Review: Protein Transport and Localization

- You discover a drug that *inhibits the activity of Ran-GAP* (GTPase Activating Protein).

- What do you expect to happen to the nuclear concentration of **Ran** following prolonged exposure to this drug? Why?

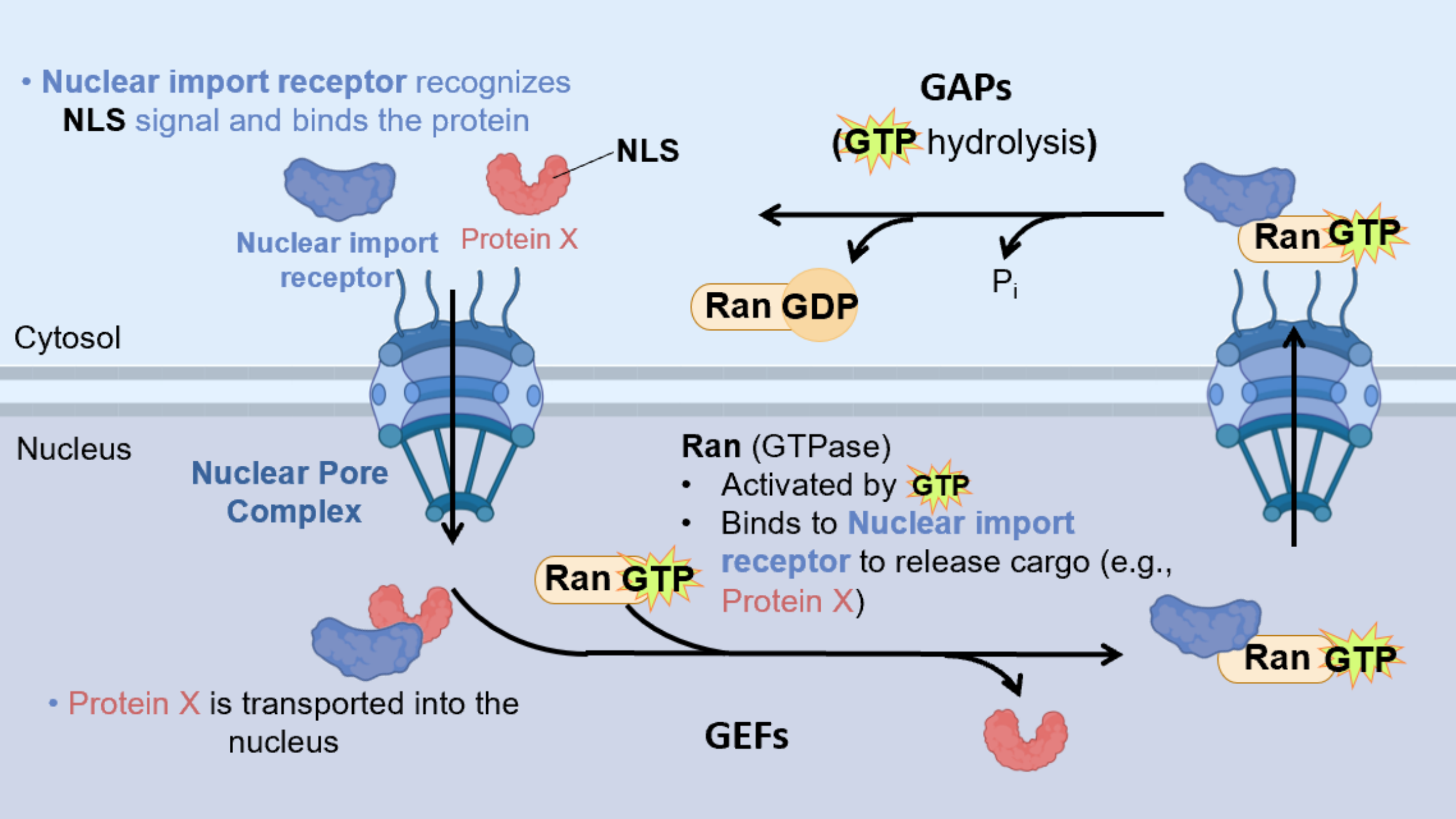
- What are some cellular consequences that you could foresee following exposure to this drug?

Review: Protein Transport and Localization

- You discover a drug that *inhibits the activity of Ran-GAP* (GTPase Activating Protein).

- What do you expect to happen to the nuclear concentration of **Ran** following prolonged exposure to this drug? Why?
  - We would expect Ran to reduce in the nucleus because GTP-hydrolysis would not occur under Ran-GAP inhibition and so GTP would remain bound to Ran, sequestering it to the cytosol. Over time, the Ran-GDP:Ran-GTP ratio would decrease as GEFs continue to function but GAPs are inhibited.

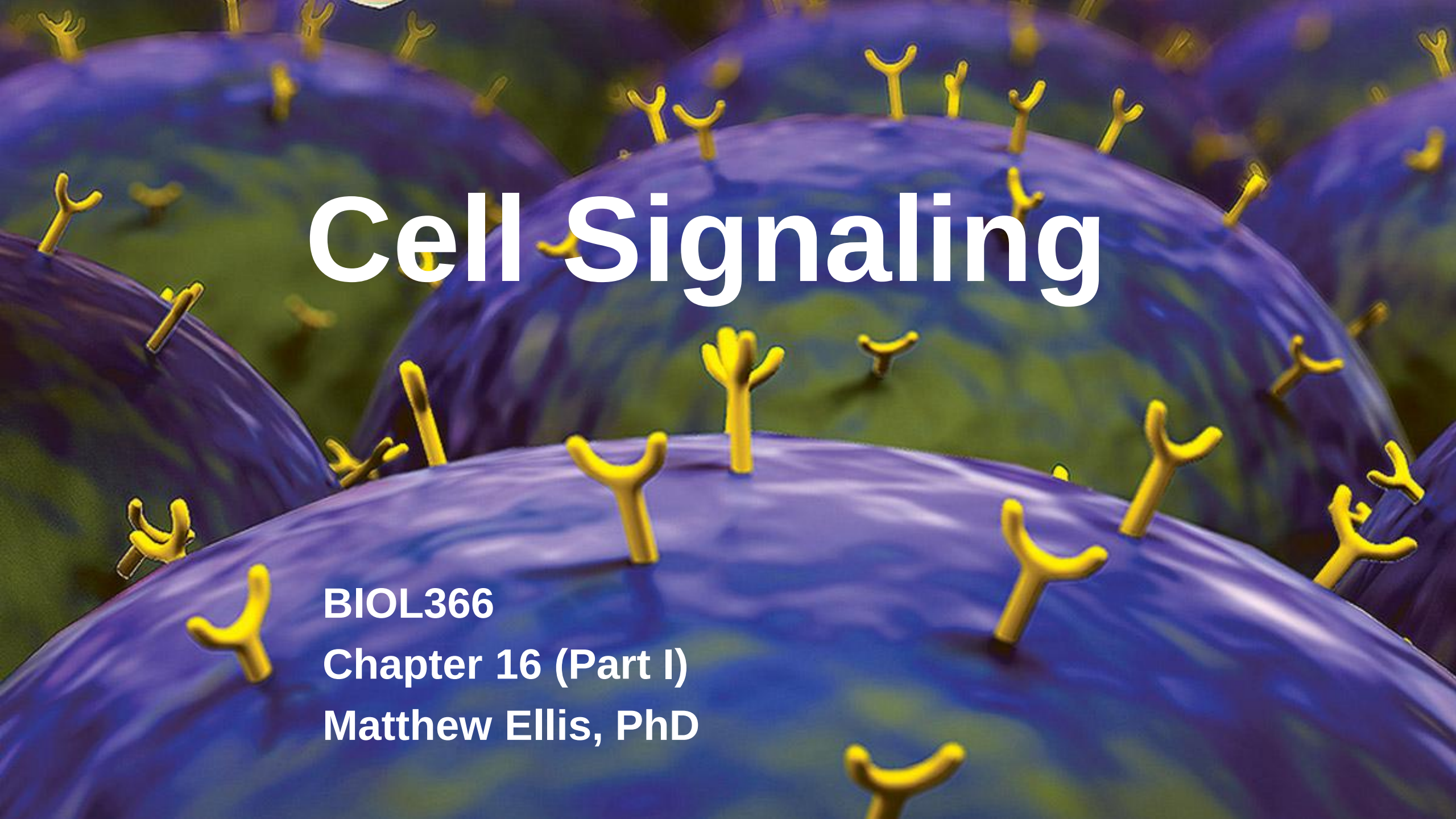
- What are some cellular consequences that you could foresee following exposure to this drug?
  - Nuclear import would be affected as nuclear import factors would remain bound to Ran-GTP and not available to facilitate nuclear entry for proteins that are meant to enter the nucleus. This would have significant effects on gene expression and cell division.


• Nuclear import receptor recognizes NLS signal and binds the protein
NLS
Nuclear import receptor
Protein X
GAPs
(GTP hydrolysis)
Ran GTP
Ran GDP
Pi
Cytosol
Nucleus
Nuclear Pore Complex
Ran (GTPase)
• Activated by GTP
• Binds to Nuclear import receptor to release cargo (e.g., Protein X)
Ran GTP
Ran GTP
• Protein X is transported into the nucleus
GEFs

Syllabus Change Update

- Group Activity #3 will now be **next class period** April 8th. This is to allow for an extra opportunity for Office Hours and additional time to review before Exam #3 the week afterwards. Groups will be made on Canvas prior to class and will be found under the “People” tab
- Today’s lecture will be covered on Exam #3, but not the lecture on April 10th

Tues.	April 8	**Group activity #3 (In class)** **Due at the end of class**	Dr. Ellis
Thurs.	April 10	Cell Signaling (Ch16 continued) (start of Exam 4 material-not tested in Exam 3) CH 11-15 Chapter Reading Quizzes due at 11:59pm!	Dr. Ellis
Tues.	April 15	**Exam 3 (In-class)** **Chapters 11-15**	Dr. Ellis



Cell Signaling

BIOL366

Chapter 16 (Part I)

Matthew Ellis, PhD

Learning Objectives for Today's Lecture:

Upon completing this module, **you should be able to**:

- Describe the general principles of cellular **signal transduction**
- Understand the role of **second messengers** in relaying and amplifying signals from receptors
- Appreciate the complexity and interplay of cell signaling using **GPCR** (G Protein Coupled Receptor) and **RTK** (Receptor Tyrosine Kinase) pathways

Key Terms

- **Signal Transduction:** The ability of a cell to respond to ligand-receptor binding by altering its behavior or gene expression
- **Second messenger:** A signaling intermediate often stimulates the production of many molecules needed for the next step
- **Effector protein:** Downstream proteins that carry out the cellular response to a signaling pathway
- **G-protein:** A protein which is activated by the binding of GTP (e.g., Ras, Ran, G_{α})
- **Autophosphorylation:** The process by which a protein phosphorylates itself

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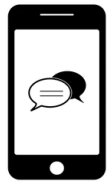
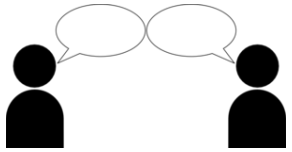
There are multiple different means we use to communicate

Texting 

Calling 

Voice memos 

In-person 

Writing 

Some offer additional cues that are absent in others

- Vocal inflection
- Body language

We may use different styles of communication depending on who we speak to

Depending on our cells needs, they may utilize specific forms of signaling to most effectively and efficiently reach the desired outcome

The basic principle of each method of communication is the same

Texting

Calling

Voice memos

In-person

Writing

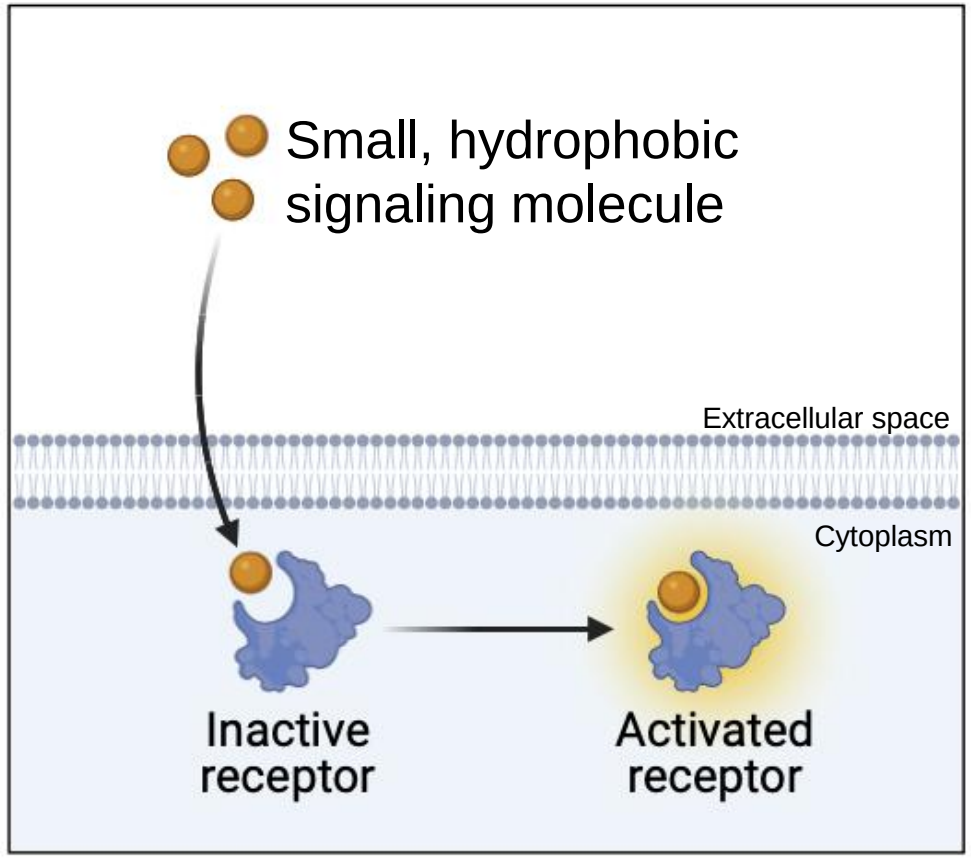
Sender

Transmit information

Receiver

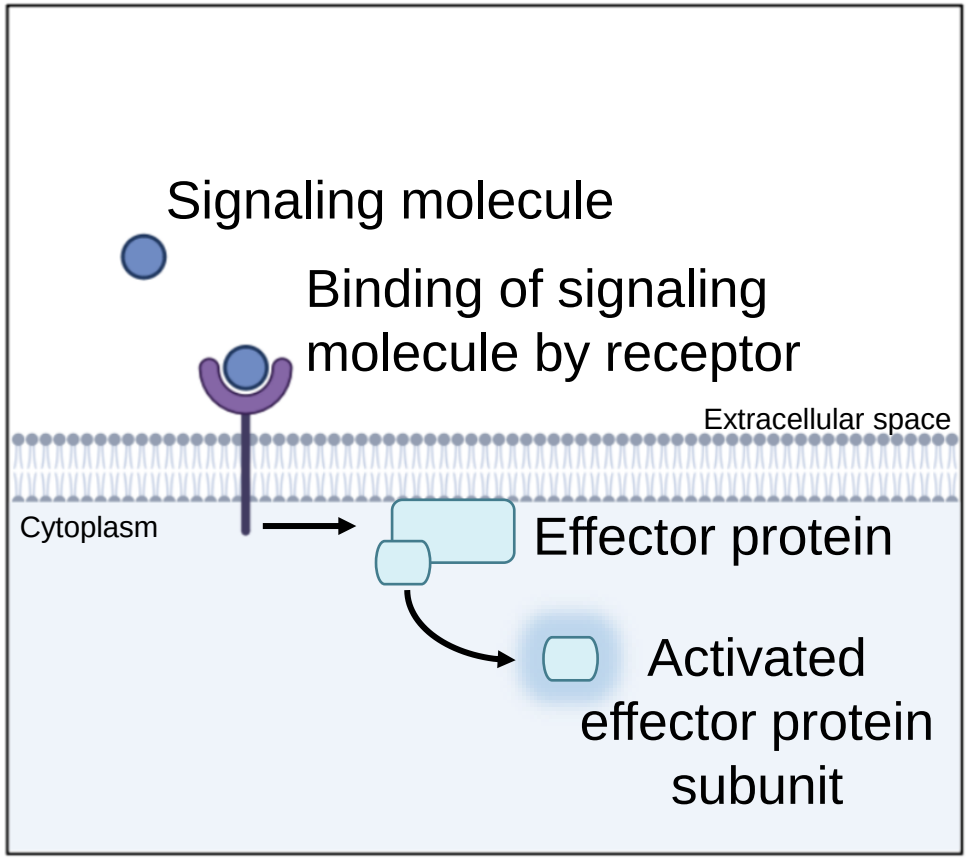
Extracellular signaling molecules can bind either Intracellular or Surface receptors

Intracellular receptors



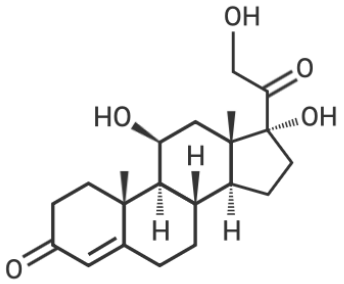
(e.g., steroids)

Surface receptors

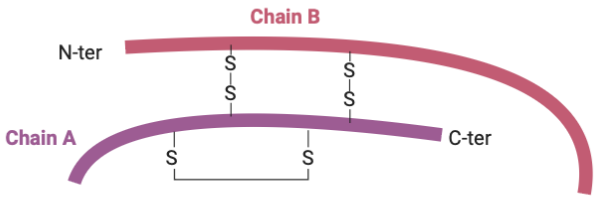


Examples of signaling molecules

Hormones

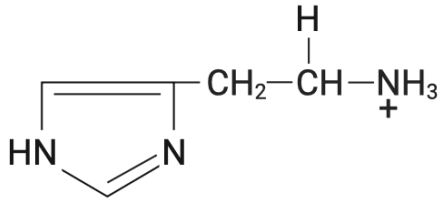


Cortisol



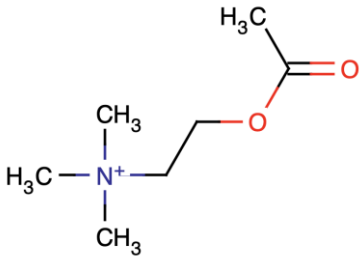
Insulin

Local mediators

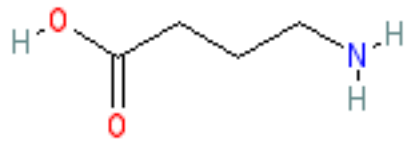


Histamine

Neurotransmitters



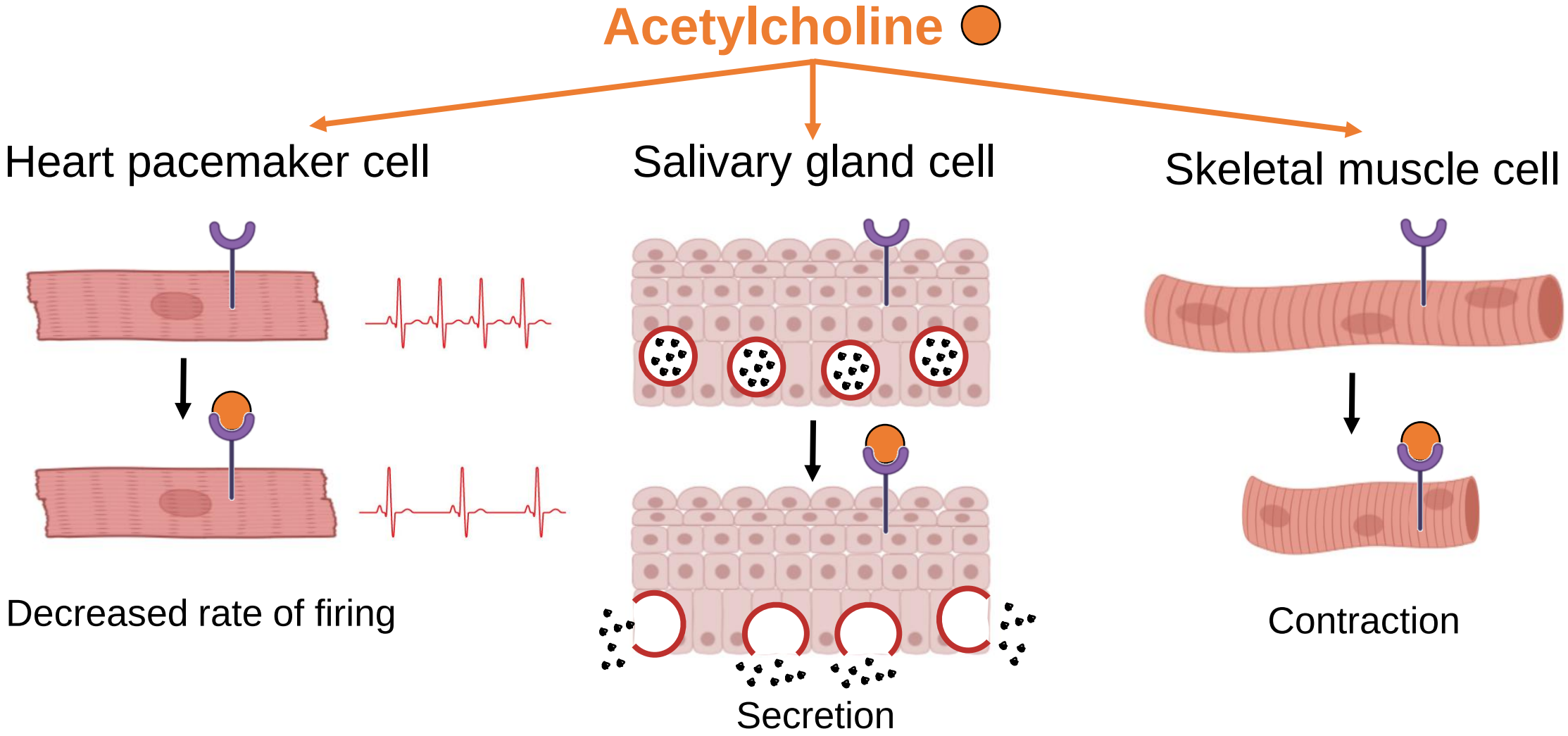
Acetylcholine



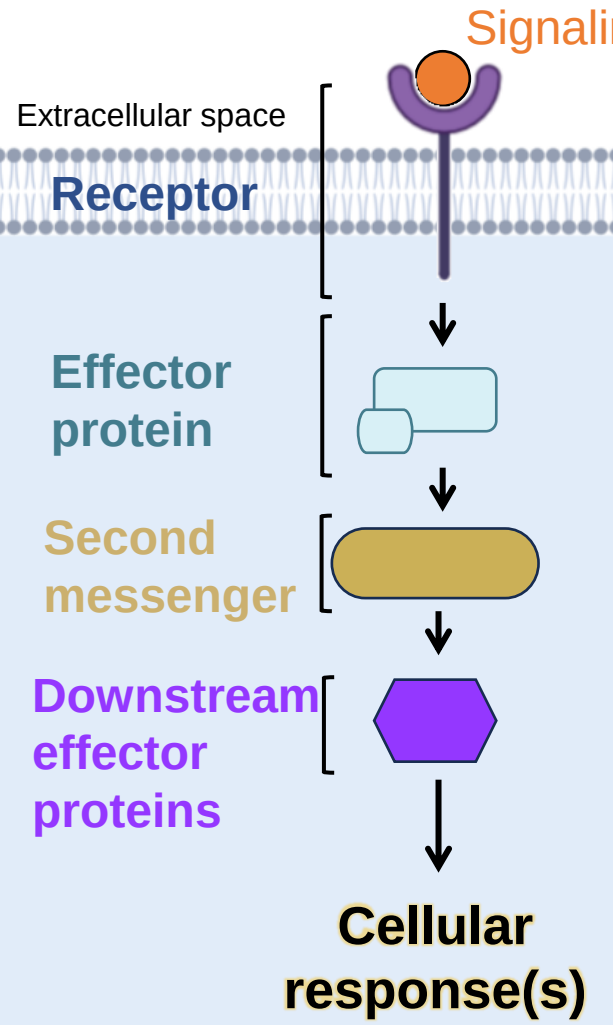
Gamma-aminobutyric acid (GABA)

- Ligand: a molecule that binds to a receptor to send signals within cells
  - **Ligand = signaling molecule = chemical messengers**

A single signaling molecule can induce different responses in different target cells



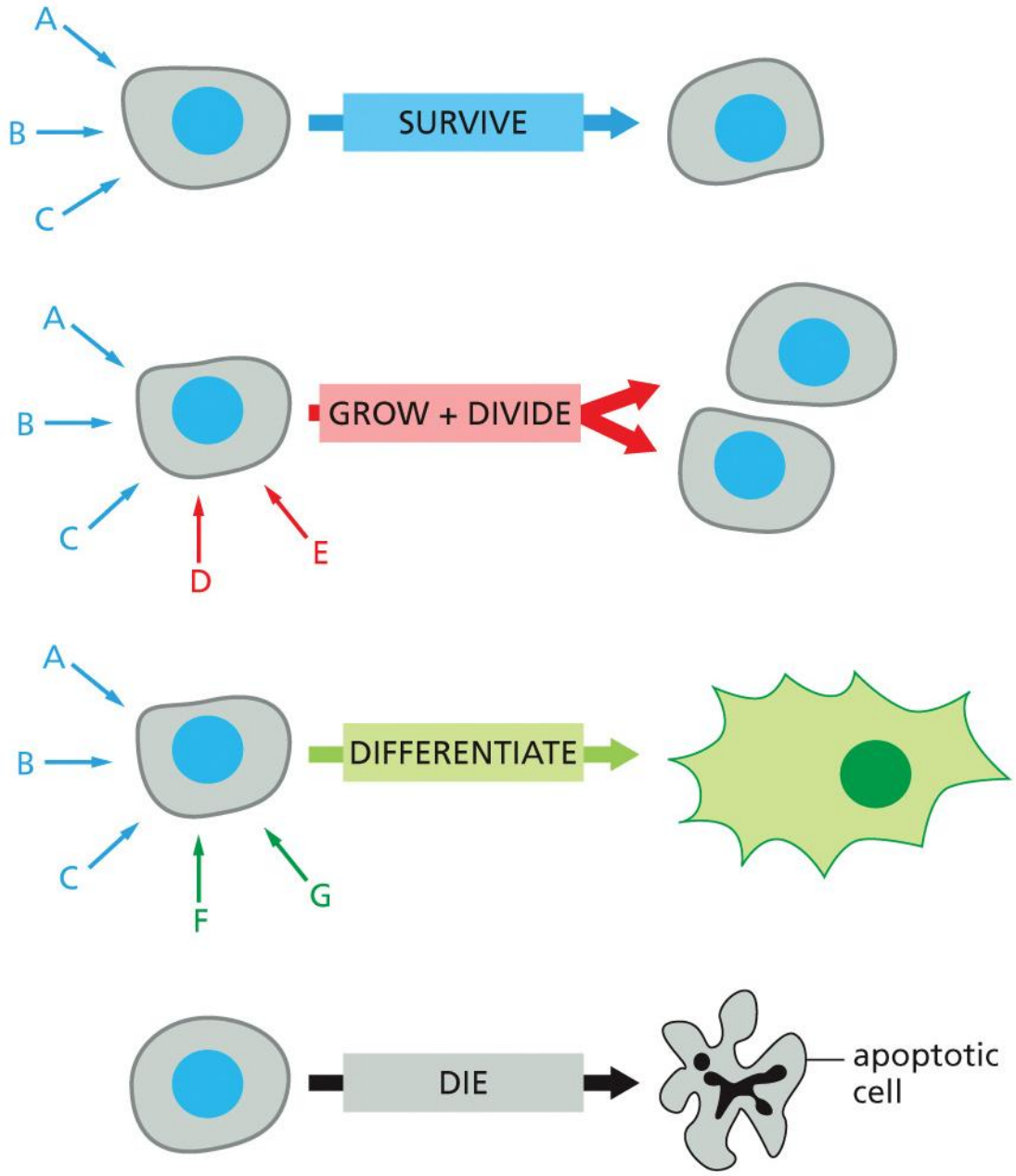
Signal transduction: converting external signals into intracellular responses



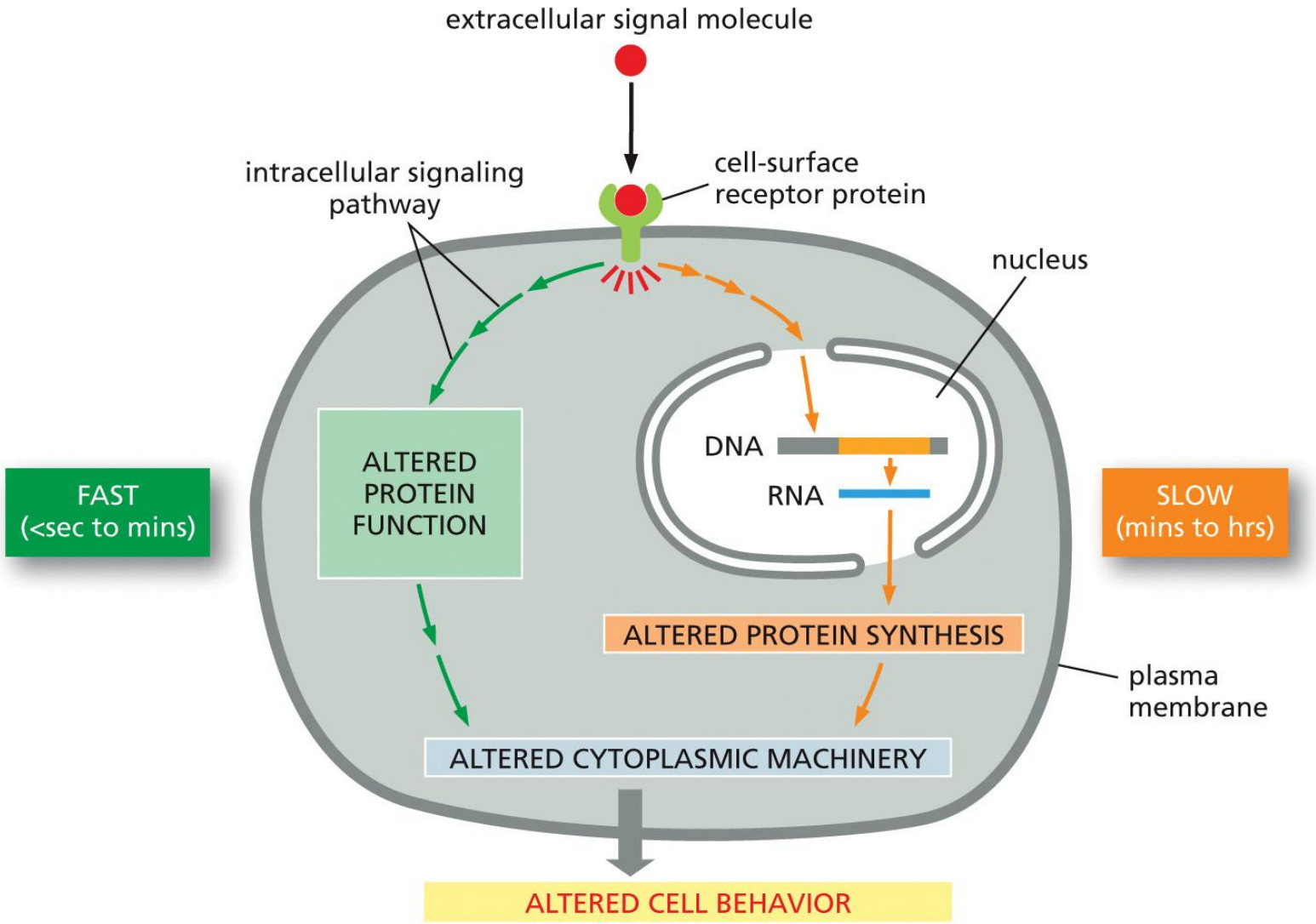
- Effector proteins carry out the cellular response to a signaling pathway
- Second messengers amplify the strength of the signal received from the receptor
  - (e.g. cAMP, IP_3, Ca^{2+})

- Changes in **cellular response(s)** include **altered metabolism**, **gene expression**, or **cell shape and movement**

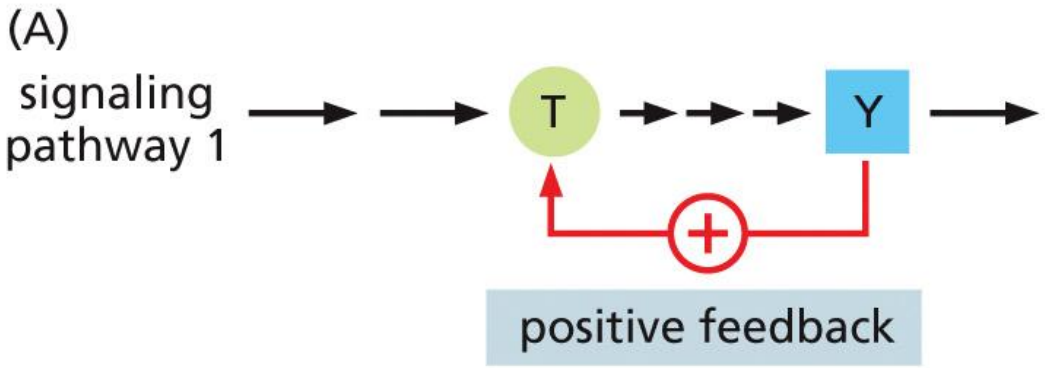
Cellular response depends on multiple extracellular signals

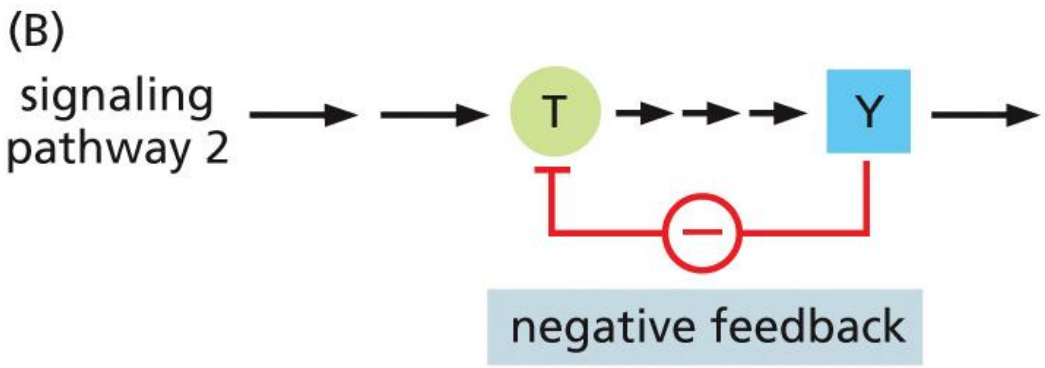


Cell signals can act slowly or rapidly



Feedback regulation adjusts cellular response to signal





Positive feedback occurs when activation of effector protein Y sends a signal upstream to ***activate even more*** of protein Y

Negative feedback occurs when activation of effector protein Y sends a signal upstream to ***inhibit future activation*** of protein Y

Depending on cellular needs at any given time the balance of feedback regulation will shift (i.e., dynamic process)

Squarecap Q#1-2

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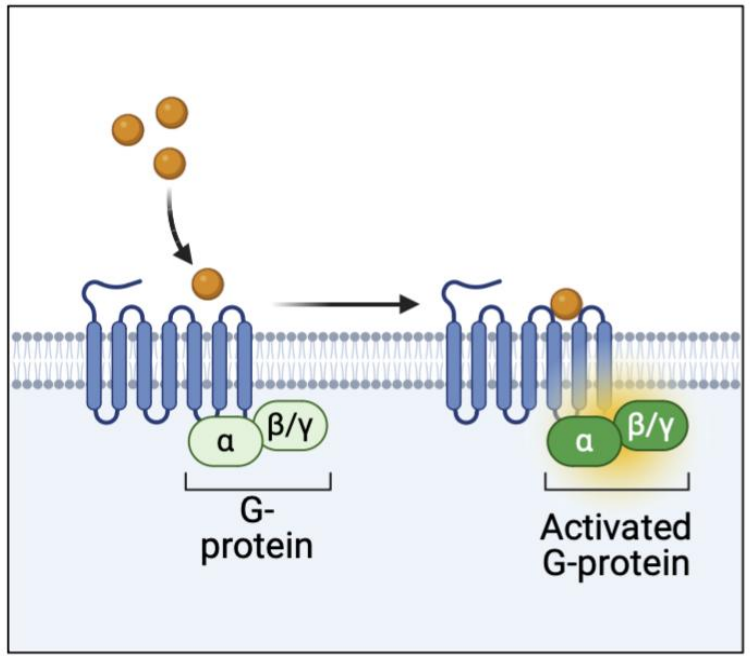
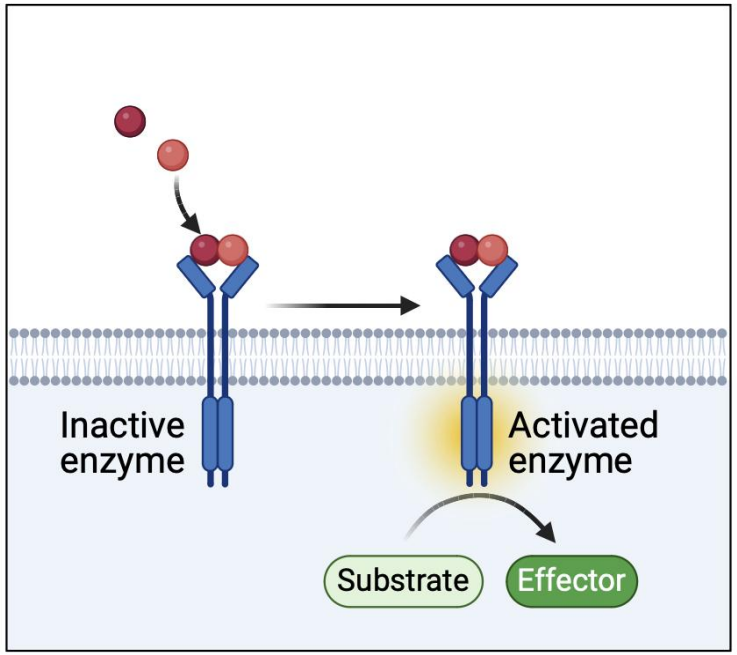
There are 3 main types of cell surface receptors

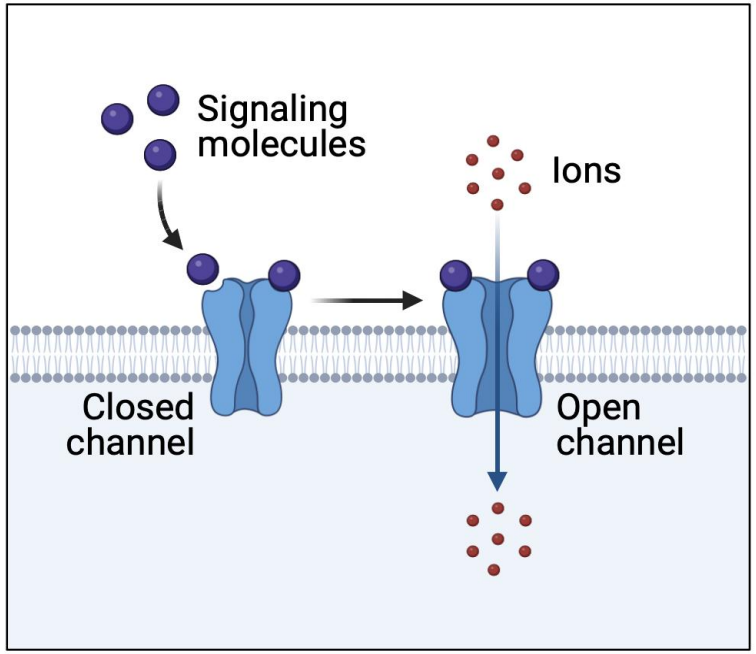
G-protein-coupled receptors



Enzyme-linked receptors



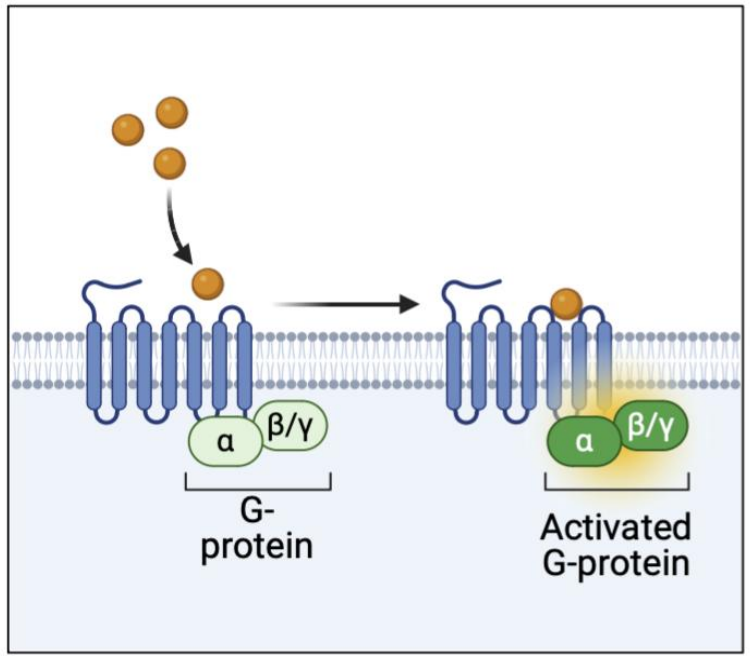
Ion channel-linked receptors



All are activated by signaling molecules (ligands) that trigger intracellular signaling pathways

There are 3 main types of cell surface receptors

G-protein-coupled receptors



Enzyme-linked receptors

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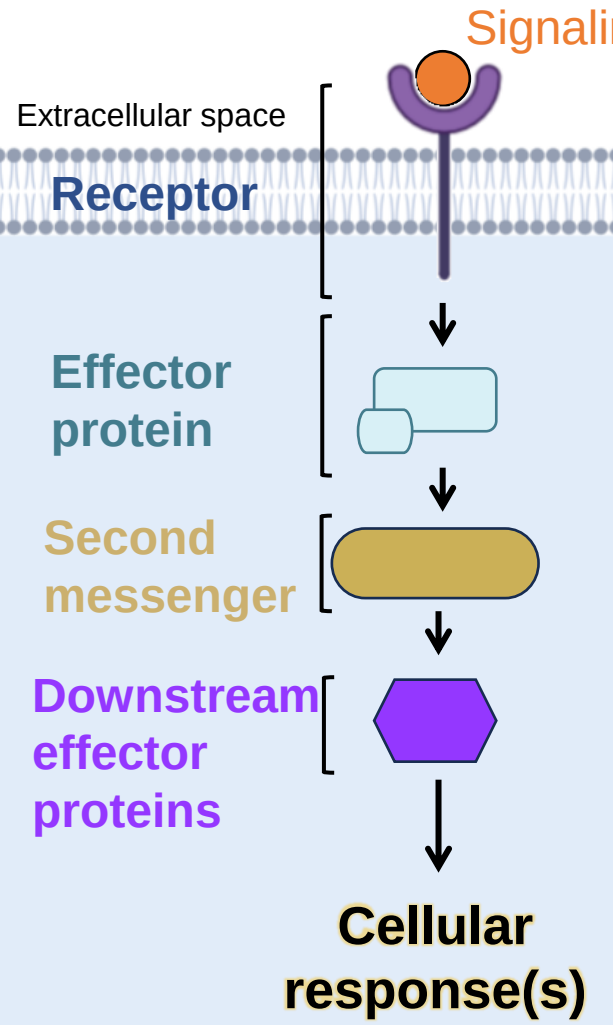
Signal transduction: converting external signals into intracellular responses



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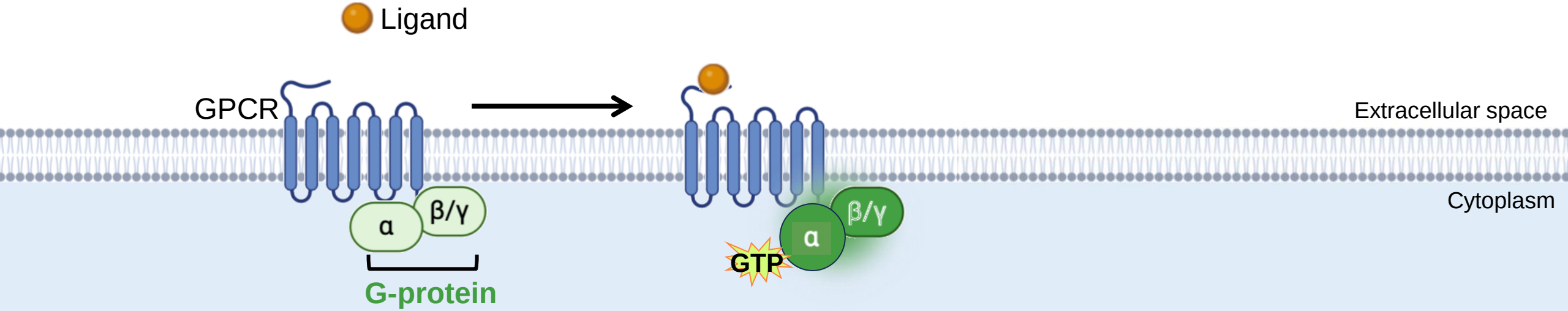
  - (e.g. cAMP, IP_3, Ca^{2+})

- Changes in **cellular response(s)** include **altered metabolism**, **gene expression**, or **cell shape and movement**

G-protein-coupled receptors (GPCRs) are activated by ligands

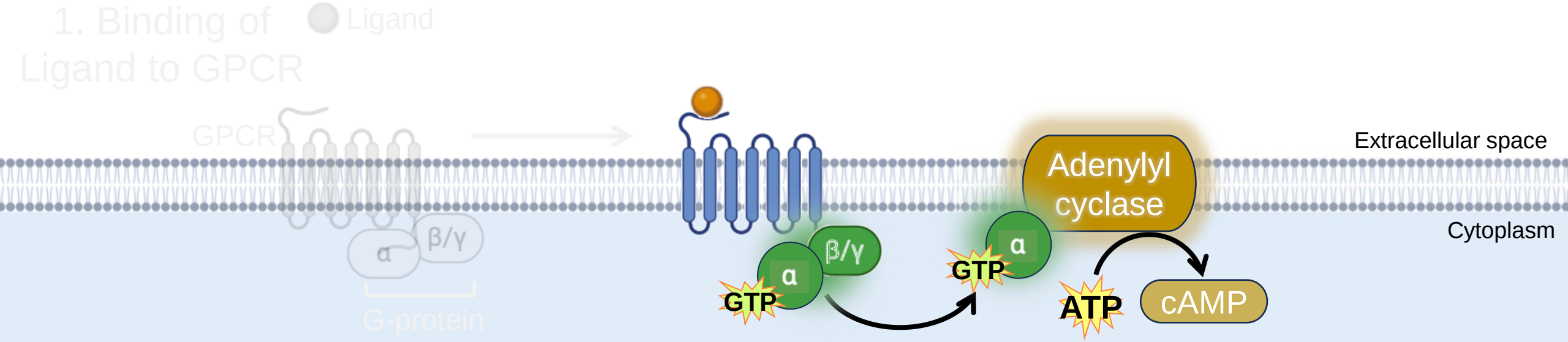
Unbound GPCR



Ligand binding leads to a change in **G-protein** conformation of the **alpha subunit (G*α*)**.

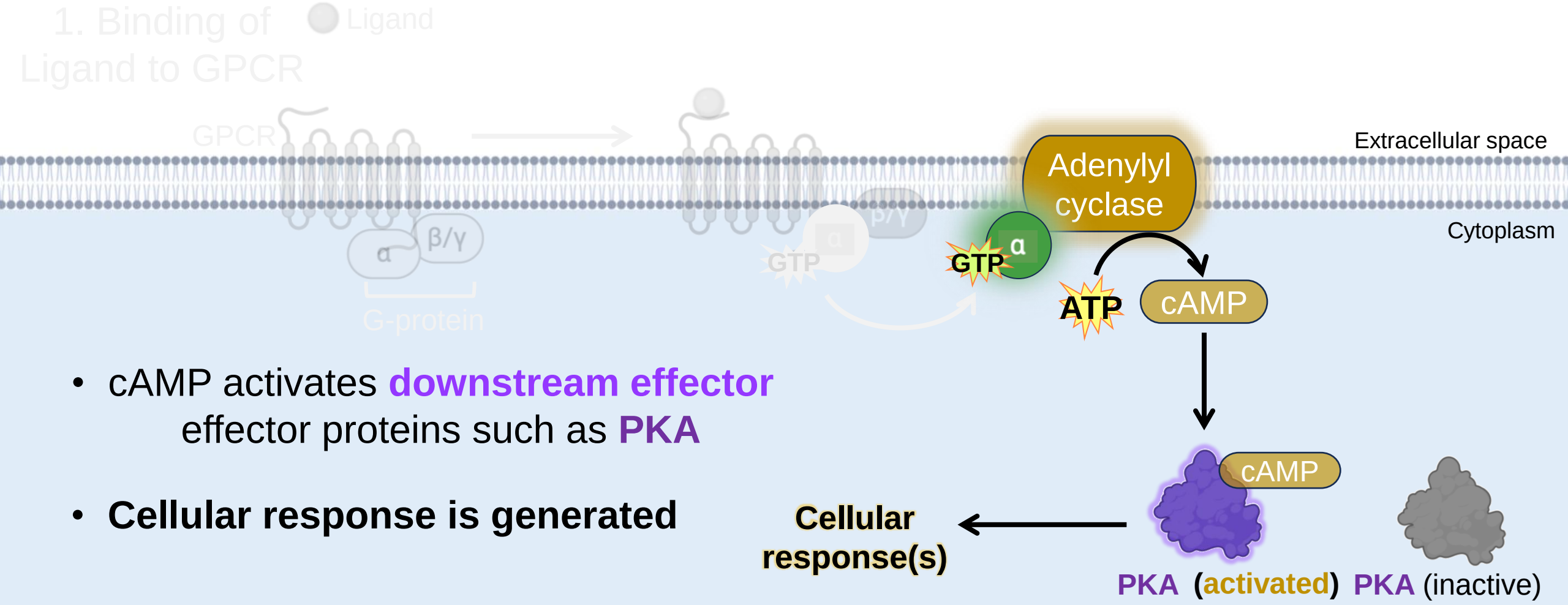
This allows **GTP** to bind **G*α*,** and it becomes **activated**

GPCR activation triggers the production of cyclic adenosine monophosphate (cAMP)



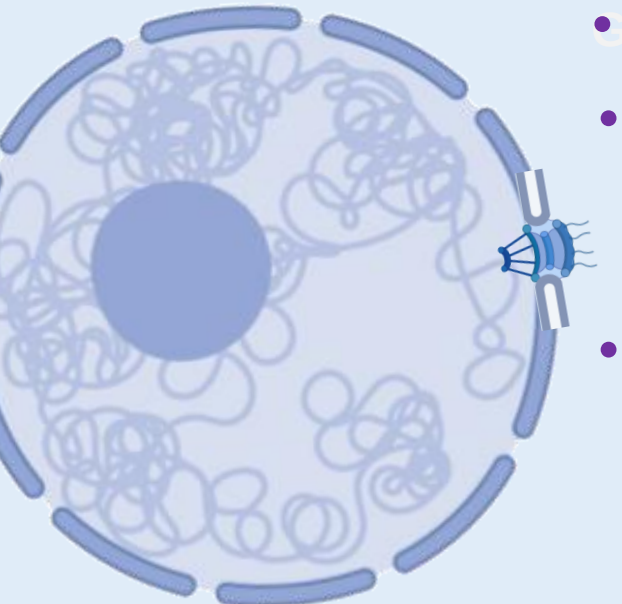
- Activated Gα activates Adenylyl cyclase (effector protein)
- Activated Adenylyl cyclase converts ATP to **cyclic AMP (cAMP)**
- **cAMP is a second messenger molecule**

Production of cAMP activates Protein Kinase A (PKA)

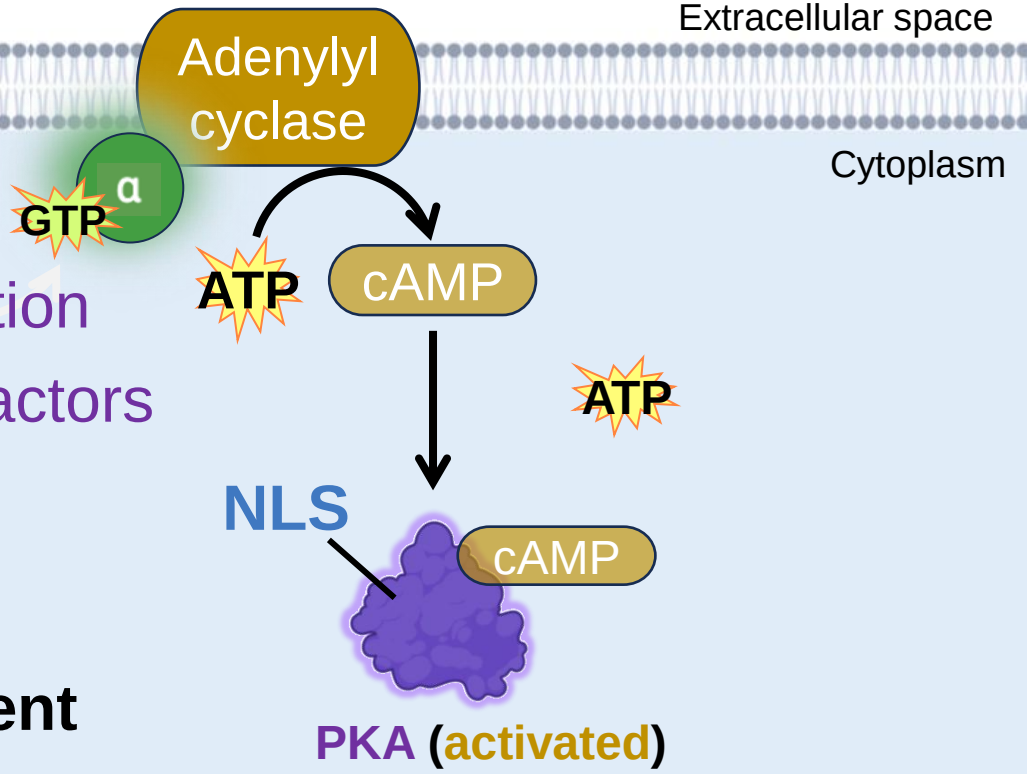


- cAMP activates **downstream effector** effector proteins such as **PKA**
- **Cellular response is generated**

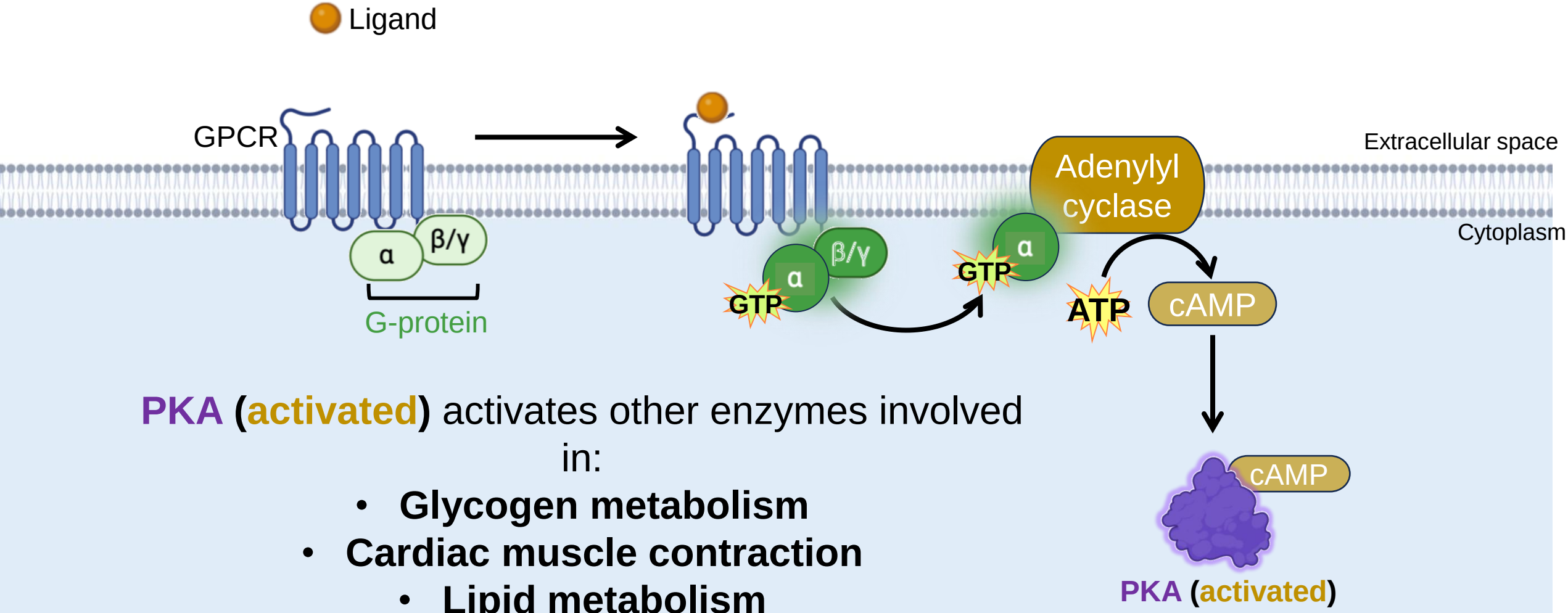
Production of cAMP activates Protein Kinase A (PKA)



- **PKA** is important for Transcription
- Phosphorylates transcription factors (proteins that control gene expression)
- **PKA <u>translocation</u> to the nucleus is primarily dependent on the binding of** cAMP



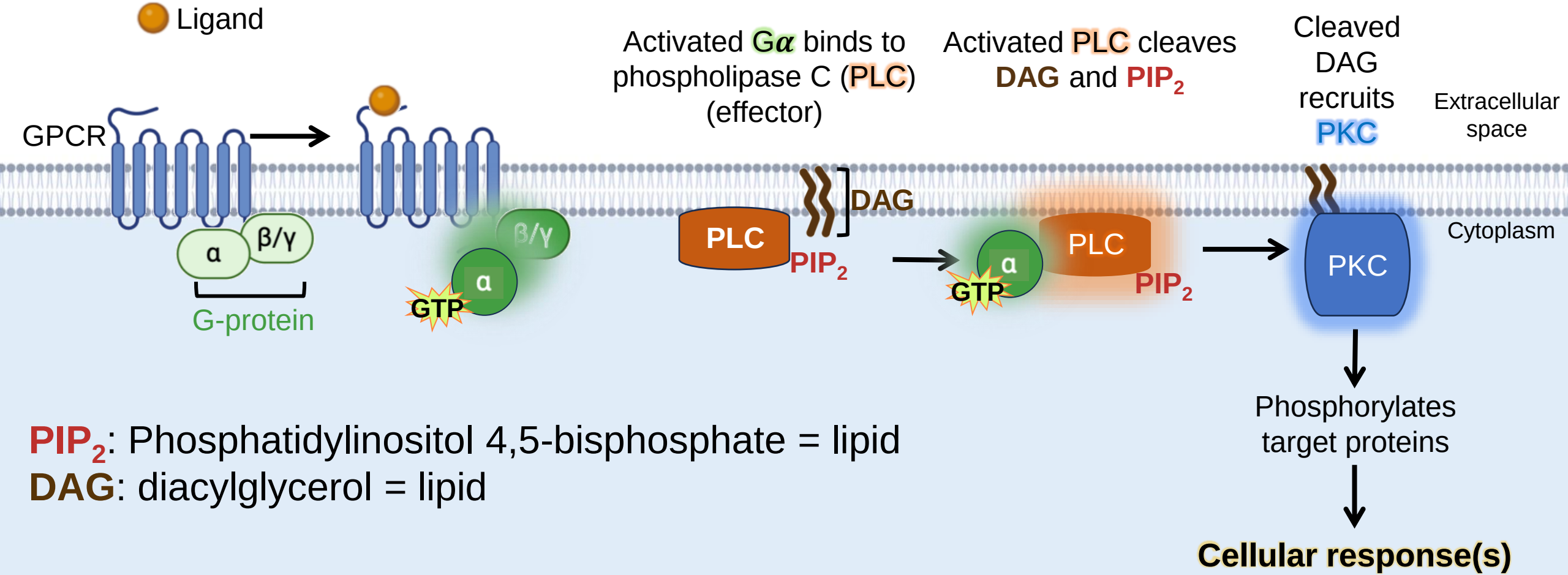
GPCR activation triggers the production of cAMP which activates PKA



PKA (activated) activates other enzymes involved in:

- **Glycogen metabolism**
- **Cardiac muscle contraction**
- **Lipid metabolism**
- **Cell proliferation**

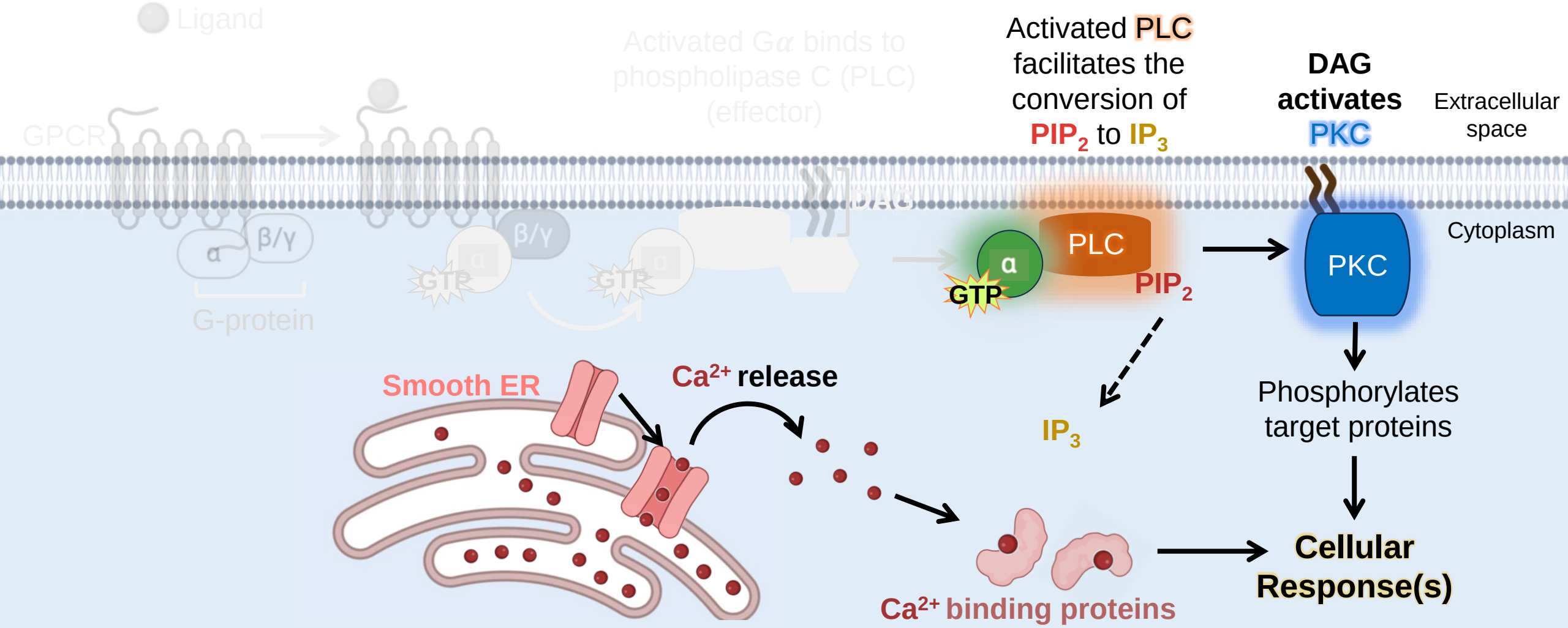
Activation of GPCRs can additionally stimulate Protein Kinase C (PKC) activity



PIP_2: Phosphatidylinositol 4,5-bisphosphate = lipid

DAG: diacylglycerol = lipid

GPCRs stimulate IP_3 to trigger Ca^{2+} release

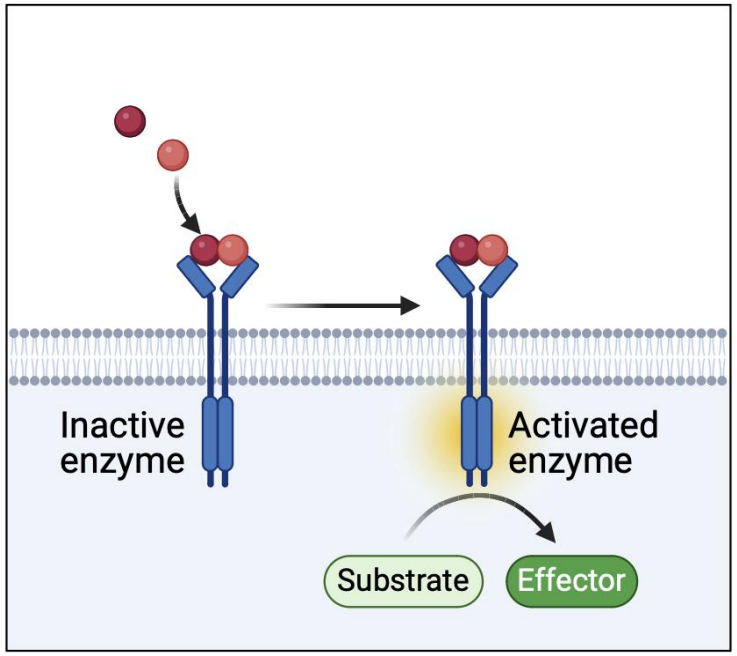


IP_3 = Inositol 1,4,5-trisphosphate; **second messenger**

There are 3 main types of cell surface receptors

G-protein-coupled receptors

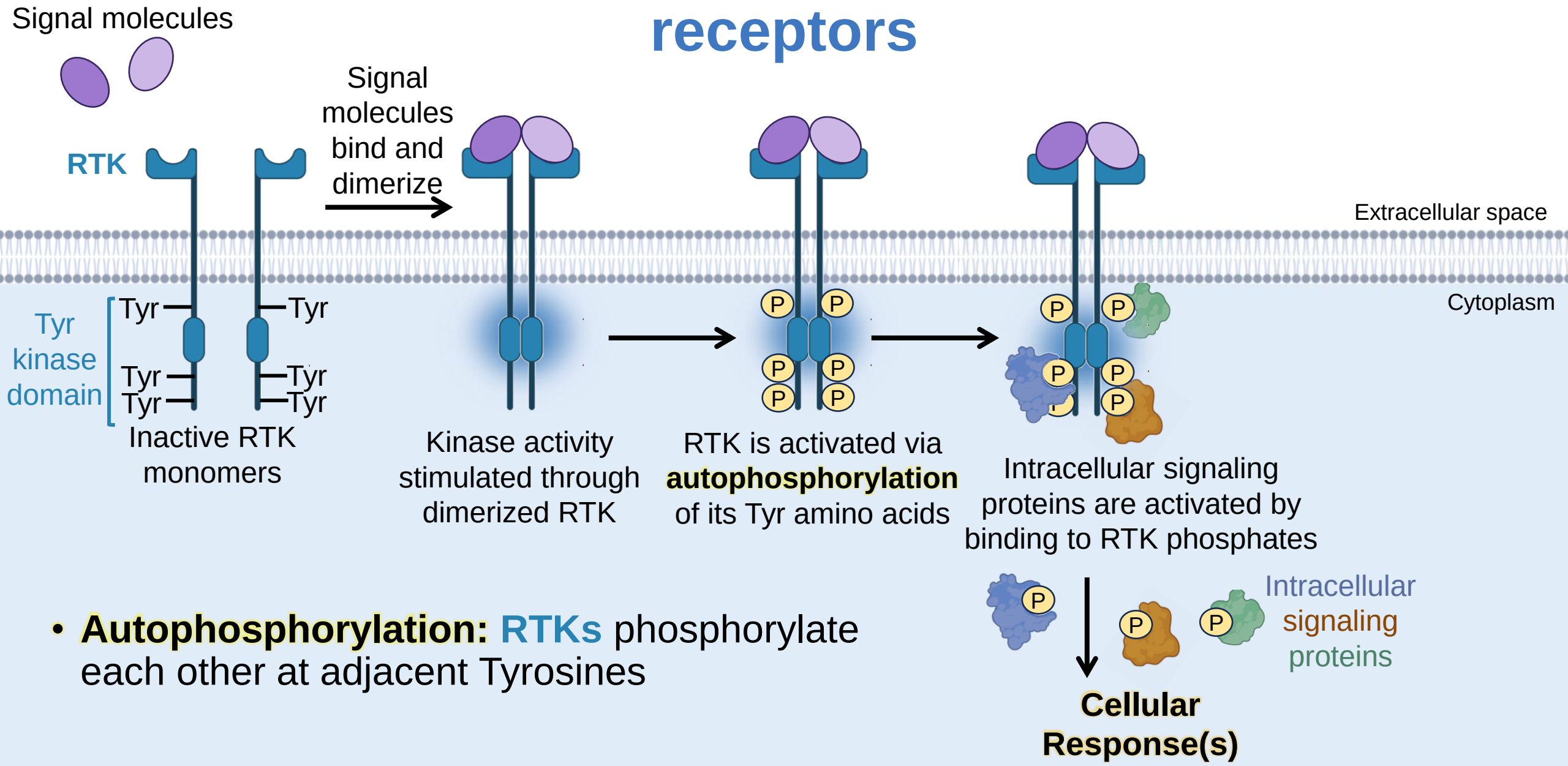
Enzyme-linked receptors



Ion channel-linked receptors

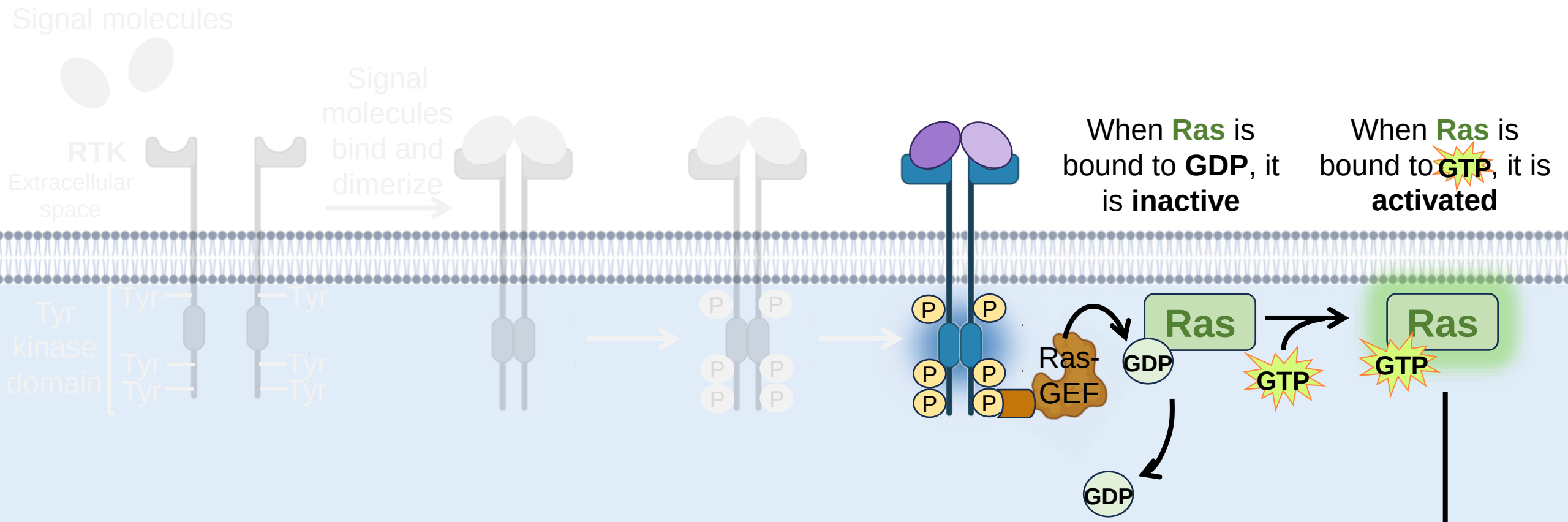
All are activated by signaling molecules (ligands) that trigger intracellular signaling pathways

Receptor tyrosine kinases (RTKs) are enzyme-linked receptors



- **Autophosphorylation:** RTKs phosphorylate each other at adjacent Tyrosines

RTKs recruit and activate the Ras G-protein



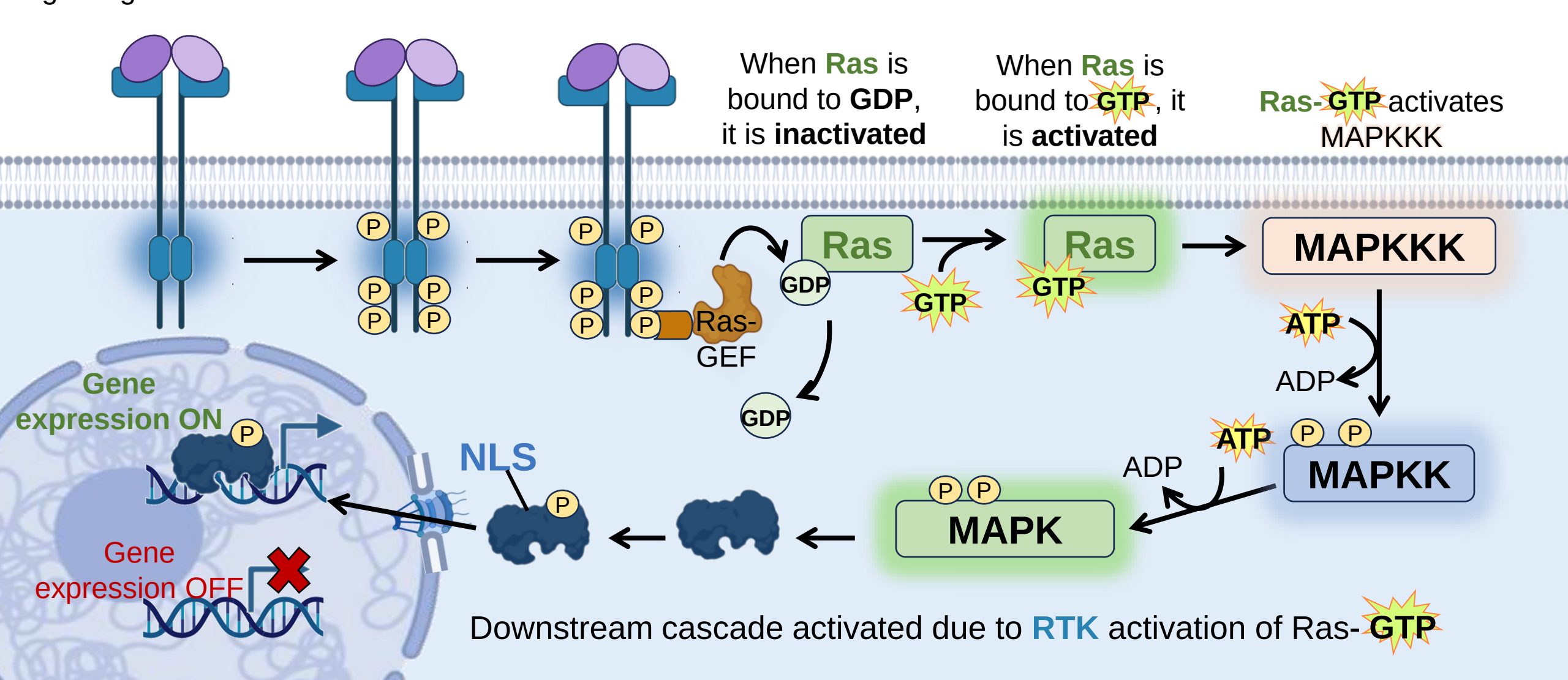
Ras is a GTPase and functions similarly to Ran who we saw in nuclear import

Ras-GEF (GEF = Guanine nucleotide exchange factor) is recruited by phosphorylated **RTK** and removes GDP from **Ras**

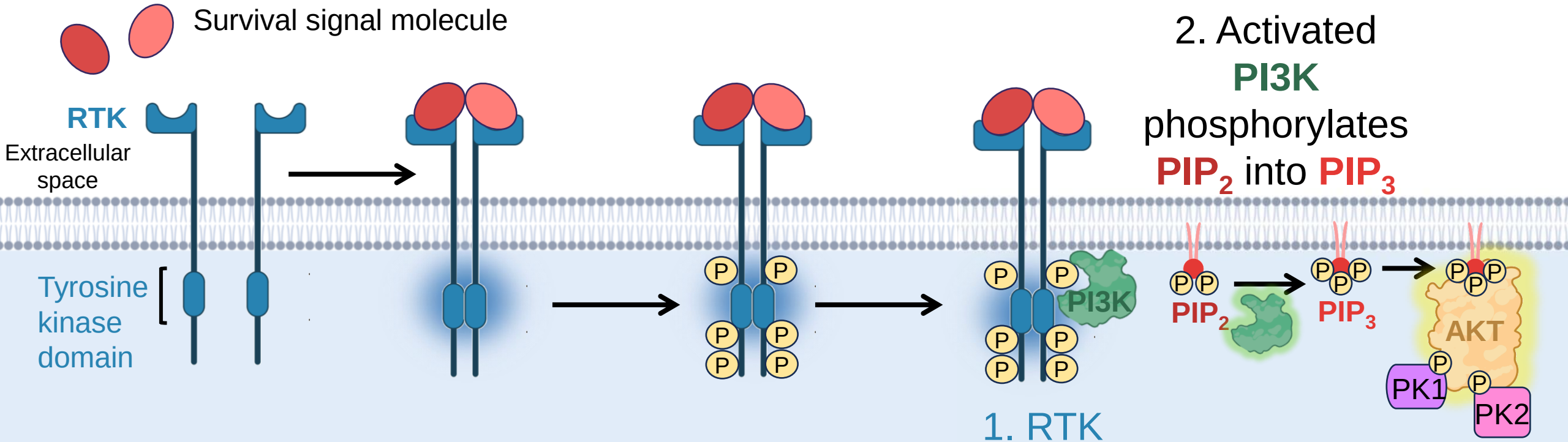
GTP binds to **Ras** → **Ras-GTP**

Ras is considered to be a “molecular switch” in many signaling pathways

Ras-GTP activates the mitogen activated protein kinase (MAPK) pathway to activate gene expression



RTKs activate the PI3K-AKT pathway



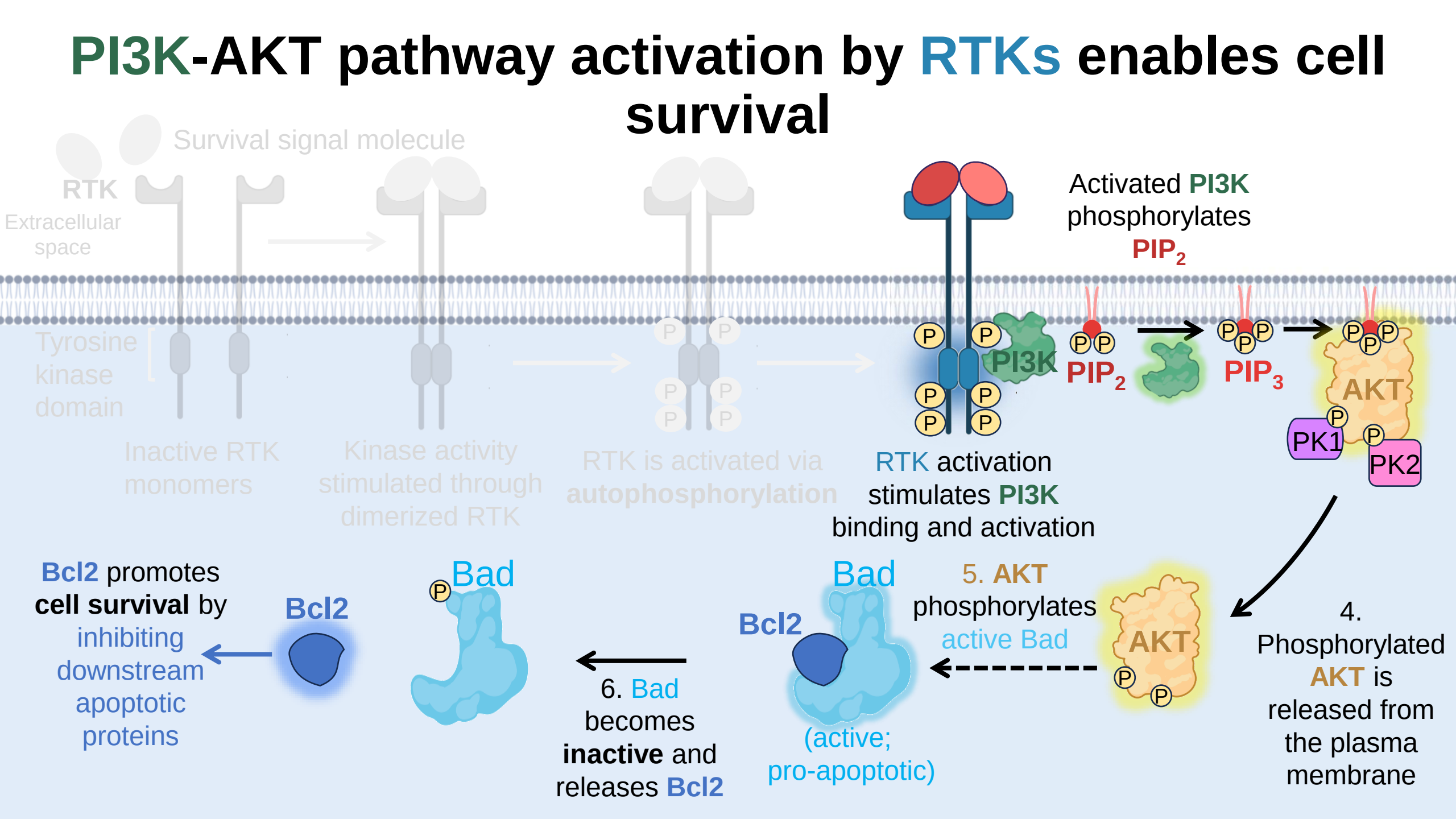
1. RTK activation stimulates **PI3K** binding and activation

2. Activated **PI3K** phosphorylates PIP_2 into PIP_3

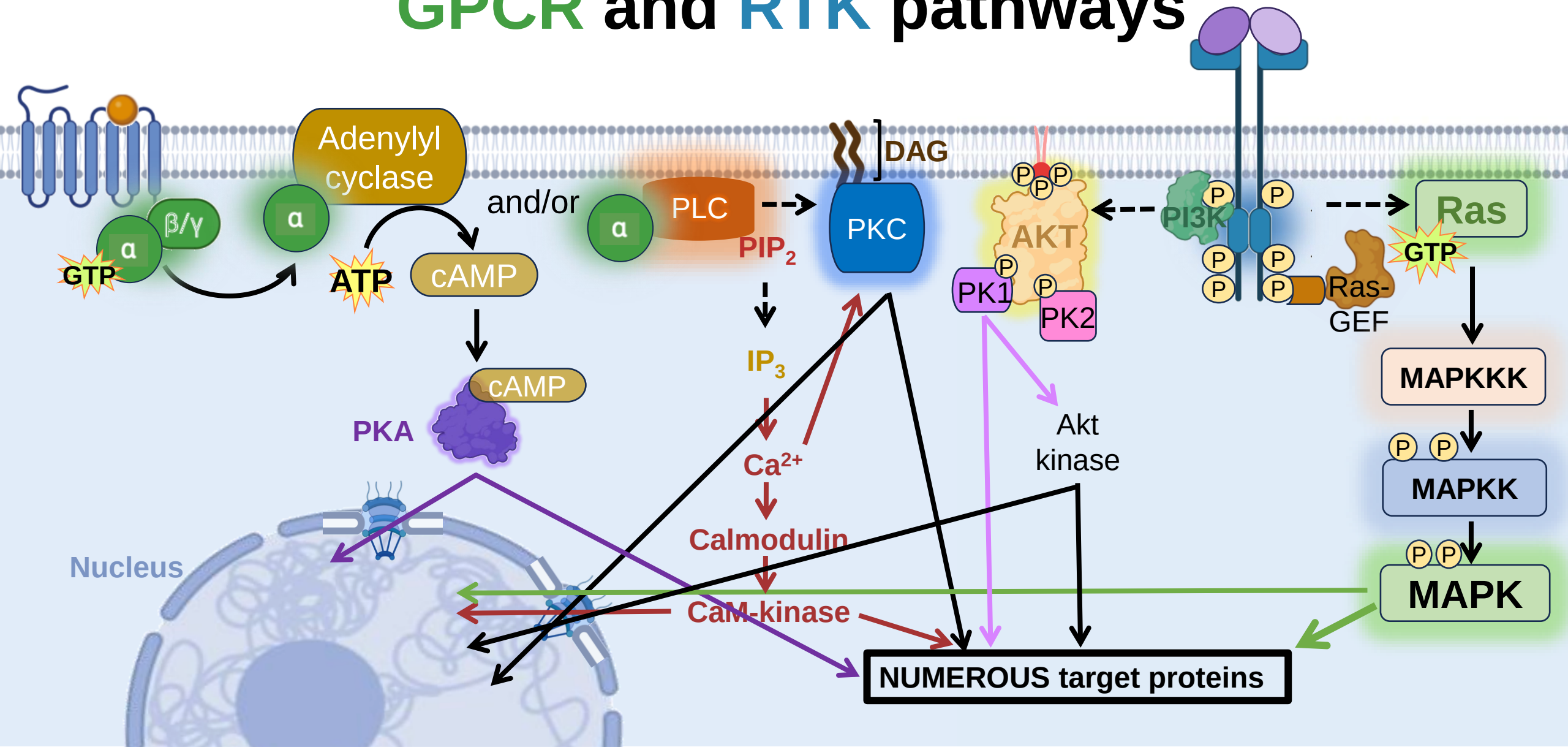
3. **AKT** is recruited to PIP_3 and activated via phosphorylation by PK1 and PK2

- **PI3K** = Phosphoinositide 3-kinase
- PIP_2 = Phosphatidylinositol 4,5-bisphosphate
- PIP_3 = Phosphatidylinositol (3,4,5)-trisphosphate
- **AKT** is also known as Protein Kinase B

PI3K-AKT pathway activation by RTKs enables cell survival



Molecular crosstalk occurs downstream of GPCR and RTK pathways



Squarecap Q#3-4

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Metacognitive Reflection Form