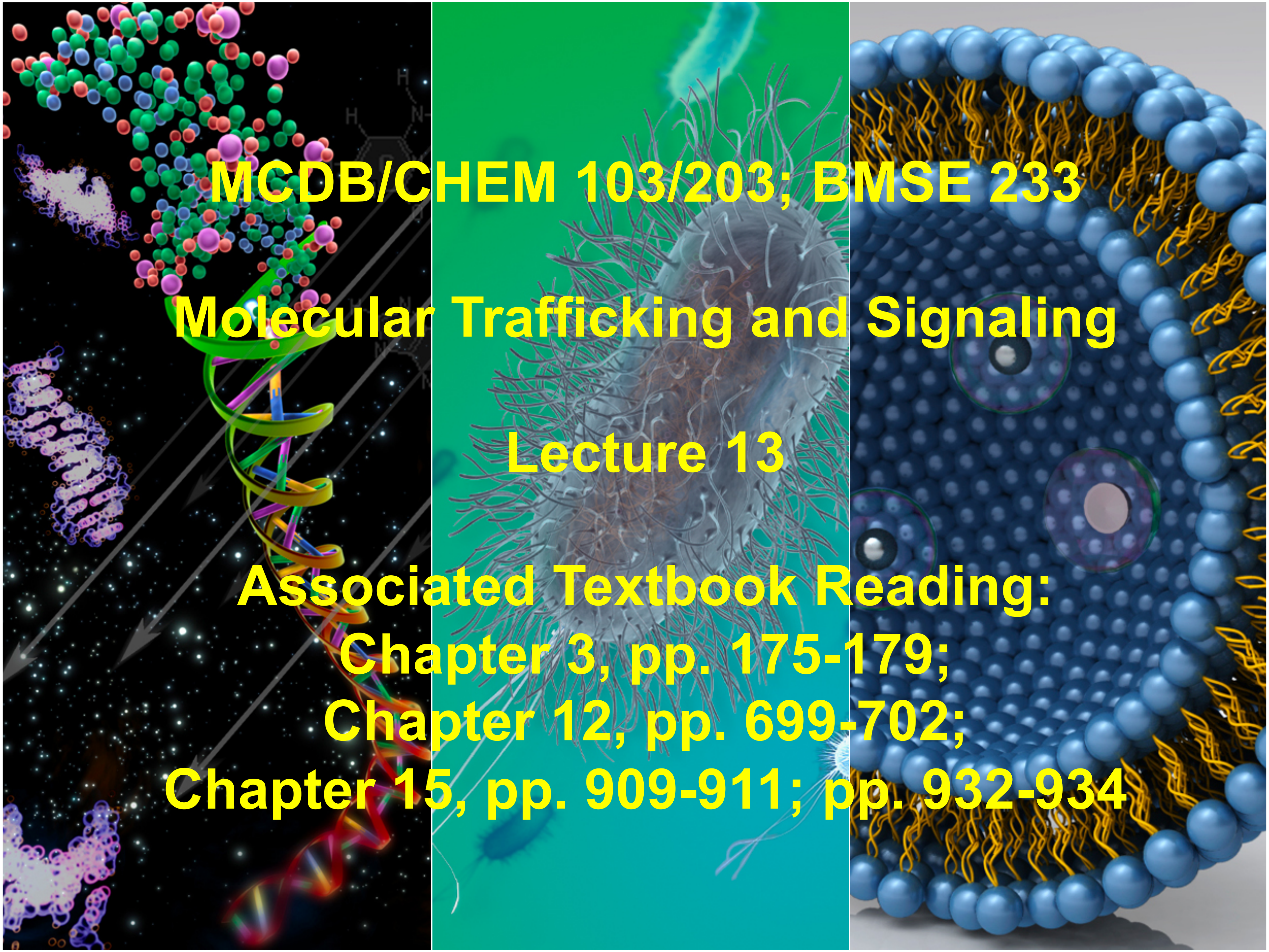


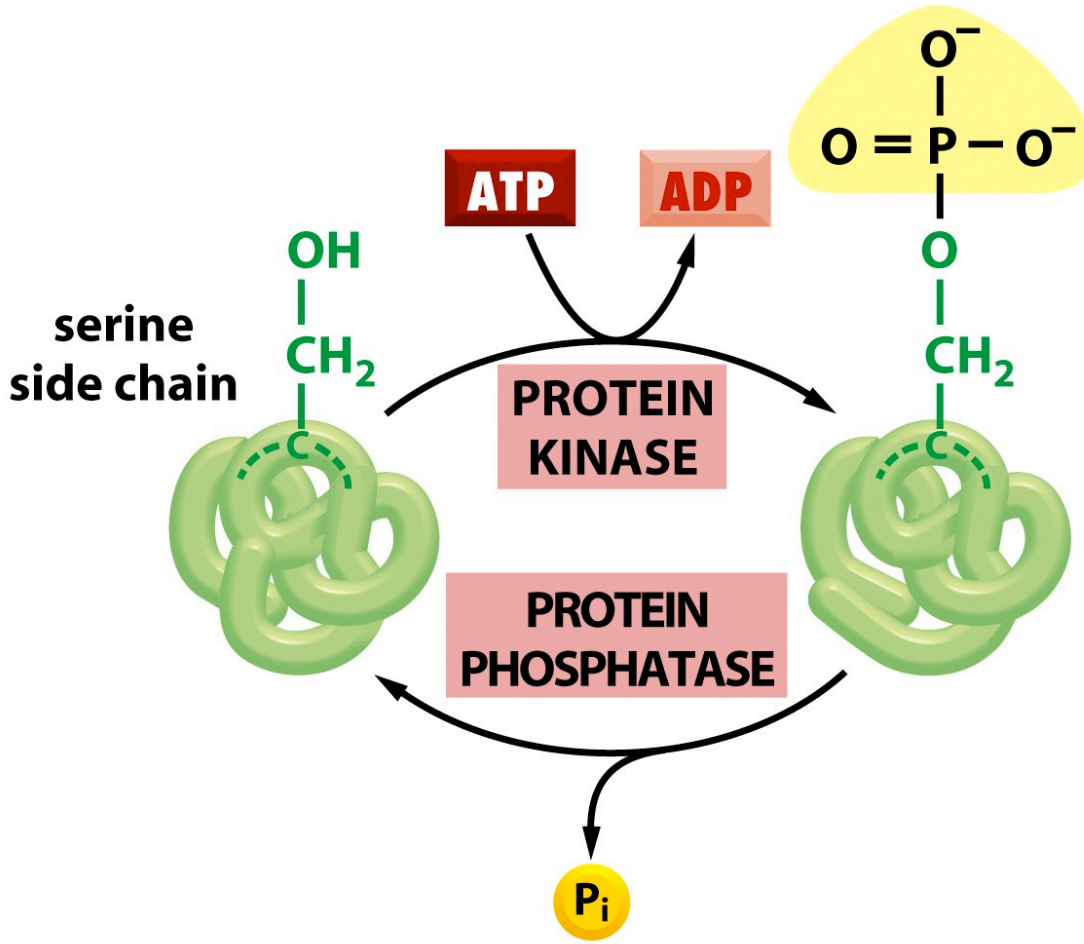
MCDB/CHEM 103/203; BMSE 233

Molecular Trafficking and Signaling

Lecture 13

Associated Textbook Reading:
Chapter 3, pp. 175-179;
Chapter 12, pp. 699-702;
Chapter 15, pp. 909-911; pp. 932-934

Protein Phosphorylation in Molecular and Cellular Signaling



One-third of all proteins present in a typical mammalian cell are believed to be covalently bound to phosphate (phosphorylated) at one time or another.

Kinases and Phosphatases Participate in Signaling by Modifying the Structures and Functions of Cellular Molecules Including Proteins and Lipids

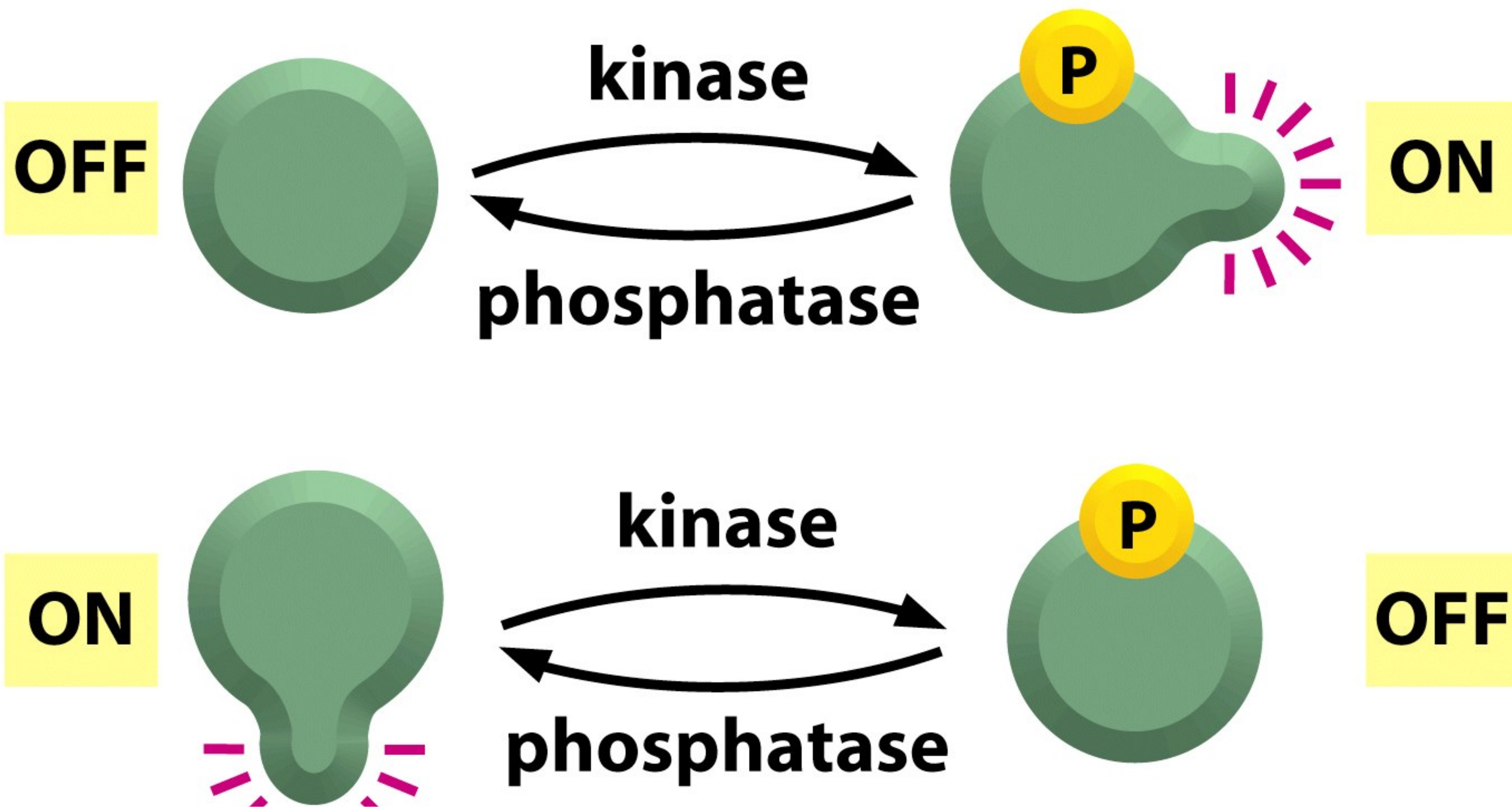


Figure 3-64b Molecular Biology of the Cell 5/e (© Garland Science 2008)

1992 Nobel Prize in Physiology or Medicine:

For their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism

Edwin G. Krebs

1918 –2009

Edmond H. Fischer

1920 – present

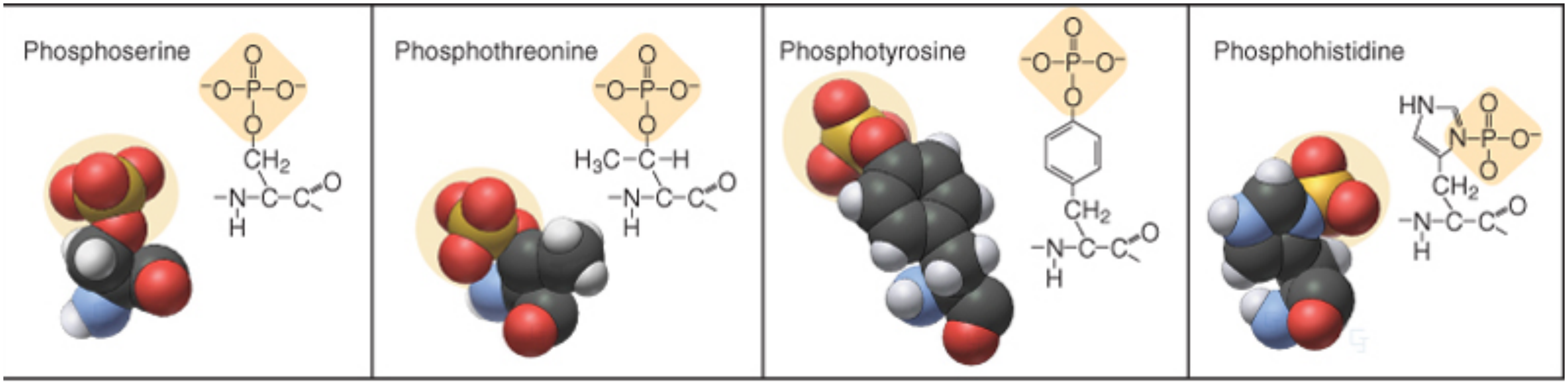
In most animals, protein kinases or phosphatases are divided into groups depending on the phosphorylated amino acid:

There are two common types:

Serine and Threonine kinases (and phosphatases) :
phosphorylate (or dephosphorylate) Ser/Thr amino acids in proteins

Tyr kinases (and phosphatases):
phosphorylate (or dephosphorylate) Tyr residues in proteins

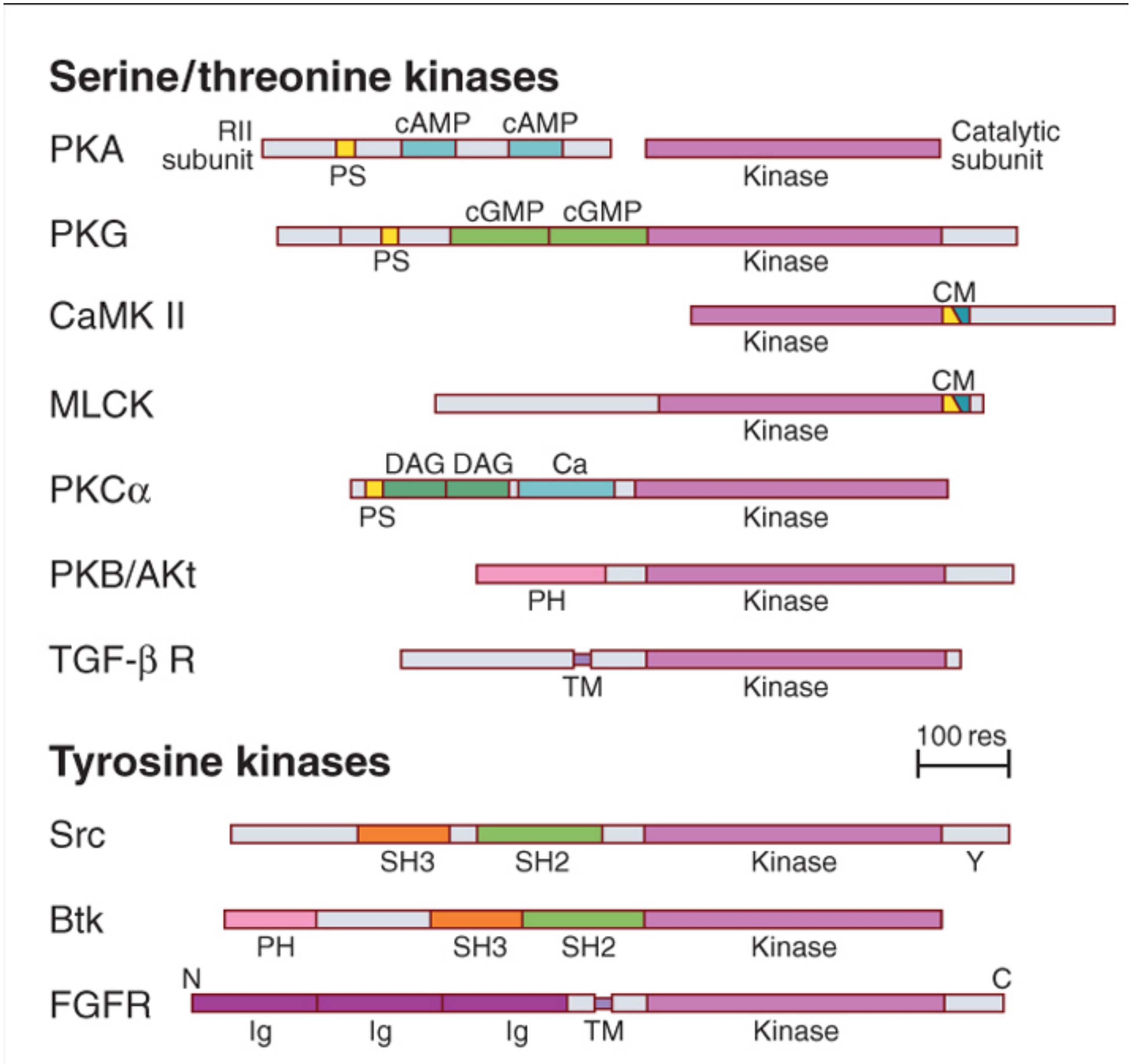
At least five amino acids can be phosphorylated among all life forms



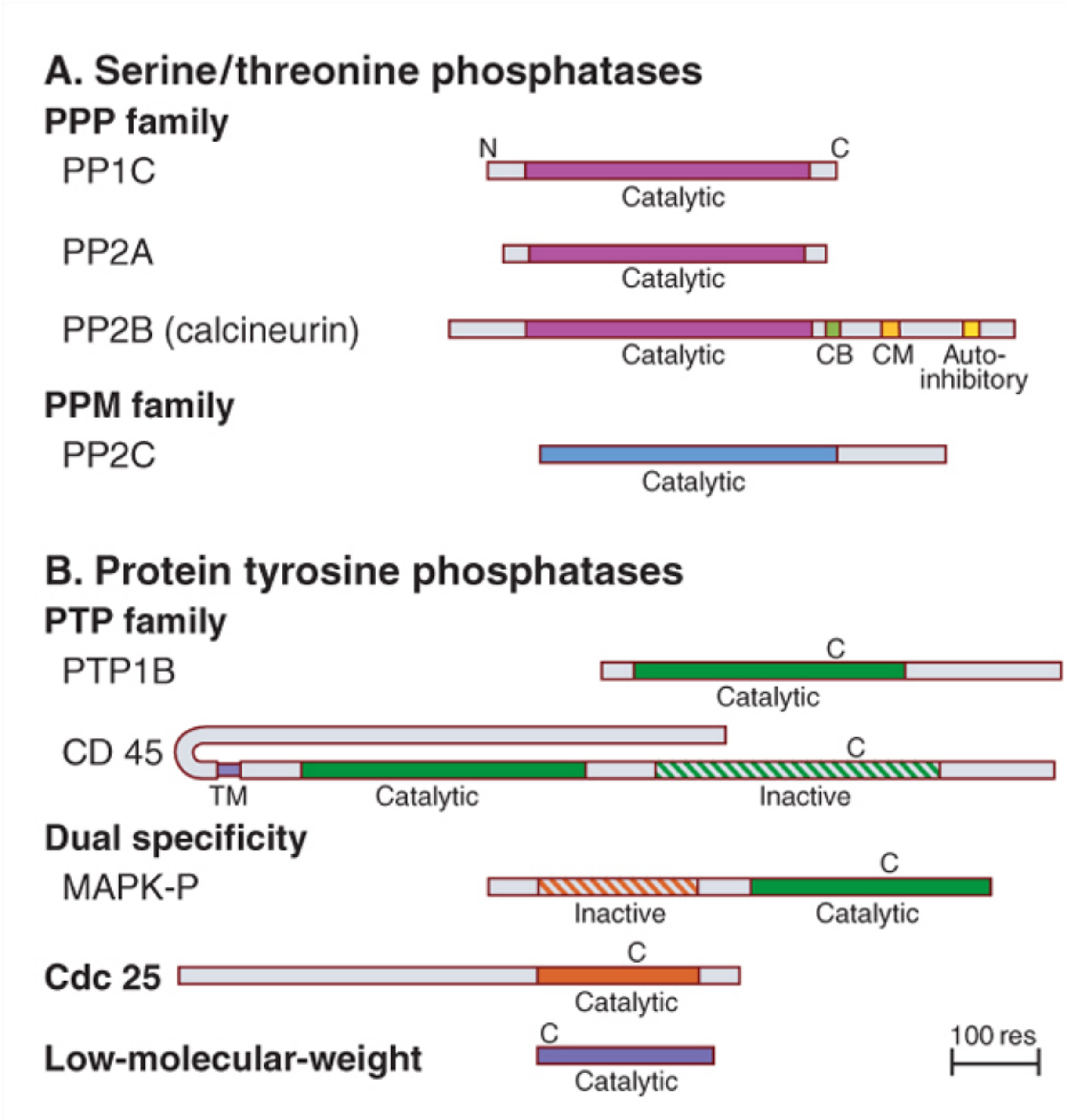
These three are most abundant and studied of the phosphorylated amino acids in proteins

Aspartate-phosphate has also been reported (not shown)

Kinases Often Have Multiple and Regulatory Domains



Phosphatases: Two Main Families



The major types of protein phosphatases (~150 total)

Protein phosphatase 1 (PPA1) (Ser/Thr phosphatase):
the most abundant phosphatase in mammalian cells
regulated by several inhibitory proteins

Protein phosphatase 2A (PP2A) (Ser/Thr phosphatase):

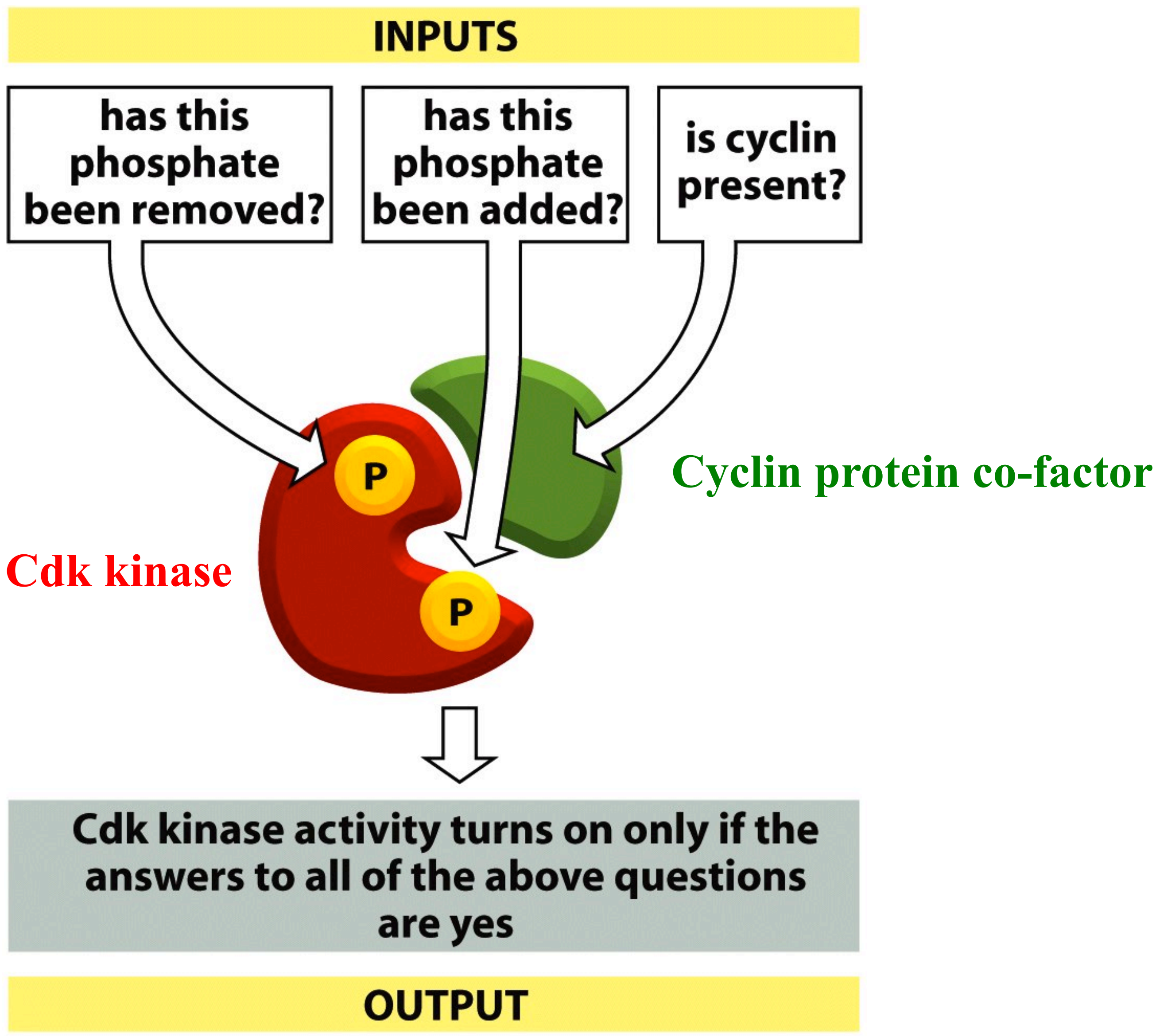
Protein phosphatase 2B (Ser/Thr phosphatase):
highly abundant in neurons
regulated by 2^{nd} messenger Ca2+/camodulin

Protein tyrosine phosphatases - 107 identified
(some with dual specificities for both Tyr-P & Ser-P/Thr-P):
CD45: first tyrosine phosphatase discovered

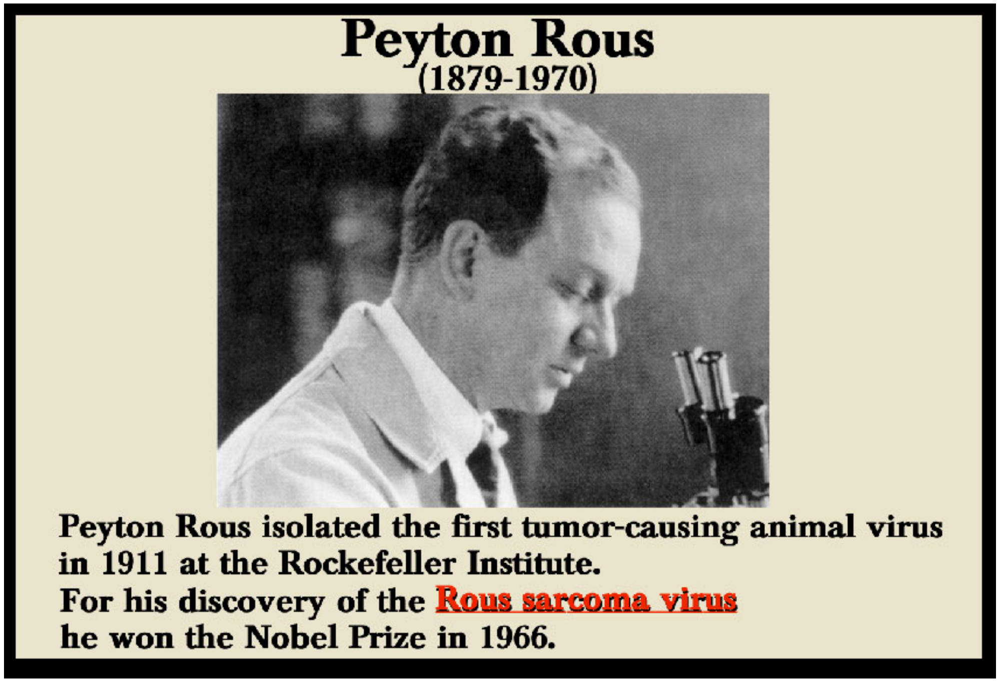
Lipid (PIP) phosphatases (different from protein phosphatases)

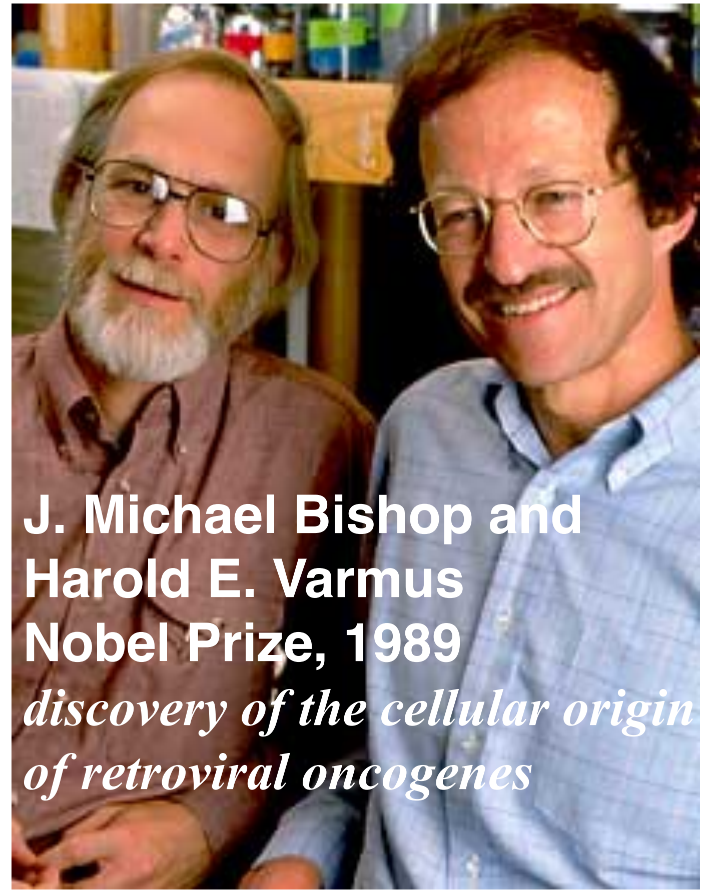
In general, protein phosphatases display less substrate specificity than protein kinases

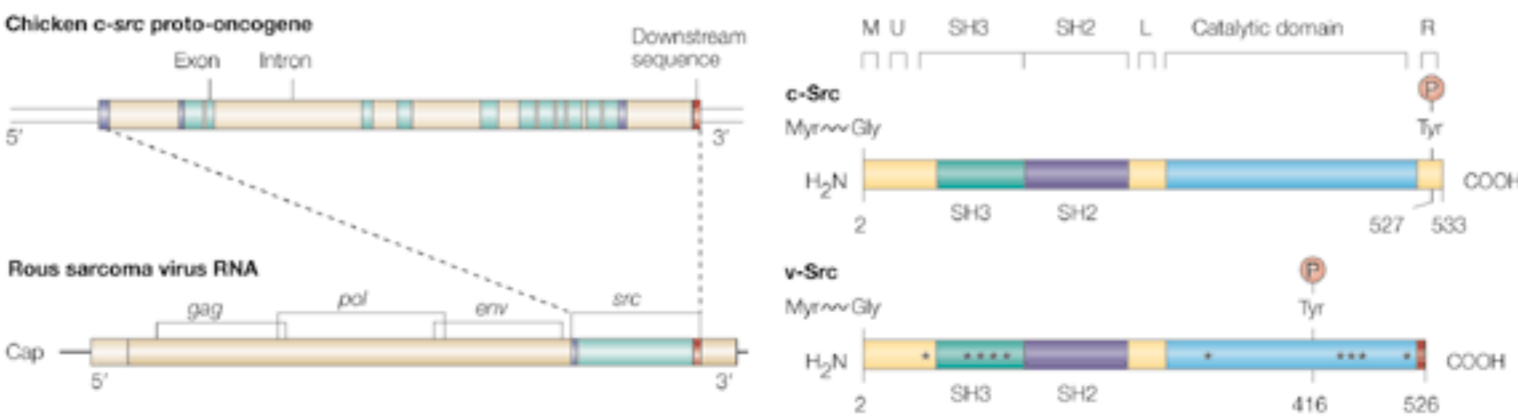
Example of How Kinases Integrate Signals (Inputs) in the Generation of an Output Signal



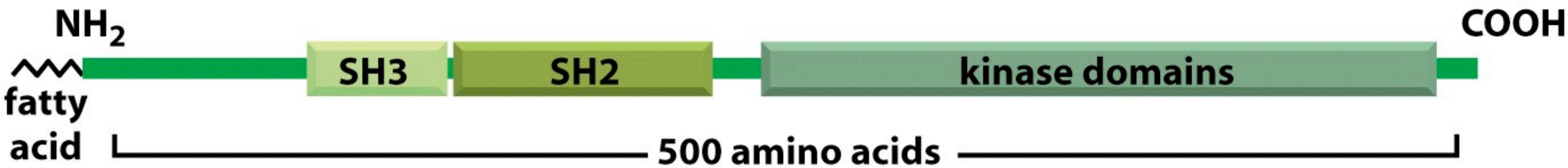
The Src Kinase and Proto-Oncogene

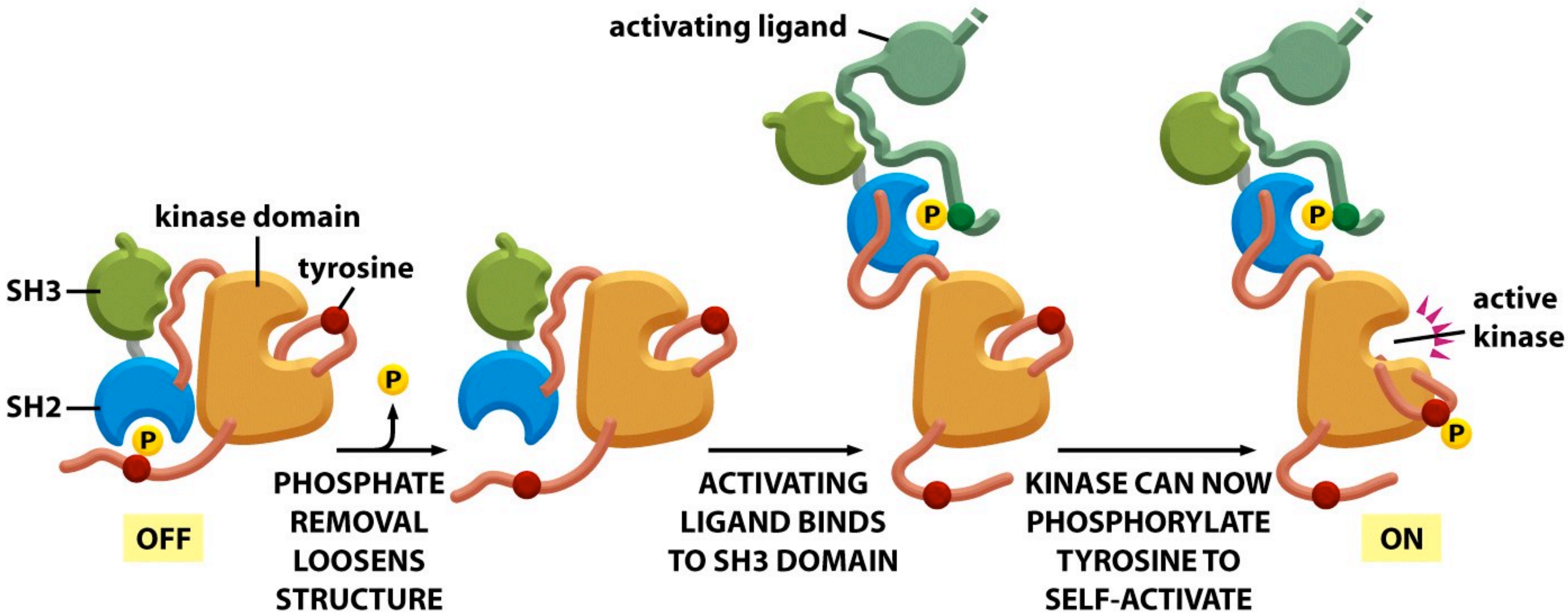




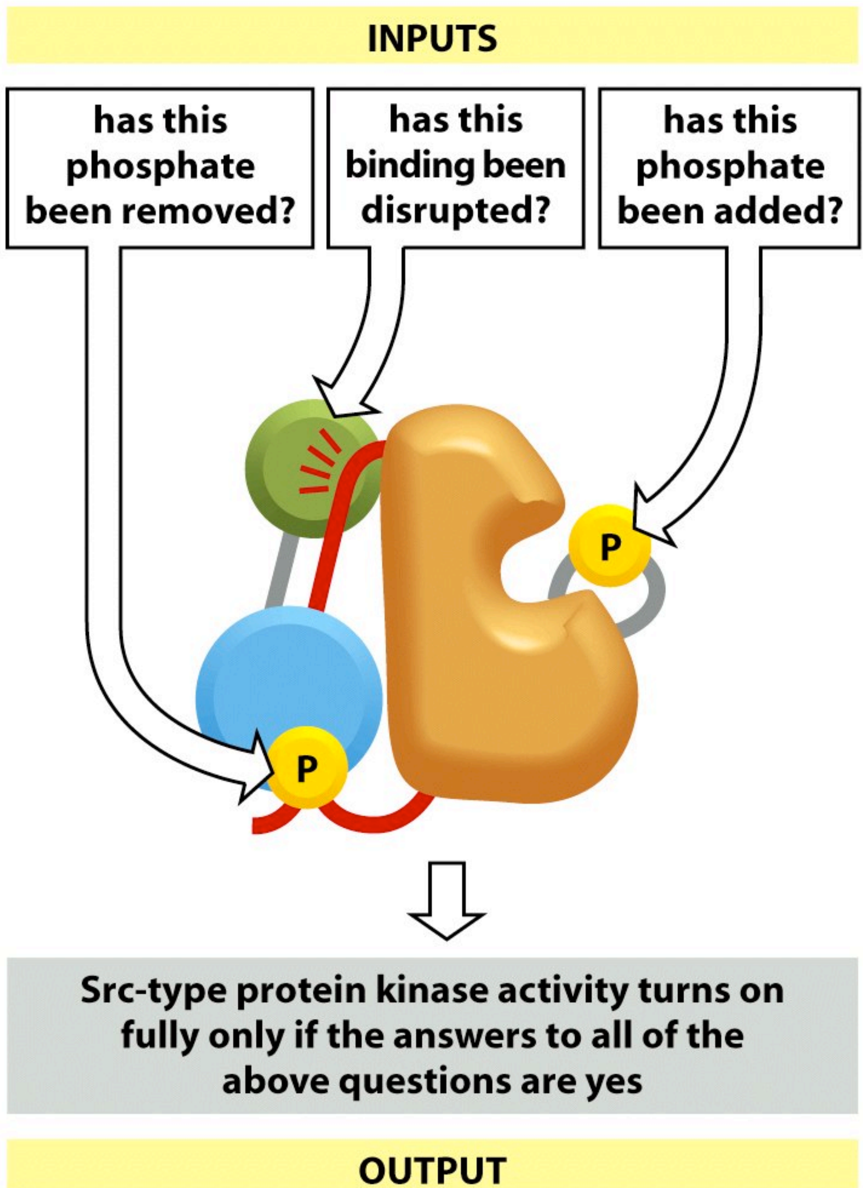


The Src Kinase and Proto-Oncogene





The Src Kinase and Proto-Oncogene



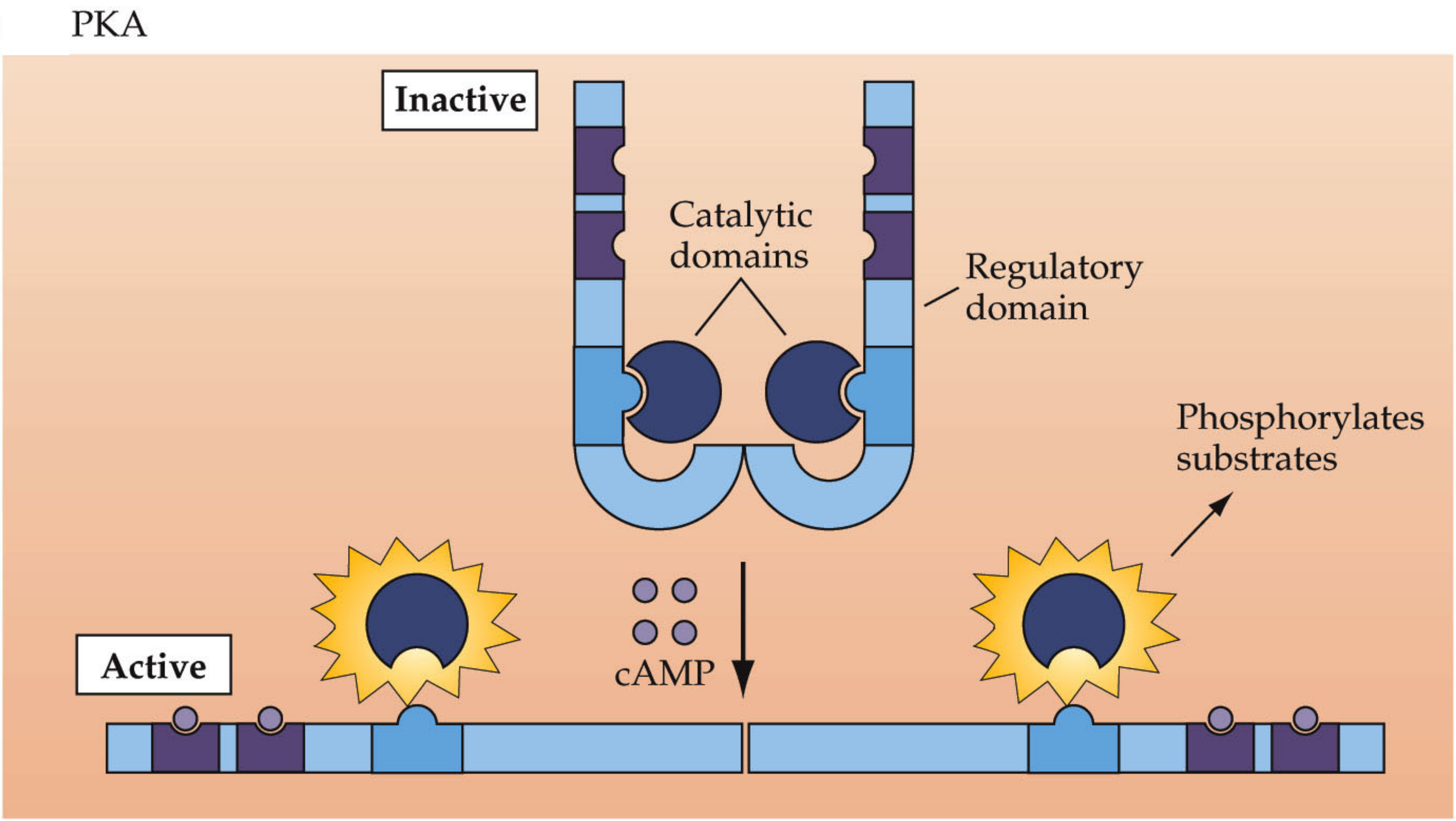
A common theme in the regulation of protein kinases

Each kinase is composed of multiple domains,
including the catalytic and regulatory domains
(sometimes, the regulatory domains are present in another subunit)

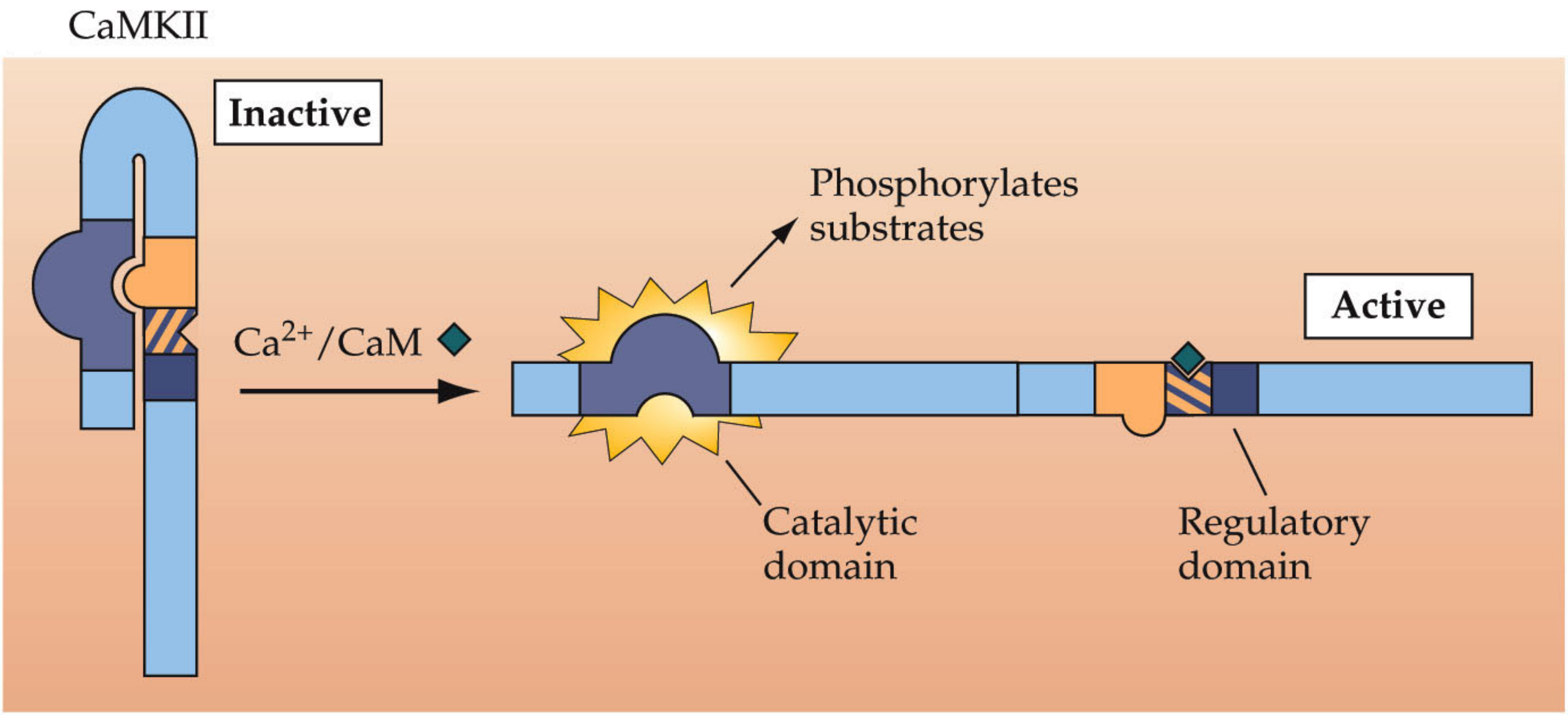
The catalytic domains of kinases are kept inactive
by the presence of a regulatory domain (called an autoinhibitory domain)
that occupies the catalytic site

The binding of signaling messengers
to the appropriate regulatory domain of the kinase
removes the autoinhibition
and allows the catalytic domain to be activated

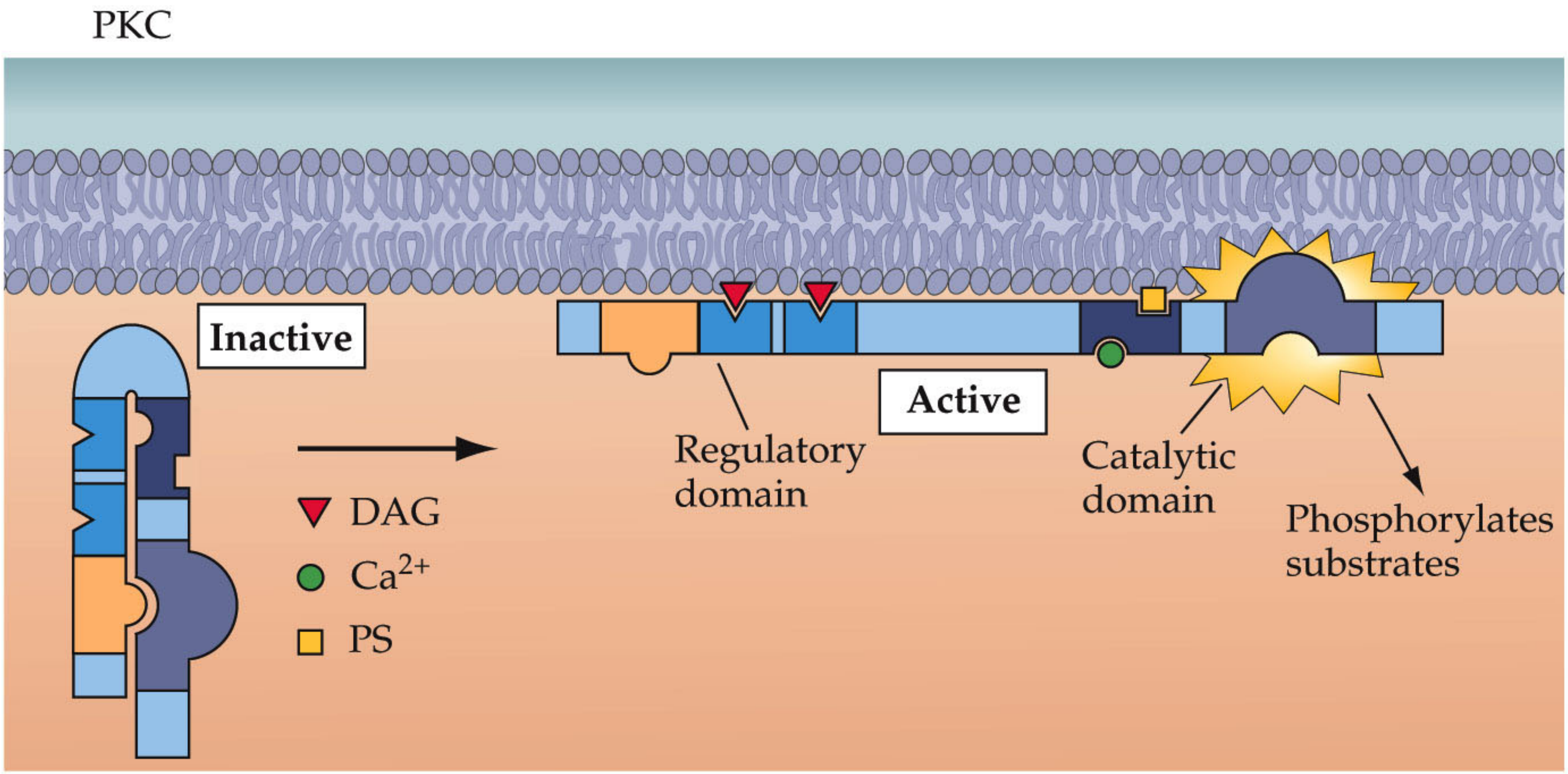
Activation of PKA by cyclic AMP (cAMP)



Activation of Calmodulin Kinase II (CaMKII) by Ca2+/camodulin



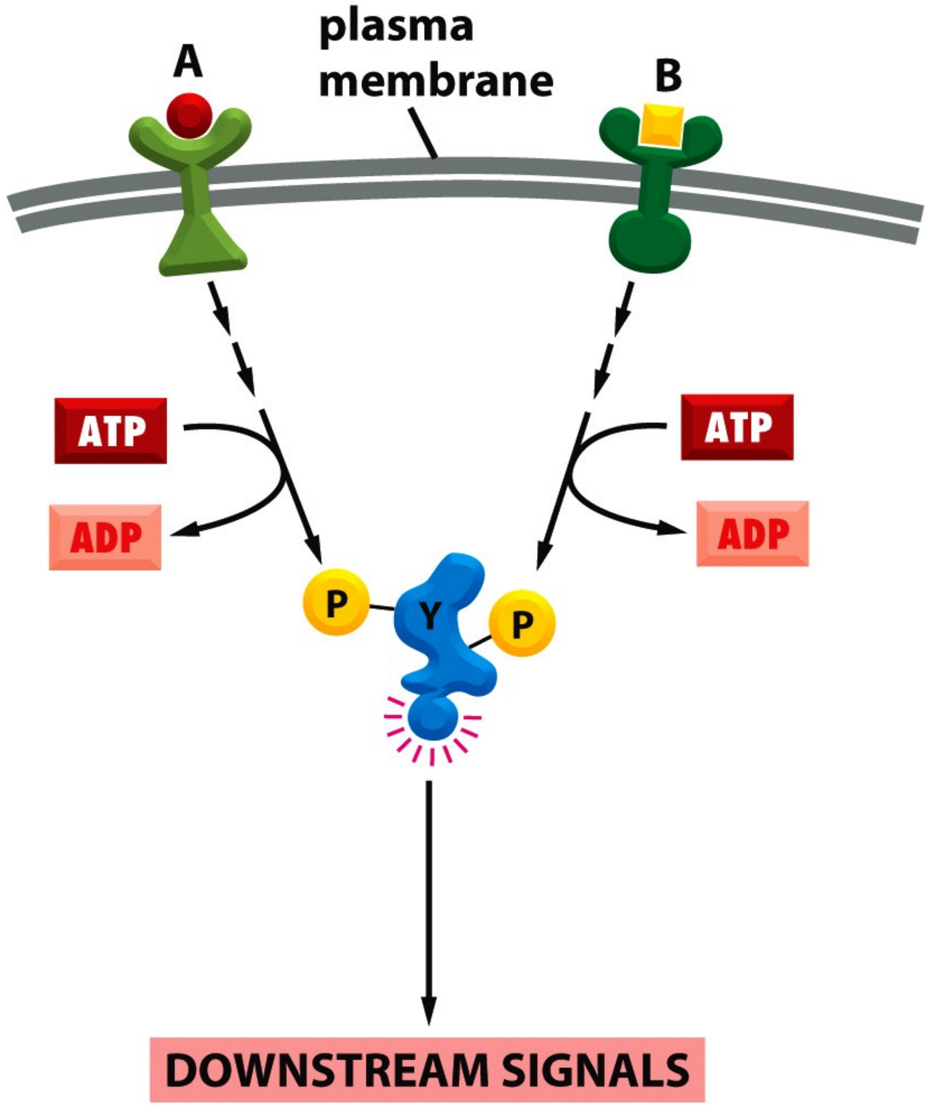
Activation of PKC by DAG, Ca2+ and phosphotidylserine (classical PKC only)



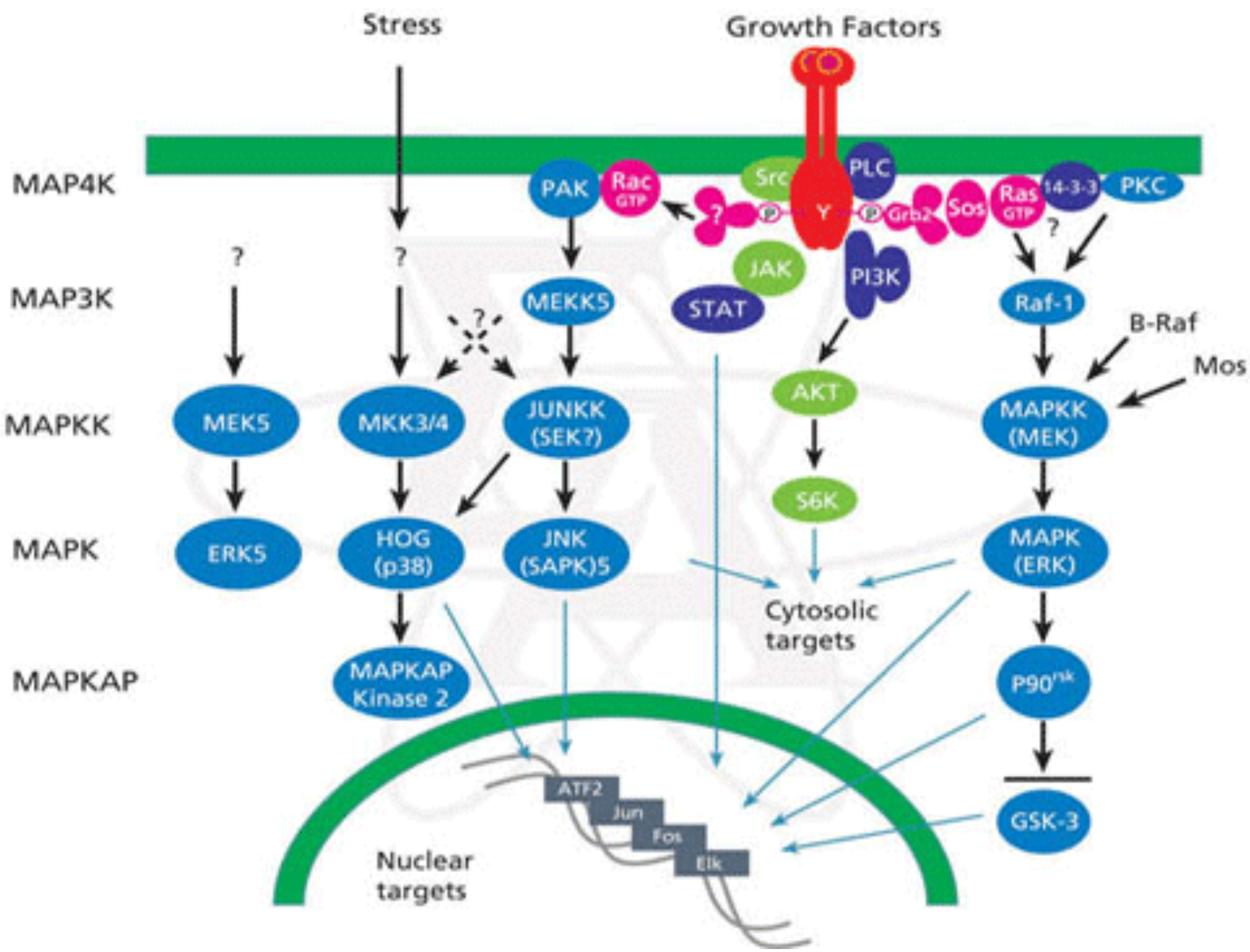
In addition to removing auto-inhibition, these 2nd messengers cause the recruitment of PKC to the PM

A similar mechanism of PKC activation occurs at other membranes

Signal Transduction: A Cascade of Events

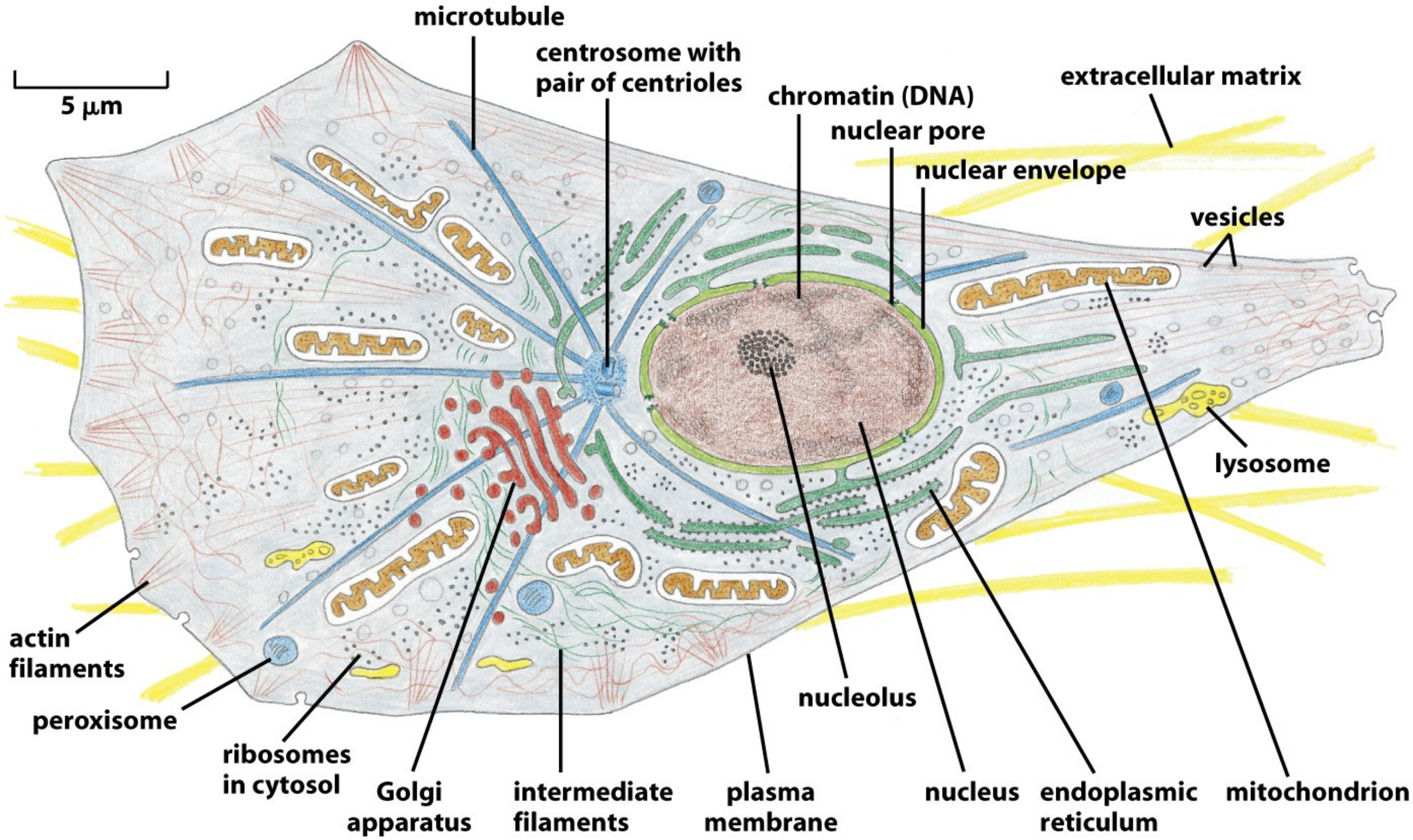


Signal Transduction Cascades Often Branch Across Multiple Pathways: Vertical and Horizontal Pathways



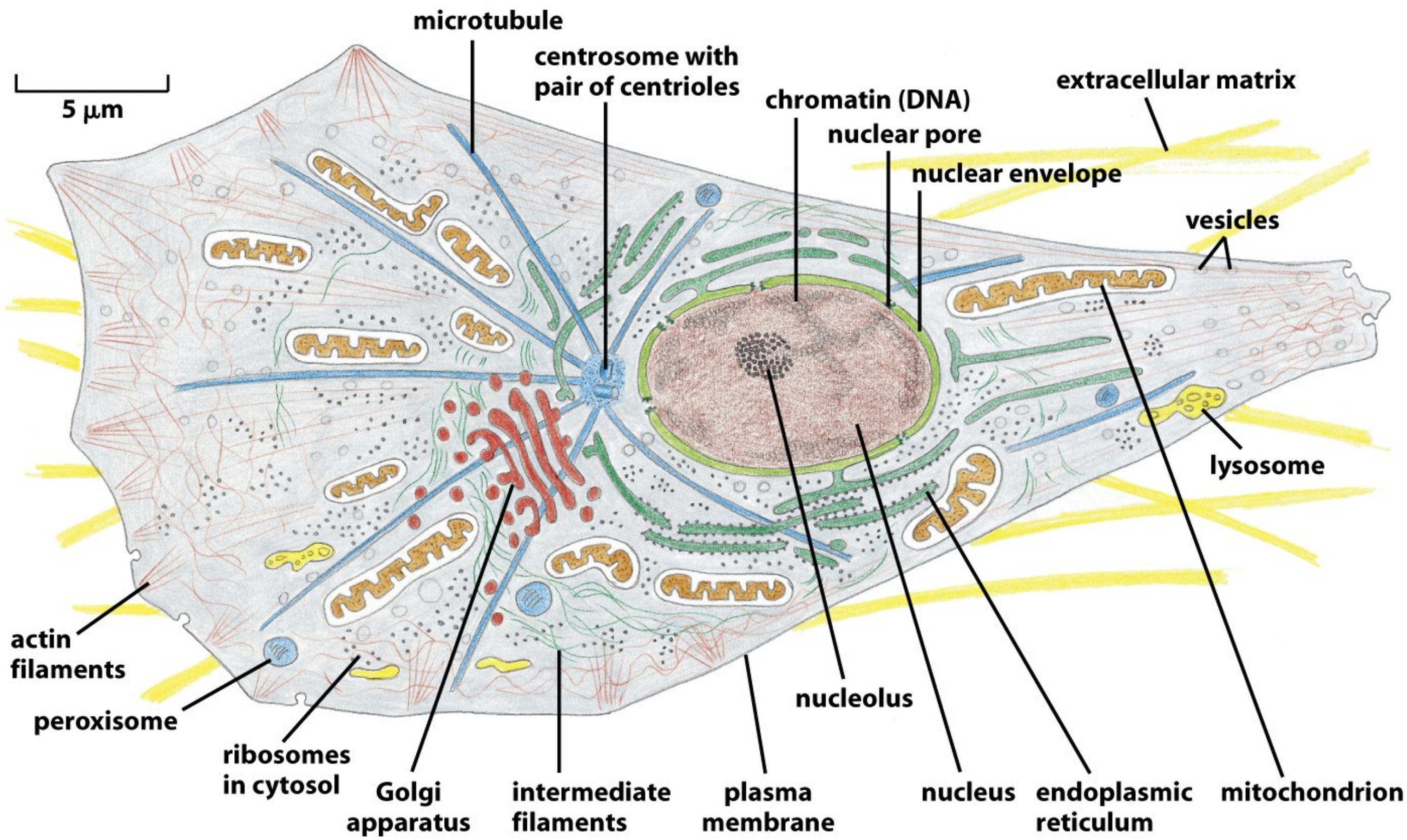
Examples of Lipid Phosphorylation and Breakdown in Signaling that Contributes to the Functional Specificity of Cell Compartments

Membranes Produce Compartments, but Membranes Alone Do Not Generate Functional Specificity



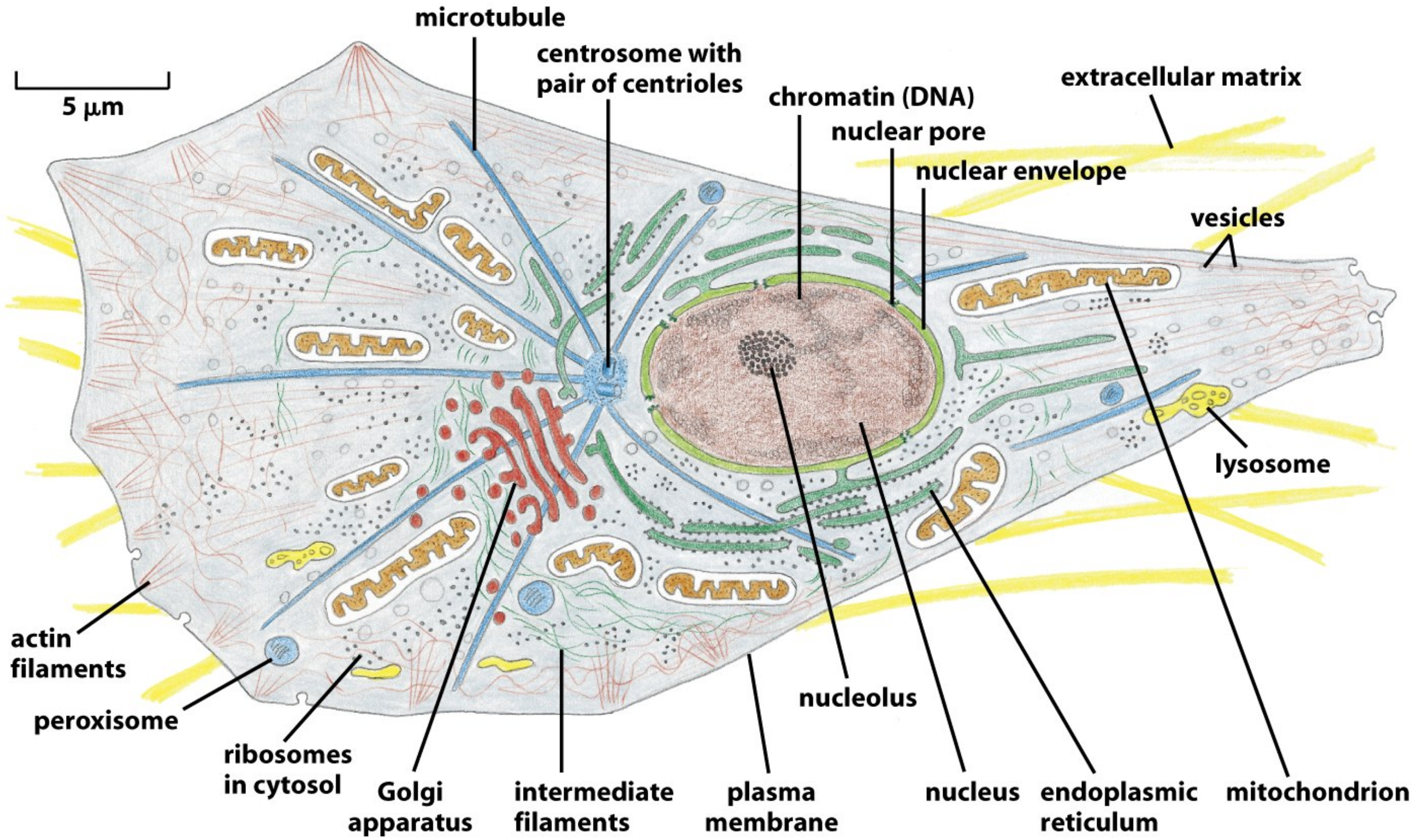
How does the cell produce both compartmental and functional specificity?

Each Intracellular Compartment has Unique Function(s) that are Conferred by Their Unique Structures.



Each compartment has its unique protein, glycan and lipid composition. How is this generated and maintained?

A Cell Must be Able to Selectively Target Proteins, Glycans, and Lipids to Different Compartments to Generate Compartmental Specificity.



How does a cell target molecules among intracellular compartments?

Lipid Phosphorylation and Breakdown In Signaling that Contributes to the Functional Specificity of Cell Compartments

Phosphatidylinositol (PI) and Phosphoinositide (PtdIns/PIP) Kinases and Phosphatases

Phospholipases

Phosphatidylinositol (PI) & Phosphoinositide (PtdIns or PIP)

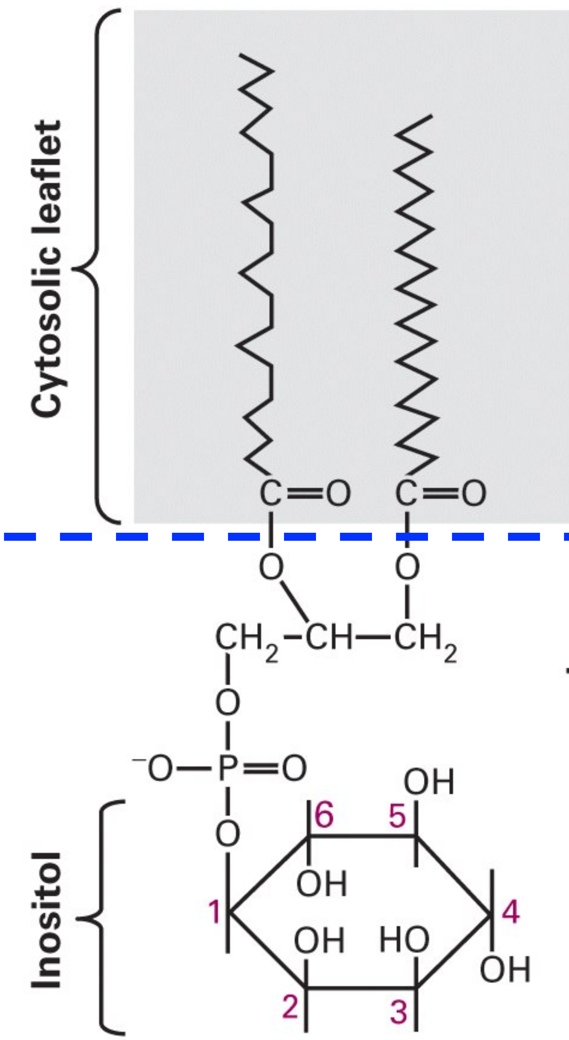
Primarily found in the cytoplasmic leaflets of plasma membrane or intracellular membranes.

Minor components of lipids (10% or less) but very effective signaling molecules.

TARGET TISSUE	SIGNAL MOLECULE	MAJOR RESPONSE
Liver	vasopressin	glycogen breakdown
Pancreas	acetylcholine	amylase secretion
Smooth muscle	acetylcholine	muscle contraction
Blood platelets	thrombin	platelet aggregation

Phosphatidylinositol (PI) is a phospholipid containing inositol as the hydrophilic head group

in the cytosolic leaflet of a lipid bilayer



Phosphatidylinositol (PI)

Tail

glycerol backbone

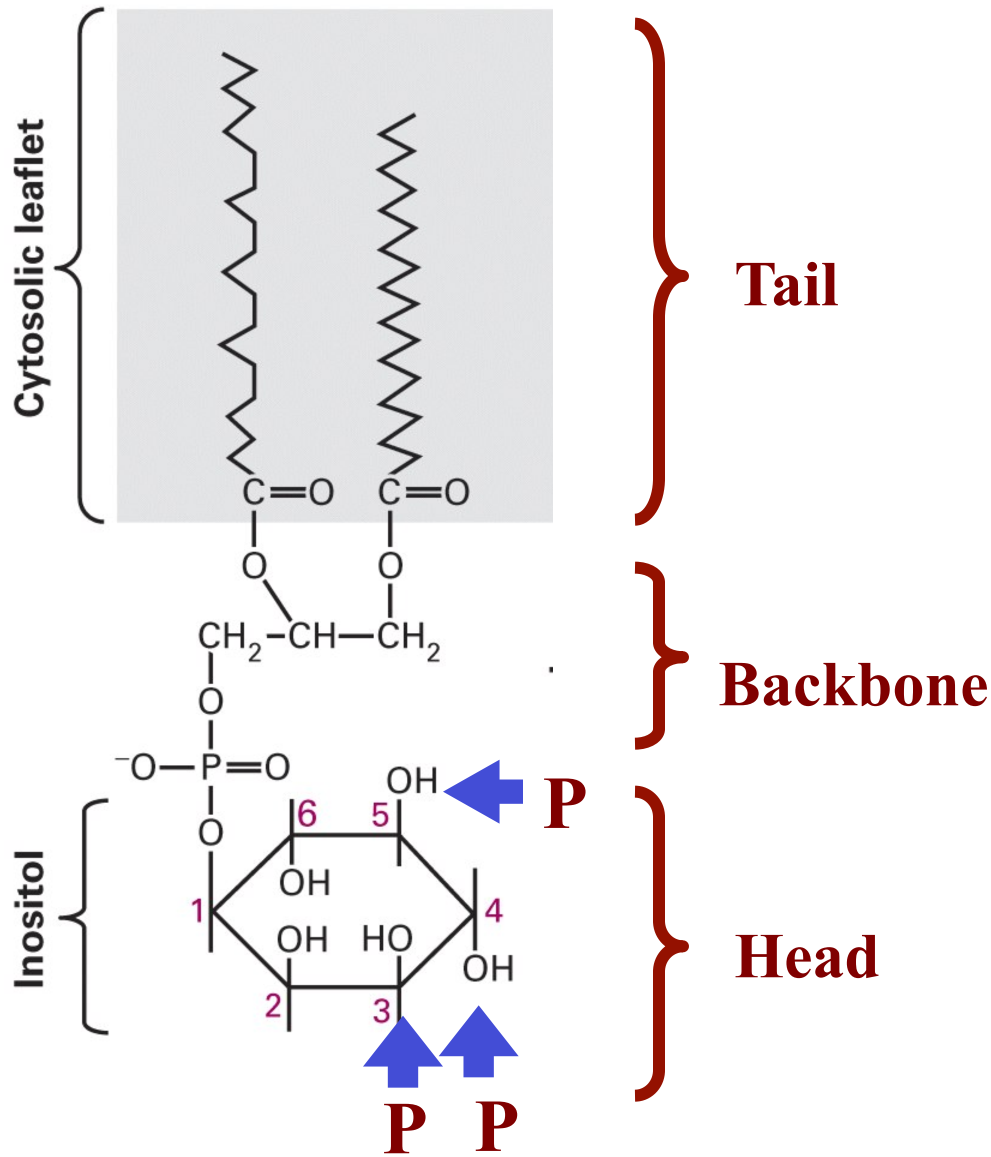
inositol head

in the cytosol

Inositol is a sugar with a ring composed of six carbons (1 to 6).

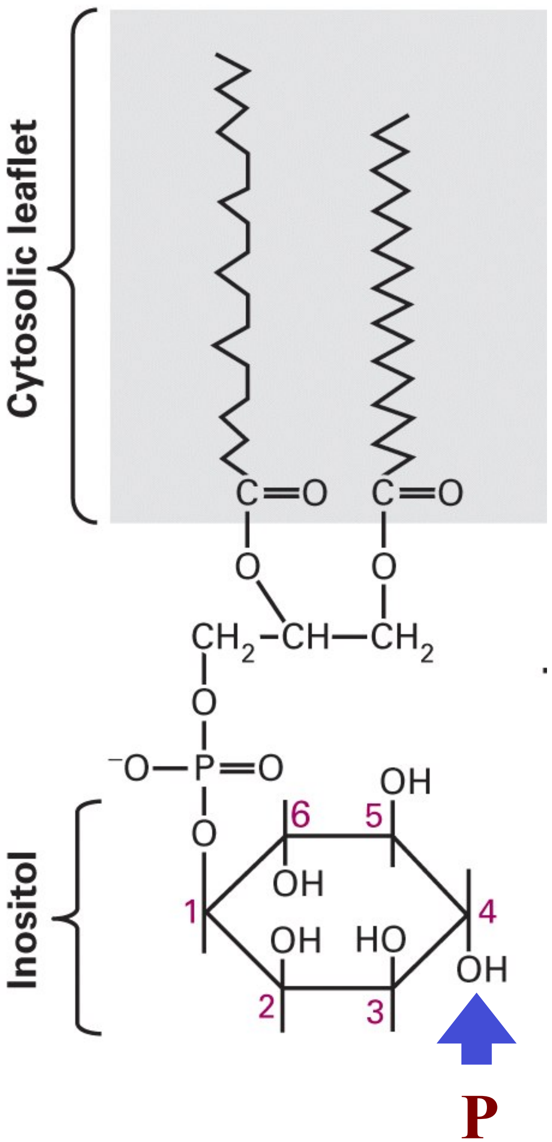
PI has the phosphate group at carbon position 1 only

Phosphoinositide (PtdIns or PIP) results from a PI phosphorylated at position 3, 4 or 5 (& various combinations) of the inositol ring, in addition to position 1

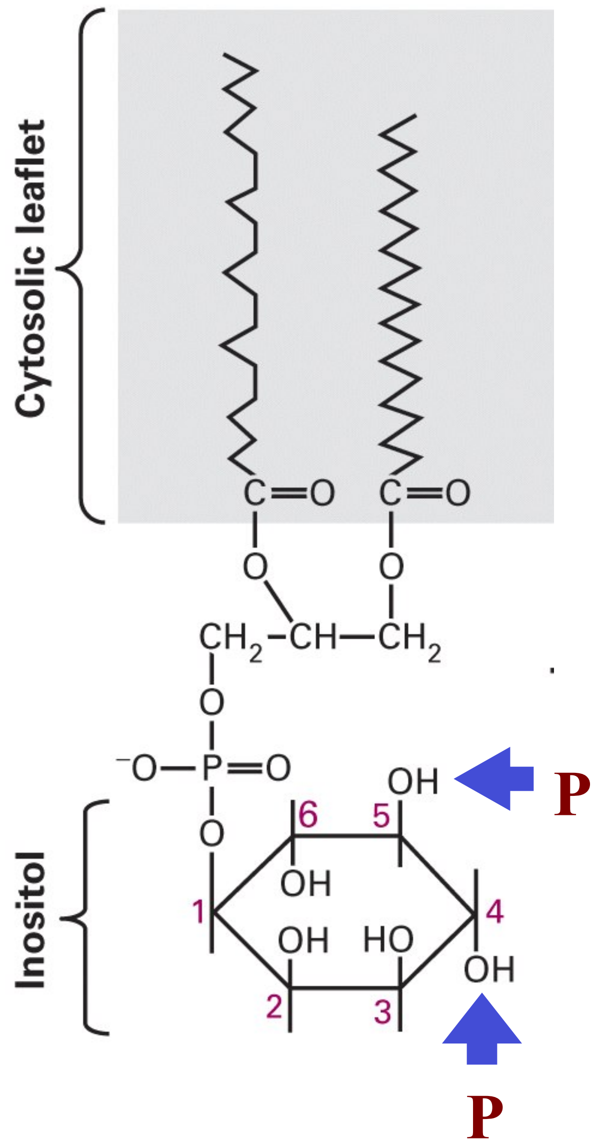


Depending on the Phosphorylated Position, there are Different Kinds of Phosphoinositides

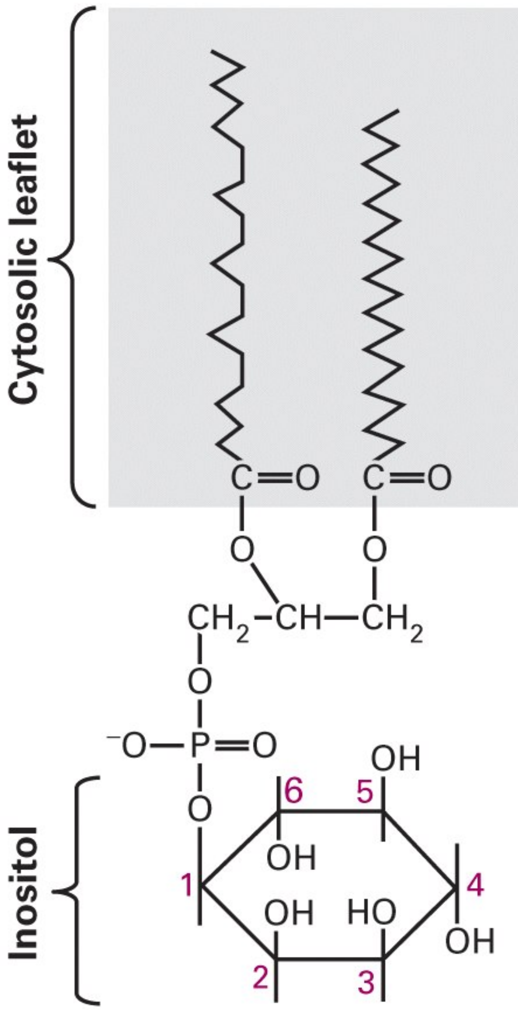
PI(4)P



PI(4,5)P2



PI/PIP Kinases and Phosphatases are Enzymes that Phosphorylate or Dephosphorylate PI/PIP, respectively

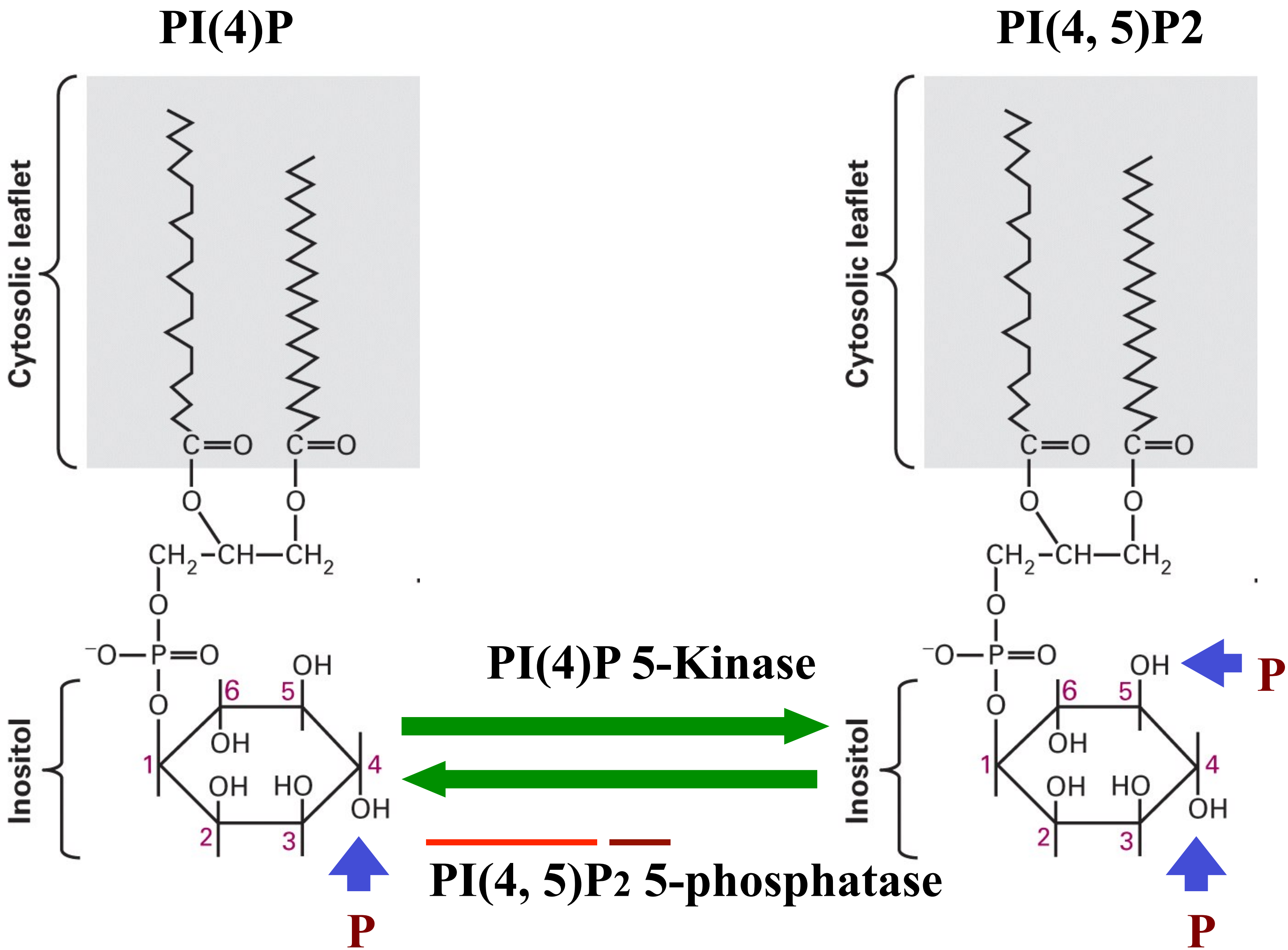


Phosphatidylinositol (PI)

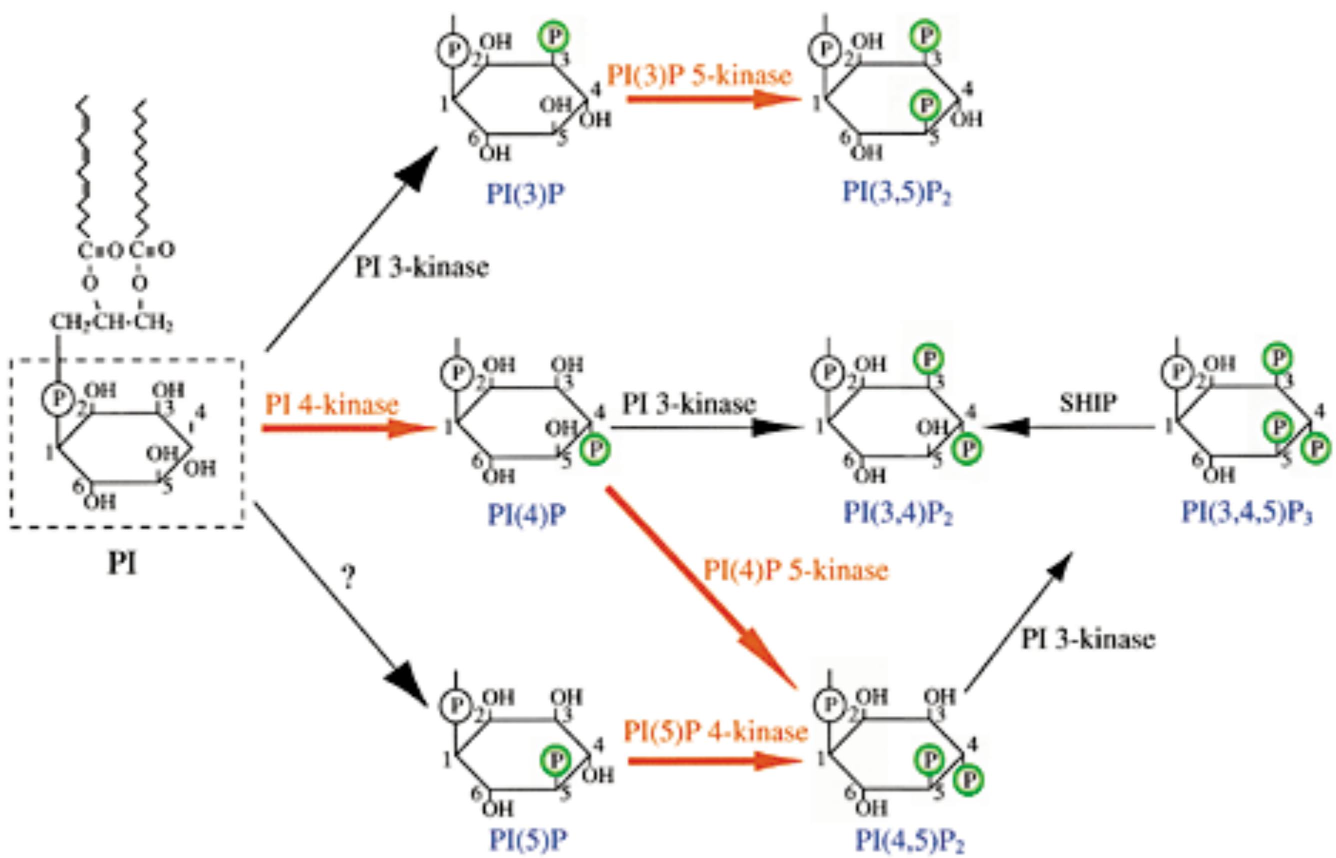
1. The substrates of PI/PIP kinases & phosphatases are lipids (not proteins).

2. Different PI/PIP kinases & phosphatases have different specificities for different carbon positions on the ring.

Different Kinases and Phosphatases Add and Remove the Phosphate Groups at Different Carbon Positions of the Inositol Ring of PI

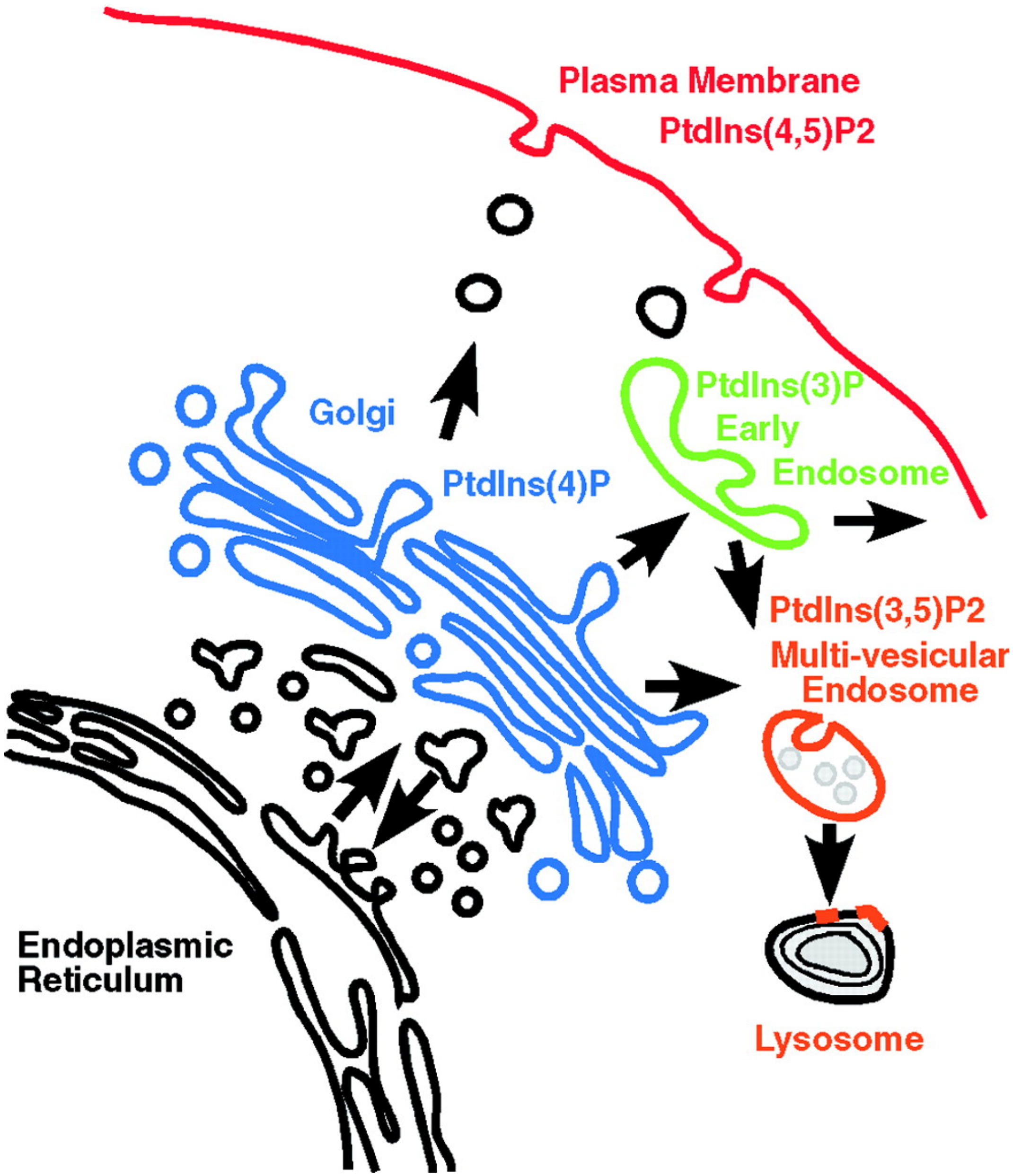


There are Seven Possible PI-Derived Phosphoinositides: The Conversions Between Them are Controlled by Different Kinases and Phosphatases (Only Kinases are Shown Here)



Different kinases recognize different substrates and add phosphate to different carbon positions of the inositol ring. The same is true for the phosphatases.

Different Kinases and Phosphatases Reside in Different Parts of the Cell: Therefore Different PIPs are Enriched in Different Parts of a Cell



How Do Phosphoinositides (PIPs) Contribute to Cell Compartment-Specific Signaling?

Different PIP species are enriched at different compartments

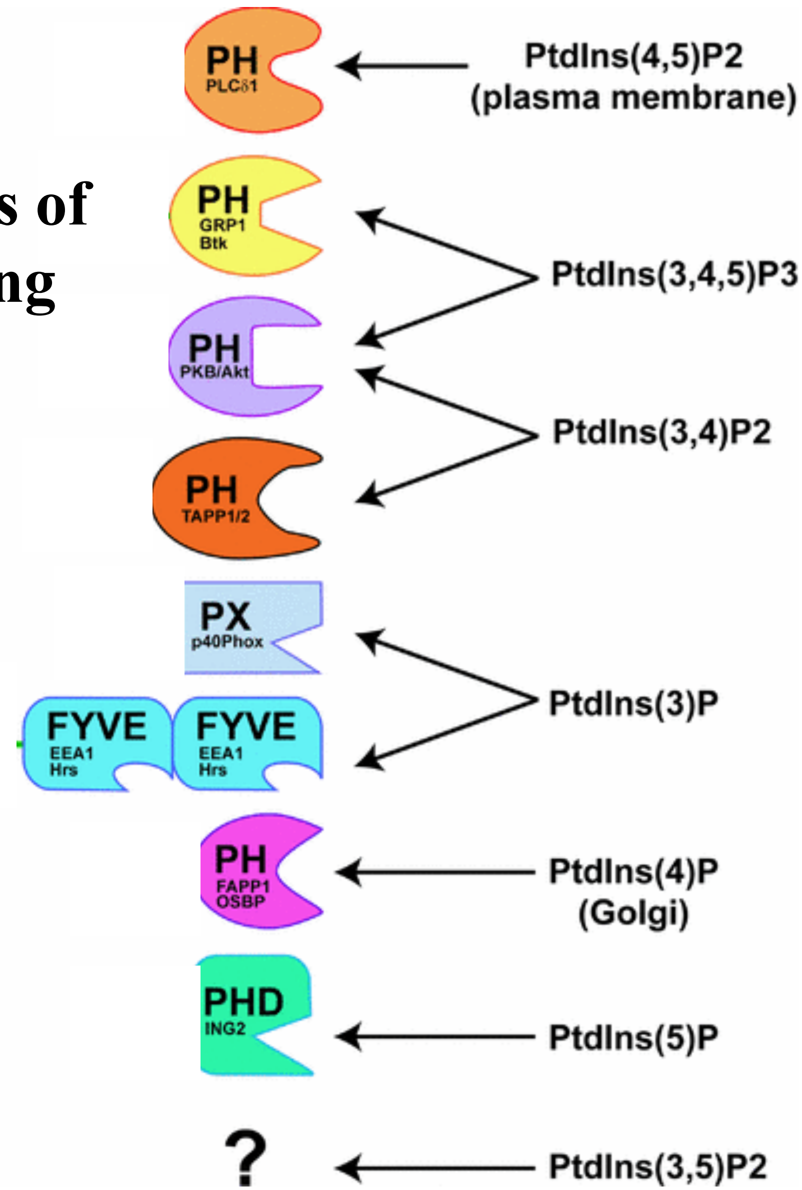
↓

Different PIP-binding proteins are recruited to different compartments to initiate different signaling events.

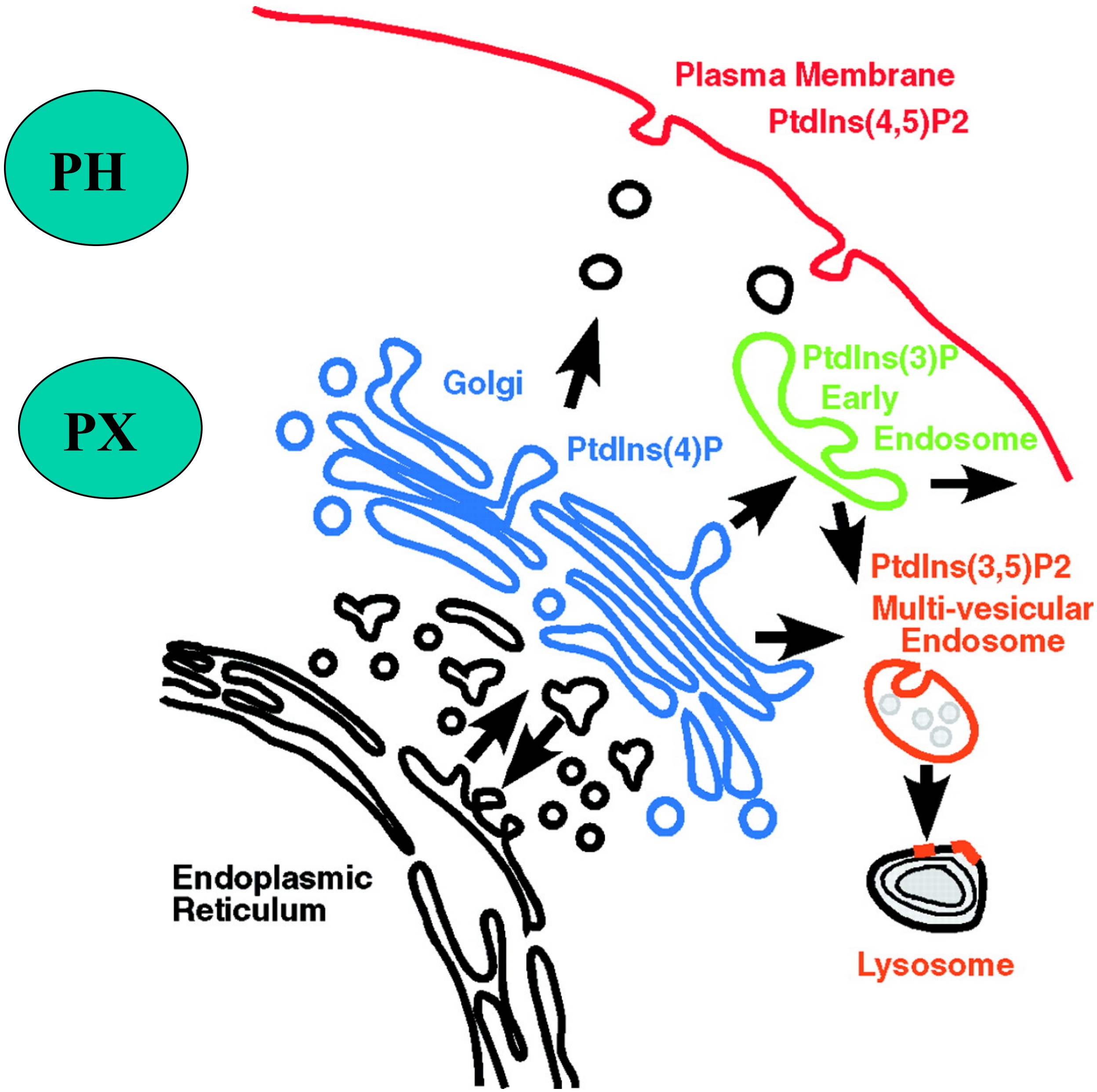
There are Many Types of PIP-Binding Protein Domains: Each Domain Recognizes a Specific Type or Types of PtdIns/PIP

PH, PX, FYVE, and PHD are names of different protein domains recognizing different species of PIP

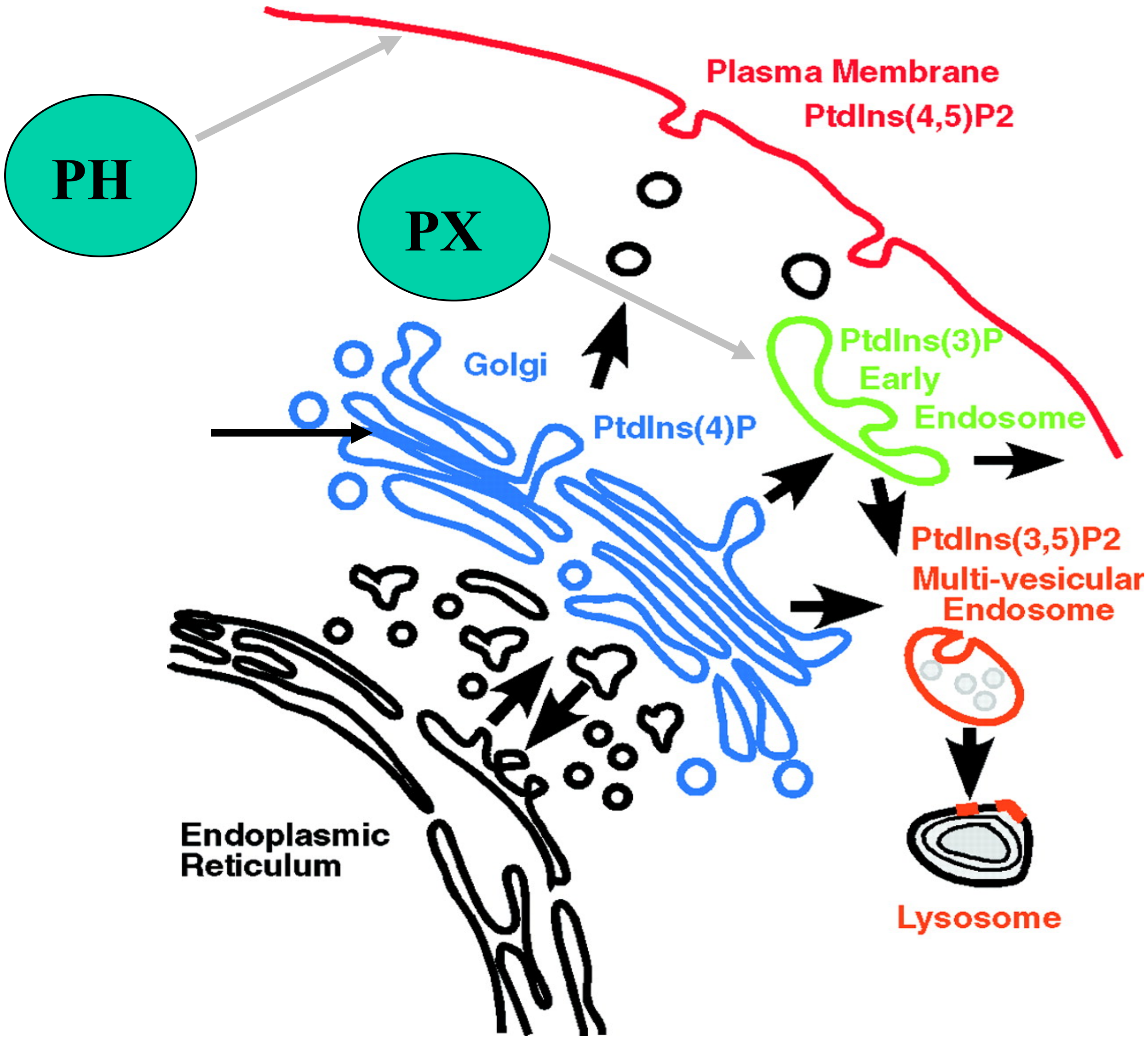
e.g. PX domain recognizes PI(3)P, whereas one type of PH domain recognizes PI(4,5)P2



Where Do You Expect to Find a Protein Containing a PH Domain Recognizing PI(4,5)P2) and a Protein Containing a PX Domain Recognizing PI(3)P)?

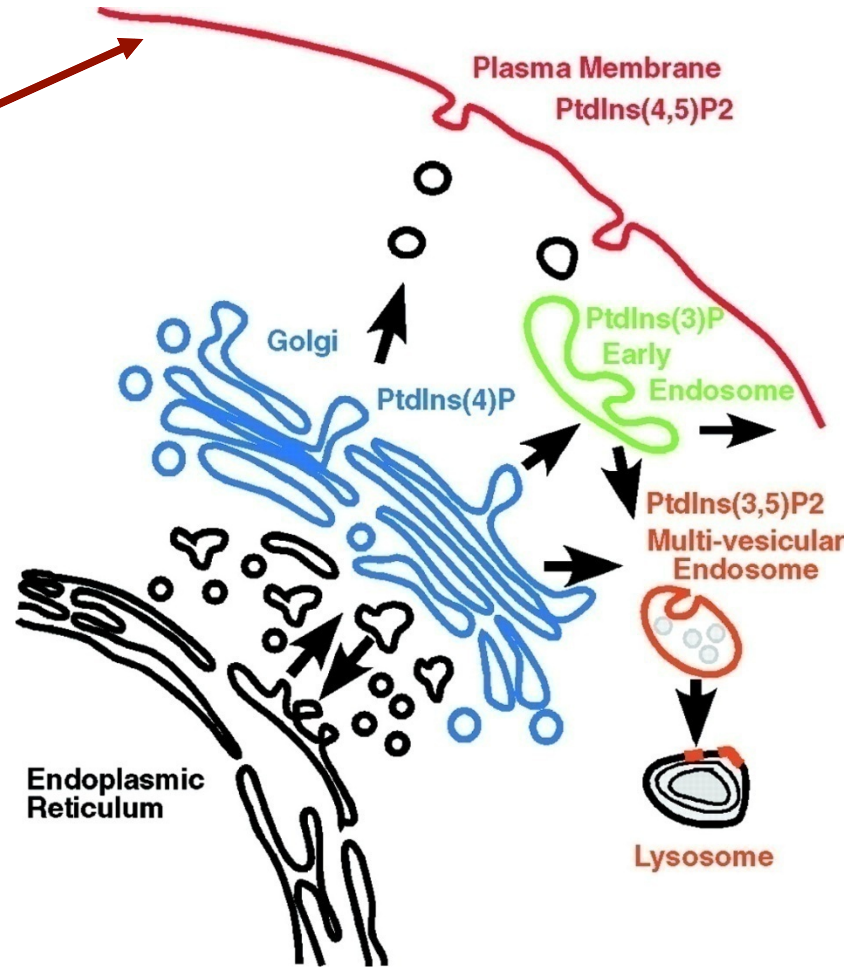


Where Do You Expect to Find a Protein Containing a PH Domain Recognizing PI(4,5)P2) and a Protein Containing a PX Domain Recognizing PI(3)P)?



Proteins with Different PIP-Binding Domains are Recruited to Different Compartments of a Cell

clathrin



Clathrin is an important protein that functions at the plasma membrane.

Clathrin is recruited to the plasma membrane because it contains a PH domain which can specifically recognize PI(4,5)P2.

PI and PtdIns/PIPs

1. Both PI and PIPs are phospholipids (not proteins).

2. PIPs are phosphorylated PI at positions 3, 4, and/or 5.

3. Both are anchored at the membrane (due to two fatty acid chains).

4. Different PI/PtdIns kinases and phosphatases have different subcellular compartmental localizations.

5. Different membranes have different compositions of PI and PIPs due to compartment-specific distribution of PI/PIP kinases and phosphatases.

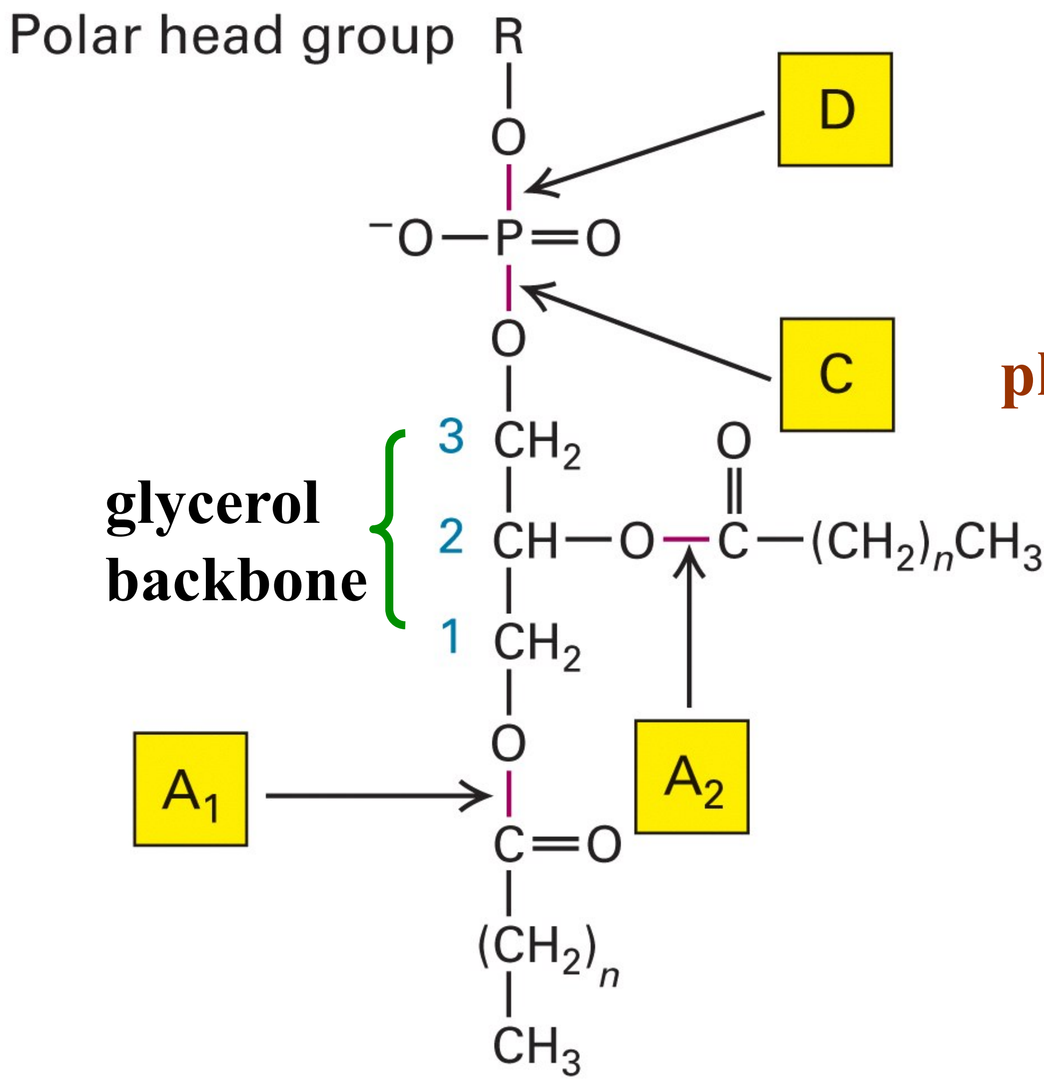
Lipid Phosphorylation and Breakdown In Signaling that Contributes to the Functional Specificity of Cell Compartments

Phosphatidylinositol (PI) and Phosphoinositide (PtdIns) Kinases and Phosphatases

Phospholipases

Phospholipases are Enzymes that Cleave and Convert Phospholipids to Fatty Acids and PIPs

Four types of phospholipases, A1, A2, C, and D (PLA1, PLA2, PLC and PLD). They hydrolyze phosphodiester or ester bonds.



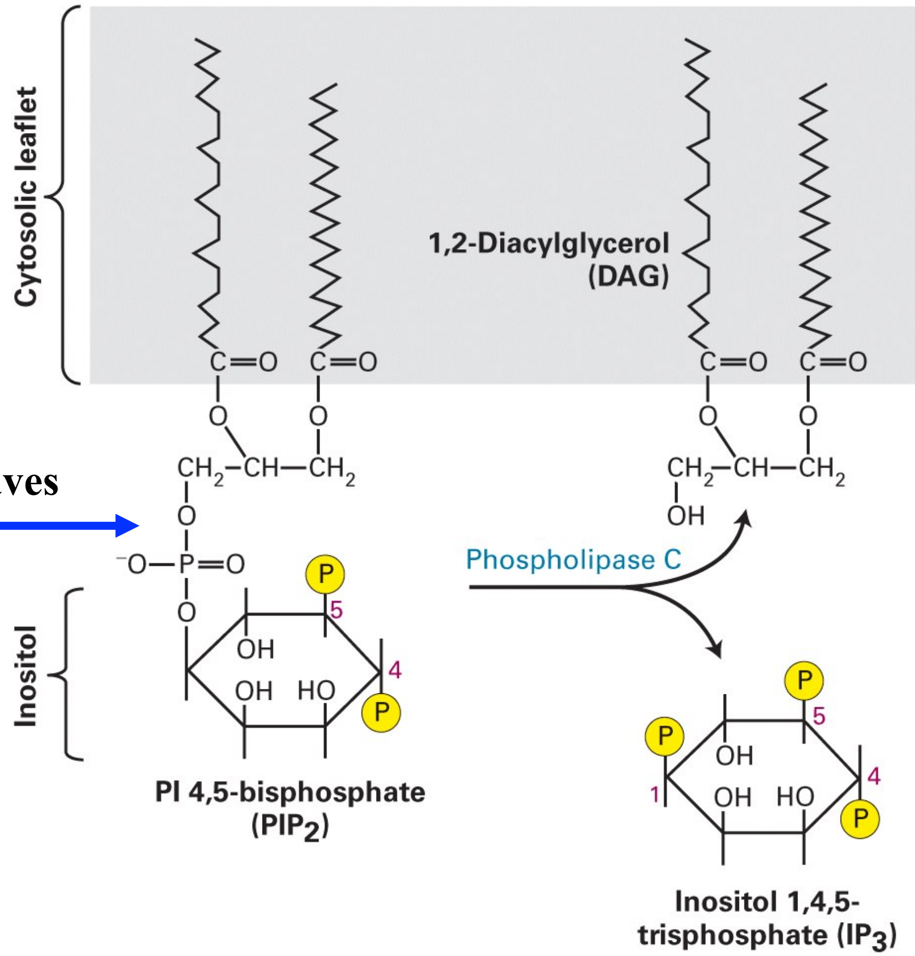
phospholipase C (PLC)

How Does PLC Mediate Signaling?

binding of ligands to specific receptors at the cell surface

PLC is activated

cleaves



signaling at the plasma membrane

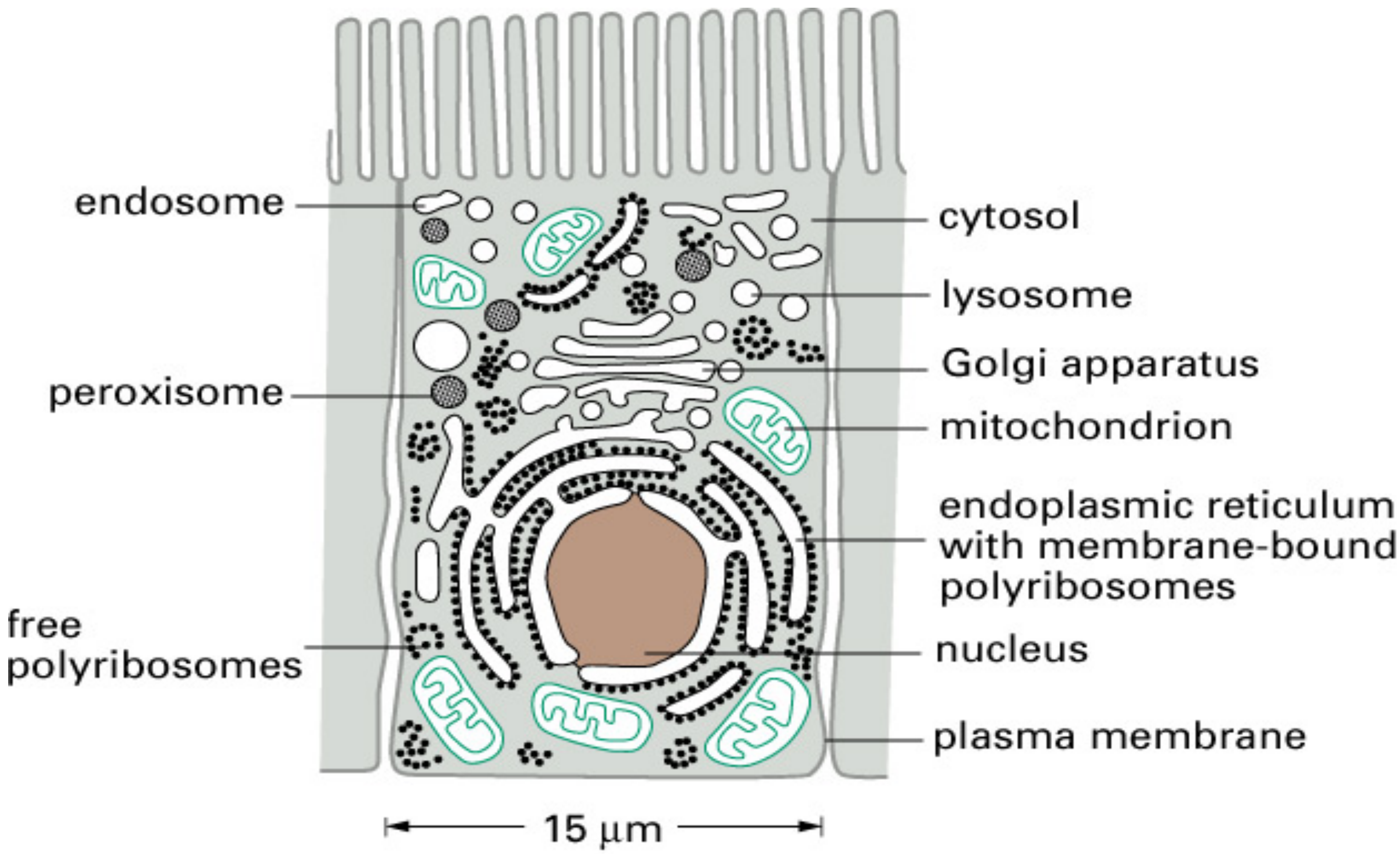
DAG is a membrane-bound signaling molecule

IP3 is a soluble freely diffused signaling molecule

signaling in the cytosol

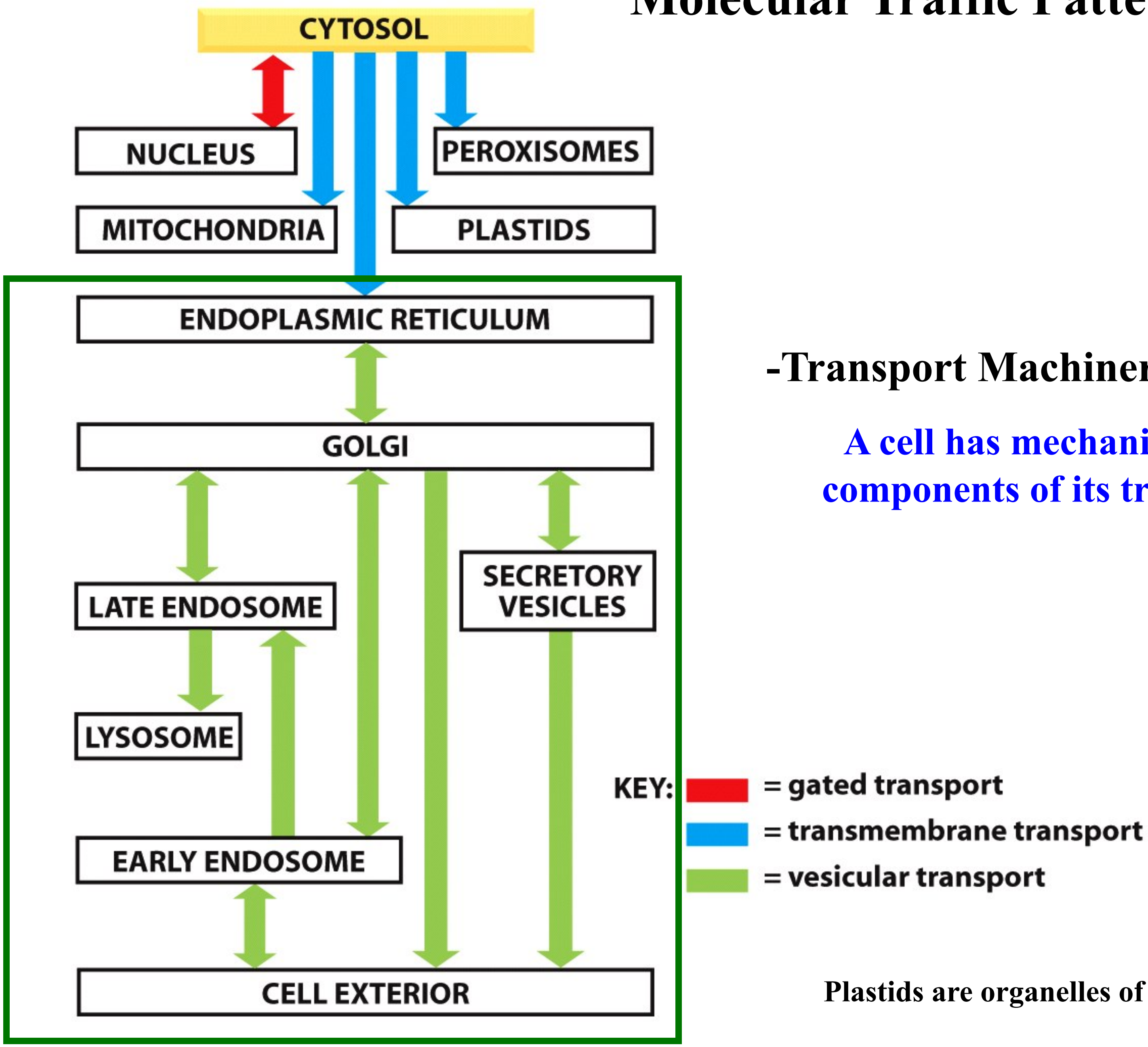
Intracellular Transport: Overview

Each Compartment of the Cell has a Unique Composition



Given that the synthesis of almost all proteins begin in the cytosol, how does a cell create different intracellular compartments via selective targeting of proteins?

Molecular Traffic Patterns in the Cell

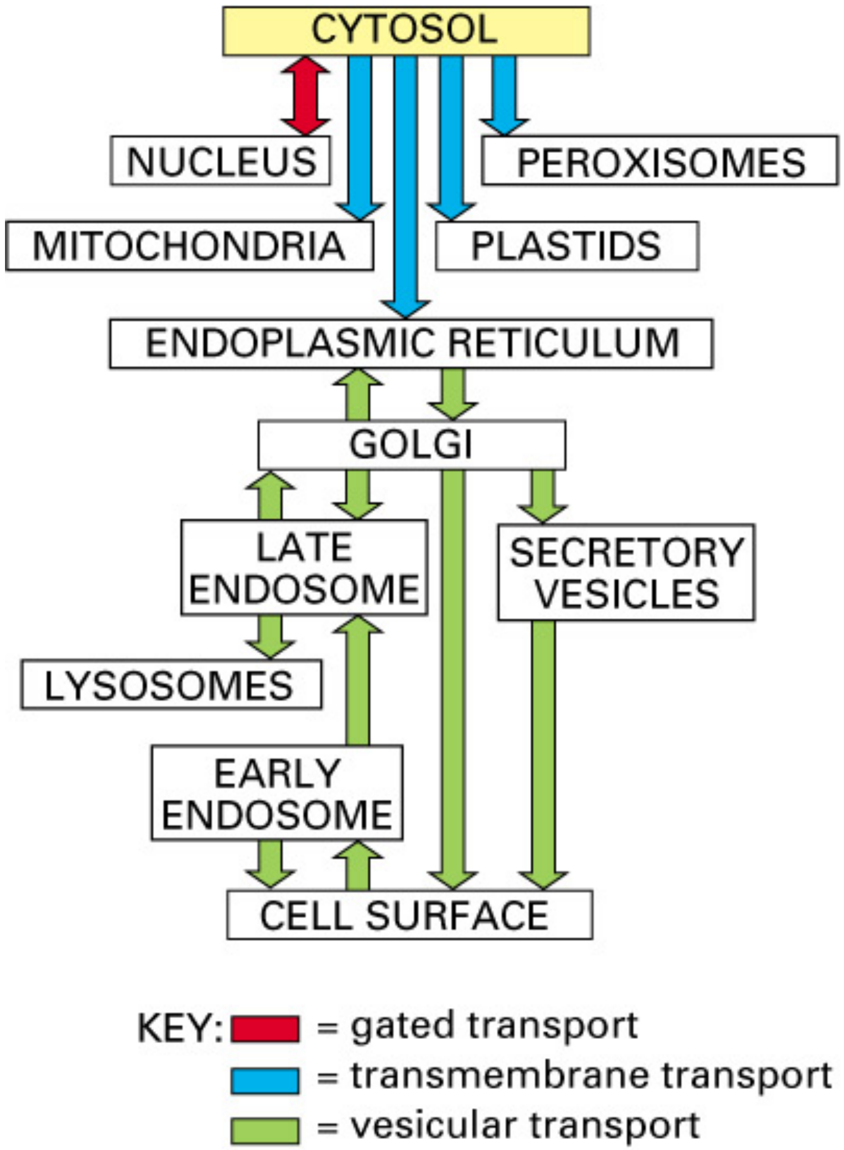


-Transport Machinery is Often Re-Used

A cell has mechanisms to recycle the components of its transport machinery

Plastids are organelles of plants and algae.

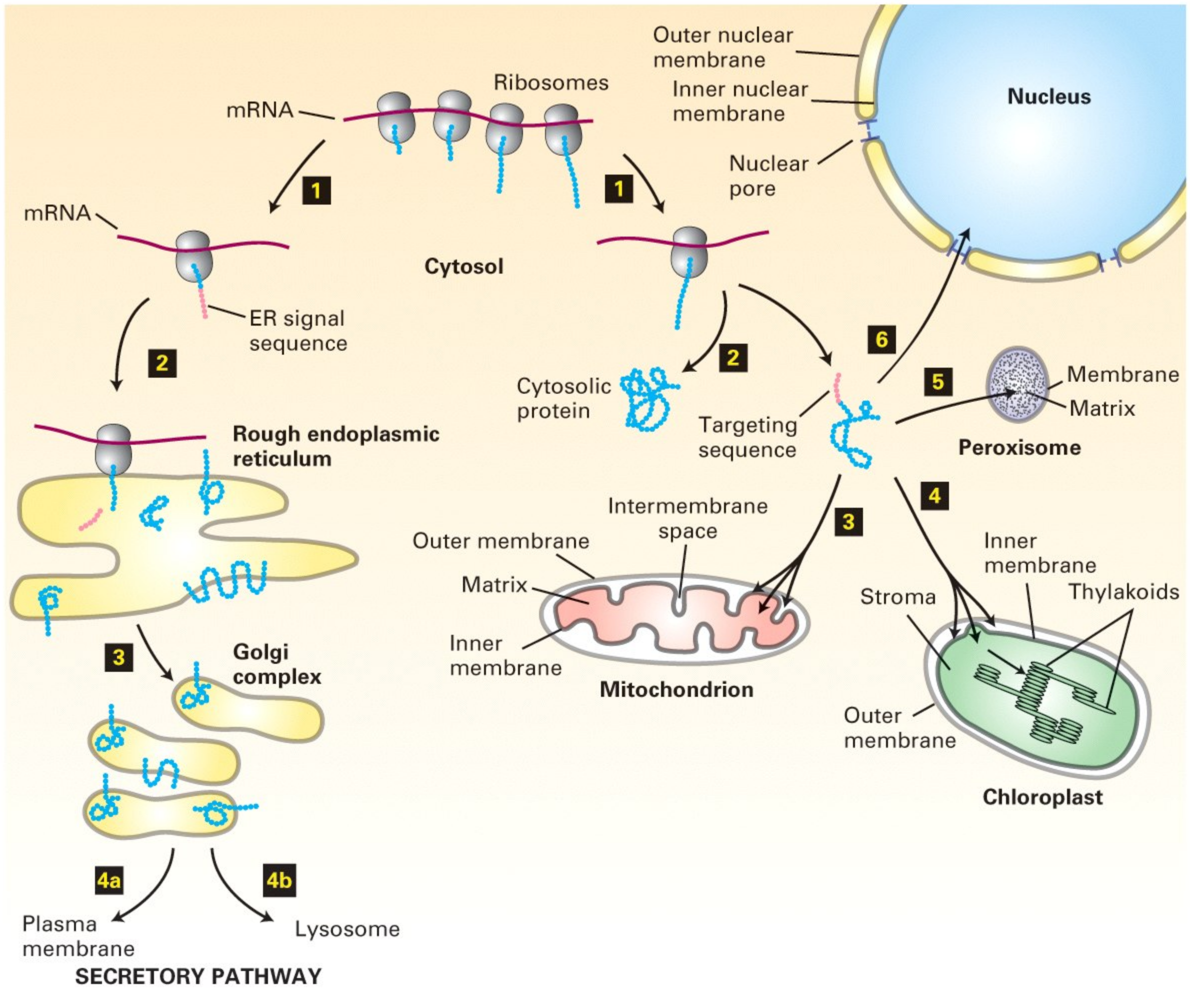
Three Mechanisms of Intracellular Transport



gated transport:
transport via a gated pore between two compartments

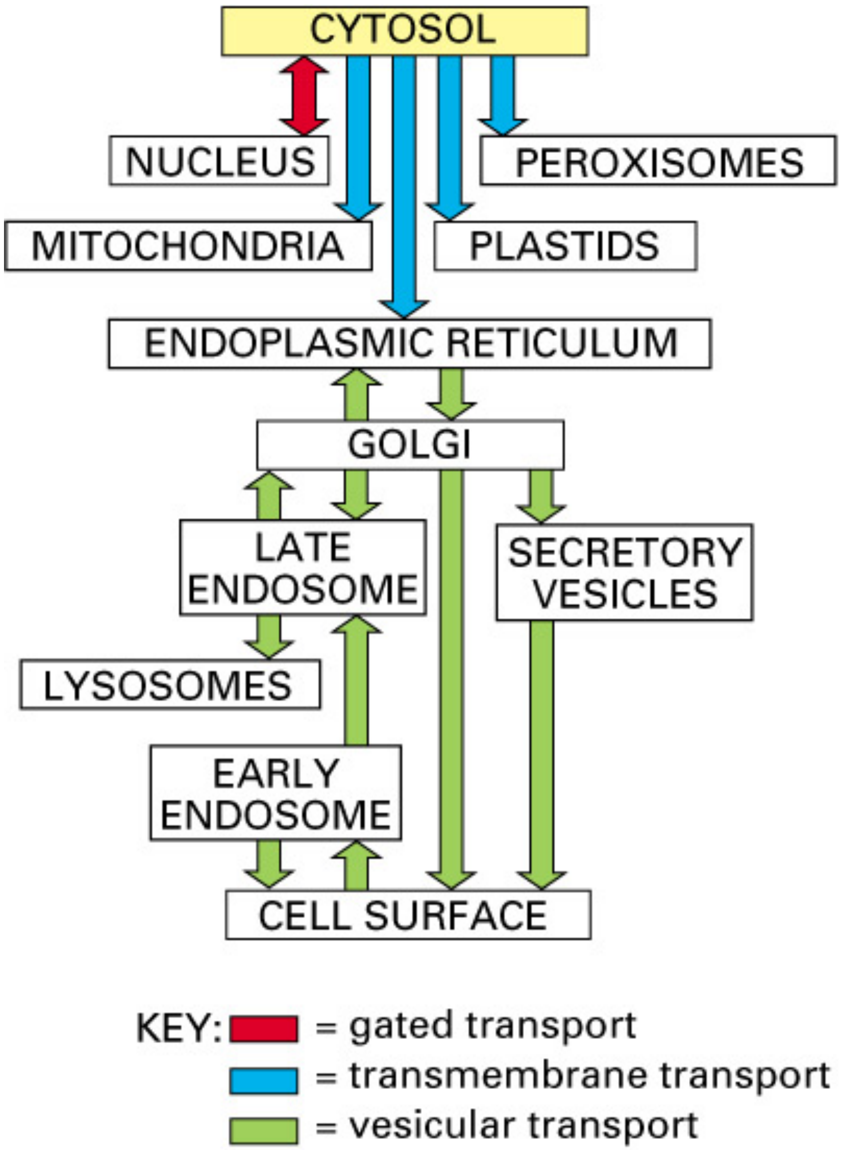
Gated Transport:

Molecular transport between the cytosol and the nucleus



NPC represents gated transport (in this case small vs large molecules)

Three Mechanisms of Intracellular Transport

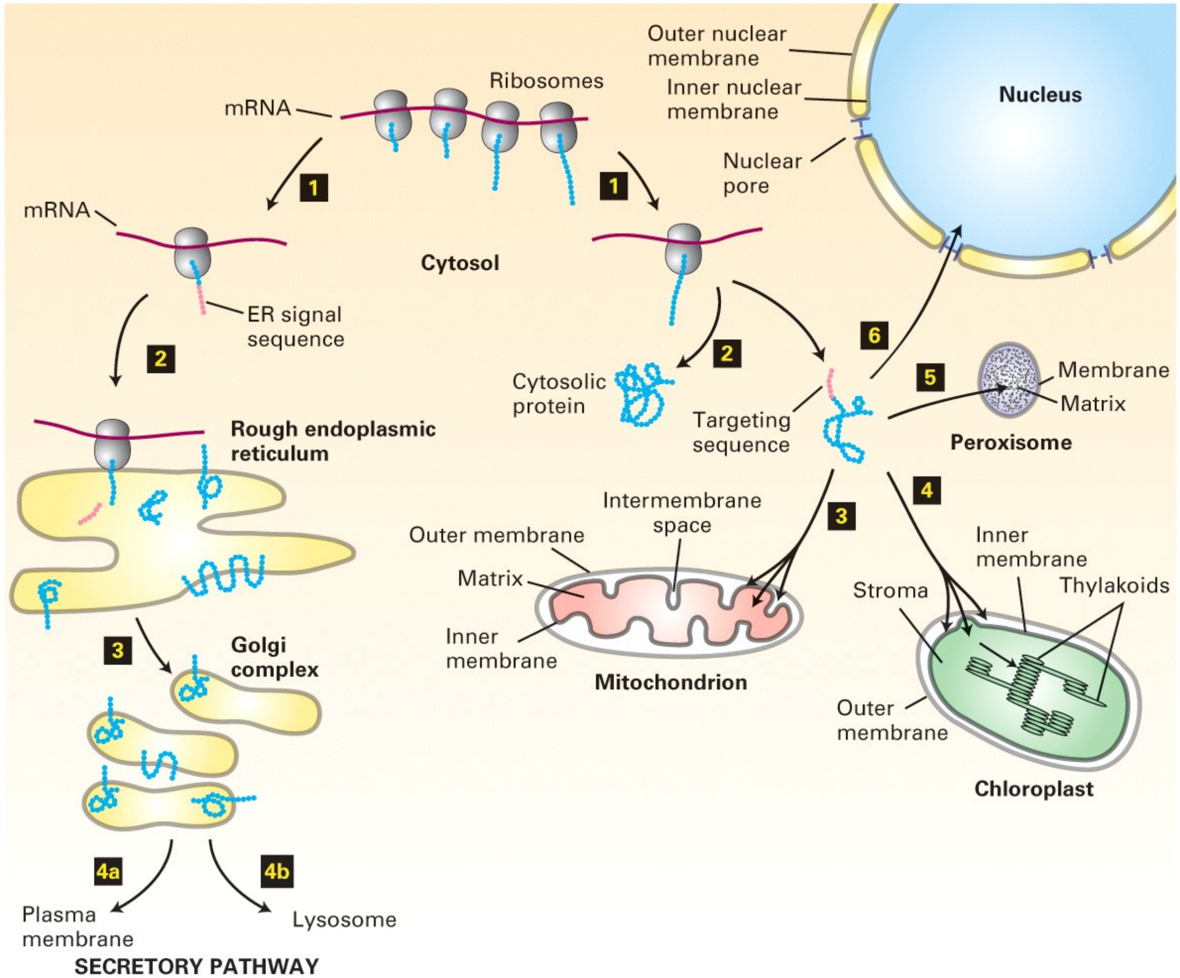


gated transport:
transport via a gated pore between two compartments

transmembrane transport:
transport between two compartments mediated by a protein or protein complex called a translocator

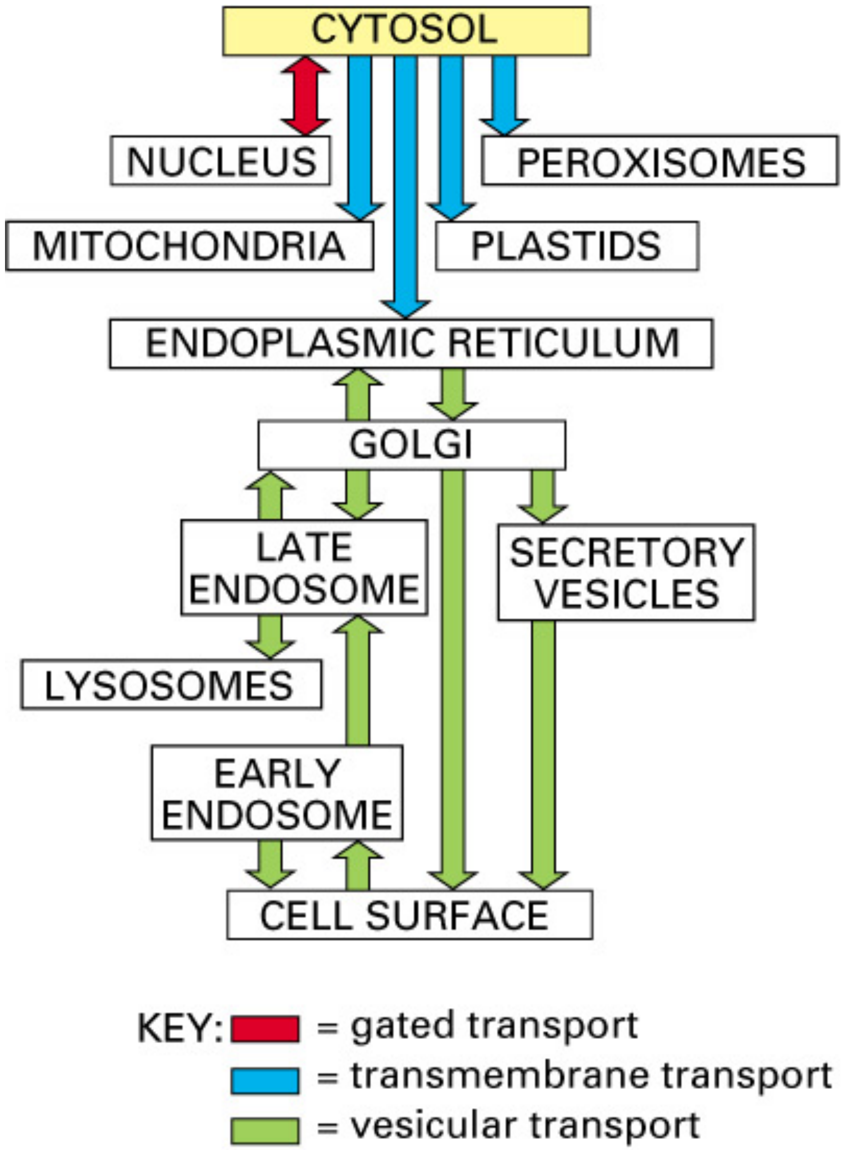
Transmembrane Transport:

between cytosol and ER, between cytosol and mitochondrion, between cytosol and peroxisome, between cytosol and chloroplast



Small hydrophilic molecules or proteins cannot move in & out freely without the help of a translocator

Three Mechanisms of Intracellular Transport

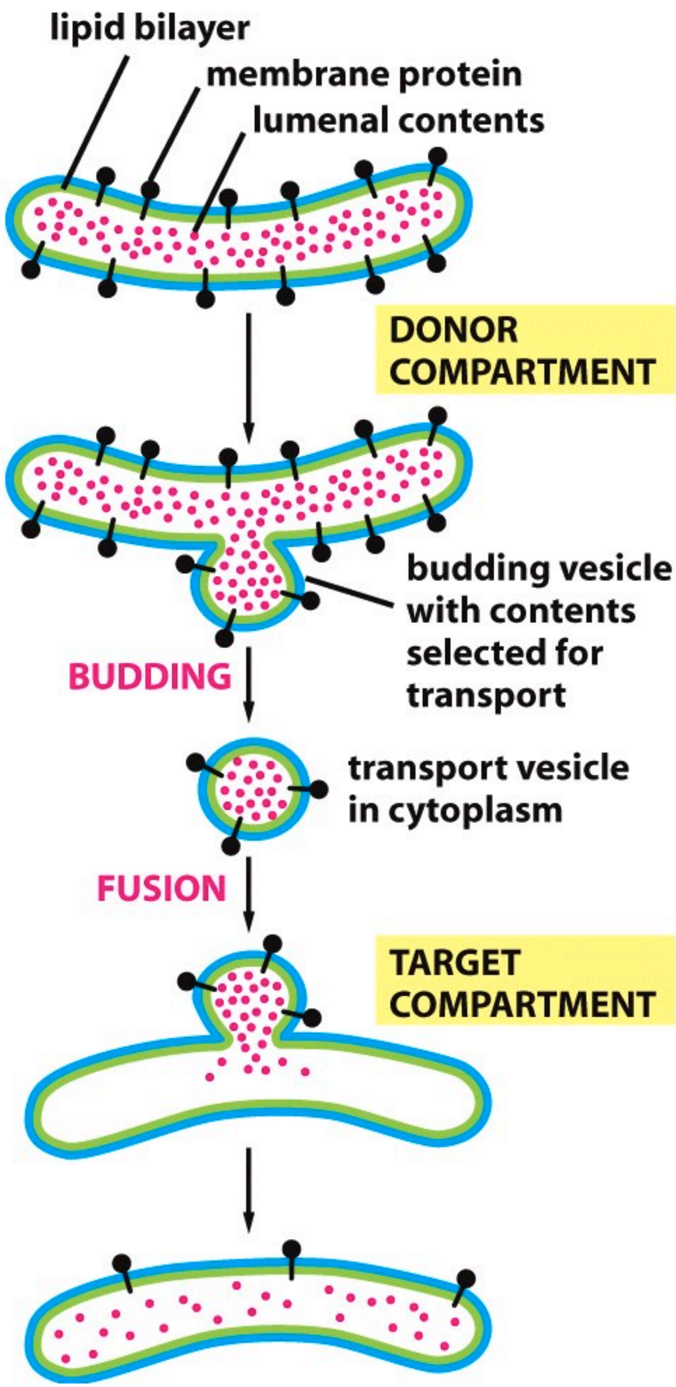


gated transport:
transport via a gated pore between two compartments

transmembrane transport:
transport between two compartments mediated by a protein or protein complex called a translocator

vesicular transport:
transport from one compartment (donor) to another compartment (target) by a membrane-enclosed transport intermediate in the form of vesicle or tubule

gated and transmembrane transports are non-vesicular transports



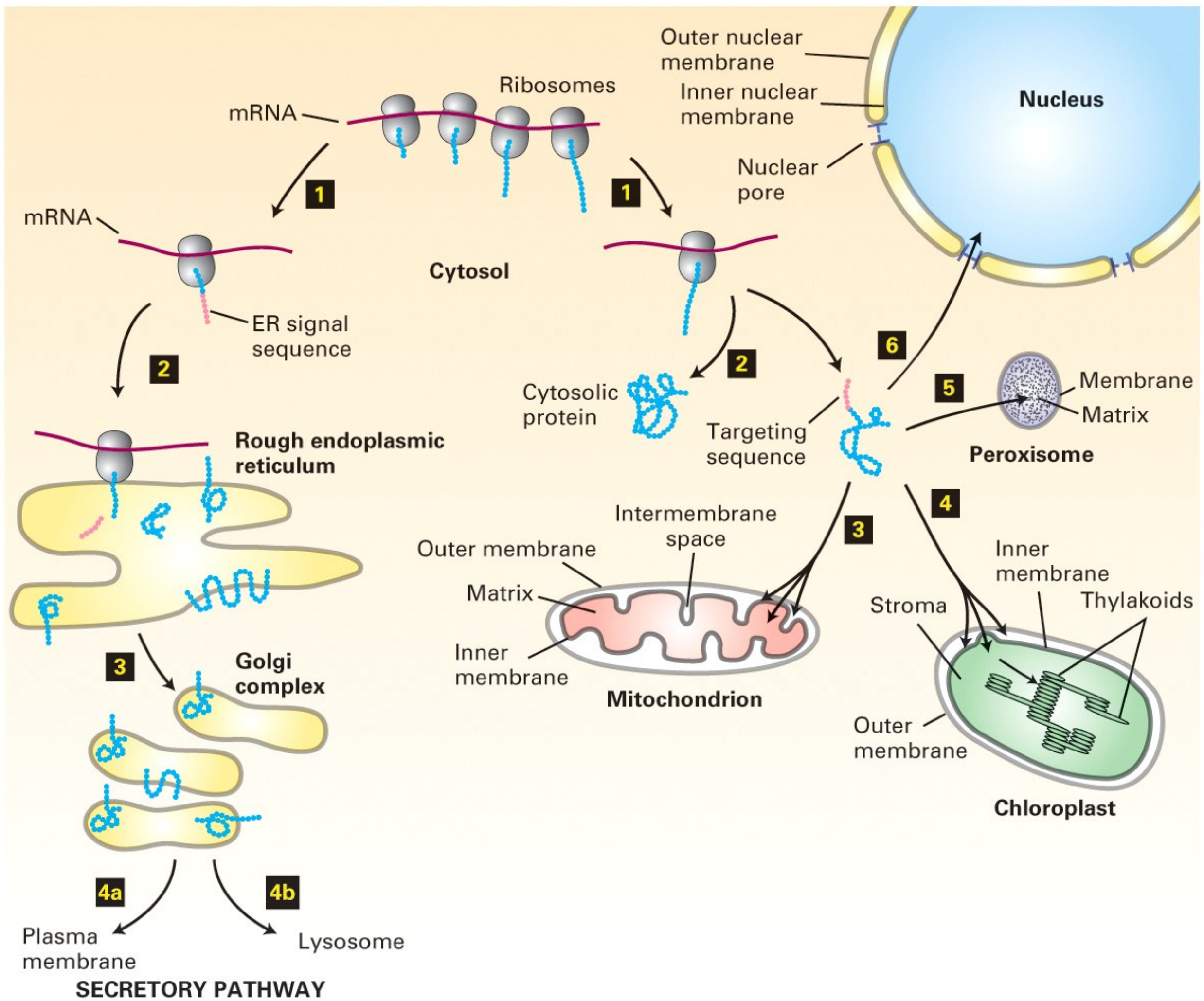
Vesicular Transport:

between cytosol and ER, between cytosol and mitochondrion, between cytosol and peroxisome, between cytosol and chloroplast

Vesicular Origins and Fate Overview

Vesicular Transport

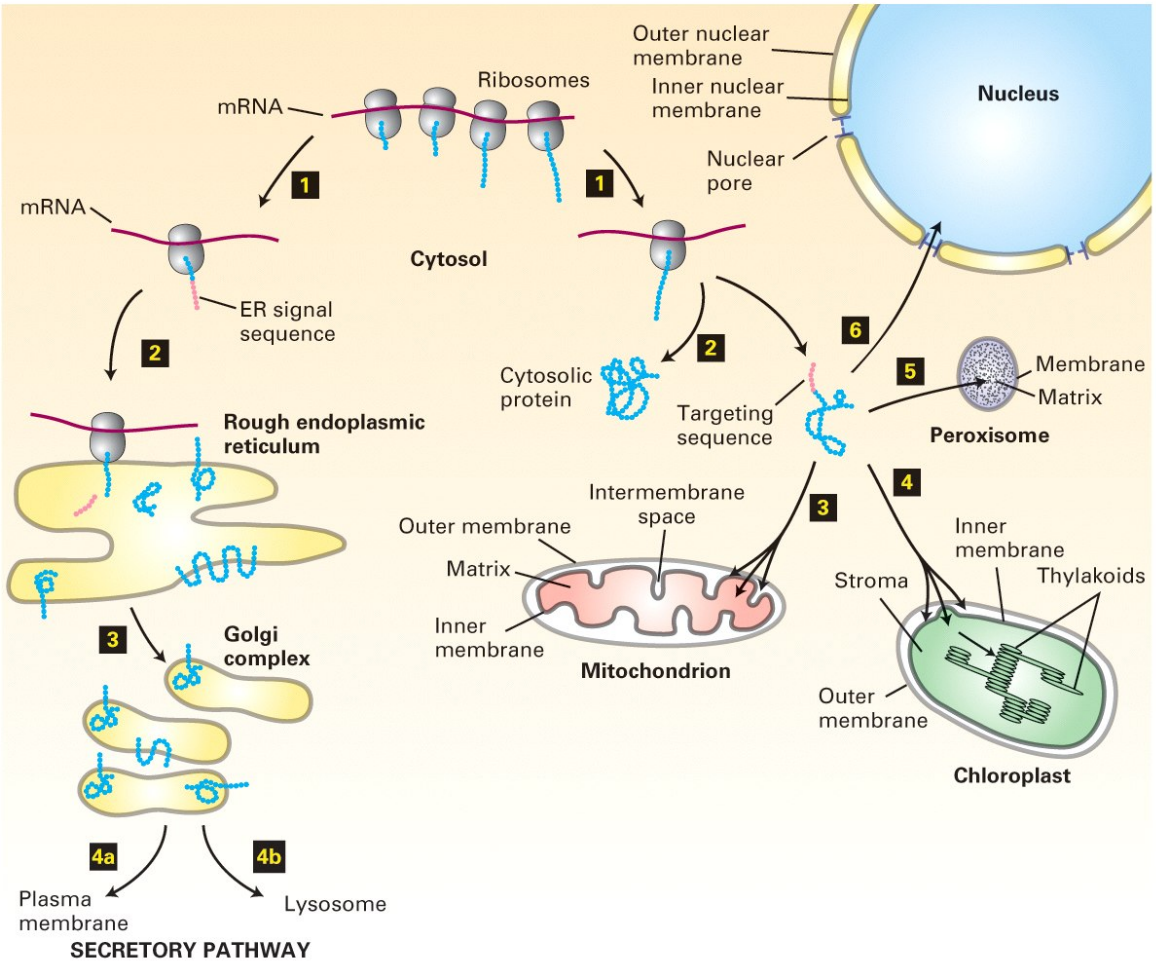
All transport events occur downstream of ER, including the transport between the following compartments ER, Golgi, plasma membrane, endosomes and lysosomes



Transport into Intracellular Organelles

originated from cytosol	Interior of nucleus:	gated transport	via nuclear pore
	Peroxisomes:	transmembrane transport	via translocators
	Mitochondria:	transmembrane transport	
	Chloroplasts:	transmembrane transport	
	ER: (including nuclear membrane)	transmembrane transport	
originated from ER	Golgi:	vesicular transport	via vesicles & tubules
	Endosomes:	vesicular transport	
	Lysosomes:	vesicular transport	
	PM	vesicular transport	
	Extracellular:	vesicular transport	

Transport Signals and Signal Receptors

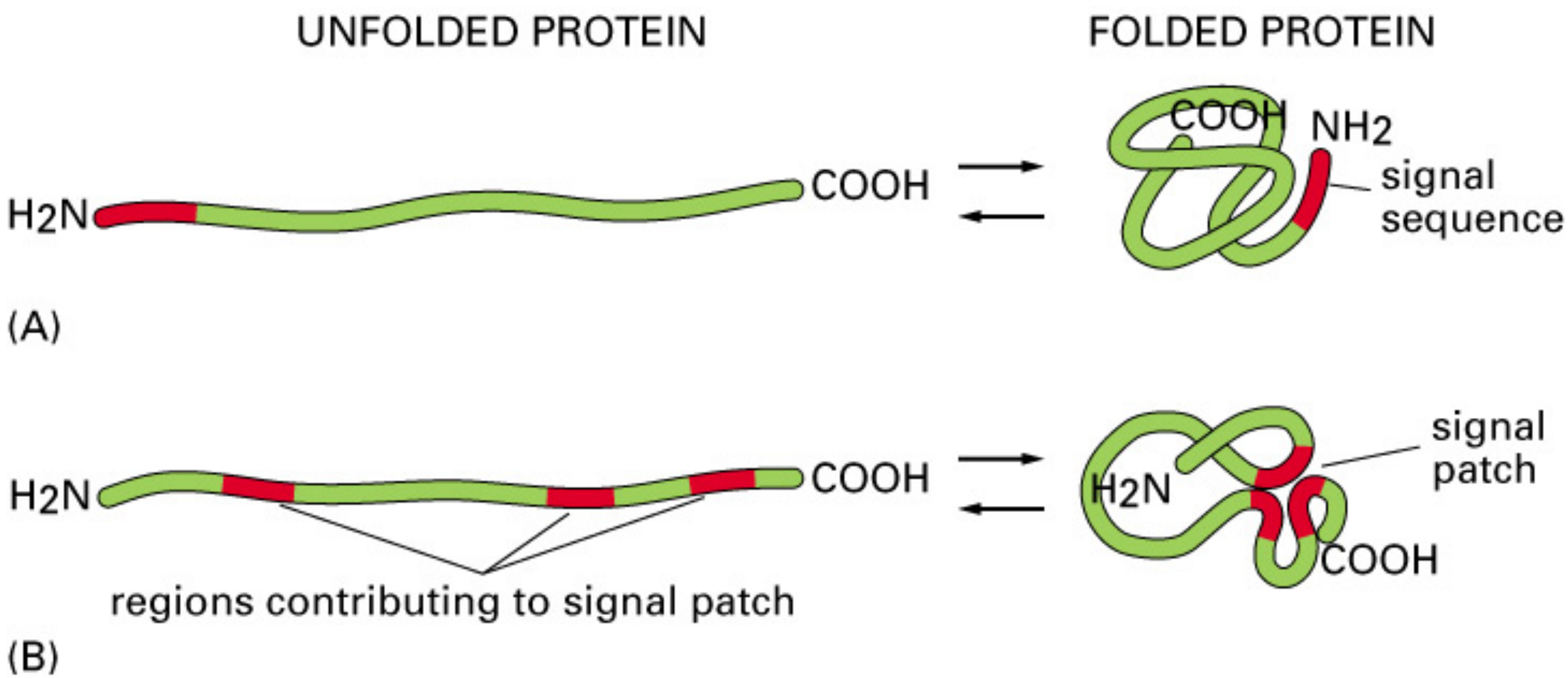


transport signals: found in a cargo protein

transport signal receptor: found on the surface of destination compartments

The interaction between signals and receptors confers transport specificity

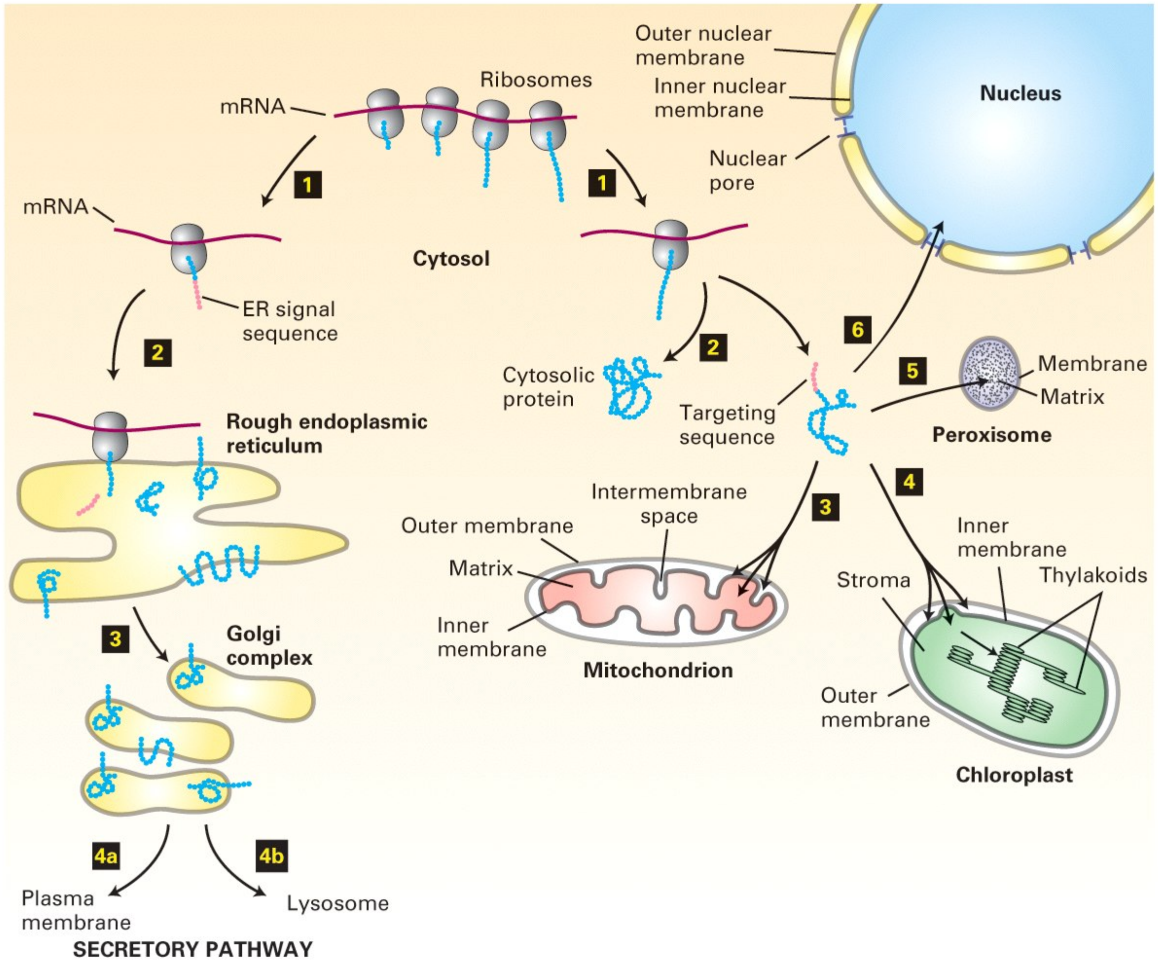
Two Types of Transport Signals



A signal sequence is a short stretch of amino acids
A signal patch is formed by the amino acids located with different parts of a cargo protein which only come into proximity in the 3-D folded state of a cargo protein.

Signal sequences are more common than signal patches

Co- and Post-Translational Transport



co-translational transport: cargo protein is in an unfolded state (associated with chaperones) during transport
post-translational transport: cargo protein can be unfolded (associated with chaperones) or folded