SEASON 2

Understanding Cancer

Lecture 9

Types of signalling pathway: normal and dysregulated PLC-γ1-PKC

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RECAP:

What you hopefully should understand so far from Lecture 8

- Following activation of EGFR or TKR, PI3K is activated.
- PI3K consists of two subunits: p110-alpha catalytic subunit and p85 regulatory subunit.
- p110 subunit produces the second messenger Phosphatidylinositol (3,4,5)-trisphosphate (PIP_3).
- AKT is a serine-threonine kinase that is activated by PDK1 and mTORC2 effector protein.
- mTORC1 signalling cascade is activated by phosphorylated AKT.
- RAS can bind to PI3K by PI3K p110 subunit.
- Inhibitors of AKT or mTOR increases expression and activity of growth factor receptors. This increases PI3K activity and RAS signalling. This correlates with advanced stage of the disease and/or poor prognosis.
- Matrix metalloproteinases (MMPs) proteolytic enzymes are regulated by AKT.

  AKT expression knockdown affects mTOR. This downregulates mRNA of MMP2 and MMP9.

  This effects invasion and metastasis

What will we learn today?

- *The structure of phospholipase C (PLC)*
- *Normal PLC-γ1-PKC signalling pathway: Receptor activation*
- *Normal PLC-γ1-PKC signalling pathway: Signal transduction*
- *Other types of signal transduction: phospholipase D (PLD).*
- *Normal PLC-γ1-PKC signalling pathway: Cellular response*
- *The link between GPCR and PLC-γ1-PKC signalling pathways*
- *Causes of dysregulated PLC-γ1-PKC signalling pathway in cancer*

GENTLE REMINDER
An ideal way of learning:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

Mini-lectures.

Approximate total time: 1 hour

Divide over 7 days at your **own pace**.

Challenge yourself with a quiz!

HN

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RECAP: How to support your learning?

- **Key facts with diagrams by HN designs presented in a simplified way.**
- **Glossary to help understand what key words mean.**
- **Summary doodle revision posters by HN designs.**
- **Quizzes to test your knowledge and reflect.**
- **Reference list for further reading.**

Acknowledgements: Special thanks to my parents, family, friends and colleagues for their support and the respected teachers and health professions who taught me and installed the passion of cancer/oncology.

The structure and function of phospholipase C (PLC)

The structure of phospholipase C (PLC)

- There are **13 isozymes of PLC in mammals** that are encoded by **different genes.**
- They have been divided into 6 families based on **structure, sequence and function.**
- They are **key signalling proteins** in response to many molecules.

Hormones

Growth factors

Neurotransmitters

Extracellular stimuli

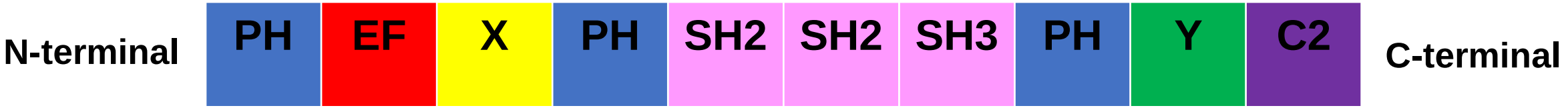
The structure of phospholipase C (PLC)

PLC isozyme	Subtypes	Function
PLCβ	1,2,3,4	• **Cardiovascular and neuronal signaling.** • **PLCβ2 negatively regulates iinflammatory responses induced by viruses. This prevents phosphoinositide from activating Transforming growth factor-β-activated kinase 1 (TAK1).** • **TAK1 is a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family.** • **TAK1 plays a part of NF-κB, c-Jun N-terminal kinase (JNK), and p38 pathways.**

Name of PLCβ domain	Abbreviation	Function
pleckstrin homology	PH	Regulatory protein domain involved in intracellular signalling.
EF		A regulatory domain or motif with a helix–loop–helix structure that bind to calcium.
X		Catalytic domain
Y		Catalytic domain
C2		A protein structural domain involved in targeting proteins to cell membranes.
PDZ-Binding Motifs	PBMs	Scaffolding protein Ion channels and proteins clustered together at synapses in the brain, photoreceptors and epithelial cells.

The structure of phospholipase C (PLC)

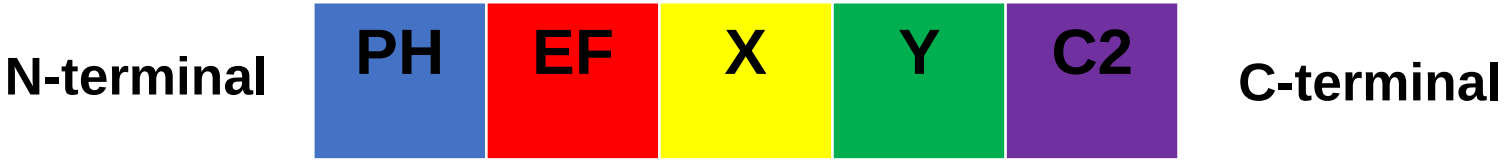
PLC isozyme	Subtypes	Function
PLCγ	1 and 2	• **Effector enzymes for membrane-bound receptors in signal transduction pathways.** **e.g.** • **It hydrolyses phosphatidylinositol-4, 5-bisphosphate (PI(4,5)P2) (PIP2) phospholipid in the membrane to produce the two second messengers:** • **Diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3).** • **Both stimulate the enzyme protein kinase C (PKC) for downstream signalling.** **PLC-γ1, the protein encoded by *Plcg1.***



Name of PLC-γ domain	Abbreviation	Function
pleckstrin homology	PH	Regulatory protein domain involved in intracellular signalling.
EF		A regulatory domain or motif with a helix–loop–helix structure that bind to calcium.
X		Catalytic domain
SH2		Interacting domains for tyrosine in receptor tyrosine kinase
SH3		It forms complex with other proteins which facilitates signal transduction.
Y		Catalytic domain
C2		A protein structural domain involved in targeting proteins to cell membranes.

The structure of phospholipase C (PLC)

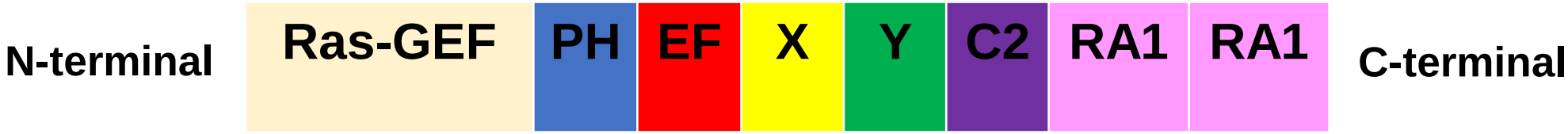
PLC isozyme	Subtypes	Function
PLCδ	**1, 3 and 4**	**It is sensitive to calcium and activated by small increases of calcium concentration only.**



Name of PLC-δ domain	Abbreviation	Function
pleckstrin homology	PH	Regulatory protein domain involved in intracellular signalling.
EF		A regulatory domain or motif with a helix–loop–helix structure that bind to calcium.
X		Catalytic domain
Y		Catalytic domain
C2		A protein structural domain involved in targeting proteins to cell membranes.

The structure of phospholipase C (PLC)

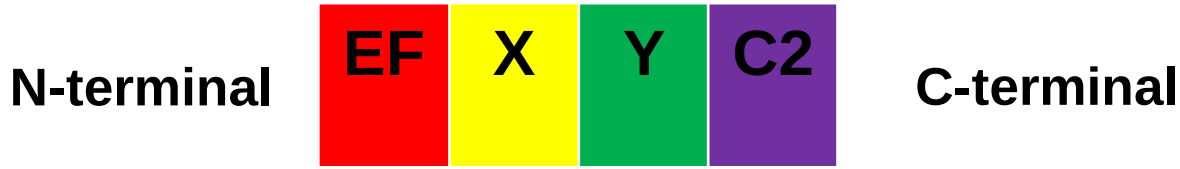
PLC isozyme	Subtypes	Function
PLCε	**1**	• **It is involved in the downstream-signalling events of G-protein coupled receptors (GPCRs).** • **They bind with RhoA and Rap1 to regulate PLCε activity.** • **Rap1 binds via its RA2 domain.**



Name of PLCε domain	Abbreviation	Function
Ras guanine nucleotide exchange factor	Ras-GEF	It is a domain found in the CDC25 family of guanine nucleotide exchange factors for Ras-like small GTPases I.e Rap1A GTPase. Ras bind to GTP and GDP and can slowly hydrolyse GTP to GDP.. Ras-GEF is encoded by a Toll-like receptor (TLR)-inducible gene TLR are expressed by macrophages to engulf pathogens. Hydrolysis of phosphatidylinositol (PI) at the space between the membranes (perinuclear) and Golgi membranes in heart muscle cells (cardiomyocytes)
pleckstrin homology	PH	Regulatory protein domain involved in intracellular signalling.
EF		A regulatory domain or motif with a helix–loop–helix structure that bind to calcium.
X		Catalytic domain
Y		Catalytic domain
C2		A protein structural domain involved in targeting proteins to cell membranes.
RA1		Ras association domains to interact with muscle-specific A-kinase anchoring protein (mAKAP) at the perinuclear membrane, and bind to activated Rap1A and Ras proteins.

The structure of phospholipase C (PLC)

PLC isozyme	Subtypes	Function
PLCζ	**1**	• **It is a sperm protein.** • **It triggers calcium ions that move back and forth (oscillations) in the eggs starts embryogenesis.**



Name of PLCζ domain	Abbreviation	Function
EF		A regulatory domain or motif with a helix–loop–helix structure that bind to calcium.
X		Catalytic domain
Y		Catalytic domain
C2		A protein structural domain involved in targeting proteins to cell membranes.

The structure of phospholipase C (PLC)

PLC isozyme	Subtypes	Function
PLCη	1 and 2	• It is the most sensitive type of PLC to calcium. • It is important during the formation and maintenance neurons in the brain postnatal (after birth).

Name of PLCη domain	Abbreviation	Function
pleckstrin homology	PH	Regulatory protein domain involved in intracellular signalling.
EF		A regulatory domain or motif with a helix–loop–helix structure that bind to calcium.
X		Catalytic domain
Y		Catalytic domain
C2		A protein structural domain involved in targeting proteins to cell membranes.
PDZ-Binding Motifs	PBMs	Scaffolding protein Ion channels and proteins clustered together at synapses in the brain, photoreceptors and epithelial cells.

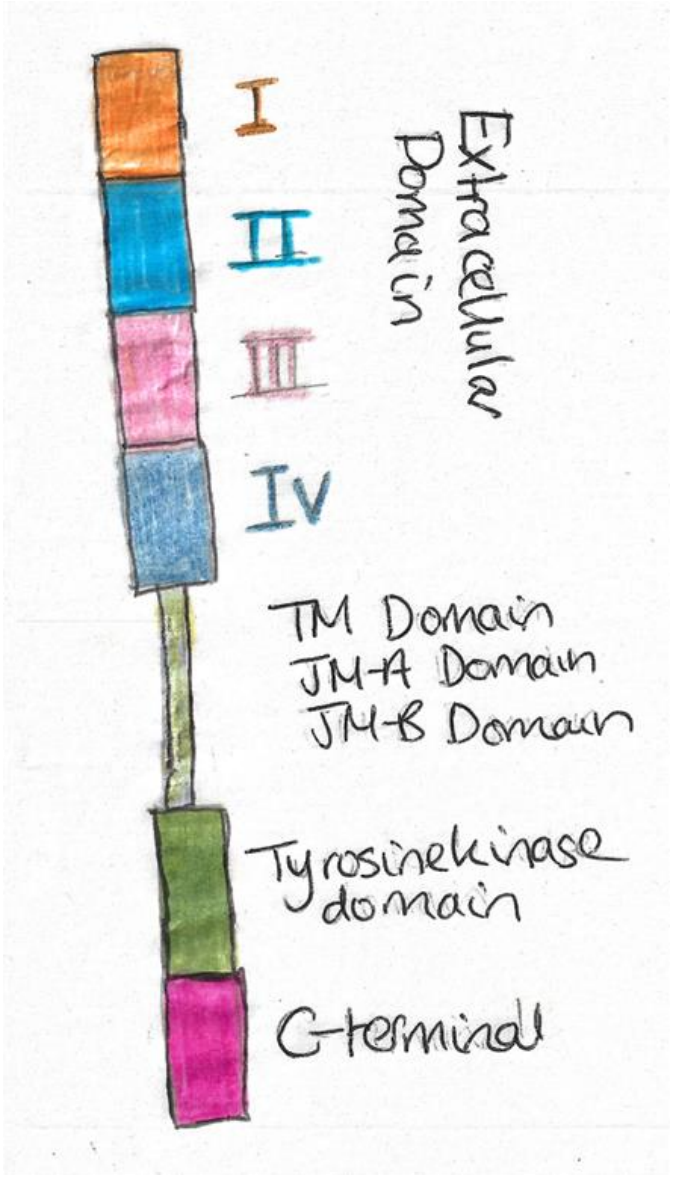
Overview

All PLCs have the following:

- **EF-hand domain**
- **C2 domain**
- **Catalytic X and Y autophosphorylation linkage domain**

Normal PLC-γ1-PKC signalling pathway: Receptor activation

RECAP: The structure of EGFR receptor.



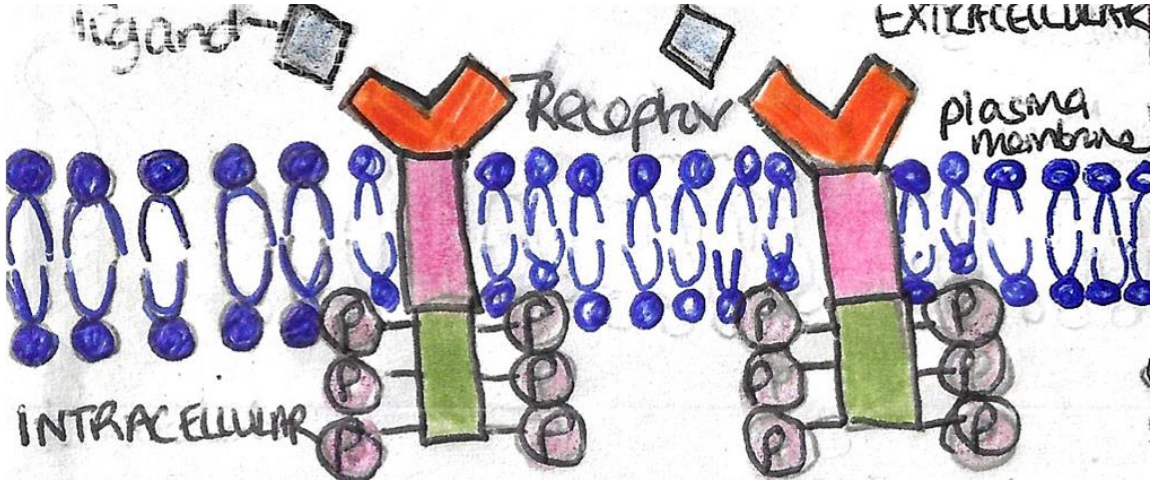
Domain	Function
I	It is rich with leucine residues and binds to the ligand.
II	It is rich with cysteine residues and contains a dimerization arm that interacts with another dimerization arm of another receptor to form a homodimer. This helps maintain EGFR signalling but does not make contact with the ligand.
III	It is rich with leucine residues and binds to the ligand.
IV	It is rich with cysteine residues and can form disulfide bonds to domain II, and links to the transmembrane domain. It does not make contact with the ligand.
Transmembrane	It firmly attaches the receptor to the membrane and is involved in the dimerization process.
C-terminal tyrosine kinase domain.	❑ It has lots of tyrosine residues involved in phosphorylation (the addition of the phosphate group) ❑ It has lots of lysine residues involved in ubiquitination (Ubiquitin is a small protein that directs proteins to the proteosome where proteins are degraded)

Step 1

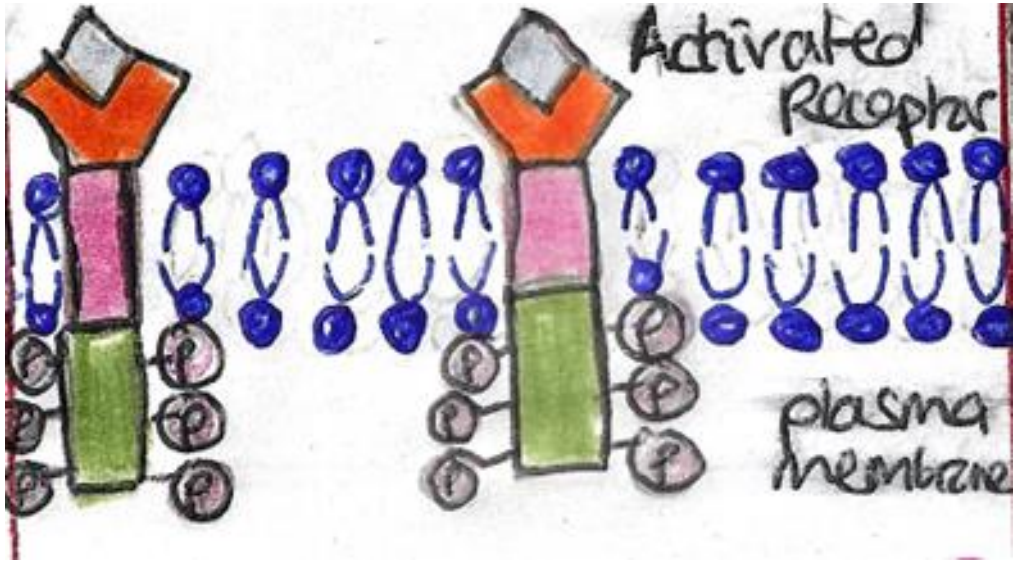
The binding of the ligand to the EGFR receptor.

- The **extracellular domain domains (I and III) to interact and bind with the ligand when** the **extracellular domain (II and IV)** are **pushed away.**

- **Each ligand specifically binds to a particular receptor.**



- The most common ligand is **Epidermal Growth Factor (EGF)** a growth factor **protein** is predominantly found in the **heart, gut (intestines), brain, teeth, reproductive tracts and eyes.**
- **It is involved in proliferation and differentiation.**



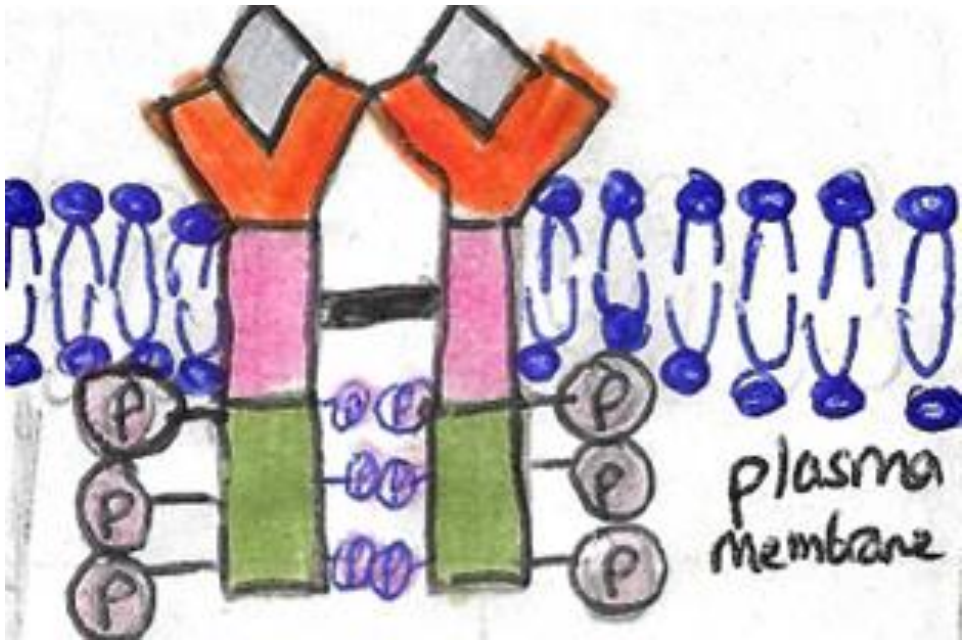
Step 2

Dimerization of the receptor.

The **dimerization arm in the extracellular domain II** interacts with another **dimerization arm of another to form a homodimer.**

Heterodimerisation between family members e.g.

EGF can induce heterodimerization of EGFR with HER2, HER3 or HER4.



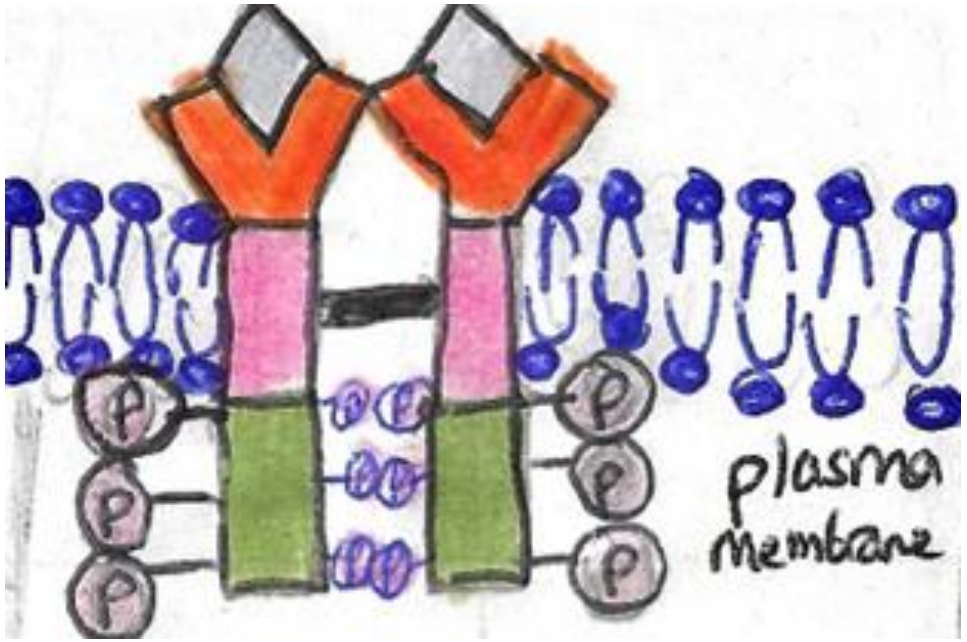
Step 3

Receptor transautophosphorylation of C-terminal domain

The **cytoplasmic domain of the intracellular region of one EGFR (N-lobe)** contains **tyrosine residues** which **phosphorylates the cytoplasmic domain of the intracellular region of the other EGFR (C-lobe).**

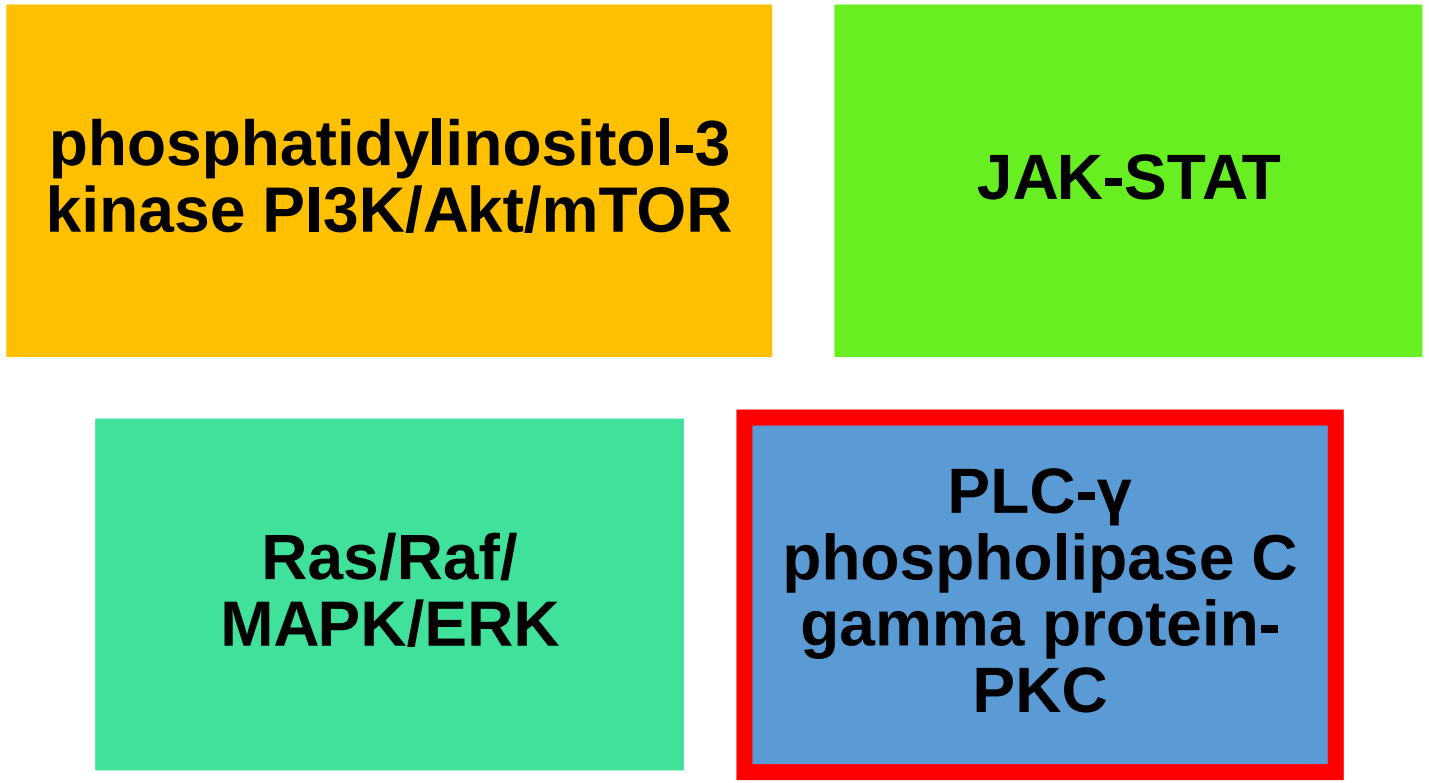
This is known as

Transautophosphorylation.



Normal PLC-γ1-PKC signalling pathway: Signal transduction

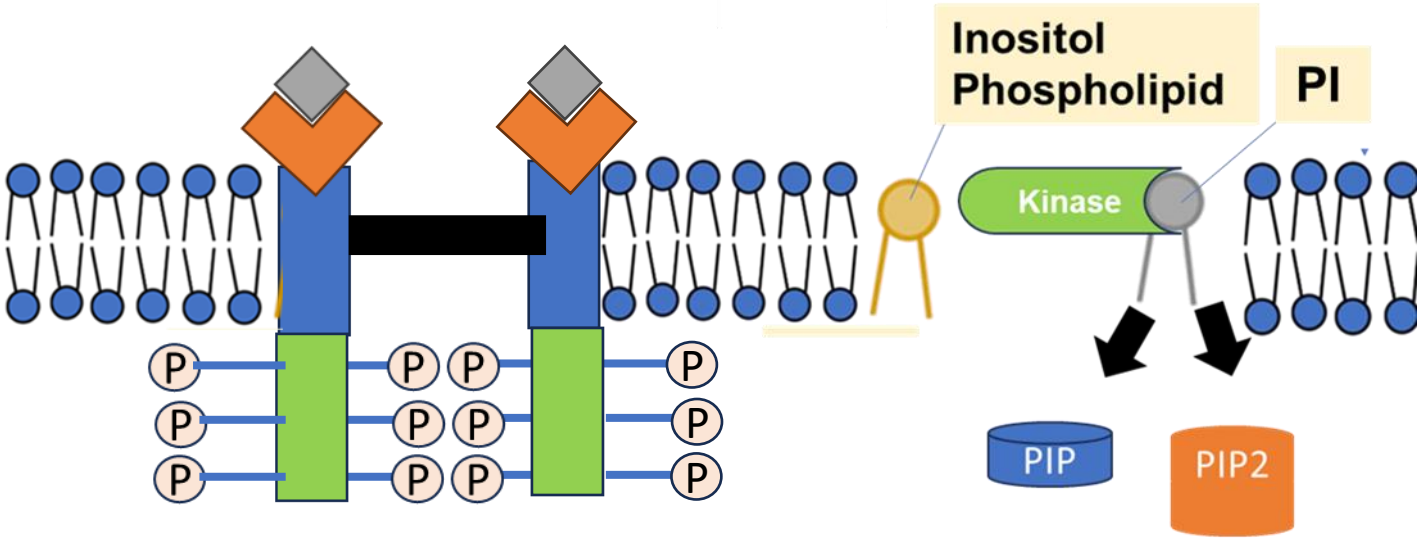
Normal PLC-γ1-PKC signalling pathway: Signal transduction



Step 4

Formation of PI-bisphosphate (PIP_2).

Phosphatidylinositol (PI) is a **phospholipid** and is **phosphorylated by kinase enzymes** to form **PI-phosphate (PIP) and PI-bisphosphate (PIP_2).**



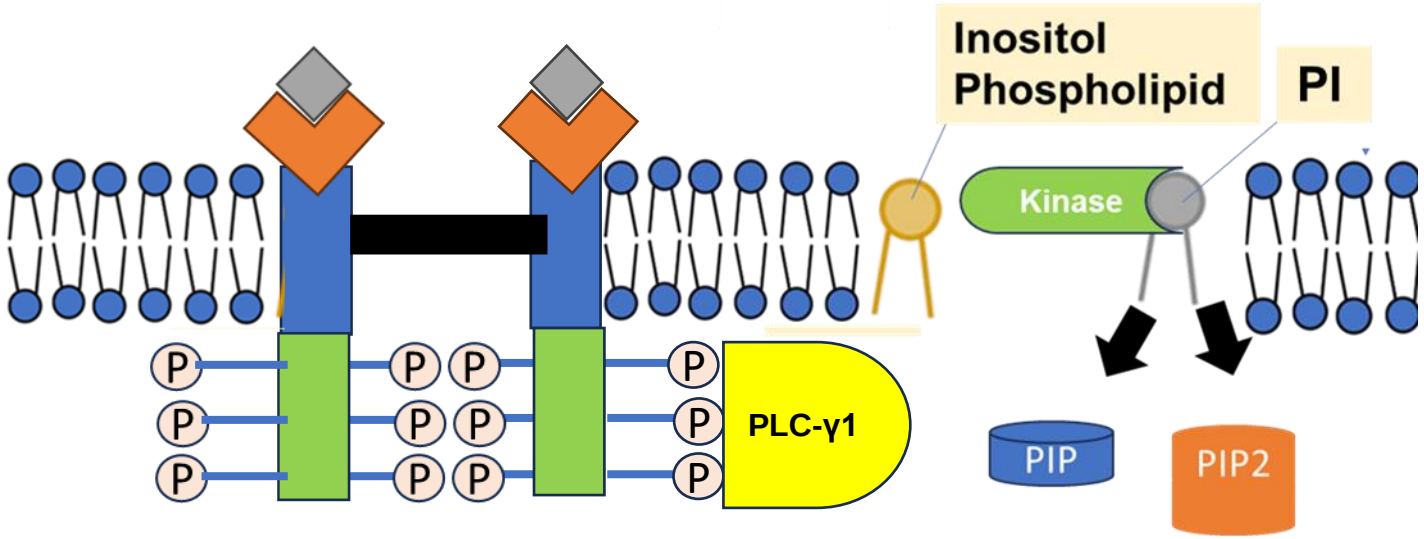
Step 5

PLC-γ1 binds to activated EGFR.

The SH2 domain of PLC-γ1 (Phospholipase C gamma-1) binds directly to activated EGFR.

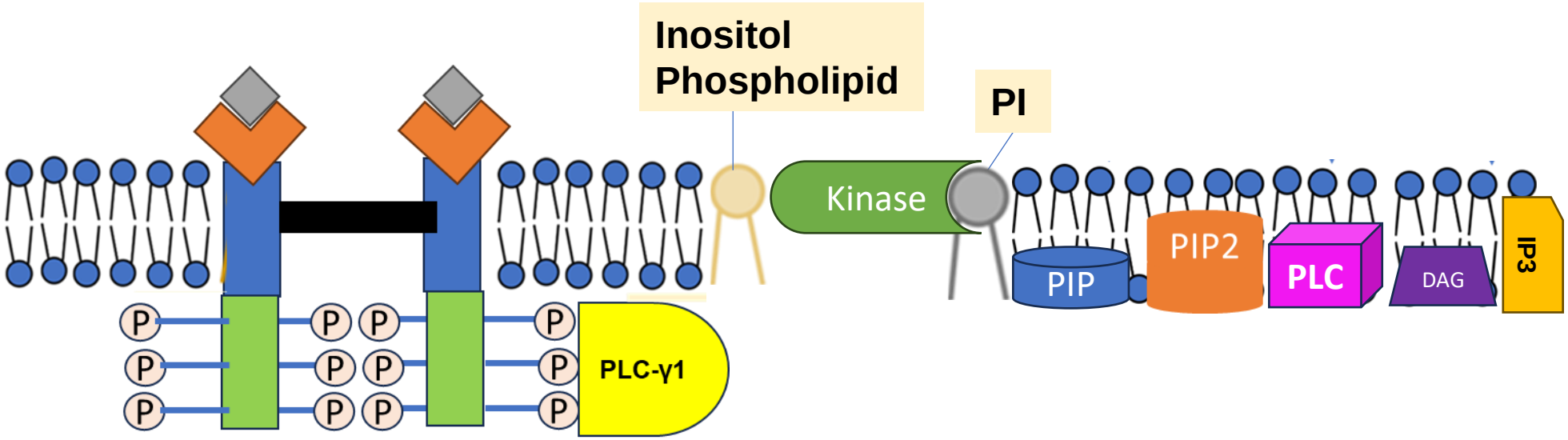
This occurs at the following tyrosine amino acid residues in EGFR:

- ❑ **Y992**
- ❑ **Y1173**



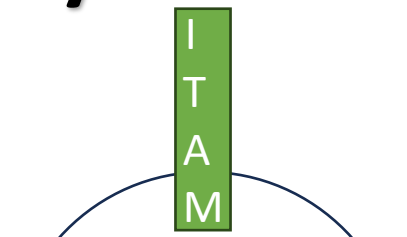
Step 6

The formation of the secondary messengers DAG and IP_3

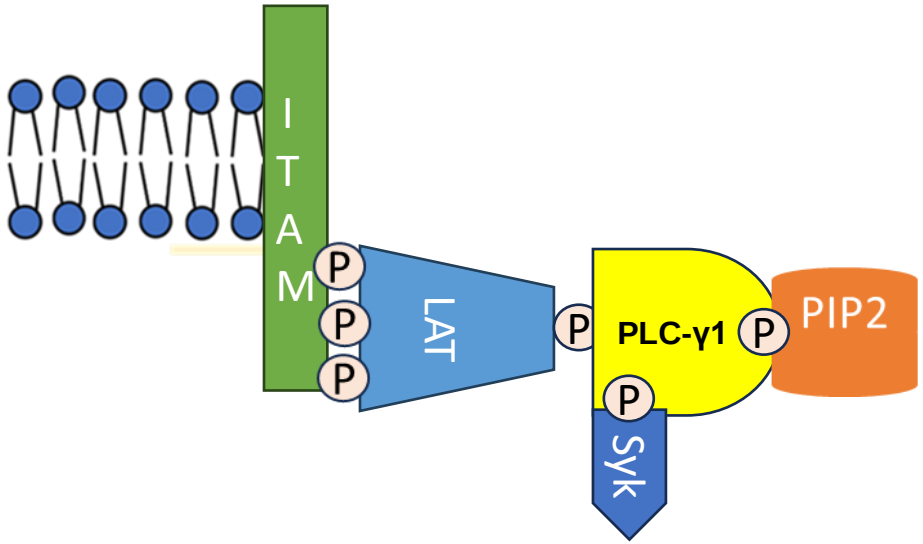


PLC cleaves PIP_2 to form two second messengers: Diacylglerol (DAG) and Inositol triphosphate (IP_3).

Did you know?



Immune cell



The immunoreceptor tyrosine-based activation motif (ITAM) receptors expressed on T cells, natural killer cells, mast cells and platelets phosphorylate tyrosine residues in the intracellular domain of the transmembrane adapter LAT (linker for activation of T cells).

The phosphorylated LAT recruits phospholipase C to the receptor.

Other adaptor proteins are Grb2 and Gads/SLP-76

PLCγ is phosphorylated by ZAP-70 or Syk proteins.

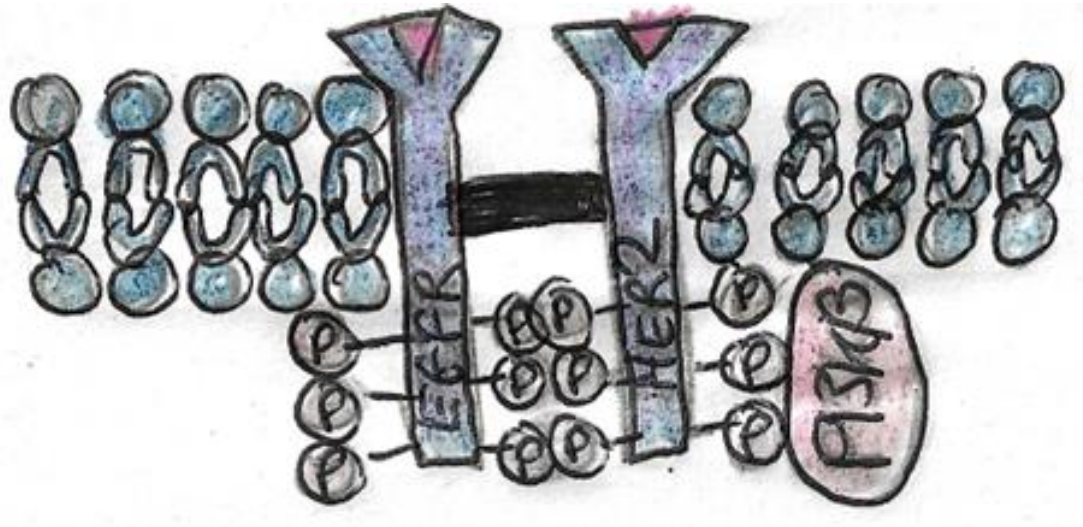
The phosphorylated PLCγ cleave the membrane lipid phophatidylinositol-4,5-bisphosphate (PIP2).

Did you know?

PI3Kβ adaptor protein phosphorylated to the receptor complex and binds to PIP_3

The PH domain of PLC-γ1 binds to PIP_3 and recruited to the plasma membrane.

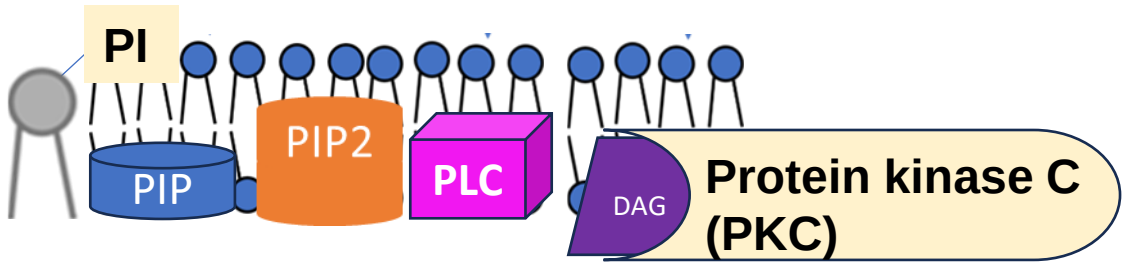
PLC-γ1 phosphorylated at Y472, Y771, Y778, Y783, and Y1254.



Step 7

Diacylglycerol (DAG) activates protein kinase C

Diacylglycerol (DAG) stays in the plasma membrane, binds and activates protein kinase C (PKC).

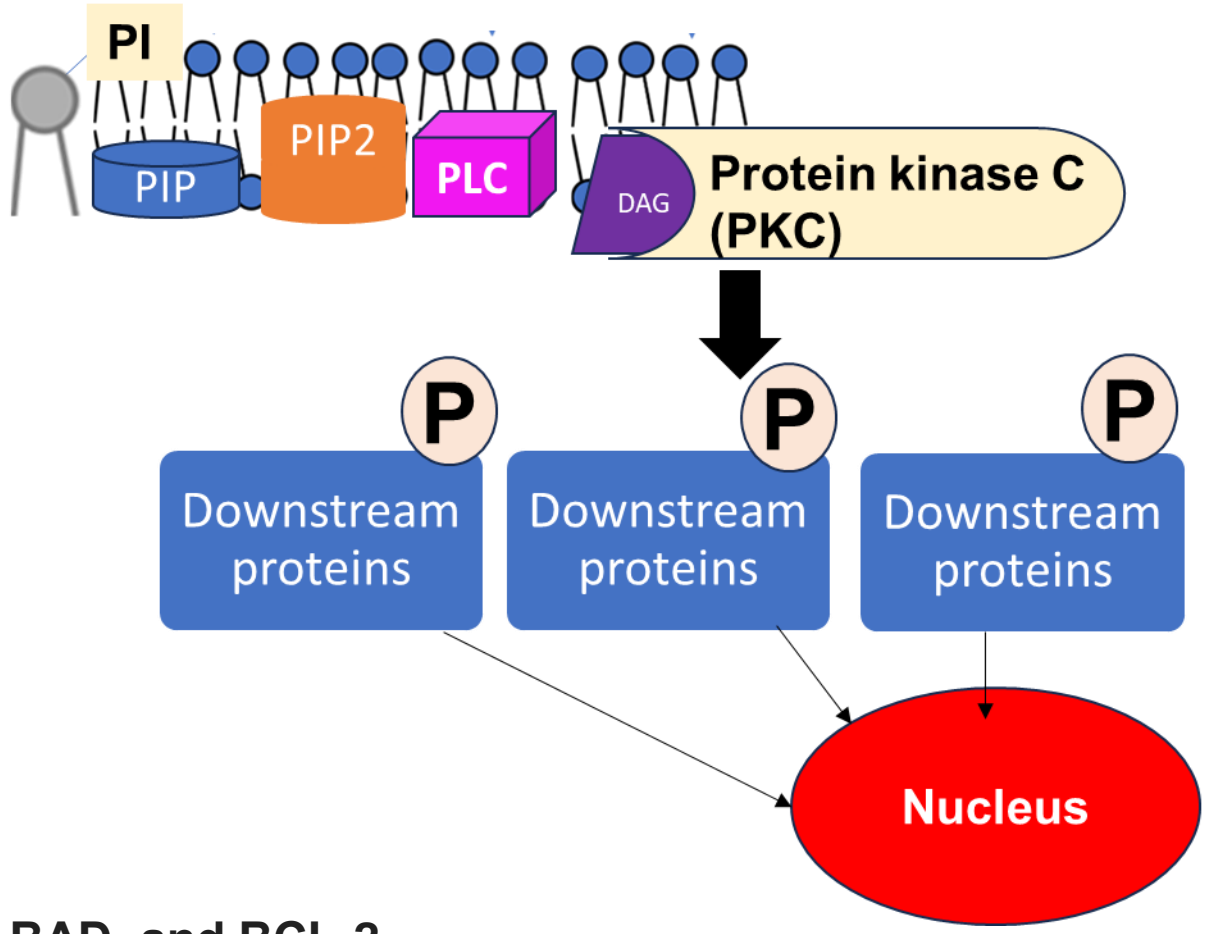


Step 8

The activated protein kinase C phosphorylates its target proteins

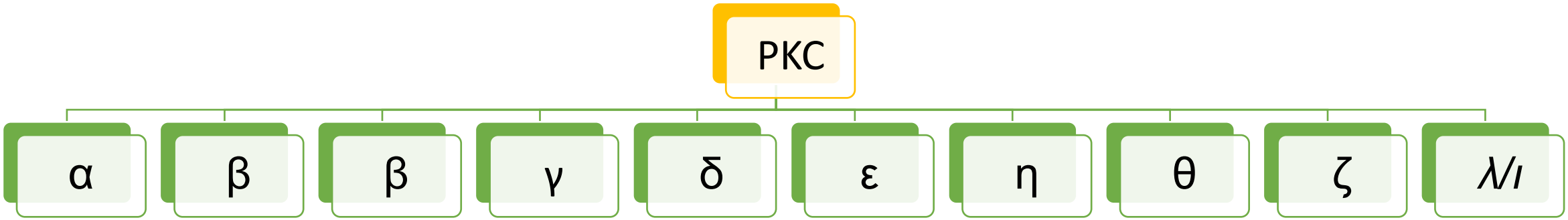
Protein kinase C phosphorylates serine and threonine amino acid residues in its target cellular proteins:

- ❑ **Cell growth**
- ❑ **Differentiation**
- ❑ **Apoptosis**



EGFR, RAF-1, H-RAS, p21, GSK-3β, RHOA, BAD, and BCL-2.

Did you know?



DAG activates a number of PKC isoforms:

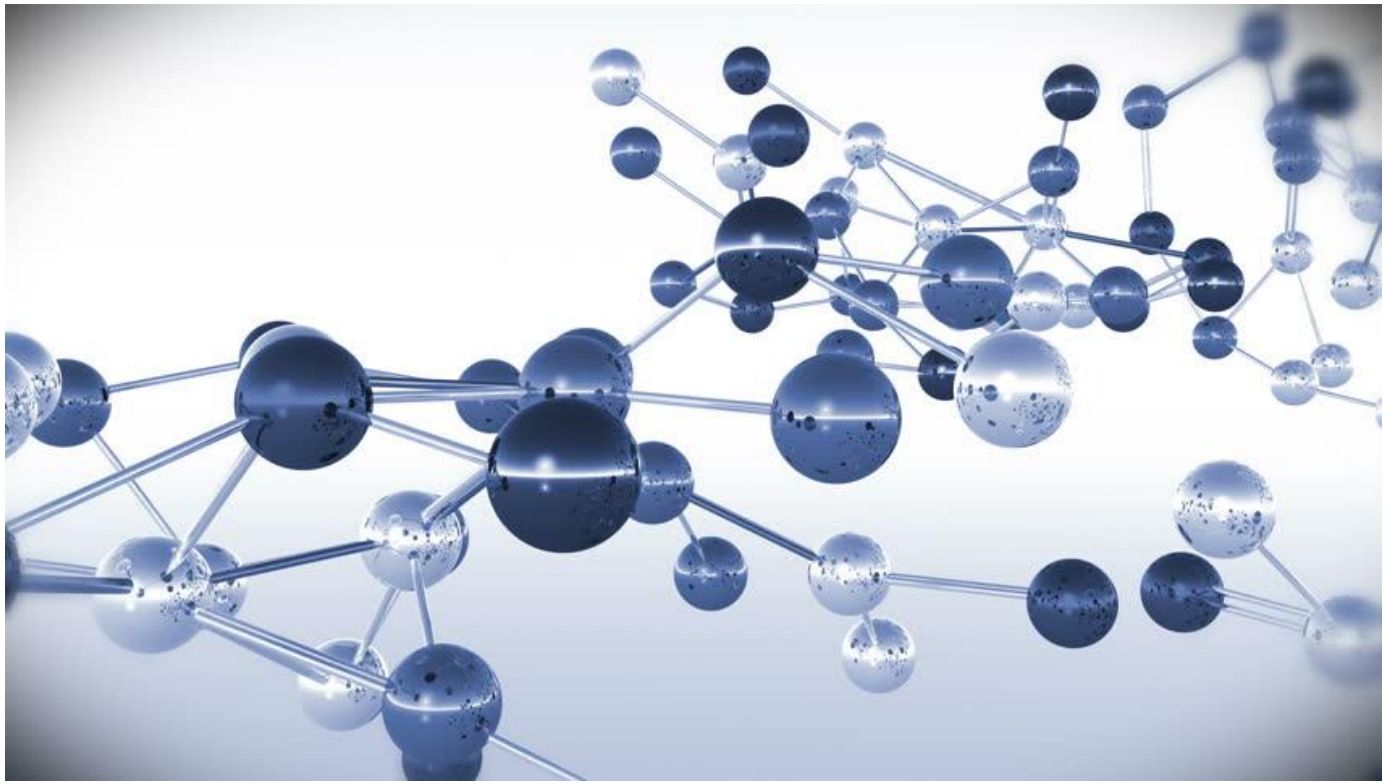
PKCα is phosphorylated by DAG at T497 residues

Isoform	Location
PKCα, βI, βII, δ, ε, η, ζ, and λ	Macrophages
PKCα, δ, ε, and ζ	TLR-induced inflammatory response
PKCθ	Macrophages but is undetectable. Lipopolysaccharide (LPS) and Interferon-gamma (IFNγ) can stimulate this form.

Did you know?

PKC can phosphorylate intracellular EGFR.

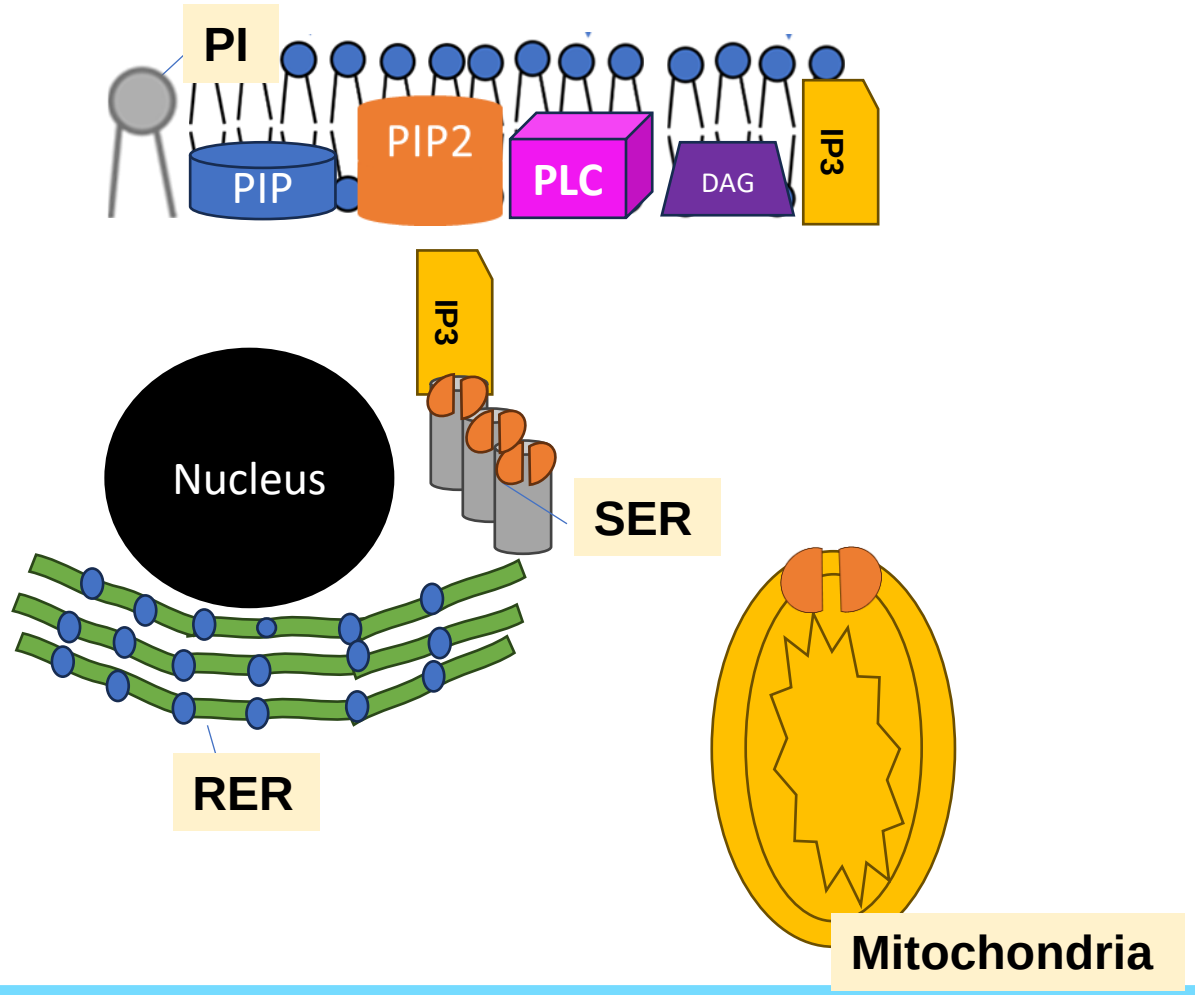
EGFR at T654 can block EGF-induced EGFR activation.

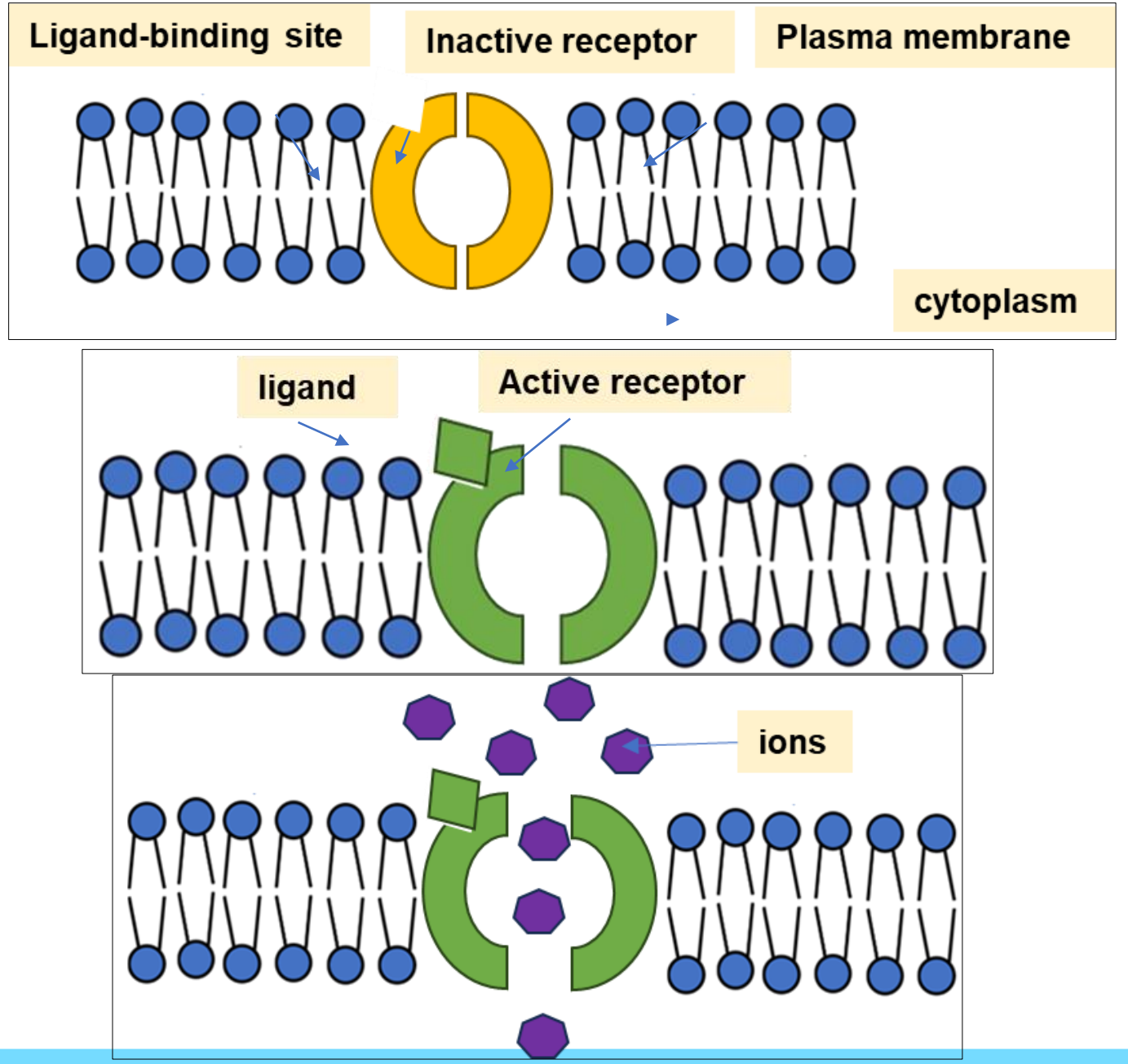


Step 9

IP_3 diffuses into the cytoplasm and binds to the calcium ligand-gated channel in the endoplasmic reticulum membrane.

IP_3 diffuses into the cytoplasm and binds to IP_3-receptors calcium channels in the endoplasmic reticulum membrane.

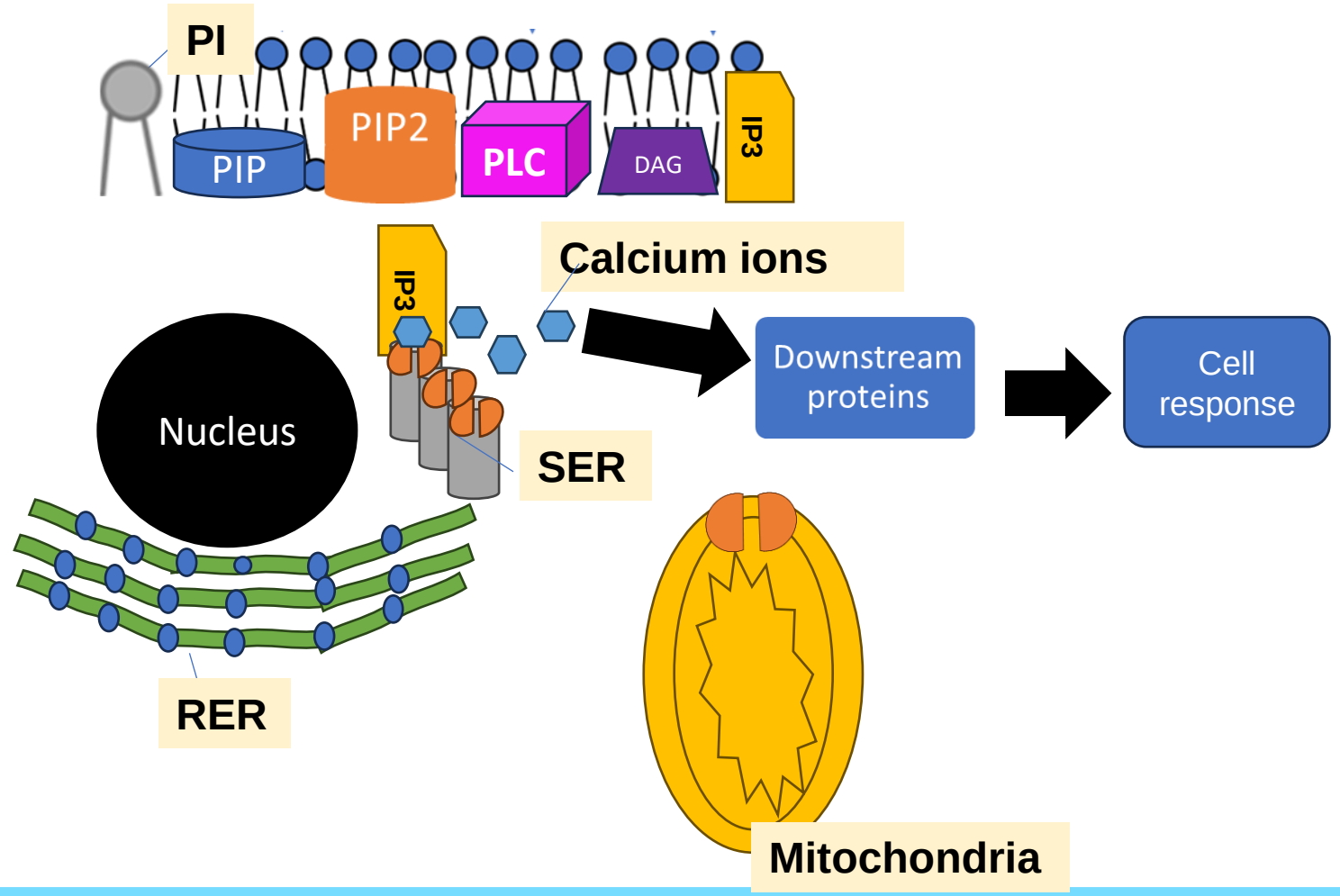



Ligand-binding site
Inactive receptor
Plasma membrane
cytoplasm
ligand
Active receptor
ions

Step 10

Calcium channels open to release calcium ions.

The binding causes the calcium channels to open and release calcium ions into the cytoplasm.



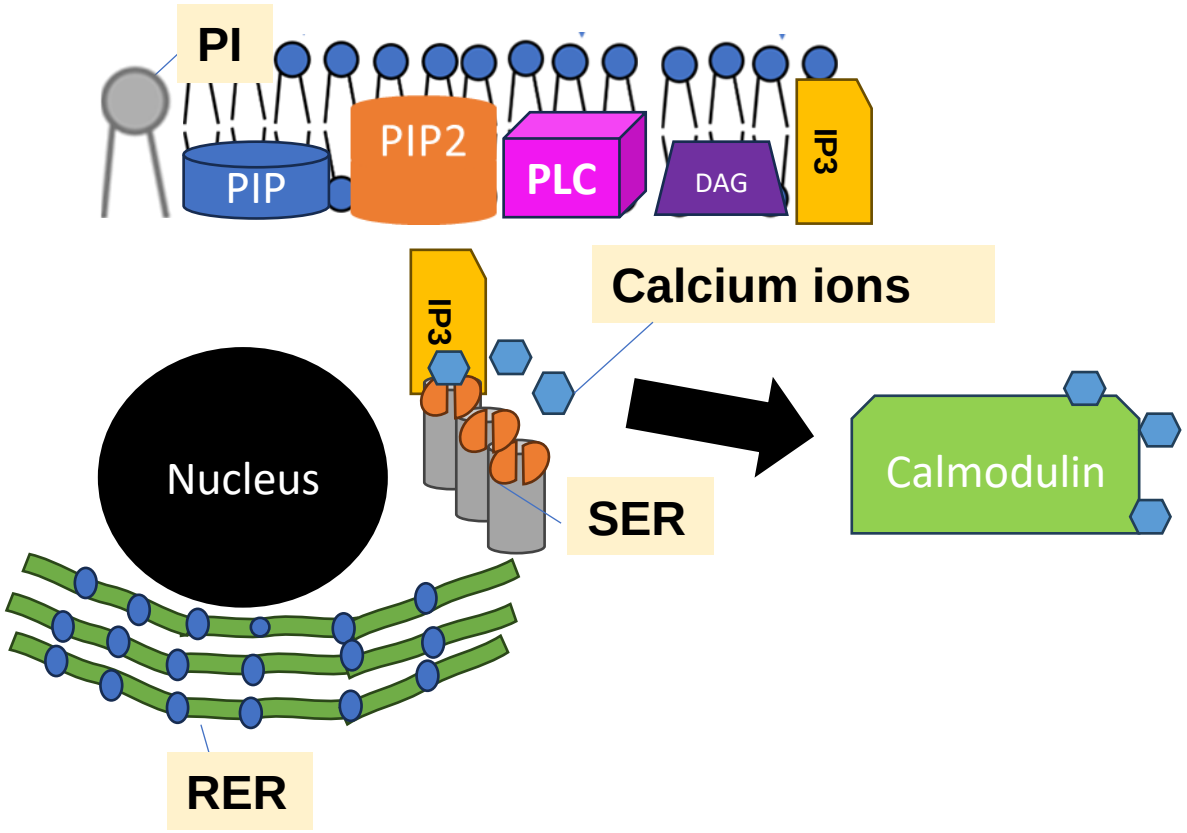
Step 11

Calmodulin binds with calcium ions.

Calmodulin (CaM) is a protein whose role is to bind to calcium and regulate calcium-dependent pathways e.g.

The calcium-calmodulin complex activates enzymes and proteins that aid with cellular response

Carbohydrate breakdown in liver cells.



Step 12

Calcium ion is a secondary messenger and controls transcription factors

The increase of calcium ions can control the activation of transcription factors such as NFAT.

NFAT can bind to:

1) Forkhead box protein P3 (FOXP3) that regulates regulatory T cell expression.

Regulatory T cells are important for homeostasis where they halt the activation of leukocytes.

2) CNS2 is linked with the central nervous system in acute lymphoblastic leukemia (ALL).

ALL is a type of blood cancer that affects white blood cells.

The presence of leukaemia cells in the cerebrospinal fluid in the brain or spinal cord.

Blast cells are immature cells also known as stem cells.

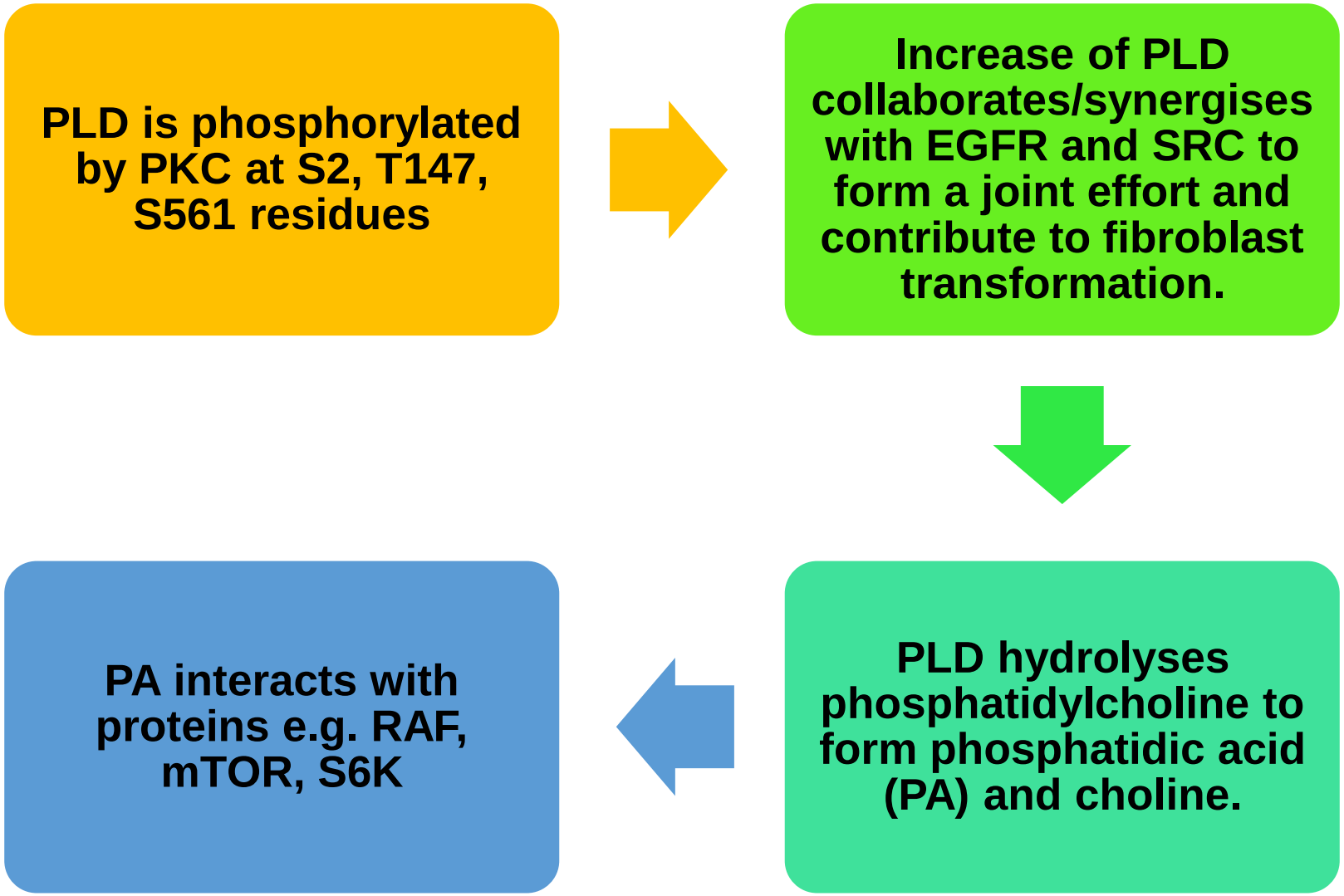
Blasts can grow and differentiate into specialized cells e.g. neuroblasts give rise to nerve cells.

CNS disease can be divided based upon the number of blasts.

Type of CNS	Presence of blasts	Number of white blood cells
CNS1	No	< 5
CNS2	Yes	< 5
CNS3	Yes	≥ 5

Other types of signal transduction: phospholipase D (PLD).

Phospholipase D (PLD).



SRC

Classification

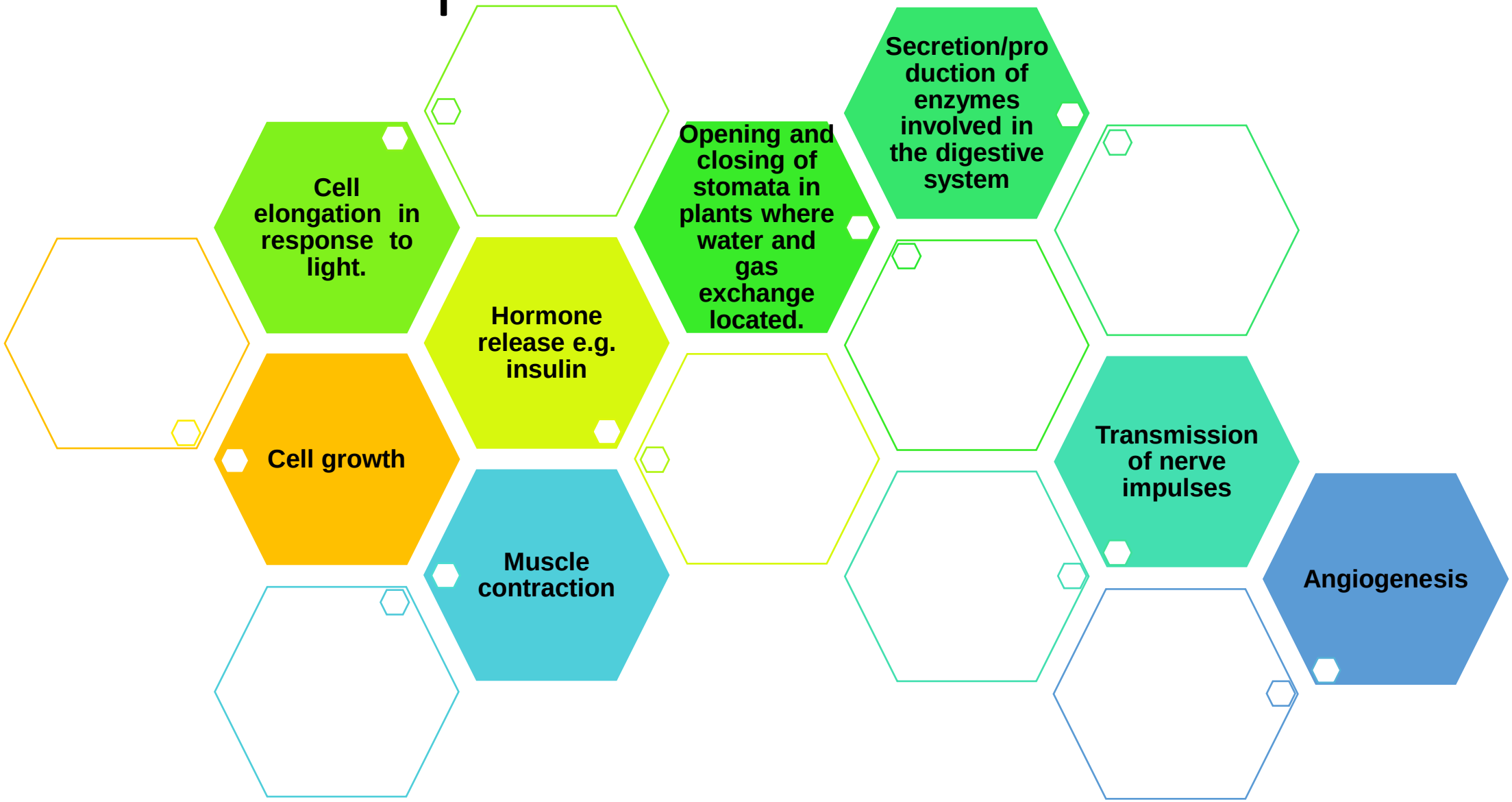
- 11 non-receptor Src Family kinases:
- c-Src, Fyn, Yes, Blk, Yrk, Frk, Fgr, Hck, Lck, and Lyn.
- c-SRC, YES, and FYN are found in most tissues.

Function

- EGFR and HER4 have binding sites for SRC.
- The binding site of EGFR does not have an autophosphorylation site.
- This is mediated by RAS and RAL.
- SRC negatively regulates RAS via phosphorylation.
- SRC positively regulates STAT (signal transducer and activator of transcription), EGFR, RAF, clathrin etc.

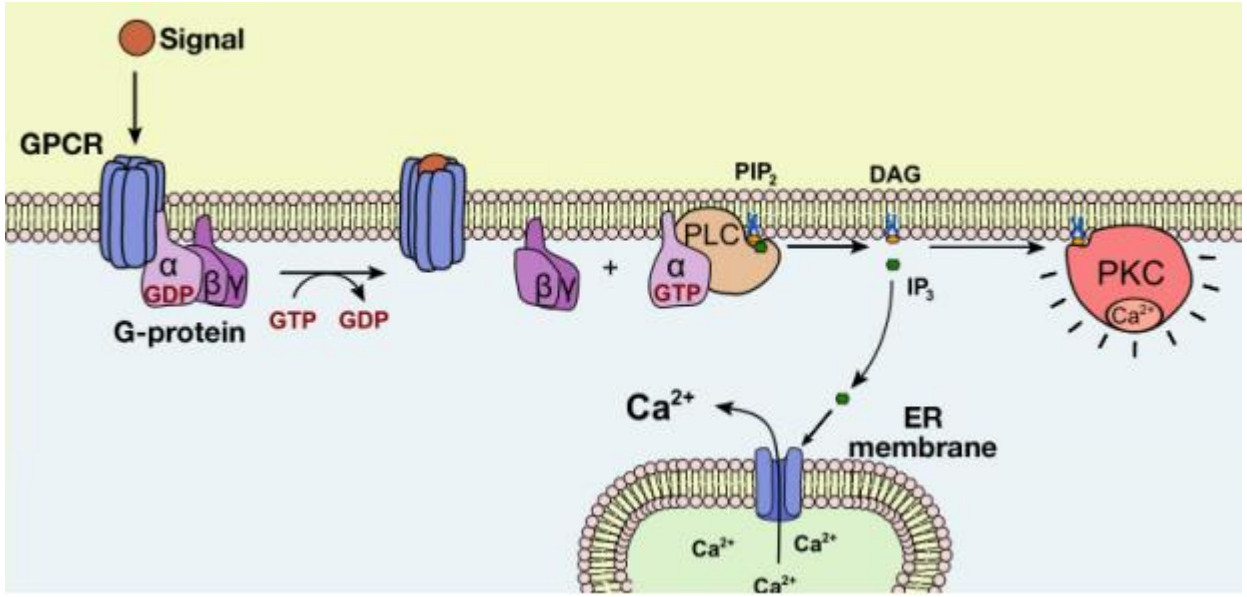
Cellular response

Cellular response



The link between GPCR and PLC

The link between GPCR and PLC



GPCR Pathway (Creative Commons, 2023)

When the ligand binds to the G protein coupled receptor activates to G protein

One of the isoforms of the alpha subunit of G-protein, Gαq, activates PLC and G12/13

PLC hydrolyses phosphatidylinositol 4,5-biphosphate into diacylglycerol and inositol triphosphate

PLC activates PKC

The causes of dysregulated PLC-γ1-PKC signalling pathway in cancer

The role of calcium ions in cancer

Angiogenesis

Sustained proliferative signaling

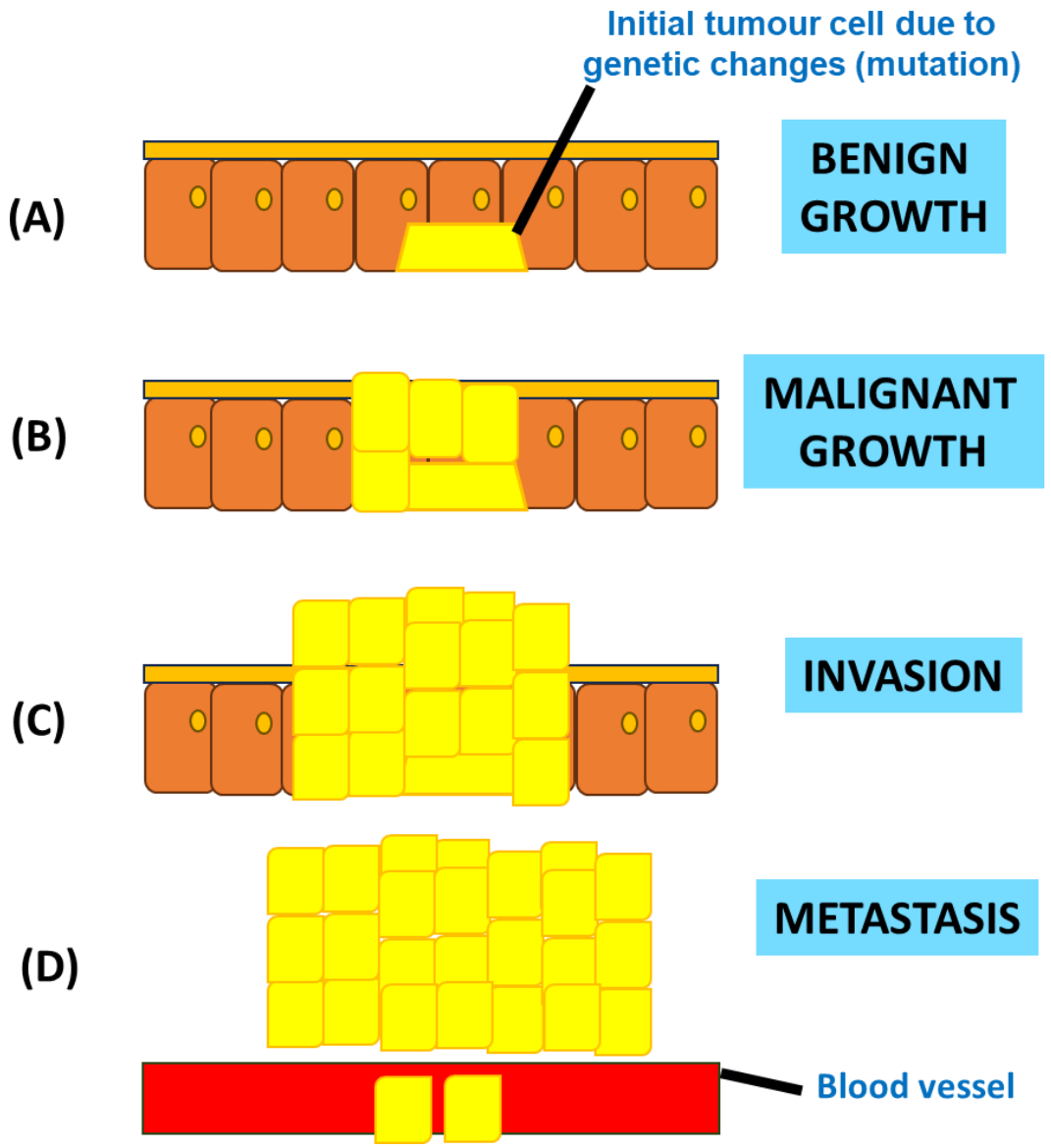
Invasion and metastasis

Evading apoptosis

Insensitivity to anti-growth signals

The role of PLC-γ1-PKC in cancer

PLC-γ1 facilitates cell migration, invasion and metastasis in cancer.



The role of SRC in cancer

C-SRC mutation (Y845F) in SRC potentiates EGFR kinase activity without the need of a ligand. This helps increase tumour progression.

Key examples:

Head And Neck Squamous Cell Carcinoma

Non-small Cell Lung Cancer (NSCLC)

Colorectal Cancer

By the end of this lecture, you should understand

- **Phospholipase C-gamma (PLC-γ) is an adaptor protein that binds to the activated EGFR-EGF receptor complex.**
- **PLC-γ hydrolyses phosphatidylinositol-4, 5-bisphosphate (PI(4,5)P2) (PIP_2) phospholipid in the membrane to produce the two second messengers: Diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3).**
- **Diacylglycerol (DAG) stays in the plasma membrane, binds and activates protein kinase C (PKC).**
- **IP_3 diffuses into the cytoplasm and binds to IP_3-receptors calcium channels in the endoplasmic reticulum membrane. The binding causes the calcium channels to open and release calcium ions into the cytoplasm.**
- **PKC phosphorylates its target cellular proteins: cell growth, differentiation and apoptosis.**
- **Mutation in PLC causes various hallmarks of cancer: angiogenesis, evade apoptosis, migration, invasion and metastasis.**

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SEASON 2

Understanding Cancer

Lecture 10

Types of signalling pathway: Transforming growth factor β (TGF-β)

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