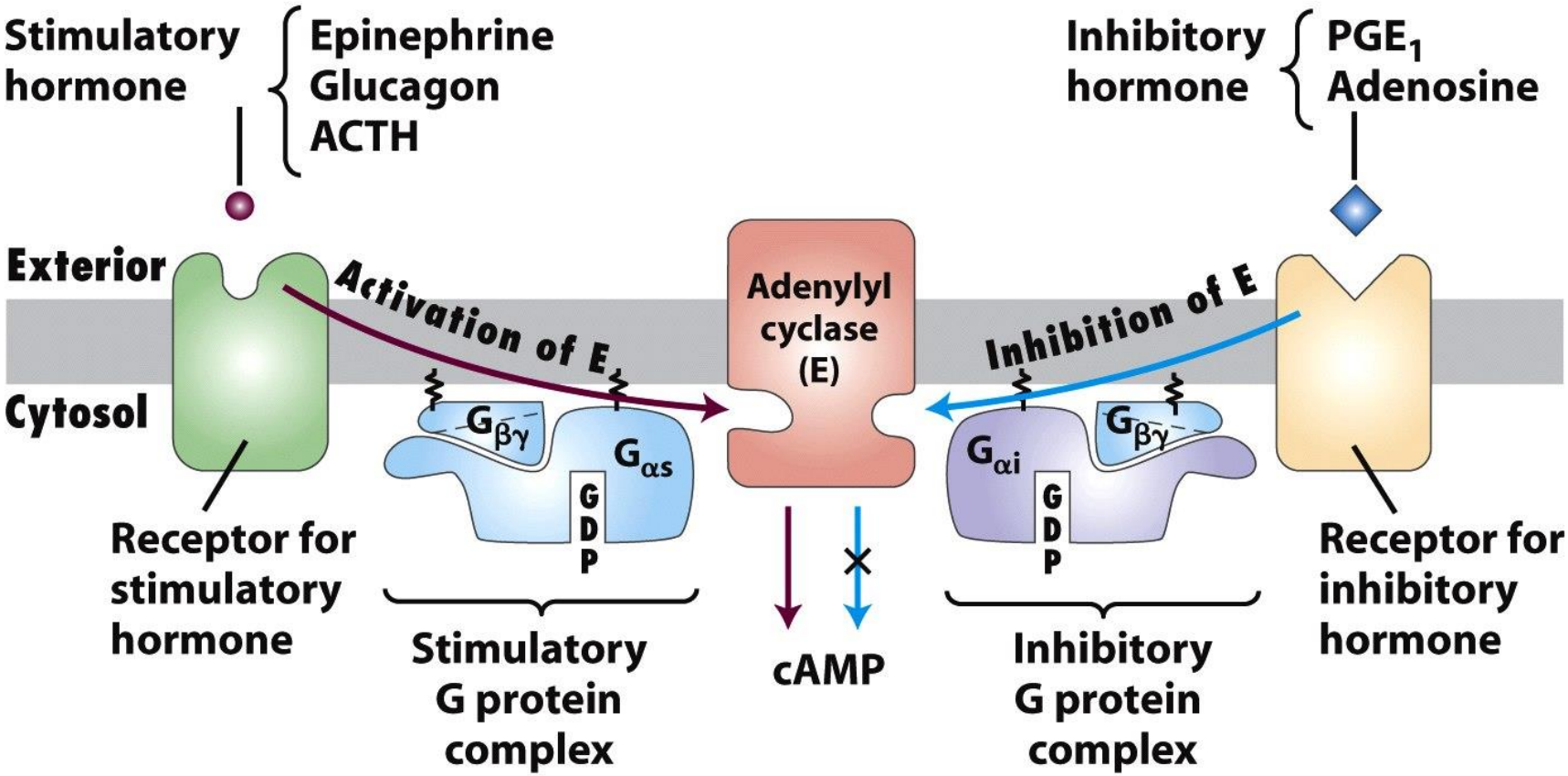
Chapter 15
Lecture 11

G protein Signaling

Protein Kinase A and cAMP pathway

G protein coupled activation or inhibition of Adenylyl cyclase



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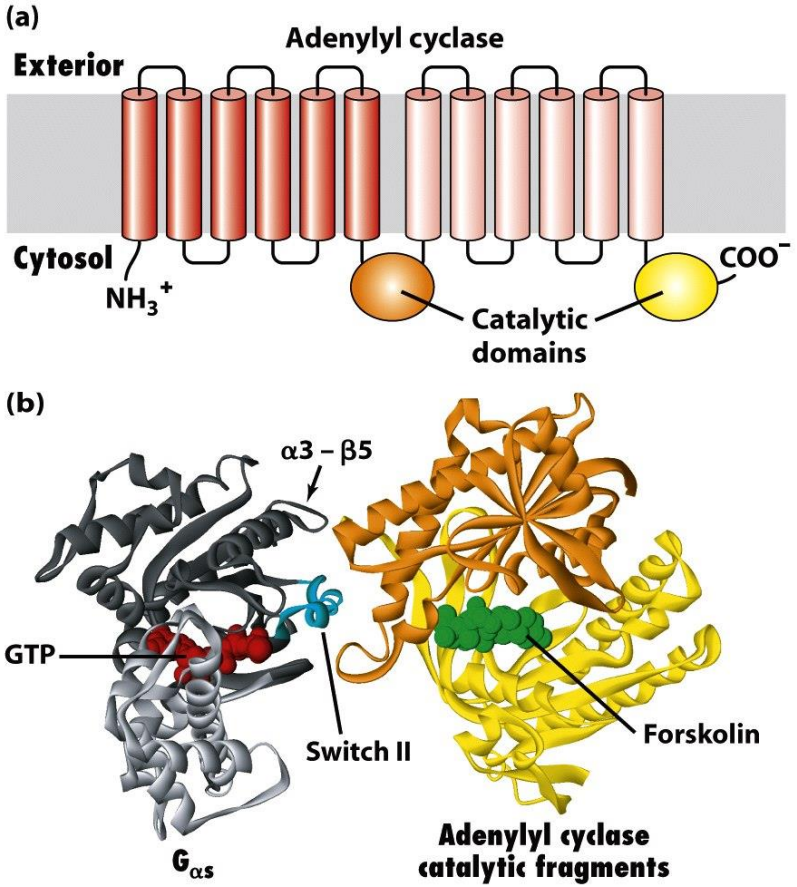
Activation or inhibition of adenylyl cyclase – what are the consequences?

- G protein coupled receptor activates adenyl cyclase
- Adenyl cyclase causes an increase in cyclic-AMP levels
- cAMP activates protein Kinase A
- Protein Kinase A phosphorylates specific target proteins
- G protein coupled receptor that inhibits adenyl cyclase A causes a decrease in cAMP levels and does not activate Protein Kinase A

Mammalian Adenyl Cyclase



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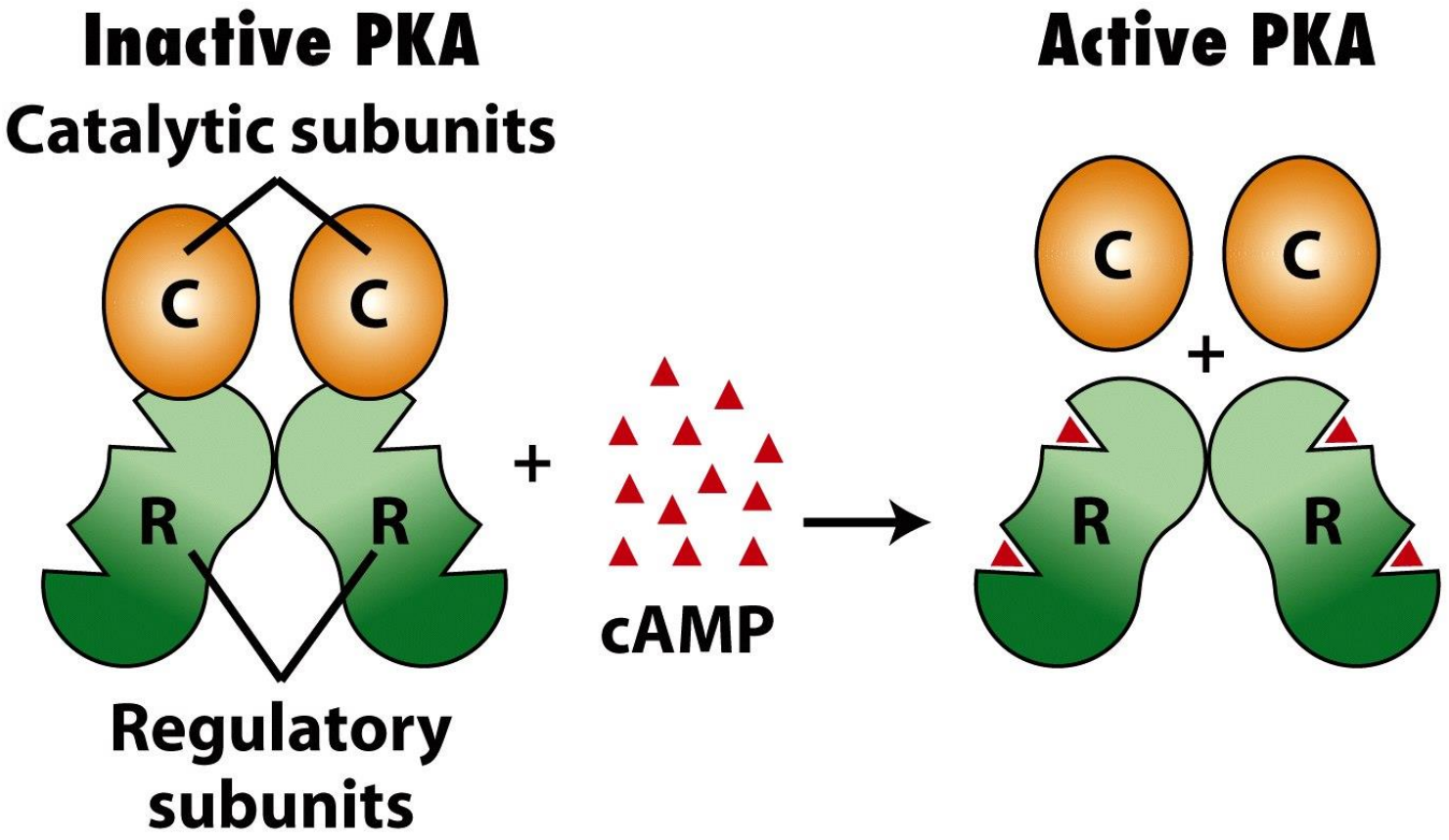
Activation of Protein Kinase A by cyclic AMP



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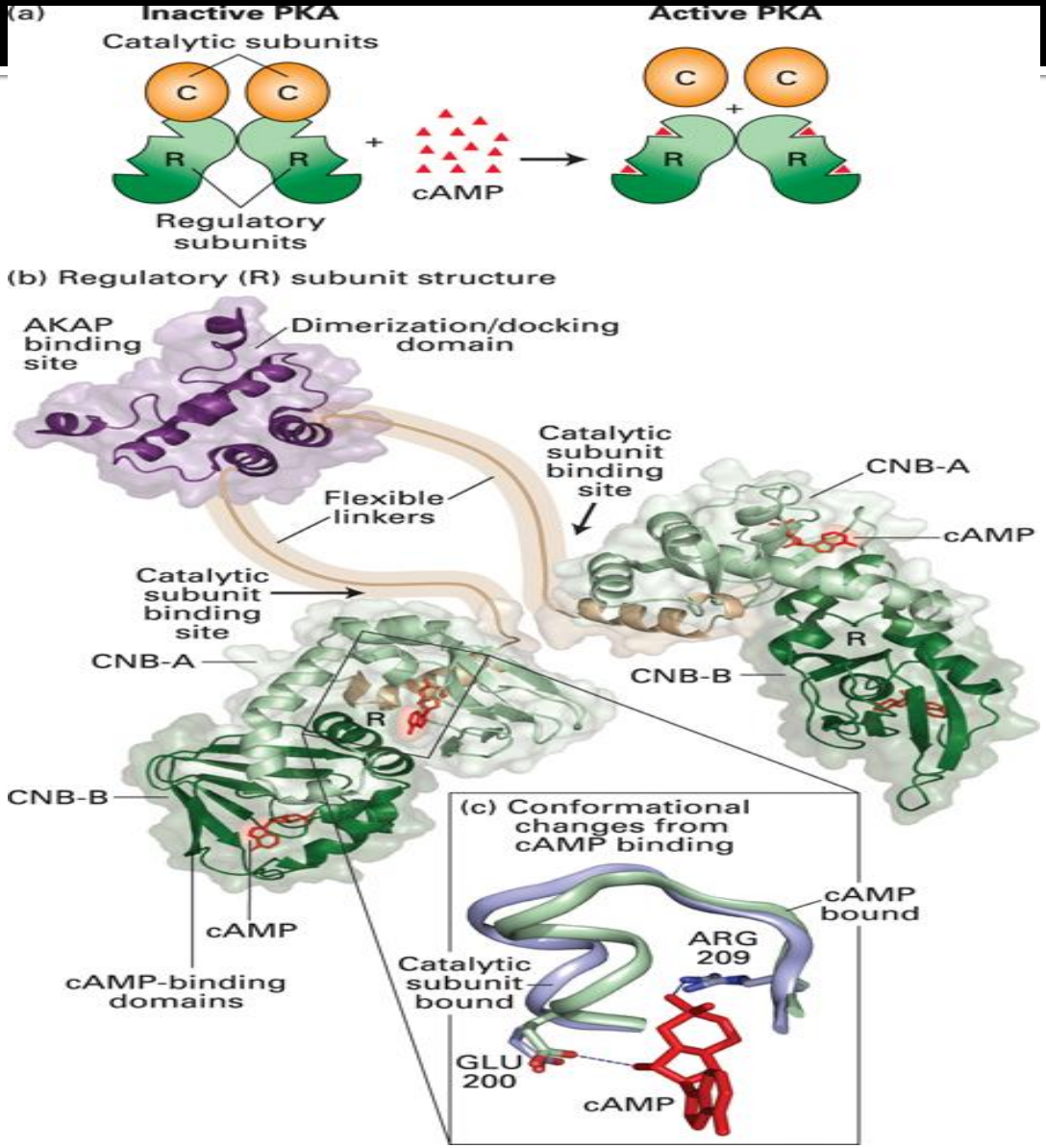
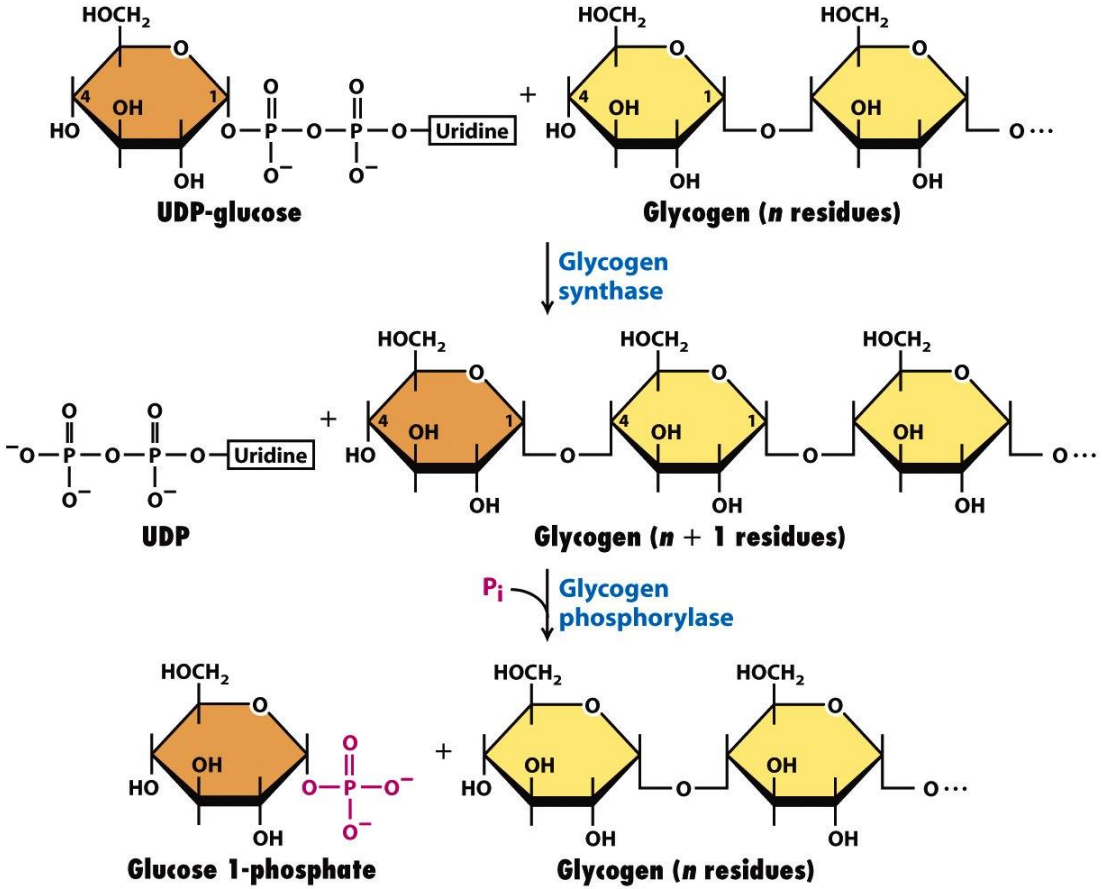
Protein Kinase A



Glycogen Metabolism



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Regulation of Glycogen metabolism in liver and muscle by cAMP

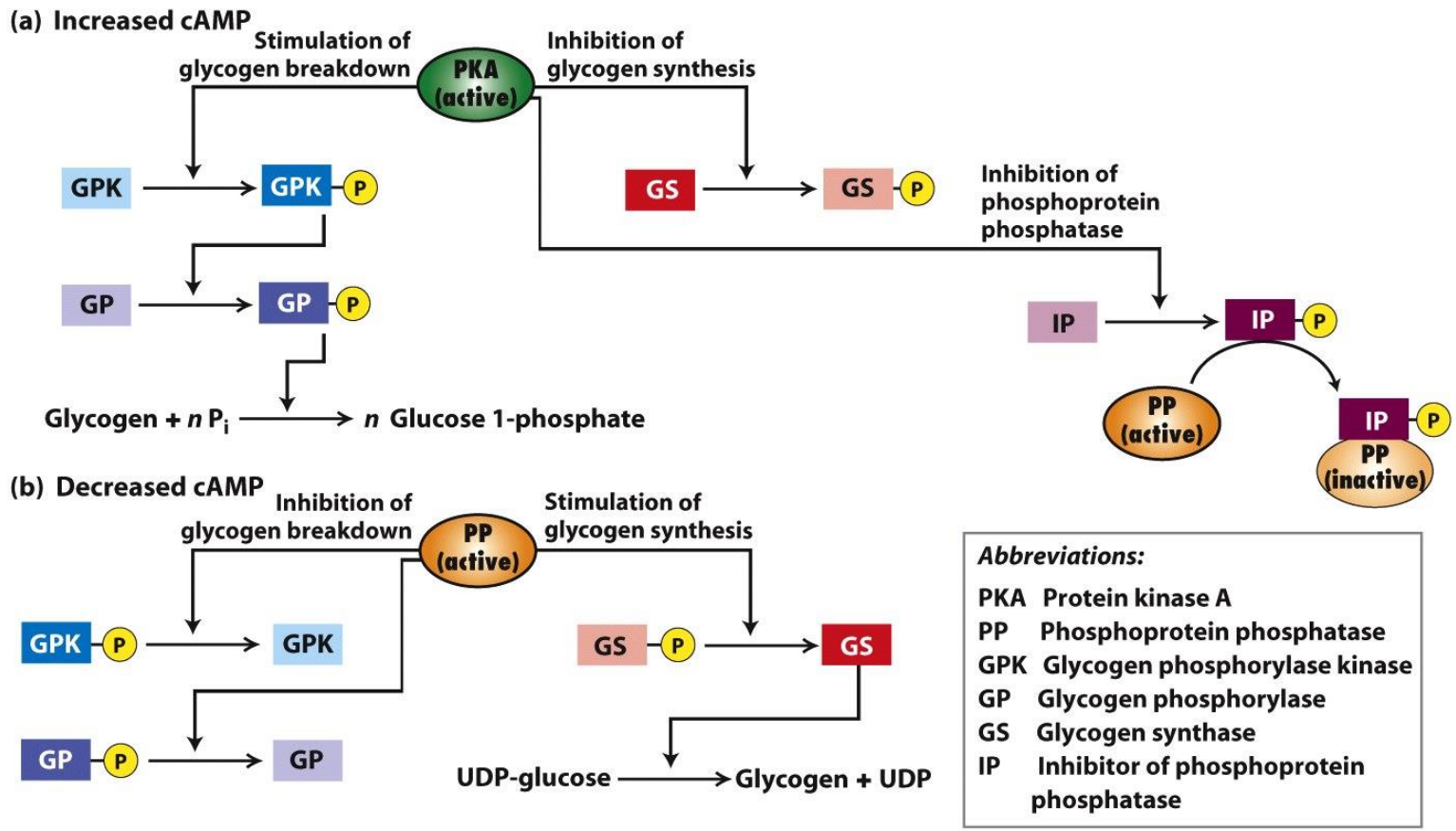

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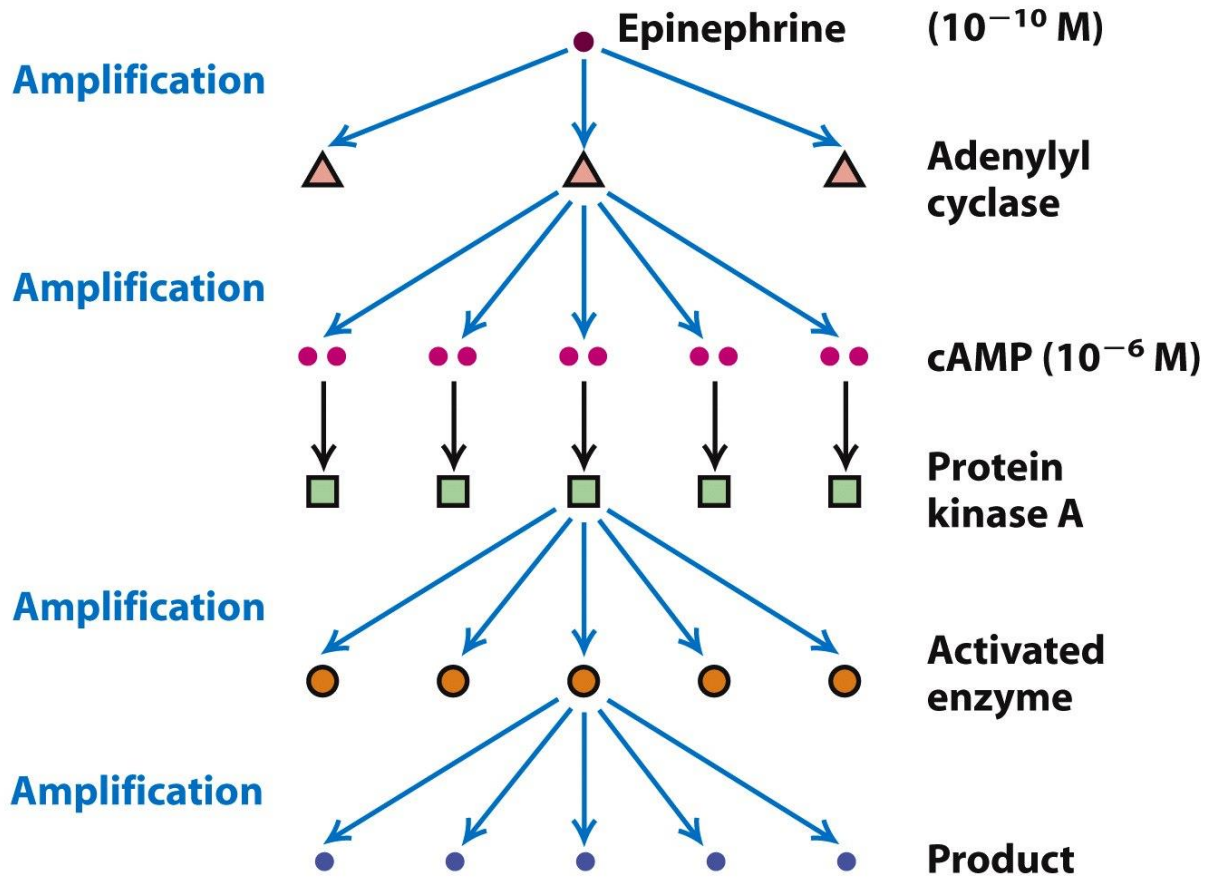
TABLE 15-2 Cellular Responses to Hormone-Induced Rise in cAMP in Various Tissues*

Tissue	Hormone Inducing Rise in cAMP	Cellular Response
Adipose	Epinephrine; ACTH; glucagon	Increase in hydrolysis of triglyceride; decrease in amino acid uptake
Liver	Epinephrine; norepinephrine; glucagon	Increase in conversion of glycogen to glucose; inhibition of glycogen synthesis; increase in amino acid uptake; increase in gluconeogenesis (synthesis of glucose from amino acids)
Ovarian follicle	FSH; LH	Increase in synthesis of estrogen, progesterone
Adrenal cortex	ACTH	Increase in synthesis of aldosterone, cortisol
Cardiac muscle	Epinephrine	Increase in contraction rate
Thyroid gland	TSH	Secretion of thyroxine
Bone	Parathyroid hormone	Increase in resorption of calcium from bone
Skeletal muscle	Epinephrine	Conversion of glycogen to glucose-1-phosphate
Intestine	Epinephrine	Fluid secretion
Kidney	Vasopressin	Resorption of water
Blood platelets	Prostaglandin I	Inhibition of aggregation and secretion

*Nearly all the effects of cAMP are mediated through protein kinase A (PKA), which is activated by binding of cAMP.
SOURCE: E. W. Sutherland, 1972, *Science* **177**:401.

Amplification of an external signal



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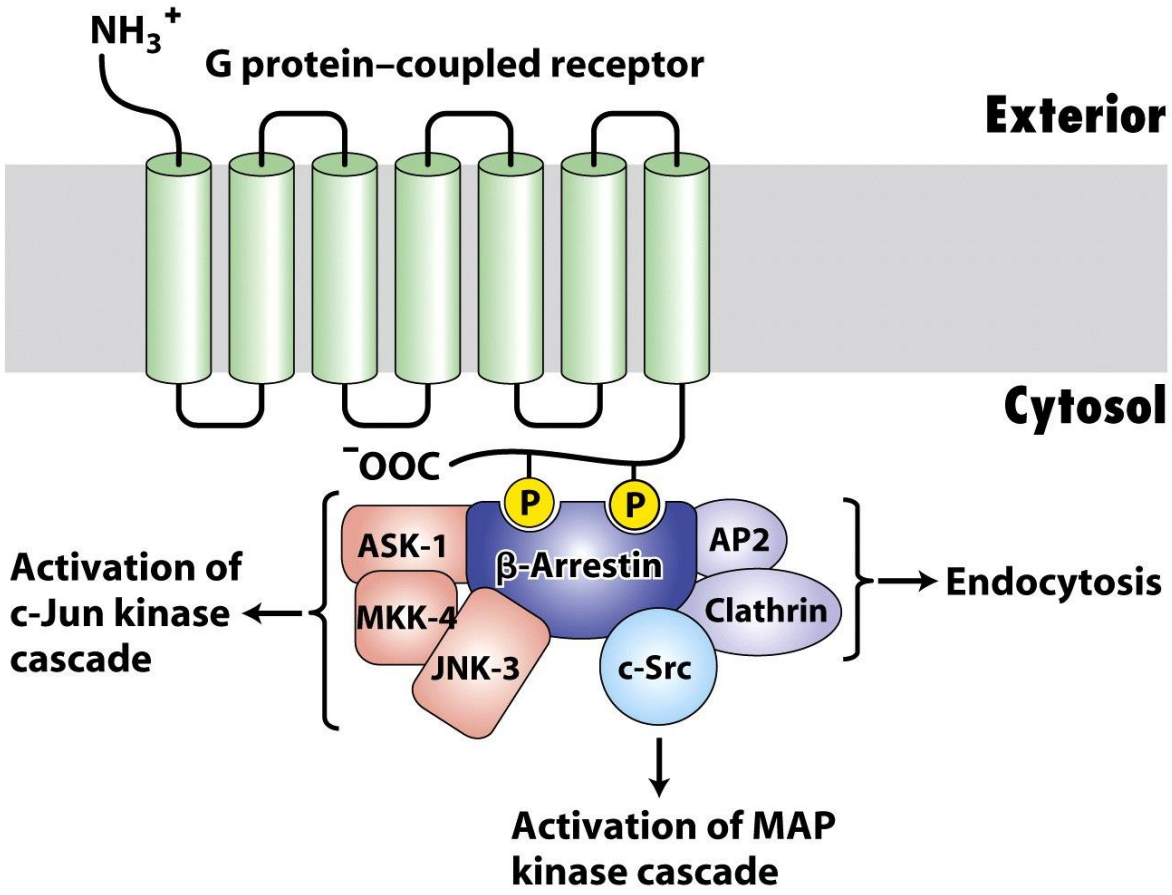
Regulation of G protein coupled receptors

- Ligand affinity decreases when the GDP bound to Gαs is replaced by GTP
- Intrinsic GTPase activity of Gαs converts the GTP to GDP
- cAMP phosphodiesterase hydrolyzes cAMP to 5'-AMP
- Receptors are phosphorylated by Protein Kinase A. This phosphorylated receptor cannot activate the G protein but can bind the ligand
- Prolonged exposure to one ligand desensitizes not only its receptor but also other G protein coupled receptors. Other ligands that activate this G protein will not be able to activate this pathway– heterologous desensitization

Regulation (cont'd)

- Homologous desensitization – receptors in their active conformation (bound to ligand) are subject to inactivaion by phosphorylation eg. Beta-adrenergic receptor kinase (BARK) which can phosphorylate different residues on the cytosolic side of the beta2-adrenergic receptors
- Beta-arrestin binds to phosphorylated beta-adrenergic receptors and prevents activation of coupled G proteins and promotes endocytosis of the receptors

β-Arrestin



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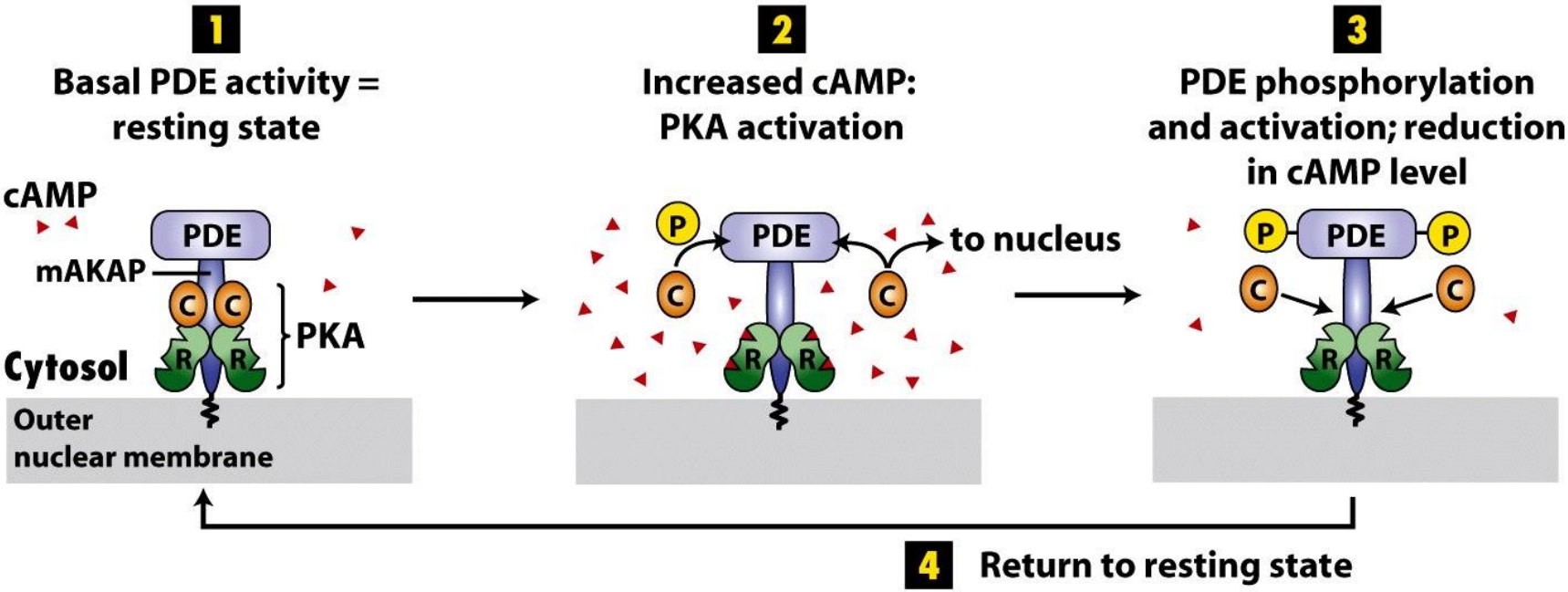
Subcellular localization of the effects of cAMP



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A family of proteins is involved in subcellular localization of the effects of Protein Kinase A . mAKAP anchors both protein Kinase A and cAMP phosphodiesterase to the nuclear membrane. AKAP – A kinase associated proteins

Other AKAP proteins

AKAP 15 is anchored to the cytosolic face of the membrane. It is located near a Ca^{2+} channel protein in heart muscle cells.
When beta-adrenergic receptors, are bound by epinephrine, there is phosphorylation of these calcium channels by PKA.
These channels open to allow calcium to enter and thereby increase the rate of contraction of the heart.

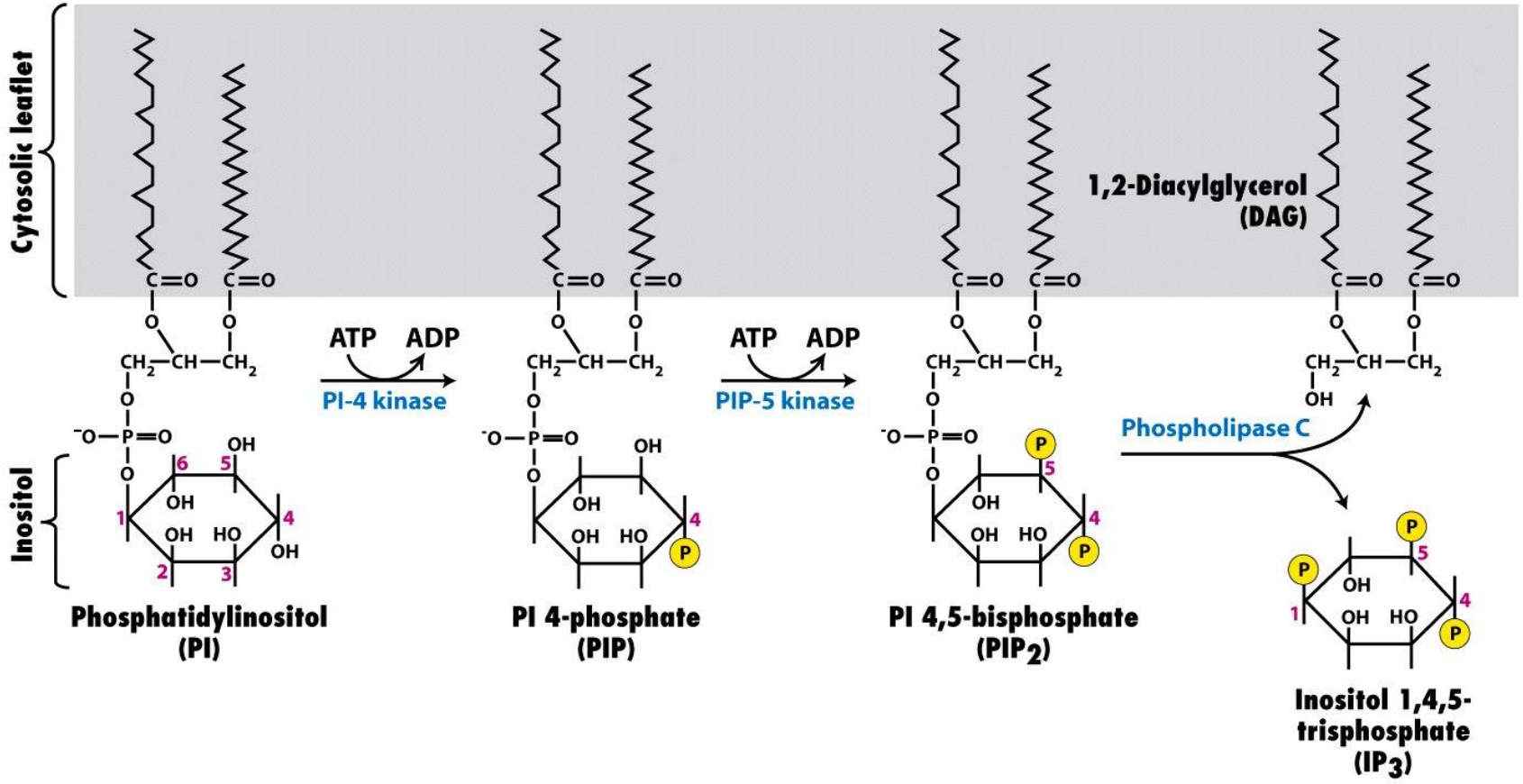
The binding of AKAP15 to PKA localizes it close to the site of action.

Phospholipase C and IP3-DAG pathway

G protein coupled receptors that activate Phospholipase C

- Phospholipase C is the effector protein in this signaling pathway
- The G proteins involved are G α_0 and Gαq
- The effector protein generates two second messengers – Phospahtidyl inositol derivatives (IP_3 and DAG)
- These second messengers activate protein kinase C and elevate $Ca2^+$ concentration

How does the effector protein generate the second messengers?



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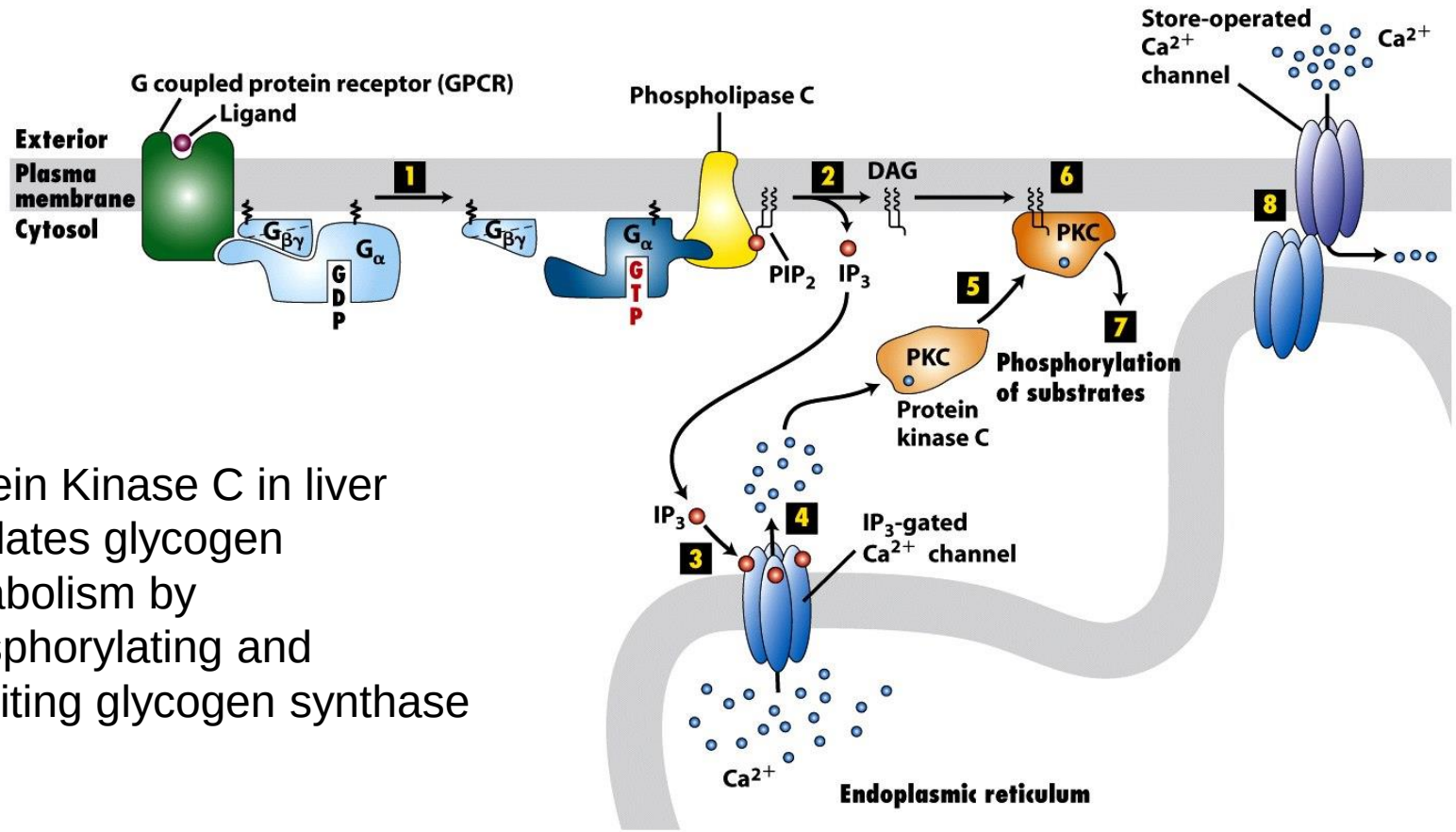
Inositol trisphosphate / Diacyl glycerol pathway



Protein Kinase C in liver regulates glycogen metabolism by phosphorylating and inhibiting glycogen synthase

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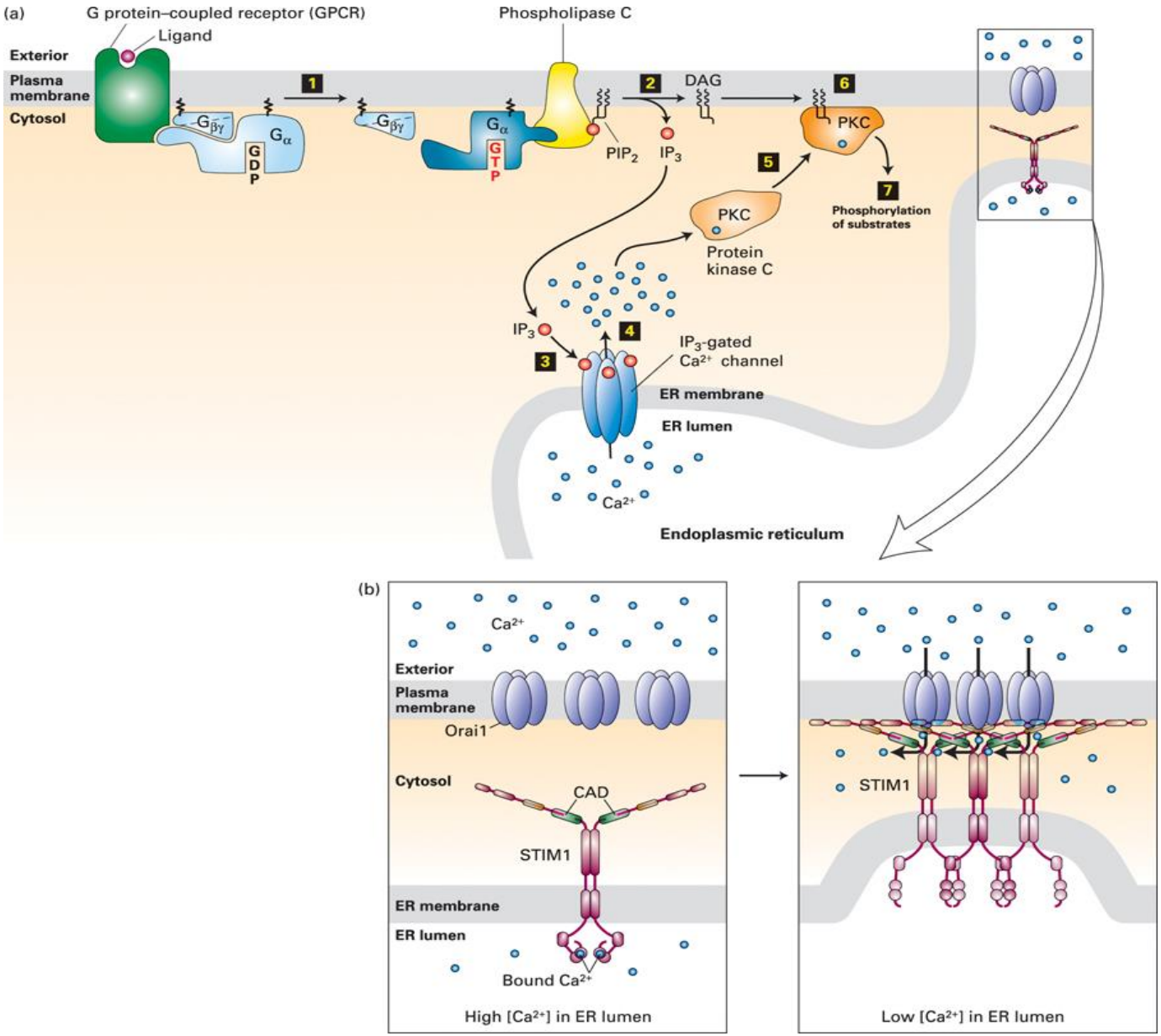
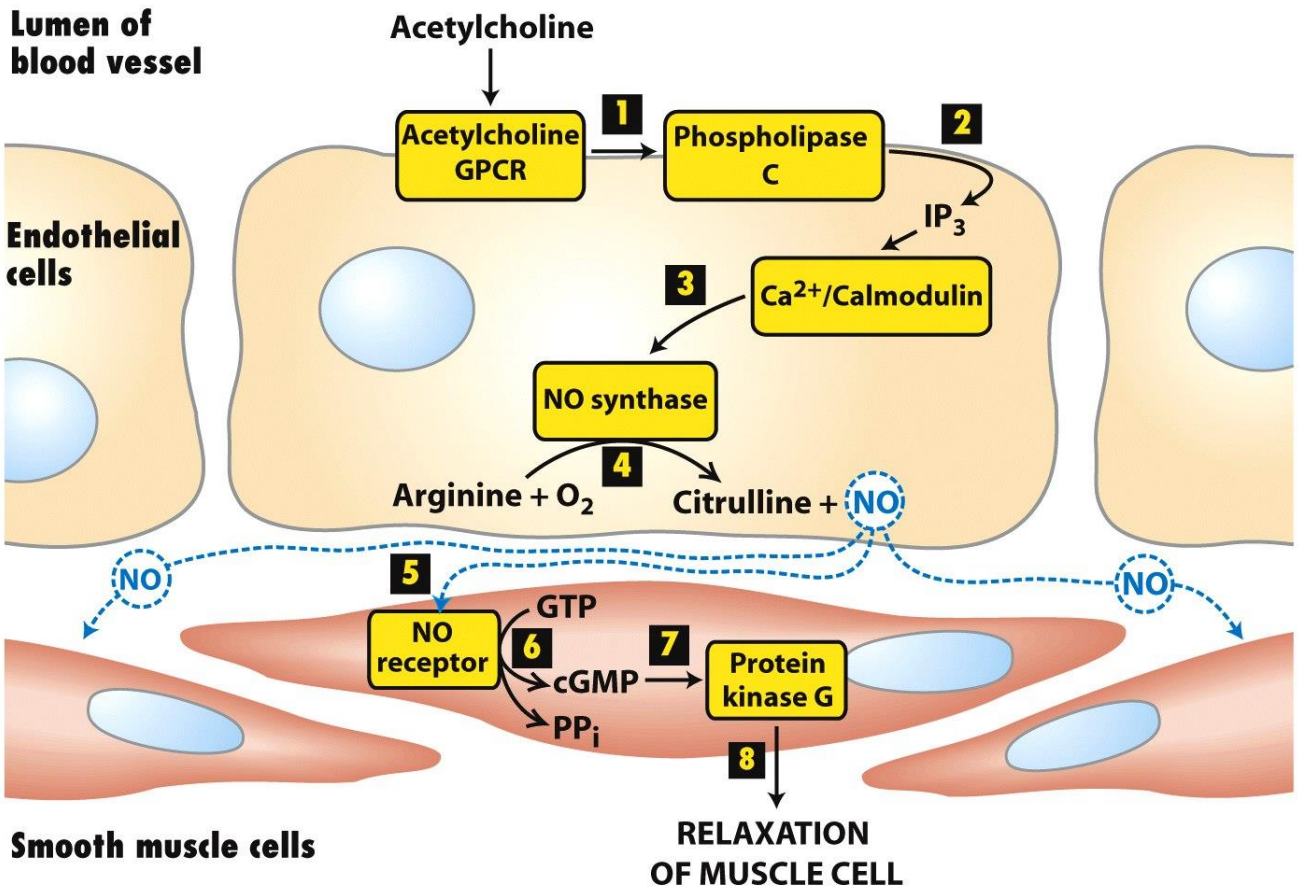

(a)
G protein–coupled receptor (GPCR)
Ligand
Phospholipase C
Exterior
Plasma membrane
Cytosol
1
2
DAG
6
PKC
PIP2
IP3
5
PKC
Protein kinase C
7
Phosphorylation of substrates
IP3
3
4
IP3-gated Ca2+ channel
ER membrane
ER lumen
Ca2+
Endoplasmic reticulum
(b)
Ca2+
Exterior
Plasma membrane
Orai1
Cytosol
CAD
STIM1
ER membrane
ER lumen
Bound Ca2+
High [Ca2+] in ER lumen
STIM1
Low [Ca2+] in ER lumen

Calmodulin

- Small cytosolic protein
- $Ca2^{+}$ binds to all four sites in calmodulin and activates it
- $Ca2^{+}$/Calmodulin complex activate other enzymes and proteins
- Examples are myosin light chain kinase and cAMP phosphodiesterase
- Activates kinases or phosphatases that modulate the function of other proteins

- Nitric oxide and cGMP pathway

Nitric oxide / cGMP pathway



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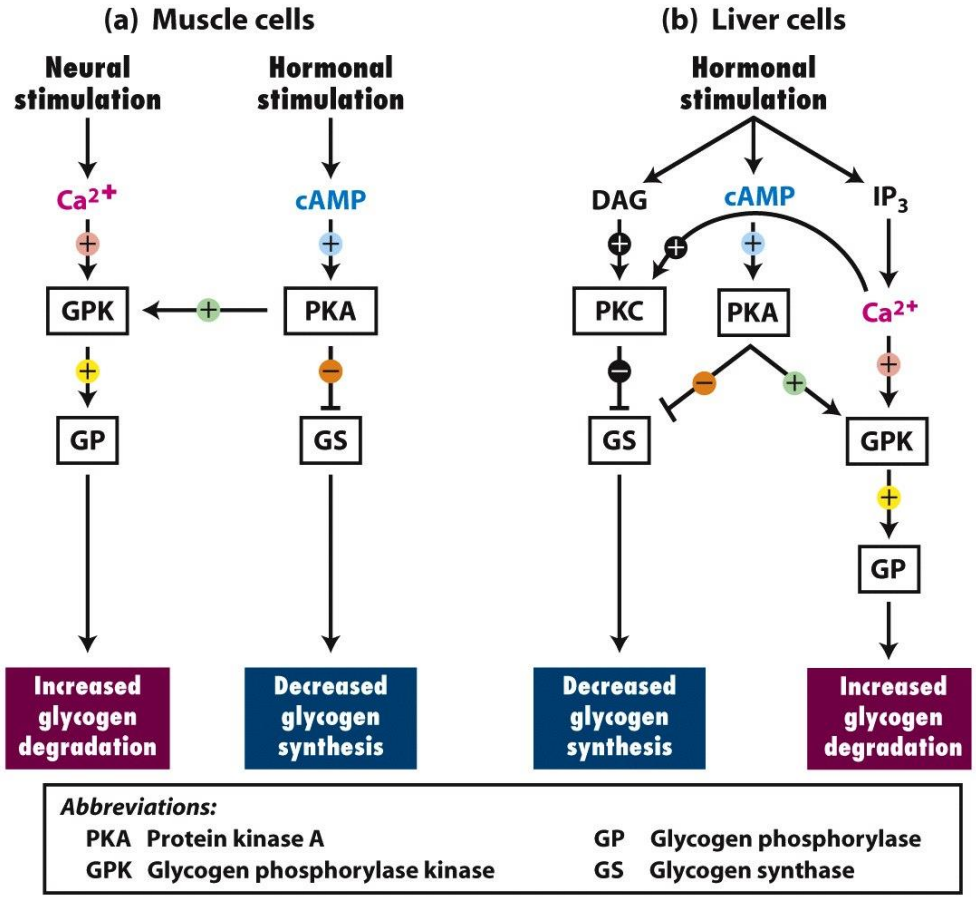
Integrated regulation of glycogen breakdown



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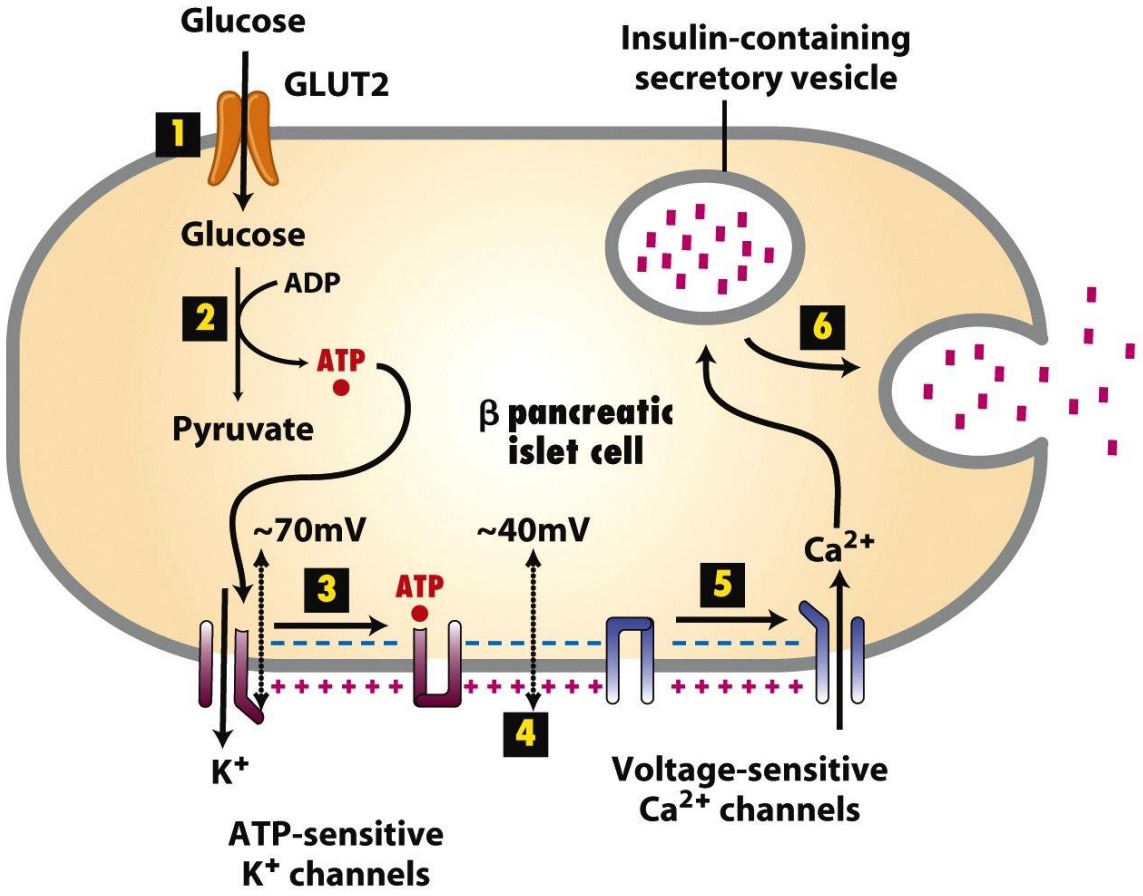
How is insulin secreted in response to rise in blood glucose? (A pancreatic beta cell)



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How insulin and glucagon work together to maintain blood glucose levels?

- Alpha cells of pancreas secrete glucagon
- Beta cells of pancreas secrete insulin
- In response to high blood glucose, insulin is secreted and it binds to insulin receptors on muscle, adipocytes and liver cells
- In muscle and fat cells, this insulin-receptor complex activates Protein Kinase B which causes fusion of vesicles containing Glut-4 molecules with the plasma membrane – This causes uptake of glucose form the blood

Insulin and glucagon (continued)

- In liver, it promotes glycogen synthesis from glucose.
- When blood glucose levels fall below normal, glucagon is secreted into the blood.
- The glucagon binds to its receptor on liver cells and through activation of Protein Kinase A inhibits glycogen synthesis and promotes glycogenolysis (breakdown of glycogen). Liver cells convert glucose-1-phosphate to glucose and release it into the blood….raising blood glucose to normal levels