SIGNAL TRANSDUCTION

Membrane receptors

- 7-pass receptors
- Tyrosine kinase receptors
- Serine-treonine kinase receptors
- Cytochine receptors
- Chanel receptors

We will focus our attention on specific signal transduction pathways:

- Adenylyl ciclase
- Phospholipase C (β and γ)
- RAS-MAPK
- PI3-kinase

Membrane receptors

- **7-pass receptors**
- Tyrosine kinase receptors
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We will focus our attention on specific signal transduction pathways:

- Adenylyl ciclase
- Phospholipase C (β and γ)
- RAS-MAPK
- PI3-kinase

7 pass-transmembrane receptors: G Protein Coupled Receptors (GPCR)

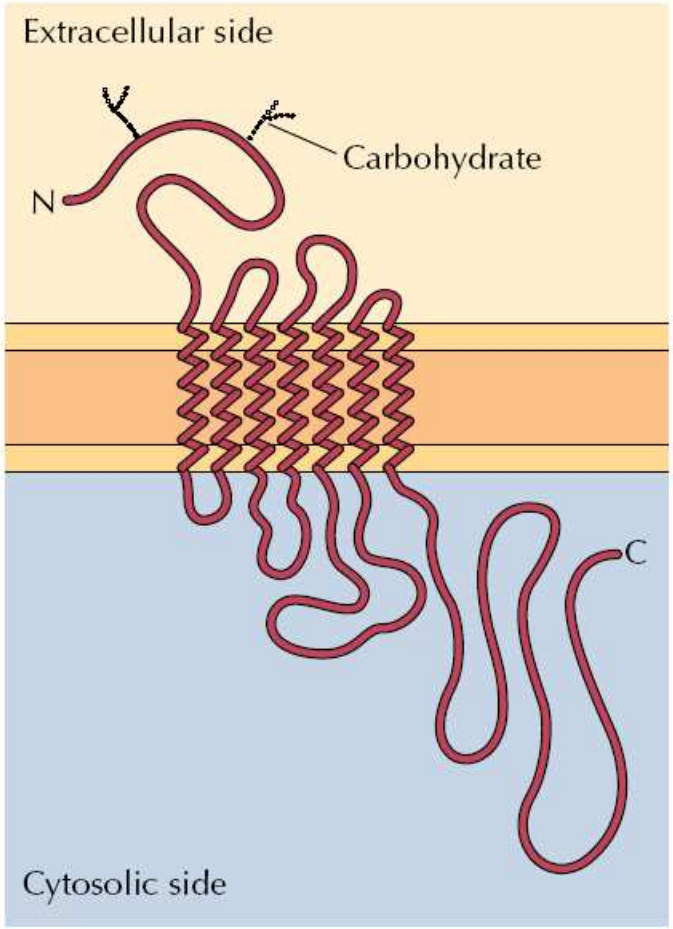


Figure 13.10 Structure of a G protein-coupled receptor
The G protein-coupled receptors are characterized by seven transmembrane α helices.

Structure of G-protein-linked receptors

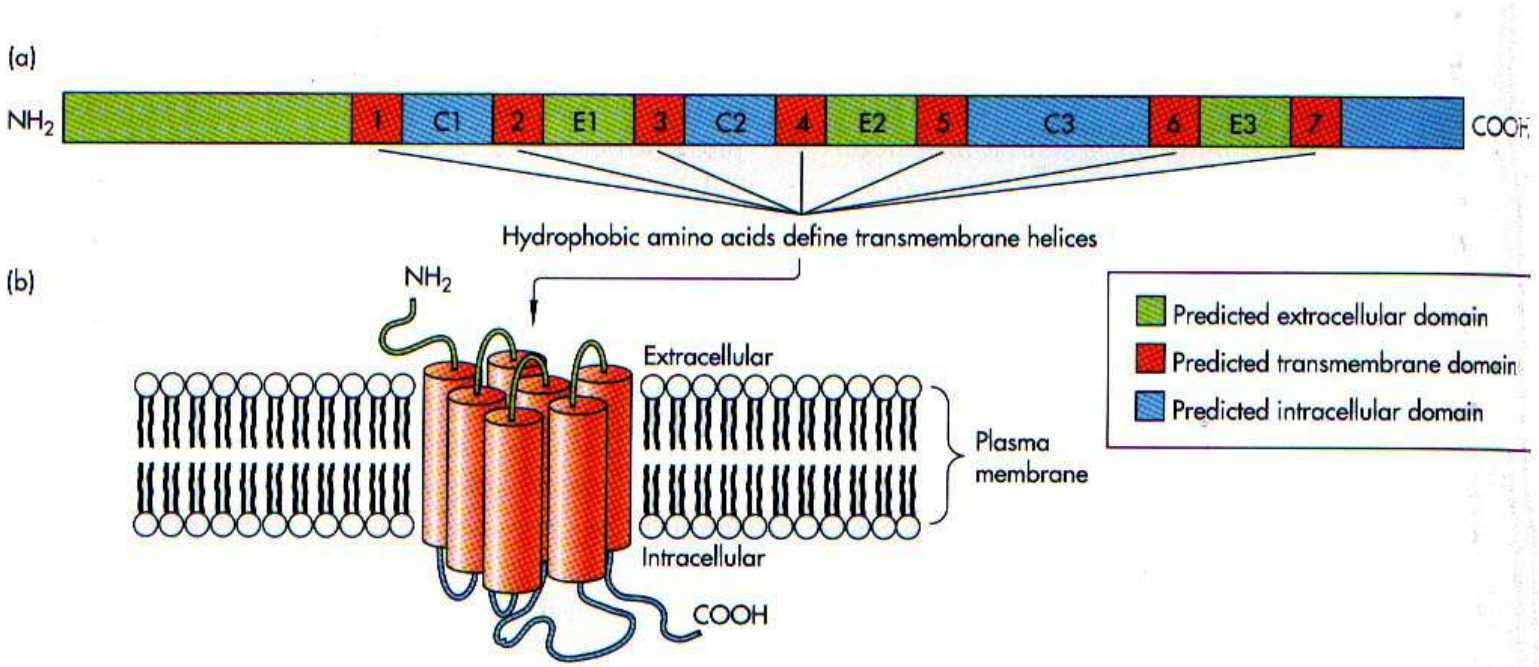
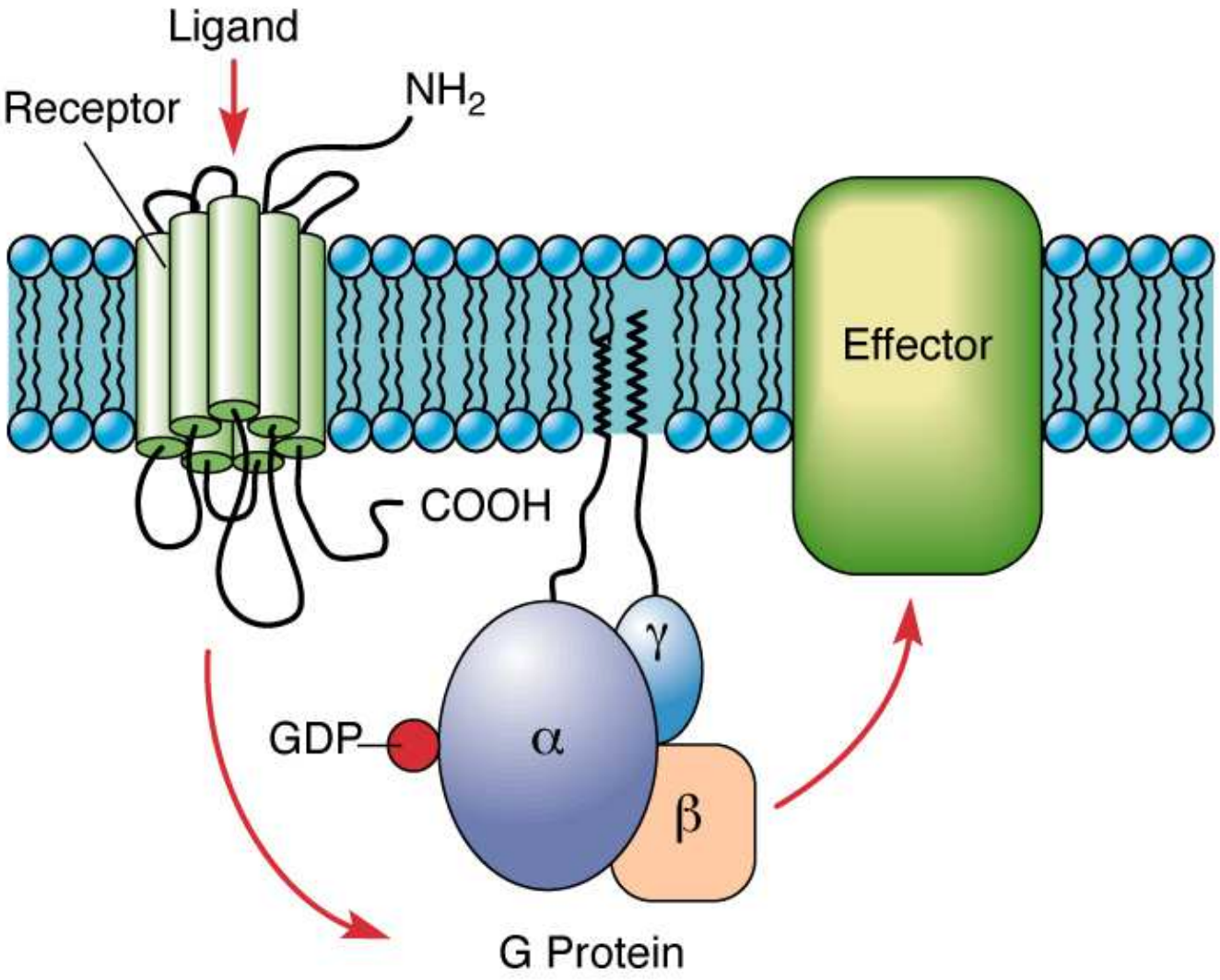


FIGURE 17-3
Structural organization of seven-helix receptors. (a) A linear representation of the receptor structure. Seven blocks of hydrophobic amino acids (1–7) form helices that are predicted to span the membrane. The helices are linked by loops that are alternatively outside (E) or inside (C) the cell. Similarly, the amino-terminal tail is outside, whereas the carboxy-terminal tail is inside. (b) A view of the proposed folded structure of the receptors.

G-protein-linked receptors are coupled to trimeric G-proteins



G protein activity regulation

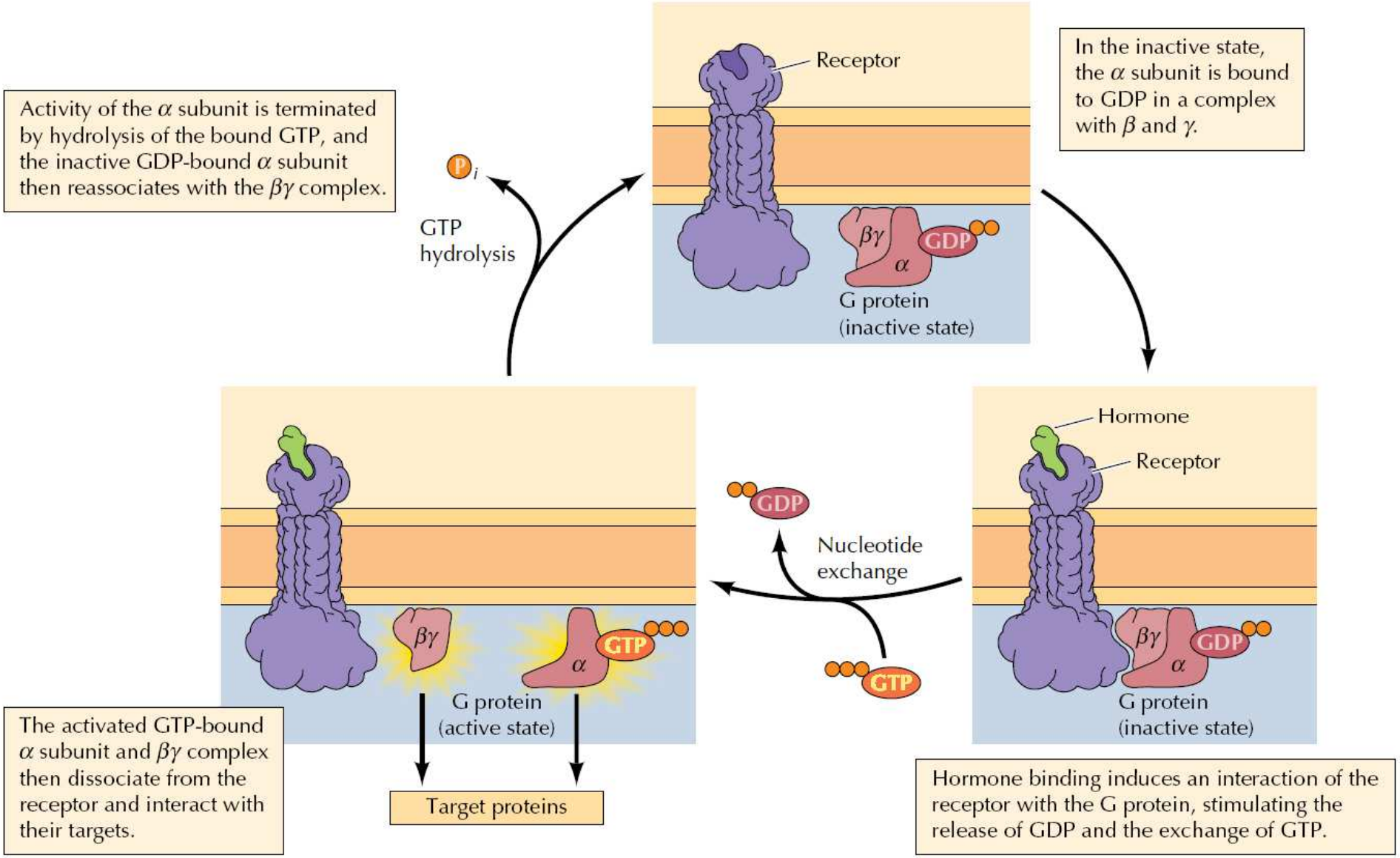
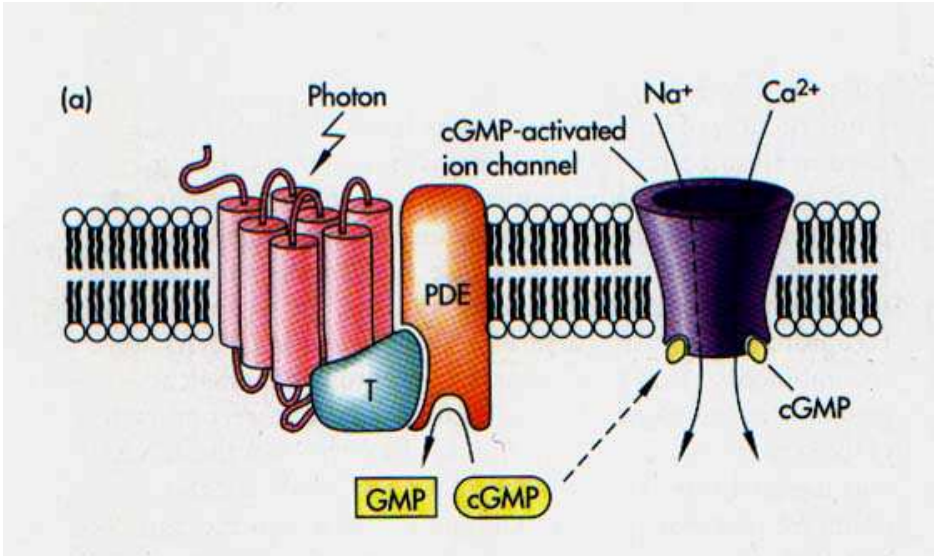
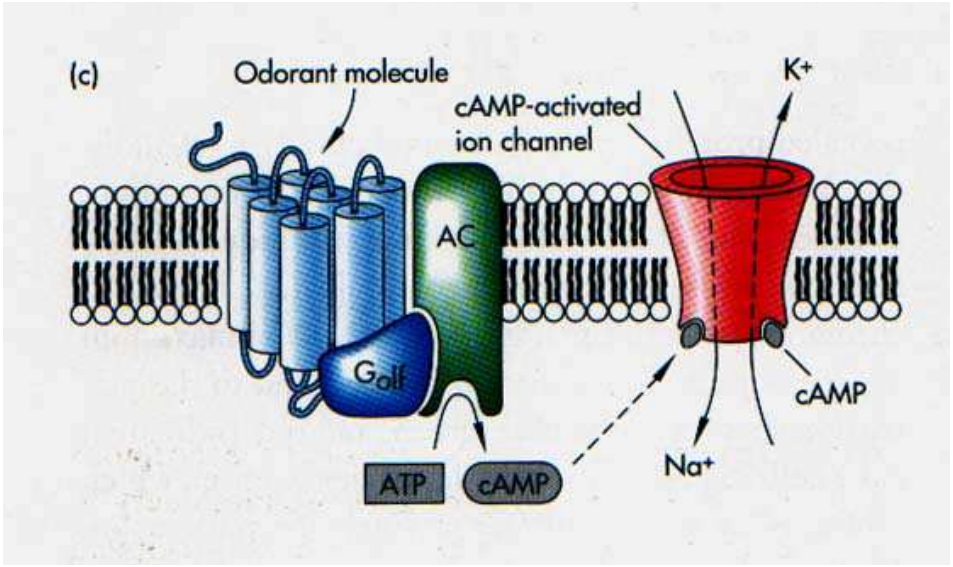


Table 7.1 Endogenous ligands for G-protein-coupled receptors

Small molecules	Glycoproteins	Peptides
Acetylcholine	Lutropin	Angiotensin
Adenosine	Thyrotropin	Bombesin
Adrenaline	FSH	Bradykinin
Cannabinoids		C5a
Dopamine		Calcitonin
Histamine		Cholecystokinin
Leukotrienes		Endothelin
Prostaglandins		f-MetLeuPhe
Retinal		Glucagon
Serotonin		Neurokinins
		Neuropeptide Y
		Neurotensin
		Opioids
		Oxytocin
		Parathyroid hormone
		Somatostatin
		Thrombin (amino-terminal cleavage peptide)
		Vasopressin

Bersagli delle proteine G		
	PROTEINA EFFETTRICE	
Proteina G	Stimolata	Inibita
G_s G_{olf}	Adenilato ciclasi	
G_i (3) G_o G_z	Canale per il K^+, PI 3-chinasi	Adenilato ciclasi
G_{gus}	Altri canali cationici	
G_t (2)	GMP ciclico fosfodiesterasi	
G_q (4)	Fosfolipasi-Cβ	
G_{12} G_{13}	Rho GEF	





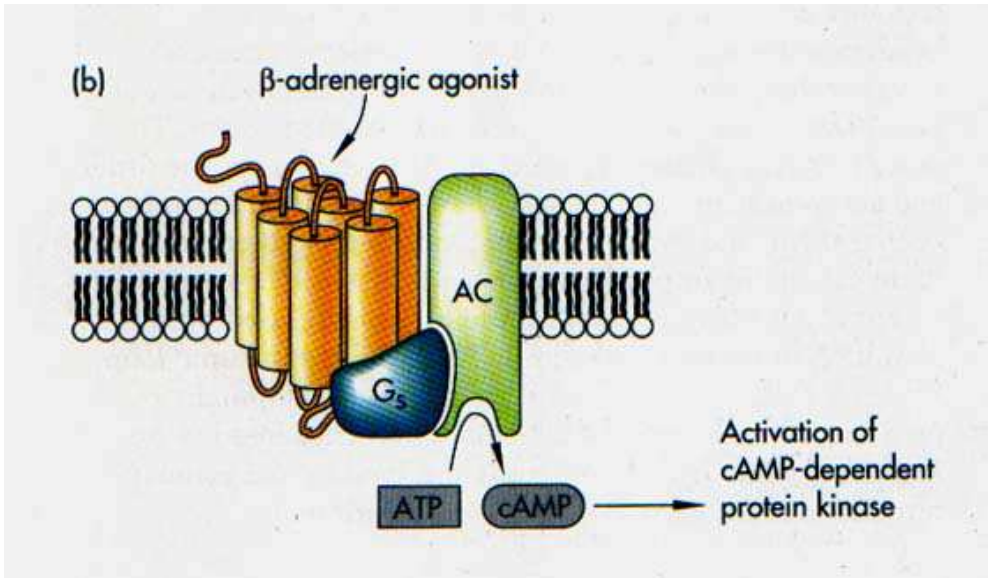
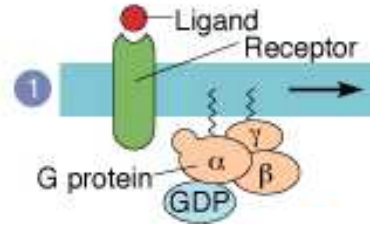


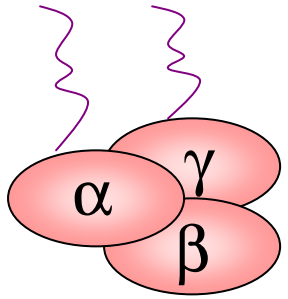
Table 15–3 **The Major Families of Trimeric G proteins***

Family	Some Family Members	α Subunits	Functions	Modified by Bacterial Toxin
I	G_s	α_s	activates adenylyl cyclase; activates Ca^{2+} channels	cholera activates
	G_{olf}	α_{olf}	activates adenylyl cyclase in olfactory sensory neurons	cholera activates
II	G_i	α_i	inhibits adenylyl cyclase; activates K^+ channels	pertussis inhibits
	G_o	α_o	activates K^+ channels; inactivates Ca^{2+} channels; activates phospholipase C-β	pertussis inhibits
	G_t (transducin)	α_t	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors	cholera activates and pertussis inhibits
III	G_q	α_q	activates phospholipase C-β	no effect

*Families are determined by amino acid sequence relatedness of the α subunits. Only selected examples are shown. About 20 α subunits and at least 4 β subunits and 7 γ subunits have been described in mammals.



Different G-proteins:



- αs: stimulates adenyl cyclase

  ATP ⟶ AMPc ↗

- αi: inhibits adenyl cyclase

  ATP ⟶ AMPc ↘

- αt: stimulates cGMP phosphodiesterase

  GMPc ↘ → 5'-GMP

- αq, αo: stimulates PLC-β

  PIP2 ⟶ IP3+DAG

- Adenylyl ciclase
- phosphodiesterase
- Phospholipase C-β

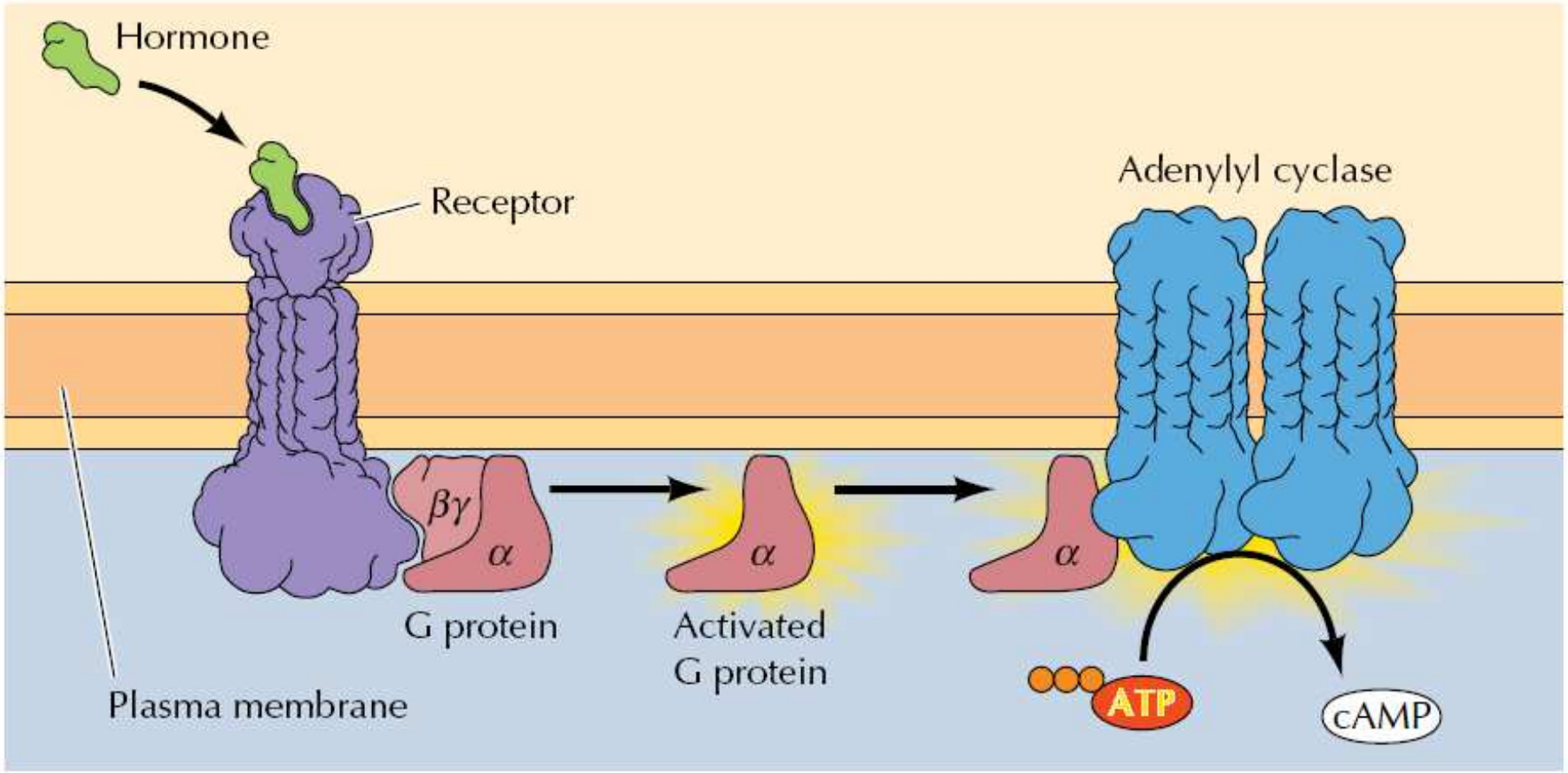
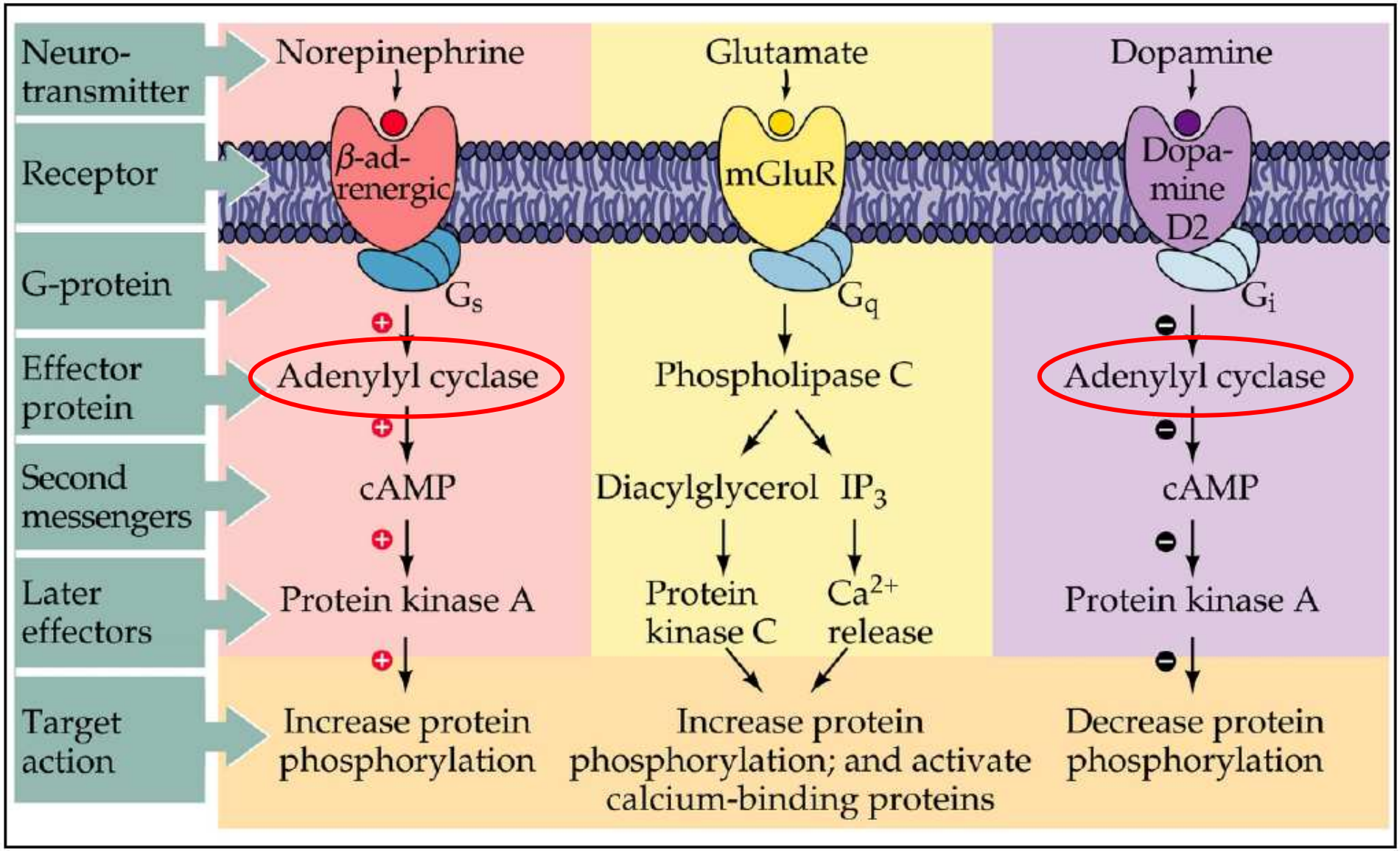


Figure 13.11 Hormonal activation of adenylyl cyclase
Binding of hormone promotes the interaction of the receptor with a G protein. The activated G protein α subunit then dissociates from the receptor and stimulates adenylyl cyclase, which catalyzes the conversion of ATP to cAMP.



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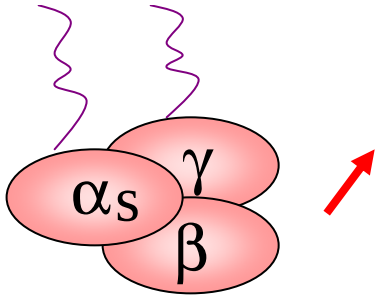
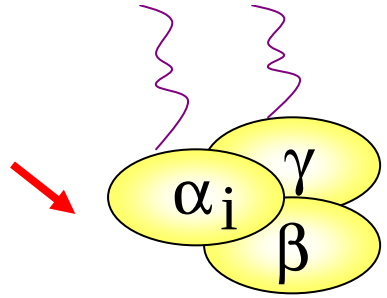
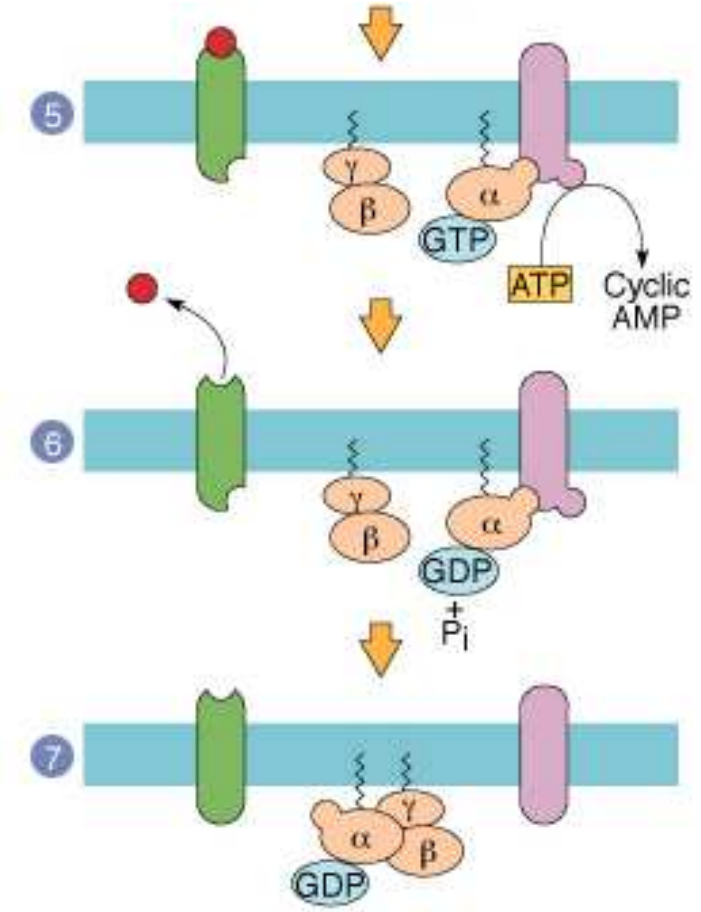
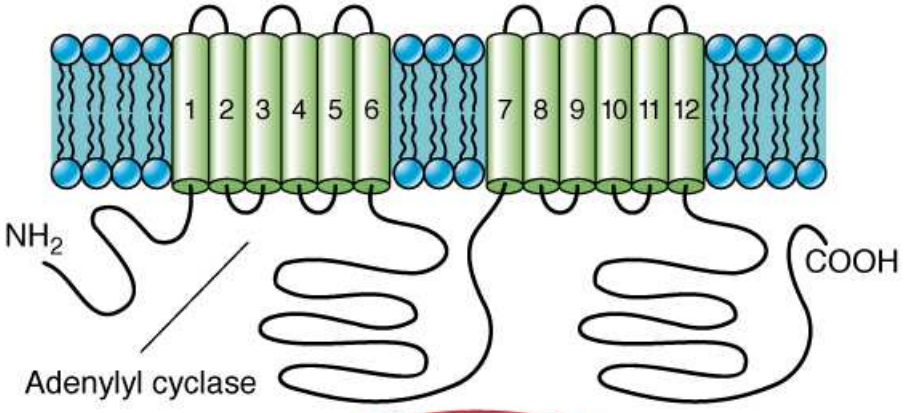
Adenyl cyclase pathway

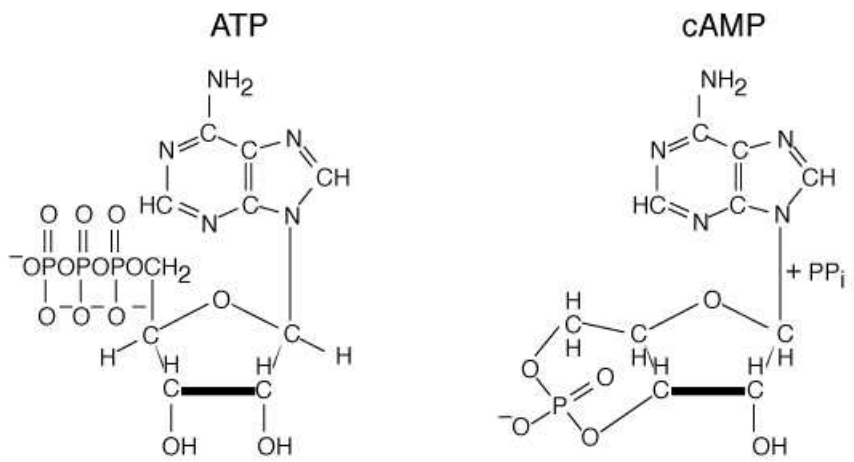


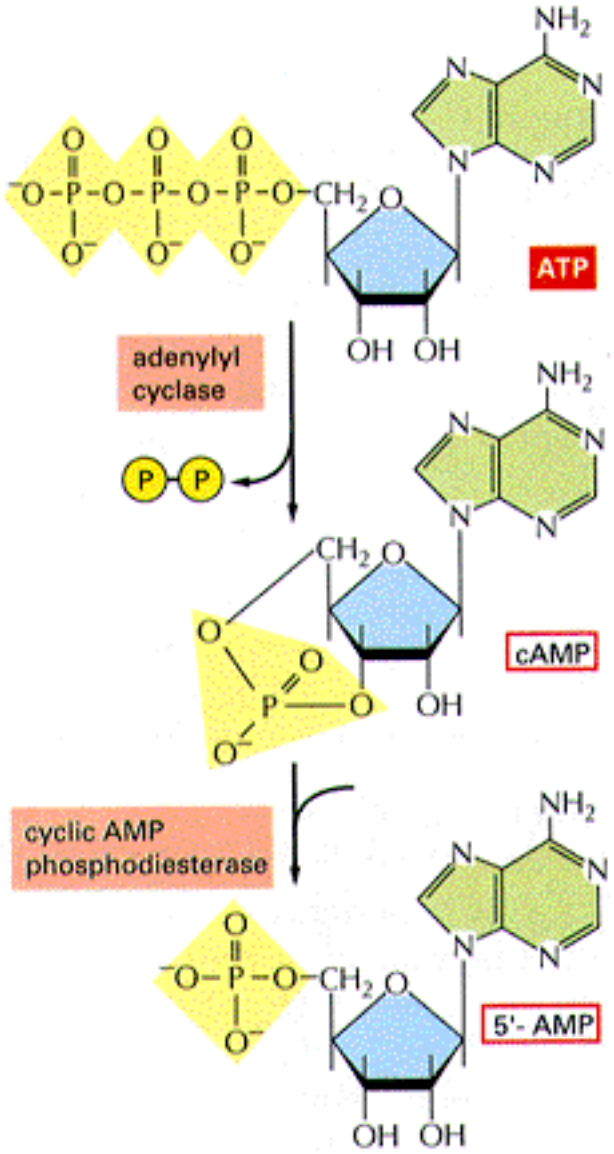
ATP ⟶ cAMP











ATP

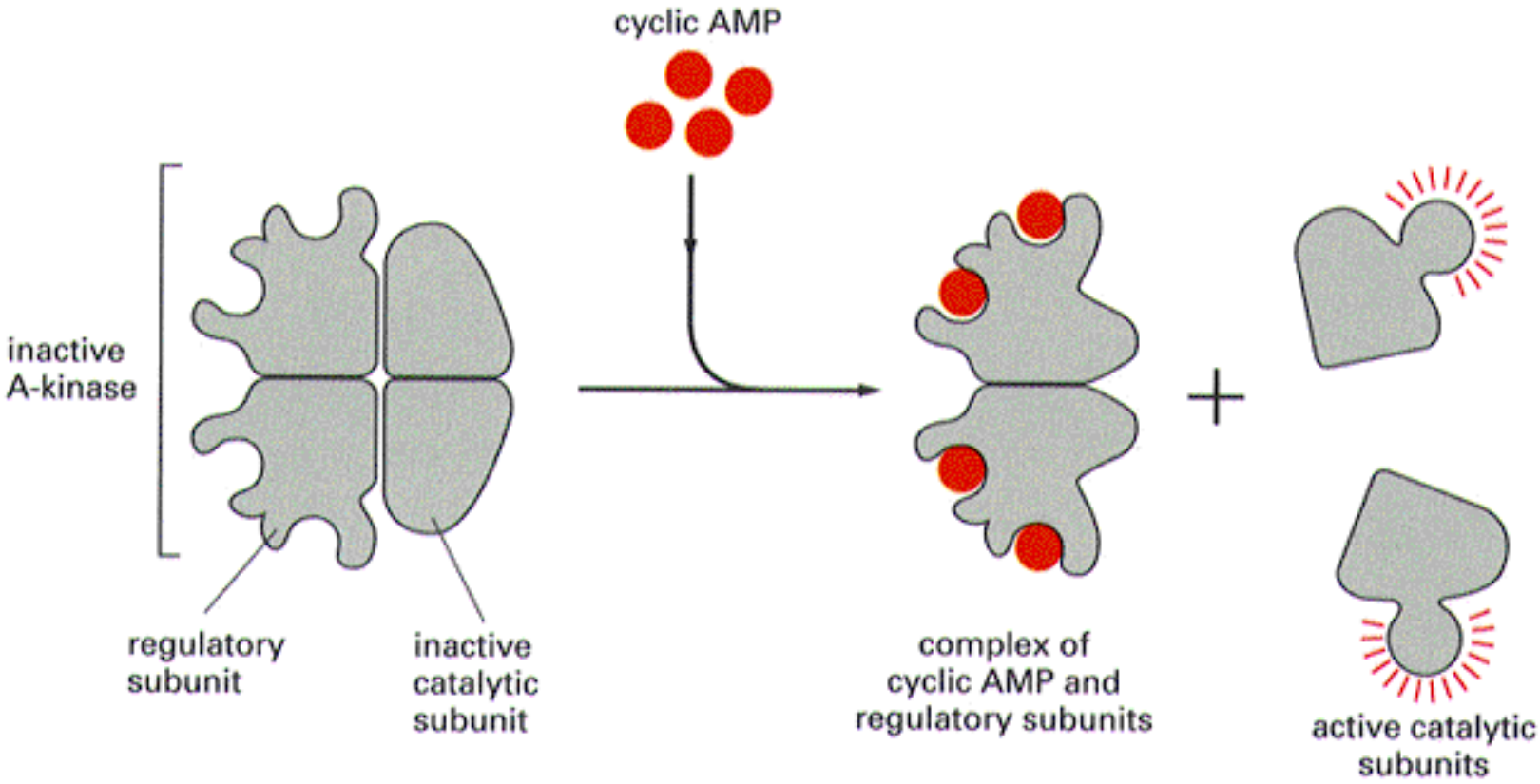
adenyl cyclase

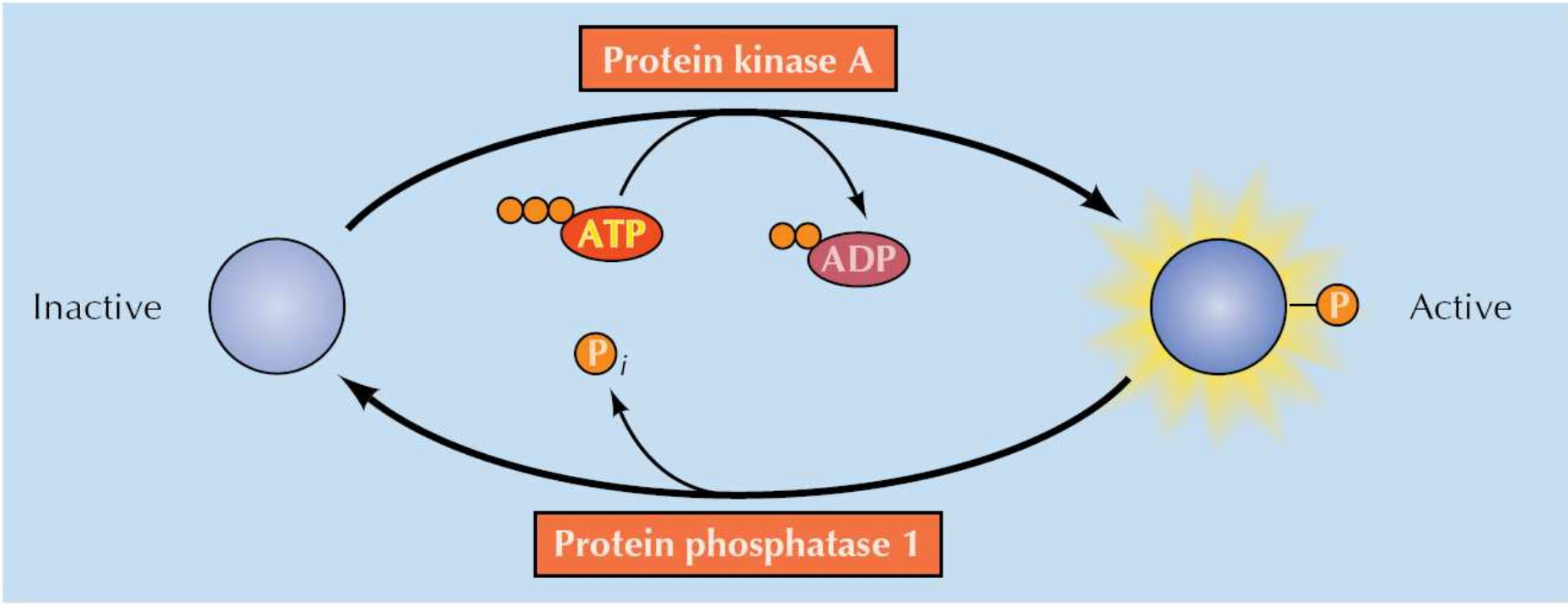
cAMP

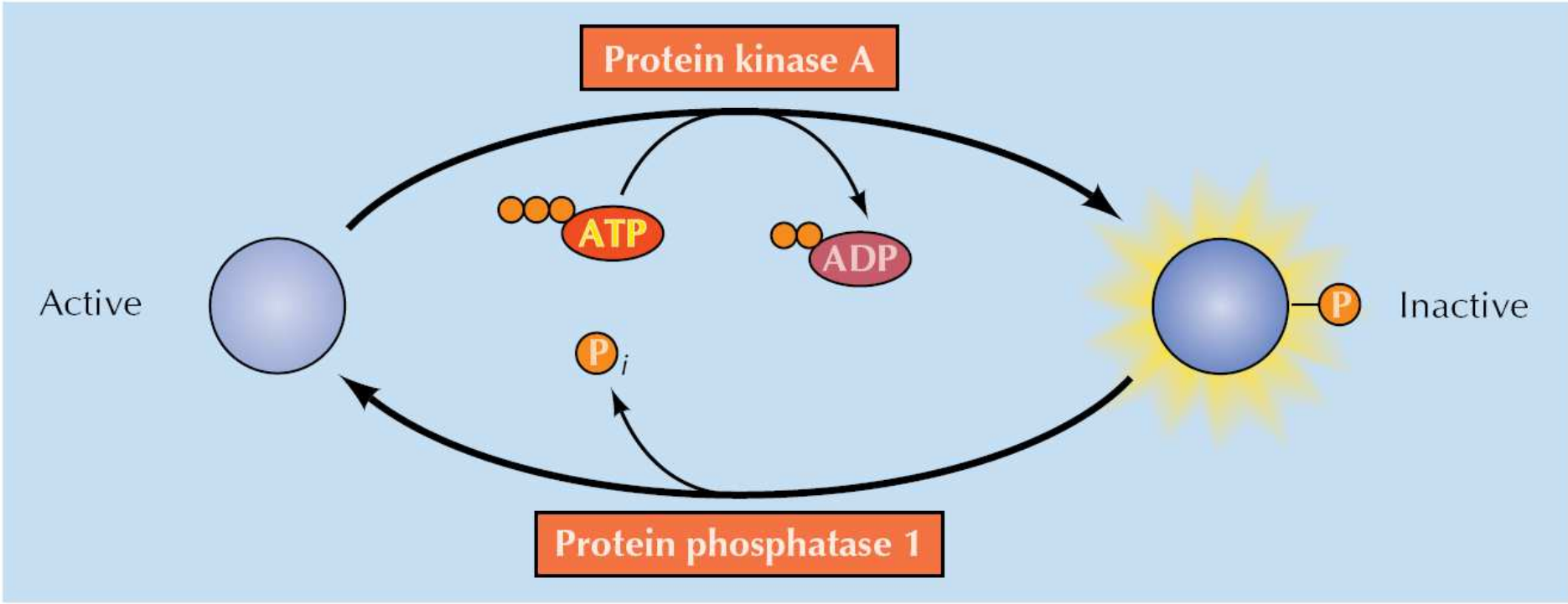
cAMP phosphodiesterase

5’-AMP

cAMP activates the protein kinase A (PKA)







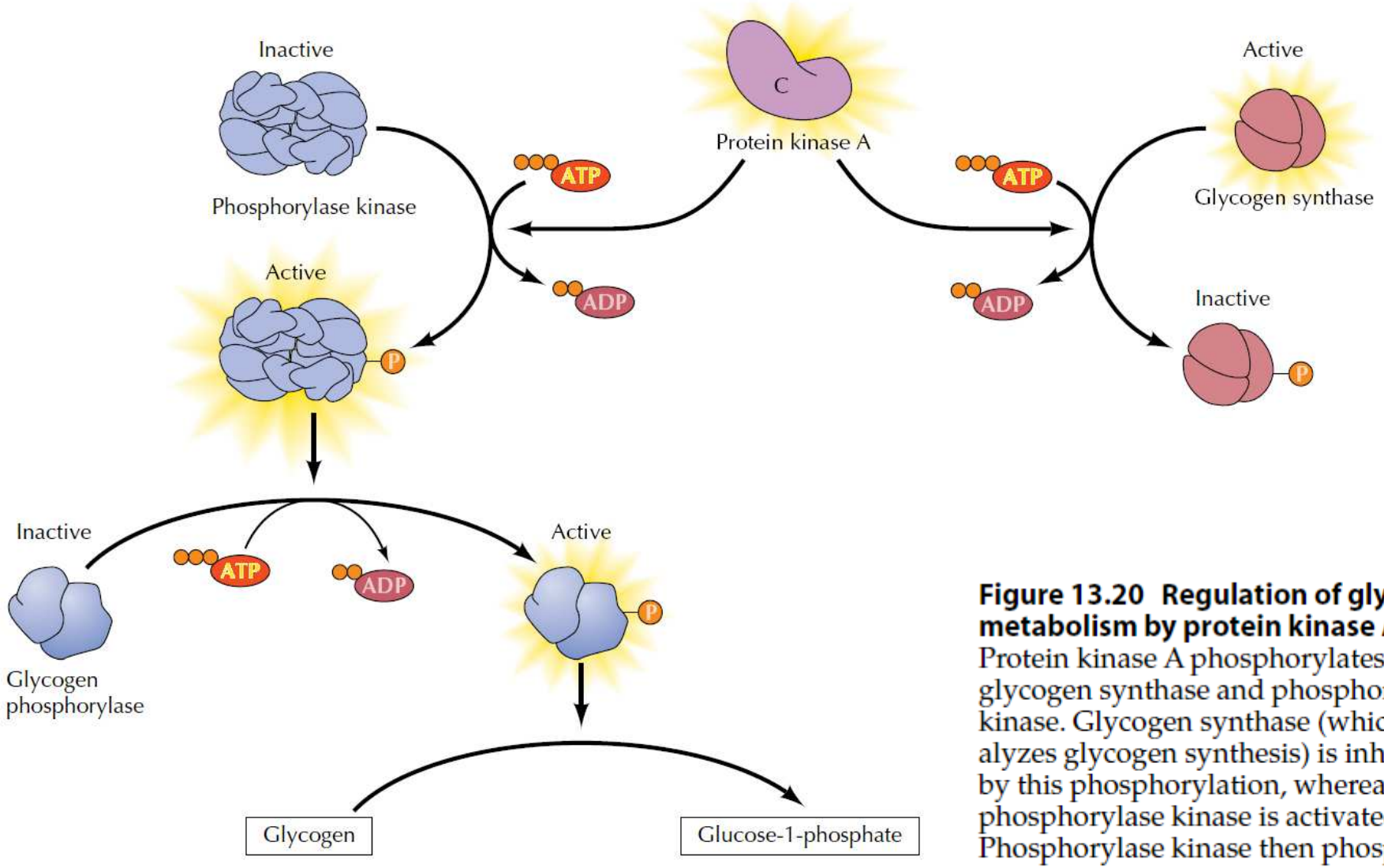
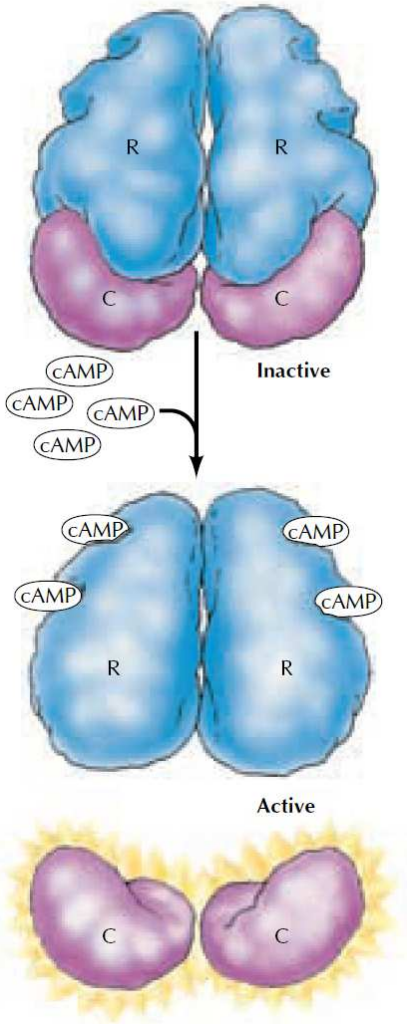
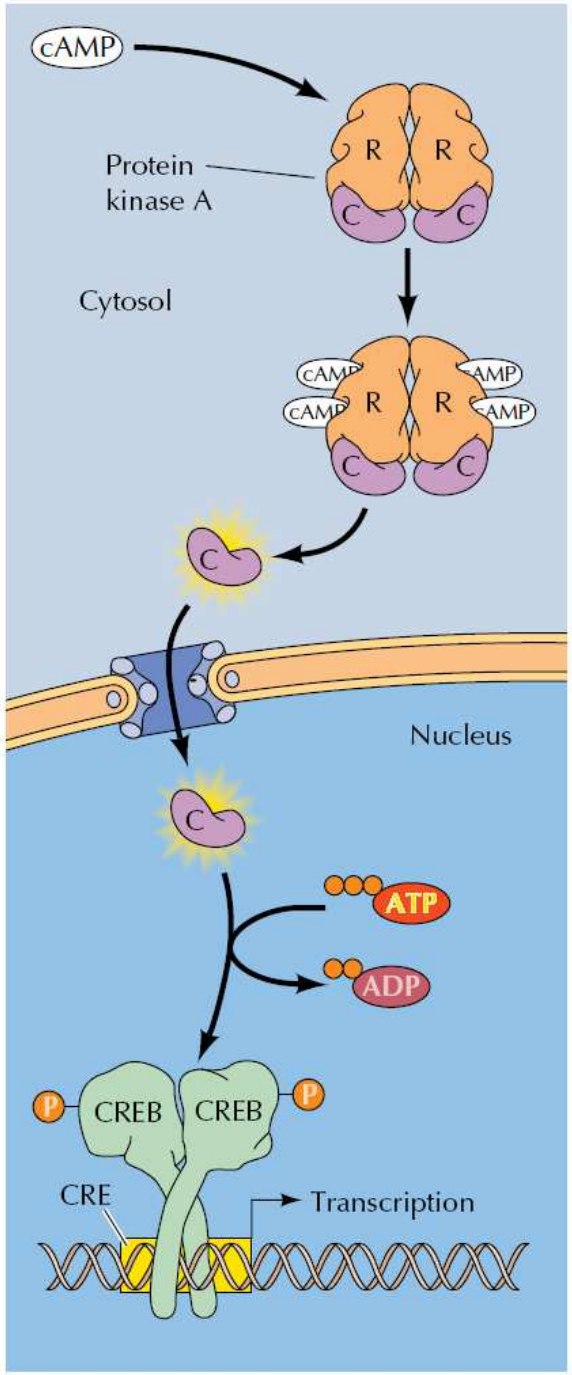


Figure 13.20 Regulation of glycogen metabolism by protein kinase A
Protein kinase A phosphorylates both glycogen synthase and phosphorylase kinase. Glycogen synthase (which catalyzes glycogen synthesis) is inhibited by this phosphorylation, whereas phosphorylase kinase is activated. Phosphorylase kinase then phosphorylates and activates glycogen phosphorylase, which catalyzes the breakdown of glycogen to glucose-1-phosphate.





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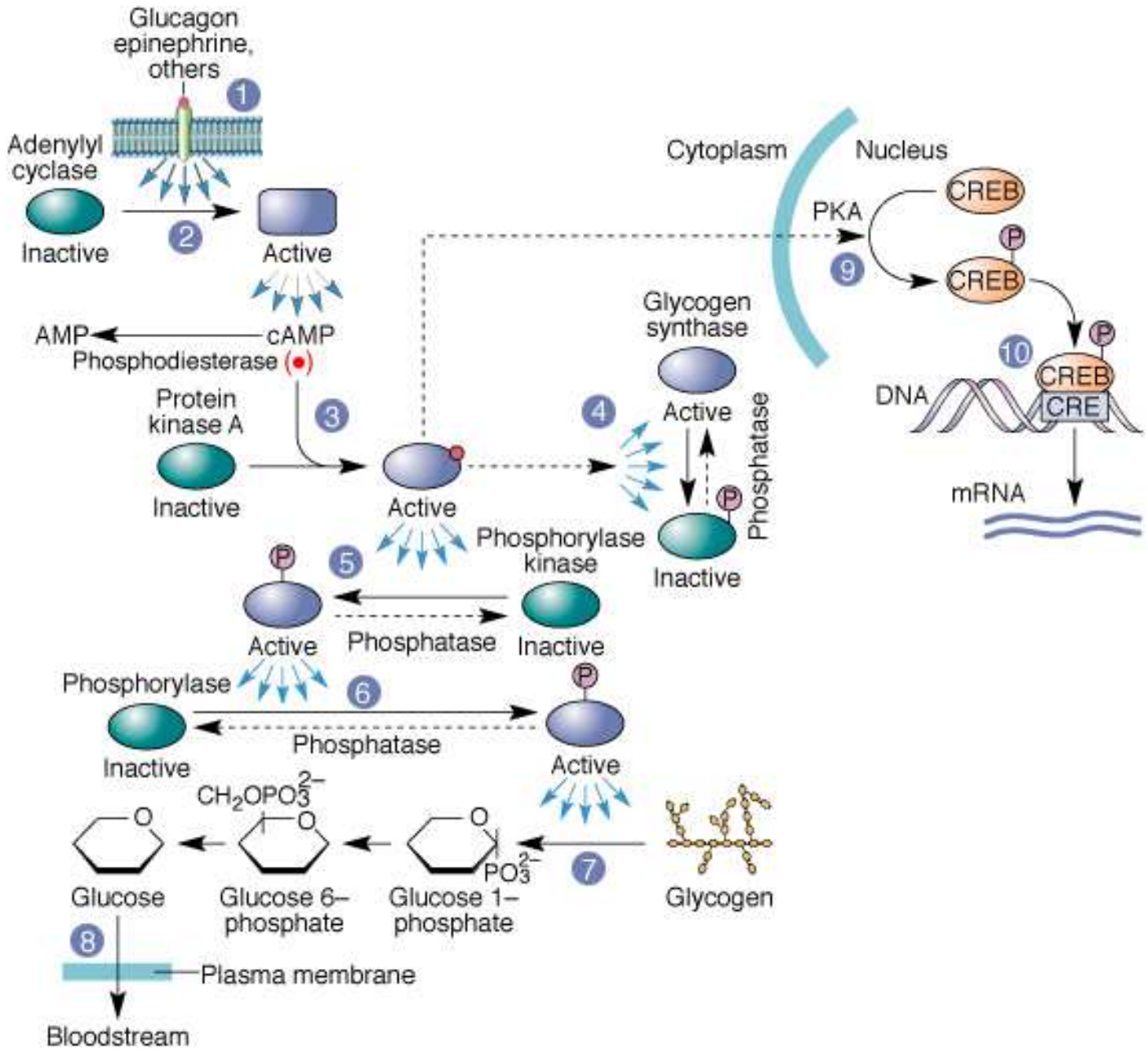
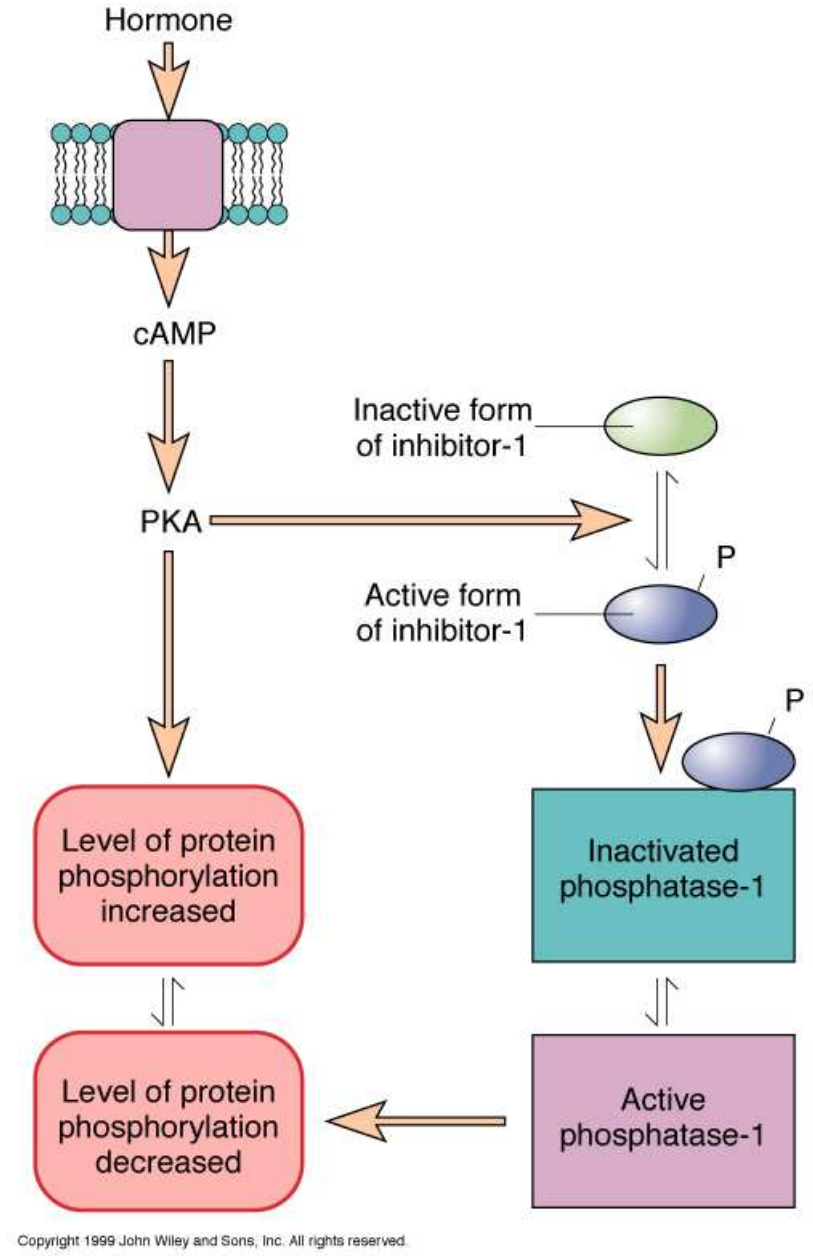
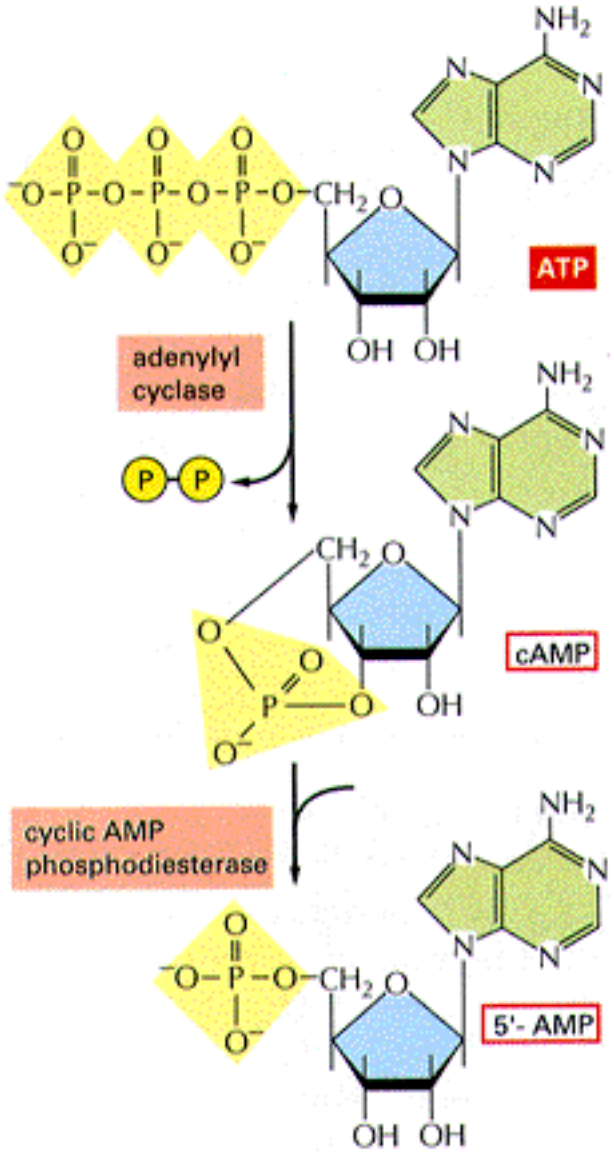


PKA is a
serine-threonine kinase



- Adenylyl ciclase
- **Phosphodiesterase**
- Phospholipase C



ATP

adenyl cyclase

cAMP

cAMP phosphodiesterase

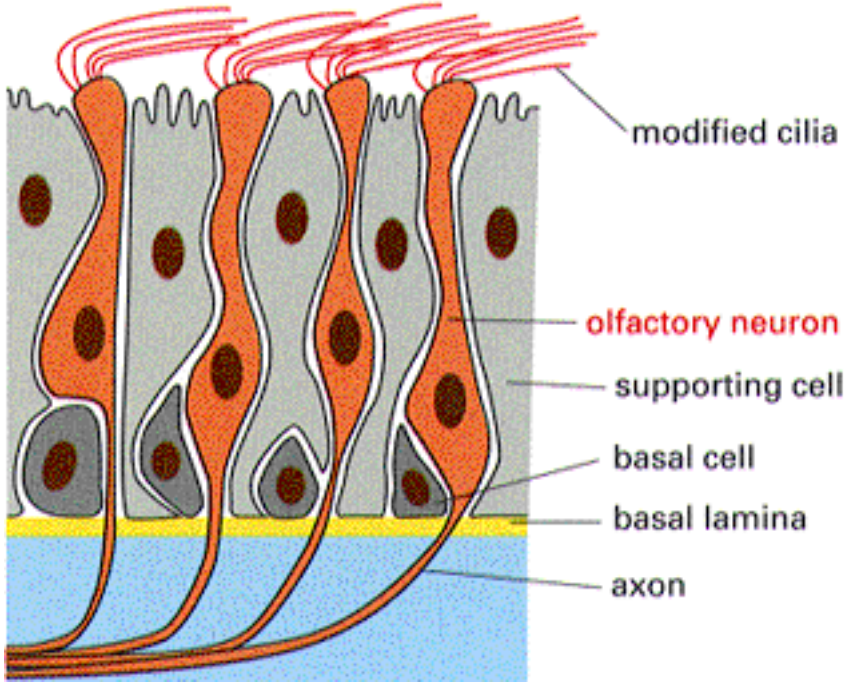
5’-AMP

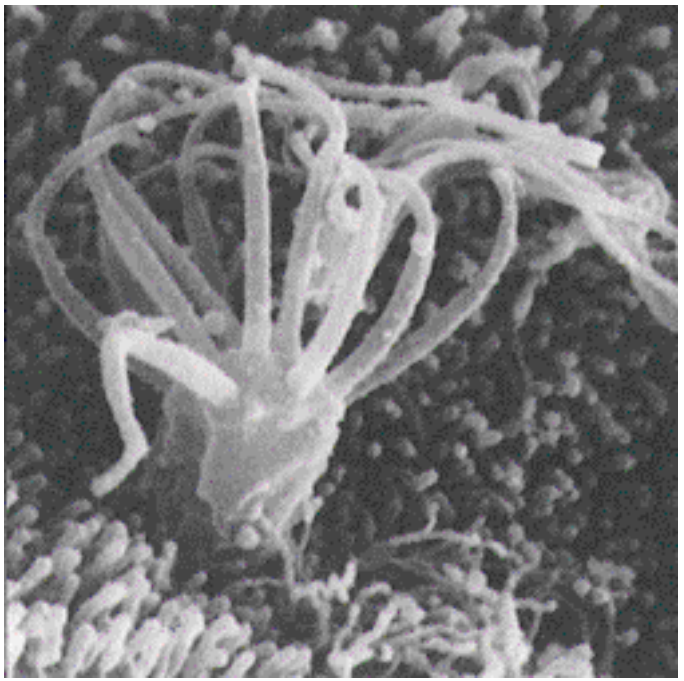
Olfaction and vision depend on G-protein-linked receptors

Olfactory receptor

↓

Golf

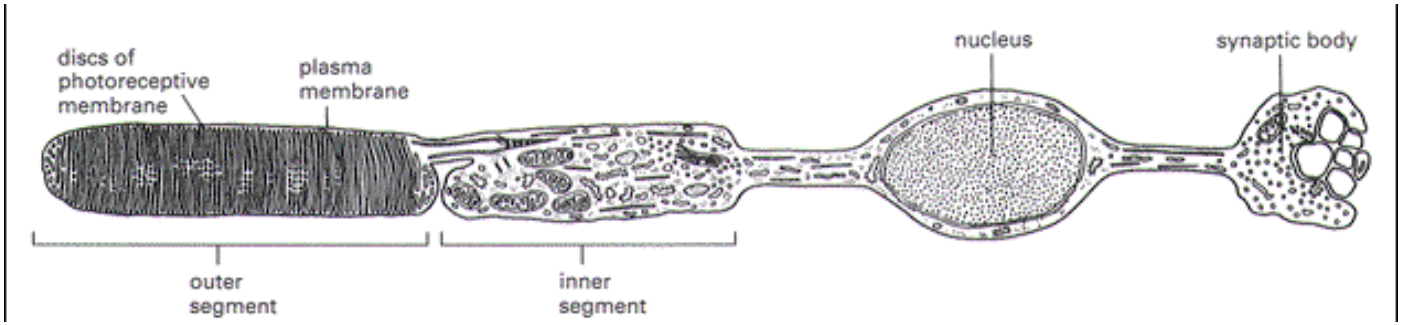


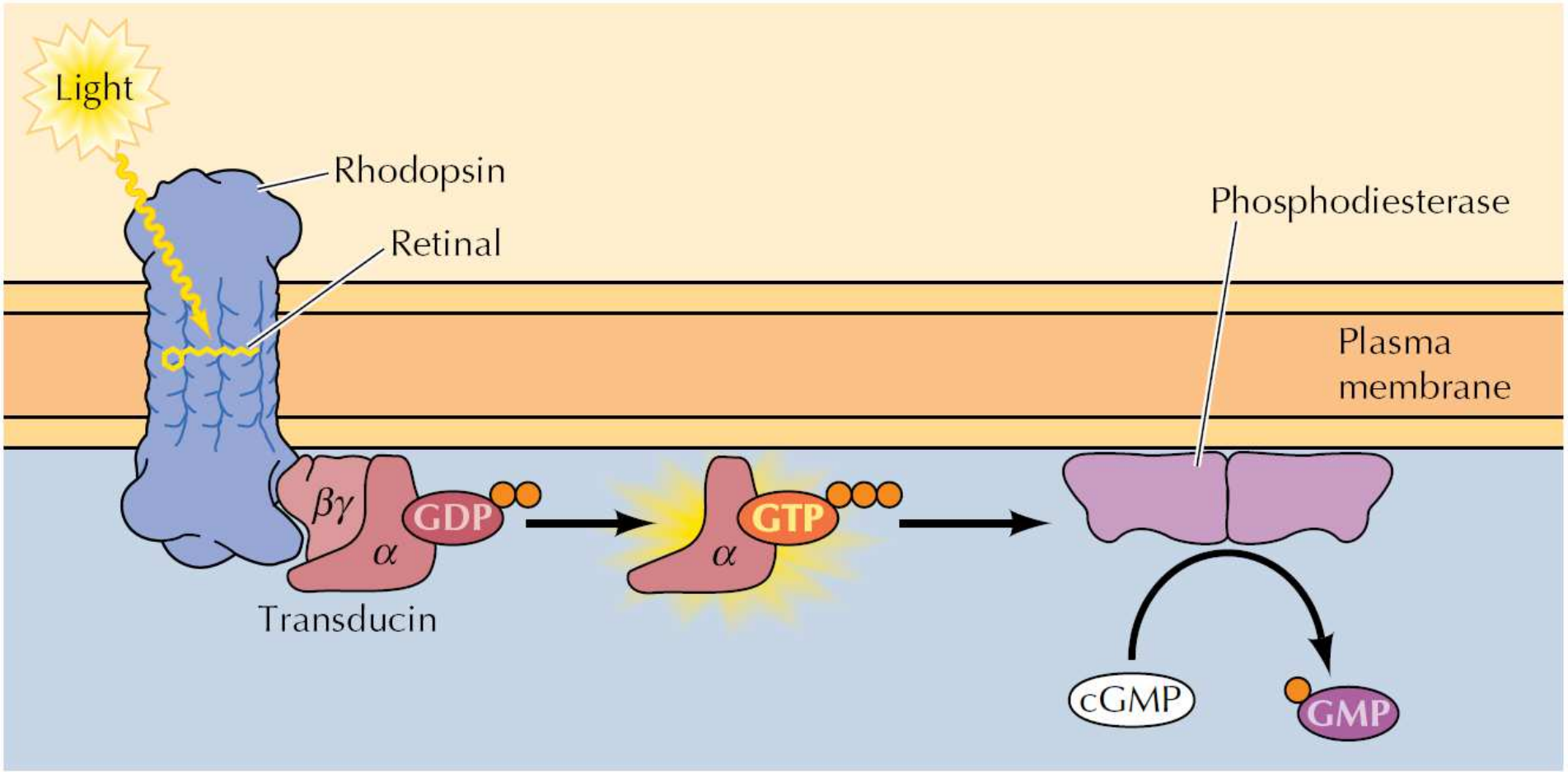


Rhodopsin

↓

transducin





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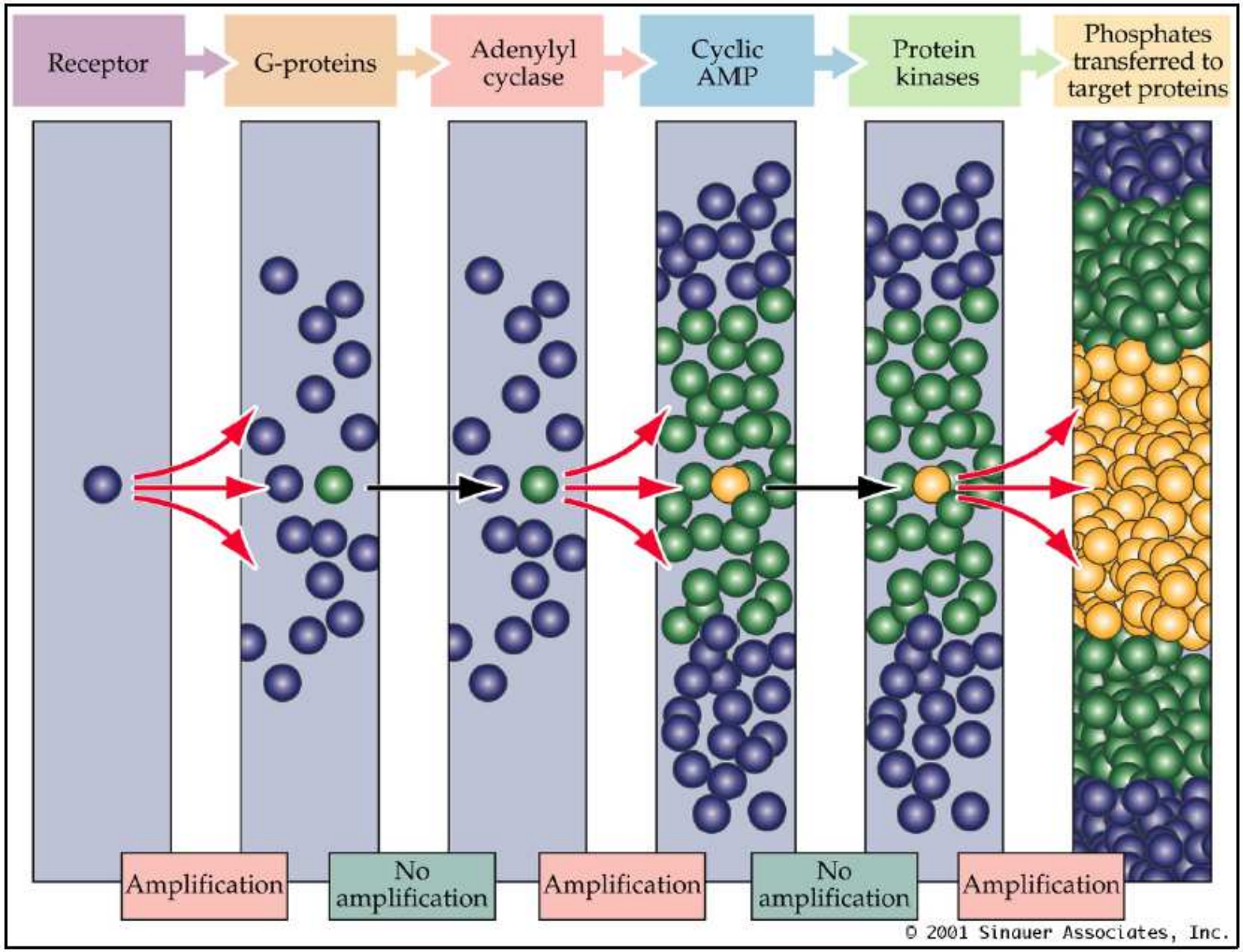
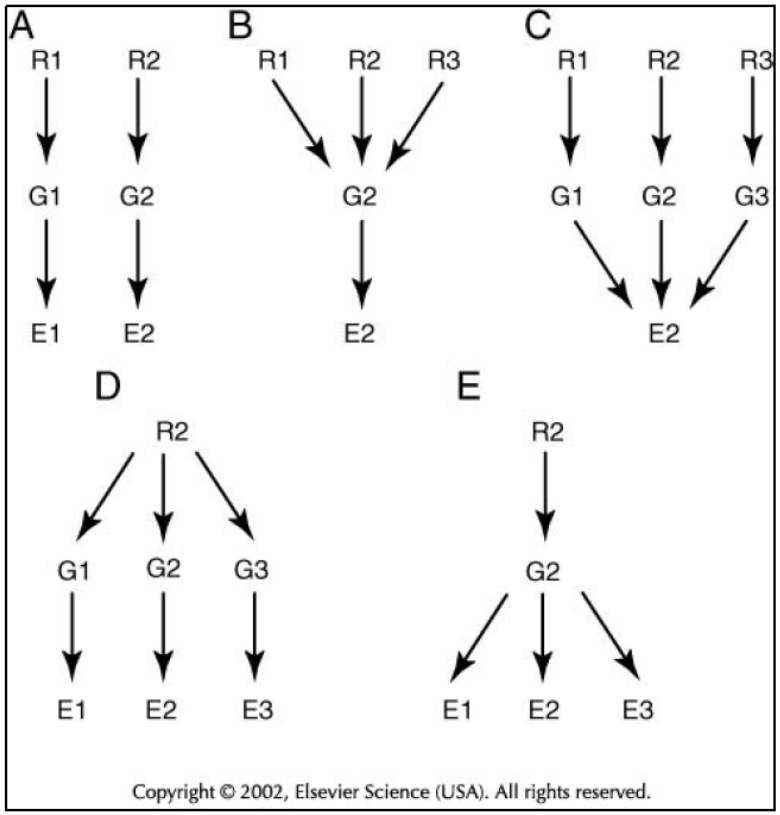
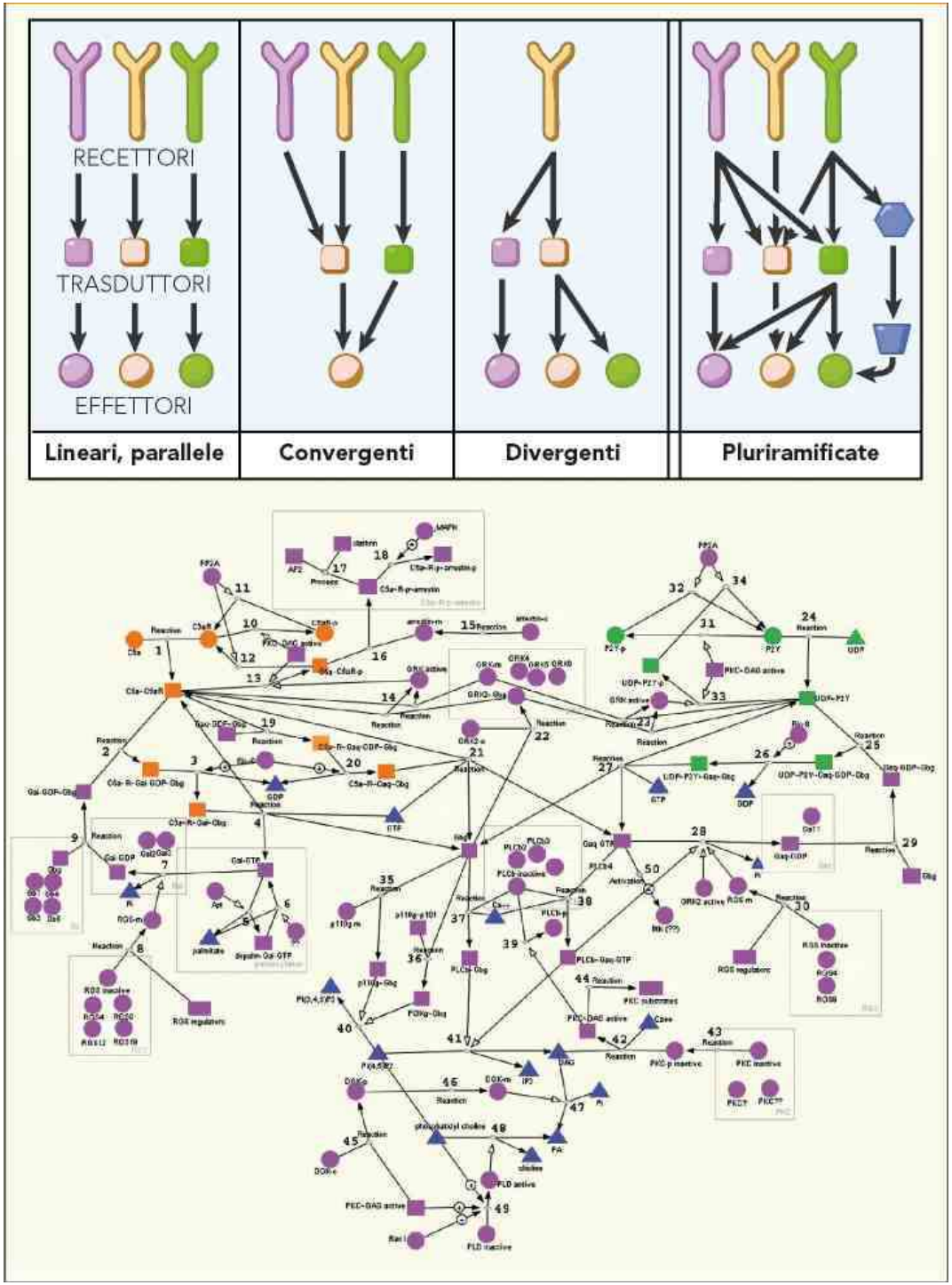
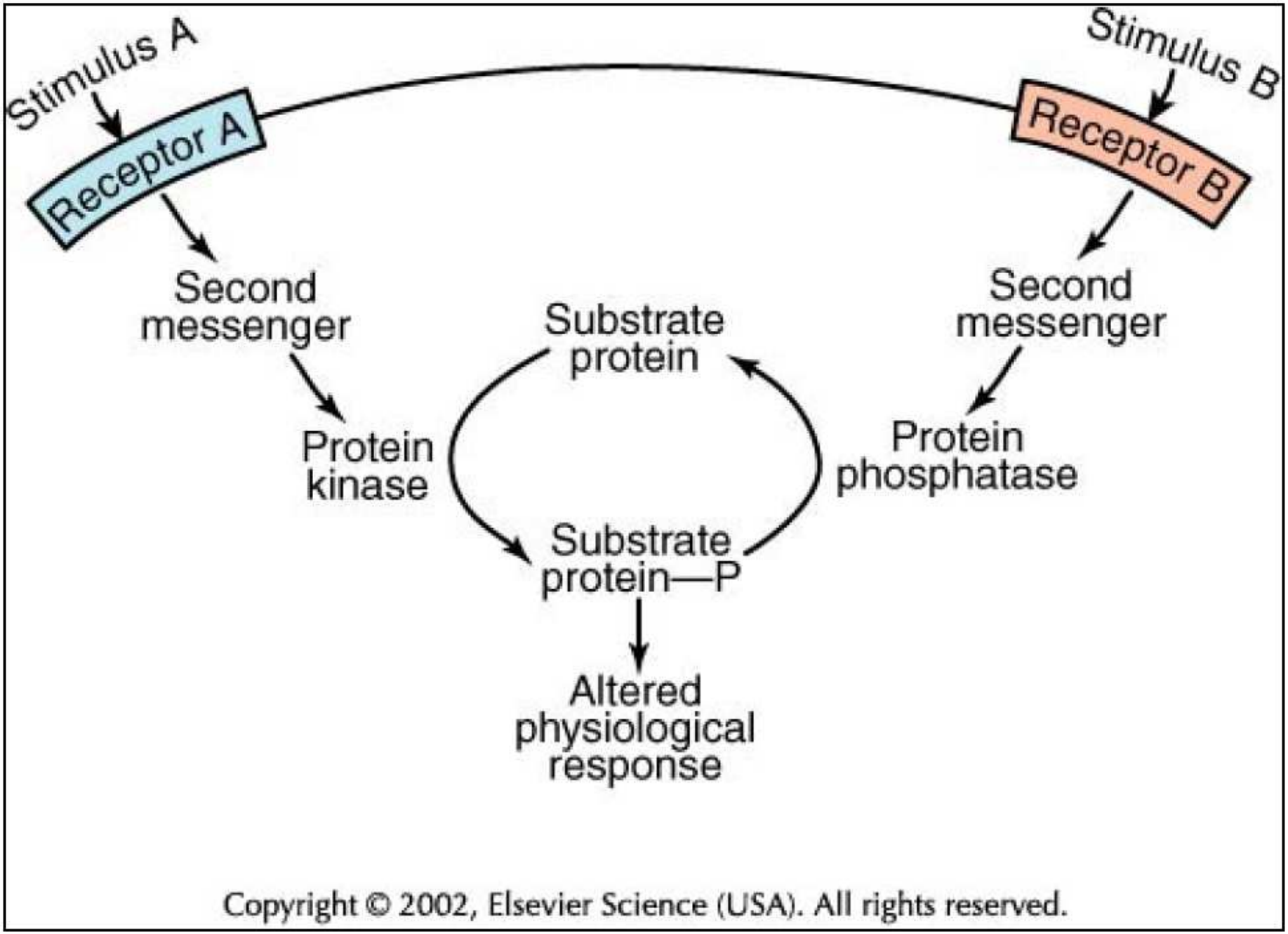
Signal amplification



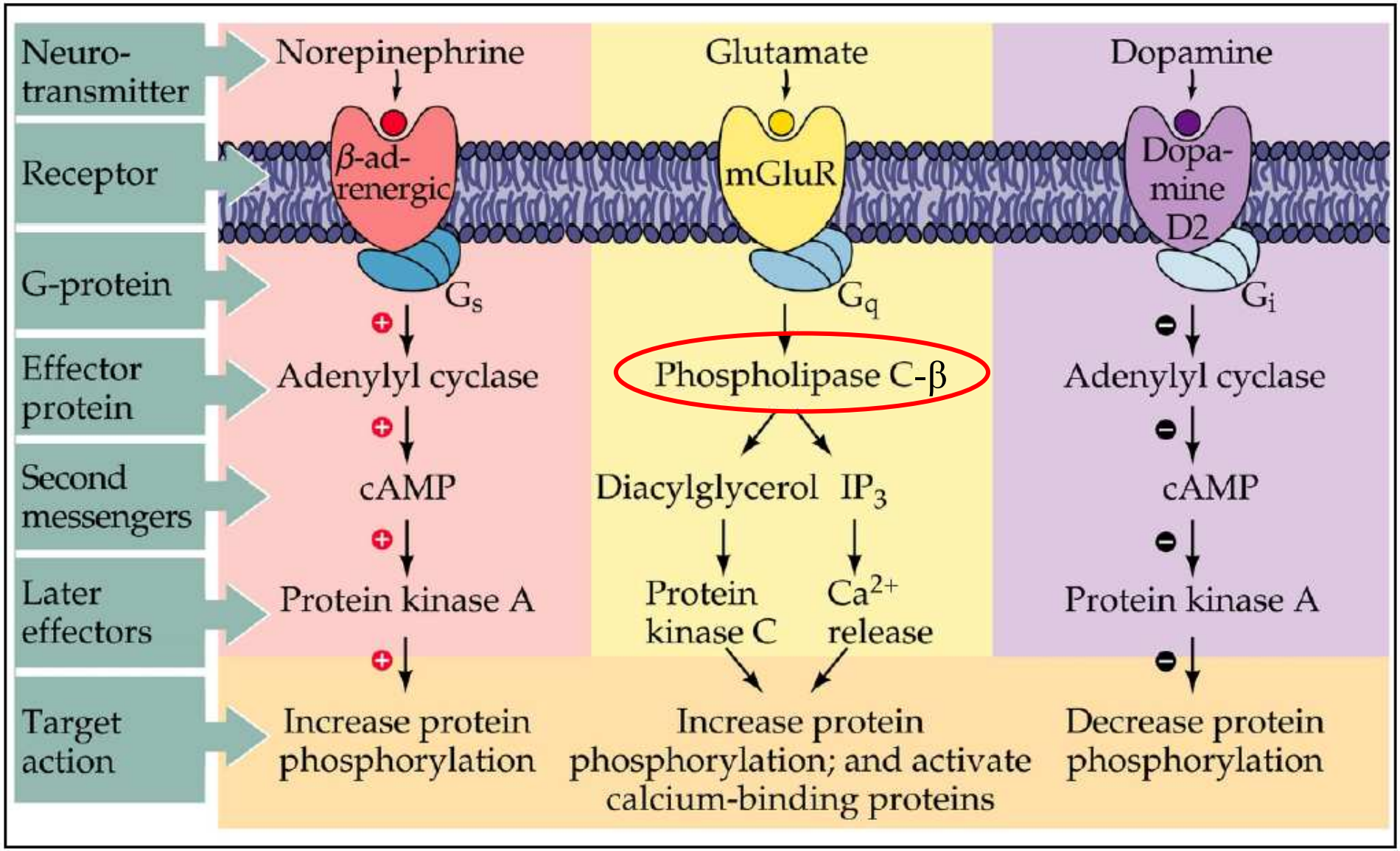
Vie di segnalazione convergenti e divergenti







- Adenylyl ciclase
- Phosphodiesterase
- **Phospholipase C-β**



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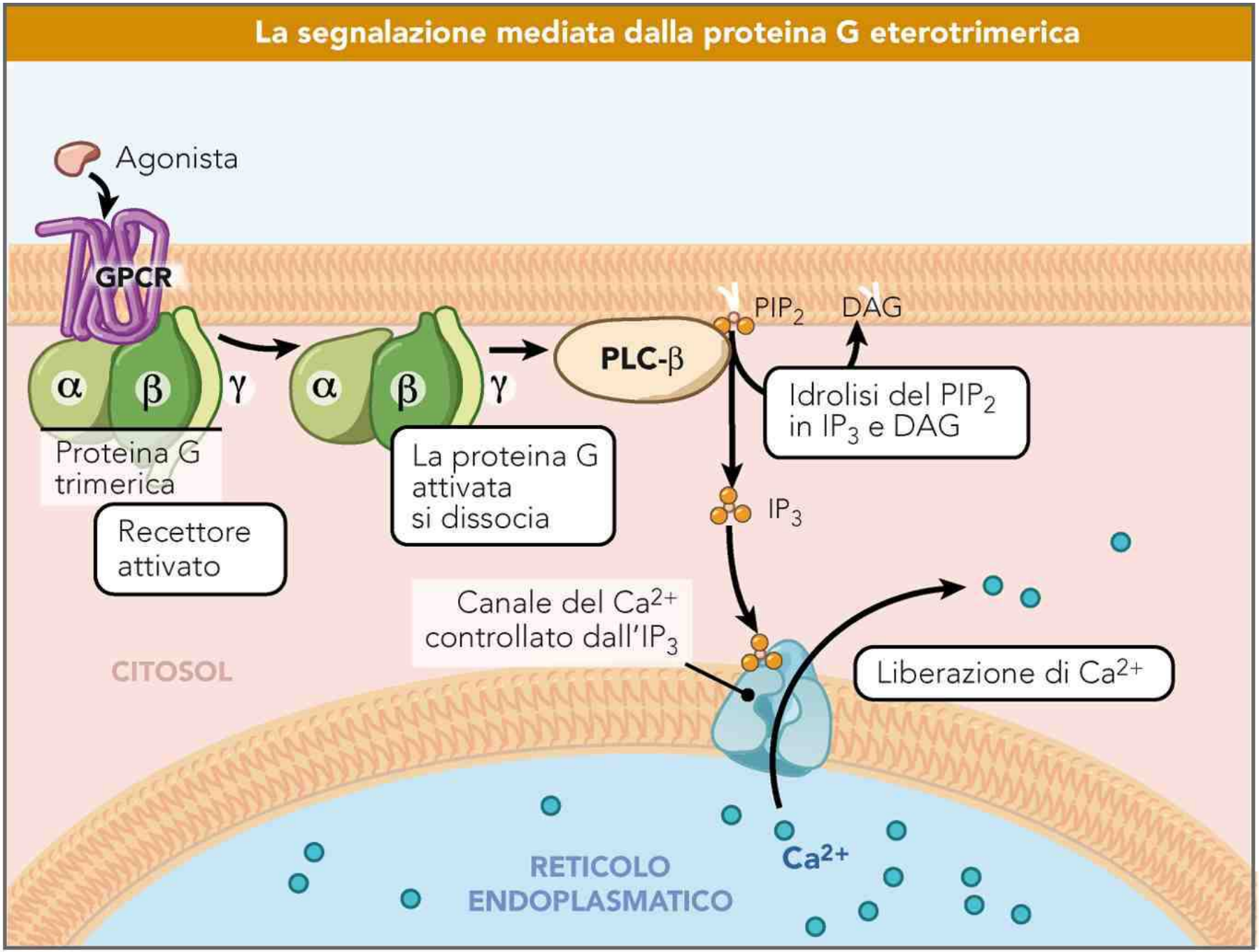
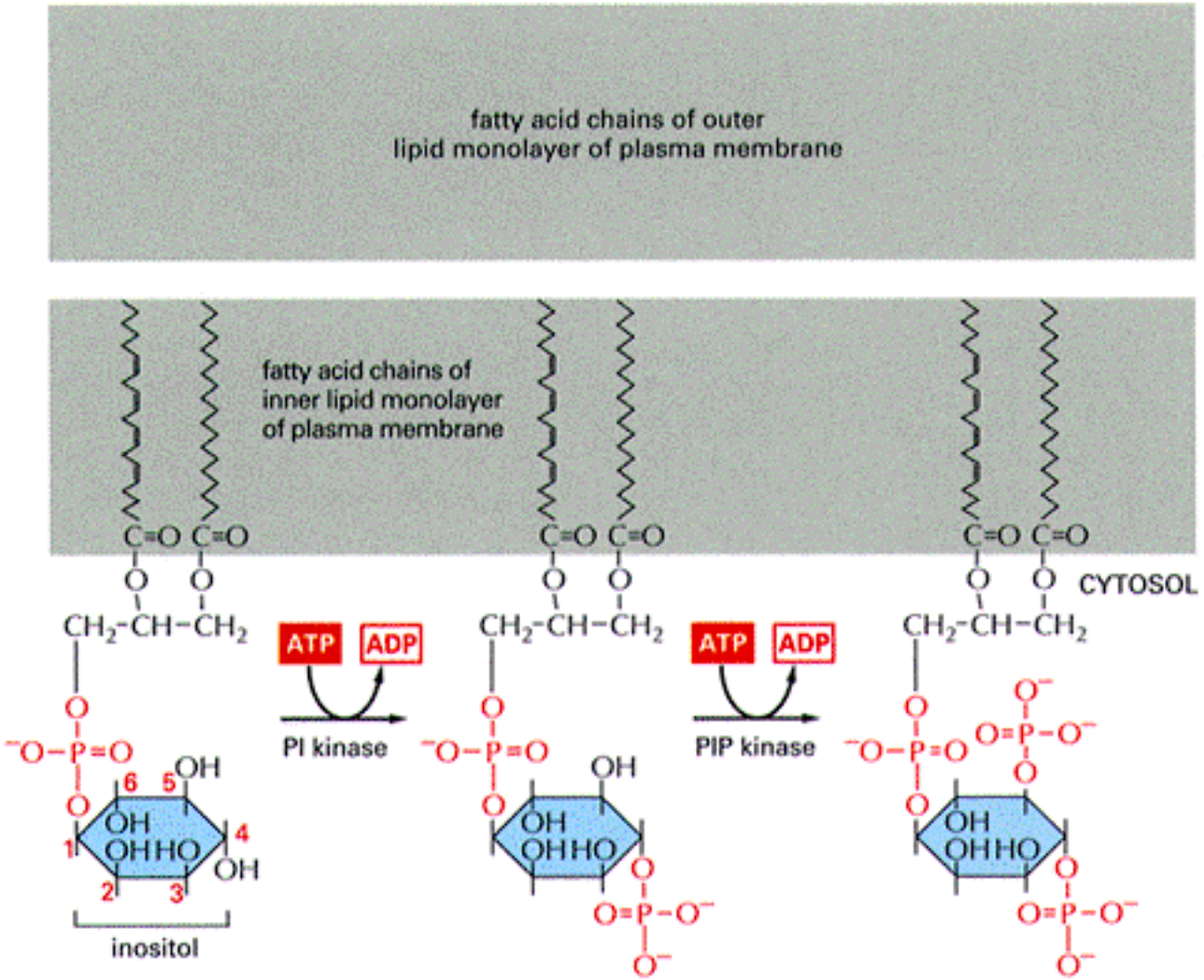


Phosphatidyl inositids



Phospatidylinositol (PI)

PI 4-phosphate (PIP)

PI 4,5-bisphosphate (PIP_2)

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Phospholipase C-β Pathway

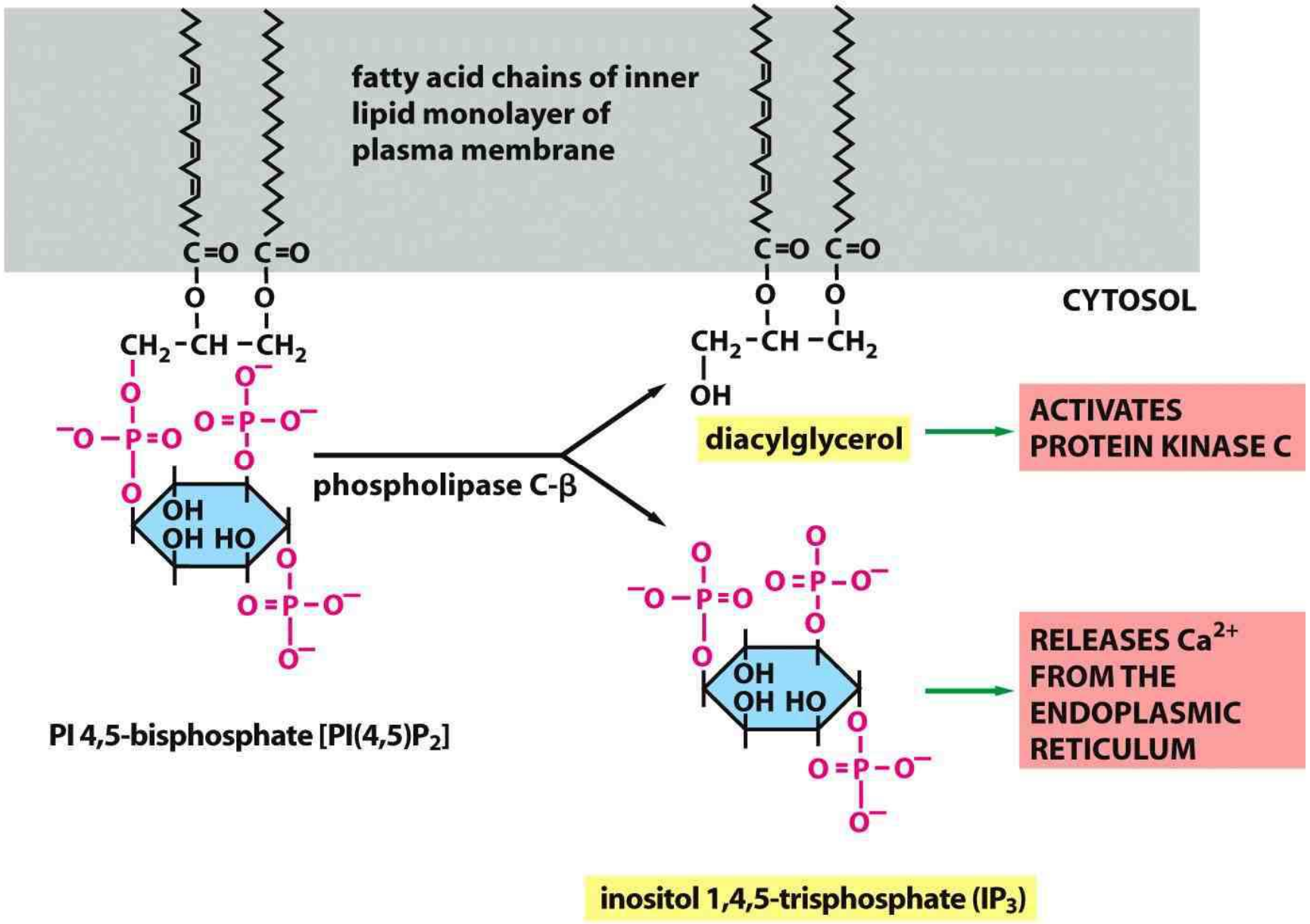
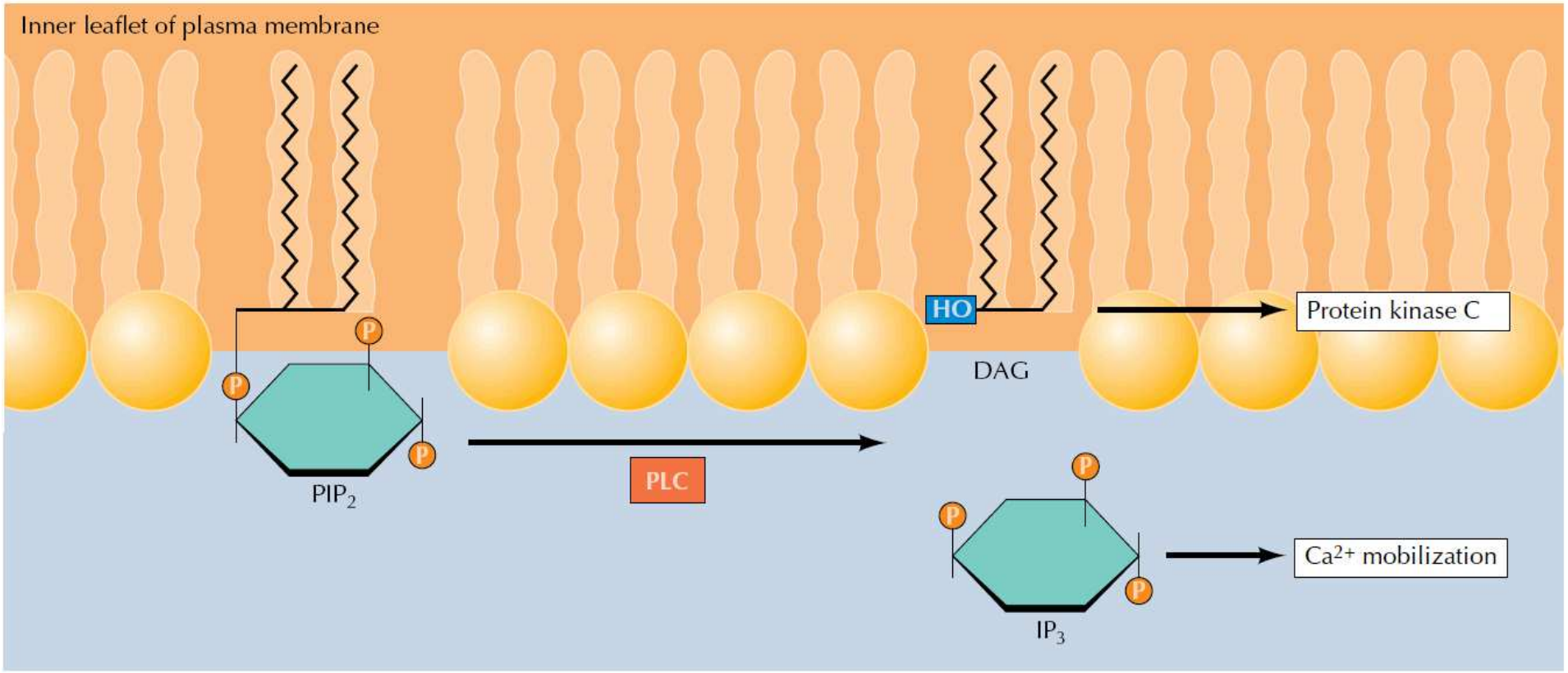
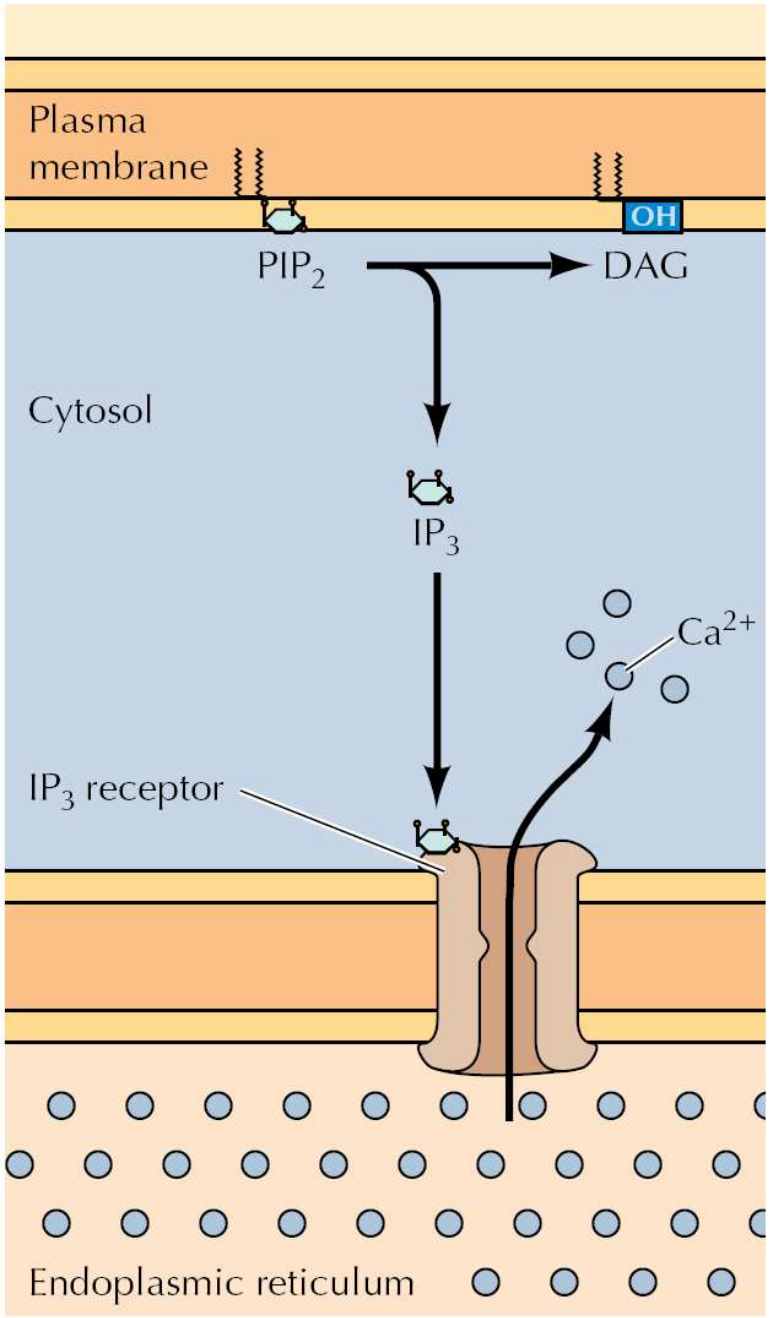
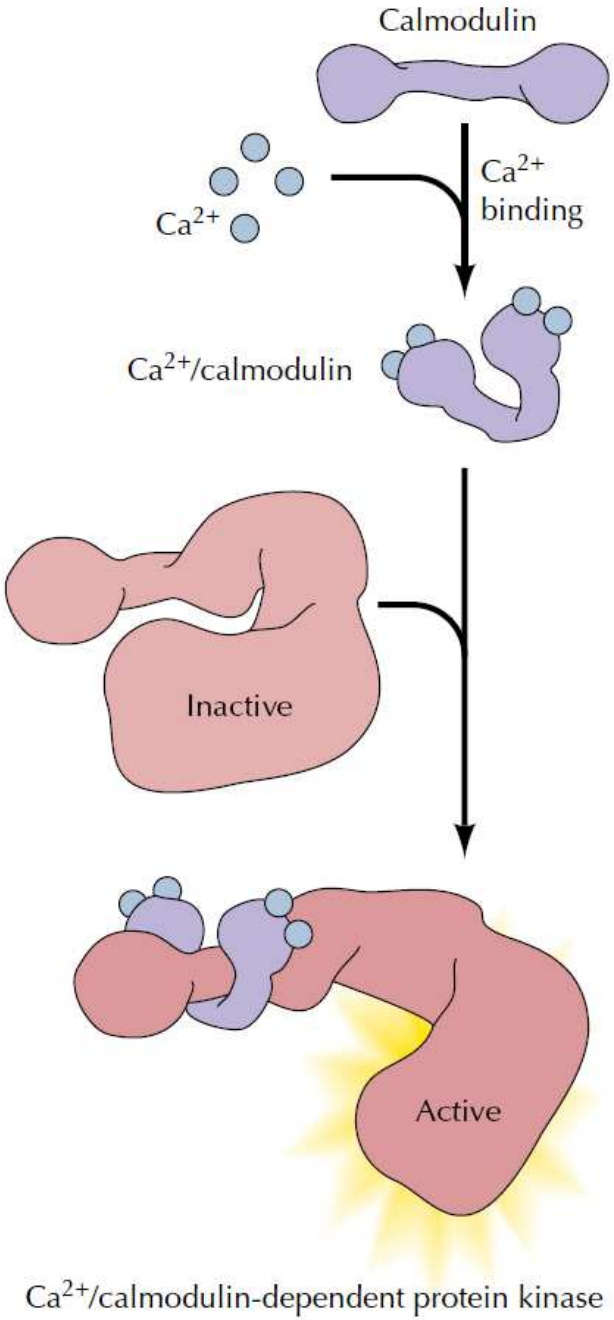


Figure 15-38 *Molecular Biology of the Cell* (© Garland Science 2008)







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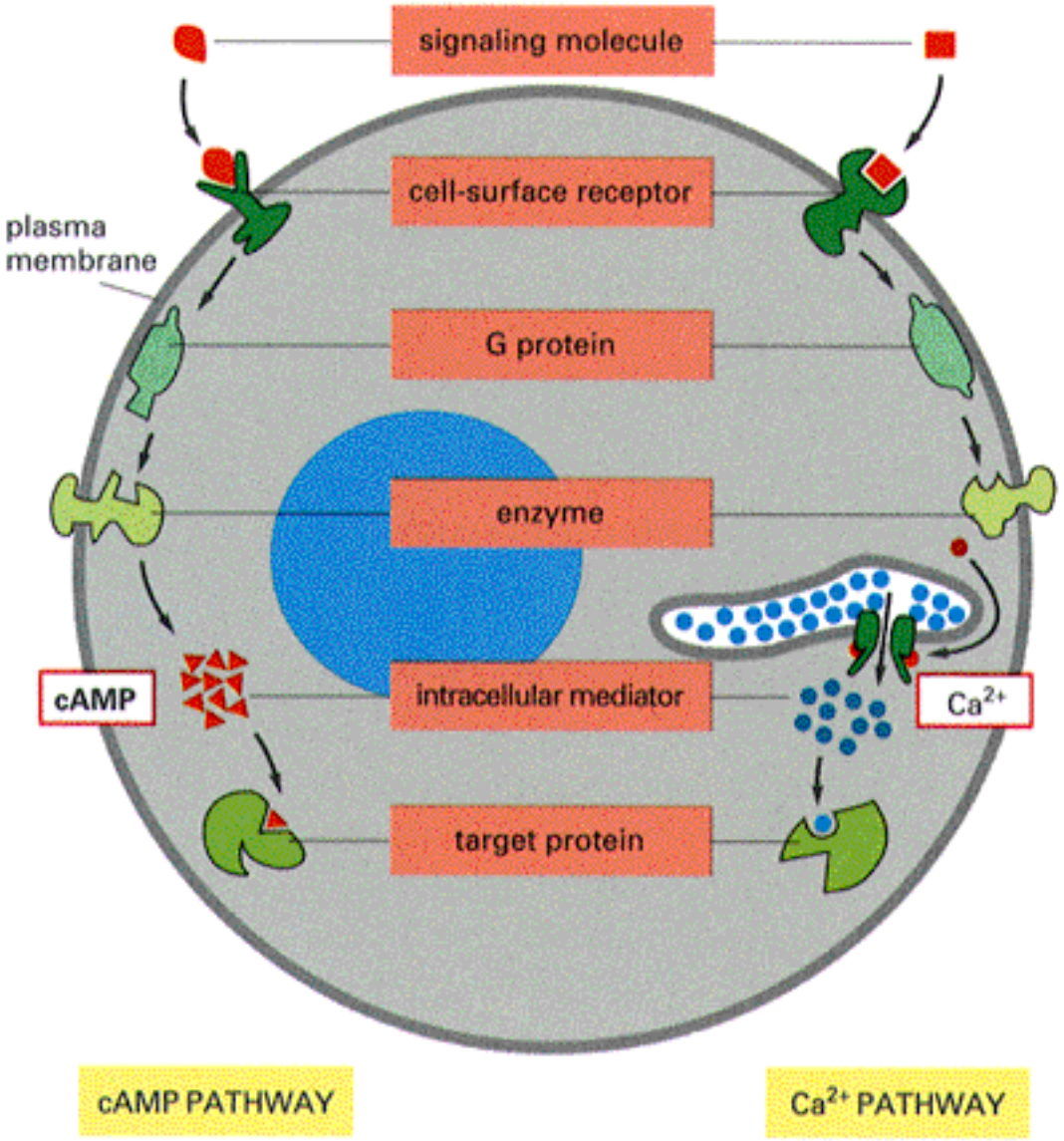
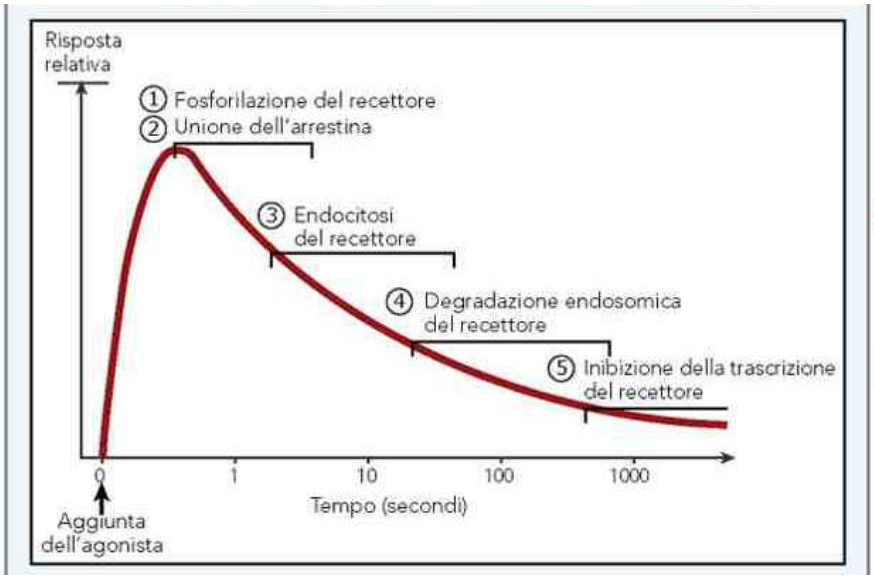
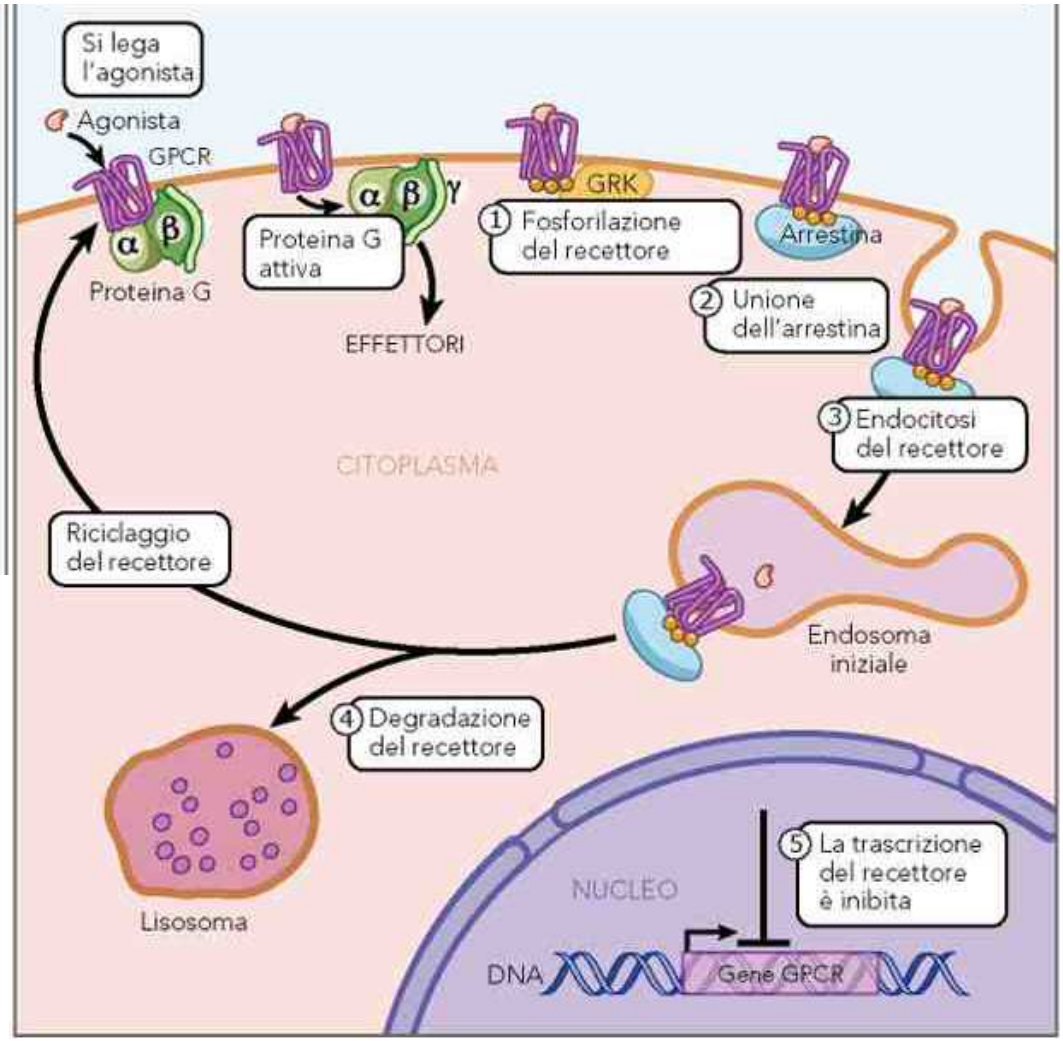


Dopo uno stimolo si verificano molti processi di adattamento





Membrane receptors

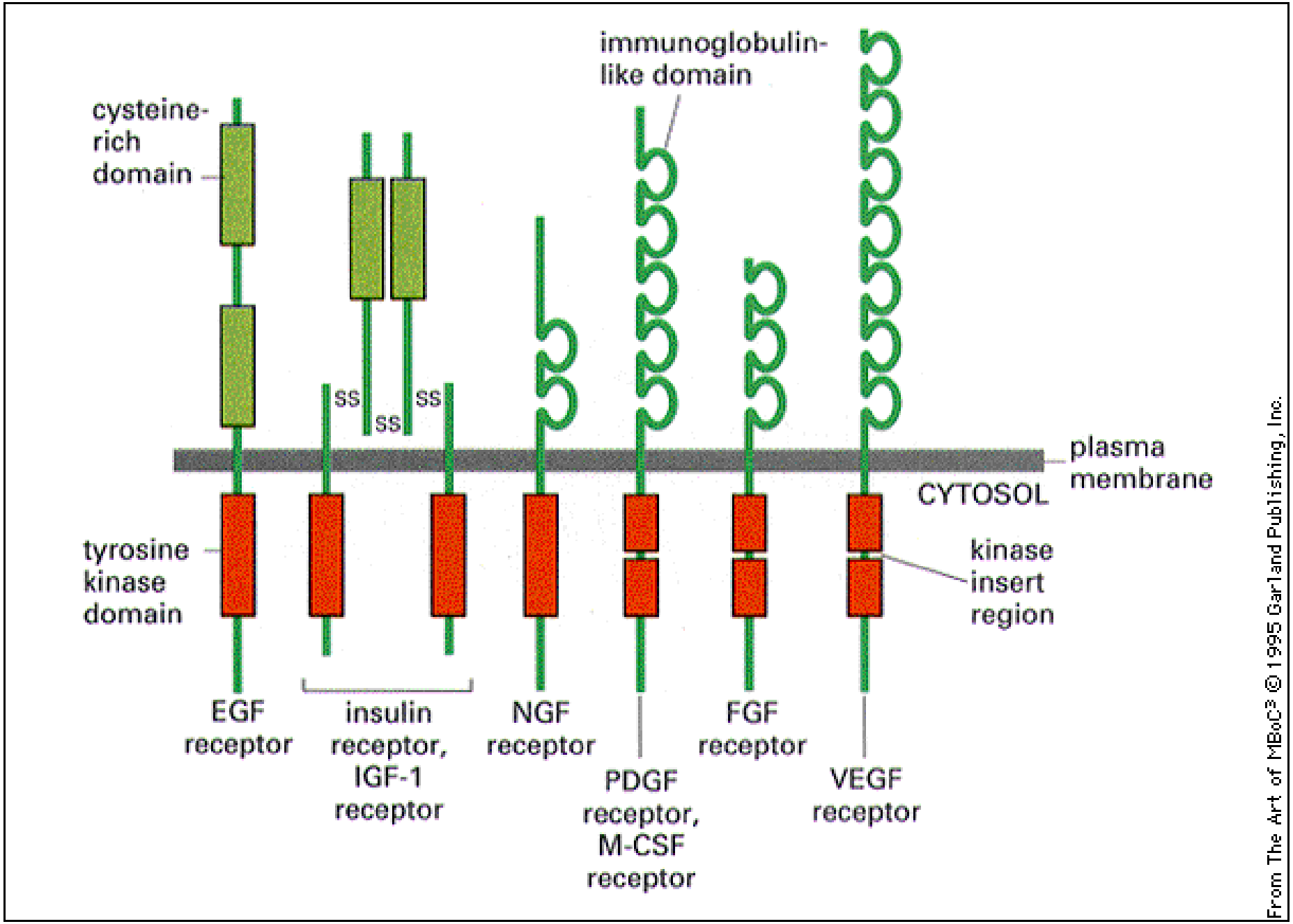
7-pass receptors

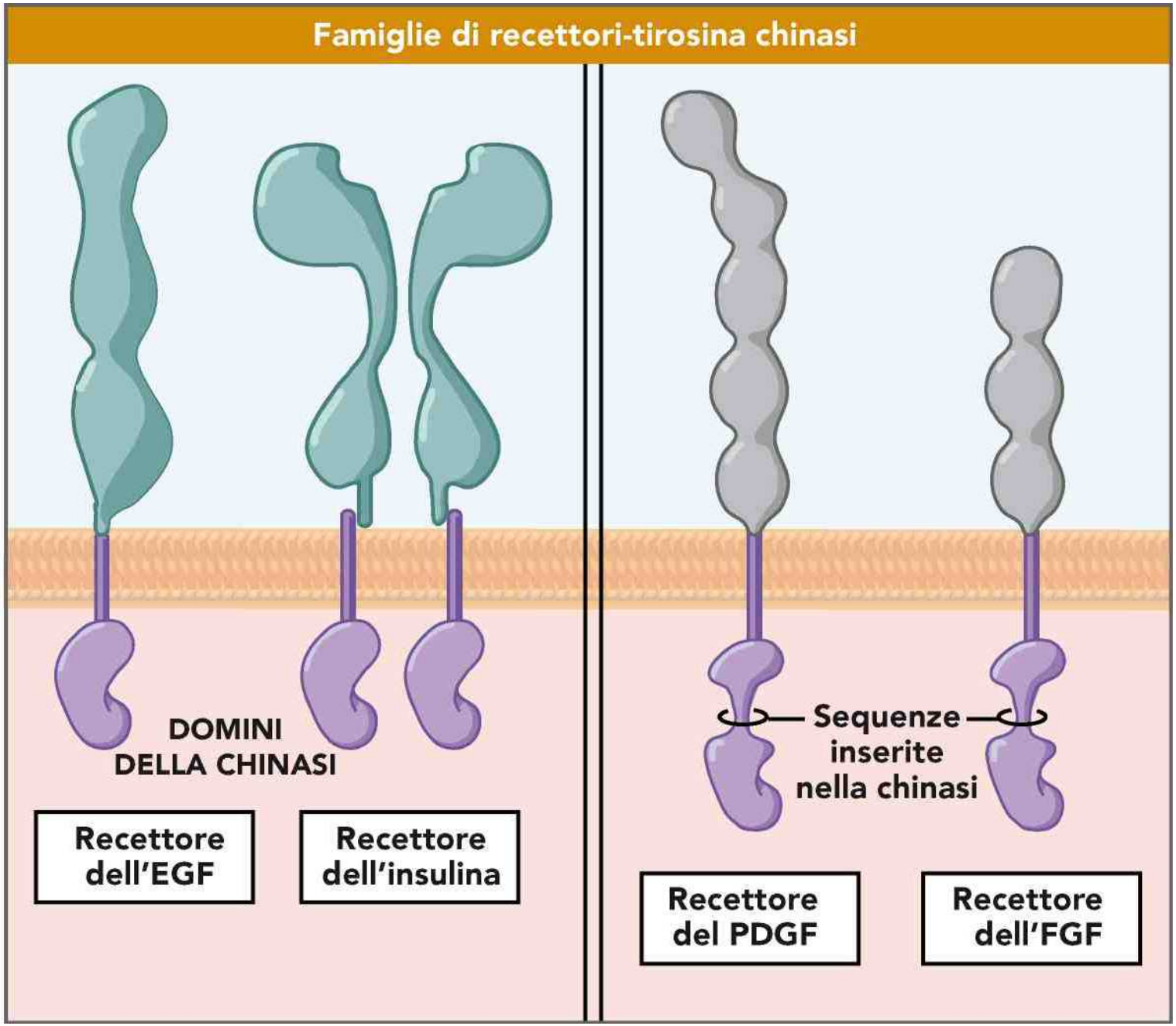
Tyrosine kinase receptors

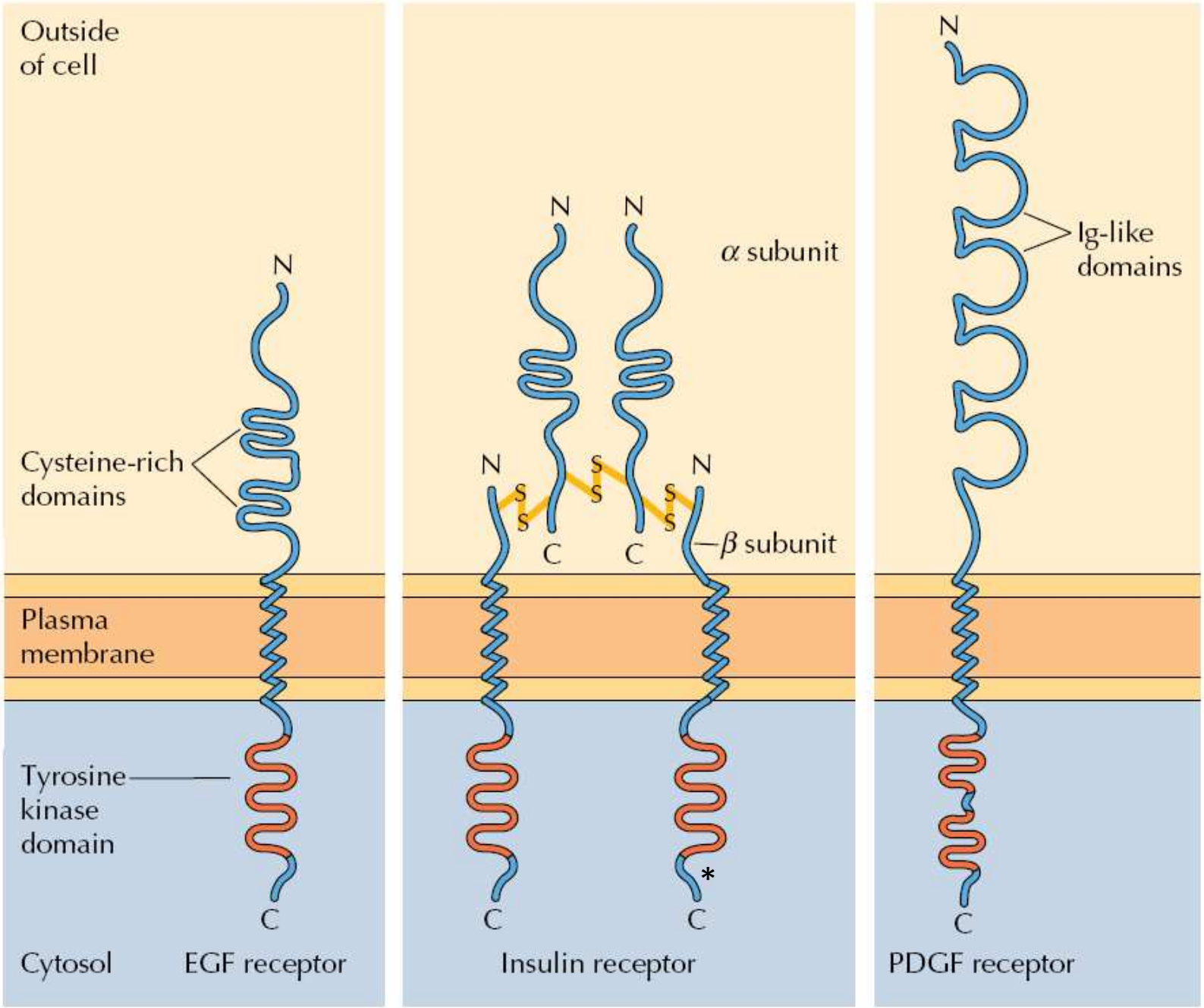
Serine-treonine kinase receptors

Cytochine receptors

Chanel receptors







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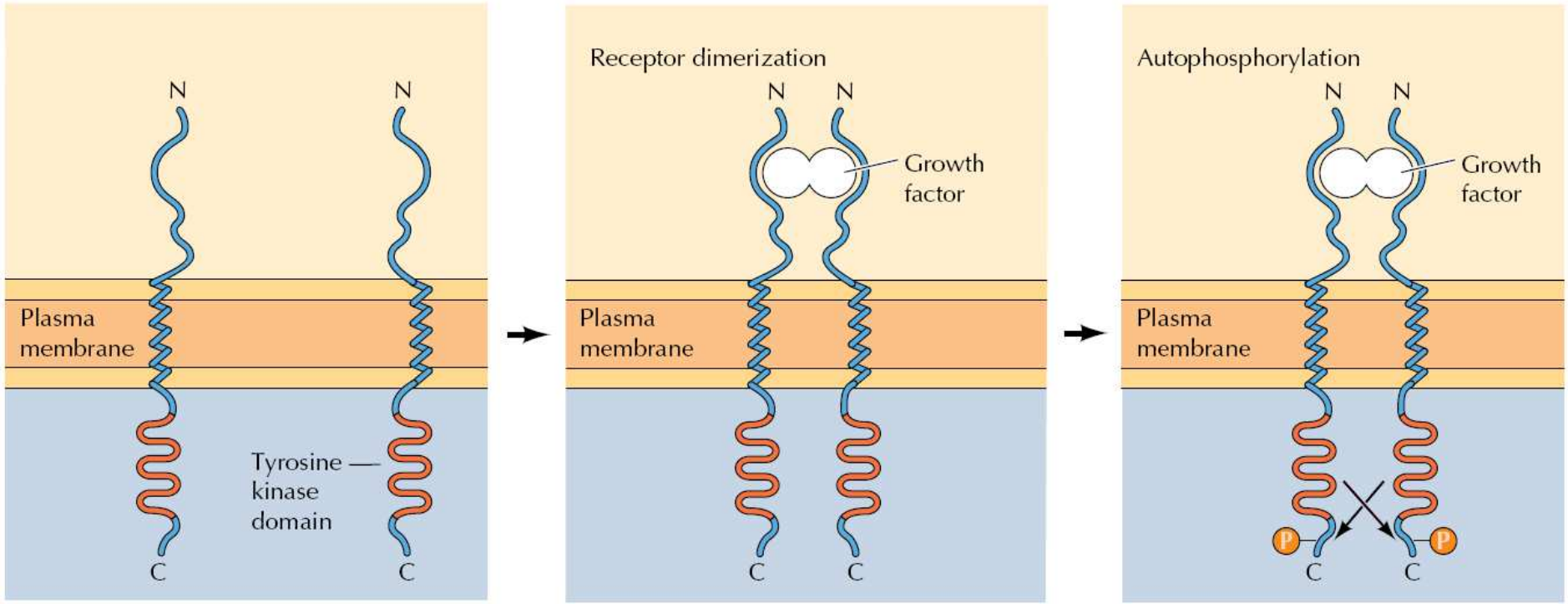
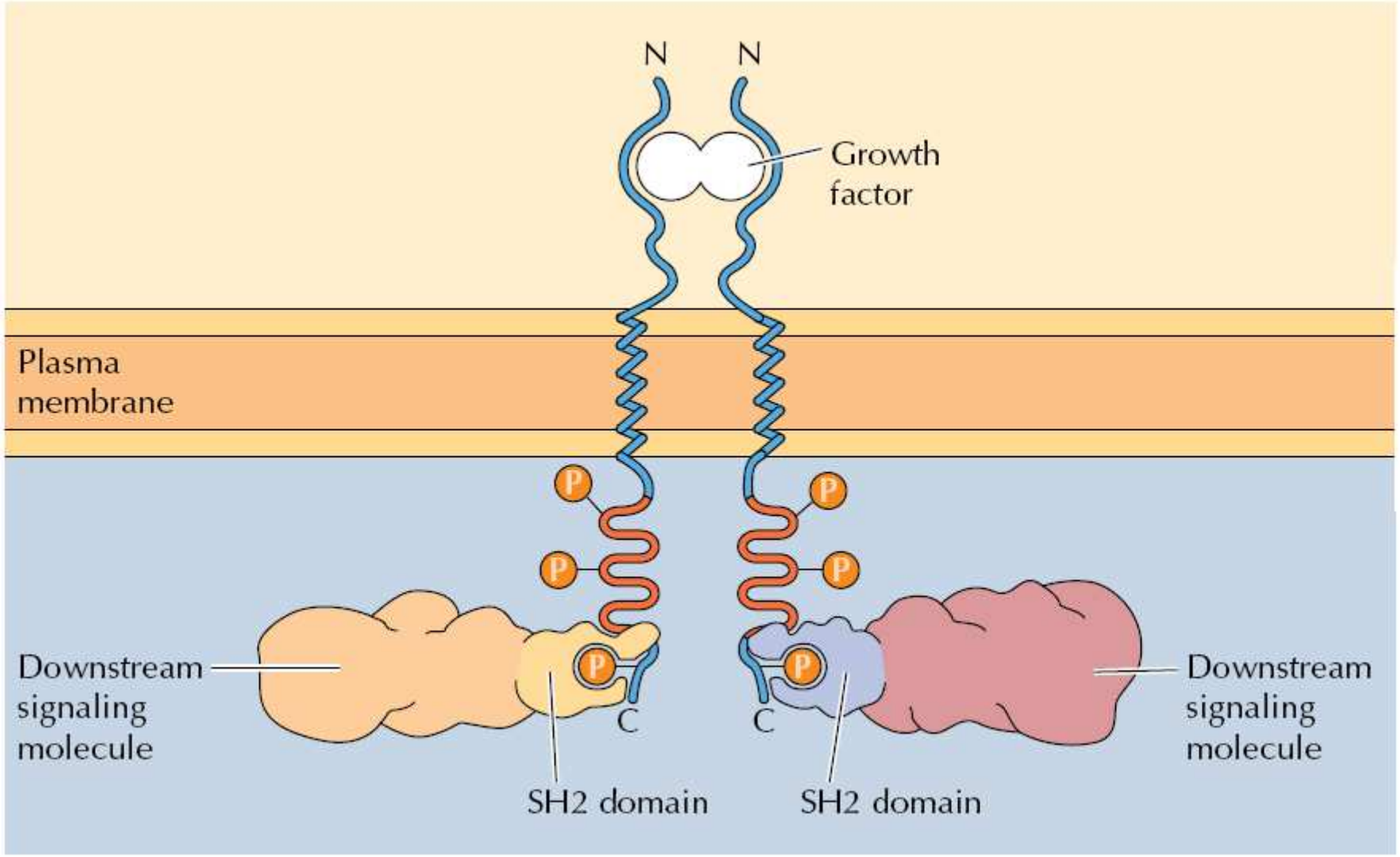
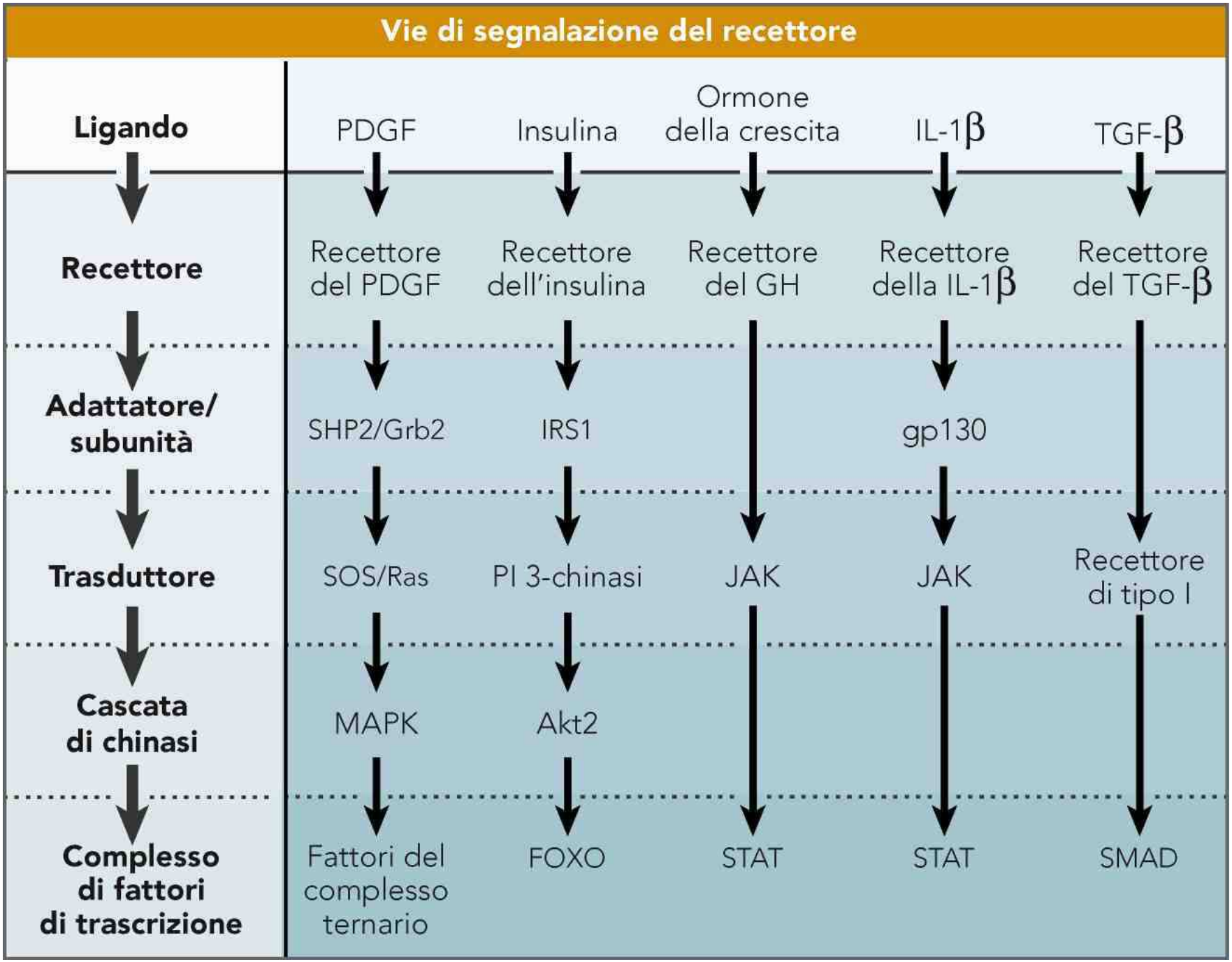
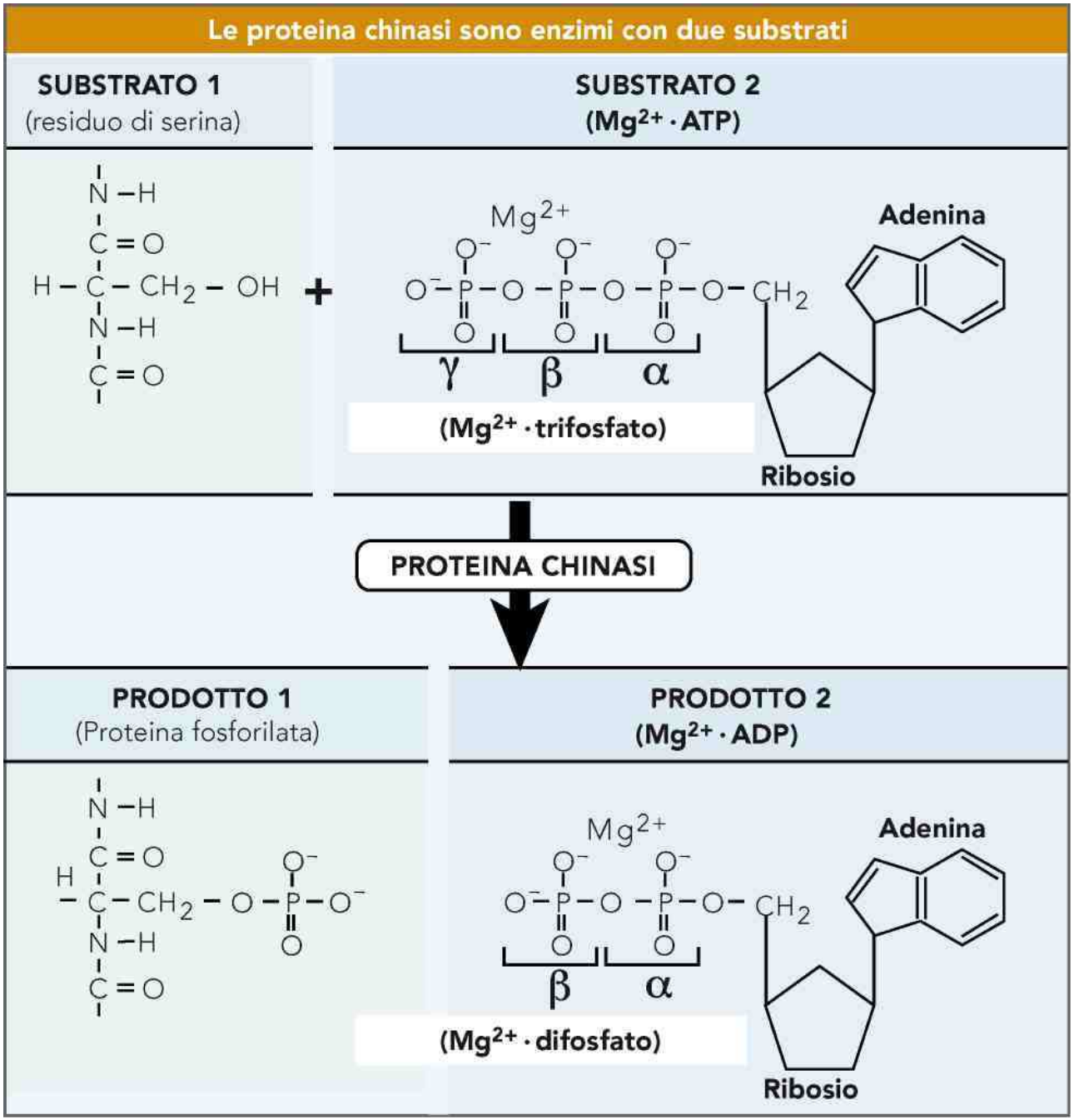


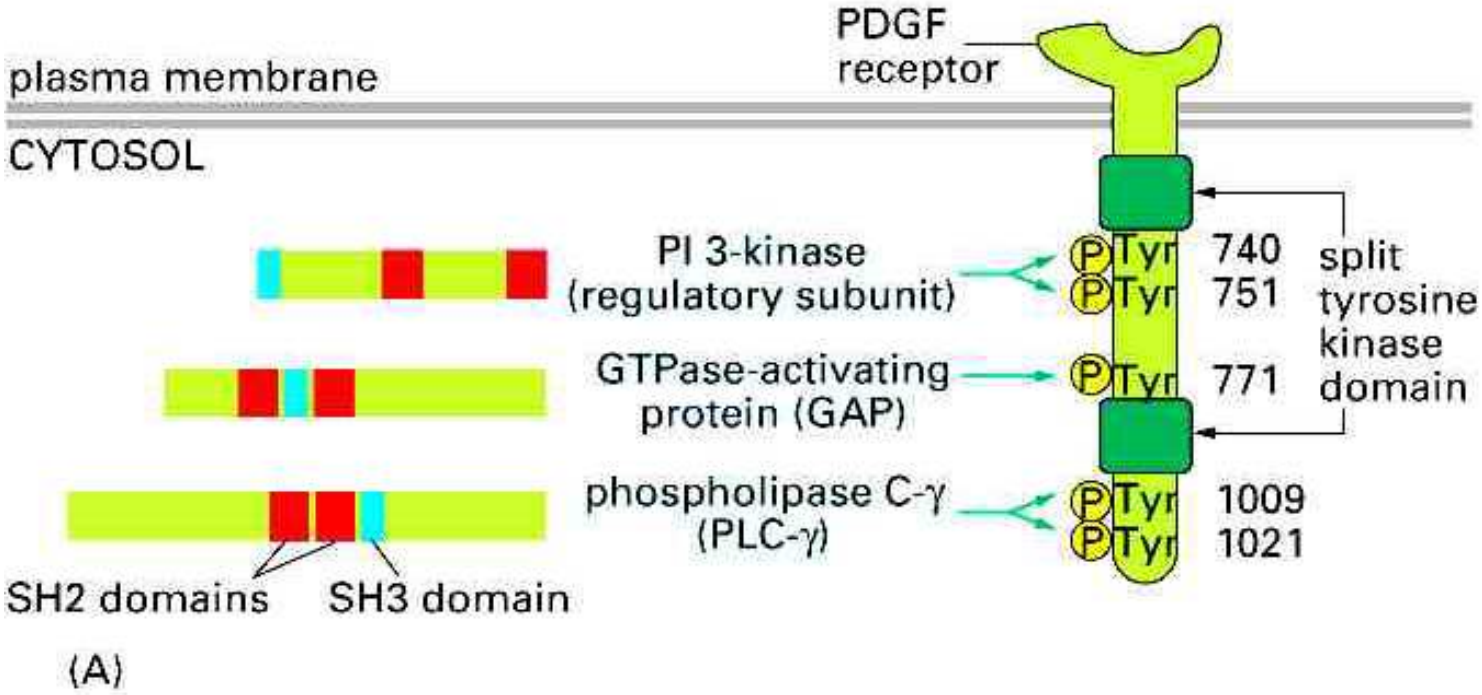
Quale esperimento può aiutarci a dimostrare che avviene la transfosforilazione?







Domini SH2 legano specifiche pTyr
Domini SH3 legano zone ricche di proline

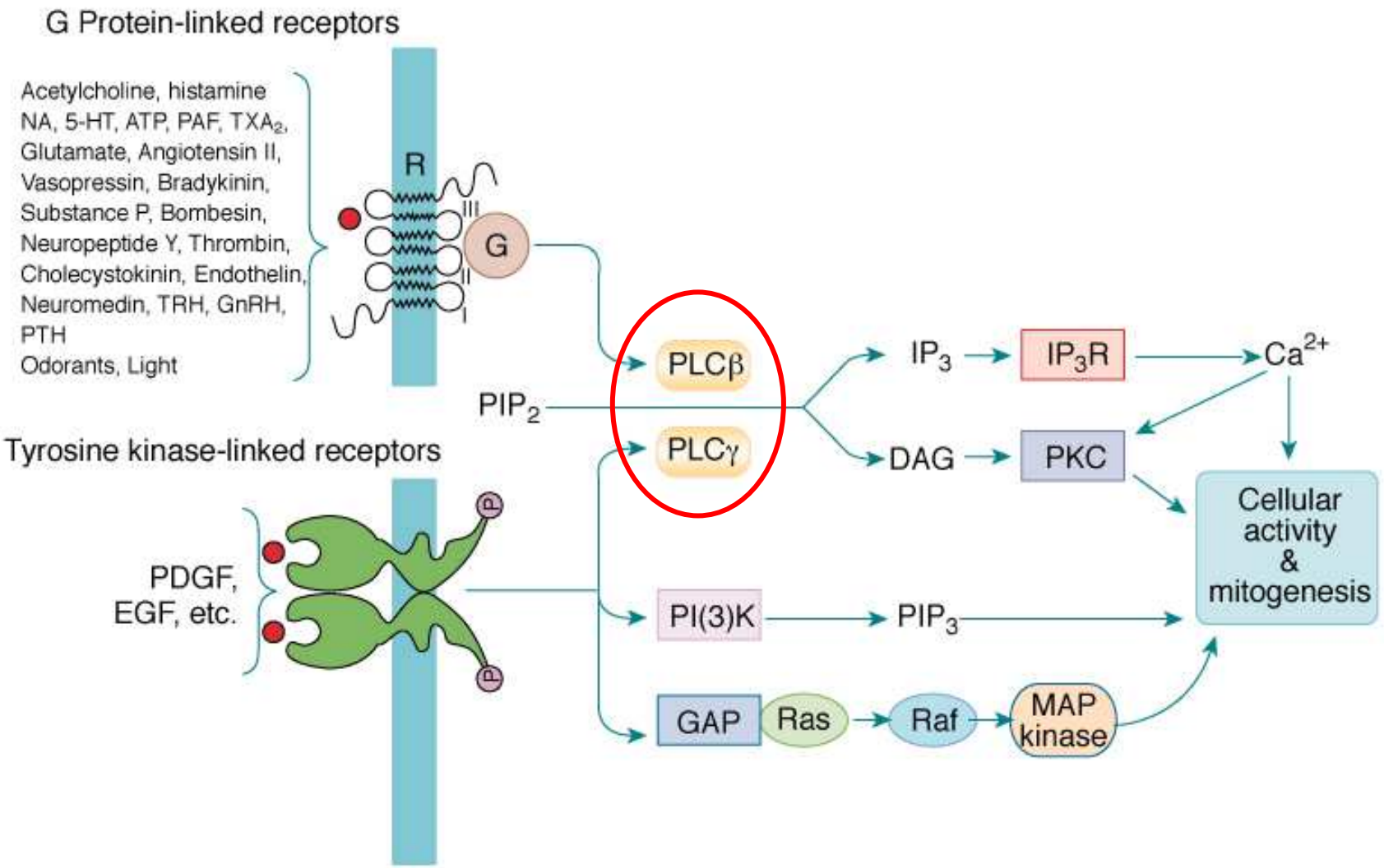


La specificità di riconoscimento dei domini SH2 è dovuta agli aminoacidi che circondano la pTyr

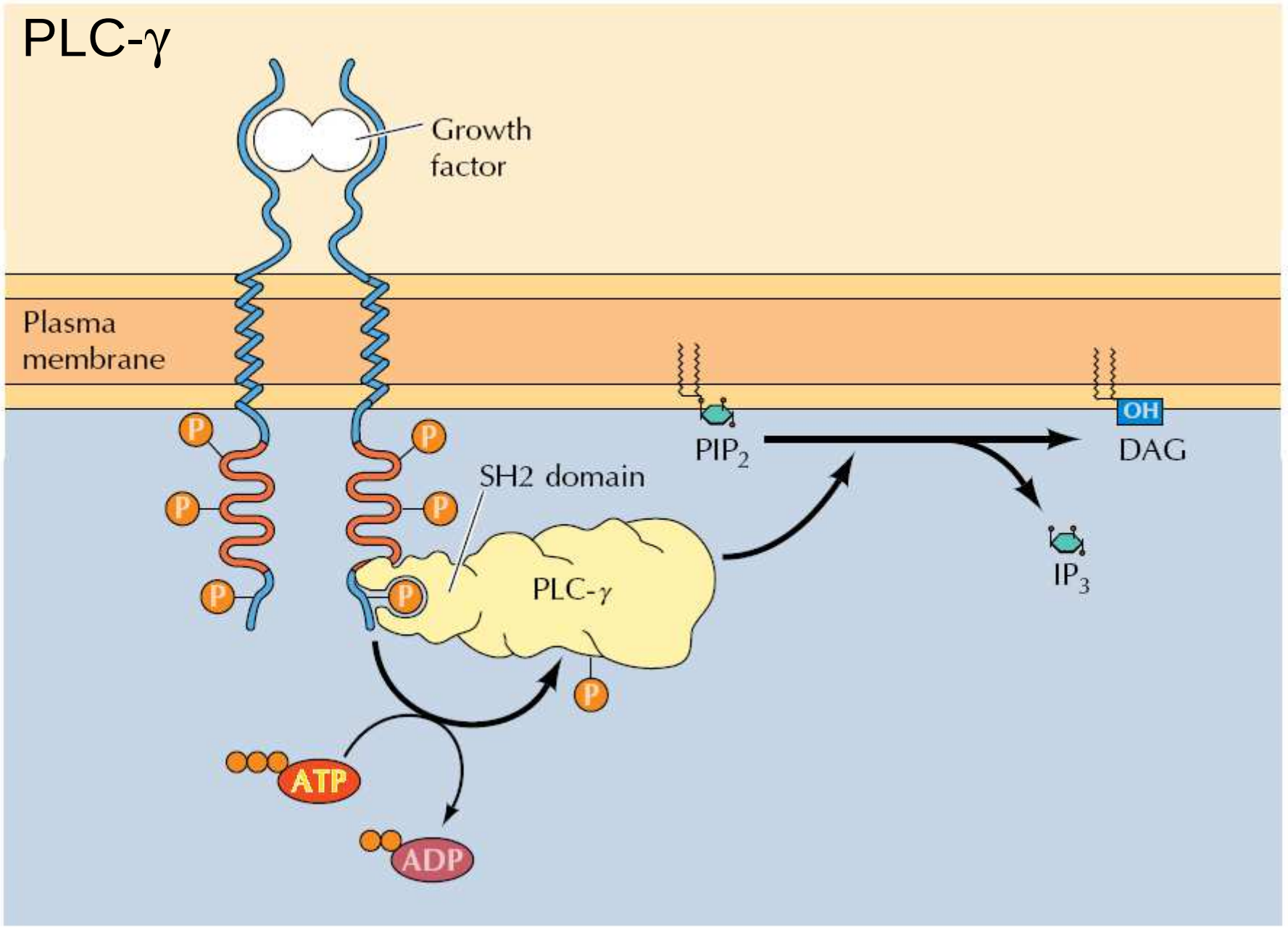
SH2 Domain Binding Motif References

Abl	[pY] [E/T/M] [N/E/D] [P/V/L]	(Songyang et al., 1993)
Blnk	[pY] [D/Q] [D] [V]	(Kabak et al., 2002)
Cbl	[N] [X] [pY] [S/T] [X] [X] [P]	(Lupher et al., 1997)
Crk	[pY] [D/K/N] [H/F/R] [P/V/L]	(Songyang et al., 1993)
Csk	[pY] [T/A/S] [K/R/Q/N] [M/I/V/R]	(Songyang et al., 1994)
Fes	[pY] [E] [X] [V/I]	(Songyang et al., 1994)
Fgr	[pY] [E/Y/D] [E/N/D] [I/V]	(Songyang et al., 1993)
Fyn	[pY] [E/T] [E/D/Q] [I/V/M]	(Songyang et al., 1993)
Grb2	[pY] [I/V] [N] [I/L/V]	(Rodriguez et al., 2004)
(SEM-5)	[pY] [L/V/I] [N] [V/P]	(Songyang et al., 1993)
Grb7	[F/Y] [pY] [E/T/Y/S] [N] [I/L/V/P/T/Y/S]	(Rodriguez et al., 2004)
Grb10	[F/Y] [pY] [E/T/Y/S] [N] [I/L/V/P/T/Y/S]	(Rodriguez et al., 2004)
Itk	[pY] [A/E/V] [Y/F/E/S/N/V] [P/F/I/H]	(Bunnell et al., 2000)
Lck	[pY] [E/T/Q] [E/D] [I/V/M]	(Songyang et al., 1993)
Nck	[pY] [D] [E] [P/D/V]	(Songyang et al., 1993)
PI3K	[pY] [M/I/V/E] [X] [M]	(Songyang et al., 1993)
PLCG1_N	[pY] [V/I/L] [E/D] [L/I/V]	(Songyang et al., 1993)
PLCG1_C	[pY] [V/I/L] [E/D] [P/V/I]	(Songyang et al., 1993)
PTPN6_N	[pY] [F] [X] [F/P/L/Y]	(Songyang et al., 1994)
PTPN6_C	[X] [X] [pY] [Y] [M] [K/R]	(Beebe et al., 2000)

PTPN11_C	[T/V/I/y] [X] [pY] [A/s/t/v] [X/ [I/v/l)	(Sweeney et al., 2005)
Rasa_N	[pY] [I/L/V] [X] [φ]	(Holland et al., 1997)
Rasa_C	[pY] [X] [X] [P]	(Holland et al., 1997)
SH2D1A	[T] [I] [pY] [X] [X] [V/I]	(Poy et al., 1999)
SH2D1B	[T] [I] [pY] [X] [X] [V/I]	(Poy et al., 1999)
SH3BP2	[pY] [E/M/V] [N/V/I] [X]	(Songyang et al., 1994)
SHB	[pY] [T/V/I] [X] [L]	(Karlsson et al., 1995)
Shc1	[pY] [I/E/T [X] [I/L/M]	(O'Bryan et al., 1996)
Shc2	[pY] [I/M/T/D/L] [M/I] [M/F/I/Y/V]	(O'Bryan et al., 1996)
Shc3	[pY] [L/M/I/Q] [M/Y] [I/L/M/V]	(O'Bryan et al., 1996)
SHIP	[pY] [Y/S/T/v] [L/y/n/l/e/f] [L/N/l/e/I]	(Sweeney et al., 2005)
Src	[pY] [EDT] [ENY] [IML]	(Songyang et al., 1993)
STAT1	[pY] [D/E] [P/R] [R/P/Q]	(Wiederkehr-Adam et al., 2003)
STAT3	[pY] [X] [X] [Q]	(Stahl et al., 1995)
Syk_C	[pY] [Q/T/E] [E/Q] [L/I]	(Songyang et al., 1994)
Tns	[pY] [E] [N] [F/I/V]	(Songyang and Cantley, 1995)
Vav1	[pY] [M/L/E] [E] [P]	(Songyang et al., 1994)



PLC-γ



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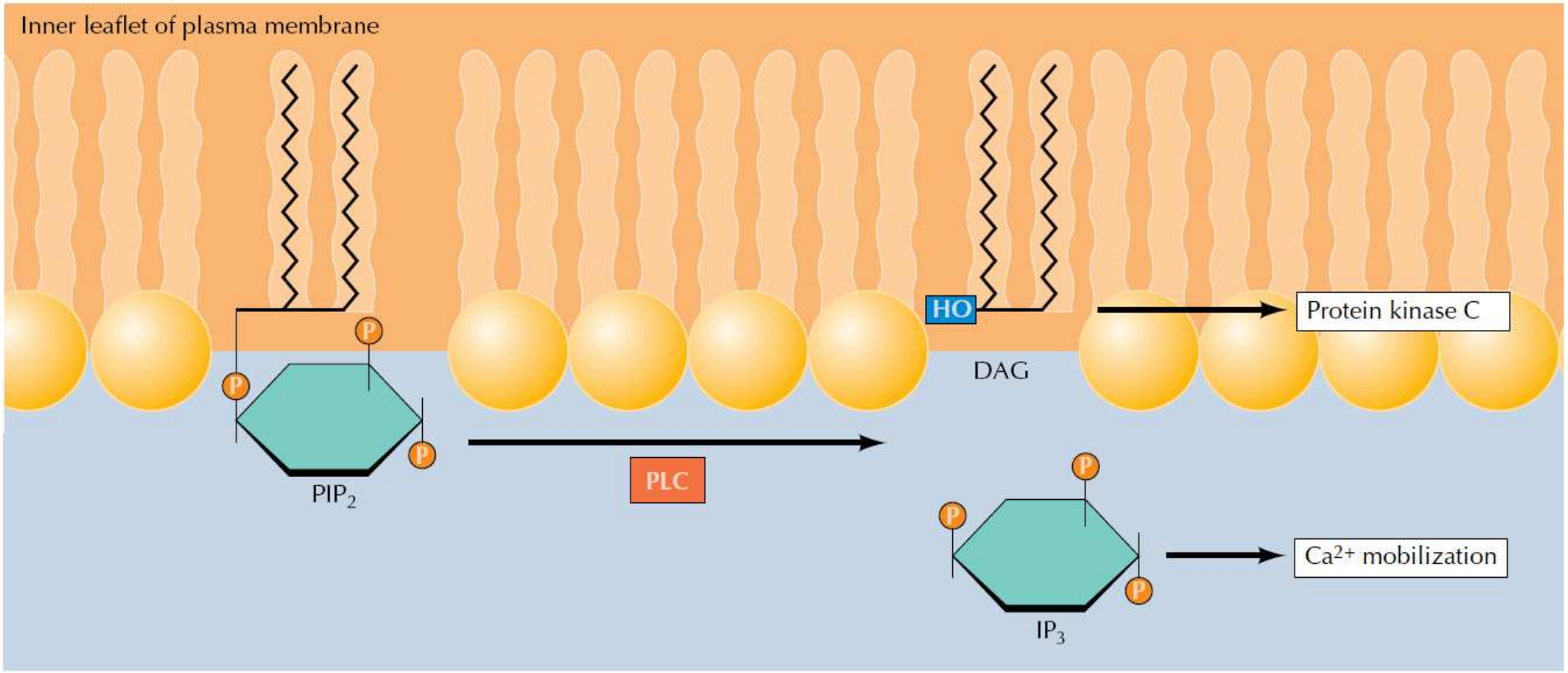

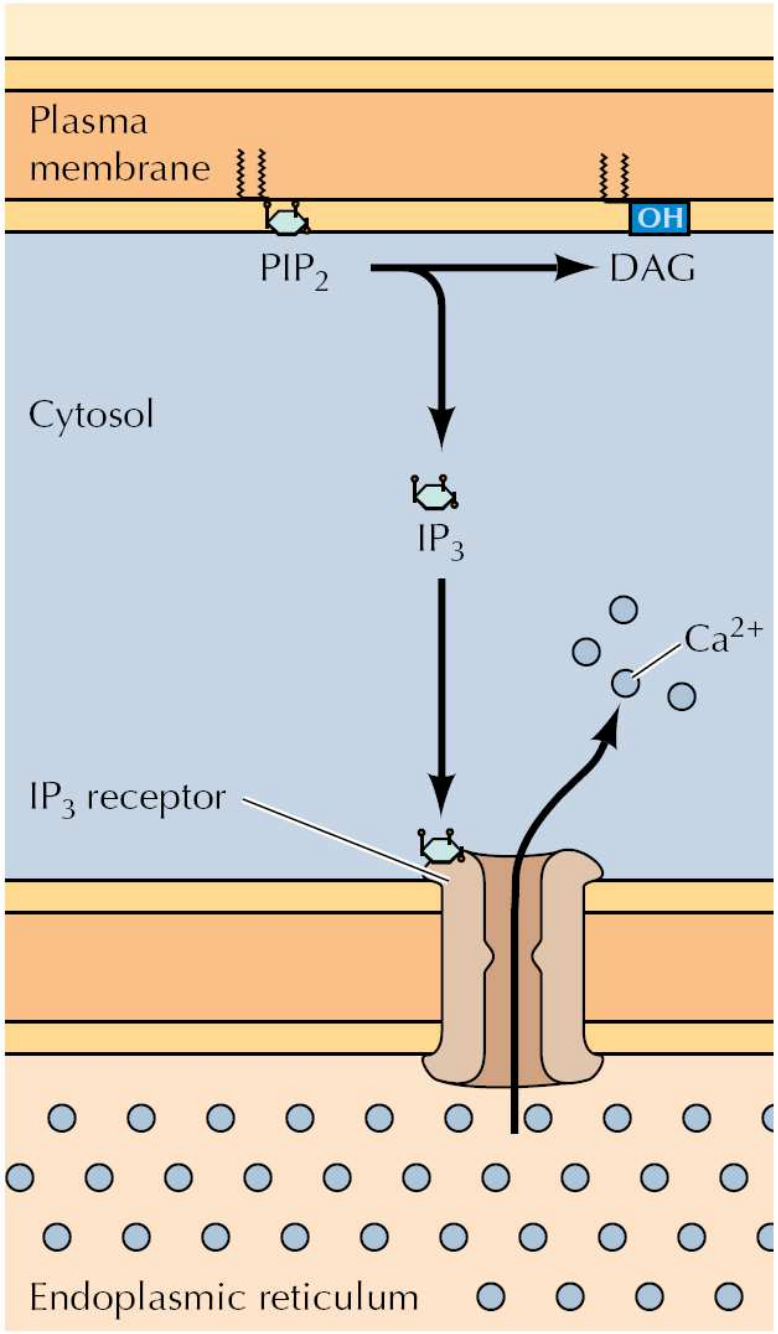

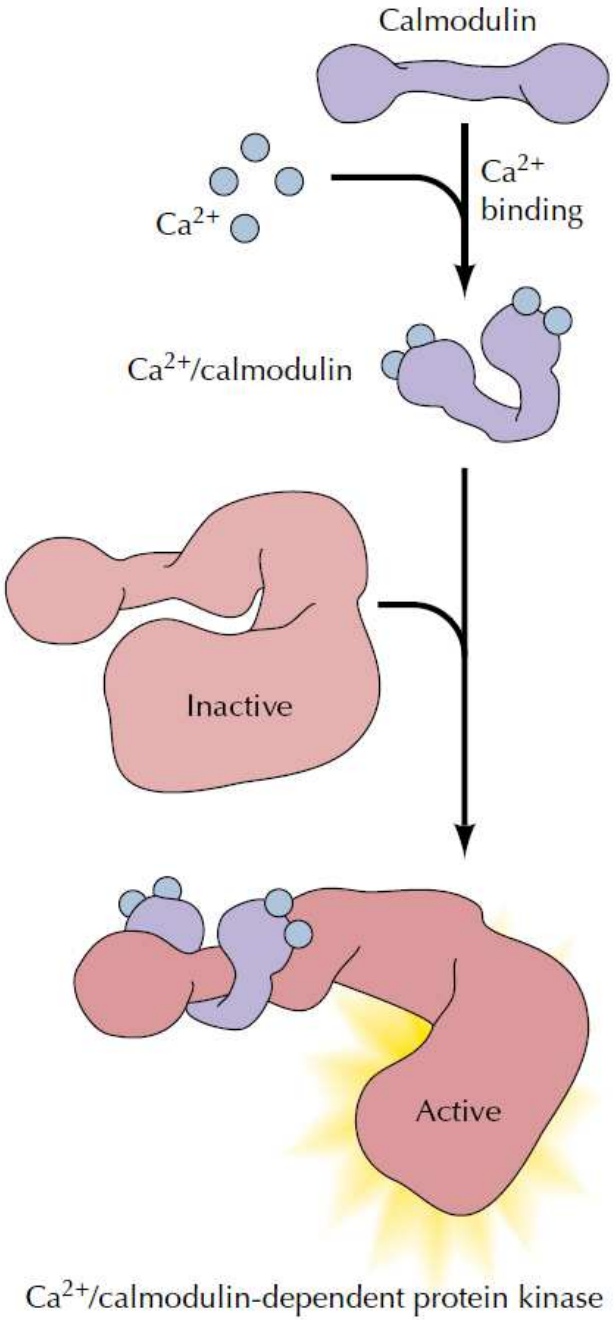

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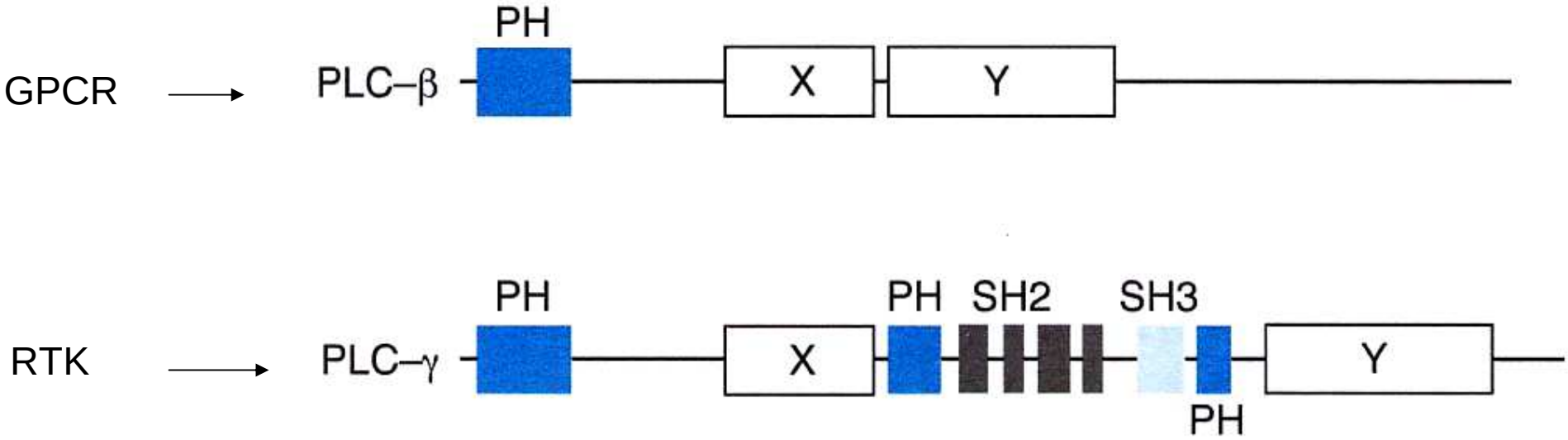


A cosa serve il dominio PH?
Cosa riconosce?
Conosci altri domini con funzione simile?
Qual è la differenza?
Cosa riconosce il dominio SH3?

The hydrolysis of PIP2 is activated downstream of both G protein-coupled receptors and tyrosine kinase receptors. This occurs because one form of phospholipase C (PLC-β) is stimulated by G proteins, whereas a second (PLC-γ) contains SH2 domains that mediate its association with activated receptor protein-tyrosine kinases. This interaction localizes PLC-γ to the plasma membrane as well as leading to its tyrosine phosphorylation, which increases its catalytic activity.

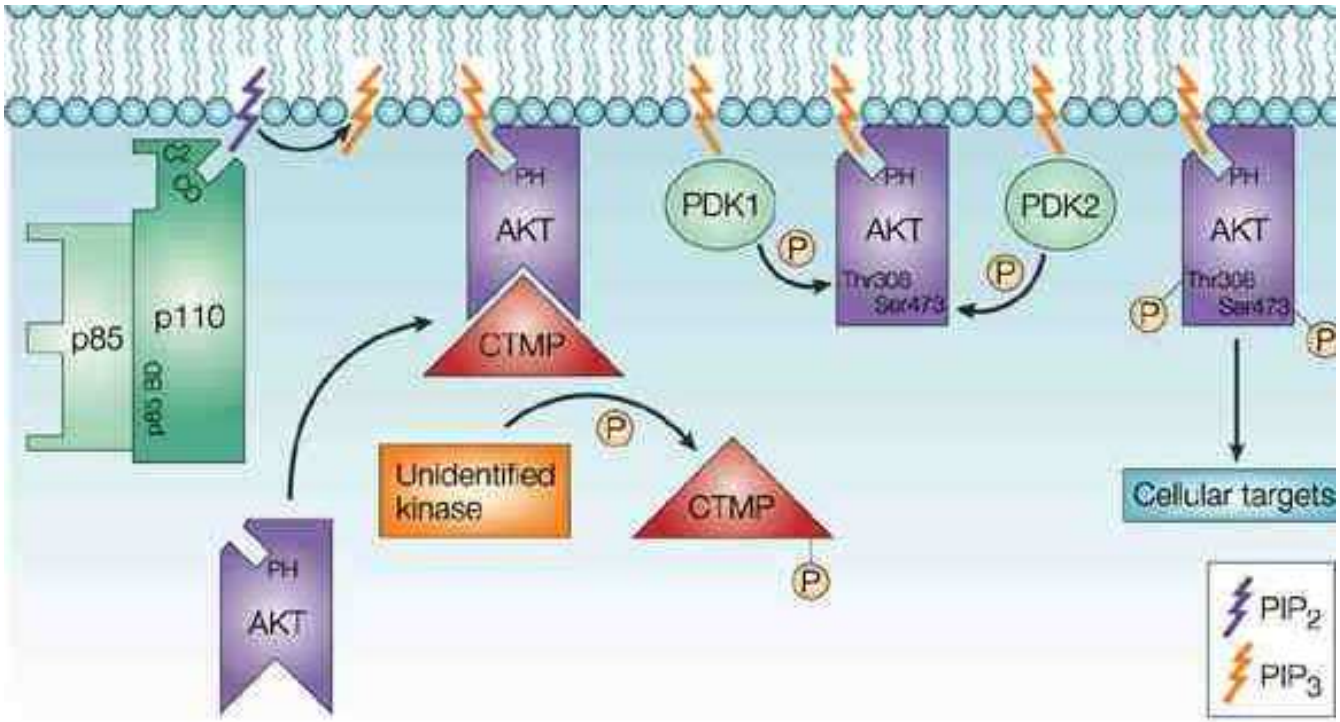
Pleckstrin homology domain (PH domain)

- protein domain of approximately 120 amino acids that occurs in a wide range of proteins involved in intracellular signaling or as constituents of the cytoskeleton.

- this domain can bind phosphatidylinositol lipids within biological membranes and proteins such as the βγ-subunits of heterotrimeric G proteins and protein kinase C.

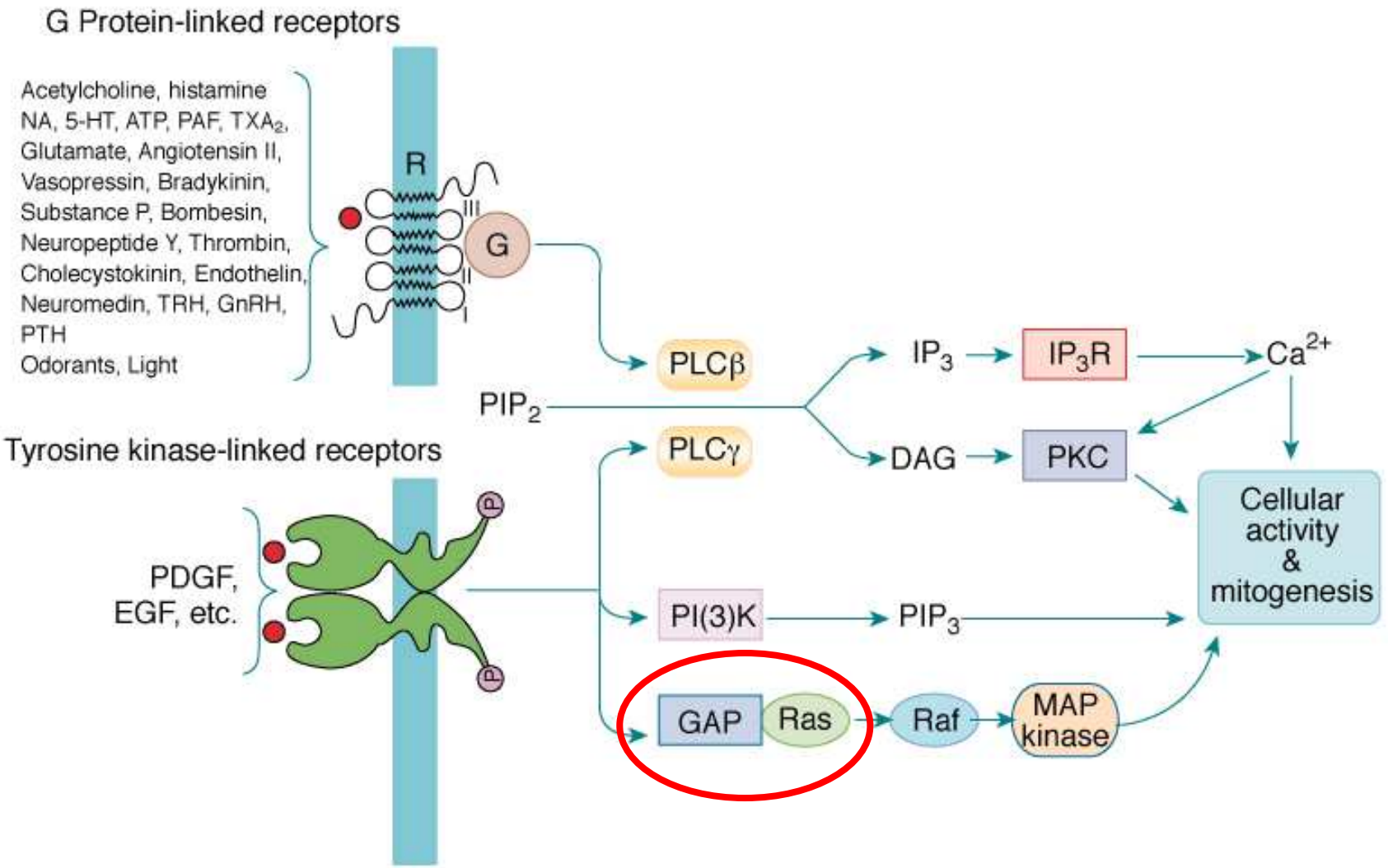
- through these interactions, PH domains play a role in recruiting proteins to different membranes, thus targeting them to appropriate cellular compartments or enabling them to interact with other components of the signal transduction pathways.

Fosfatidil inositidi fosforilati in posizione 3 servono da sito di aggancio per proteine che contengono domini PH (plextrin homology).

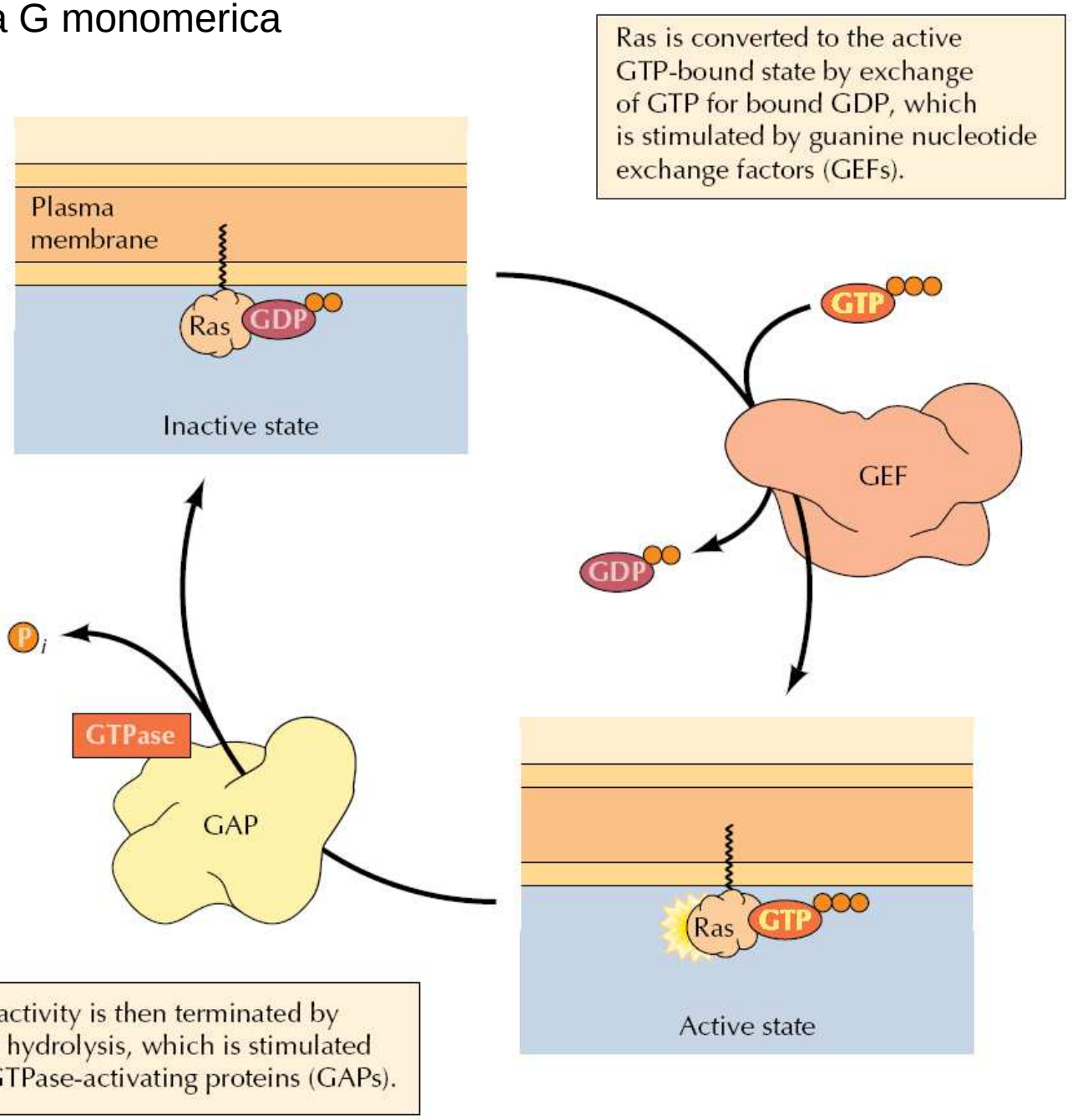


Let’s focus our attention on specific signal transduction pathways:

- Phospholipase C-γ
- **RAS-MAPK**
- PI3-kinase



Ras: proteina G monomerica



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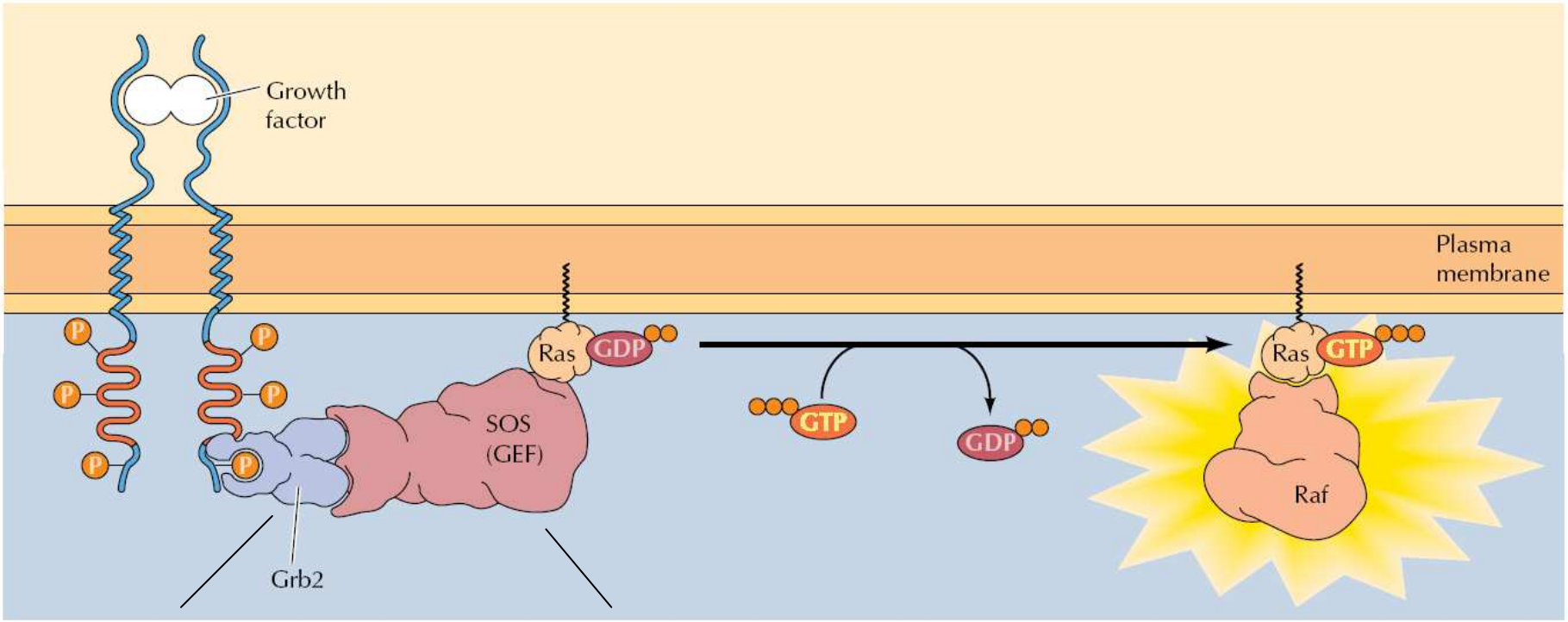
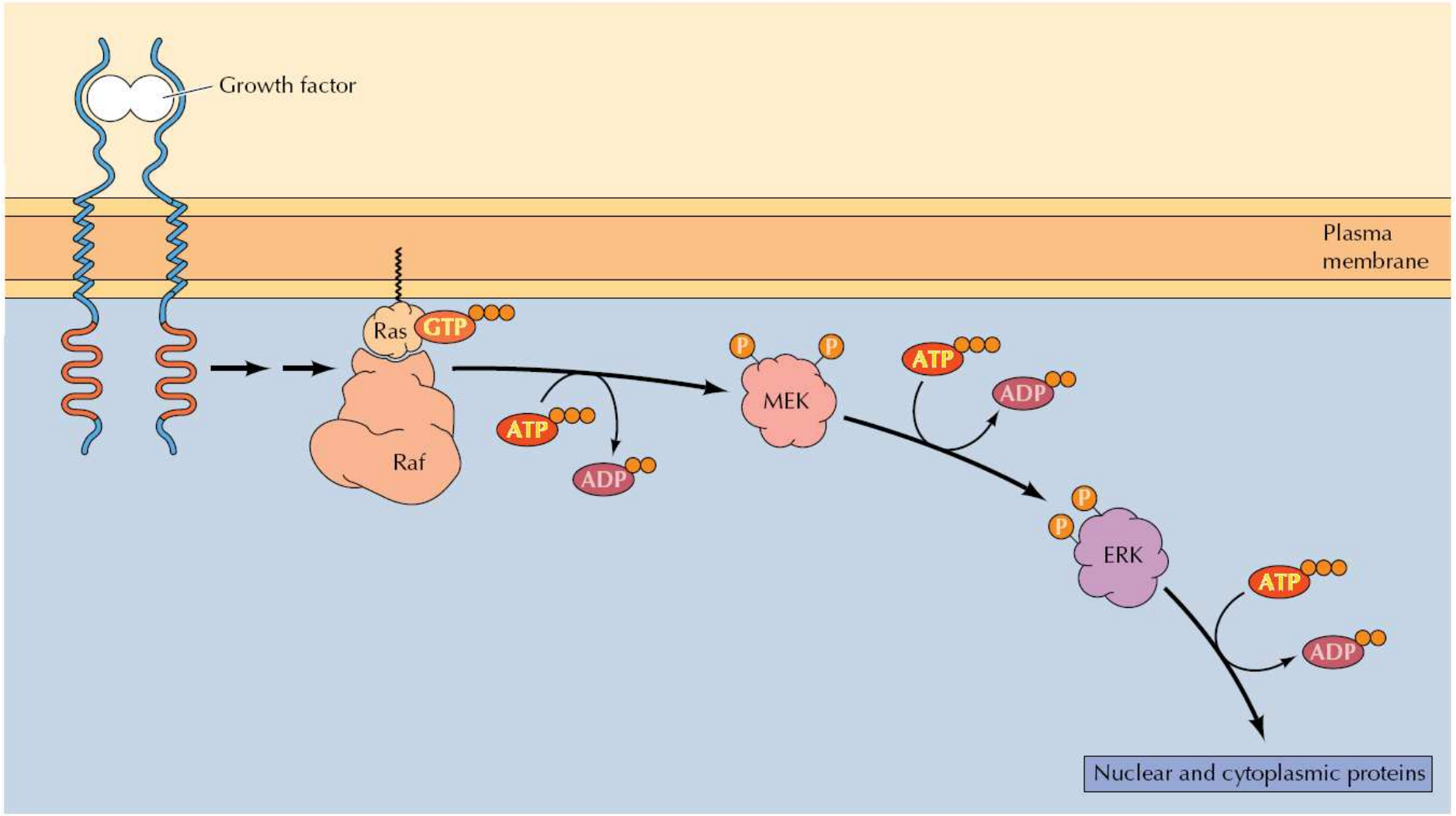


proteina adattatrice proteina che attiva Ras



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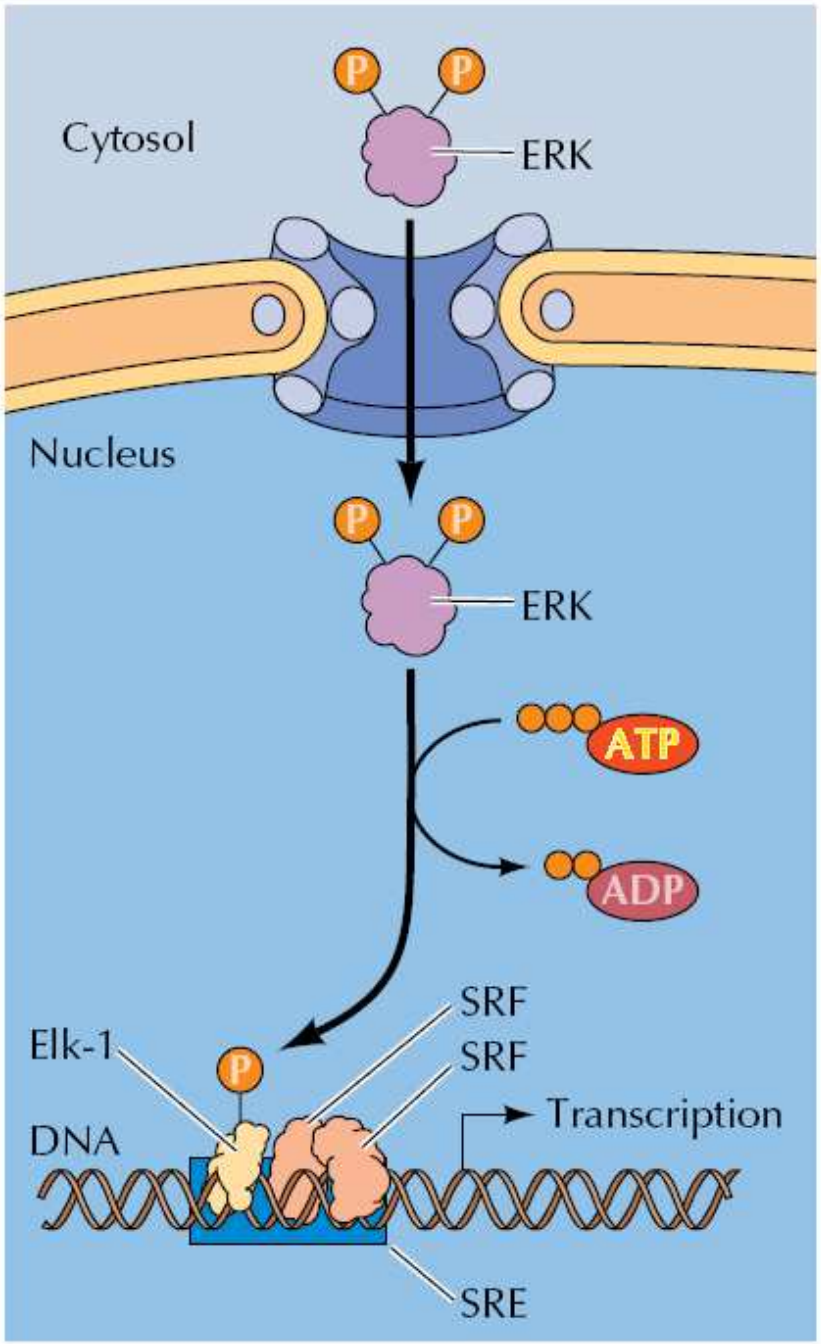

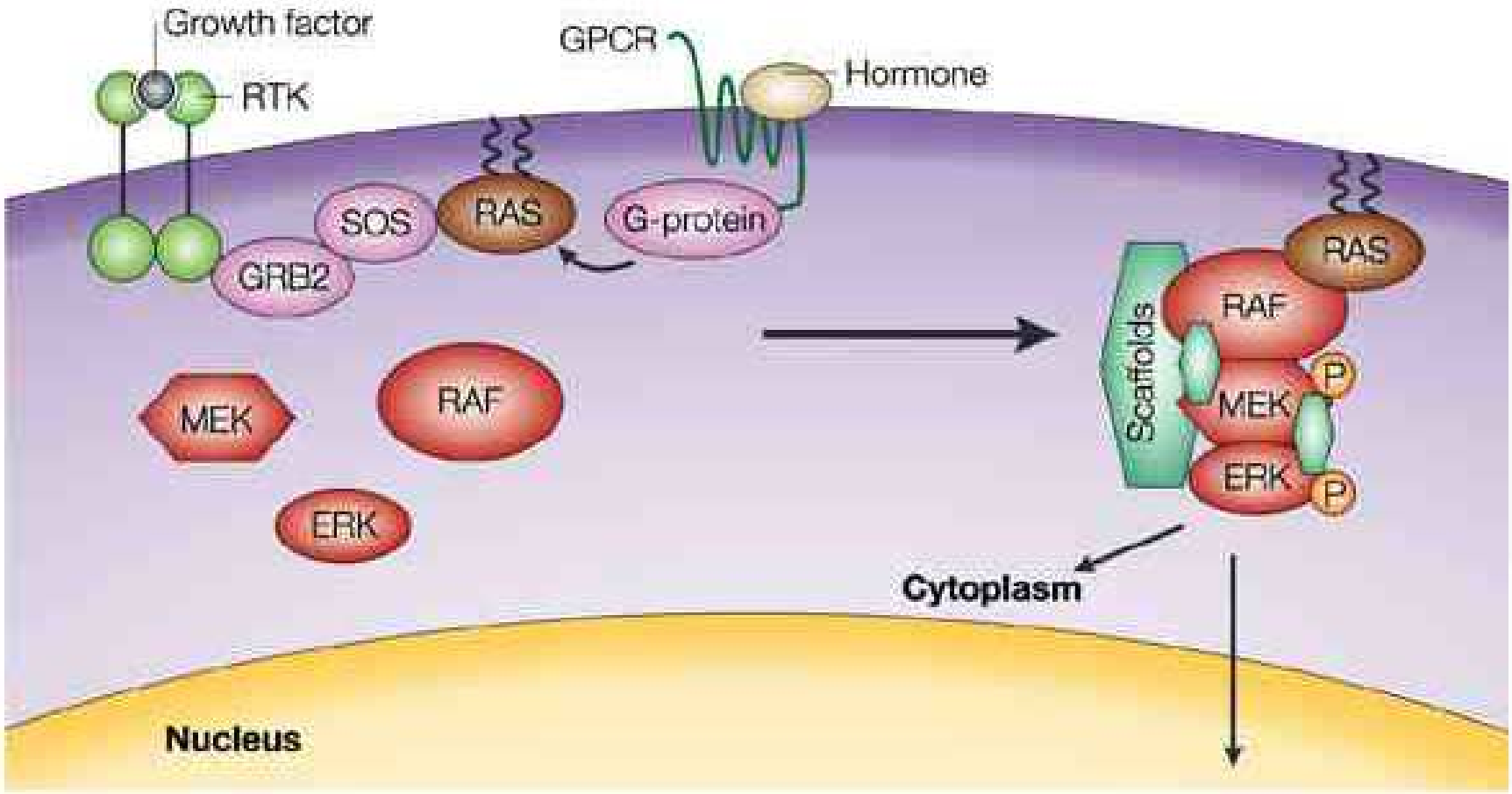

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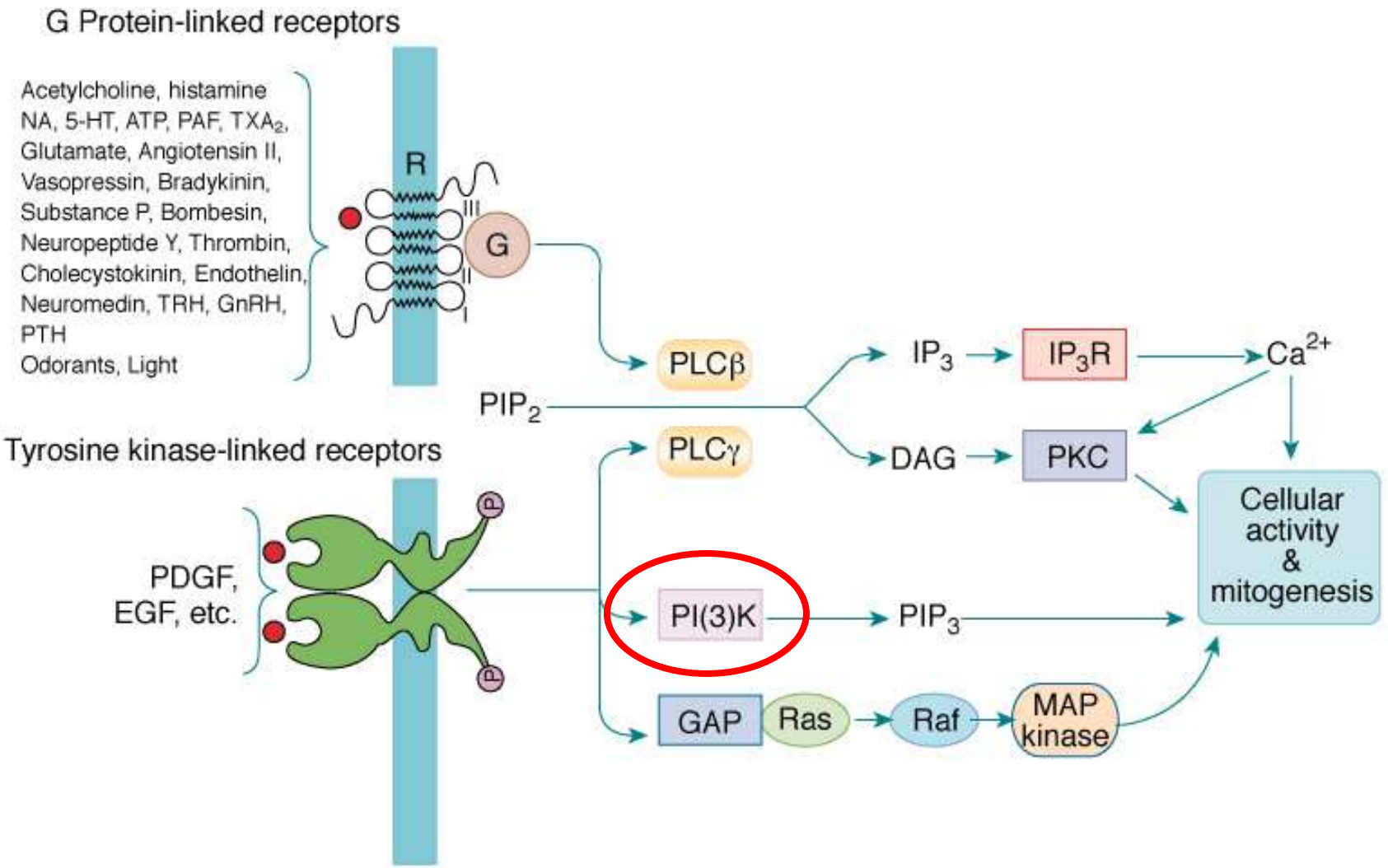
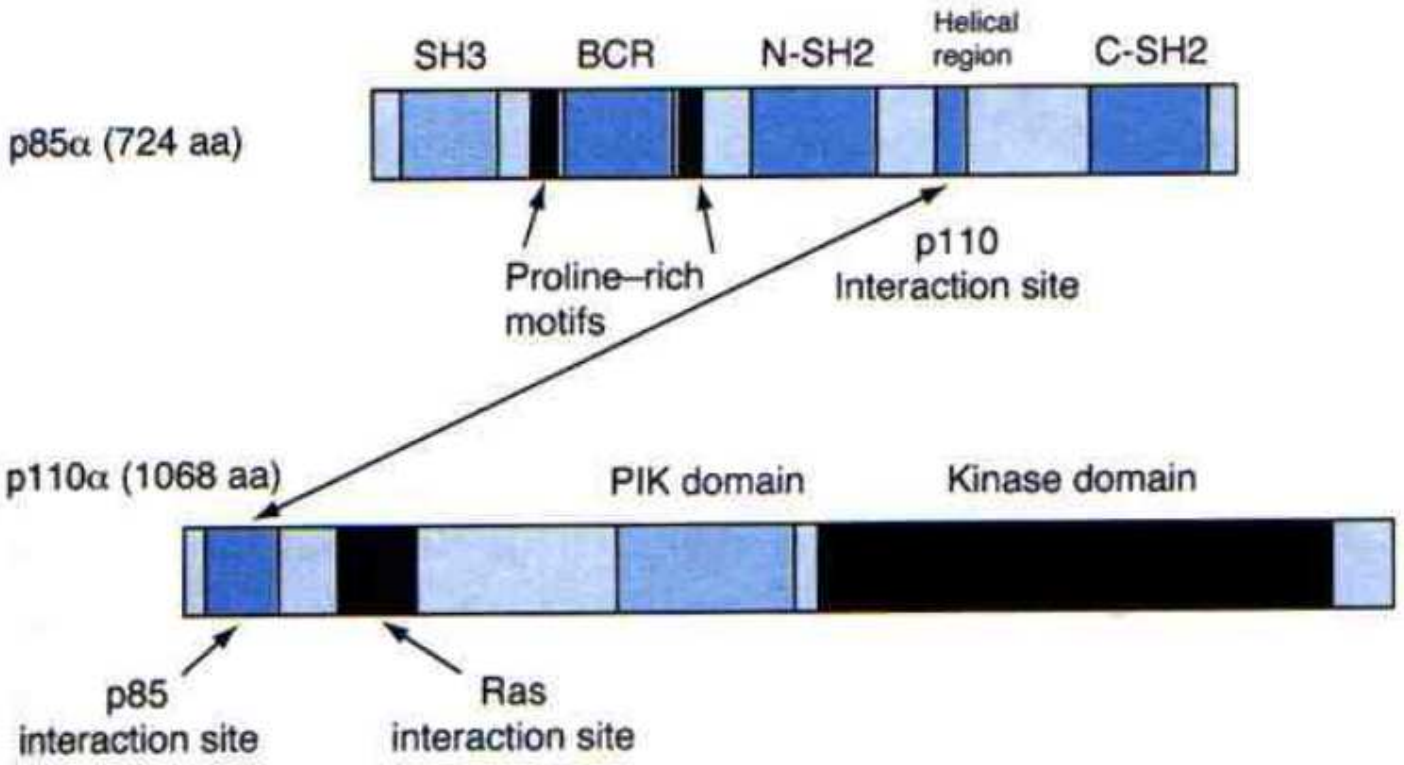
- Phospholipase C-γ
- RAS-MAPK
- **PI3-kinase**

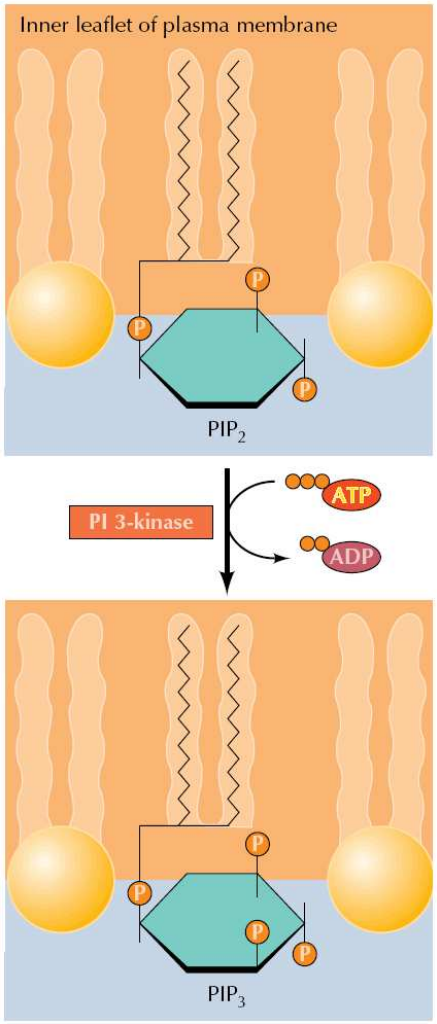


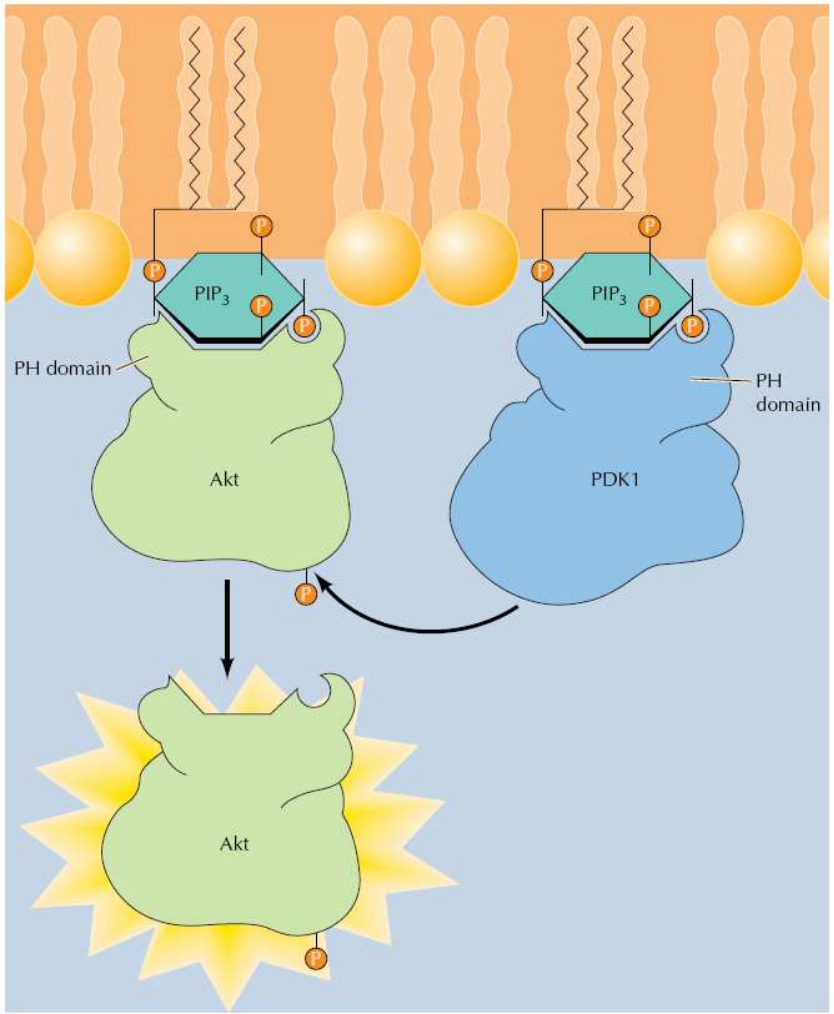
PI3K: un dimero composto da una subunità regolativa (p85)
ed una subunità catalitica (p110)



Fosfatidil inositidi fosforilati in posizione 3 servono da sito di aggancio per proteine che contengono domini PH (plextrin homology).

PI3K → PIP3 → aggancio di PDK1 e AKT/PKB → PDK1 fosforila e attiva AKT/PKB





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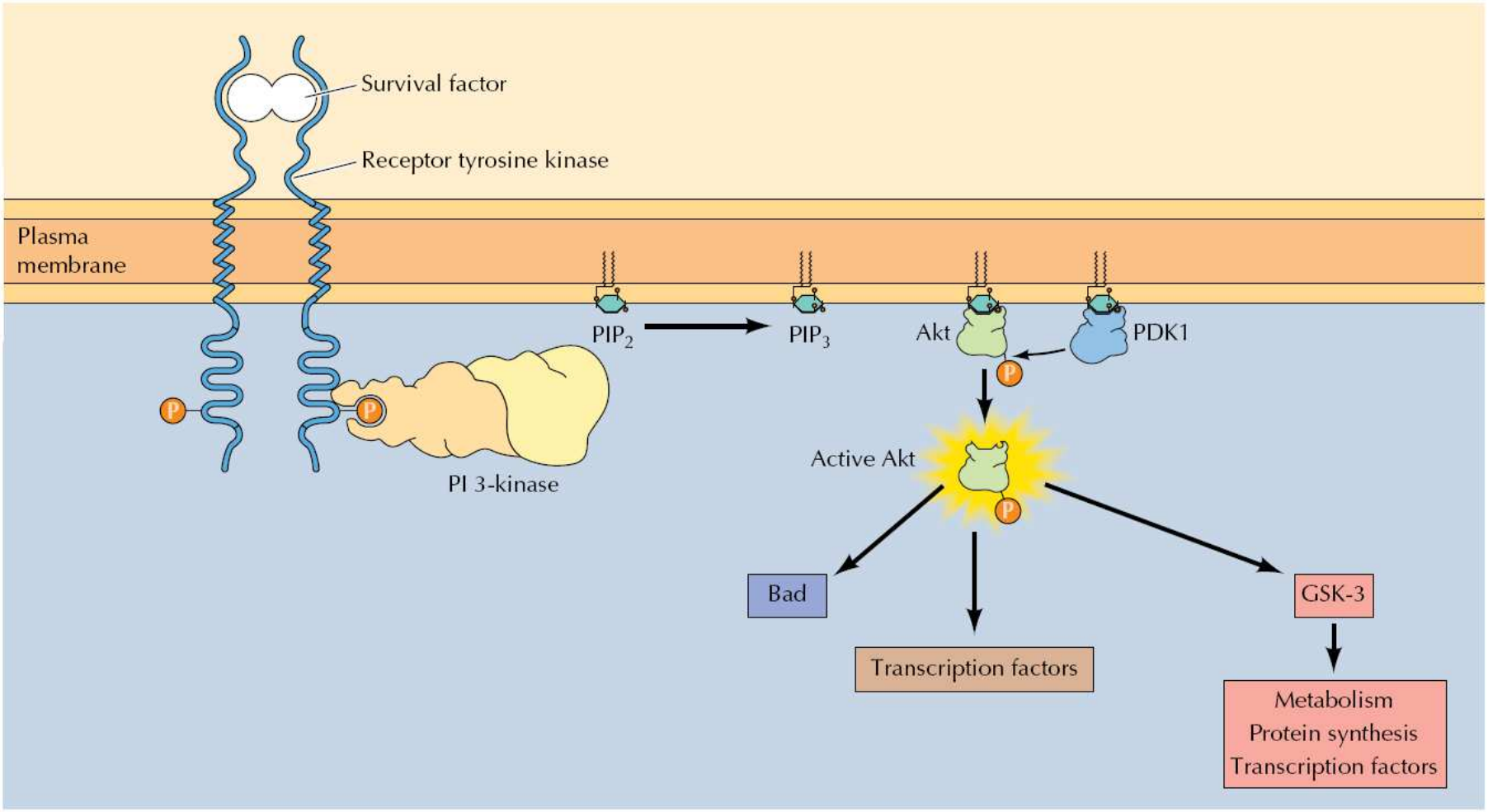
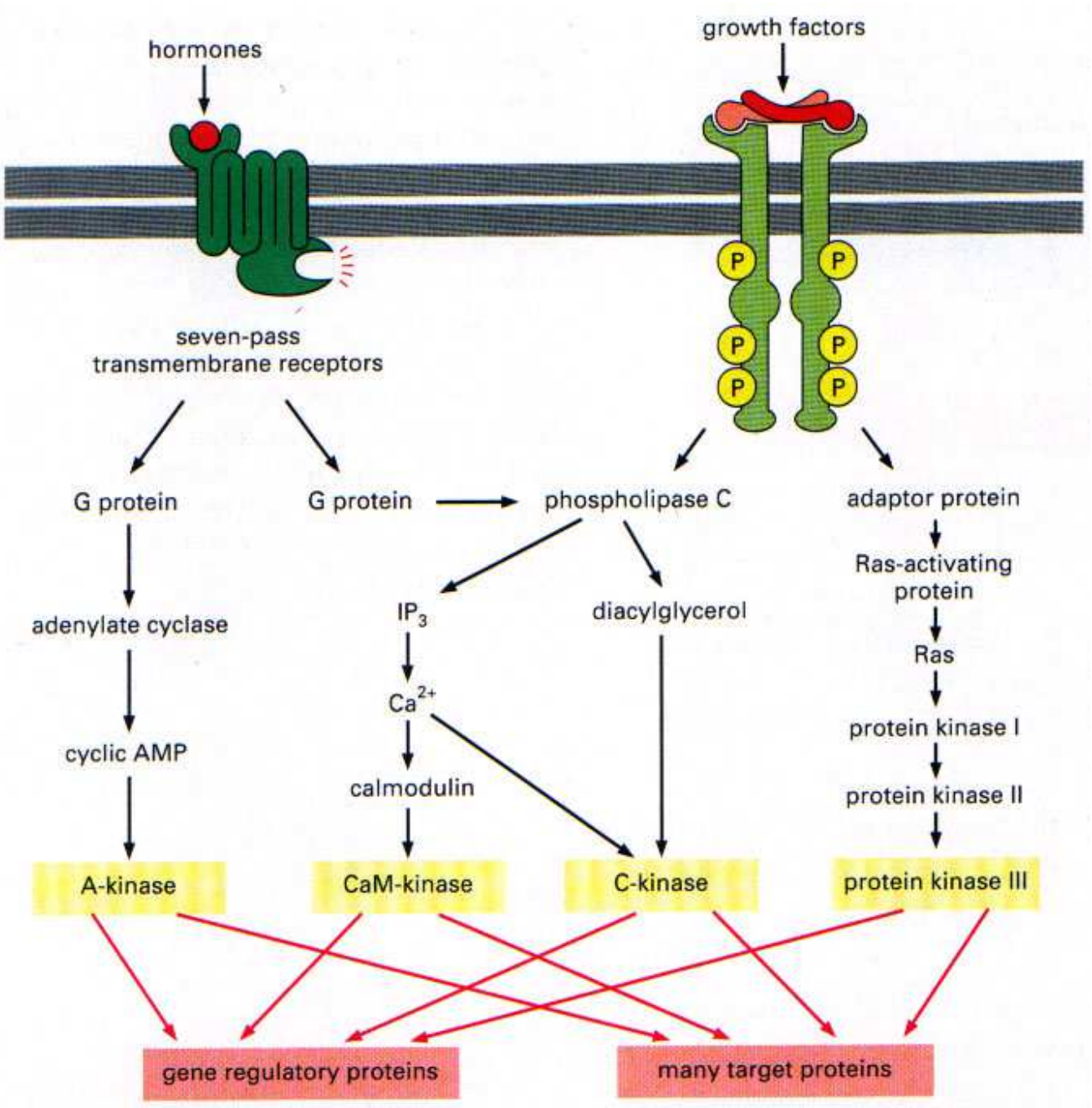
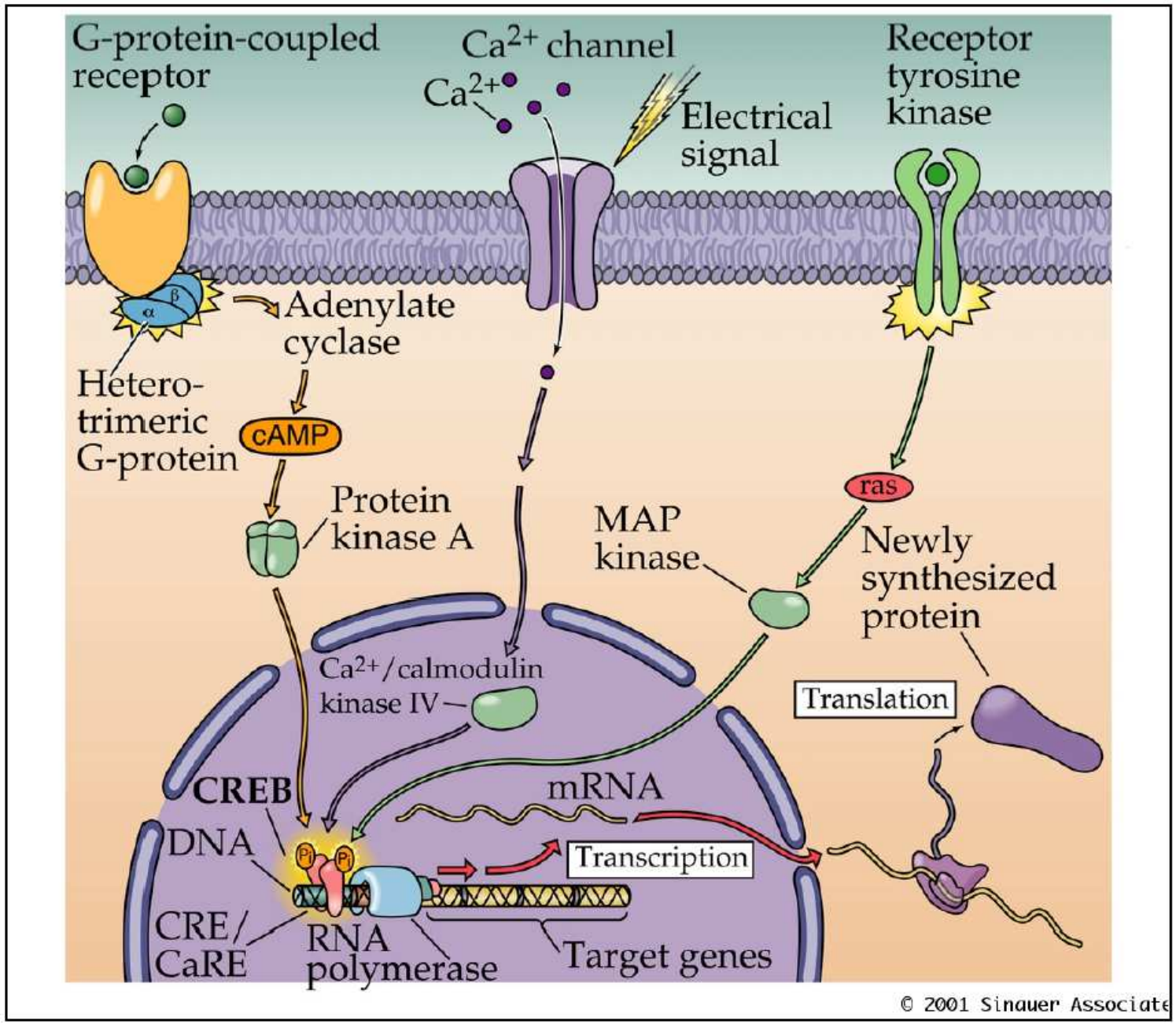
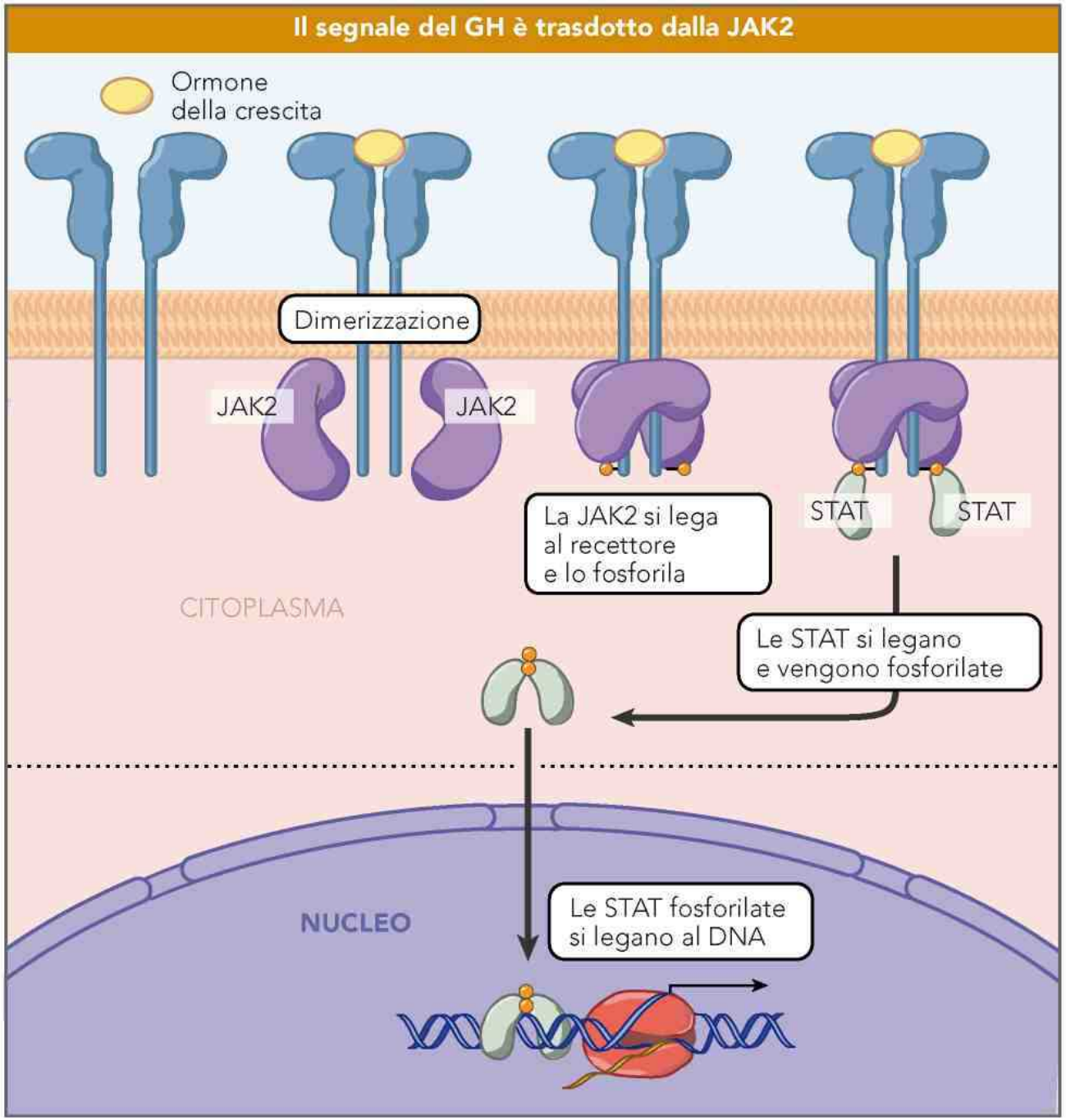






non-receptor tyrosine kinases
Jak and Src


Il segnale del GH è trasdotto dalla JAK2
Ormone della crescita
Dimerizzazione
JAK2
JAK2
La JAK2 si lega al recettore e lo fosforila
STAT
STAT
CITOPLASMA
Le STAT si legano e vengono fosforilate
NUCLEO
Le STAT fosforilate si legano al DNA

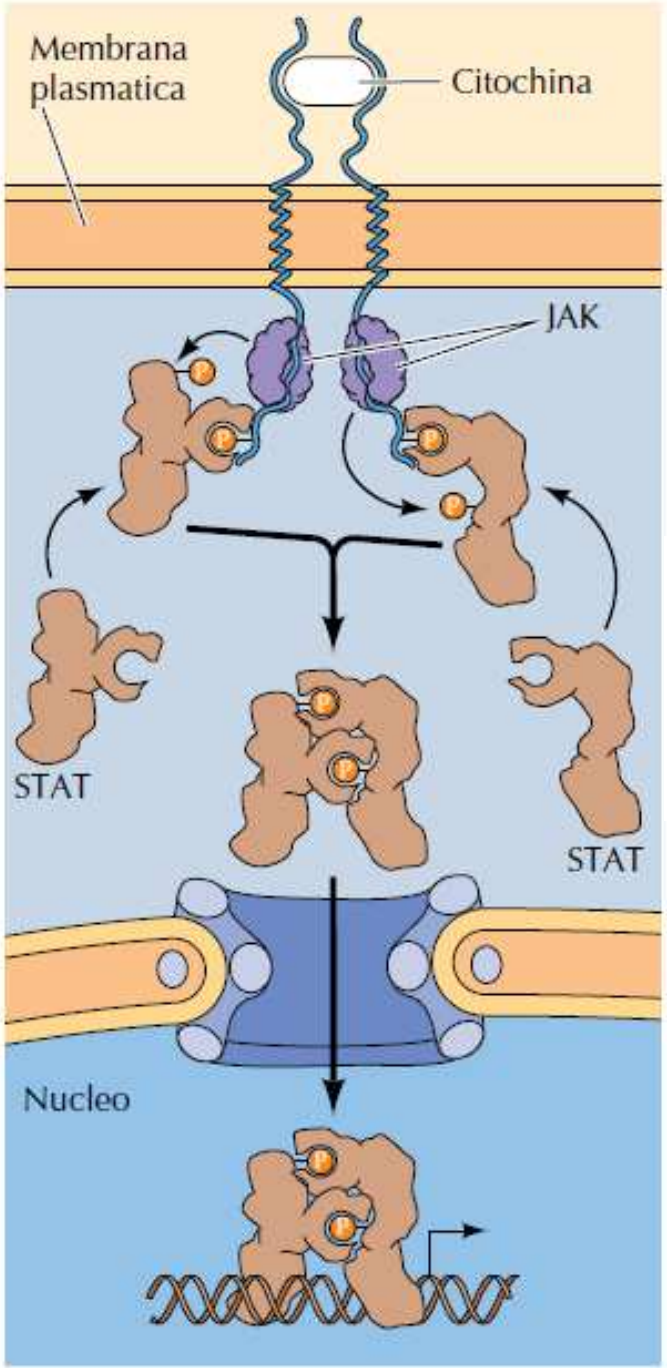
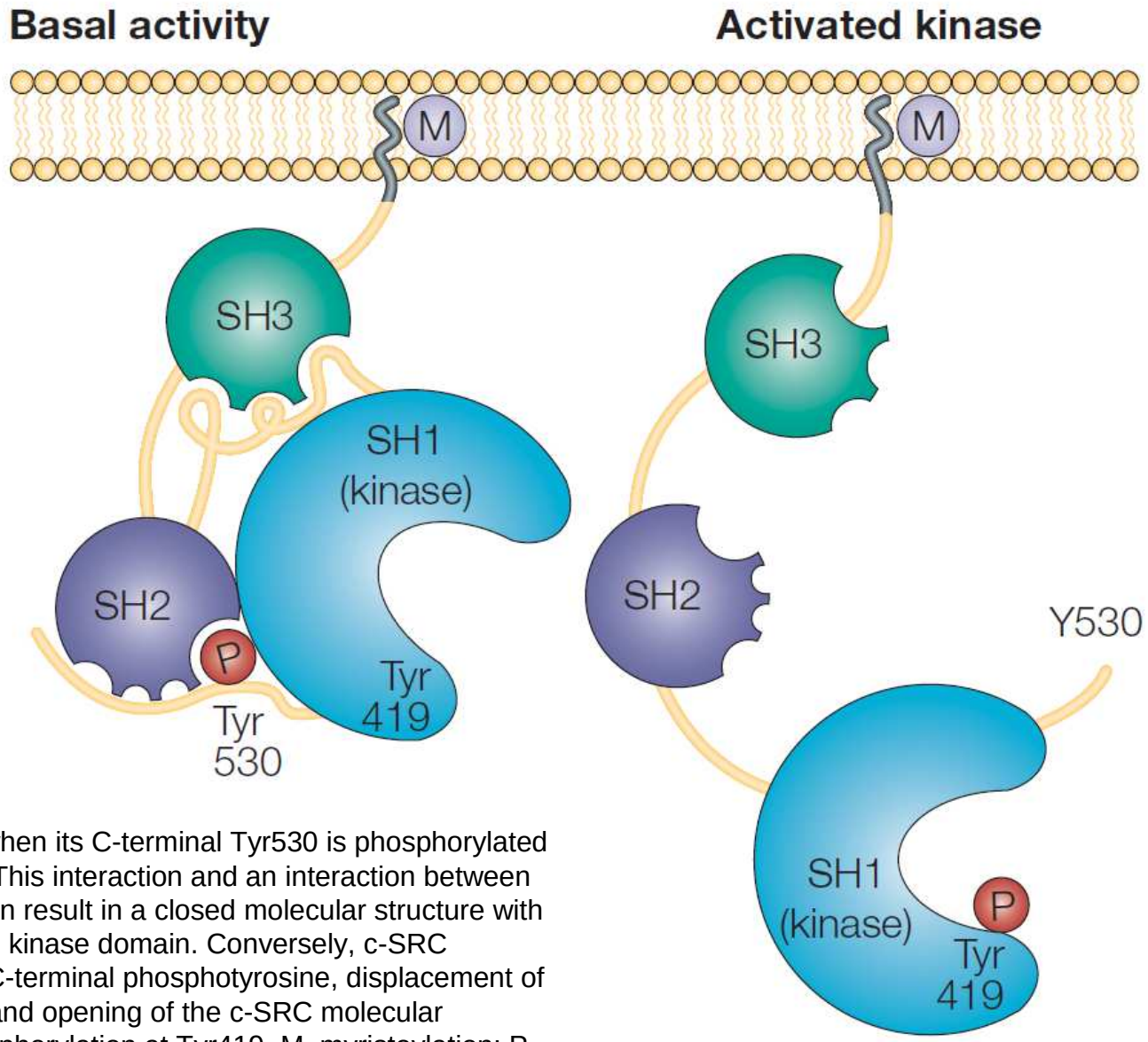


FIGURA 15.40 La via di segnalazione JAK/STAT Le proteine STAT sono fattori di trascrizione che contengono domini SH2 che mediano il loro legame con le sequenze contenenti fosfotirosine. Nelle cellule non stimolate, le proteine STAT sono inattive nel citosol. La stimolazione dei recettori per le citochine induce il legame con proteine STAT, che vengono fosforilate dalle proteine ad attività proteina-tirosina chinasi JAK associate ai recettori. In seguito, le proteine STAT fosforilate dimerizzano e traslocano nel nucleo, dove attivano la trascrizione dei geni bersaglio.

Tyrosine phosphorylation = activation or inhibition?

SRC proteins



Inactivation of human c-SRC occurs when its C-terminal Tyr530 is phosphorylated and it binds back to the SH2 domain. This interaction and an interaction between the SH3 domain and the kinase domain result in a closed molecular structure with diminished access of substrates to the kinase domain. Conversely, c-SRC activation occurs with removal of the C-terminal phosphotyrosine, displacement of inhibitory intramolecular interactions, and opening of the c-SRC molecular structure. Full activation involves phosphorylation at Tyr419. M, myristoylation; P, phosphorylation.

NATURE REVIEWS | CANCER doi:10.1038/nrc1366

• v-*src* was the first of numerous viral oncogenes to be identified and is among the best studied of these.

The cellular counterpart of this oncogene — c-*SRC* — is implicated in a range of human cancers.

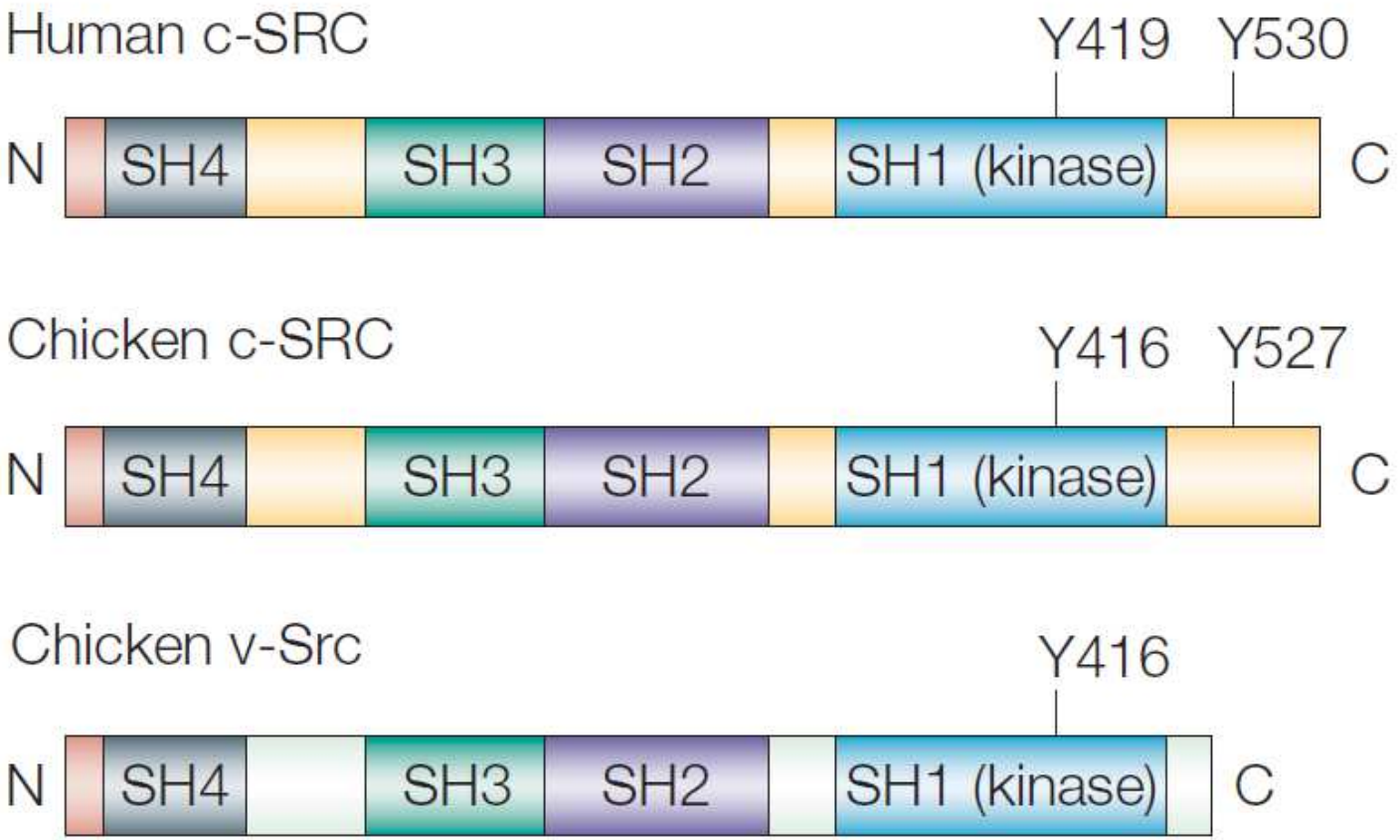
• In addition to cell proliferation, SRC proteins regulate three main cellular functions that ultimately control the behaviour of transformed cells:
- adhesion
- invasion
- motility.

These functions might also contribute to tumour progression and metastasis.

NATURE REVIEWS | CANCER doi:10.1038/nrc1366

(materiale di supporto allo studio, non proiettato a lezione)

Comparison of the molecular structures of human c-SRC, chicken c-SRC and chicken v-Src



Perchè chicken v-src è oncogenica?

NATURE REVIEWS | CANCER doi:10.1038/nrc1366

Attivazione di SRC:
1 - competizione (di un’altra proteina con dominio SH2)
2 - defosforilazione (un ruolo importante è giocato dalle tirosina fosfatasi)

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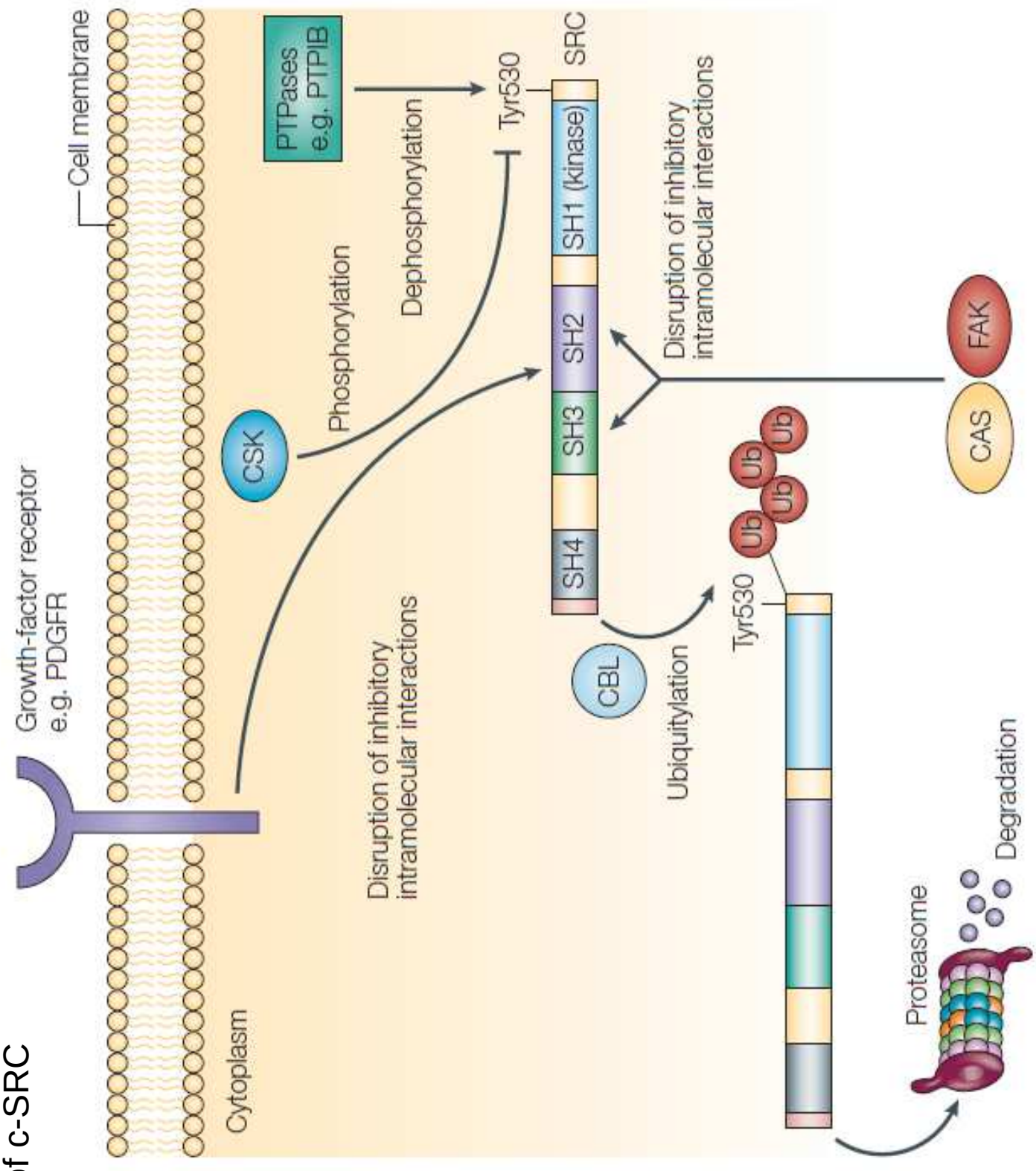
Regulation of c-SRC



Regulation of c-SRC

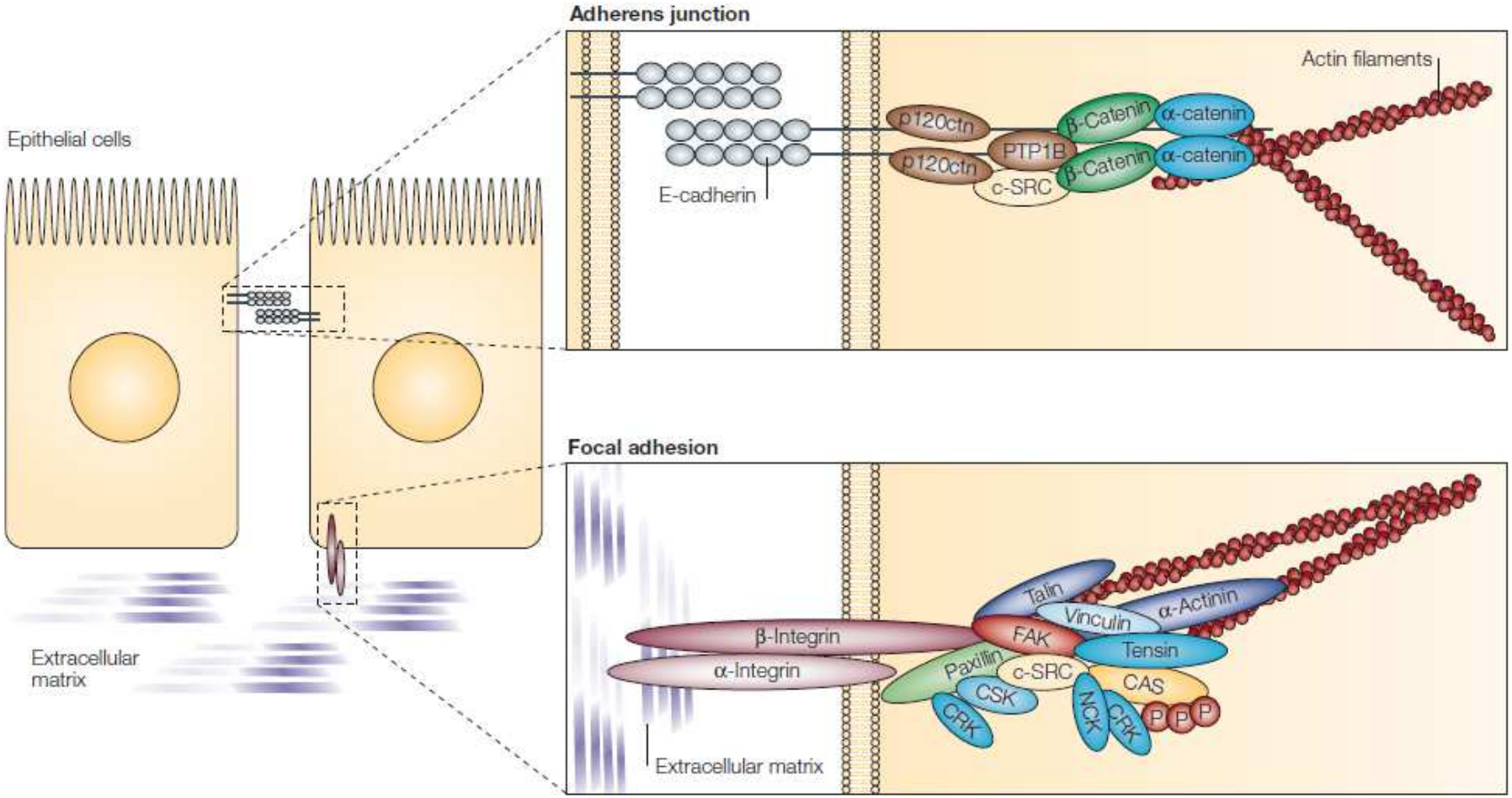
• c-SRC is regulated in terms of both protein levels and levels of activity by a range of mechanisms.

• inactivation of c-SRC is carried out by c-SRC tyrosine kinase (CSK), which phosphorylates a conserved tyrosine residue in the c-SRC carboxy-terminal domain (Tyr530). This is reversed by phosphatases such as protein tyrosine phosphatase 1B (PTP1B), which leads to c-SRC activation.

• activation of growth-factor receptors leads to their association with the c-SRC SRC homology 2 (SH2) domain, which disrupts inhibitory intramolecular interactions to promote c-SRC activation.
Other proteins, such as CRK-associated substrate (CAS) and FAK, bind to the c-SRC SH2 and SH3 domains to promote c-SRC activation by a similar mechanism.

• Levels of c-SRC protein are negatively regulated by the E3 ubiquitin ligase CBL, which leads to c-SRC ubiquitylation and subsequent degradation by the proteasome.

Cell adhesion at adherens junctions and focal adhesions



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