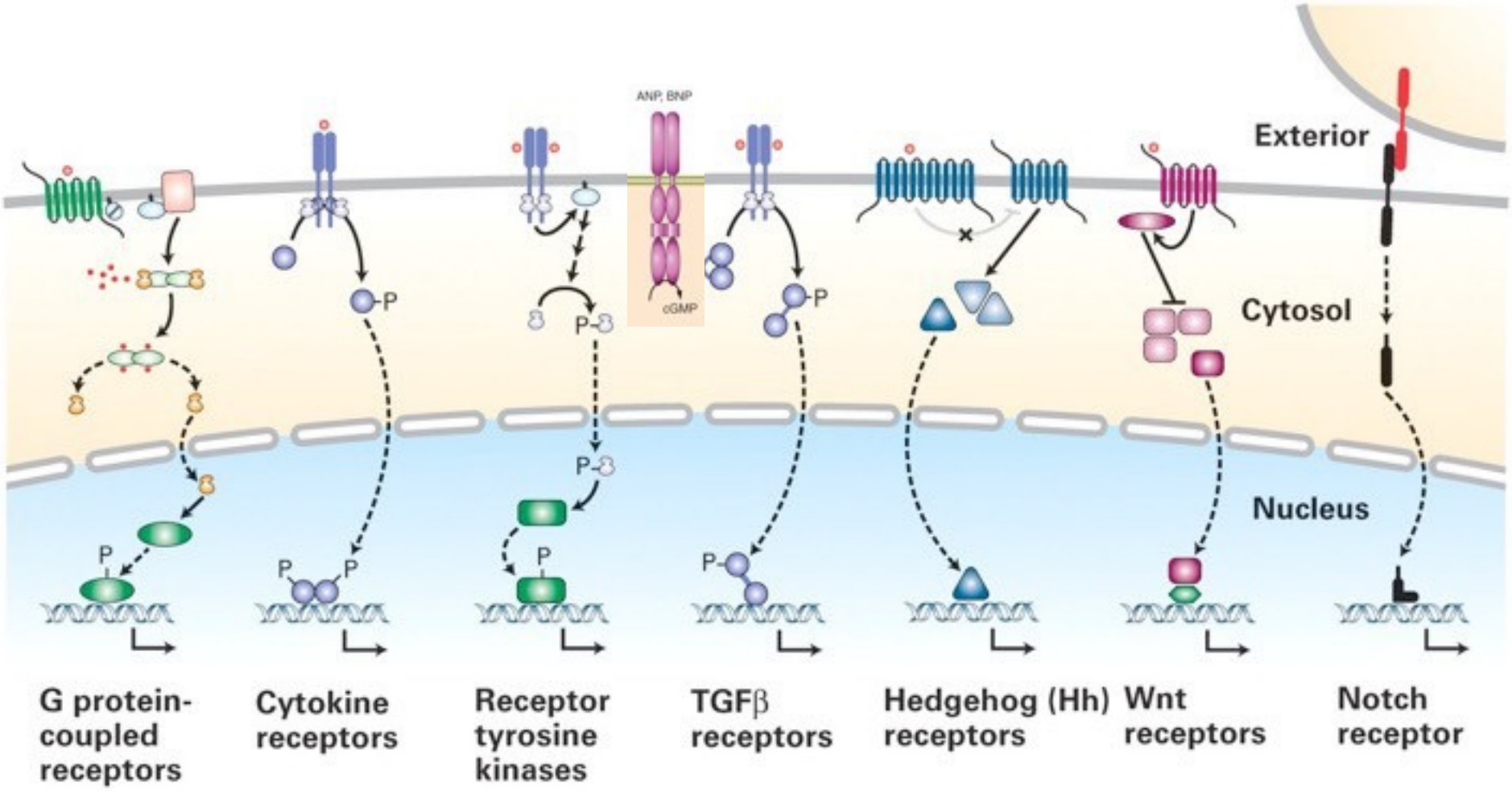
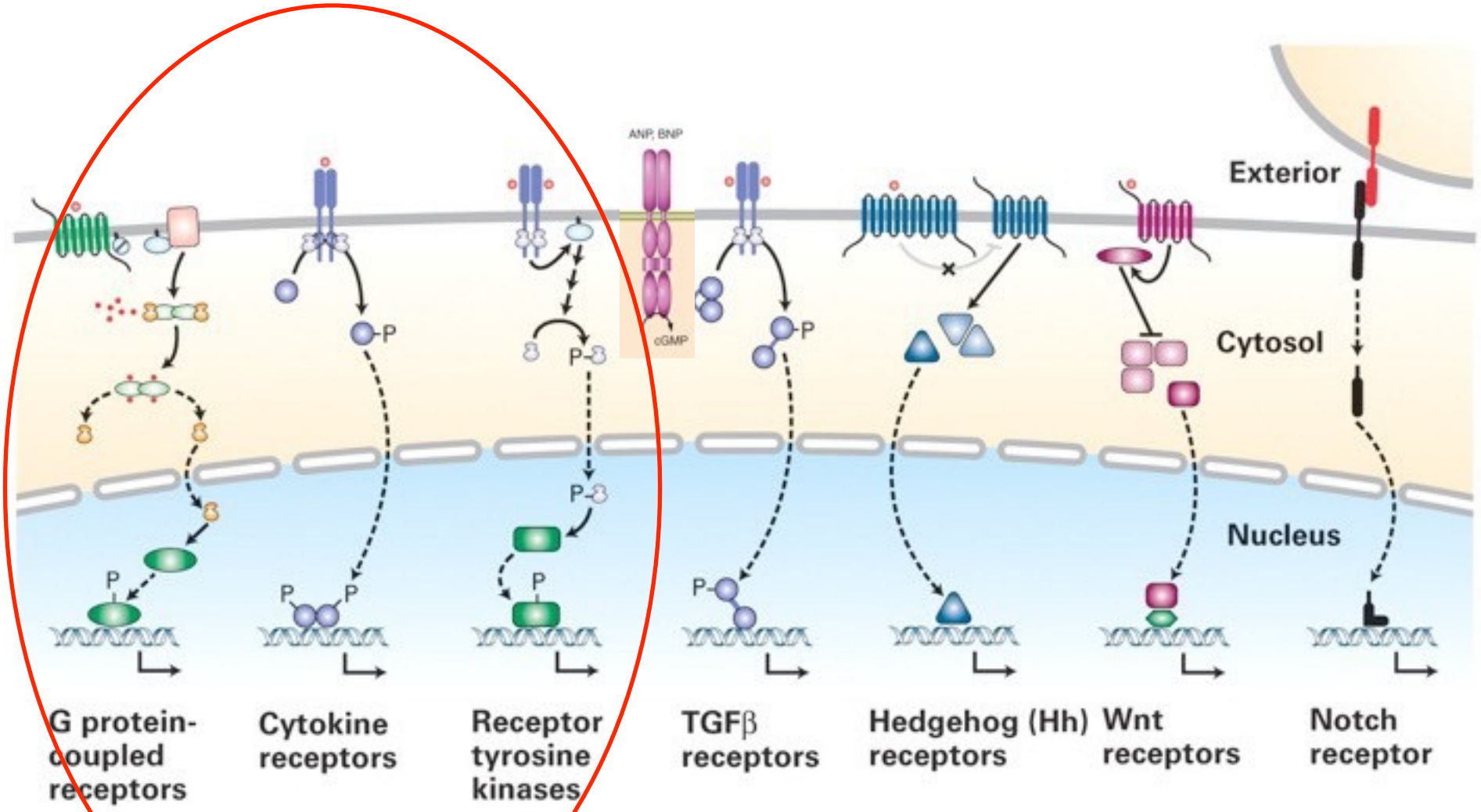
Cell membrane spanning receptors



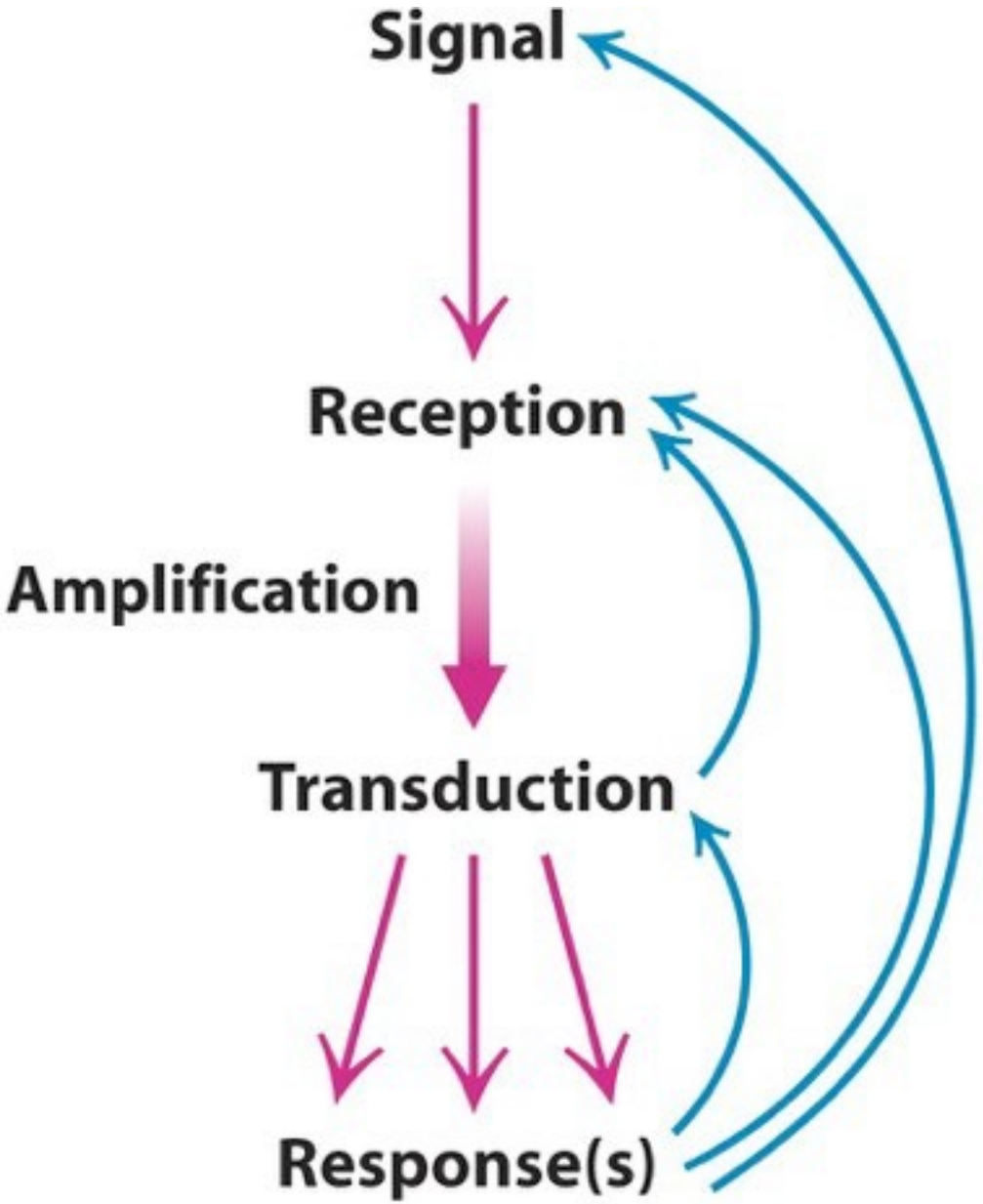
Guanylyl Cyclase receptors

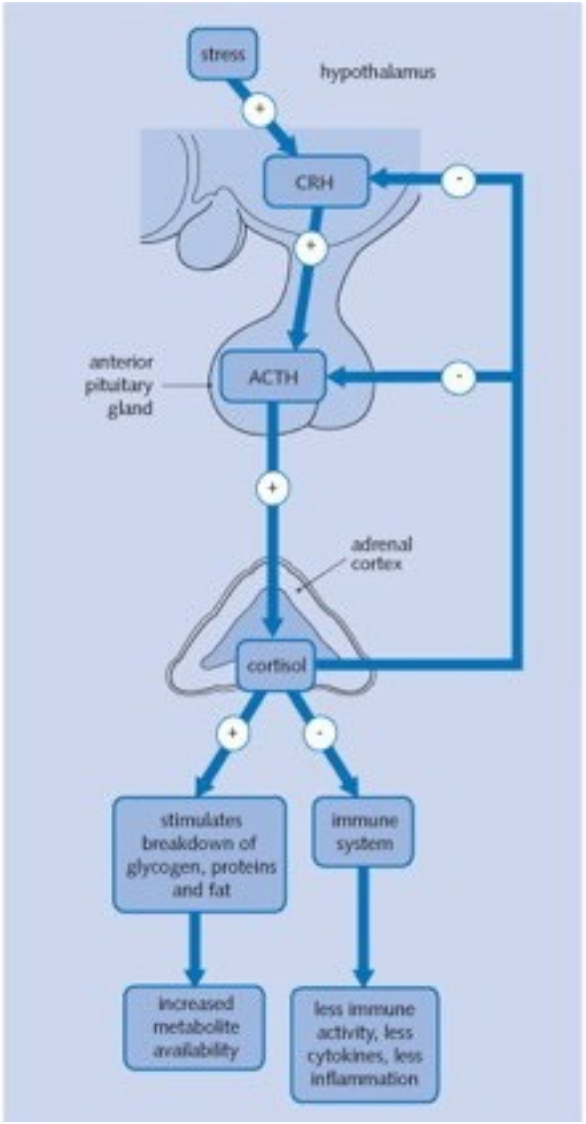
Atrial natriuretic peptide ANP

Cell membrane spanning receptors



Guanylyl Cyclase receptors


Signal
Reception
Amplification
Transduction
Response(s)


stress
hypothalamus
CRH
anterior pituitary gland
ACTH
adrenal cortex
cortisol
stimulates breakdown of glycogen, proteins and fat
immune system
increased metabolite availability
less immune activity, less cytokines, less inflammation

Signal transduction: general principles

- Interaction of ligand and receptor alters tertiary or quartenary structure of the receptor, including the intracellular parts.

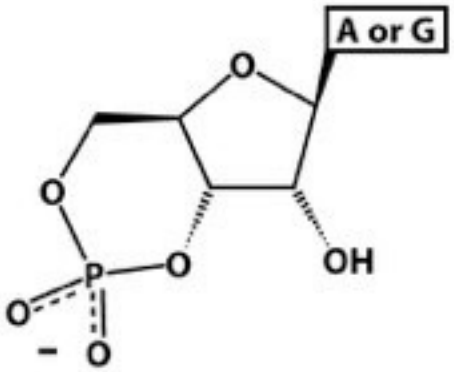
- Second messengers
  - Small intracellular molecules
  - Relay information from receptor ligand complex
  - Change in concentration in response to extracellular signal
    - cAMP, cGMP, Ca^{2+}, inositol 1,4,5-triphosphate (IP_3), diacylglycerol (DAG)

What's the point?

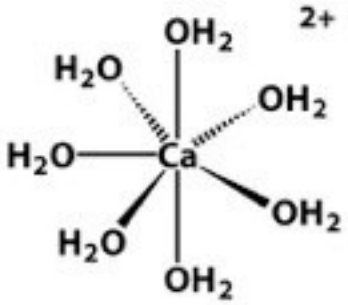
Second messengers

- Amplify the signal
- Can transmit the signal to other parts of the cell and other cellular compartment
- Allow for crosstalk and fine tuning
  - If the same second messenger is used in multiple pathways

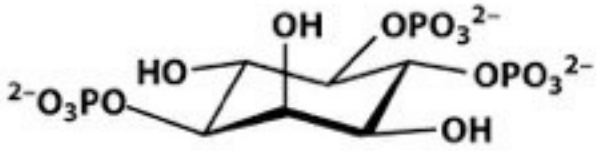
Second messengers



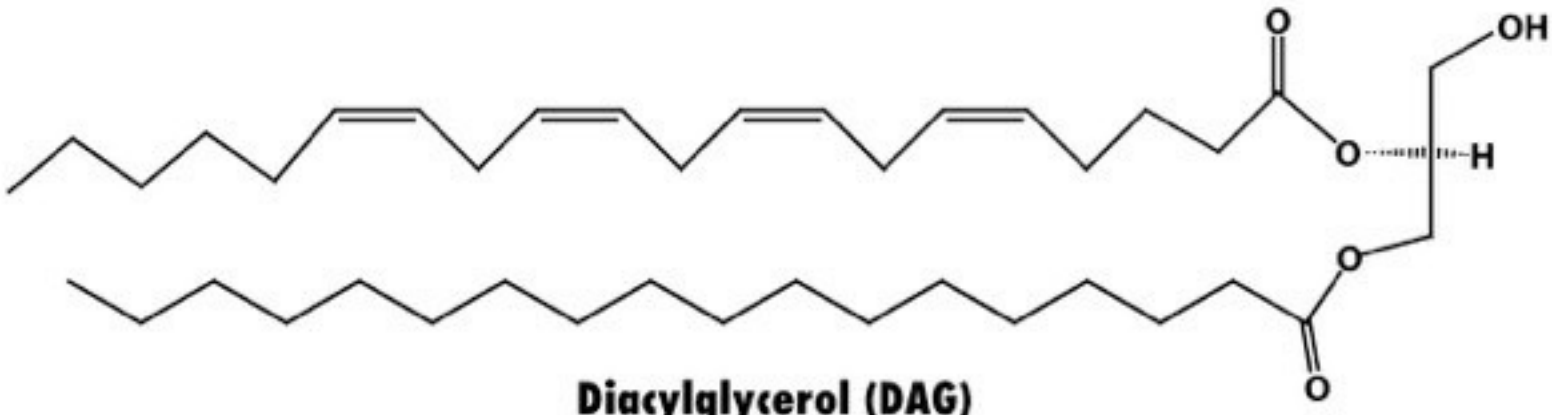
cAMP, cGMP



Calcium ion



Inositol 1,4,5-trisphosphate (IP_3)



Diacylglycerol (DAG)

Signal transduction: general principles-2

- activation of effectors
  - pumps
  - enzymes
  - transcription factors
  - physiologic response

- termination of the signal
  - once the cell has completed its response to a signal, the signaling process must be terminated or the cell loses its responsiveness to new signals

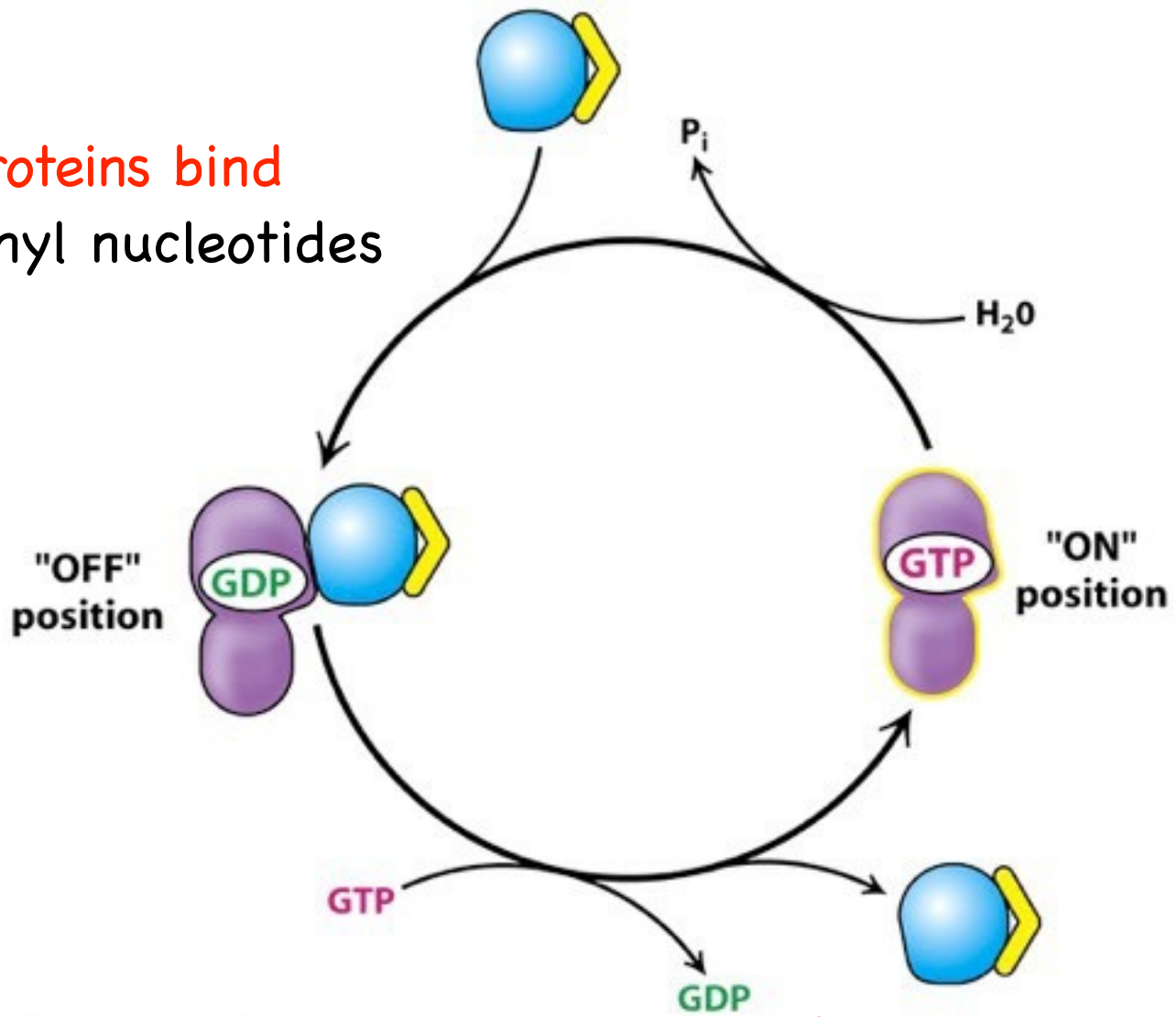
1. G-protein coupled receptors/7 transmembrane domain receptors

Biological functions mediated by GPCR/7TMRs

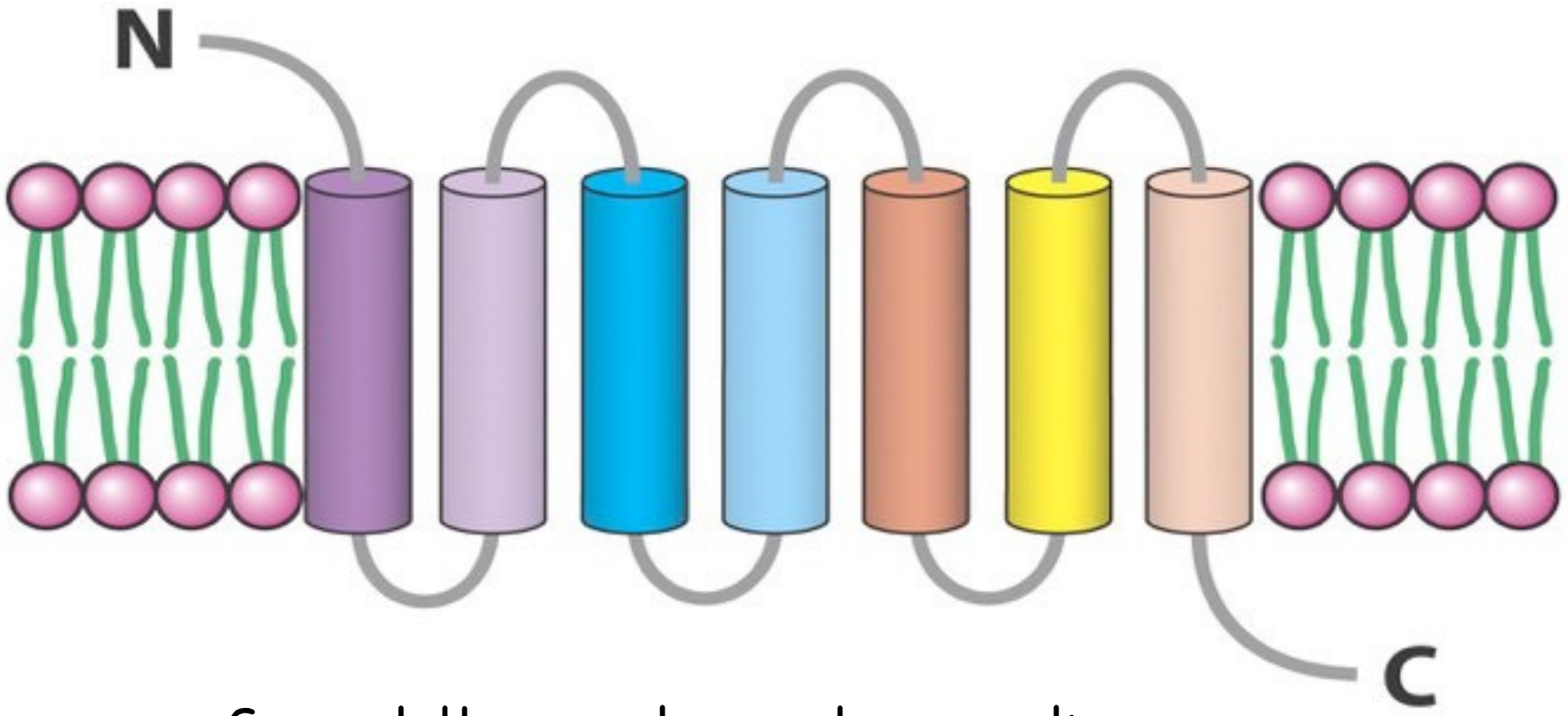
- Hormone action
- Hormone secretion
- Neurotransmission
- Chemotaxis
- Exocytosis
- Control of blood pressure
- Embryogenesis/development
- Cell growth and differentiation
- Smell
- Taste
- Vision
- Viral infection

Heterotrimeric G-protein

G-proteins bind
Guanyl nucleotides

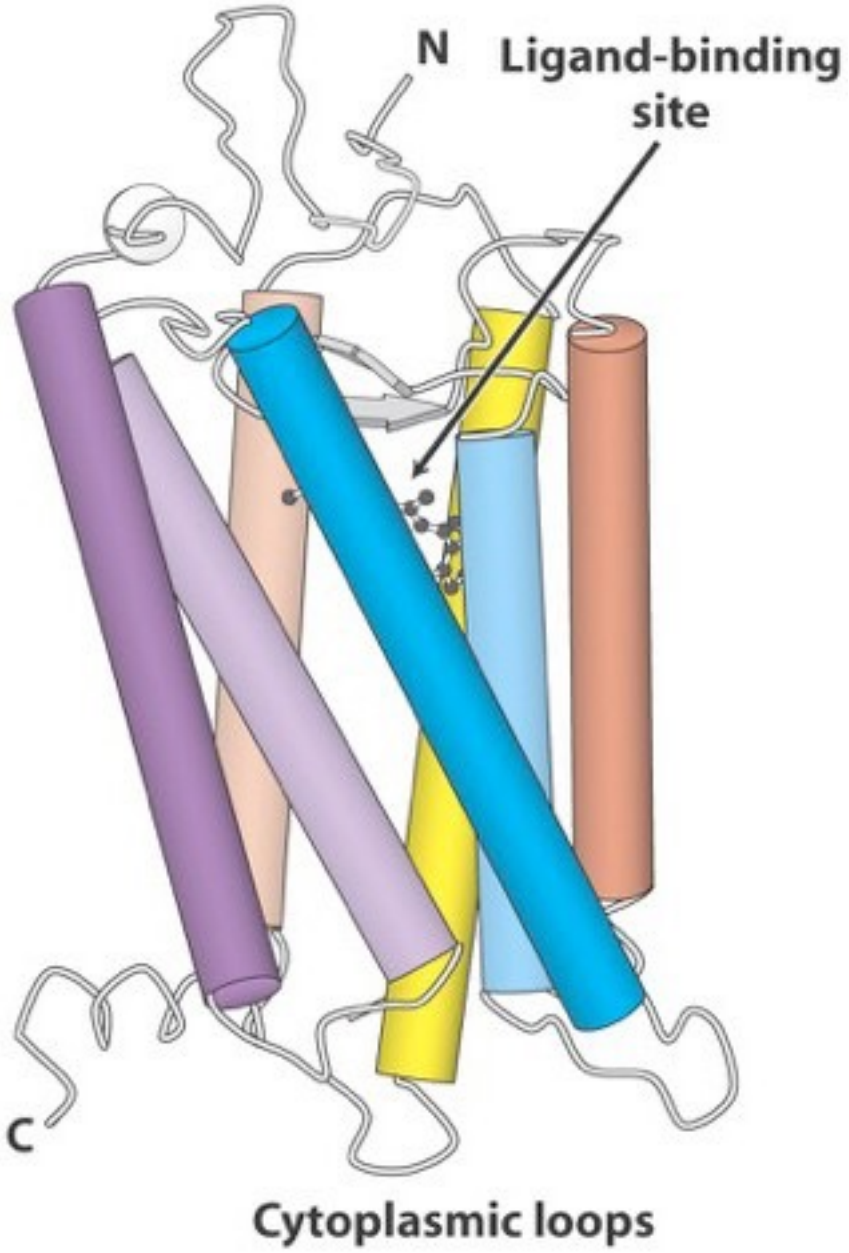


Seven-transmembrane-helix receptors: "serpentine receptors"



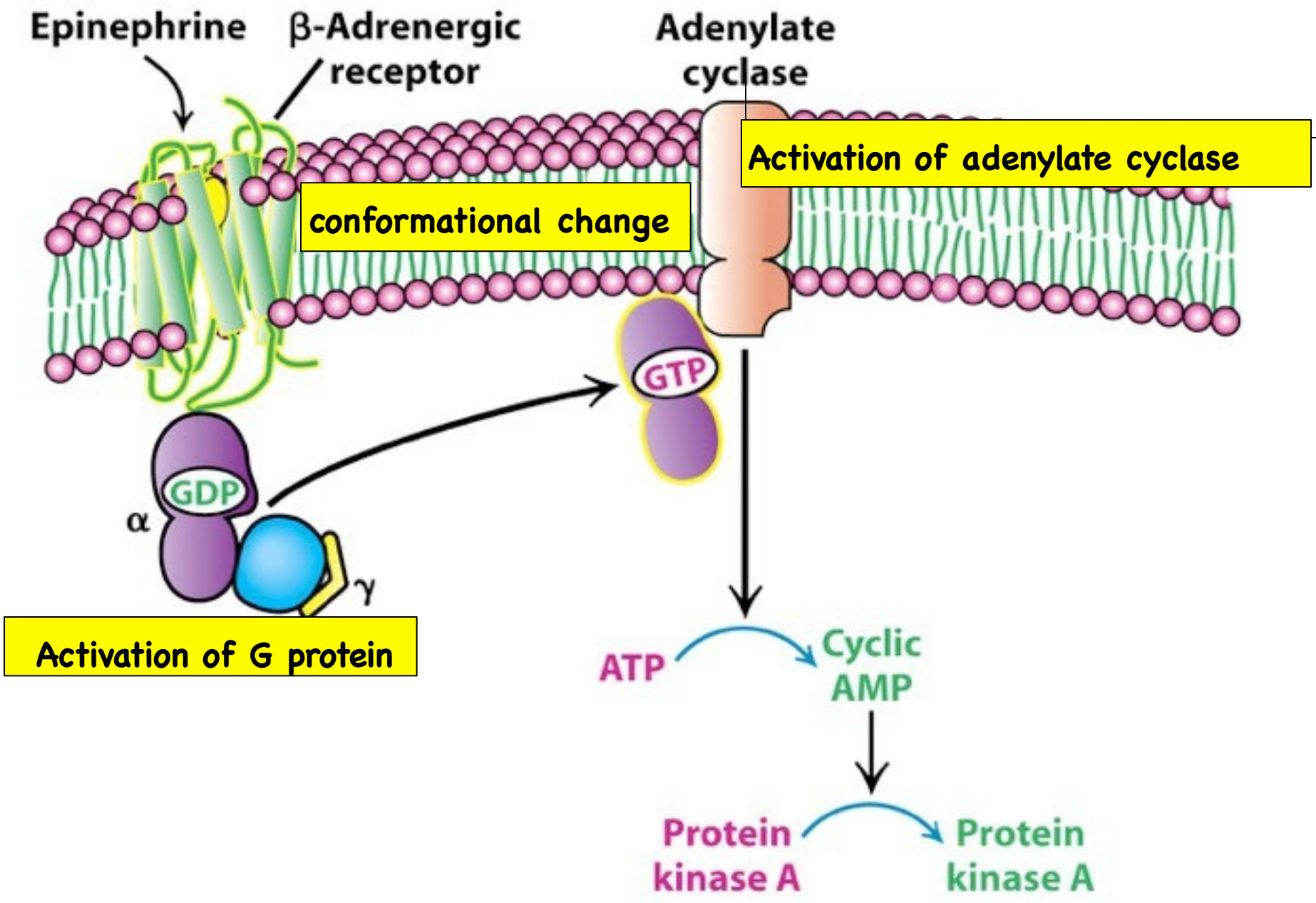
Several thousand receptors are known

50% of all drugs target 7TM receptors

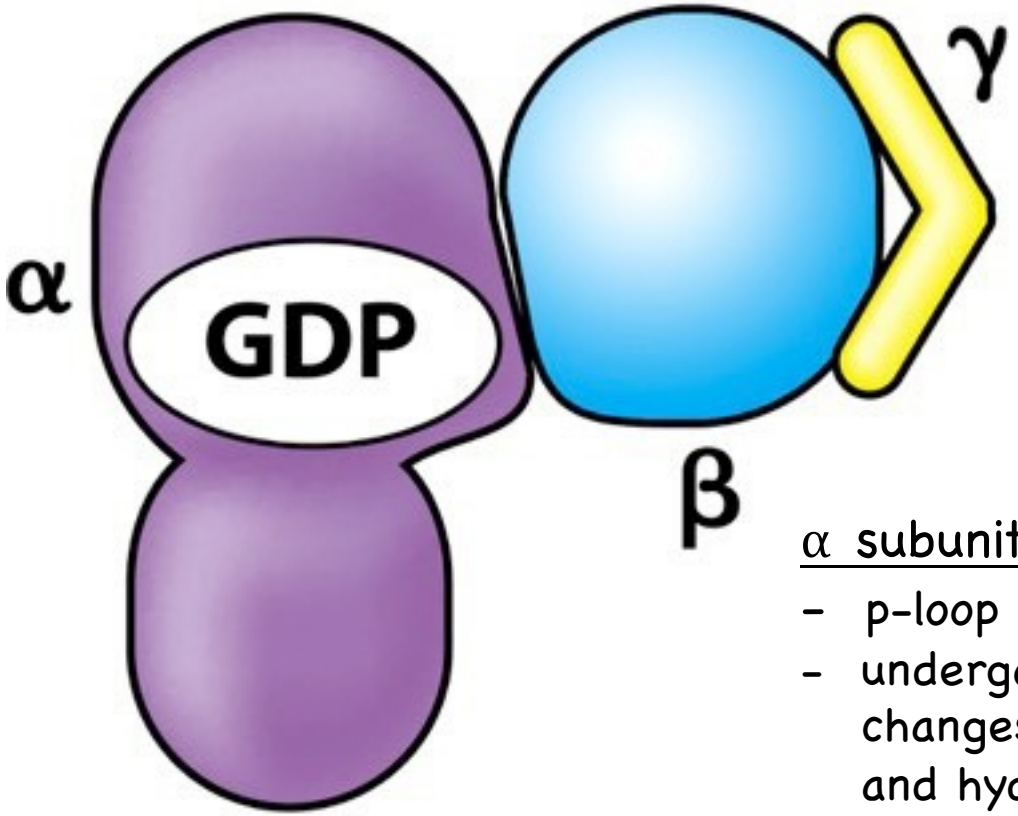


7TM receptor structure

- Ligand binding induces conformational change in the receptor's cytoplasmic domain


Epinephrine
β-Adrenergic receptor
Adenylate cyclase
Activation of adenylate cyclase
conformational change
GTP
GDP
α
γ
Activation of G protein
ATP
Cyclic AMP
Protein kinase A
Protein kinase A

G protein



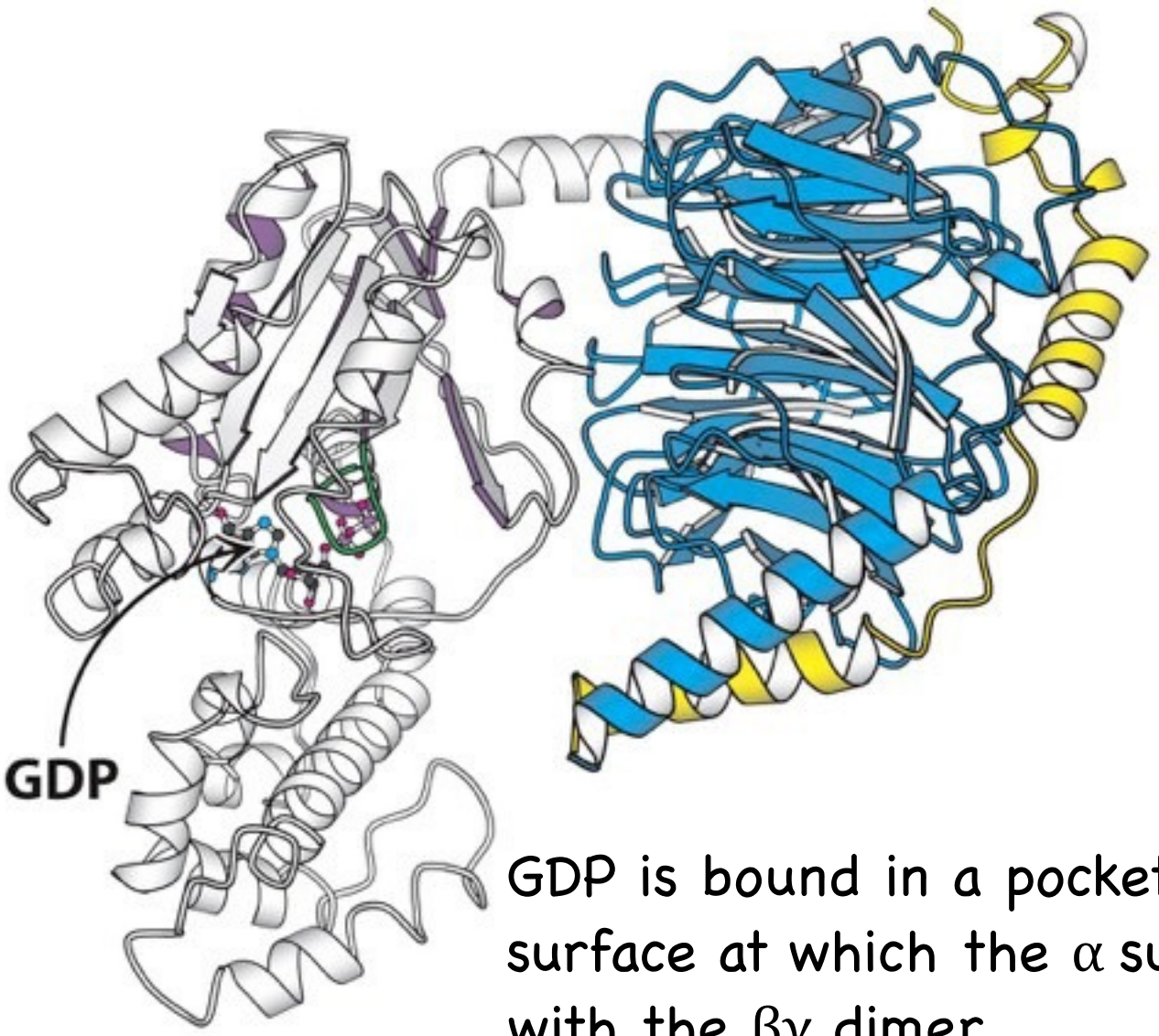
α subunit:

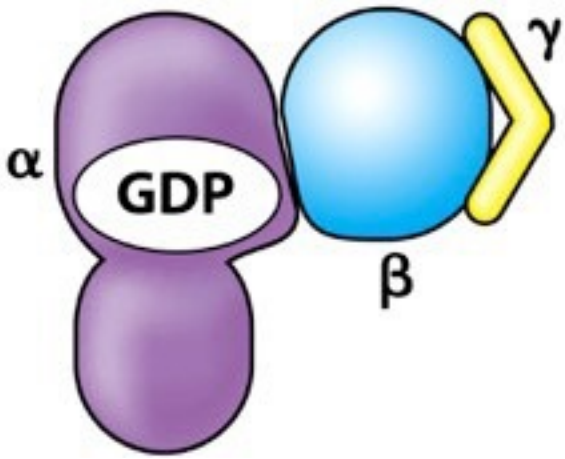
- p-loop NTPase
- undergoes substantial conformational changes on nucleoside triphosphate binding and hydrolysis
- participates in nucleotide binding

β and γ subunits:

are anchored to the membrane by covalently linked fatty acids

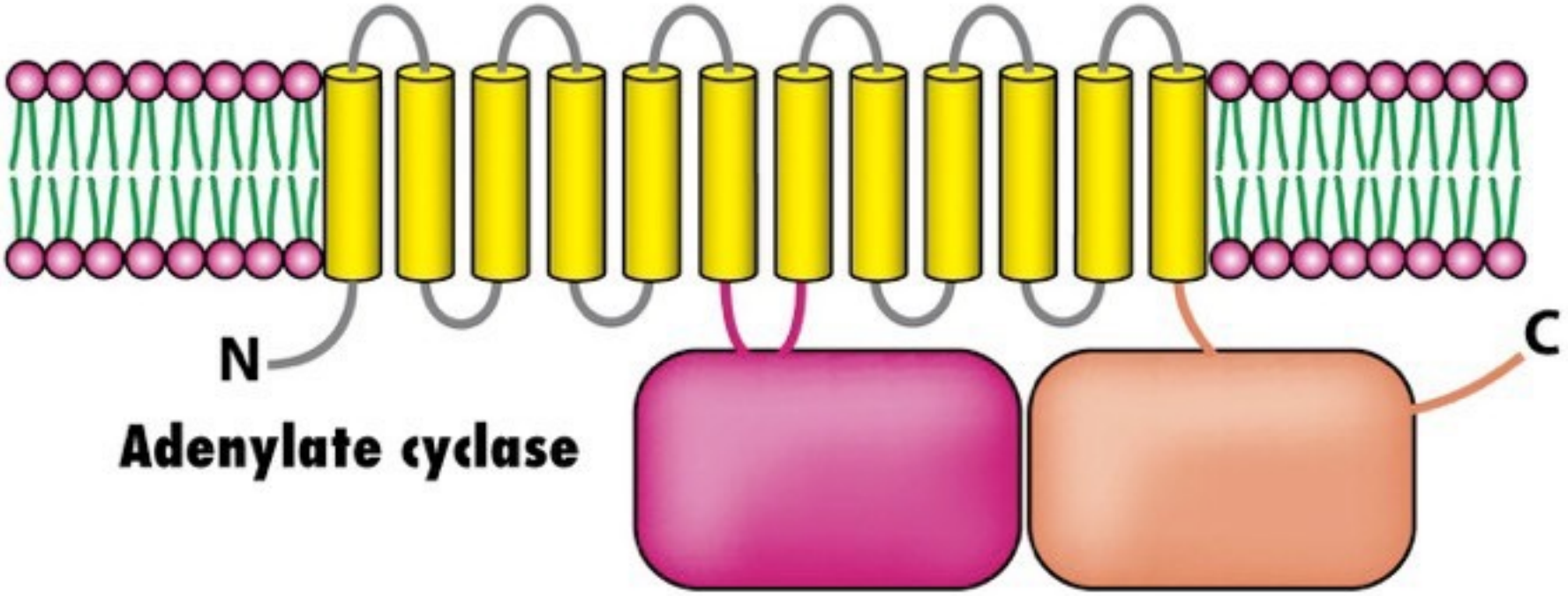
- hormone-bound receptor catalyzes the exchange of GTP for GDP
- it opens the nucleotide-binding site
- GDP can depart and GTP can bind, α subunit dissociates simultaneously from the $\beta\gamma$ dimer and now binds adenylate cyclase instead
- interaction of $G_{\alpha s}$ with adenylate cyclase favors a more catalytically active conformation
- one hormone-receptor complex can stimulate nucleotide exchange in many G-protein heterotrimers





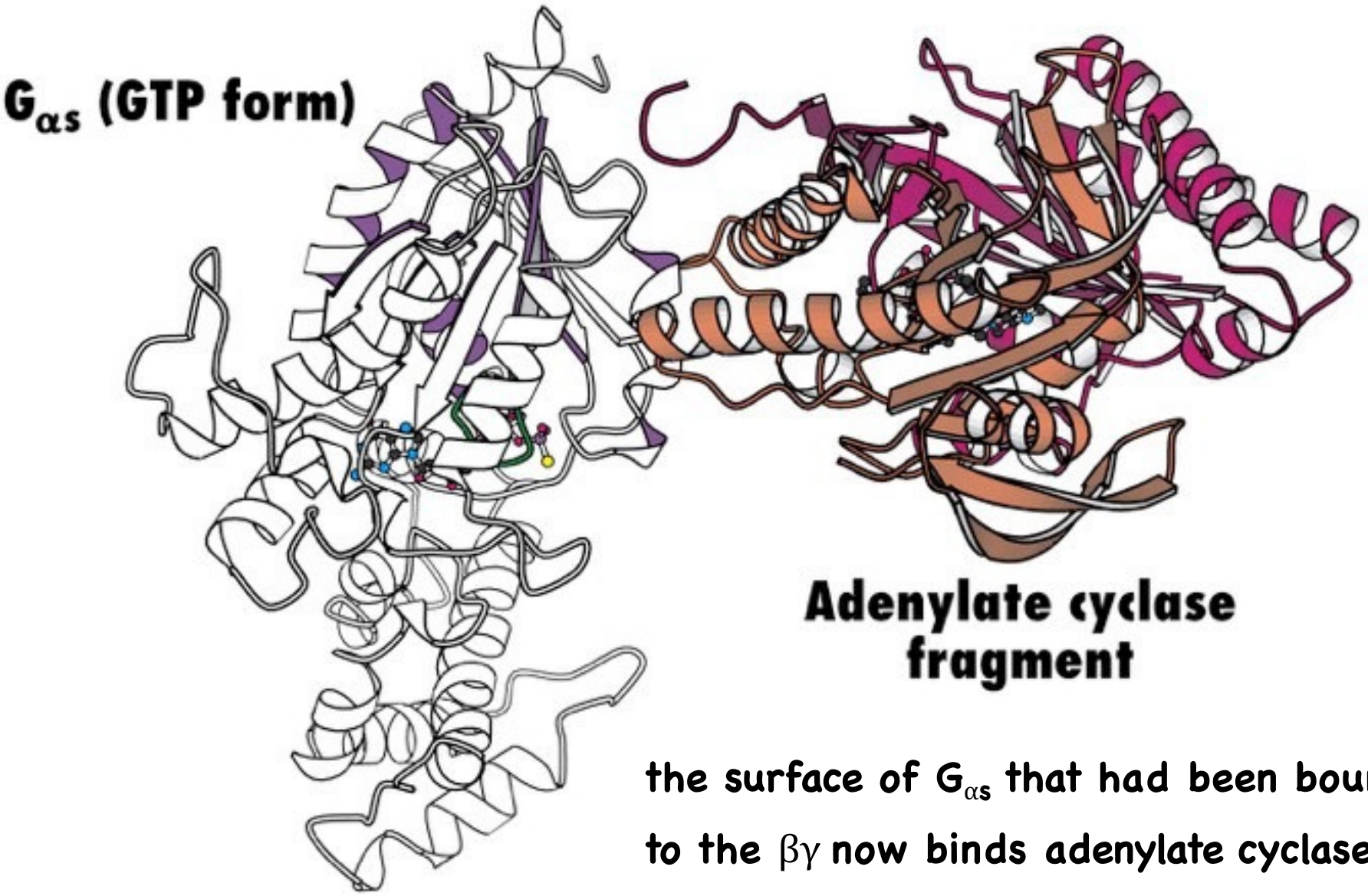
GDP is bound in a pocket close to the surface at which the α subunit interacts with the βγ dimer

12 membrane spanning helices

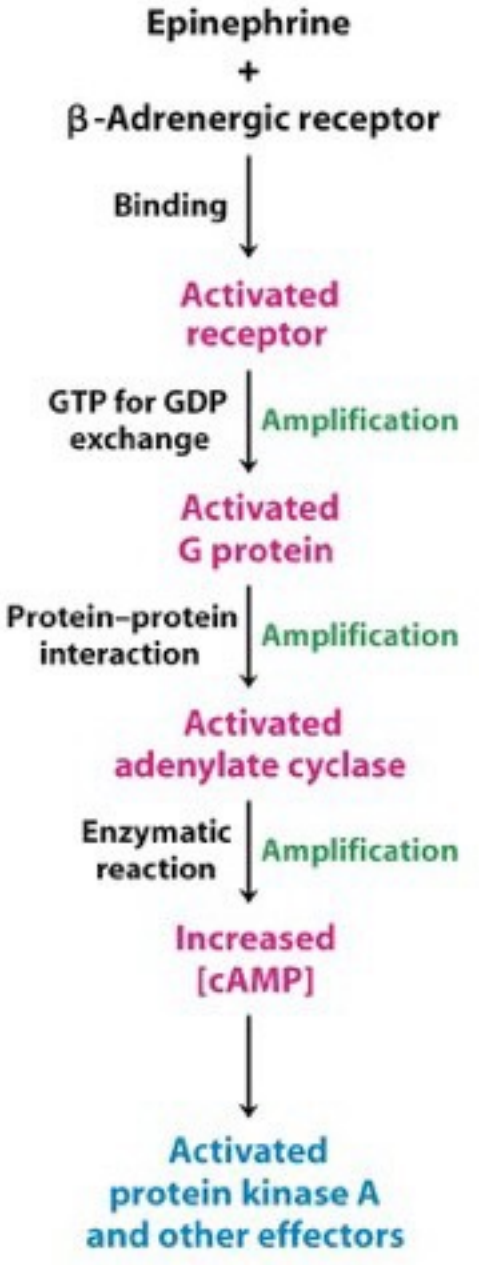


α subunit dissociates simultaneously from the $\beta\gamma$ dimer and now binds adenylate cyclase instead

- interaction of $G_{\alpha s}$ with adenylate cyclase favors a more catalytically active conformation



the surface of $G_{\alpha s}$ that had been bound to the $\beta\gamma$ now binds adenylate cyclase

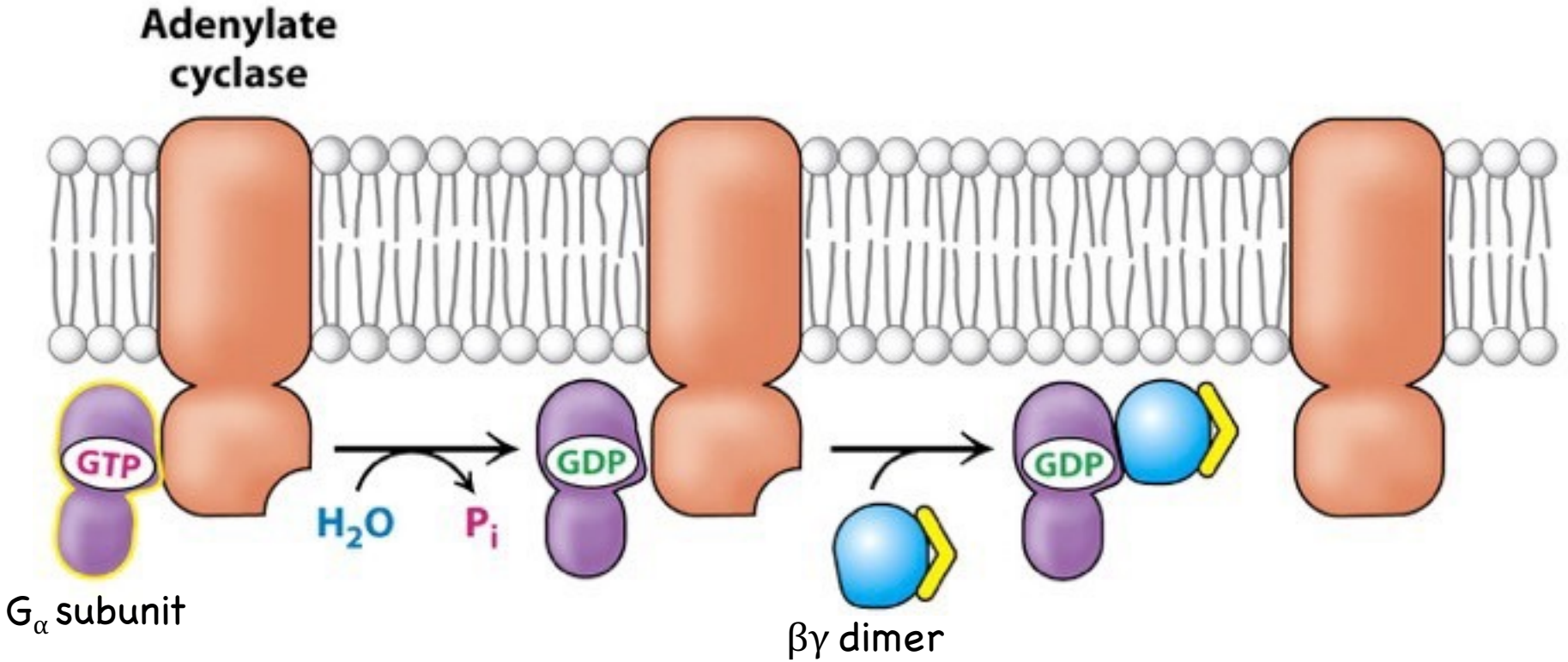


- most effects of cAMP are mediated by the activation of protein kinase A

Protein kinase A (PKA)

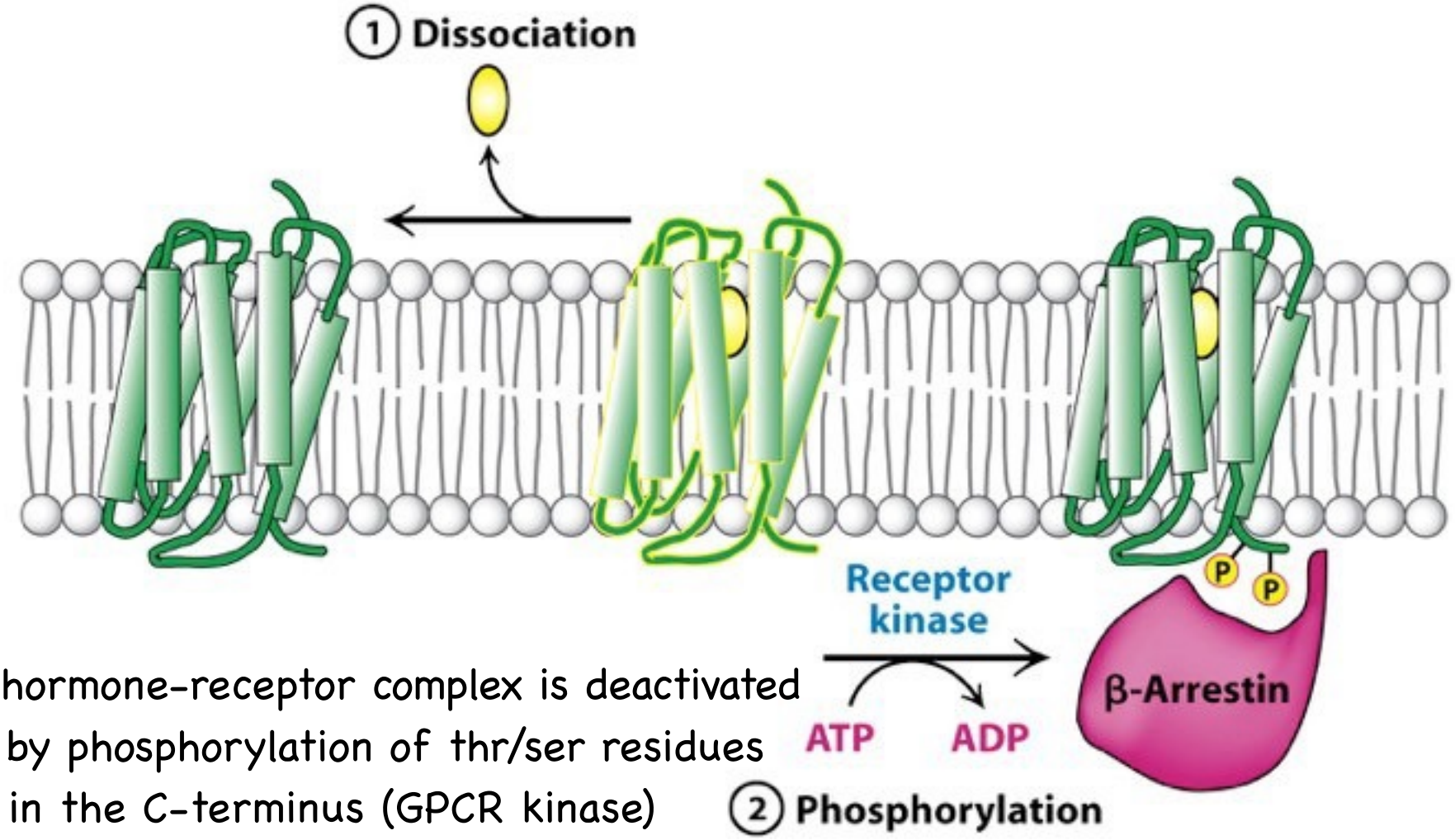
- consists of two catalytic and two regulatory chains (R_2C_2)
- binding of 4 cAMP to the regulatory chains releases the catalytic chains which are catalytically active on their own
- serine/threonine kinase
- phosphorylation of the cAMP-response element binding (CREB) protein leads to changes in gene expression

Signal termination-1



- G_α subunits have intrinsic GTPase activity
- upon hydrolysis of the bound GTP, G_α reassociates with the $\beta\gamma$ dimer to form the heterotrimeric G protein
- activation of adenylate cyclase is terminated

Signal termination-2



- hormone-receptor complex is deactivated by phosphorylation of thr/ser residues in the C-terminus (GPCR kinase)

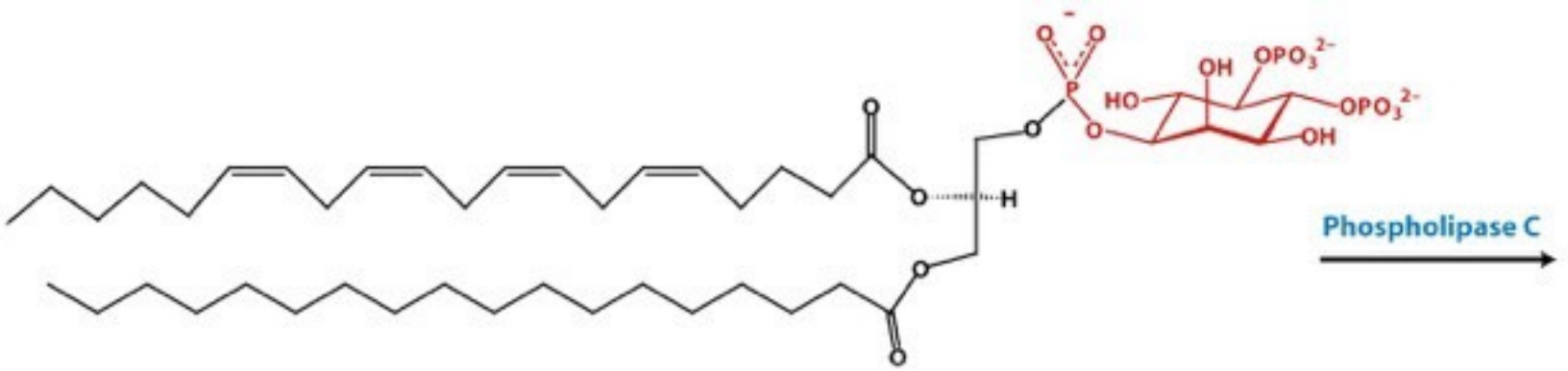
- β-arrestin binds and further diminishes its ability to activate G proteins

7TM receptors/ GPCR

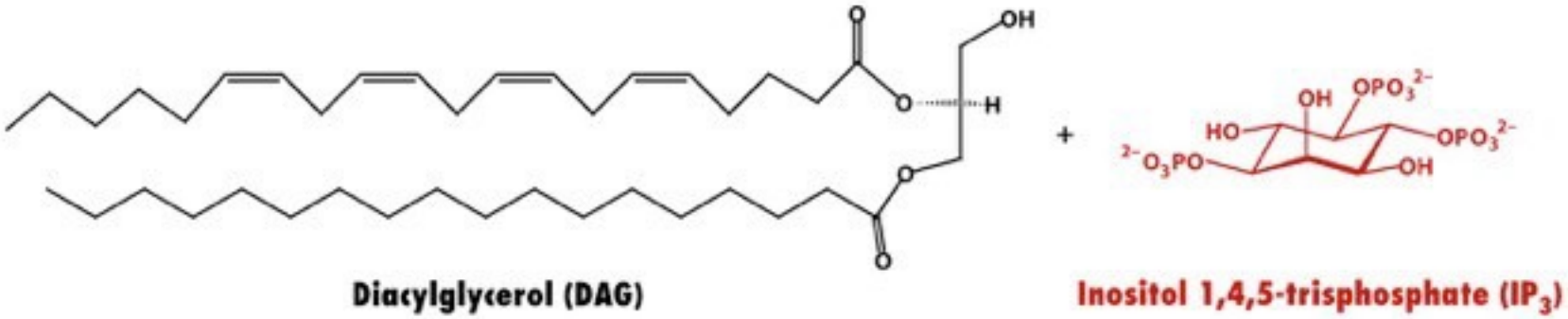
- cAMP/protein kinase A (PKA)
- β-adrenergic receptor
- Phospholipase C (PLC)
- Angiotensin II R

Phosphoinositide cascade

- $G_{\alpha q}$ subunit activates β-isoform of protein kinase C
- Phospholipase C cleaves the membrane lipid phophatidylinositol 4,5-biphosphate into two second messengers:
  - diacylglycerol: remains in the membrane
  - inositol 1,4,5-triphosphate: diffuses away from the membrane

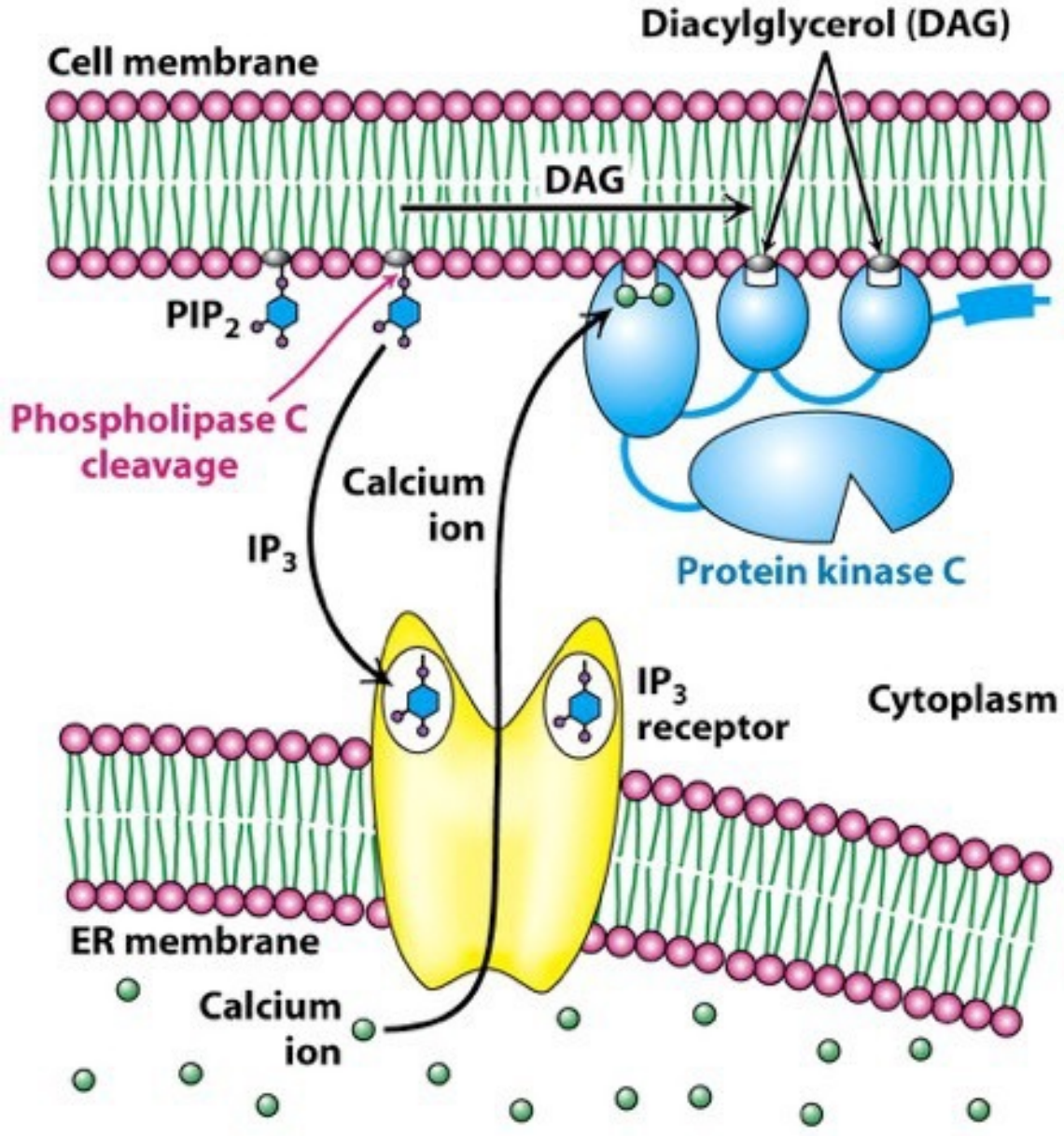


Phosphatidylinositol 4,5-bisphosphate (PIP_2)



Diacylglycerol (DAG)

Inositol 1,4,5-trisphosphate (IP_3)



Protein kinase C

ser/thr kinase

DAG

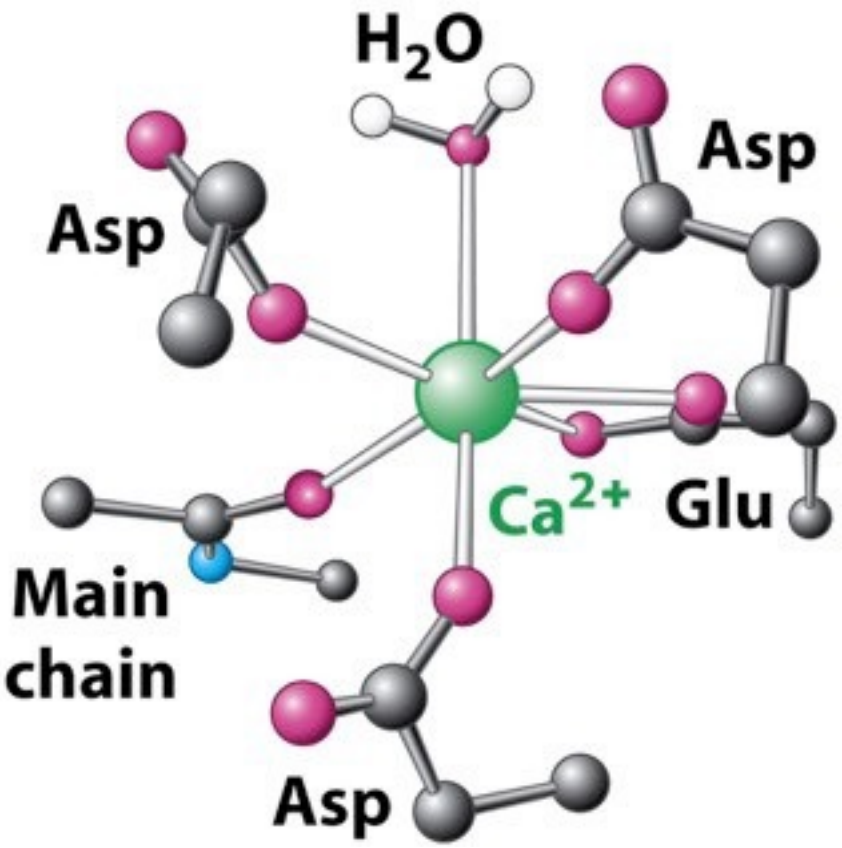
binding domain requires bound calcium

remains in the plasma membrane

=> IP_3 and DAG coactivate protein kinase C

both IP_3 and DAG act transiently because they are converted into other species by phosphorylation

Calcium-binding site

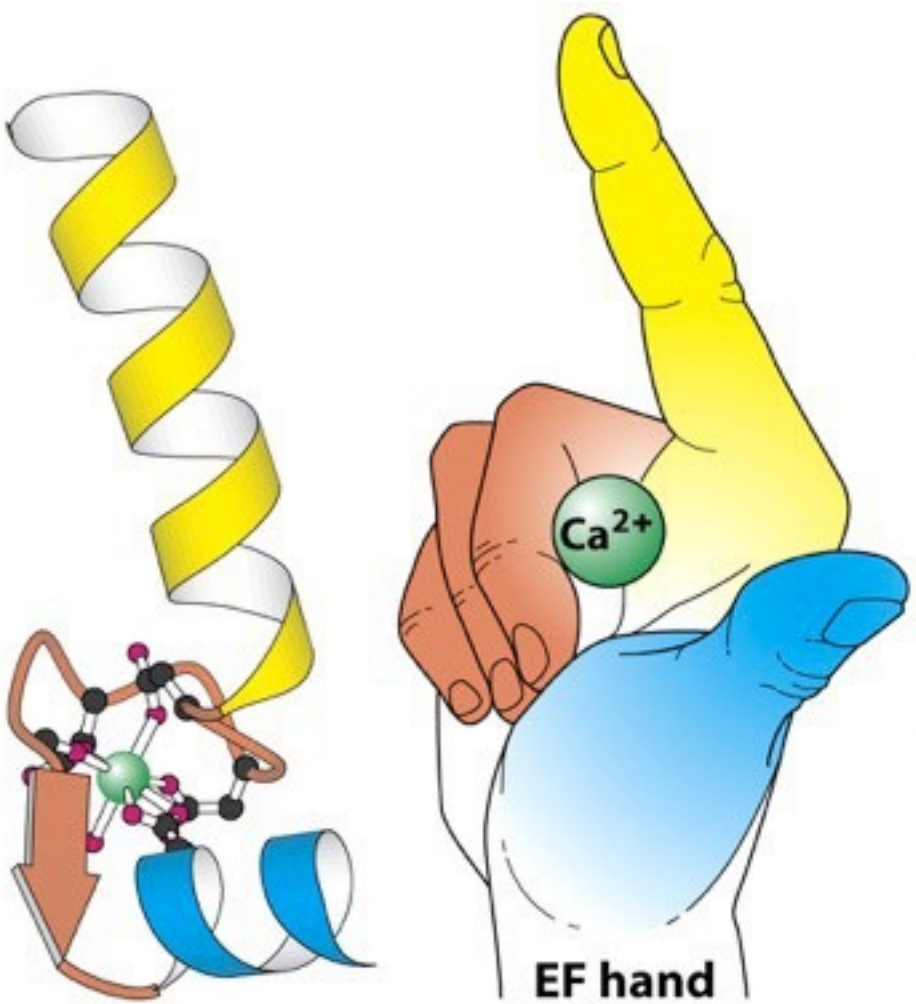


- Common mode of calcium binding: the ion is coordinated to six oxygen atoms from a protein and one of water
- The capacity to be coordinated to multiple ligands enables Ca^{2+} to cross-link different segments of a protein and induce significant conformational changes

Calmodulin (CaM)

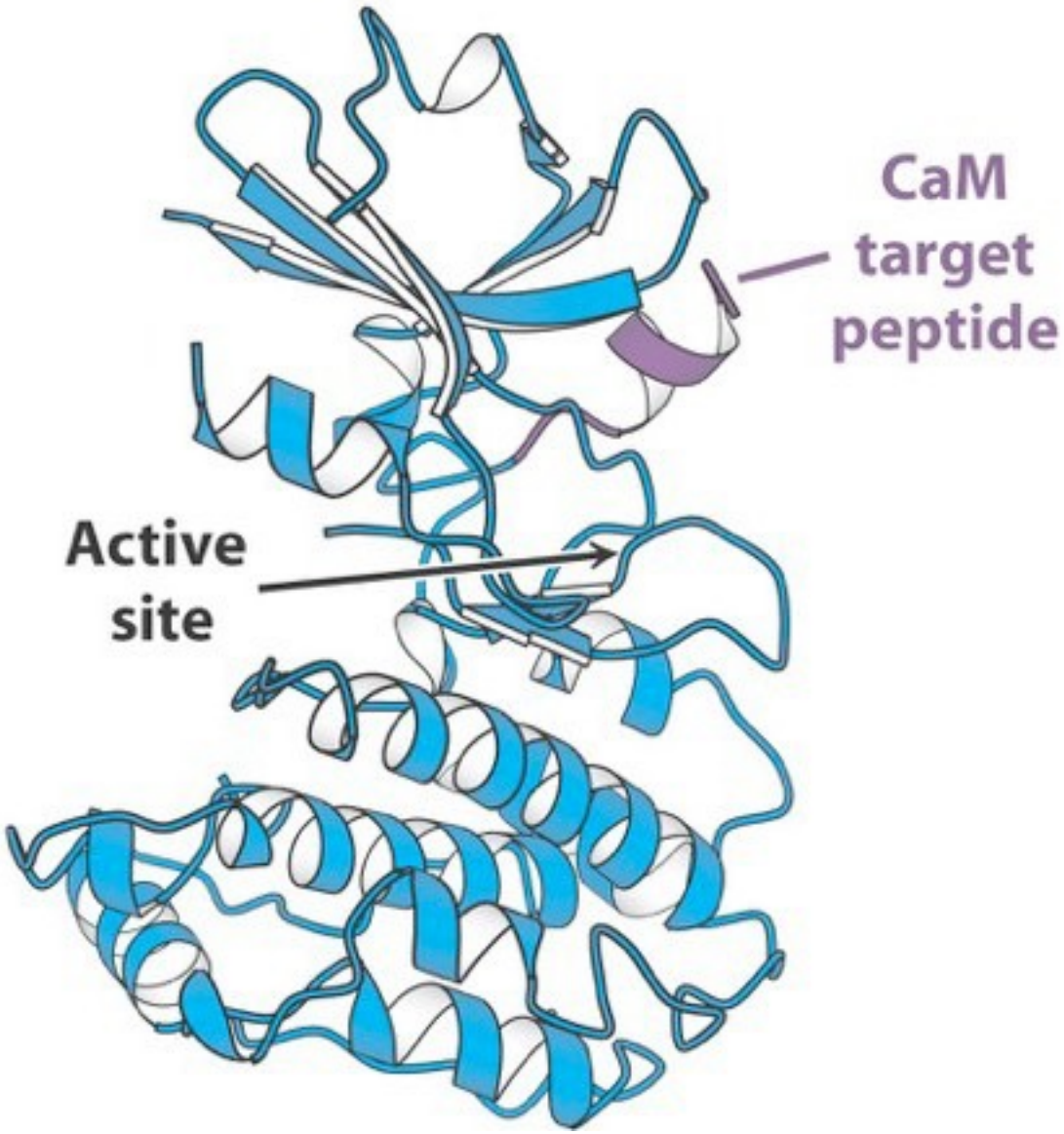
- 17-kd protein
- four Ca^{2+} binding sites
- serves as calcium sensor in nearly all eukaryotic cells
- activated by binding of Ca^{2+} when the cytoplasmic Ca^{2+} levels > 500 nM
  - usually 100 nM, several orders of magnitude lower than in the extracellular medium => transient increases can readily be sensed
- member of the EF-hand protein family

EF hand

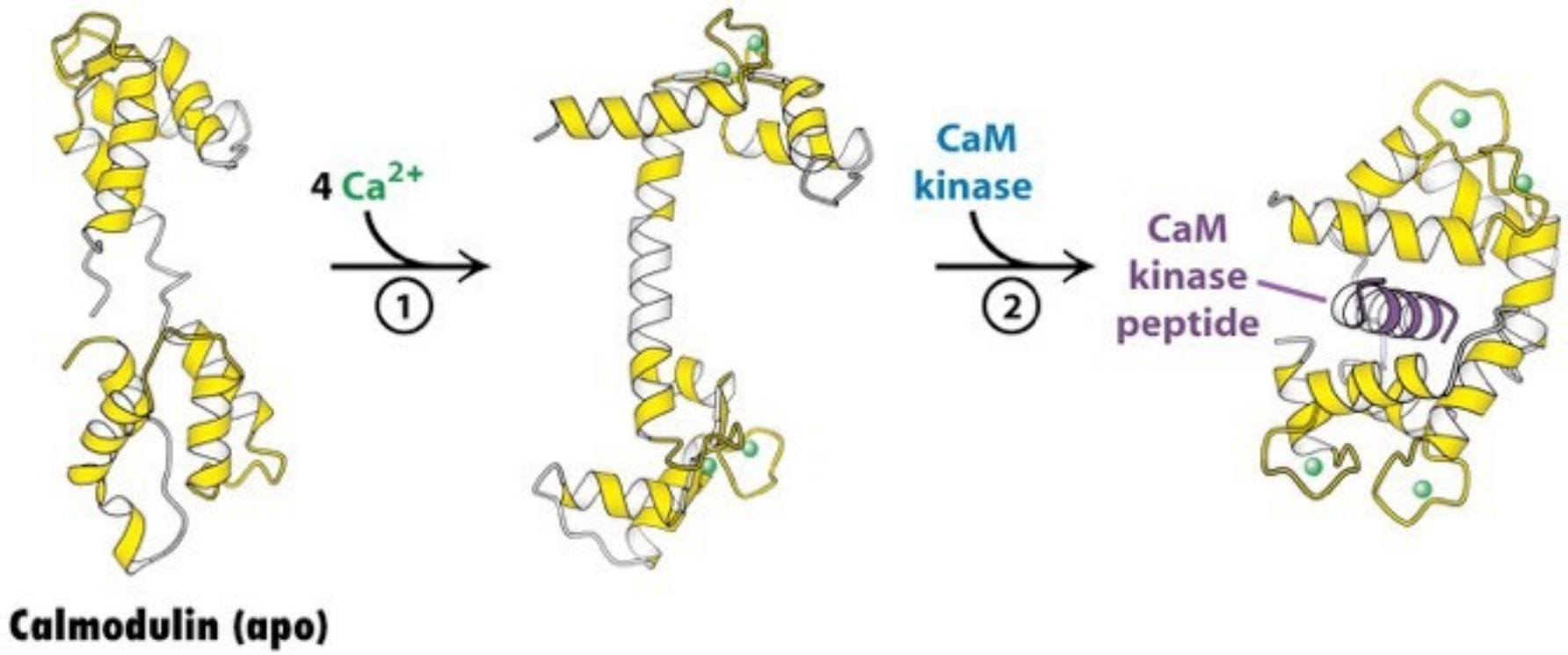


- discovered in parvalbumin
- Ca^{2+} is bound in a loop connecting 2 nearly perpendicular helices
- binding of Ca^{2+} induces substantial conformational changes
- hydrophobic surfaces are exposed that are used to bind other proteins
- using its two sets of two EF hands, calmodulin clamps down around specific regions of target proteins, usually exposed α helices with appropriately positioned hydrophobic and charged groups

CaM kinase



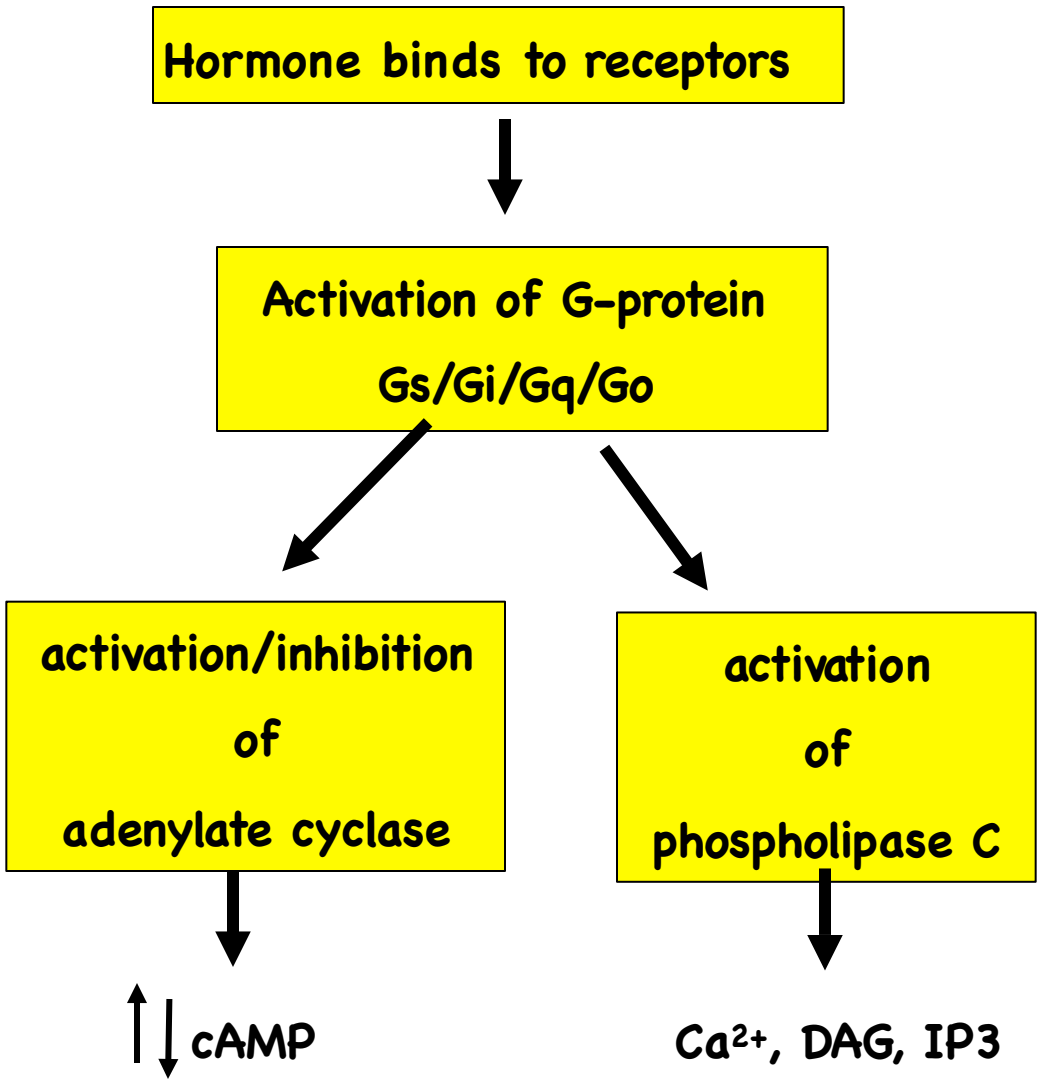
controls effectors


4 Ca2+
1
CaM
kinase
2
CaM
kinase
peptide
Calmodulin (apo)

Subfamilies of G protein subunits

- $G_s\alpha$: activates adenylate cyclase
- $G_i\alpha$: inhibits adenylate cyclase
- $G_q\alpha$: activates phospholipase C (PLC)
- $G_o\alpha$: activates ion channels

Hormonal activation of G-protein-coupled receptors



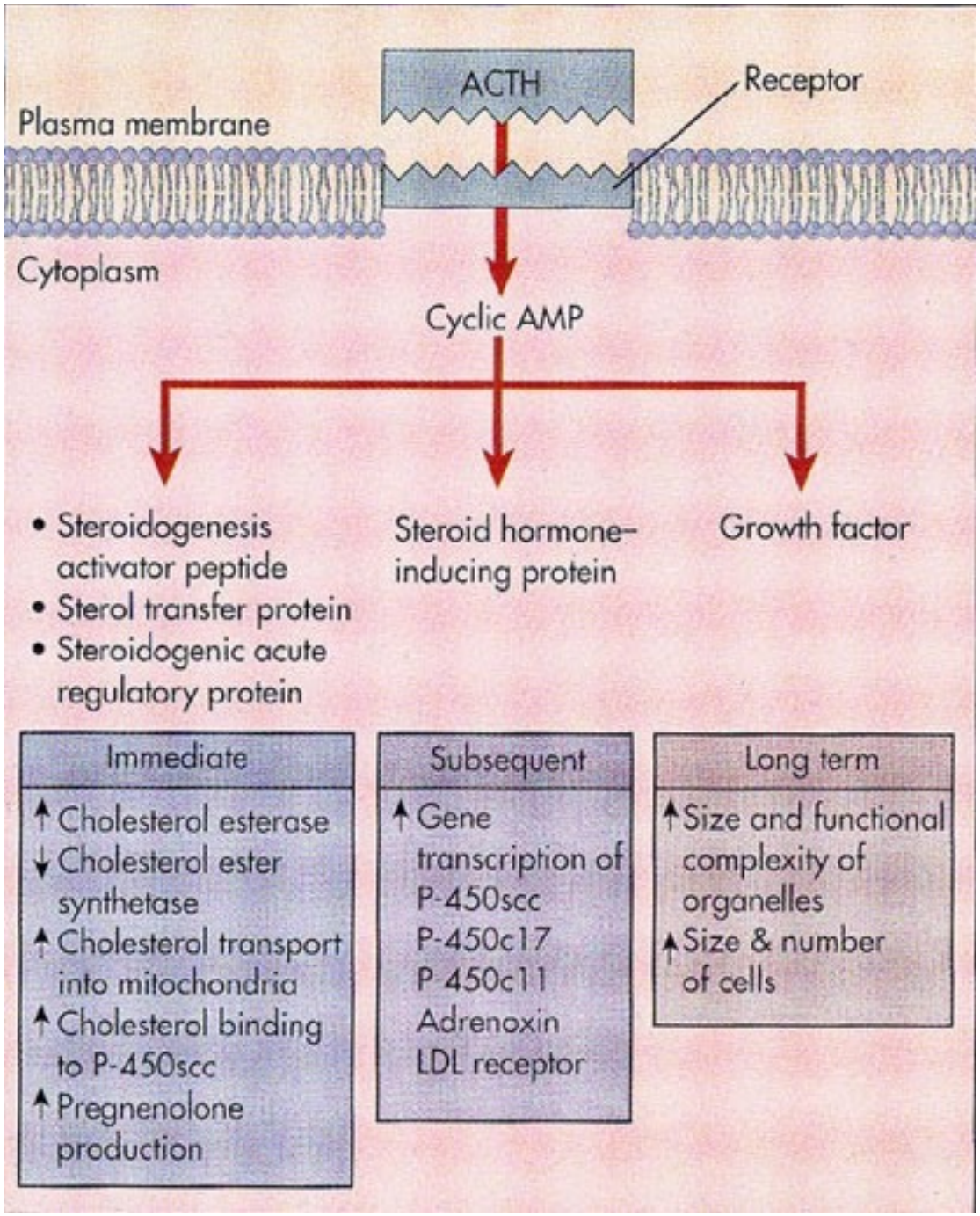
Hormone receptor

G-protein

Catalytic subunit

Second messenger

Thyrotrophin-releasing hormone (TRH)	$G_q\alpha$
Corticotrophin-releasing hormone (CRH)	$G_s\alpha$
Gonadotrophin-releasing hormone (GnRH)	$G_q\alpha$
Somatostatin (SS)	$G_i\alpha/G_q\alpha$
Thyroid-stimulating hormone (TSH)	$G_s\alpha/G_q\alpha$
Luteinizing hormone (LH)/human chorionic gonadotrophin (hCG)	$G_s\alpha/G_q\alpha$
Follicle-stimulating hormone (FSH)	$G_s\alpha/G_q\alpha$
Adrenocorticotrophic hormone (ACTH)	$G_s\alpha$
Oxytocin	$G_q\alpha$
Arginine vasopressin (AVP)	$G_s\alpha/G_q\alpha$
Catecholamines (β-adrenergic)	$G_s\alpha$
Angiotensin II (AII)	$G_i\alpha/G_q\alpha$
Glucagon	$G_s\alpha$
Calcium	$G_i\alpha/G_q\alpha$
Calcitonin	


ACTH
Receptor
Plasma membrane
Cytoplasm
Cyclic AMP
• Steroidogenesis activator peptide
• Sterol transfer protein
• Steroidogenic acute regulatory protein
Steroid hormone–inducing protein
Growth factor
Immediate
↑ Cholesterol esterase
↓ Cholesterol ester synthetase
↑ Cholesterol transport into mitochondria
↑ Cholesterol binding to P-450scc
↑ Pregnenolone production
Subsequent
↑ Gene transcription of
P-450scc
P-450c17
P-450c11
Adrenoxin
LDL receptor
Long term
↑ Size and functional complexity of organelles
↑ Size & number of cells

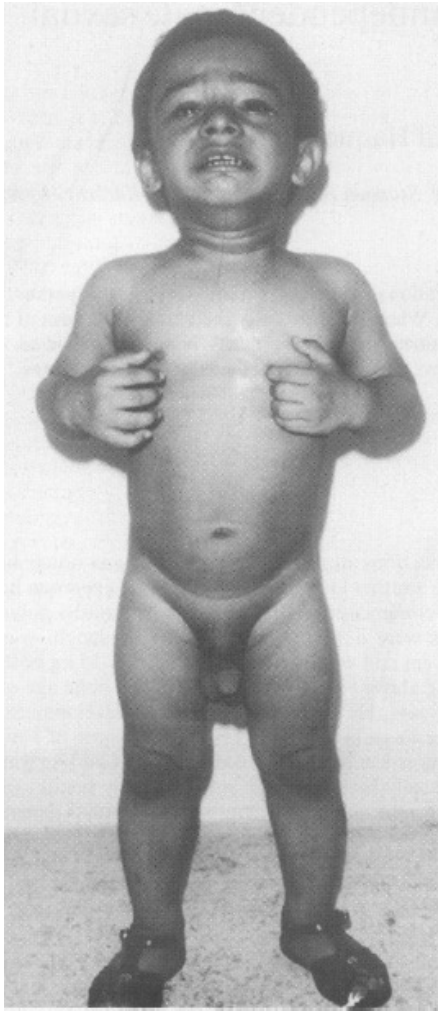
Defects in the GPCR/G-protein signaling pathways

- Gain of function
  - LH receptor:
    - Male precocious puberty "testotoxicosis"
  - TSH receptor:
    - Toxic thyroid adenomas
  - $G_s\alpha$:
    - McCune-Albright syndrome
      - some cases of acromegaly and some autonomous thyroid nodules
- Loss of function
  - Vasopressin receptor
    - nephrogenic diabetes insipidus
  - TSH receptor
    - resistance to TSH
  - $G_s\alpha$:
    - pseudohypoparathyroidism
    - Albright's hereditary osteodystrophy

LH receptor:

Male precocious puberty

"testotoxicosis"



2-year old boy

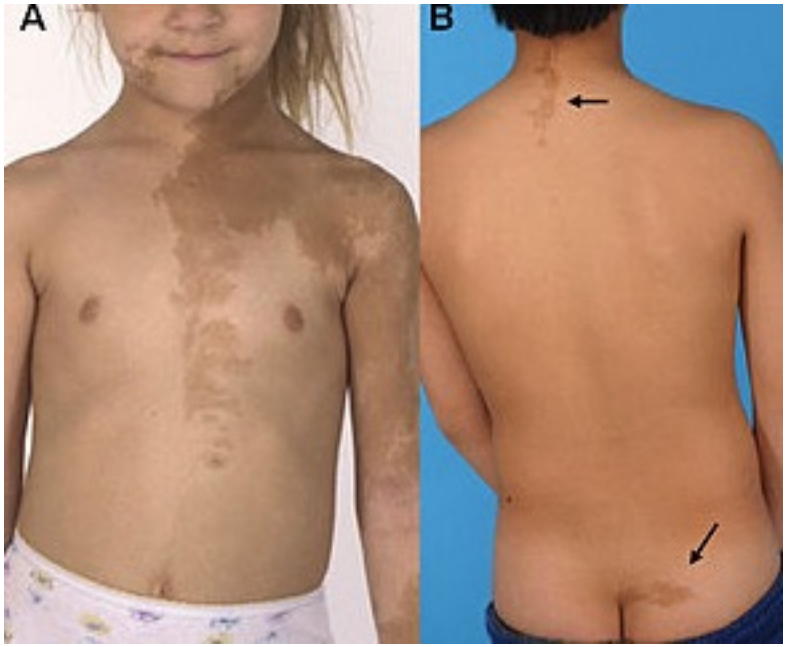
- $G_s\alpha$:
  - McCune-Albright syndrome

  GNAS1 gene

  -polyostotic fibrous dysplasia of bone

  -*café-au-lait* skin pigmentation

  -peripheral precocious puberty

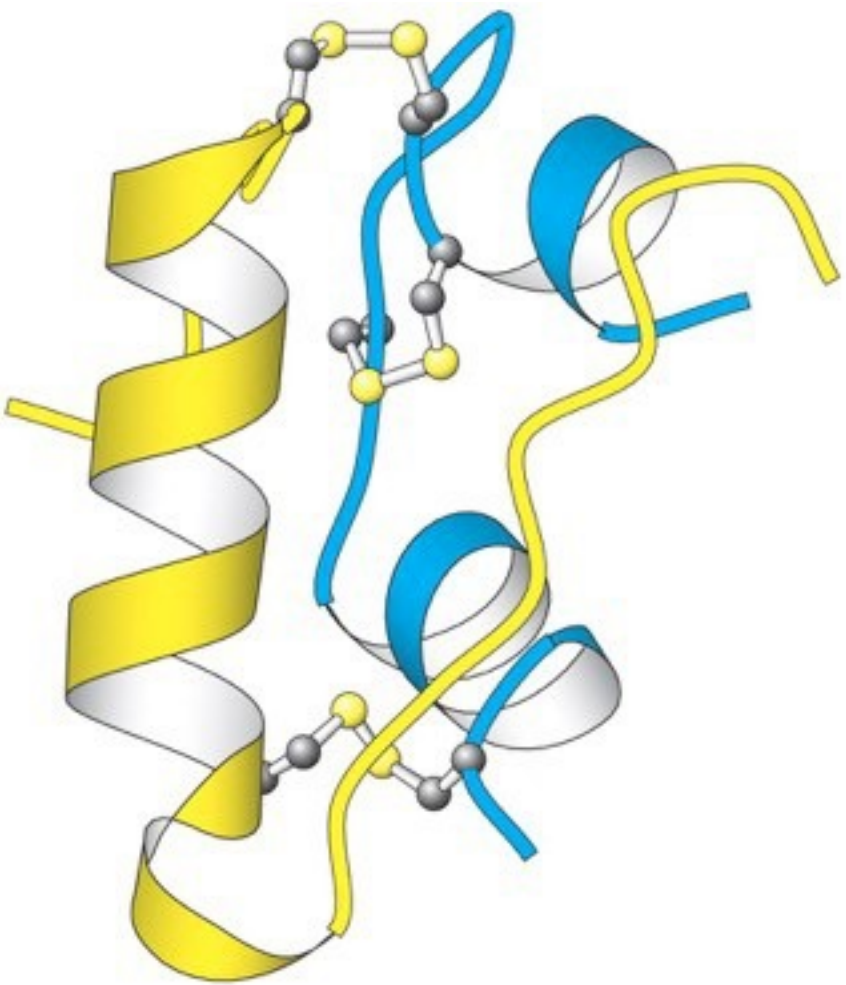


2. Receptor Tyrosine Kinases

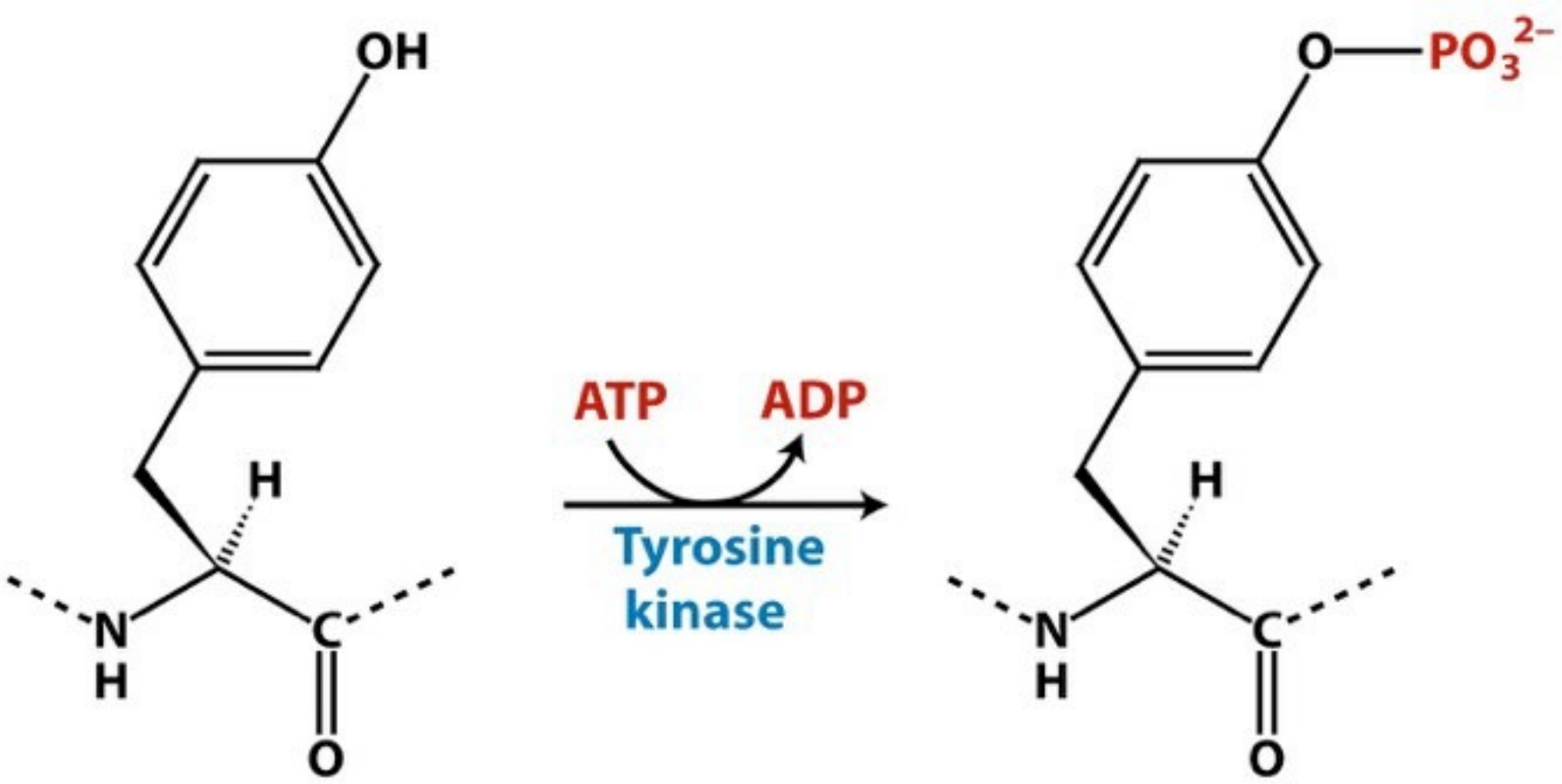
Insulin signaling

- after a meal: insulin is released
- mobilizes glucose transporters that allow the cell to take up the glucose that is in the bloodstream after a meal

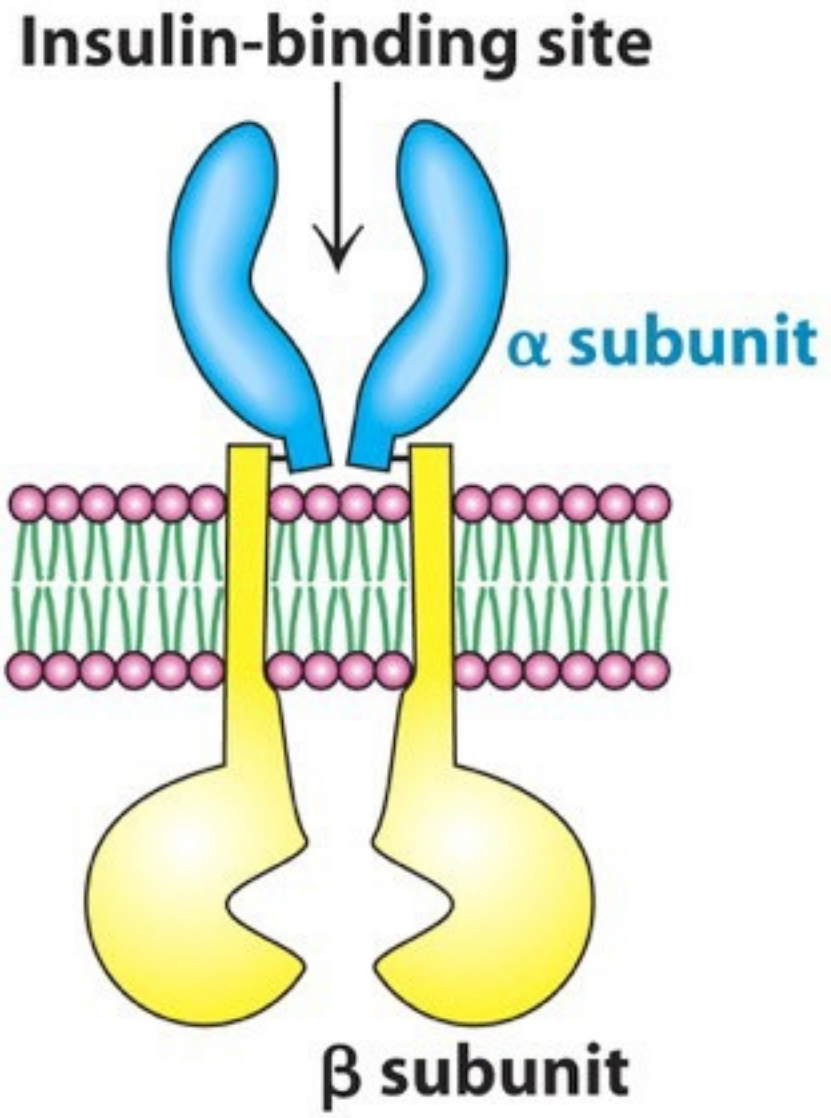
insulin



- peptide hormone
- 2 chains linked by 3 disulfide bonds


OH
H
N
H
C
O
ATP
ADP
Tyrosine
kinase
O
PO3 2–
H
N
H
C
O

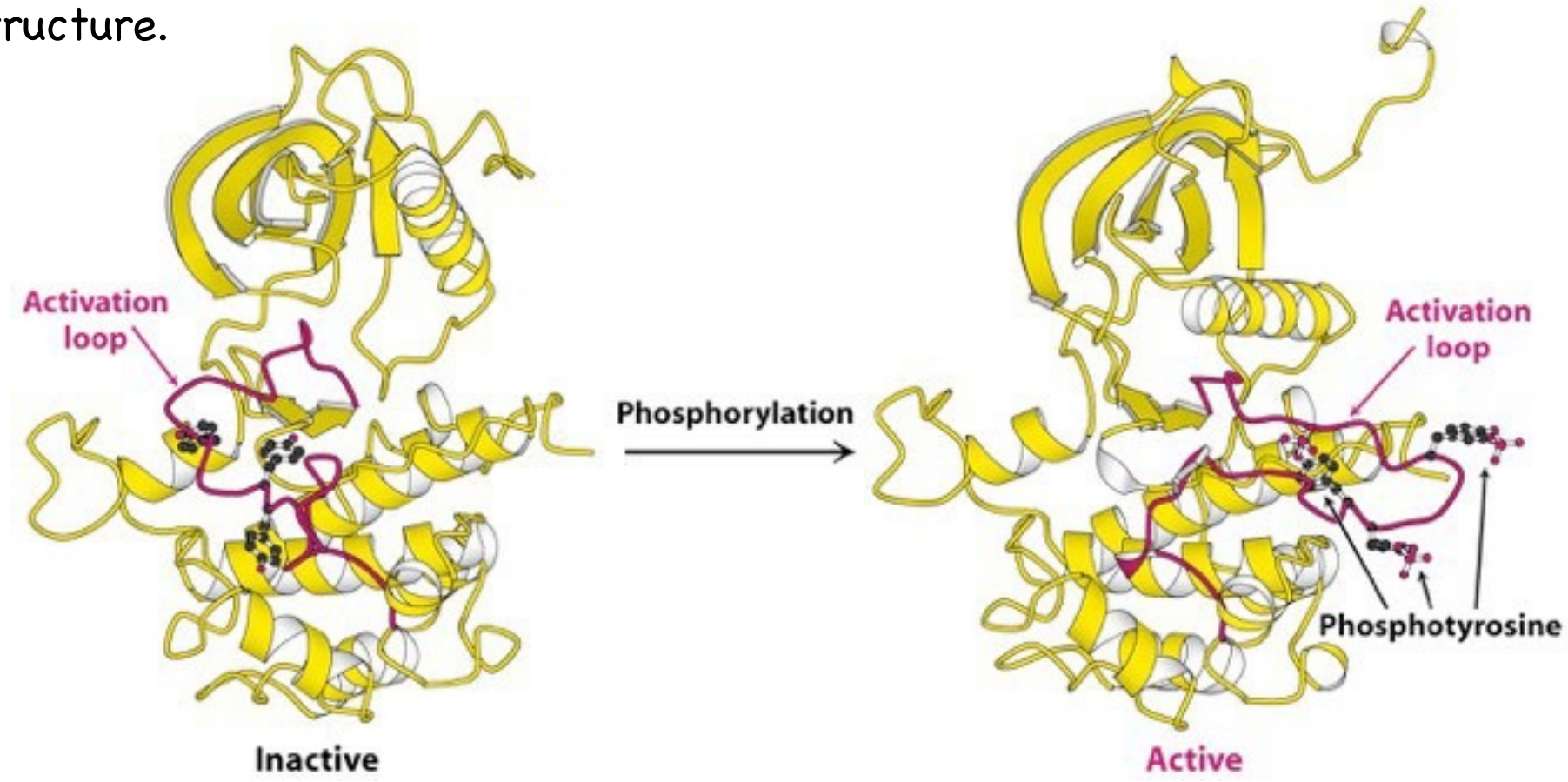
Insulin Receptor



homodimer

- each unit has a α and β subunit that are linked by a single disulfide bond
- a single insulin molecule interacts with two identical insulin receptor chains
- the moving together of the dimeric units in the presence of the ligand activates signaling
- each β subunit consists primarily of a protein kinase domain

The insulin receptor kinase is in an inactive conformation when the kinase domain is not covalently modified : the activation loop lies in the center of the structure.



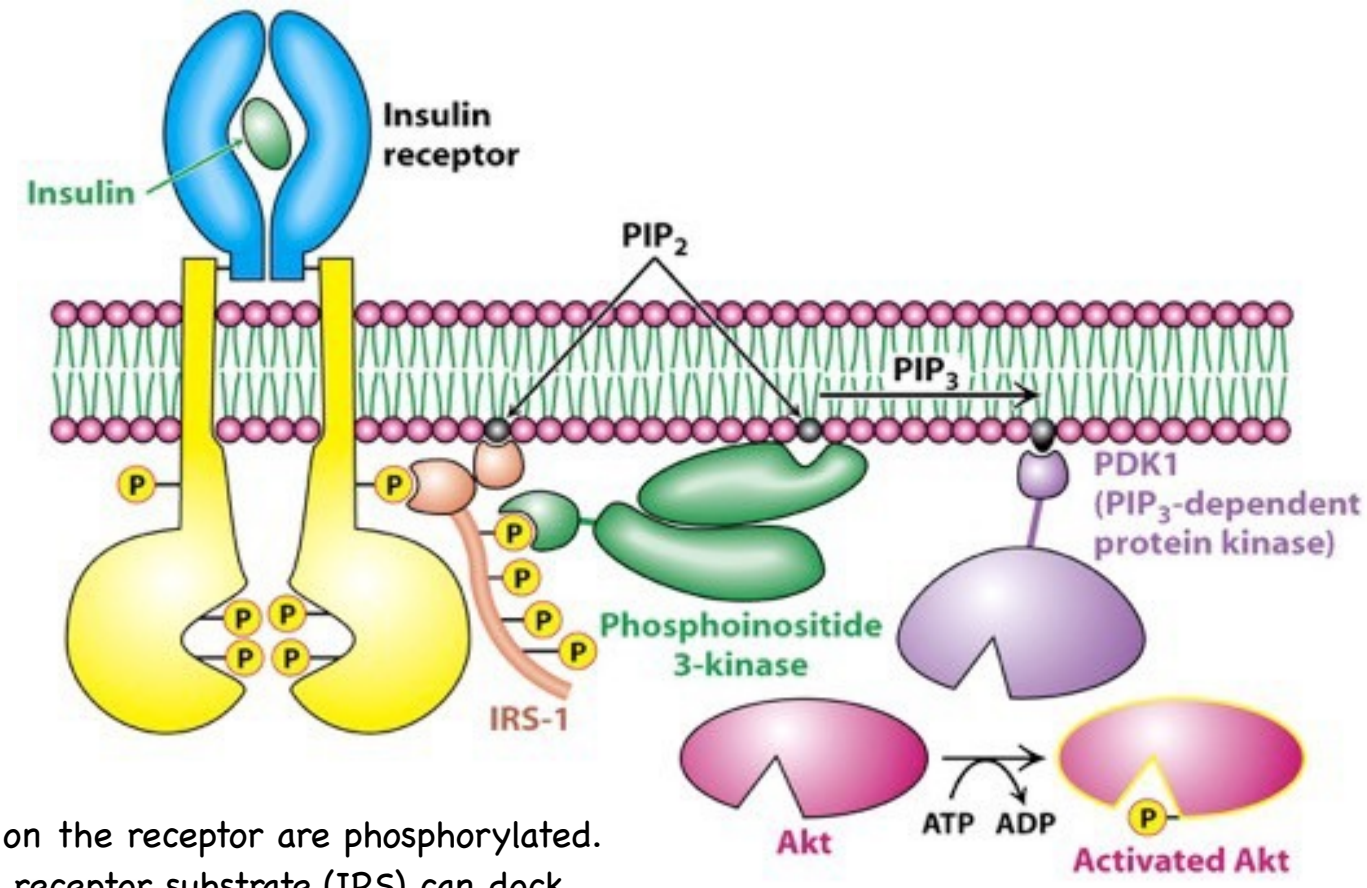
When 3 tyrosine residues in the activation loop are phosphorylated, the activation loop swings across the structure.

The new conformation is catalytically active.

Insulin Receptor Signaling -Overview

1.insulin binding moves the two subunits together

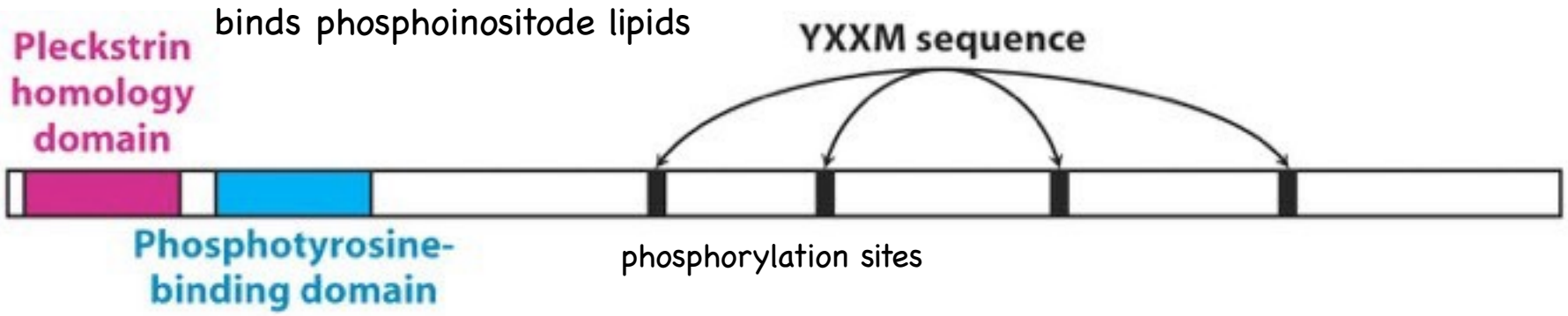
2. The flexible activation loop of one kinase fits into the activation loop of the other kinase subunit. With the two β subunits forced together, the kinase domains catalyze the addition of phosphoryl groups from ATP to tyrosine residues in the activation loops. This converts the kinase into an active conformation.



3. Additional sites on the receptor are phosphorylated.
insulin-receptor substrate (IRS) can dock

From the IRS protein, the signal is conveyed to a series of membrane-anchored molecules to a protein kinase that finally leaves the membrane

IRS-1 and IRS-2



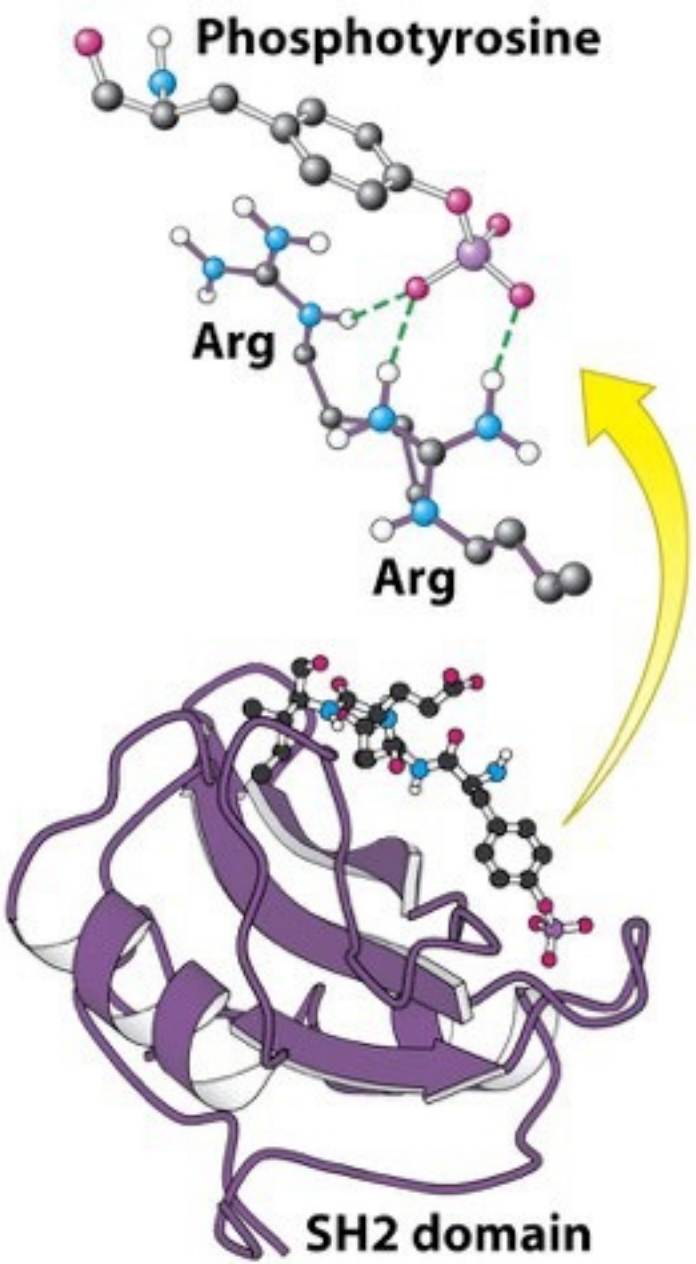
Pleckstrin homology domain and phophotyrosine binding domain anchor the IRS to the insulin receptor and the membrane

pleckstrin homology domain binds phosphoinositide

Act as adaptor: once phosphorylated they bind the lipid kinase, bring it to the membrane so that it can act on its substrate a membrane lipid

Adaptor domains

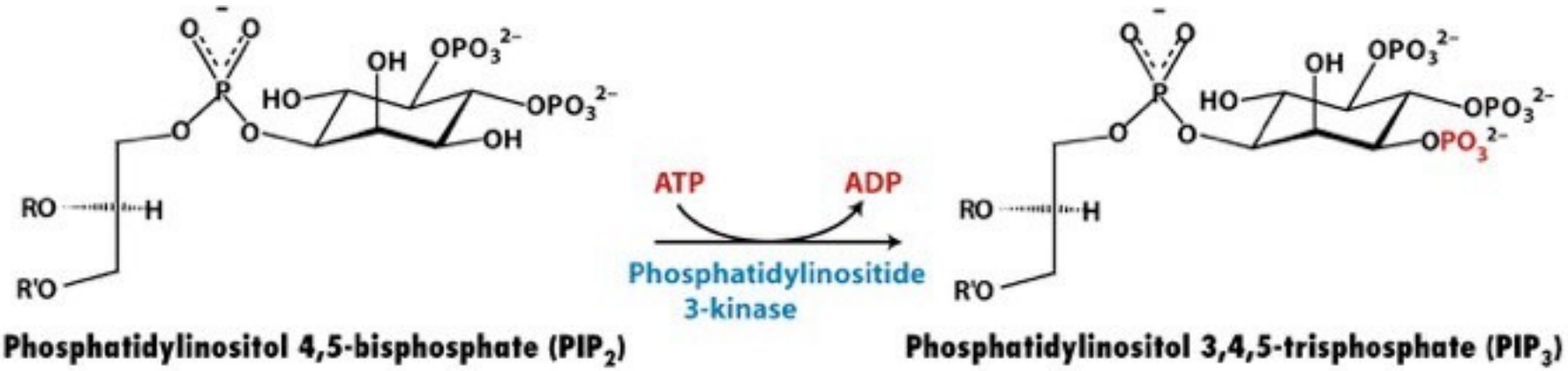
- Usually recognize specific classes of molecules and help transfer information from one protein to another



SH2 domain structure

- Negatively charged phosphotyrosine residue interacts with two arginine residues that are conserved in all SH2 domains

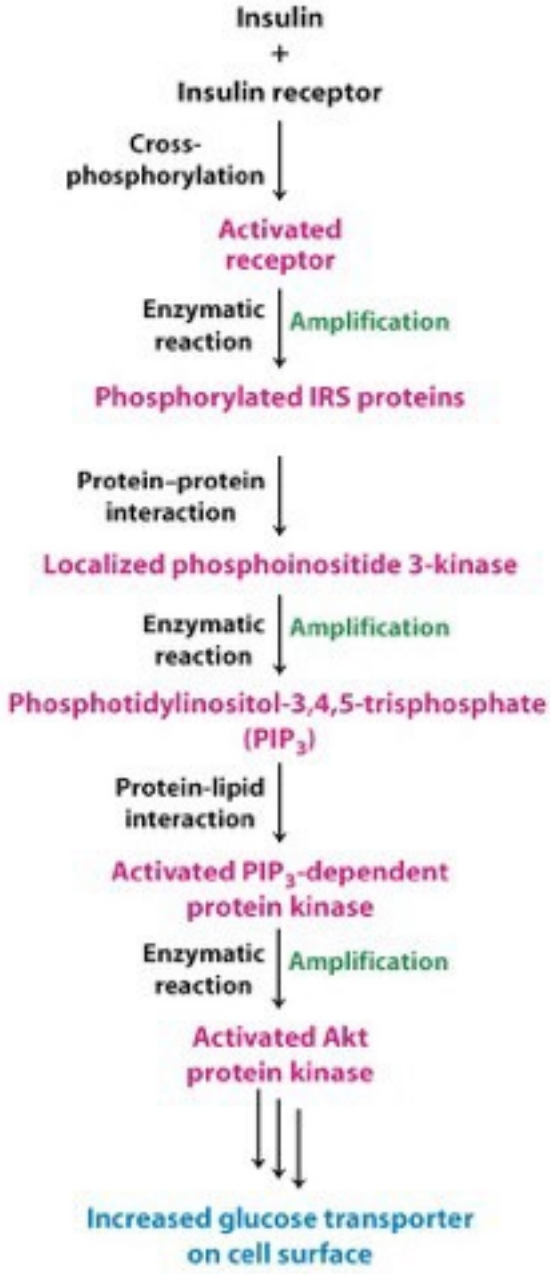
PI3 kinase



- Heterooligomer: 110-kd catalytic subunits

  85-kd regulatory subunits, contains SH2 domain

- PIP3 activates PDK1 via pleckstrin domain present in this protein kinase
- PDK1 phosphorylates Akt: non-membrane bound kinase
- Akt phosphorylates targets throughout the cell
  - Components that control trafficking of the glucose receptor
  - Enzymes that stimulate glycogen synthesis


Insulin
+
Insulin receptor
Cross-phosphorylation
Activated receptor
Enzymatic reaction
Amplification
Phosphorylated IRS proteins
Protein–protein interaction
Localized phosphoinositide 3-kinase
Enzymatic reaction
Amplification
Phosphotidylinositol-3,4,5-trisphosphate (PIP_3)
Protein-lipid interaction
Activated PIP_3-dependent protein kinase
Enzymatic reaction
Amplification
Activated Akt protein kinase
Increased glucose transporter on cell surface

Termination of insulin signaling

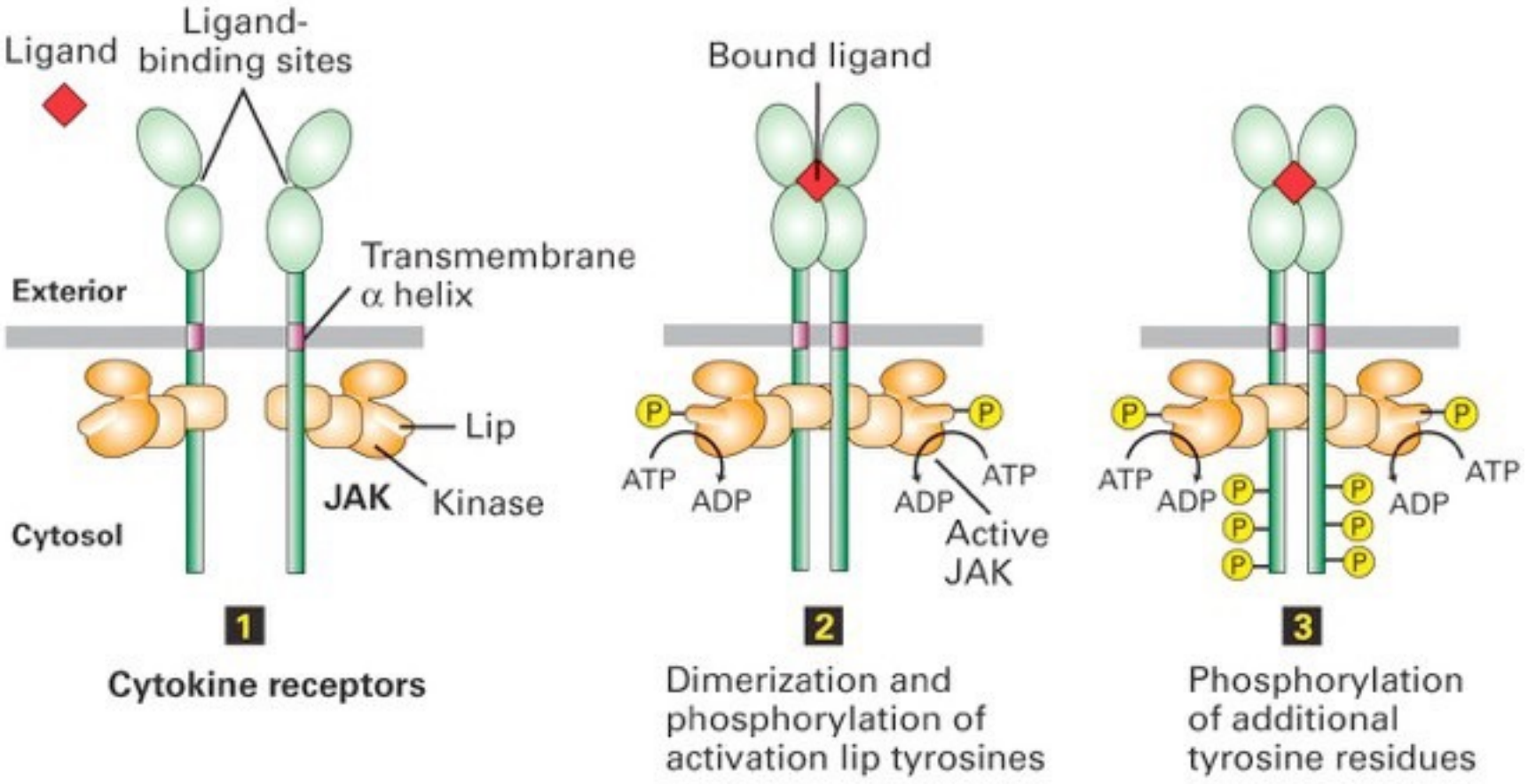
- Ser/thr or tyr phosphorylations are kinetically stable

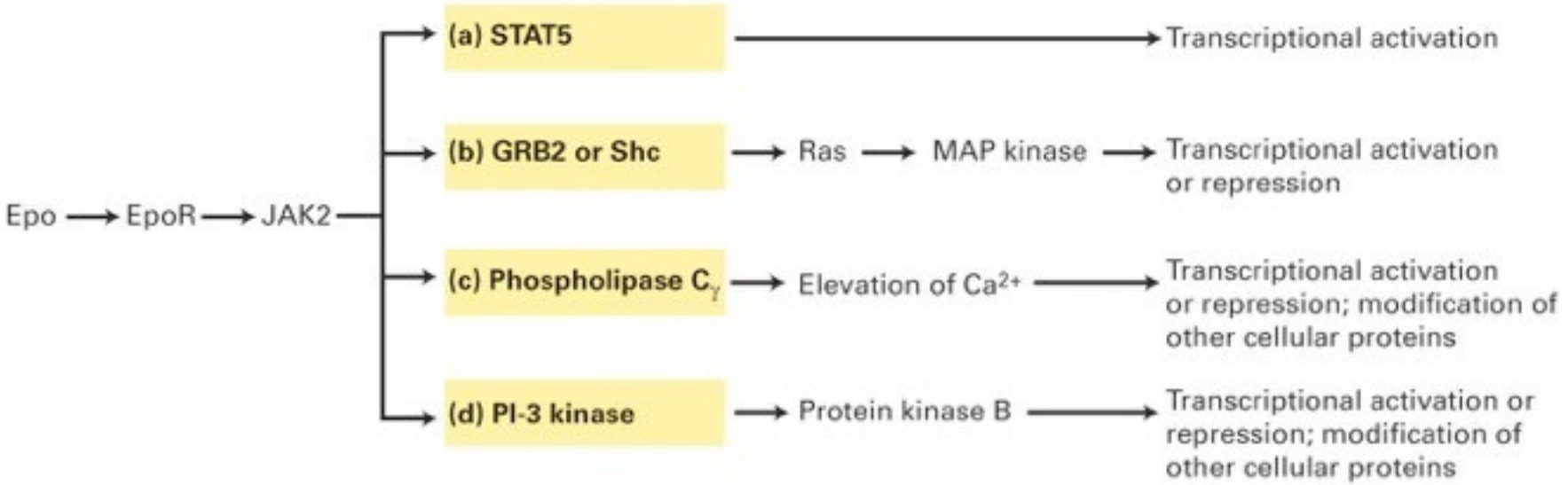
- Phosphatases:
  - Protein tyrosine phoshatases
  - Protein serine phosphatases
  - Lipid phosphatases

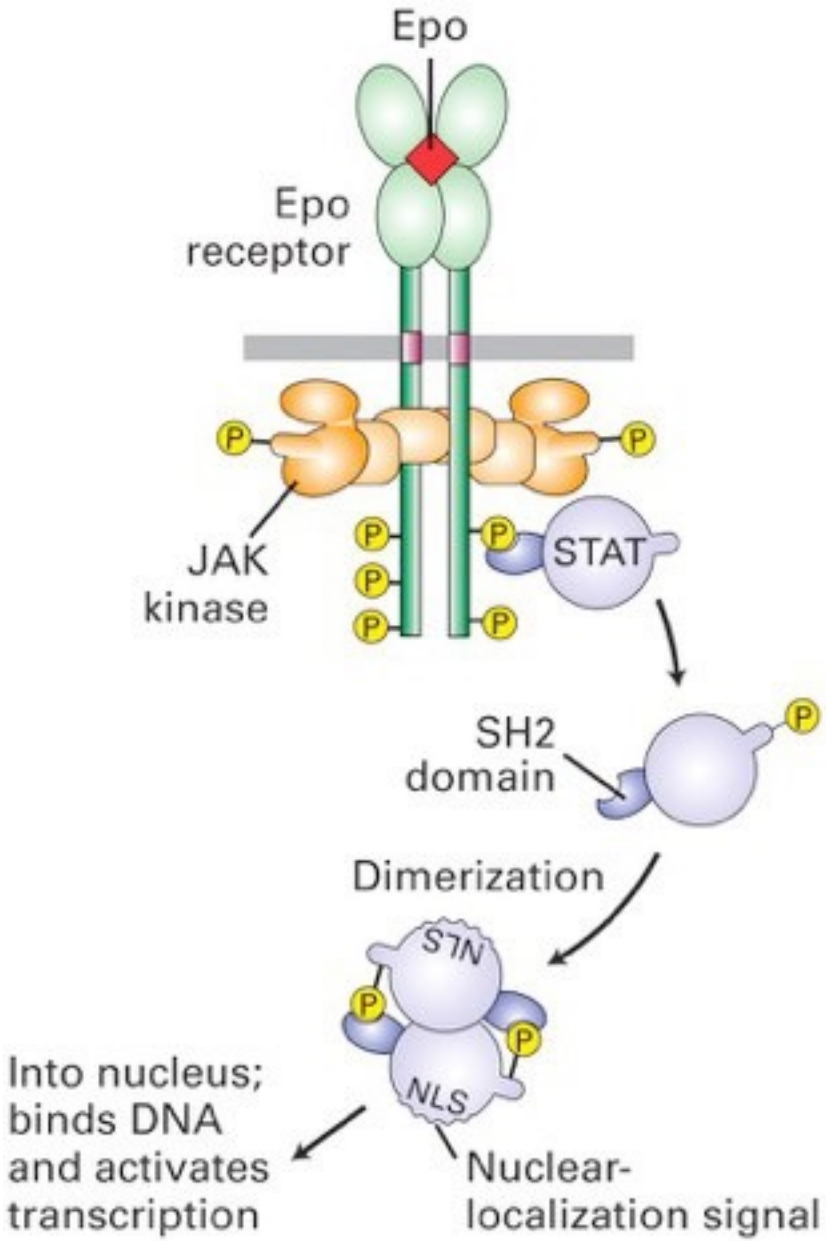
- Many of these phosphatases are activated or recruited as part of the response to insulin

=> Binding of the initial signal sets the stage for the eventual termination of the response

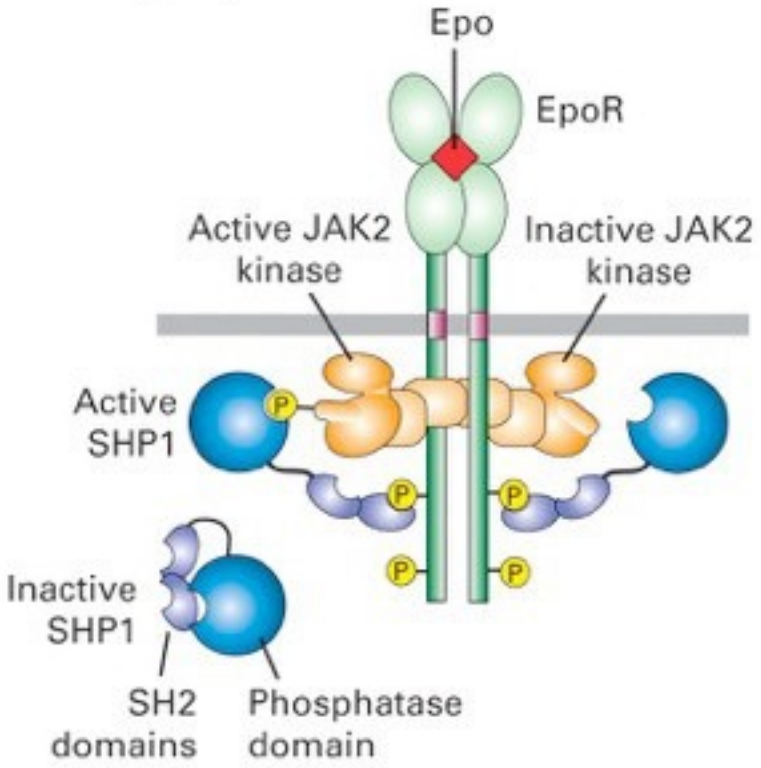
3. Cytokine receptor signaling



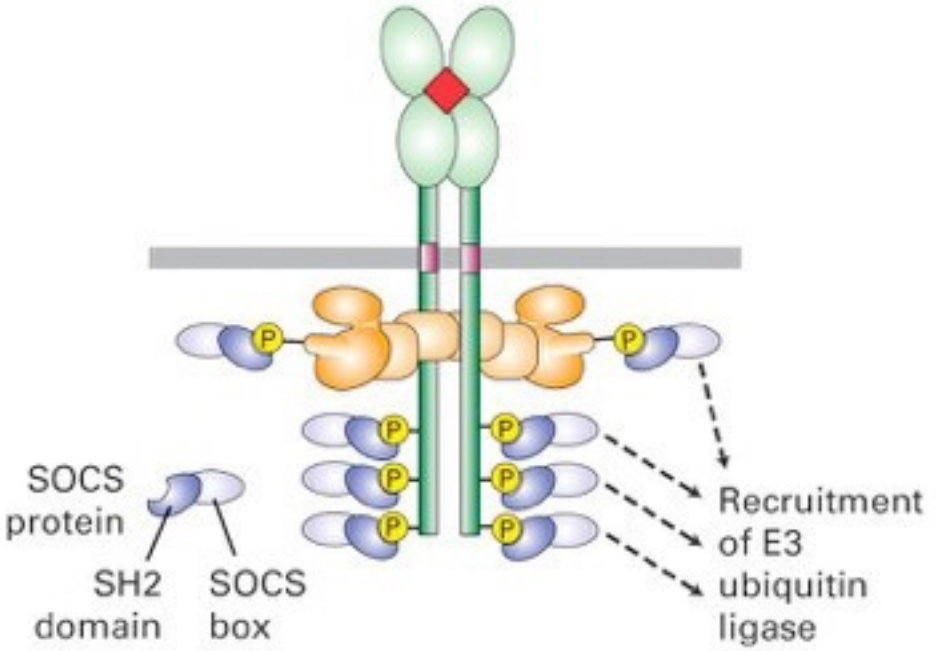

Epo
EpoR
JAK2
(a) STAT5
Transcriptional activation
(b) GRB2 or Shc
Ras
MAP kinase
Transcriptional activation or repression
(c) Phospholipase C_γ
Elevation of Ca^{2+}
Transcriptional activation or repression; modification of other cellular proteins
(d) PI-3 kinase
Protein kinase B
Transcriptional activation or repression; modification of other cellular proteins

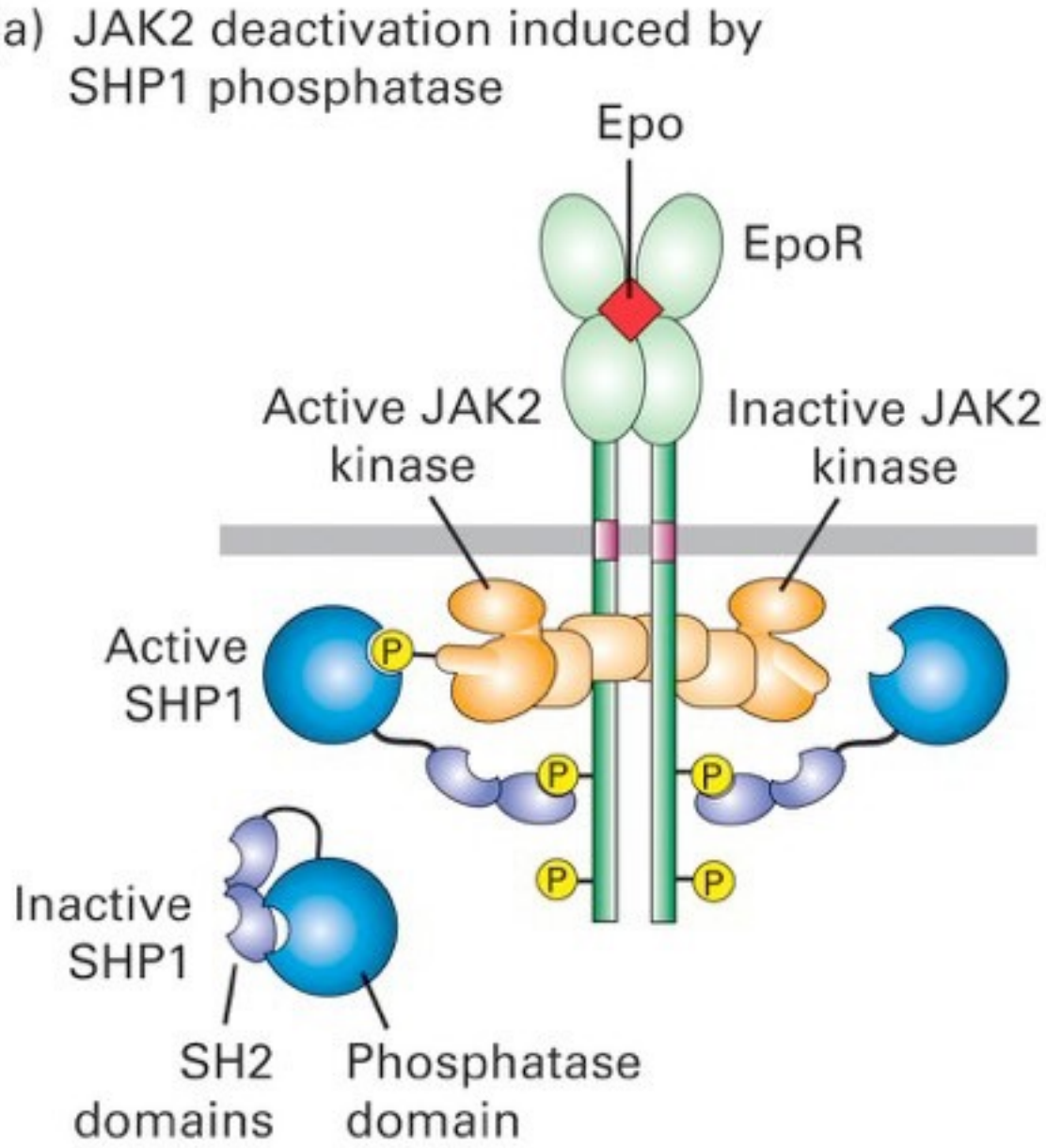

Epo
Epo receptor
JAK kinase
STAT
SH2 domain
Dimerization
NLS
Into nucleus; binds DNA and activates transcription
Nuclear-localization signal

(a) JAK2 deactivation induced by SHP1 phosphatase

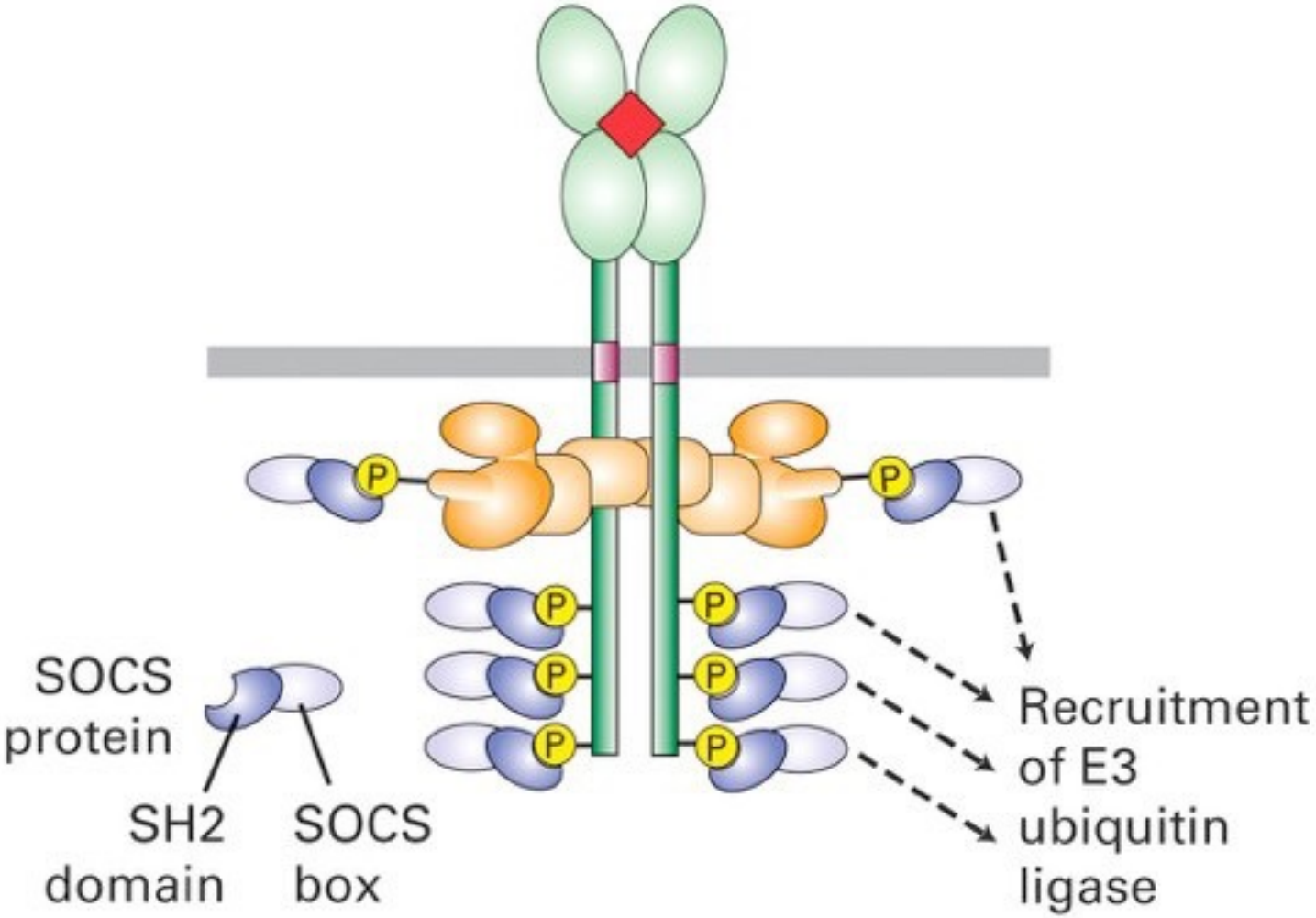


(b) Signal blocking and protein degradation induced by SOCS proteins

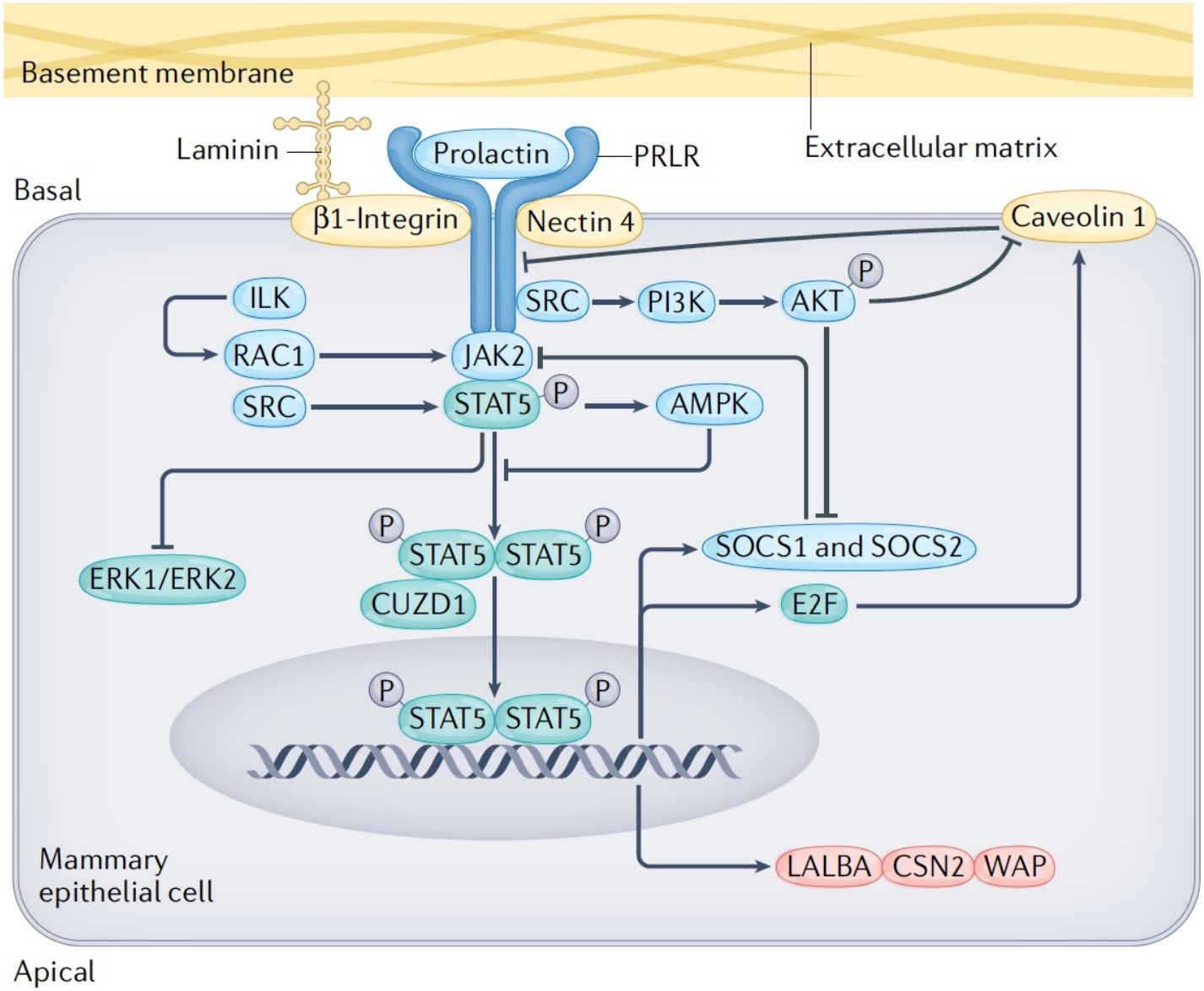



(a) JAK2 deactivation induced by SHP1 phosphatase
Epo
EpoR
Active JAK2 kinase
Inactive JAK2 kinase
Active SHP1
P
Inactive SHP1
SH2 domains
Phosphatase domain

(b) Signal blocking and protein degradation induced by SOCS proteins



Prolactin receptor signaling in a mammary epithelial cell



Receptor Classes and Signaling pathways

- G-protein coupled receptors
  - Ligands: epinephrine, glucagon, vasopressin, ACTH
  - 2nd messengers: cAMP or IP3/DAG
  - MAP kinase
- Receptor tyrosine kinases
  - Ligands: IGF-1, insulin
  - PI3 kinase
  - IP3/DAG
  - Ras/MAPK
- Cytokine receptors
  - Ligands: GH, prolactin
    - STAT activation
    - PI3 kinase
    - IP3/DAG
    - Ras/MAPK