بسم الله الرحمـٰن الرحيم

(وَفوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | Lecture 13

Cell Signaling pt.2

Written by : DST

Reviewed by : NST

Signaling pathways involving enzyme-linked receptors

Types of enzyme linked receptors:

Receptor Tyrosine kinases (RTK)
NON Receptor Tyrosine kinases = signaling pathway tyrosine kinase

SIMILARITIES:
BOTH : Tyrosine kinase will cause phosphorylation for Signaling molecules such as (effectors , adaptives)

DIFFRENCES:
RTK : Enzymatic activity (kinase enzymatic activity ,phosphorylation) is in the Receptor it self

NRTK : the Receptor away (isolated) from Tyrosine kinase (TK associate NON-Covalently with the receptor)
(Without ligand it will not be bound to the receptor but when ligand come it will associate with it non covalently)

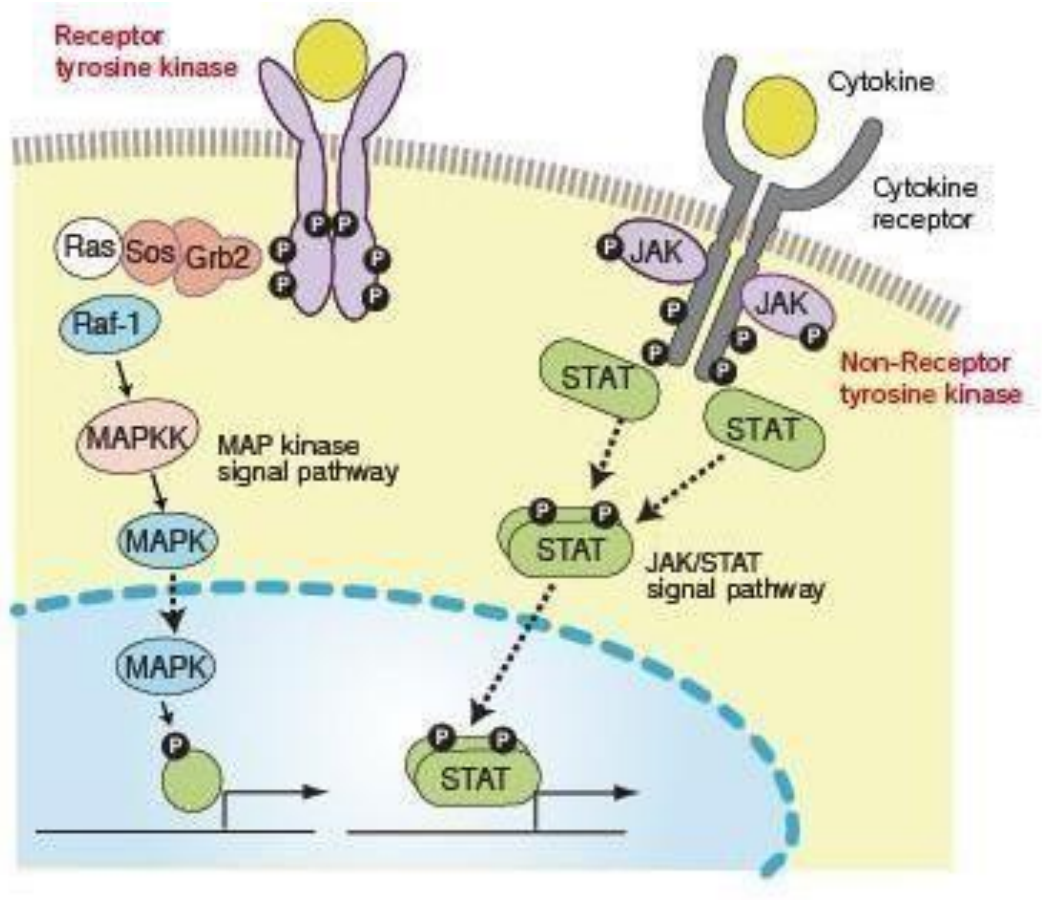
Receptor + tyrosine kinases (RTK)

- Some receptors either
  - Receptor tyrosine kinases (have an intrinsic tyrosine kinase activity)

OR

  - Nonreceptor tyrosine kinases (directly and noncovalently associated with tyrosine kinases.

Binding of ligands extraellularly activates the kinase activity resulting in a phosphorylation cascade.

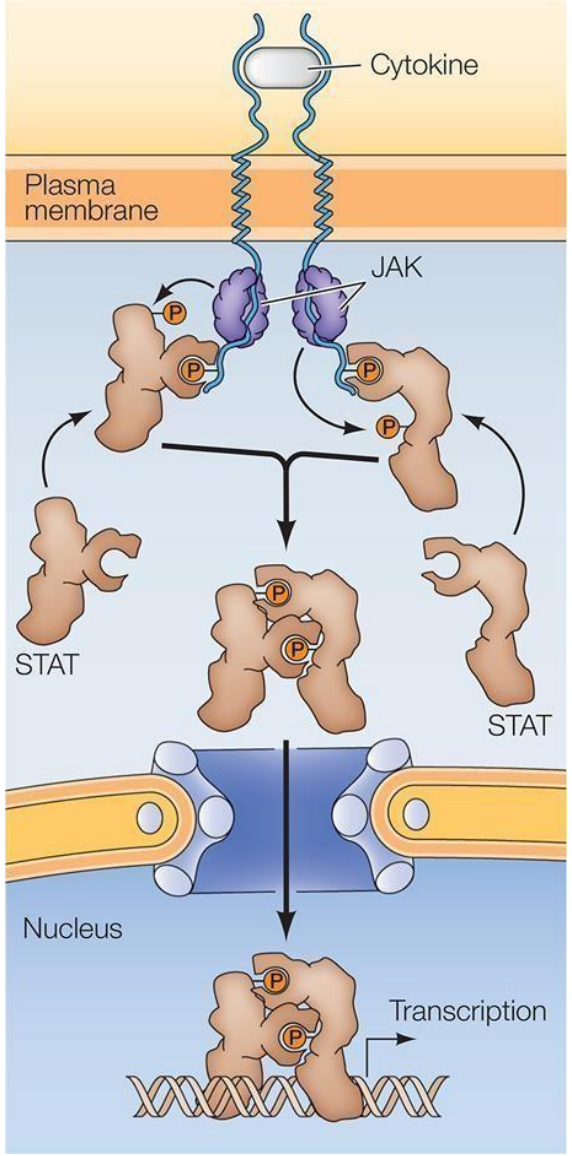


The JAK/STAT pathway

Ligand is Cytokines mainly

Cytokines are small proteins (ligand) that signal for the control of the growth and activity of immune cells and blood cells.

- Stimulation of cytokine receptors leads to the binding of the transcription factor, STAT, to phosphotyrosine-binding sites on the cytokine receptor
- The STAT proteins are phosphorylated by the receptor-associated JAK tyrosine kinases.
- The phosphorylated STAT proteins then dimerize and translocate to the nucleus, where they activate the transcription of target genes.



Jack stat pathway

Jack : is a Kinase, associate with Receptor , may phosphorylate it self in addition to phosphorylation STAT

Summery

ligand come (cytokine) and bind to the Receptor

Dimerization Receptor

JACK protein phosphorylate a Transcription factor which is STAT

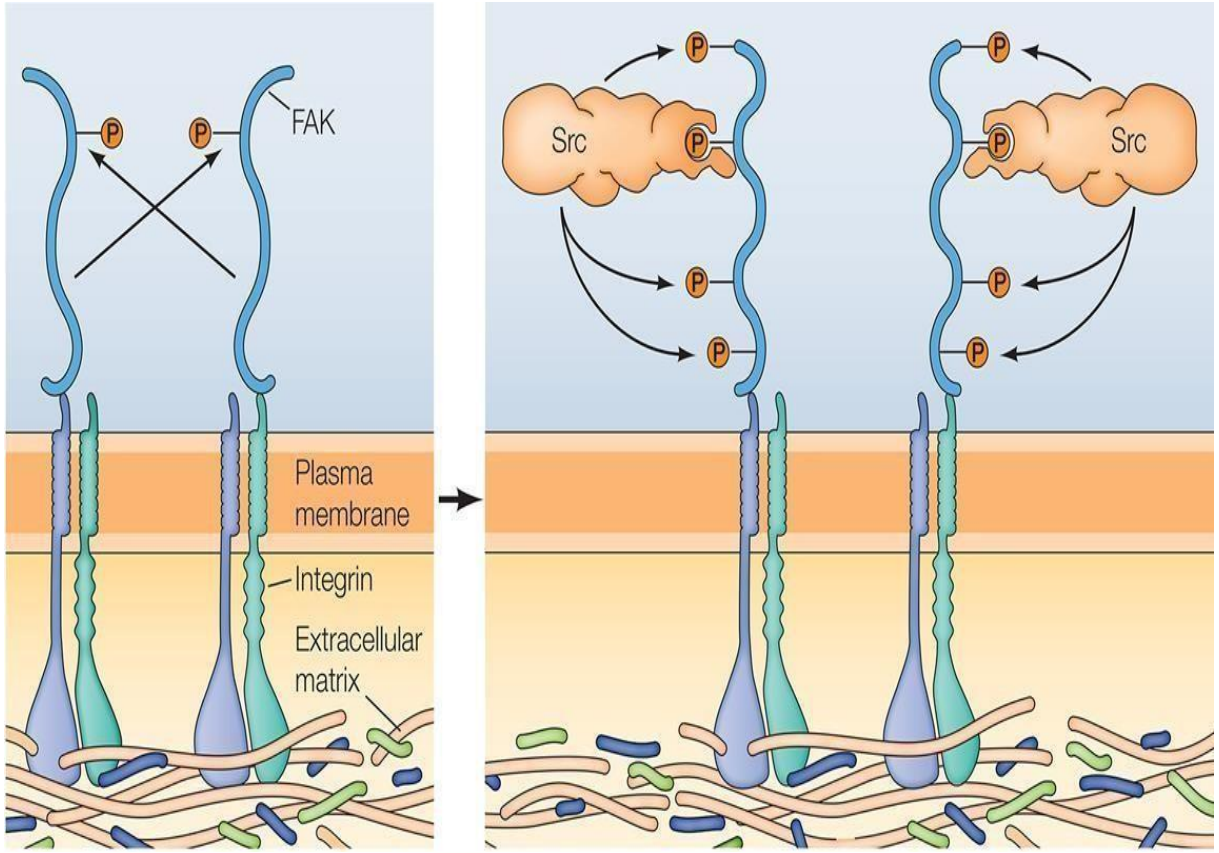
once phosphorylated STAT Dimerization happen then enter Nucleus – bind to DNA – Expression / transcription of certain gene (responsible for inflammatory response)

Integrin signaling

- Binding of integrins to the extracellular matrix leads to integrin clustering and activation of the nonreceptor tyrosine kinase FAK (focal adhesion kinase) by autophosphorylation.

- The nonreceptor tyrosine kinase, Src, then binds to the Src , FAK and phosphorylates FAK on additional tyrosine residues, which serve as binding sites for downstream signaling molecules (e.g., Ras).

- Other like-receptors: members of *the Ig superfamily* and *cadherins*



- when integrins interact with matrix protein (found in ECM) -- Dimerization Integrins happen

–- This dimerization activates focal adhesion kinase (FAK), causing FAK molecules to phosphorylate each other, which creates a docking site for a protein called Src (which is itself a kinase).

-- Src then phosphorylates the integrin receptor, further activating it.

---Src also associates with FAK (forming a concentrated complex) and can bind to other docking and signaling molecules, including those that interact with the RAS pathway.

---- This leads to further phosphorylation by Src, amplifying the signaling

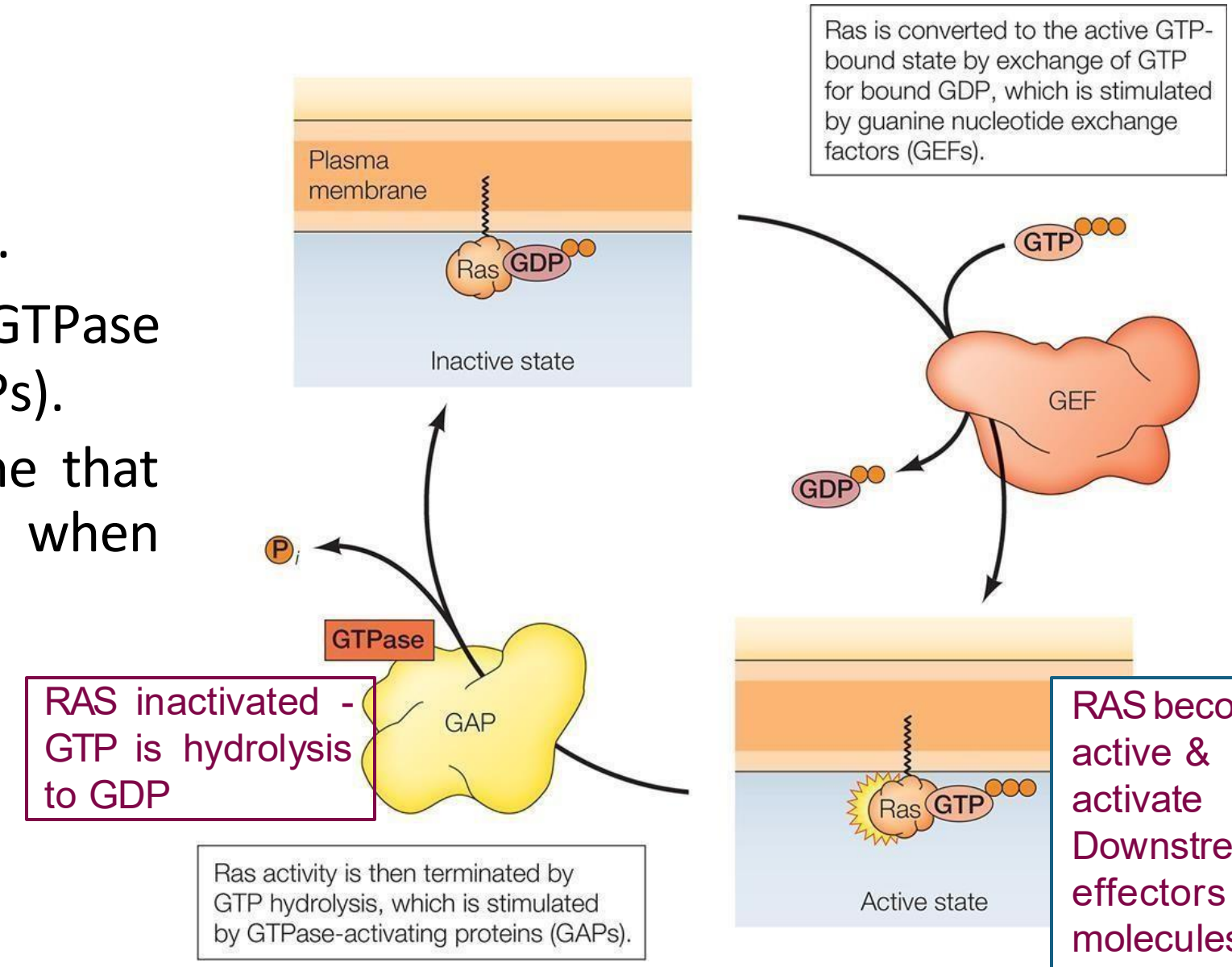
The MAP kinase pathways

MAP: mitogen-activated pathway = Mitogen activated protein

A mitogen is a small bioactive protein or peptide that induces a cell to begin cell division or enhances the rate of division (mitosis).

Let's start with Ras (*a small monomeric G protein* = activated when bind to GTP)

- Ras is stimulated by Guanine nucleotide exchange factors (GEFs).
- Ras is inhibited by GTPase activating proteins (GAPs).
- Ras is a proto-oncogene that becomes an oncogene when mutated.
- Ras is associated with colorectal cancer.
- *Onco = tumor*



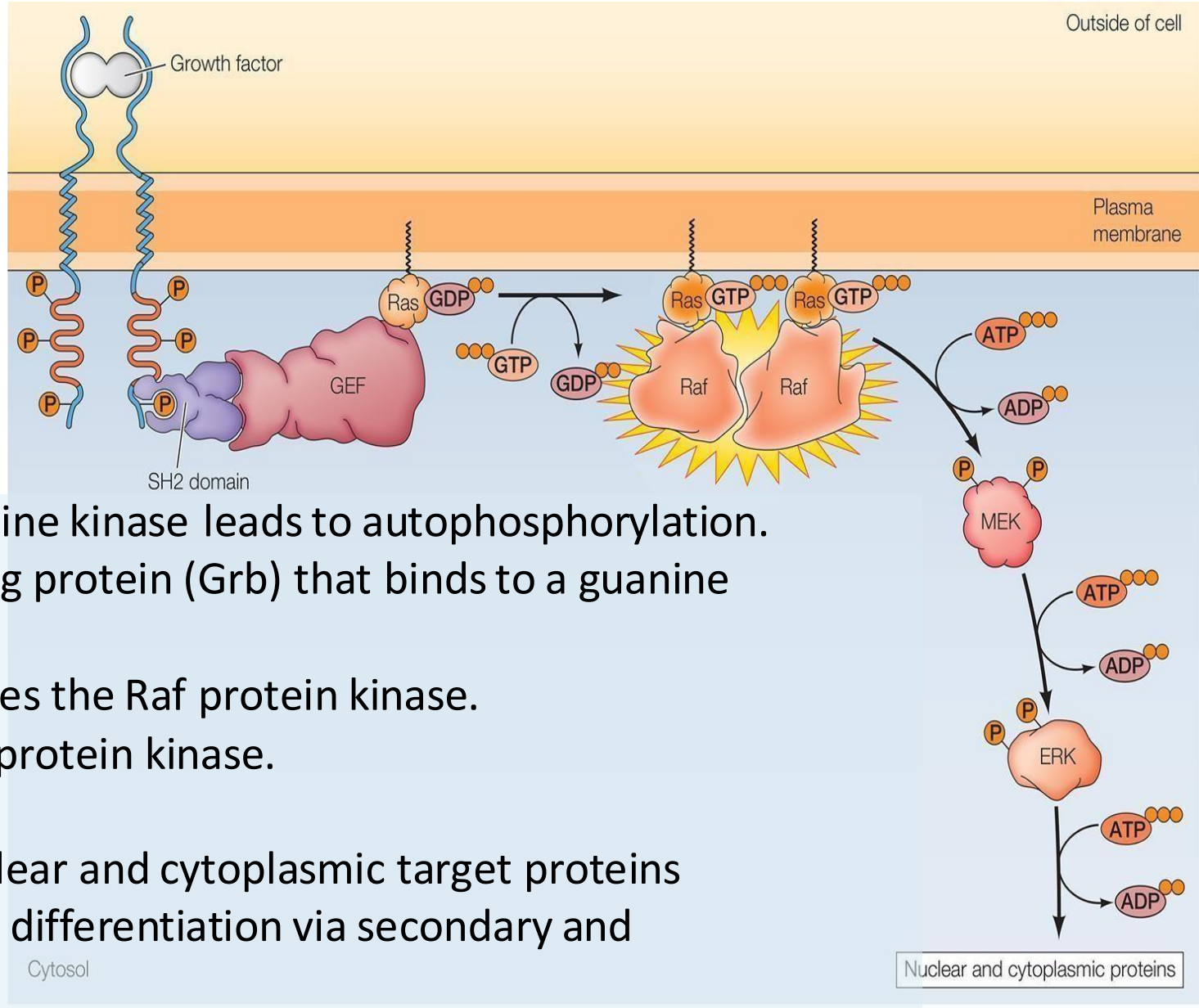
REGULATED BY : GTP binding and that happen with help of other protein (Facilitated) GNEF (loose GDP , ADD GTP)

RAS become activated when bind to GTP and once activated it can activate other protein DOWN STREAM
RAS become inhibited by GTPase activating protein activity is INTRINSIC (belong to) Ras
Inhibition happpen by a Hydrolysis reaction facilitaed or activated by GAPs (GTPase Activating protein)

A proto-oncogene is a normal gene that regulates cell growth and division. When it undergoes a mutation or becomes overexpressed, it turns into an oncogene, which promotes uncontrolled cell proliferation and can lead to cancer. In other words, proto-oncogenes are essential for normal cellular functions, but once altered, they become oncogenes that drive tumor formation. For example, the RAS gene normally helps control cell signaling, but when mutated, it becomes an oncogene that causes continuous cell division.

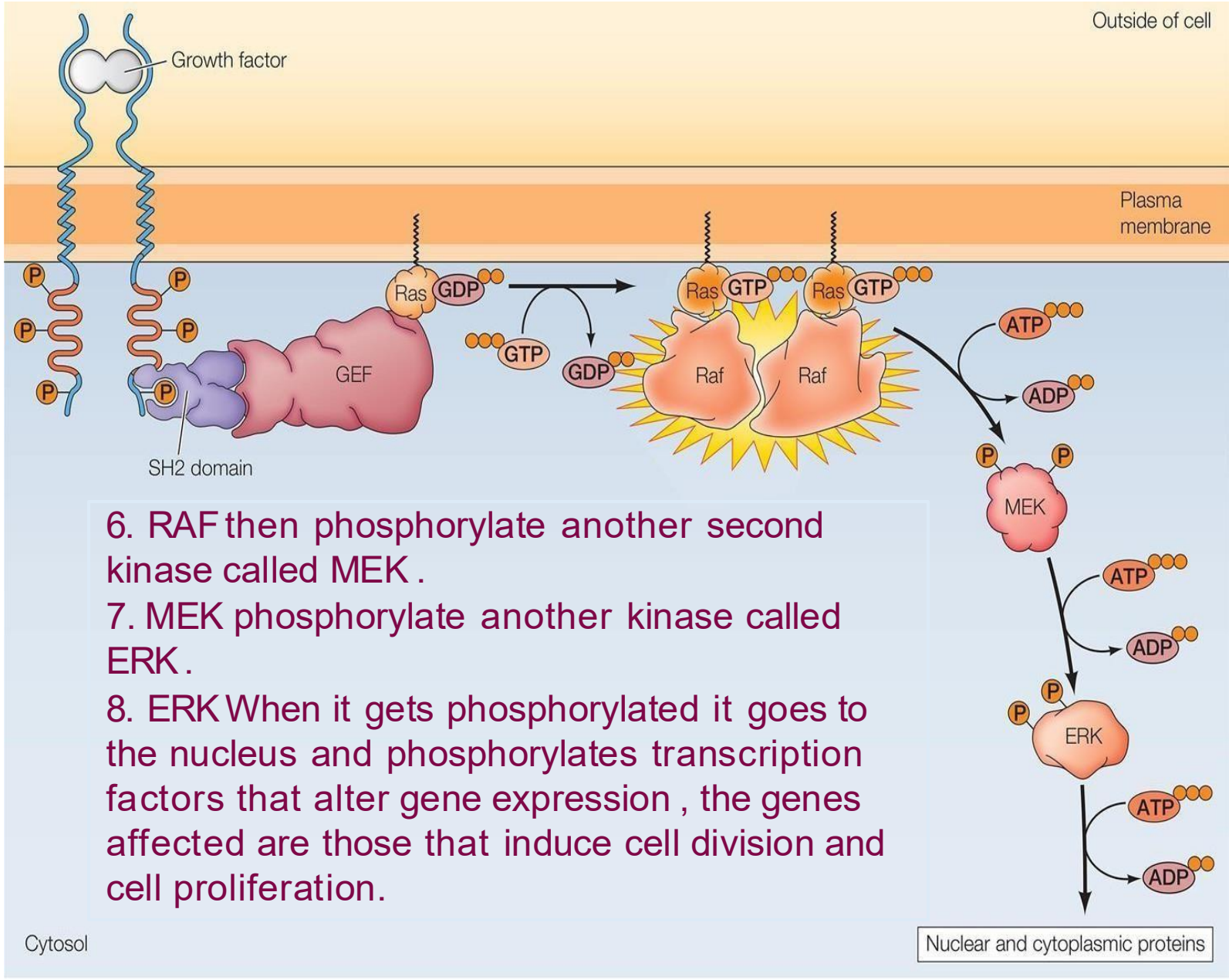
Approximately 95% of pancreatic cancers have mutations in the RAS gene, making it one of the most commonly mutated genes in this type of cancer. These mutations cause the RAS protein to remain constantly active, leading to uncontrolled cell growth. Similarly, a significant proportion of colorectal cancers also show RAS mutations, contributing to tumor development and progression. This highlights the crucial role of RAS mutations in driving cancer formation in both the pancreas and colon.

The Ras/Raf/MAPK signaling pathway



1. Growth factor binding to a receptor tyrosine kinase leads to autophosphorylation.
2. The phosphorylated sites recruit a docking protein (Grb) that binds to a guanine nucleotide exchange factor (GEF).
3. The GEF activates Ras, which then activates the Raf protein kinase.
4. Raf phosphorylates and activates MEK, a protein kinase.
5. MEK phosphorylates and activates ERK.
6. ERK then phosphorylates a variety of nuclear and cytoplasmic target proteins promoting cell proliferation, survival, and differentiation via secondary and tertiary responses.

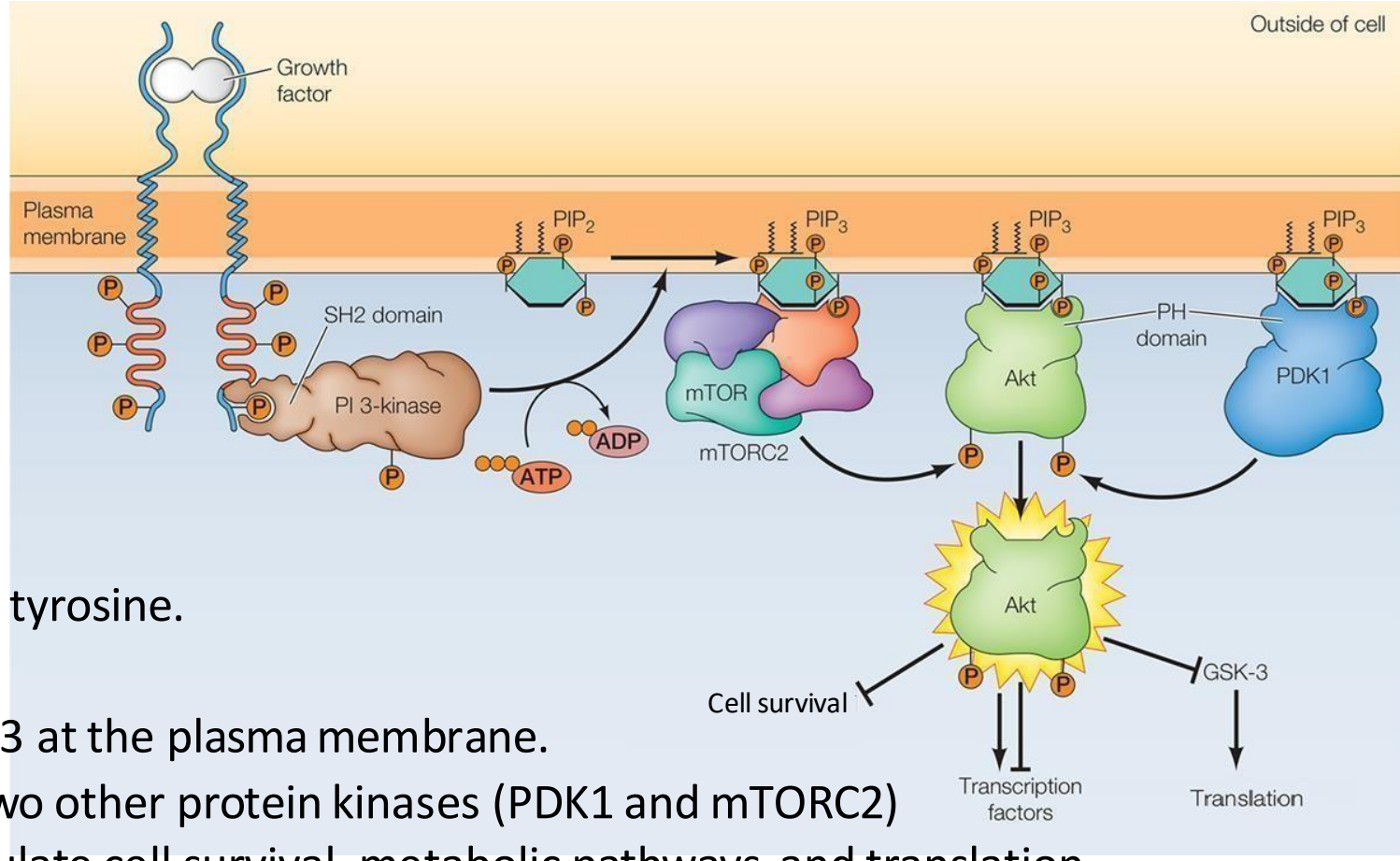
1. Ligand Binding to the Receptor
2. Receptor Dimerization:
After the ligand binds, the receptors undergo dimerization—they pair up with another receptor molecule. This is often necessary to activate the receptor.
3. Receptor Autophosphorylation:
The dimerized receptor then undergoes autophosphorylation (self-phosphorylation), where it adds phosphate groups to itself.
4. Creating Docking Sites for Proteins:
The autophosphorylated receptor creates docking sites for other proteins.
5. RAS (not an enzyme) is on the plasma membrane so when GEF bind to the receptor it becomes close to the RAS and activates it , so RAS become GTP bound and then it binds to kinase called RAF .



6. RAF then phosphorylate another second kinase called MEK .
7. MEK phosphorylate another kinase called ERK .
8. ERK When it gets phosphorylated it goes to the nucleus and phosphorylates transcription factors that alter gene expression , the genes affected are those that induce cell division and cell proliferation.

The PI 3-kinase/Akt pathway

PI 3-kinase (phosphatidylinositide (PI) 3-kinase) phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3).



- PI 3-kinase is recruited to activated receptor tyrosine.
- PI 3-kinase phosphorylates PIP2 to PIP3.
- Akt (a serine/threonine kinase) binds to PIP3 at the plasma membrane.
- It is then phosphorylated and activated by two other protein kinases (PDK1 and mTORC2)
- Akt then taphosphorylates proteins that regulate cell survival, metabolic pathways, and translation (protein translation).

The PI 3-kinase/Akt pathway

It's a pro survival pathway

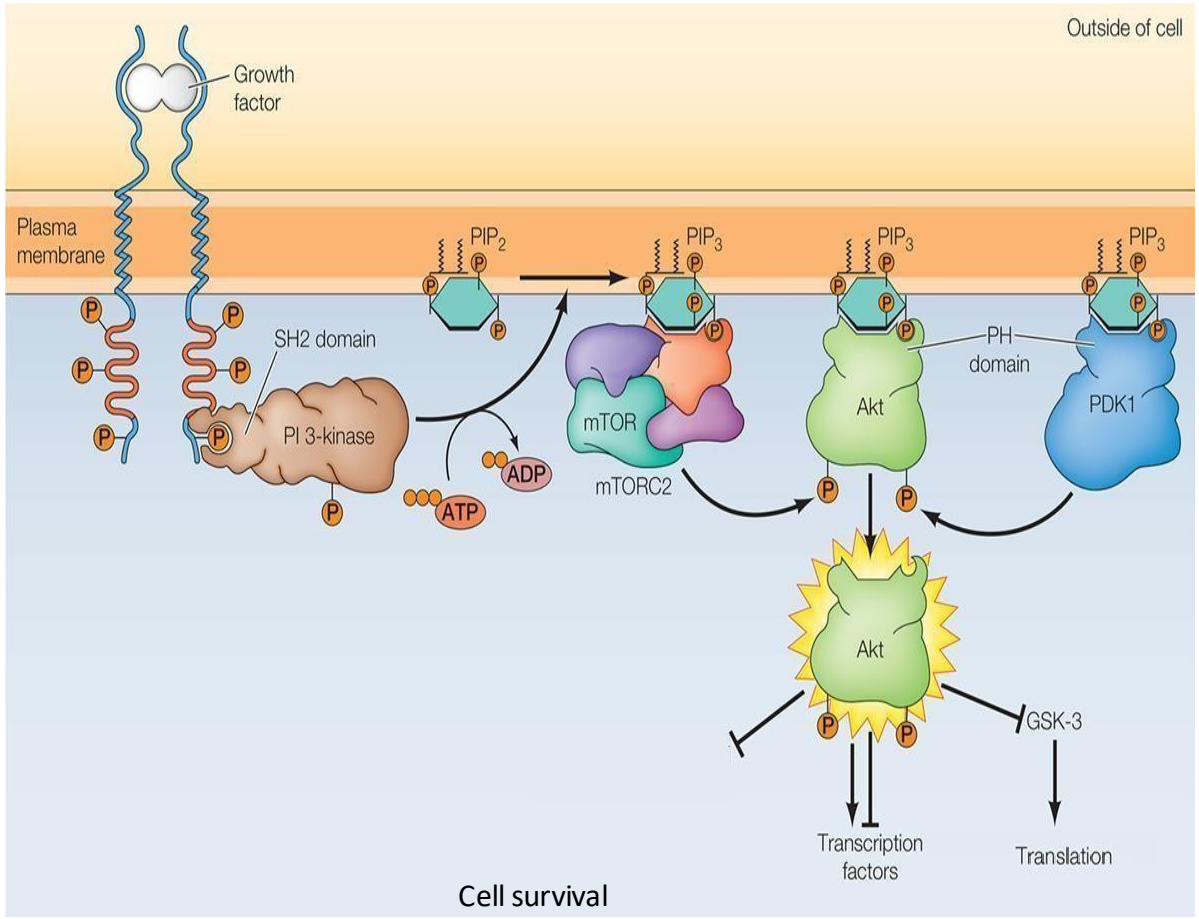
1)we have a receptor binds to the ligand, undergoes autophosphorylation , creating a docking site when PI3 kinase (has one phosphate group) interact with the receptor.

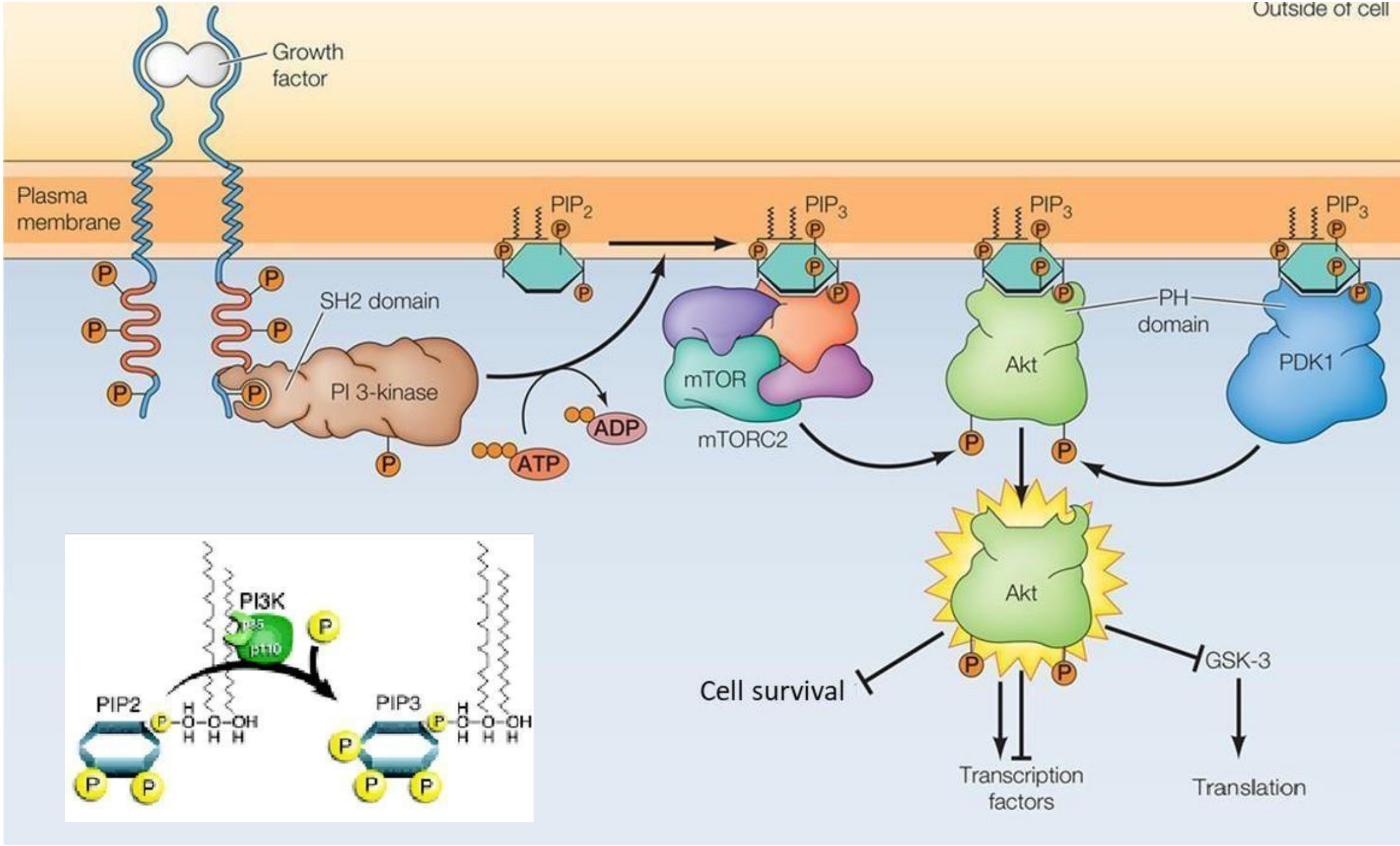
2) Once PI3 kinase is bound to the receptor, it can convert PIP2 to PIP3, PIP2 is a phosphatidylenositol bisphosphate , It has two phosphate groups (note that there is already a PIP2 in the plasma membrane).

3) This kinase adds a third phosphate group, which become PIP3 , When PIP-3 is present in the plasma membrane, it serves as a binding site for a signaling molecules called AKT , PDK1 and mTOR . When they get close to each other PDK1 and mTOR (kinases) phosphorylate AKT.

4) Once it becomes activated, AKT phosphorylates various proteins. What it does is that it inhibits cell death, so promoting cell survival. It controls a number of transcription factors, and what it does is that it activates translation of proteins.

GSK is an inhibitor of translation , GSK gets inactivated by AKT, so AKT activates translation. So it is a pro-survival molecule. Its signaling pathway is pro-survival.

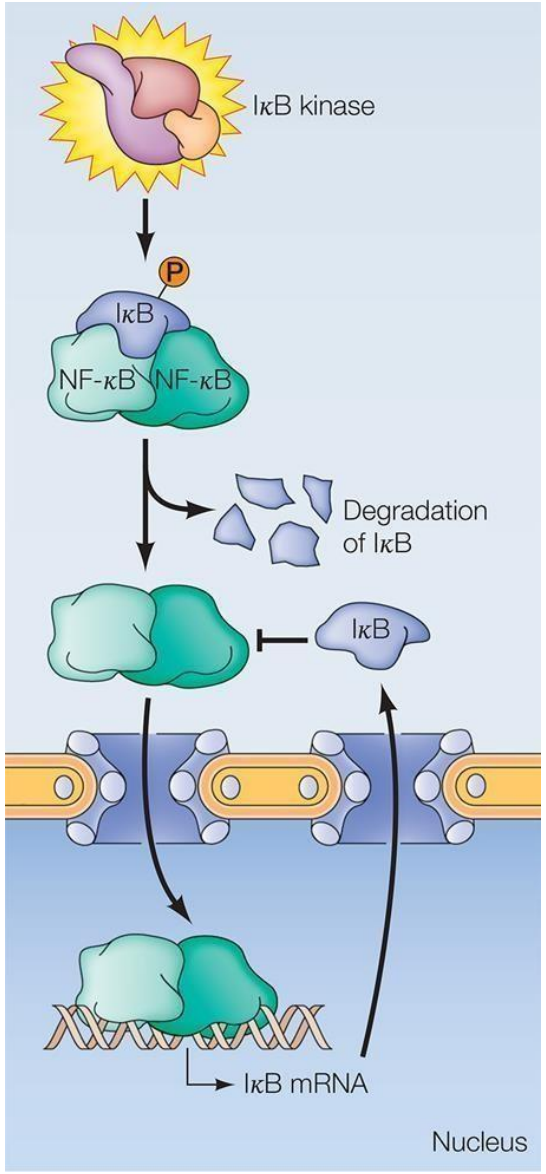



Outside of cell
Growth factor
Plasma membrane
SH2 domain
PI 3-kinase
ATP
ADP
PIP2
PIP3
PIP3
PIP3
mTOR
mTORC2
Akt
PH domain
PDK1
Akt
Cell survival
GSK-3
Transcription factors
Translation
PI3K
p85
p110
PIP2
PIP3

Signaling Dynamics and Networks

Feedback loops and signaling dynamics

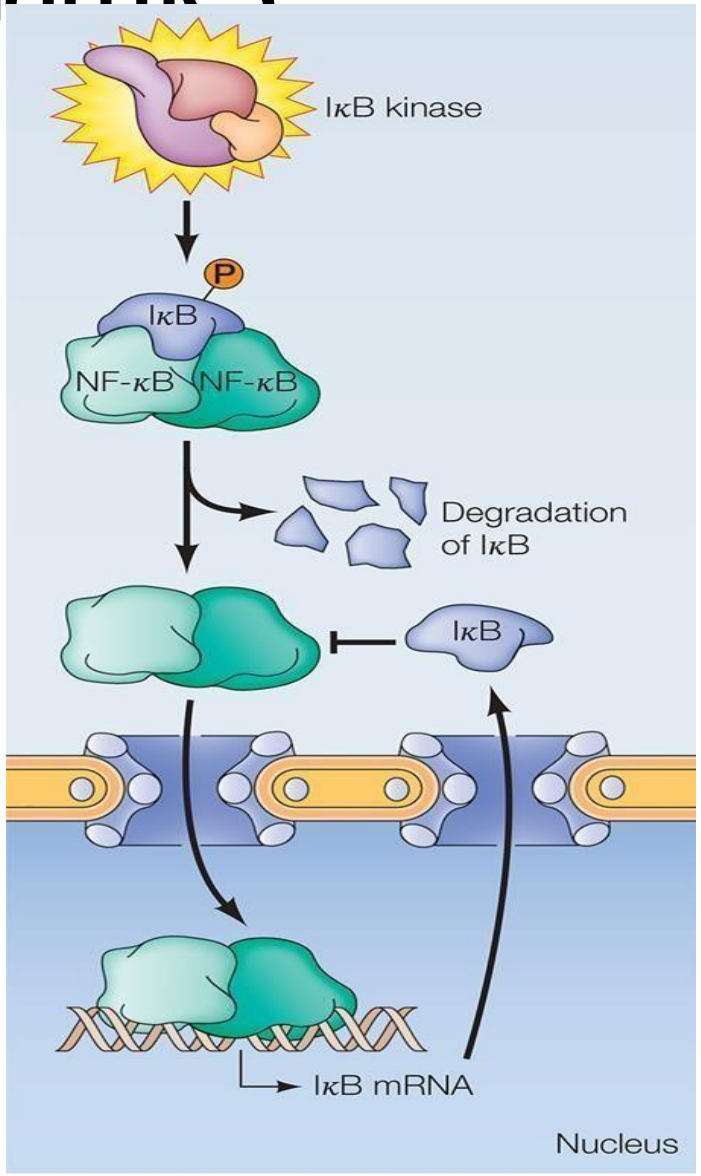
- Activation of certain signaling pathways results in the phosphorylation of IκB, an inhibitor of NF-κB (nuclear factor-kappa B).
- This causes the activation of NF-κB, which translocates into the nucleus and activates transcription of target genes.
- One of the genes activated by NF-κB encodes IκB, generating a feedback loop that inhibits NF-κB activity.



Feedback loops and signaling dynamics

When NF-kappa-B is in its free form, it can enter the nucleus and regulate gene expression. However, it needs to be tightly regulated. This regulation is often done through an inhibitor called IκB (Inhibitor of NF-kappa-B).

The inhibitor binds to NF-kappa-B and traps it in the cytoplasm, preventing it from entering the nucleus. This inhibition is maintained until a signaling pathway, such as the MAP kinase pathway, is activated , when the signaling pathway is activated, it leads to the phosphorylation of the inhibitor. Once the inhibitor is phosphorylated, it undergoes degradation, releasing NF-kappa-B. As a result, NF-kappa-B is then free to enter the nucleus and initiate the expression of various genes.

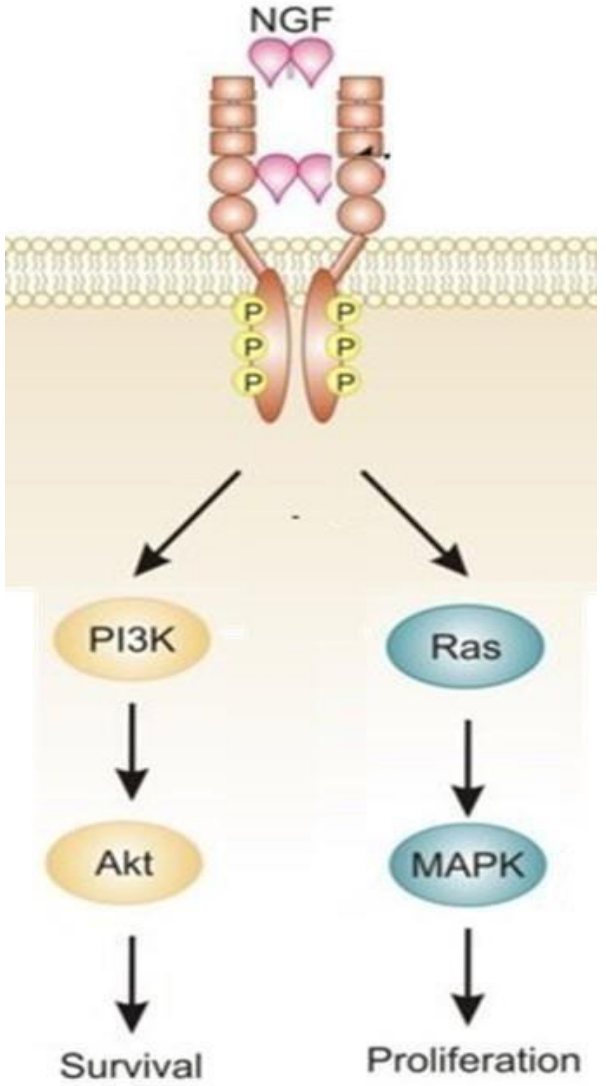


Signaling homeostasis

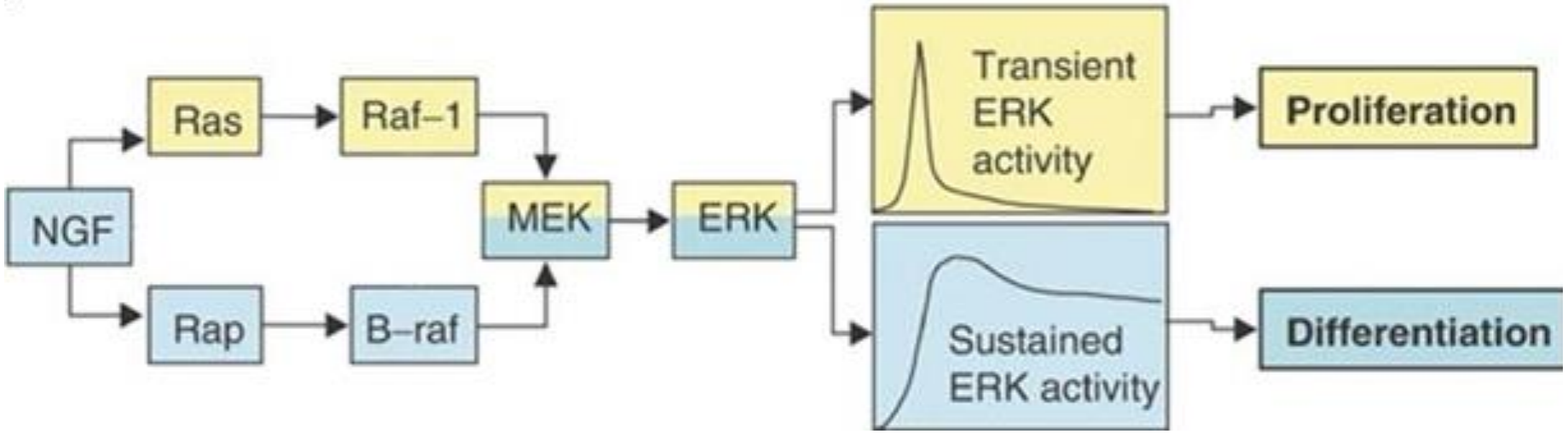
When it bind to its receptor, it activates the RAS pathway and the PI3 kinase pathway (the pro-survival pathway)

- In response to nerve growth factor (NGF), ERK signaling can lead either to cell proliferation or to neuronal differentiation depending on the duration of ERK activity.
- Activation of ERK for 30–60 minutes stimulates cell proliferation.

activation of the RAS pathway, but not the PI3 kinase pathway.



Signaling homeostasis



Activation of ERK for 30–60 minutes stimulates cell proliferation.
Sustained activation of ERK for 2–3 hours induces differentiation of the NGF-treated cells into neurons.

- Sustained activation of ERK for 2–3 hours induces differentiation of the NGF-treated cells into neurons.

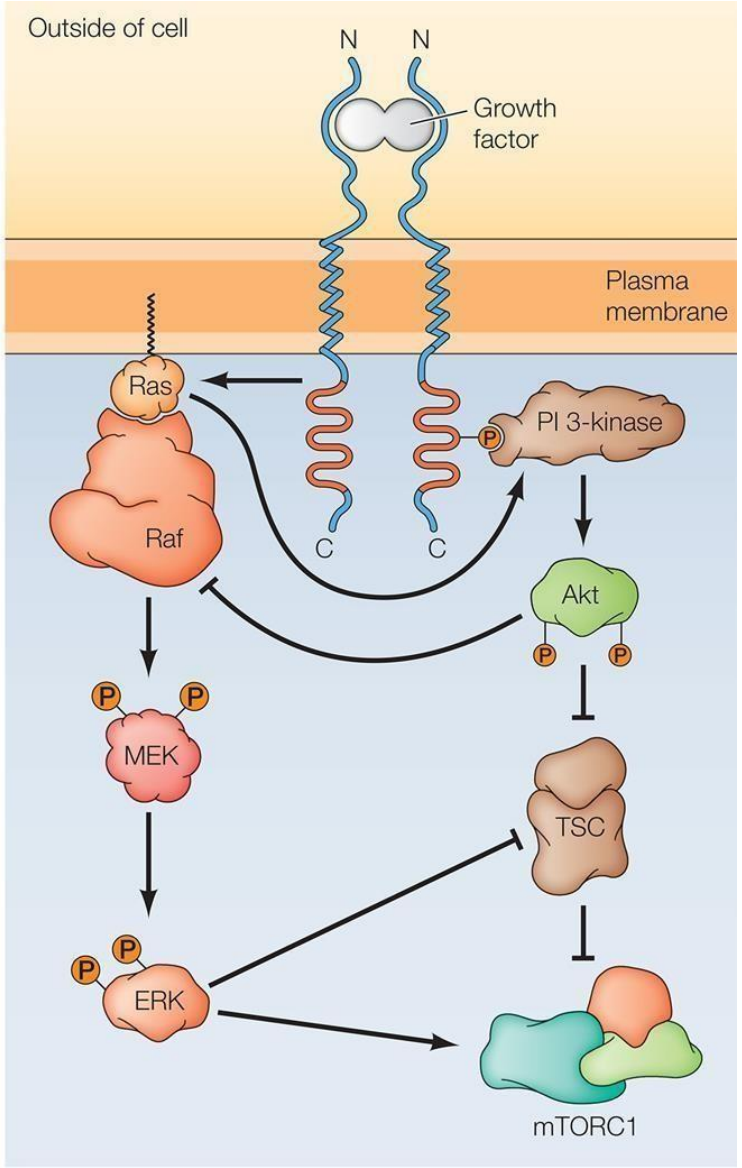
if the signal prolongs, if it becomes active for a longer time, then the other pathway, the PI3 kinase pathway, is activated, not the RAS pathway,
and this pathway induces differentiation of cells into neurons.

Crosstalk of pathways

The ERK and PI 3-kinase signaling pathways

- The Ras/Raf/MEK/ERK and PI 3-kinase/Akt/mTORC1 pathways are connected by both positive and negative crosstalk,
  - PI 3-kinase is activated by Ras.
  - Raf is inhibited by Akt.
  - mTORC1 is activated by ERK.

Crosstalk: A regulatory mechanism in which one signaling pathway controls the activity of another.

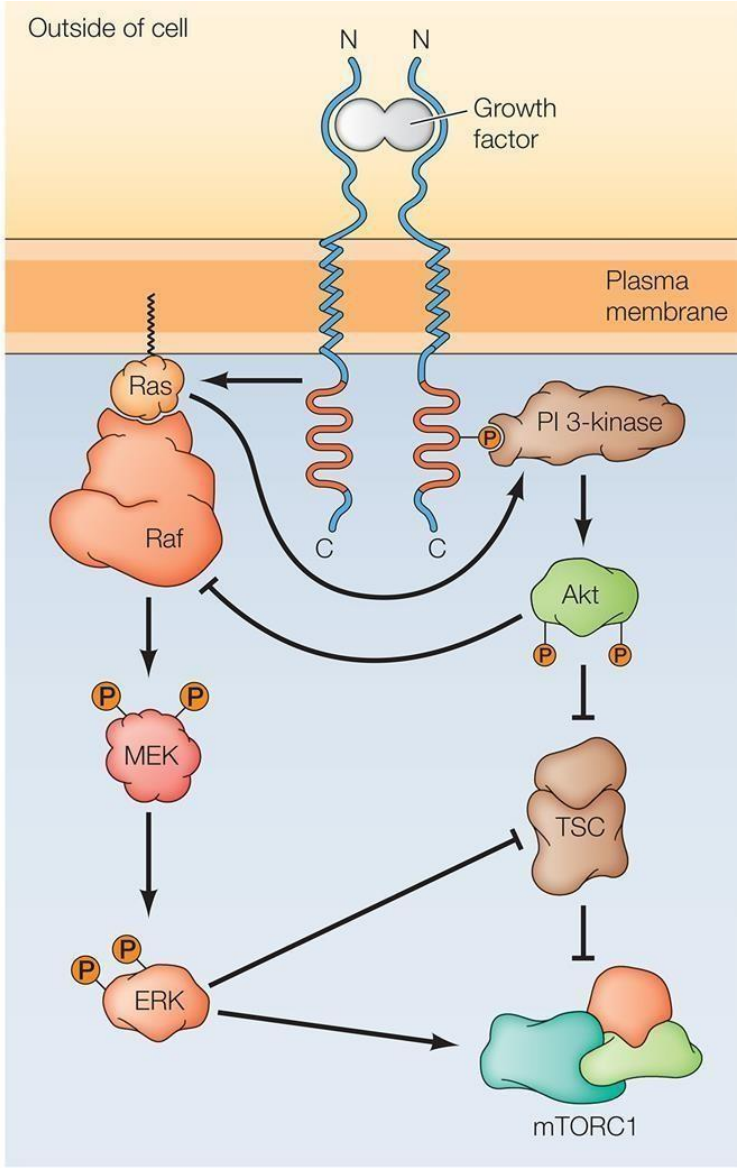


Crosstalk of pathways

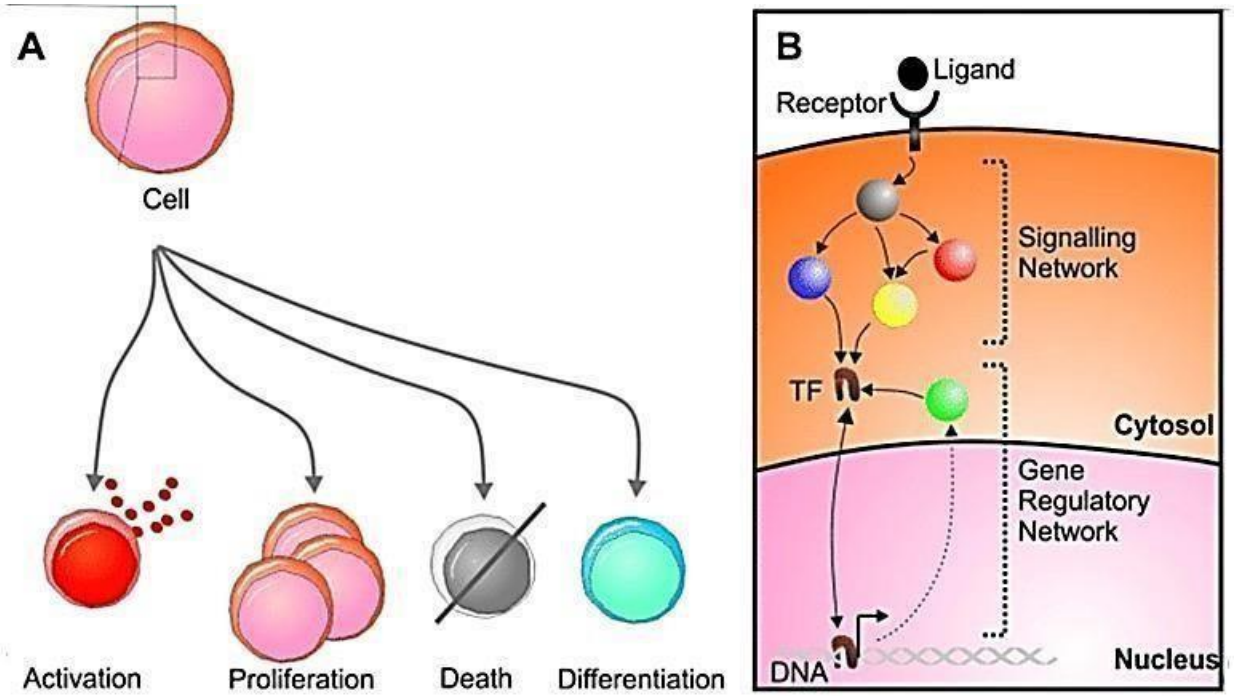
The ERK and PI 3-kinase signaling pathways

1)We have the RAS pathway and the PI3 kinase pathway. When the ligand binds to its receptor , it will activate the RAS and RAF pathway.
2) RAF activates the PI3 kinase and PI3 kinase can then activate AKT.
3) AKT in turn inhibits RAF , so there is a limit by which RAF would be activated for , so it doesn't go out of control .
4) Now, eventually, if RAF is active, it activates MEK , MEK activates ERK and ERK activates the downstream molecule down there, the mTOR, which activates the AKT as well.

So you have this complex interactions of the different molecules ,but the whole purpose of this whole thing is to balance things out.



Cell-specific response. Why?



- Cells have distinct receptors.
- Cells contain a different combination of regulatory molecules (effectors and secondary messengers) that influence the cells' behaviors.
- The final target protein must have access to its target.
  - For example, a transcription factor would bind to the DNA-binding site and activate transcription if the chromatin is packaged loosely (i.e., euchromatin). If the site is packaged tightly (i.e., heterochromatin), the complex would not be able to bind to the DNA.

Sometimes, cells can respond to the same hormone, but in different ways. Cells can have the same hormone and the same receptor, but still they respond differently. So how do cells do that? Well, there are different mechanisms.

One, you can have cells having different receptors. So you can have the same ligand acting on the same cell, except that it binds to different receptors.

Maybe cells have the same receptor, but they behave differently, they respond differently , because they have different effectors, they have different transducers, they respond to secondary messengers in a different way. So for example, you can have activation of protein kinase A that would then respond differently because it has different effectors.

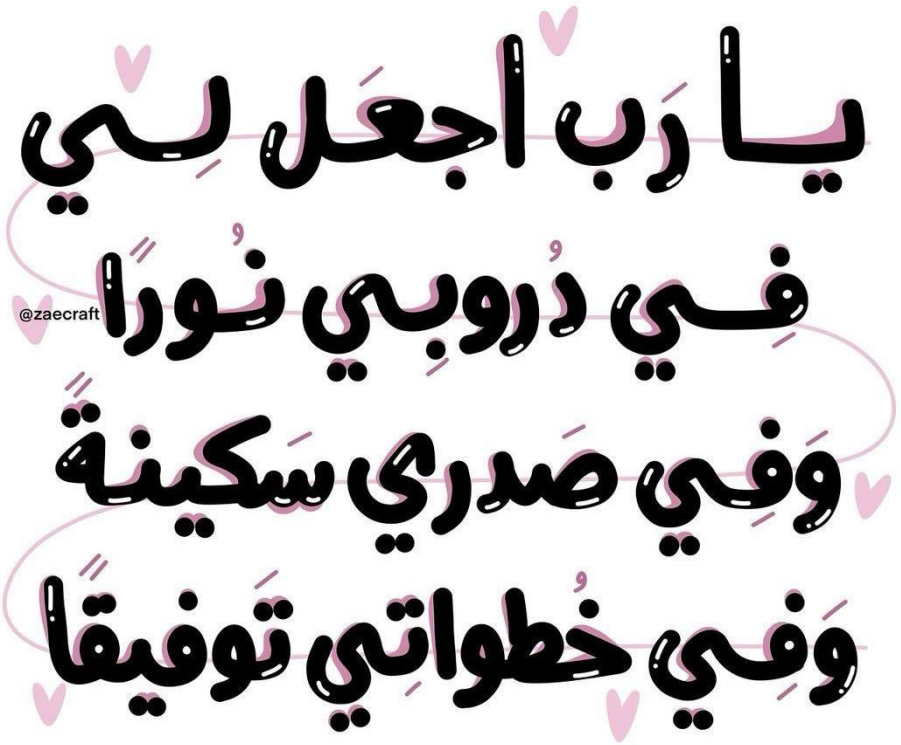
You can have the same secondary messenger like cyclic AMP, but cyclic AMP binds to different effector molecules.

Also, if we assume that the final target is the same, still DNA is not the same in all cells (Every cell has its own

molecular profile, a molecular fingerprint) , Yesthey have the same number of genes, but remember, we have

heterochromatin and we have euchromatin.

يملعلا قوفلا نم ةلاسر:



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 21		Photo & text added by the doctor
V1 → V2			