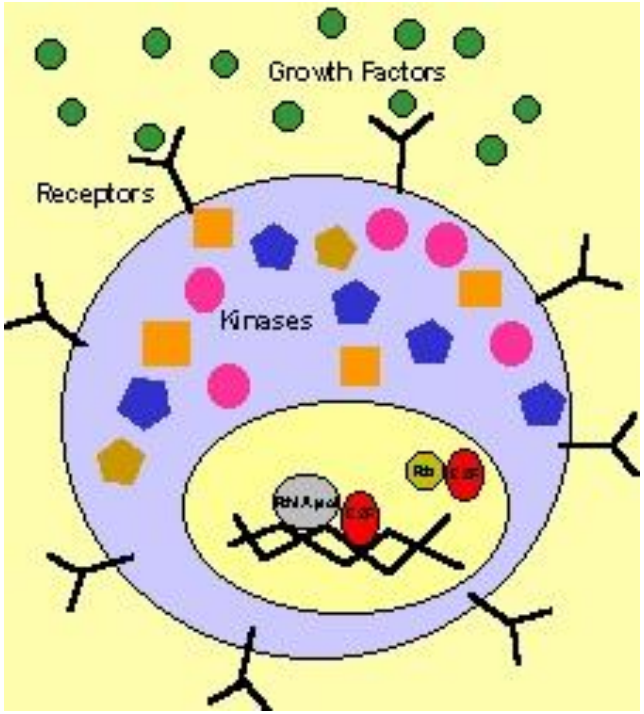


- Cell growth factors are **soluble signaling molecules** (cytokines and hormones) which achieve their effects by binding to the **specific receptors on the cell membrane.**
- The interaction between the growth factor and the specific receptor for the growth factor on the cell membrane activates the **cascade of intracellular signaling pathways** resulting in an appropriate biological response.
- The signals are distributed to different places in the cell, and finally reach the nucleus, where activated **transcription factors** modulate the **expression of target genes.**

TRANSDUCTION OF SIGNAL IN THE CELL

Receptors transmit information from the outside through the cell membrane via the numerous intracellular signaling pathways in which **cytoplasmic signaling proteins** participate.

After triggering a signal from the receptor, signaling proteins are activated by **switching from an inactive to an active conformation**, until they receive another signal that returns them to an inactive state.

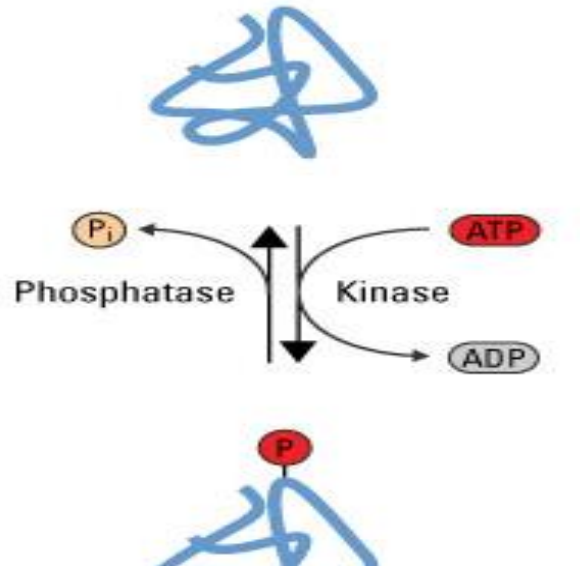


The two basic mechanisms of activation of signaling proteins are:

- **Phosphorylation** and
- **Binding of guanosine-3-phosphate (GTP)**

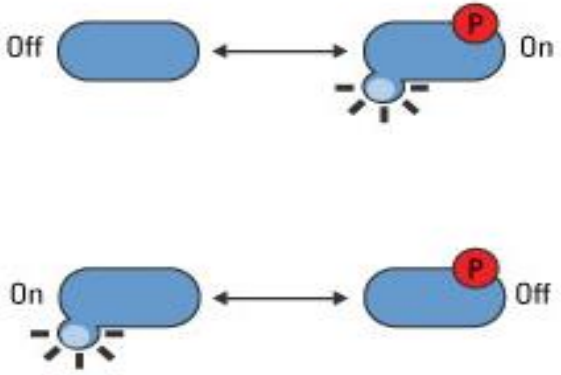
TRANSDUCTION OF SIGNAL IN THE CELL

- The largest number of signaling proteins are activated by the process of **phosphorylation** carried out by enzymes called **protein kinases** - enzymes that covalently attach one or more phosphate groups to amino acid residues of proteins (**serine/threonine** and **tyrosine kinases**).



- Many signaling proteins are protein kinases organized as signaling cascades.

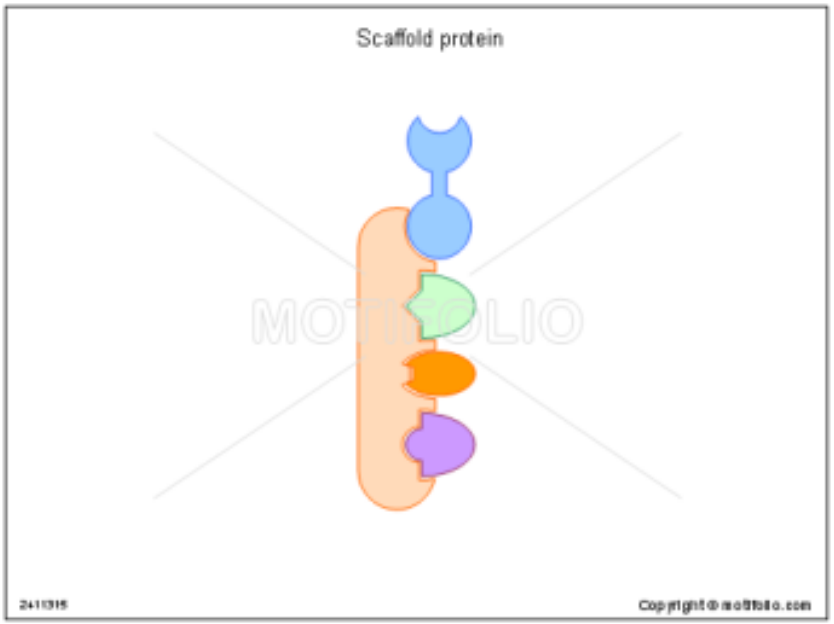
- On the contrary, protein **phosphatases** are enzymes that remove phosphate groups, i.e. perform dephosphorylation of proteins, usually returning them to an inactive state and interrupting signal transmission.



TRANSDUCTION OF SIGNAL IN THE CELL

The efficient coordination of signaling events in the cell is controlled by molecules called **adapter** and **scaffold** proteins.

These proteins integrate, connect, the intracellular components of the signaling pathway.



Through protein-protein reactions, adapter proteins connect molecules of the signaling cascade with proteins such as receptors with tyrosine kinase activity.

Scaffold proteins bind the different signaling proteins together in a functional complex and thereby enable the formation of multienzyme signaling complexes involved in certain signaling pathways.

SIGNALING PATHWAYS IN THE CELL

- Different components of the signaling cascade can become oncogenic and participate in the malignant transformation of the cell.

- **Excessive expression** or accumulation, or **hyperactivity of a certain component** of the signaling pathway, caused by gene mutation, is most often involved in the constitutive activation of the signaling pathway independently of control regulatory mechanisms.

- Similarly, inhibitory mechanisms, which block signal transduction and continuously regulate cell growth and division, can be disrupted.

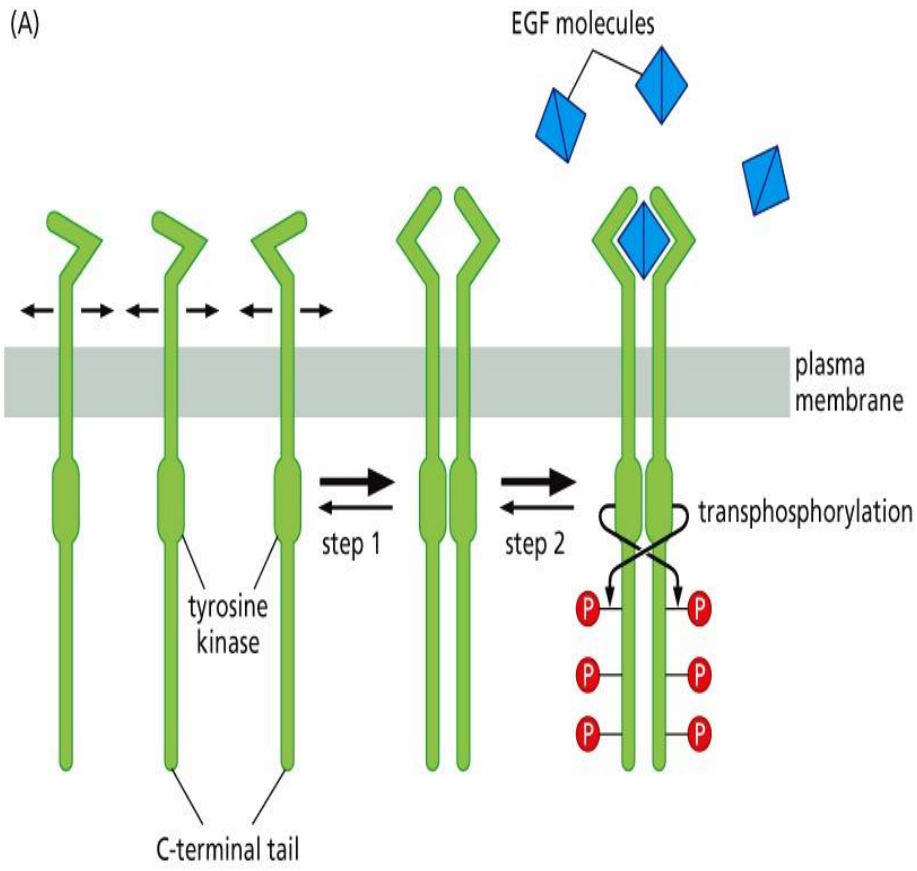
By losing strict control and regulation of growth and proliferation, the tumor cell acquires independent proliferative capacity and behavior, independent of extracellular or intracellular influences and control.

Receptors for growth factors

Several classes of receptors are involved in signal transduction in the cell:

- **receptors with tyrosine kinase activity**
- **G-protein coupled receptors**
- **cytokine receptors**

Signal transduction through receptors with tyrosine kinase activity



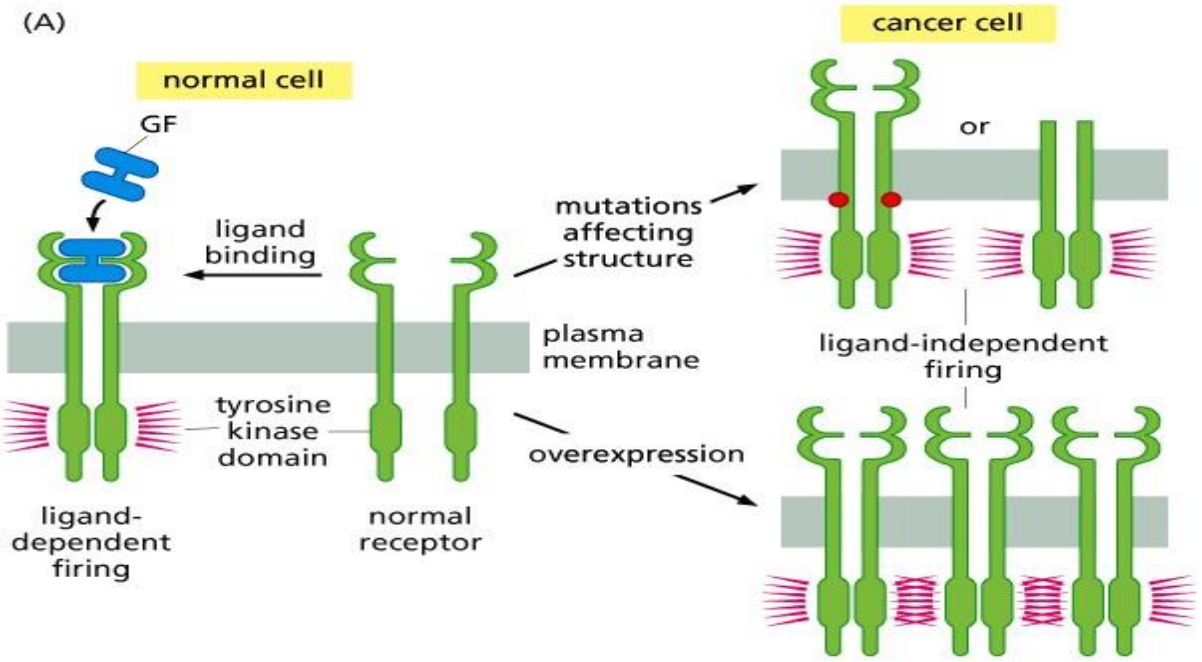
Most of the extracellular signaling proteins, including cell growth factors, achieve their effect by binding to membrane receptors that possess tyrosine kinase activity, which contain so-called tyrosine kinase domains in their intracytoplasmic part.

After ligand binding, receptors dimerize or oligomerize, which brings their tyrosine kinase domains into close proximity to each other and allows them to phosphorylate multiple tyrosines of adjacent receptor chains (transphosphorylation) or other proteins involved in signal transduction.

Signal transduction through receptors with tyrosine kinase activity

- Phosphorylated tyrosine residues further serve as anchoring sites for various downstream signaling proteins. Signaling proteins bind with high affinity to tyrosine phosphate groups using specific phosphotyrosine-binding domains.

- After binding to the activated receptor, the signaling proteins themselves are activated by phosphorylation, which initiates the formation of signaling complexes and the distribution of signals to different places in the cell.



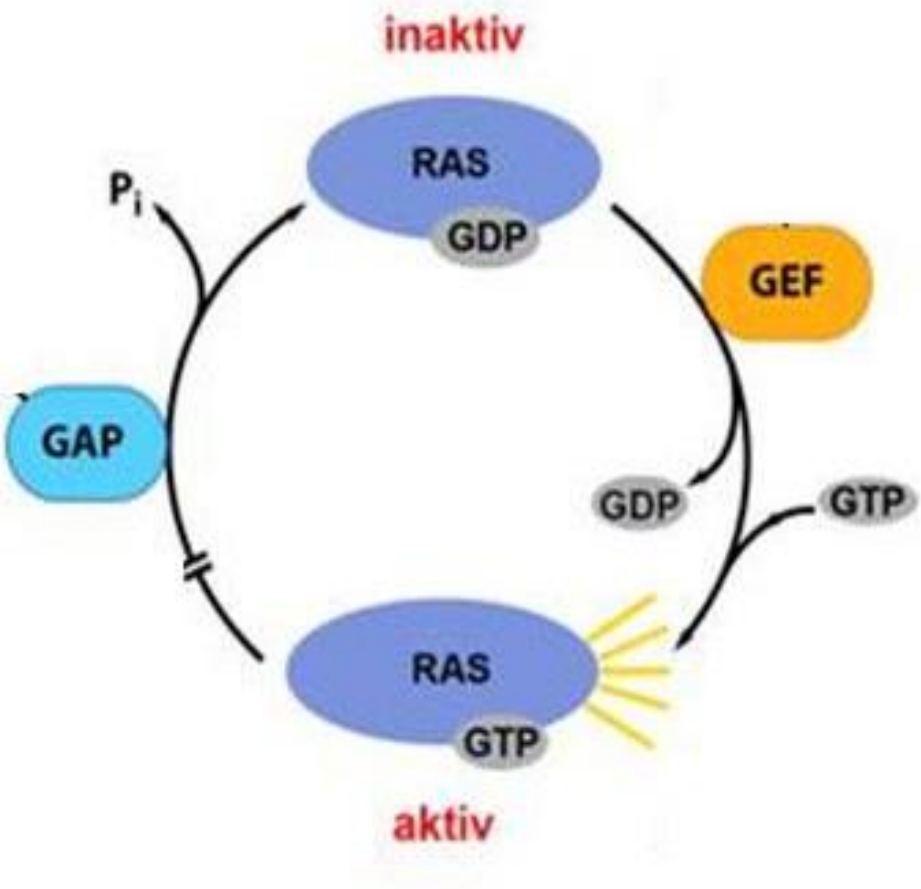
Transduction of signal through the receptors with tyrosine kinase activity

- **Ras/MAPK (Ras protein/mitogen-activated protein kinase)**
- **PI3K/Akt (phosphatidyl-inositol-3 kinase/Akt kinase)**
- **JAK/STAT (Janus Kinase/Signal Transducer and Activator of Transcription)**

Rassistant, proliferation and differentiation of the cells-oncogenesis and tumor development

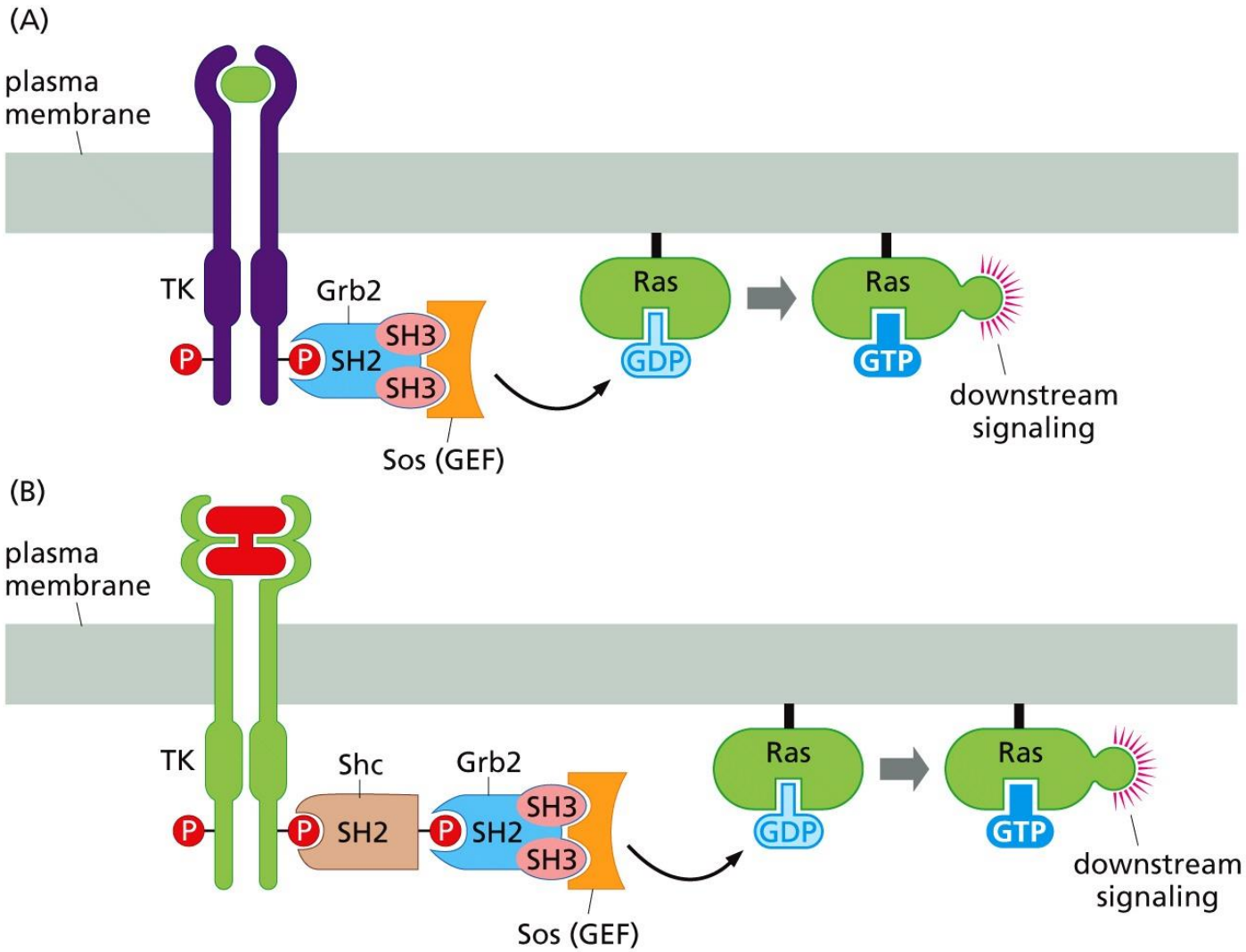
Ras protein

- Ras is a small GTP-binding protein that contains its own GTP-ase region.
- In the inactive state, the Ras protein binds GDP. When the receptor is activated by ligand binding, the **guanine-nucleotide exchange factors (GEFs)** stimulates the dissociation of GDP and the subsequent binding of GTP instead of GDP on the Ras protein.
- Over time, the GTP-ase region of the Ras protein hydrolyzes GTP to GDP, thereby converting it back into its inactive state.
- GTP-ase activity is strongly activated by molecules called **GTP-ase-activating proteins (GAPs)**.



Ras protein

- Receptors with tyrosine kinase activity activate Ras either by GEFs activation or by inactivation of GAPs following their binding to phosphorylated tyrosine residues of the activated receptor.



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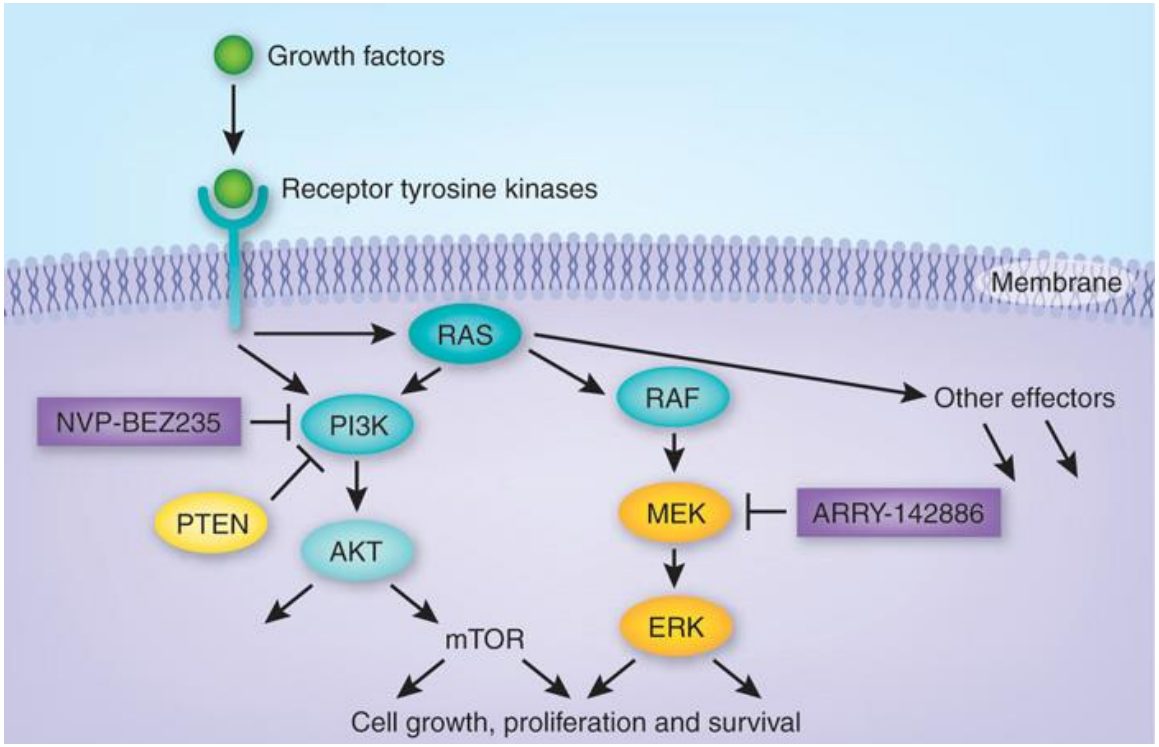
Ras protein

- Ras protein represents a hub from where, in a strictly coordinated action, a signal is generated to different places in the cell.
- Activated Ras protein can recruit a large number of effector molecules to the membrane and initiate various signaling pathways in the cell.



Depending on the cell type and the nature of the extracellular signal, the Ras protein mediates the processes of cell growth, proliferation, differentiation and survival in different ways.

The role Ras proteins in oncogenesis

- Ras protein is hyperactive in many tumor cells. Mutations in genes encoding Ras proteins are present in about a third of all tumors.

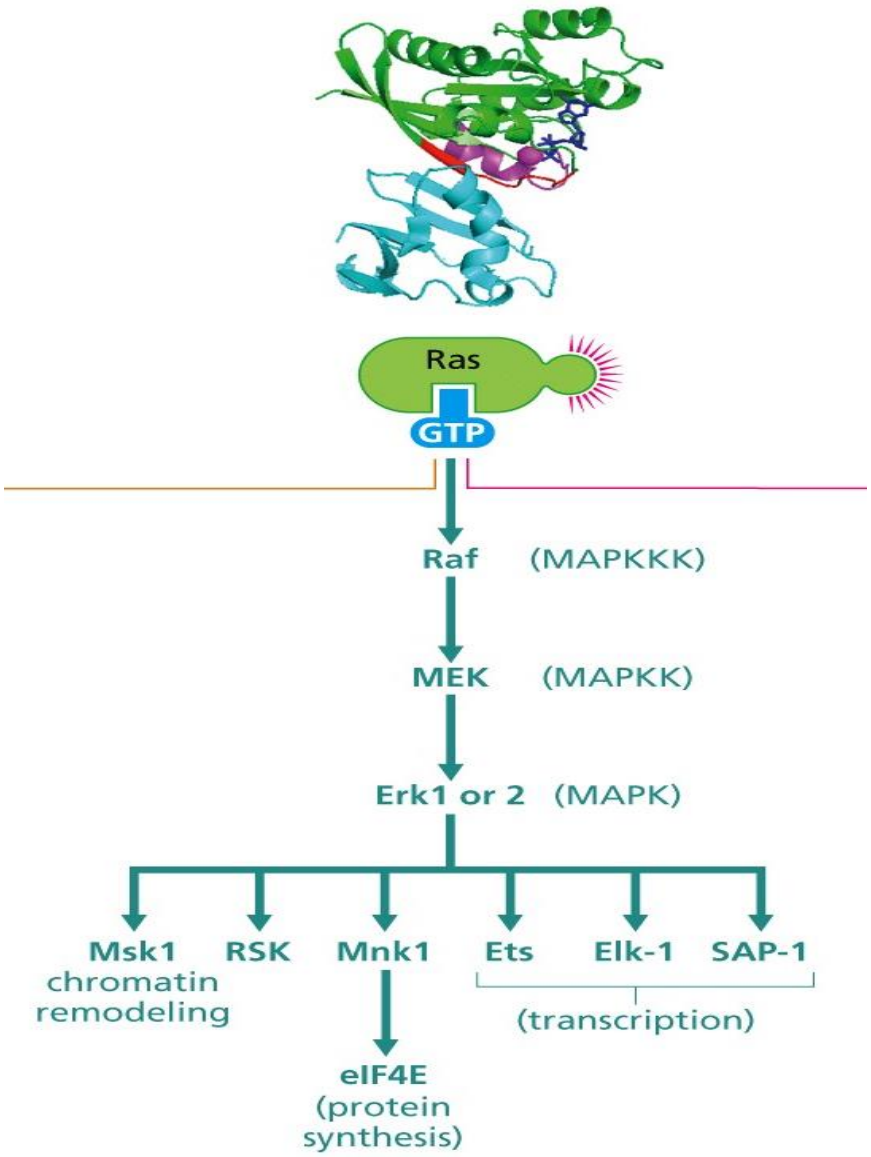
- Mutated forms of **K-Ras** protein are detected in about 90% of pancreatic adenocarcinoma, 40-45% of colon adenocarcinoma, lung cancer, melanoma and numerous other tumors.

- Mutated forms of the **H-Ras** protein are represented in a significant percentage in bladder cancer, while mutations of the gene encoding the **N-Ras** protein are mainly present in hematopoietic tissue tumors (various forms of leukemia and lymphoma), anaplastic thyroid cancer, melanoma and other tumors.

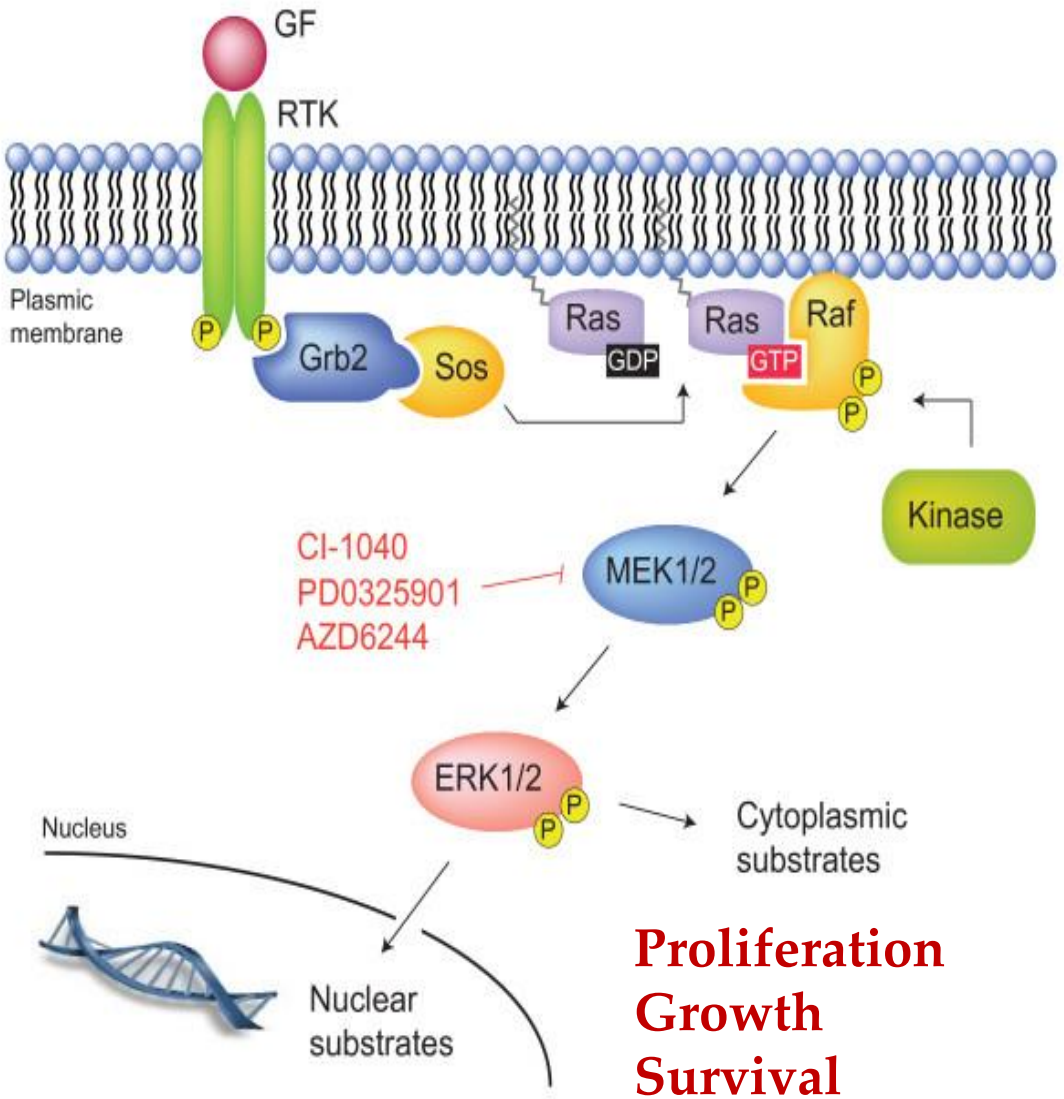
Ras/Raf/MAPK signaling pathway

- When the growth factor binds to the receptor on the target cell, a cascade of phosphorylation is triggered by the receptor, via the Ras protein and a series of mitogen-activated protein kinase kinases (MAPKs).

- These kinases enter the nucleus where they phosphorylate and activate transcription factors that regulate the expression of target genes.



Ras/Raf/MAPK signaling pathway



- MAPKs regulate the activation of several transcription factors important for cell cycle regulation.
- Among the most important transcription factors are Fos and Jun, which together form activation protein 1 (AR-1), hyperactive in many tumor cells. ERKs stimulate the transcription of genes encoding heparin-binding EGF, cyclin D1, Fos, p21.

Ras/Raf/MAPK signaling pathway

- All eukaryotic cells contain several members of the MEK (Mitogen/Extracellular signal-regulated kinase) superfamily, which phosphorylate different members of the MAPKs superfamily.

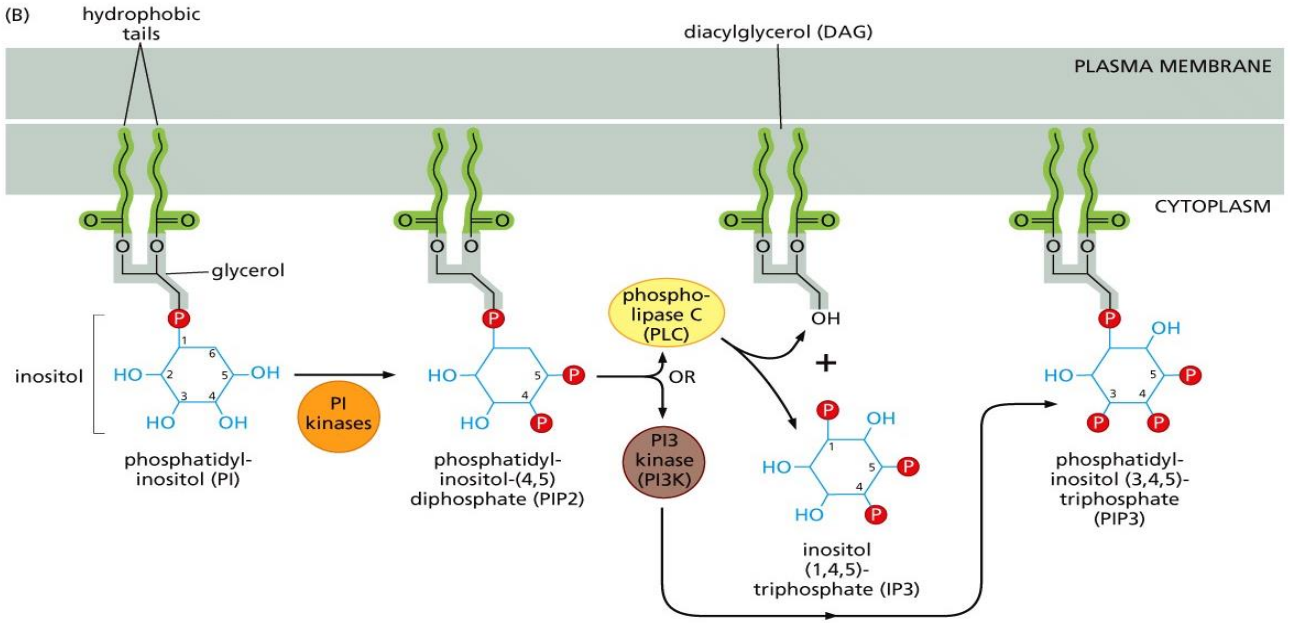
- In addition to **ERK**, there are other MAPKs, such as **JNKs (Jun N-terminal kinases)** and **p38 kinase**, which become active under stress conditions. All these enzymes are serine/threonine kinases, which are activated in the cytoplasm, in response to a specific extracellular signal, and then transported to the nucleus.

- Different MAPKs regulate different cell responses, including morphogenesis, cell death, and stress responses.

PI3K/Akt signaling pathway

- PI kinases are lipid kinases that catalyze the transfer of a phosphate group from the ATP molecule to membrane-bound phosphatidyl inositol (PI).

- The most important of them phosphorylates phosphatidyl inositol-2-phosphate (PIP2), resulting in phosphatidyl inositol-3-phosphate (PIP3).



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- One of the most important phosphatases is PTEN (Phosphatase and tensin homolog), which dephosphorylates PIP3 and thereby inhibits signal transduction.

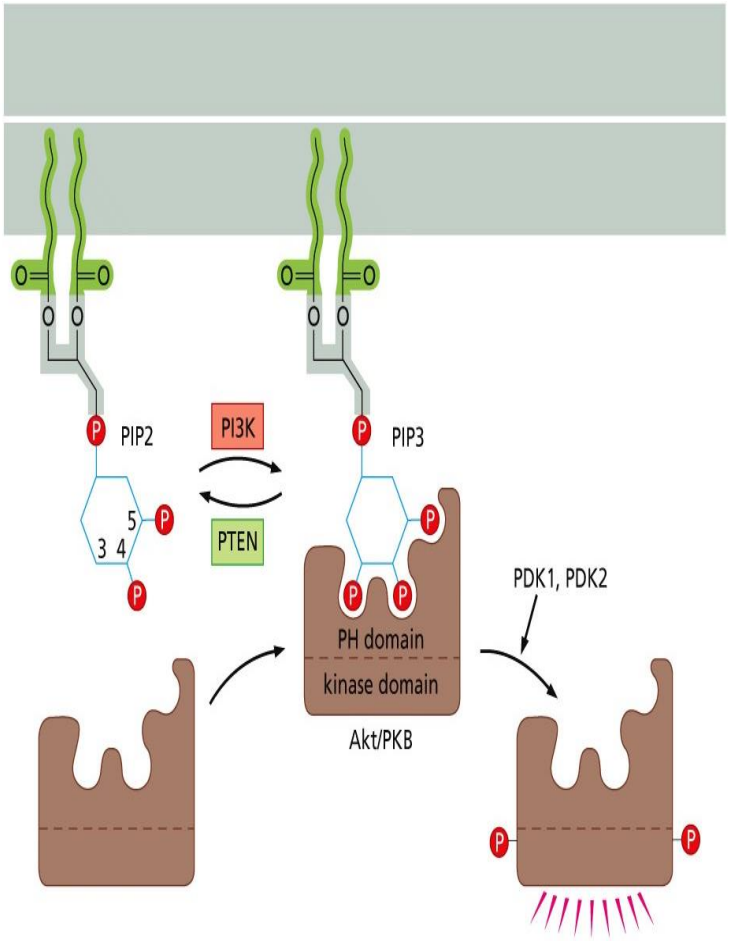
PI3K/Akt signaling pathway

- The most important cytoplasmic protein that binds to the PIP3 is the **serine/threonine kinase Akt, also known as protein kinase B (PKB)**.

- By binding to the cytoplasmic membrane and subsequent phosphorylation, the functional activation of Akt kinase occurs, which further engages numerous cytoplasmic proteins with multiple effects on the cell (survival, growth and proliferation).

- Akt kinase **prevents cells from entering programmed cell death** by inhibiting several important proapoptotic proteins.

- At the same time, Akt kinase stimulates **cell proliferation** by promoting its passage through the cell cycle.



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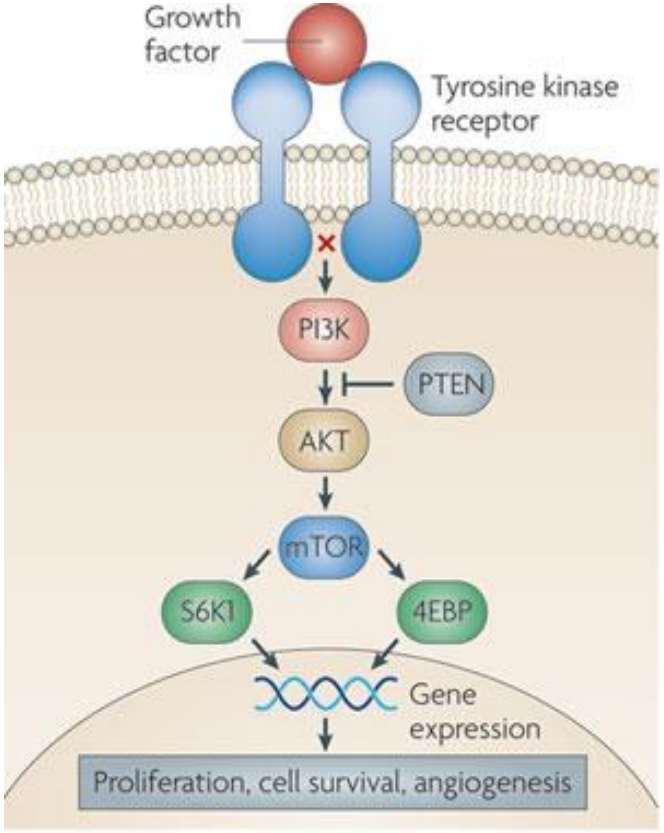
PI3K/Akt/mTOR signaling pathway

- The activated PI3K/Akt signaling pathway exerts a strong effect on the **stimulation of cell growth by stimulating protein synthesis**.
- PI3K, through the activation of Akt kinase, engages the central molecule of this signaling pathway, the serine/threonine kinase **mTOR** (Mammalian target of rapamycin).
- In human cells, mTOR kinase is present in the form of two protein complexes: mTORC1 and mTORC2. mTORC1 consists of two main subunits: mTOR kinase and a regulatory protein called Raptor.
- **mTORC1 regulates gene transcription, ribosome formation, protein synthesis, cellular metabolism, autophagy, and cell growth and division.**



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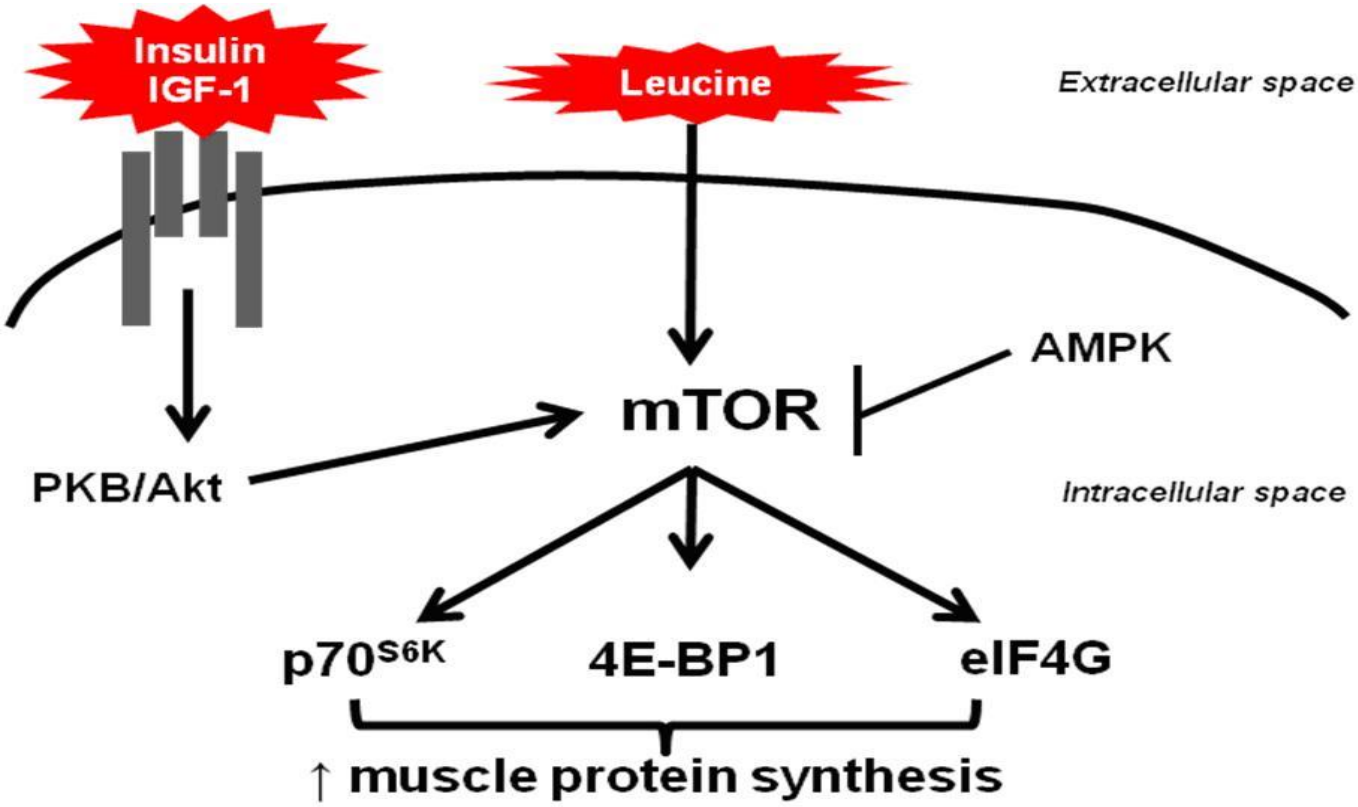
mTOR

- mTORC1 activity depends on the availability of growth factors, nutrients and energy in and around the cell. Thus, amino acids, especially leucine, directly activate mTORC1.

- Protein kinase AMPK (AMP-activated protein kinase) inhibits mTORC1. Cellular stress, hypoxia or DNA damage inhibit mTORC1 via AMPK.

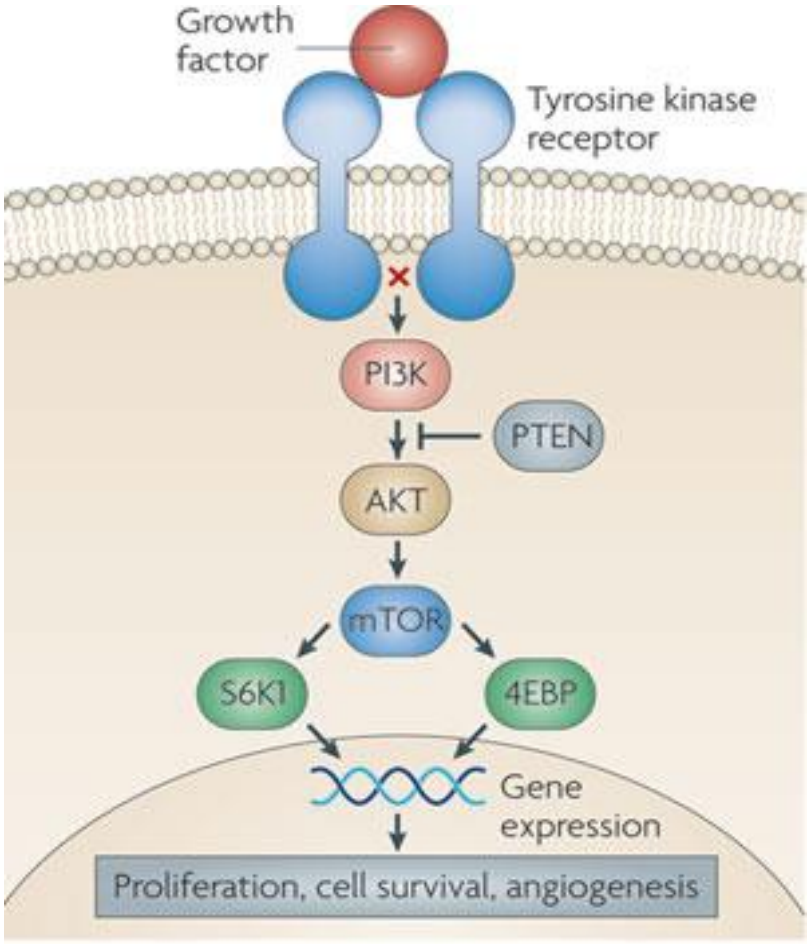


mTOR

- The main substrates of mTORC1 are **S6 kinase (S6K)** and **4E-BP (Eukaryotic translation initiation factor 4E (eIF4E)-binding protein)**.

- mTORC1 phosphorylates S6K and thus activates it. S6K increases the synthesis of ribosomal proteins, elongation factors and other proteins necessary for the translation process.

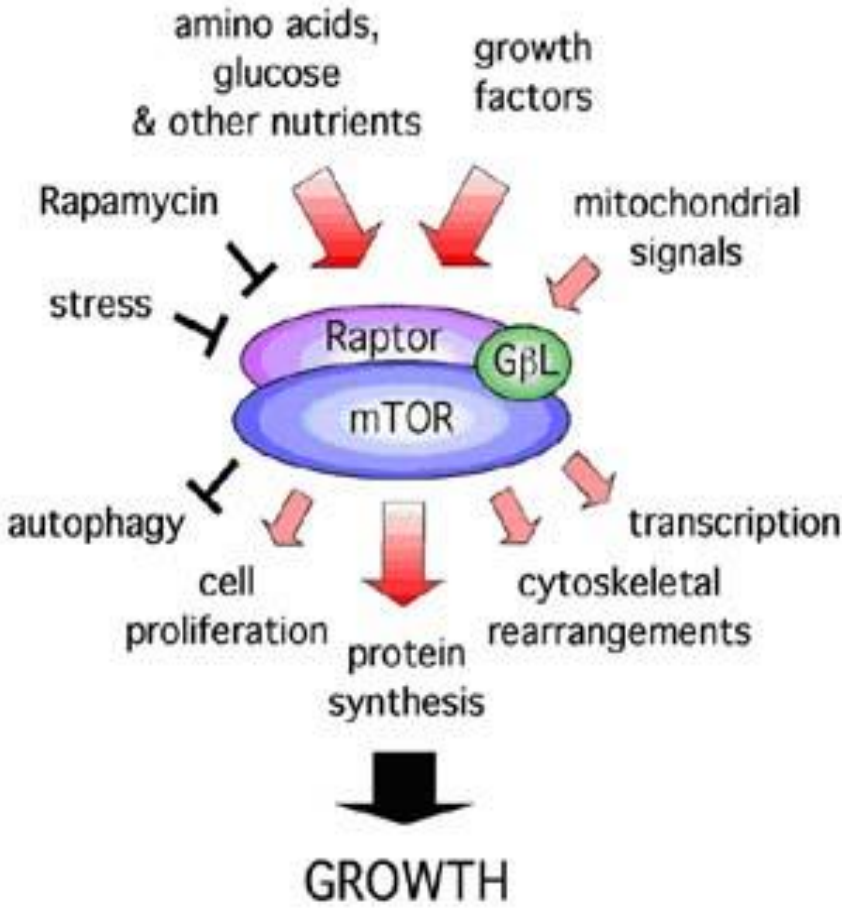
- In addition, mTORC1 phosphorylates 4E-BP and thus prevents the inhibition of eIF4E. Free eIF4E further stimulates translation.



Nature Reviews | Drug Discovery

mTOR

- mTOR regulates the transcription of genes involved in metabolic and biosynthetic processes, the uptake of glucose, amino acids, lipoproteins and iron into the cell, as well as autophagy and apoptosis.
- **mTOR stimulates protein synthesis.**
- **In addition to cell growth, mTOR kinase stimulates cell transition from G1 to S phase of the cell cycle.**



The role of PI3K/Akt signaling pathway in oncogenesis

- Various components of the PI3K/Akt signaling cascade, particularly PI3K, Akt and PTEN, are overexpressed or mutated in numerous tumors.

- In addition to mutations in genes encoding Ras proteins, mutations in genes encoding PI3K are among the most common genetic abnormalities in tumorigenesis. Mutated forms of PI3K, responsible for increased activation of the PI3K/Akt signaling pathway, are present in a number of tumors such as: colorectal and hepatocellular carcinoma, endometrial, breast and prostate tumors, glioblastoma and other.

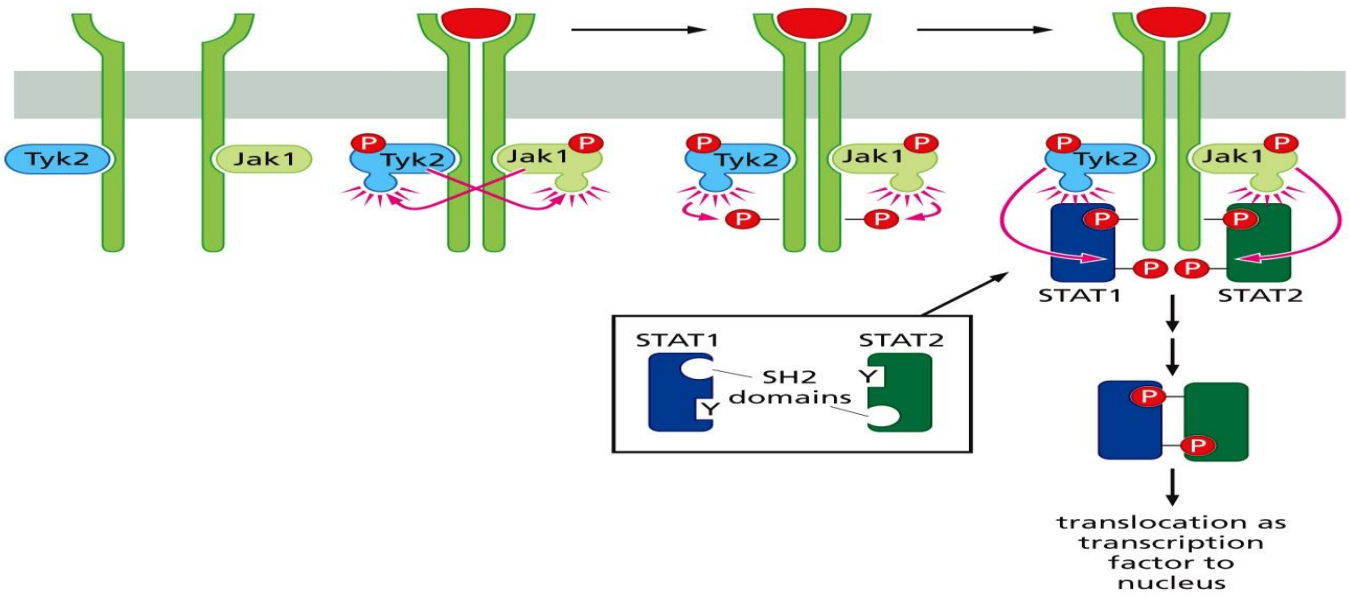
- Increased expression or activation of Akt kinase, resulting from a gene mutation, has been detected in liver tumors, pancreatic cancer, hepatocellular and colorectal cancer, glioblastoma or hematological malignancies.

- Loss of PTEN function as a result of gene mutation is responsible for the constitutive activation of this signaling pathway in prostate and endometrial tumors, gliomas, melanoma, etc.

Signal transduction from cytokine receptors JAK/STAT signaling pathway

- A large number of local mediators of intercellular communication, such as cytokines, but also some hormones and growth factors, exert their effect on the cell by activating receptors that are grouped into a large family of **cytokine receptors**.

- Cytokine receptors do not contain intracellular tyrosine kinase domains, although their activation depends on tyrosine phosphorylation. Key molecules in signaling from cytokine receptors are tyrosine kinases that belong to the group of so-called **Janus kinases (JAK)**: **JAK1, JAK2, JAK3 and TYK2.**



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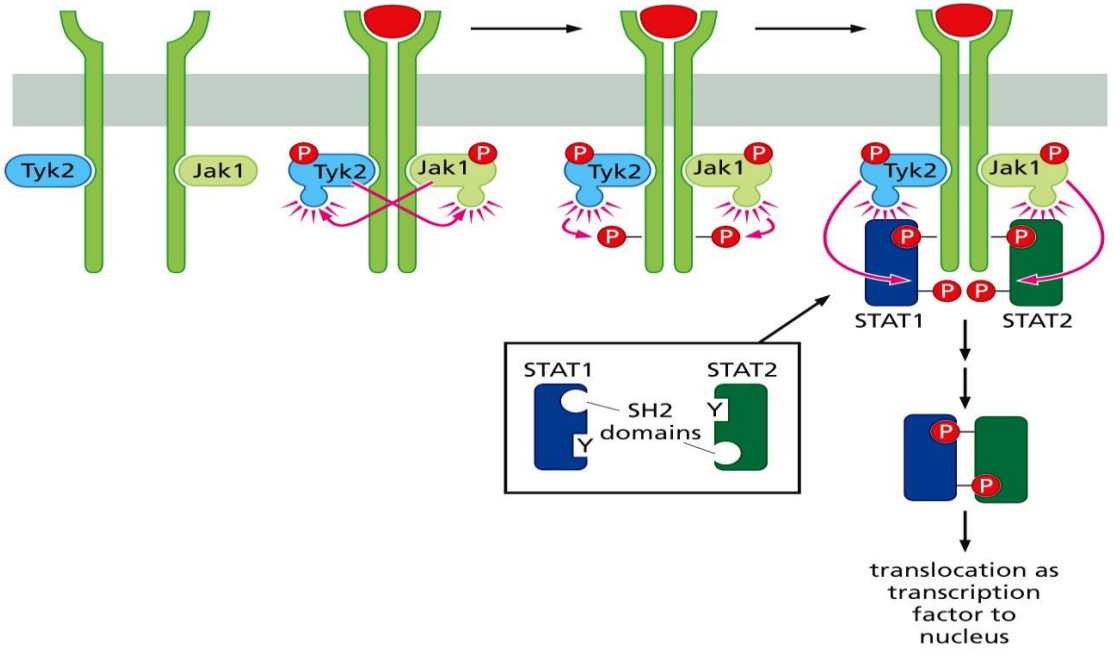
Signal transduction from cytokine receptors JAK/STAT signaling pathway

- After interacting with the ligand, cytokine receptors form dimers and activate JAKs, which perform transphosphorylation, as well as phosphorylation of the intracytoplasmic part of the receptor. The phosphorylated tyrosine residues of the receptor serve as anchoring sites for molecules called signal transducers and activators of transcription (STATs). To date, 7 STAT molecules have been identified.

- STAT molecules that form homodimers or heterodimers and are transported to the nucleus where they further regulate the expression of target genes.



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STAT molecules stimulate the transcription of several hundred genes important for the processes of cell proliferation and survival, such as **myc**, the genes encoding **cyclins D2 and D3** or the gene encoding the **antiapoptotic protein Bcl-XL**.

The role of the JAK/STAT signaling pathway in oncogenesis

Continuous activation of STAT molecules, induced by phosphorylation mediated by JAKs or other tyrosine kinases or reduced activity of phosphatases that remove phosphate groups, may be an important factor of enhanced proliferation or reduced apoptosis in the process of malignant cell transformation.

STAT3 molecule is constitutively activated in many tumors such as breast tumor, head and neck tumors, melanoma, glioblastoma and lung and stomach tumors.

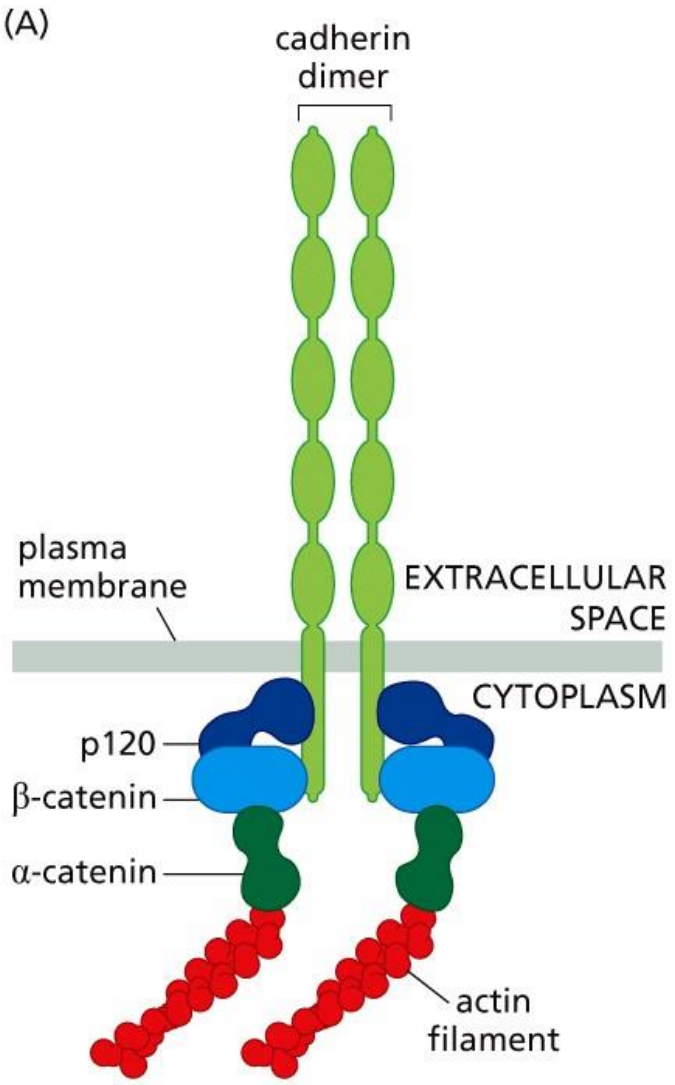
Mutation of the gene encoding JAK2 has been identified in a large number of patients with different types of myeloproliferative neoplasms.

Wnt signaling pathway

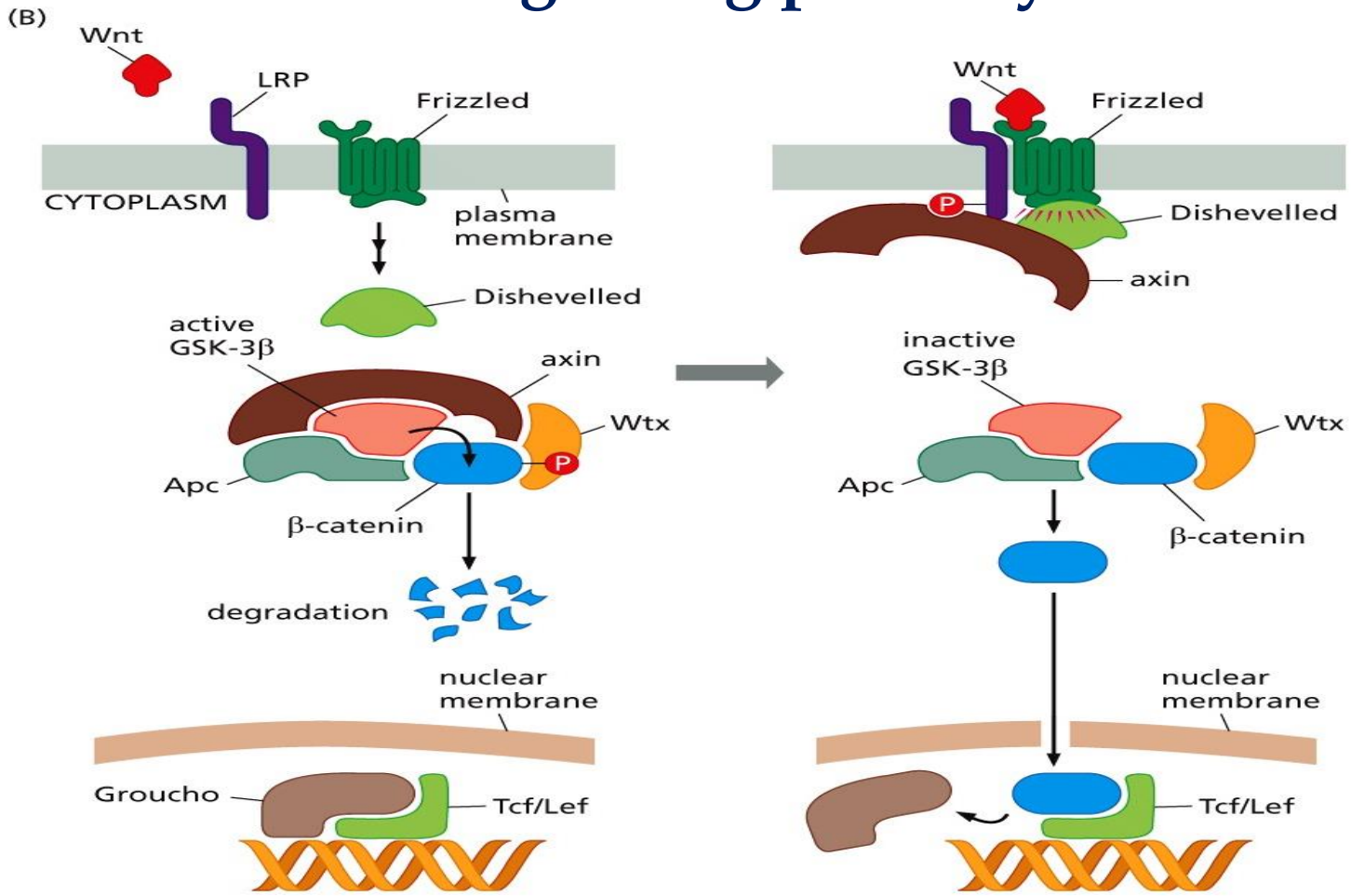
- Wnt proteins are secreted signal molecules that act as local mediators in the control of many aspects of organism development such as cell proliferation, stem cell survival and their differentiation.
- They are structurally distinctive and contain fatty acids covalently bound to the N-terminal part of the protein, which increases their ability to bind to the cell surface.
- There are 19 known Wnt proteins in the human body.
- Wnt proteins activate several signaling pathways in the cell, of which the **Wnt/β-catenin signaling pathway** is the best described.

Wnt signaling pathway

- Wnt/β-catenin signaling pathway regulates the proteolytic degradation of the multifunctional molecule β-catenin, which plays an important role in the processes of intercellular adhesion and the regulation of gene expression.
- In epithelial cells, β-catenin is bound to the transmembrane adhesive protein cadherin and participates in the formation and stabilization of intercellular connections.
- If it is not bound to cadherin, β-catenin is rapidly degraded in the proteasomes.



Wnt signaling pathway



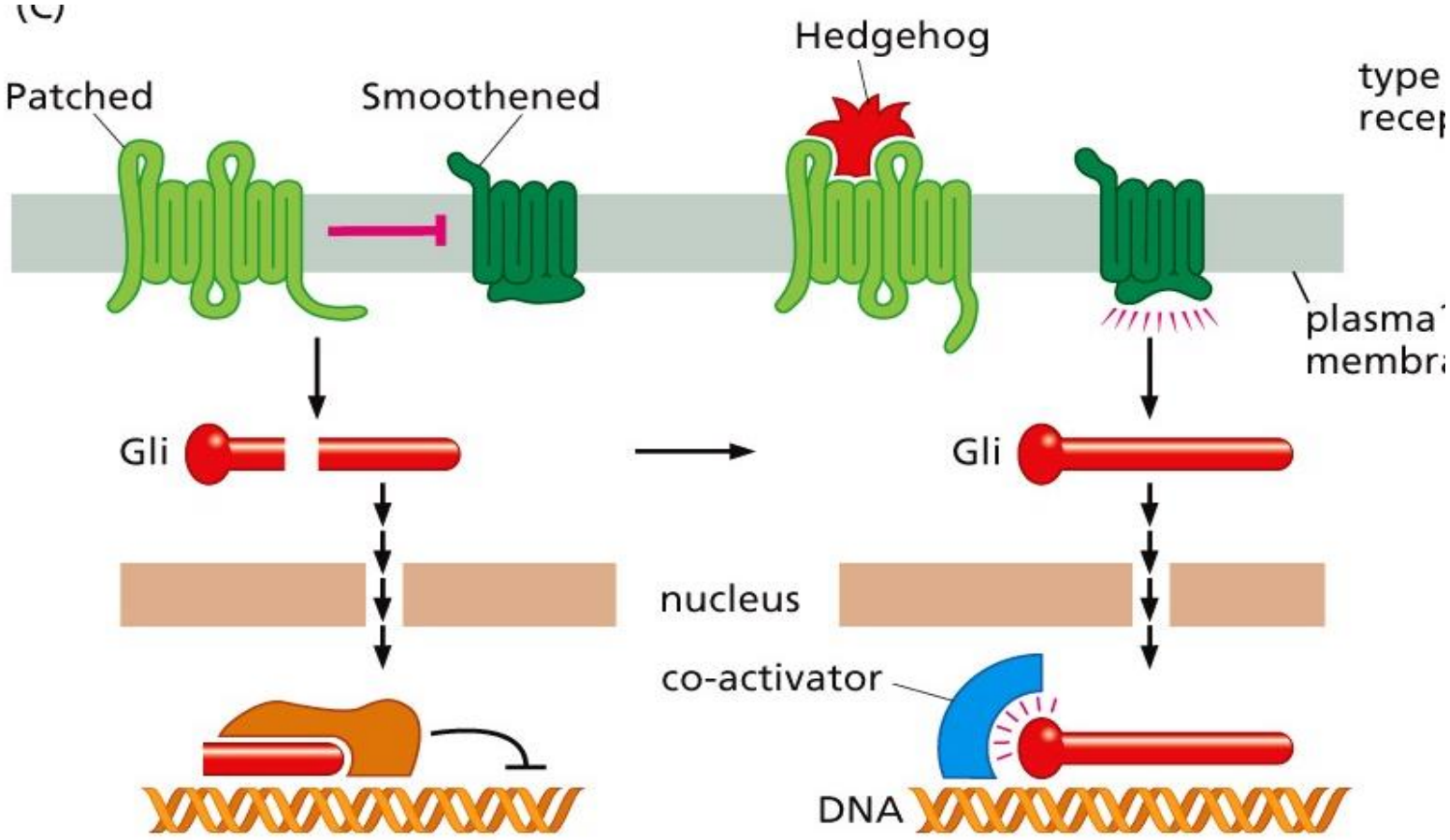
In the presence of Wnt signaling, β-catenin accumulates in the nucleus and stimulates the transcription of c-myc and other genes, which results in uncontrolled cell growth and proliferation and consequent tumor formation.

Wnt signaling pathway

- Wnt signaling pathway is constitutively autocrine activated in different types of tumors such as breast tumors, prostate cancer or sarcoma.
- Much more often, mutations in genes encoding various components of the Wnt/β-catenin signaling pathway, such as β-catenin or APC protein, may be involved in tumorigenesis.
- Hereditary mutations of the gene encoding the APC protein are responsible for the occurrence of familial adenomatosis of the colon, which is characterized by the appearance of multiple adenomatous polyps of the colorectal mucosa, which eventually undergo malignant transformation and the development of colorectal cancer.
- Mutations of the gene encoding the APC protein are also detected in 80% of cases of sporadic colon cancer.

Hedgehog signaling pathway

- Hedgehog proteins are secreted signal molecules structurally modified by covalently bound lipids.
- They represent important molecules that control the transcription of numerous genes.



Hedgehog signaling pathway

Hedgehog proteins are important stimulators of cell proliferation, so increased activation of the Hedgehog signaling pathway may represent one of the molecular mechanisms for tumor formation.

Mutations in genes encoding inactive Patched proteins are the basis for increased activation of this signaling pathway, which is often the underlying mechanism of basal cell skin cancer.

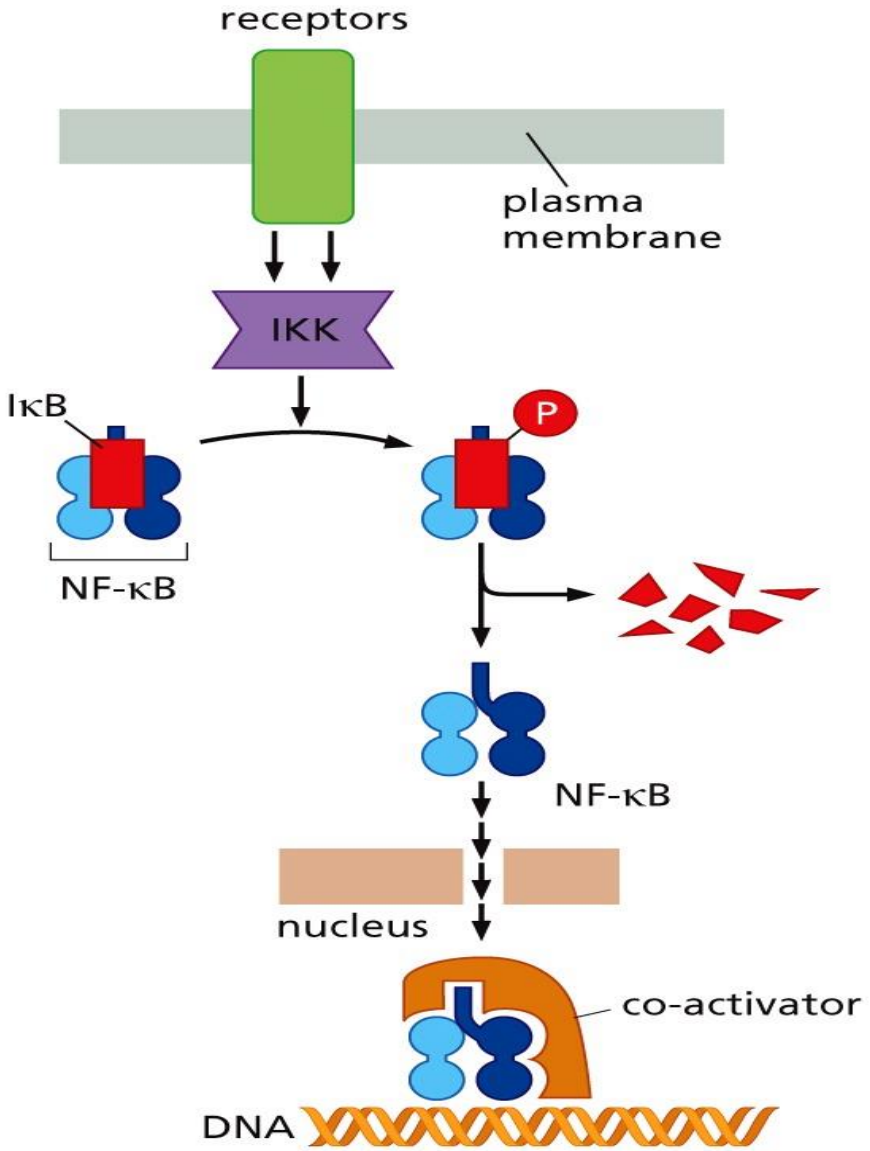
The role transcription factor NF-κB in oncogenesis

- NF-κB (Nuclear factor-κB) is a transcription factor that plays a central role in the cell's response to infection and injury.
- It regulates the expression of about 500 different genes that encode proteins involved in inflammation and the cell's response to stress.
- It is activated by numerous extracellular stimuli such as structures of microorganisms, pro-inflammatory cytokines, reactive oxygen mediators and others, by binding to specific receptors on the cell membrane.

In chronic inflammation, NF-κB participates in tumor formation by stimulating cell survival and proliferation.

The role transcription factor NF-κB in oncogenesis

- NF-κB is a heterodimer composed of subunits p65 and p50.
- In its inactive form, it is sequestered in the cytoplasm by binding to the inhibitory polypeptide IκB (inhibitor of NF-κB).
- Activation of NF-κB depends on the protein kinase IKK (IκB kinase), which performs phosphorylation and subsequent dissociation and degradation of IκB.
- NF-κB is released and transported to the nucleus where it regulates the expression of numerous genes.
- NF-κB stimulates the expression of anti-apoptotic proteins, the myc oncogene and cyclin D1 and thus prevents tumor cell apoptosis and promotes its proliferation.



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The role of transcription factor NF-κB in oncogenesis

- Constitutive activation of the NF-κB signaling pathway is common in tumors, although mutated forms of components of the NF-κB signaling cascade are rare.
- In breast tumors, the NF-κB signaling pathway is often hyperactive, which is most often the result of increased expression of IKK.
- NF-κB plays an important role in the malignant proliferation of cells of the immune system. Increased expression of NF-κB was detected in a large percentage of B and T lymphocyte lymphomas and myeloma.

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