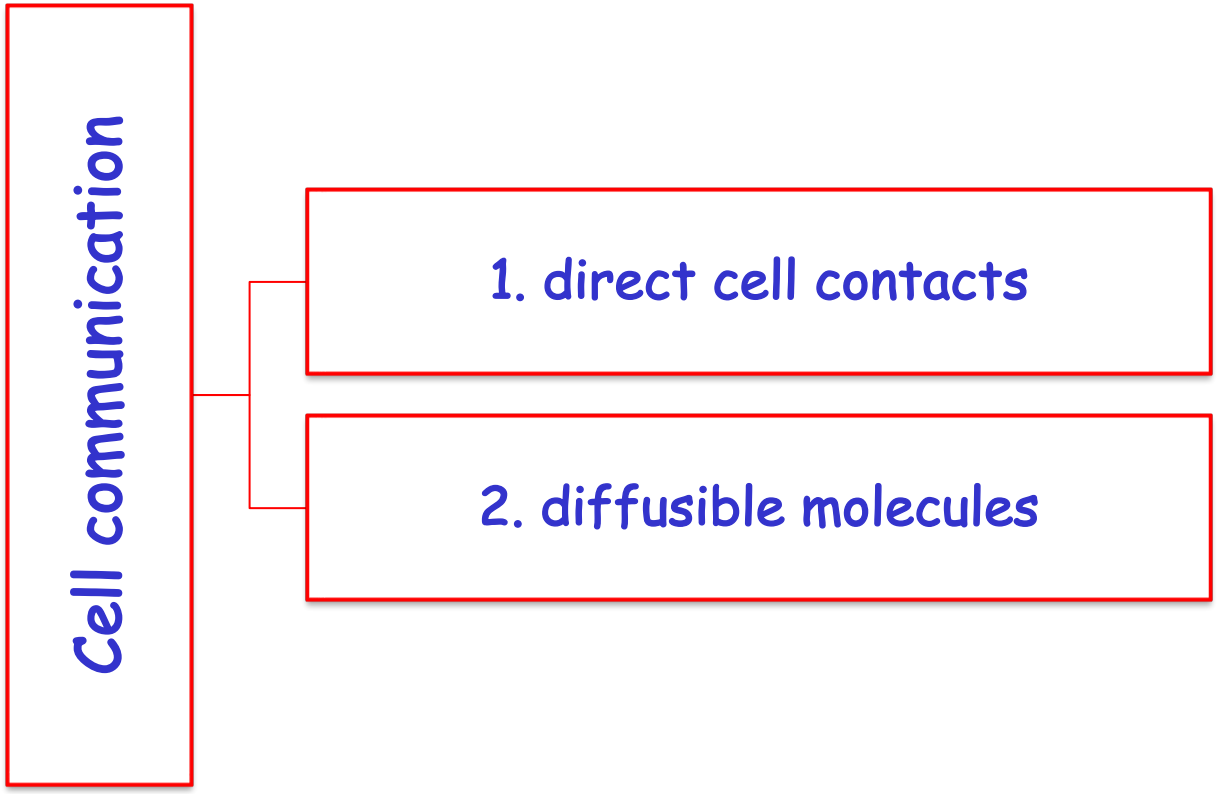
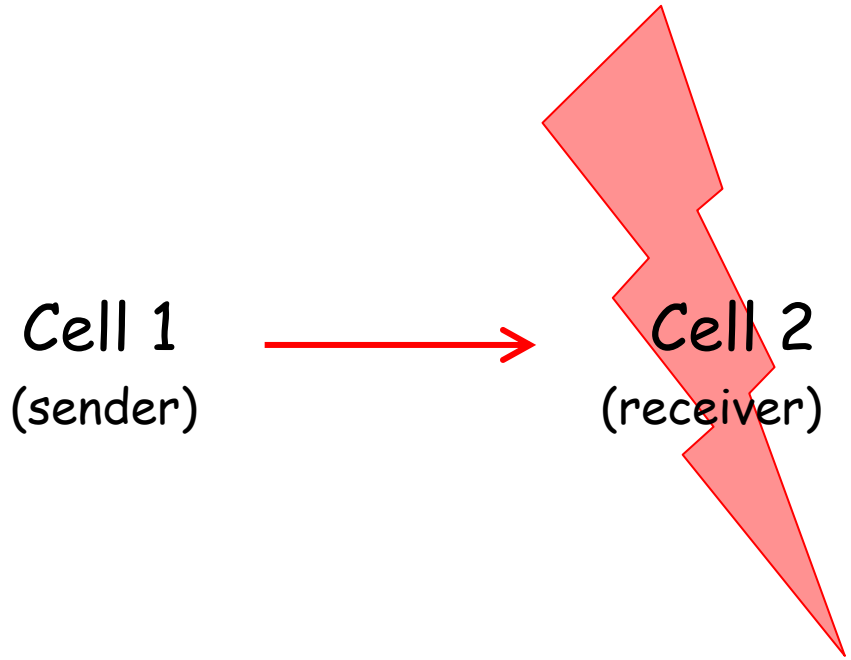
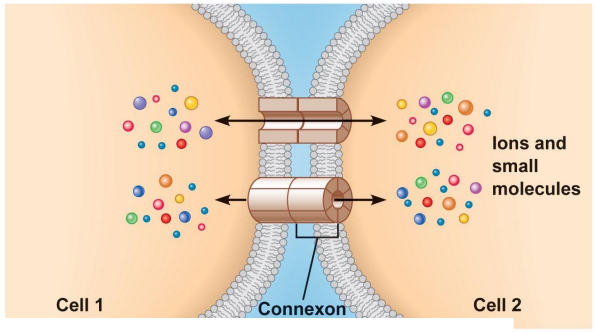
Cell communication




Cell 1
(sender)
Cell 2
(receiver)

Cell communication (1)

1. contact



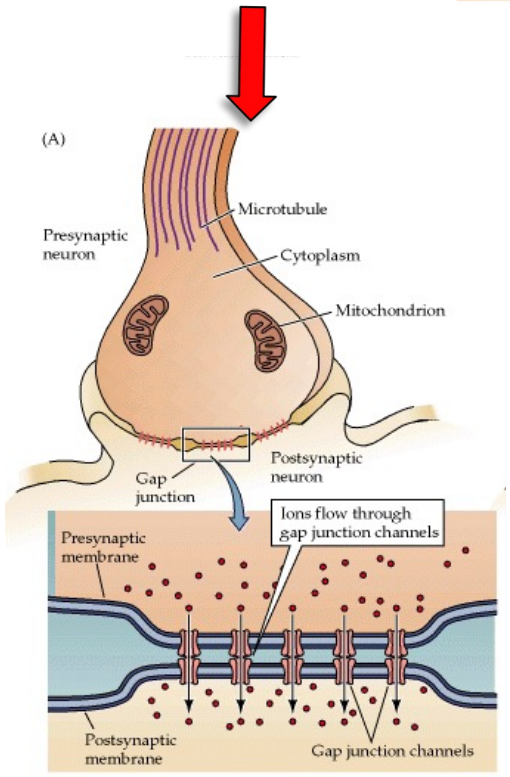
Exchange (potentially bidirectional) of ions and molecules (with low MW) between the two communicating cells

Cell 1 (sender) ⟷ Cell 2 (receiver)

Endpoint

Variation in composition of the intracellular compartment in the receiving cell

---



Electrical synapse

The communication occurs between excitable cells

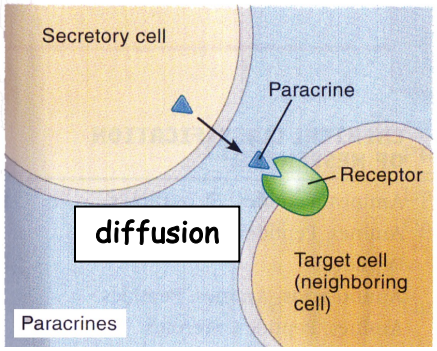
Ions are exchanged

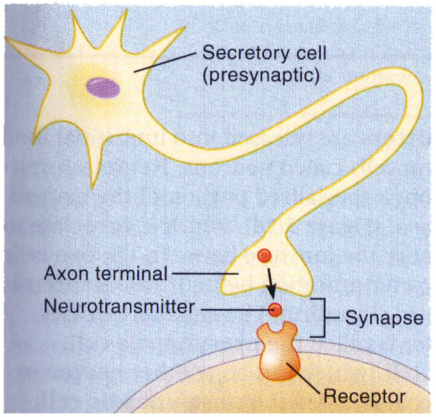
Endpoint

Change in the membrane potential (action potential)

Cell communication (2)

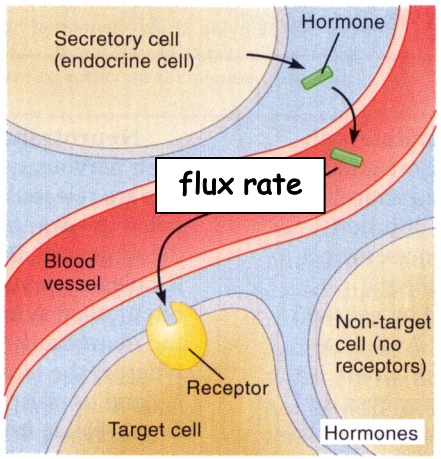
2a. short distance

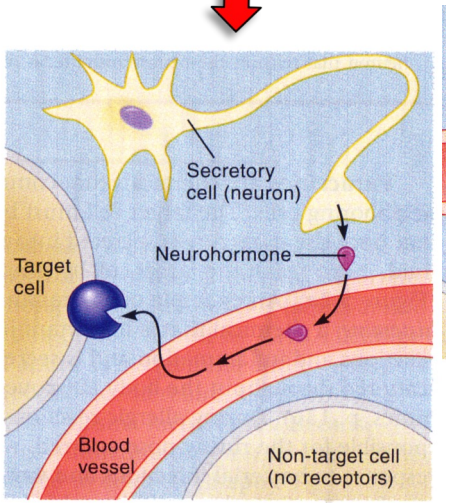




Chemical synapse

2b. long distance





Neurohormones

<u>Signalling molecule</u>
(first messenger, ligand):
extracellular molecule (with low MW)

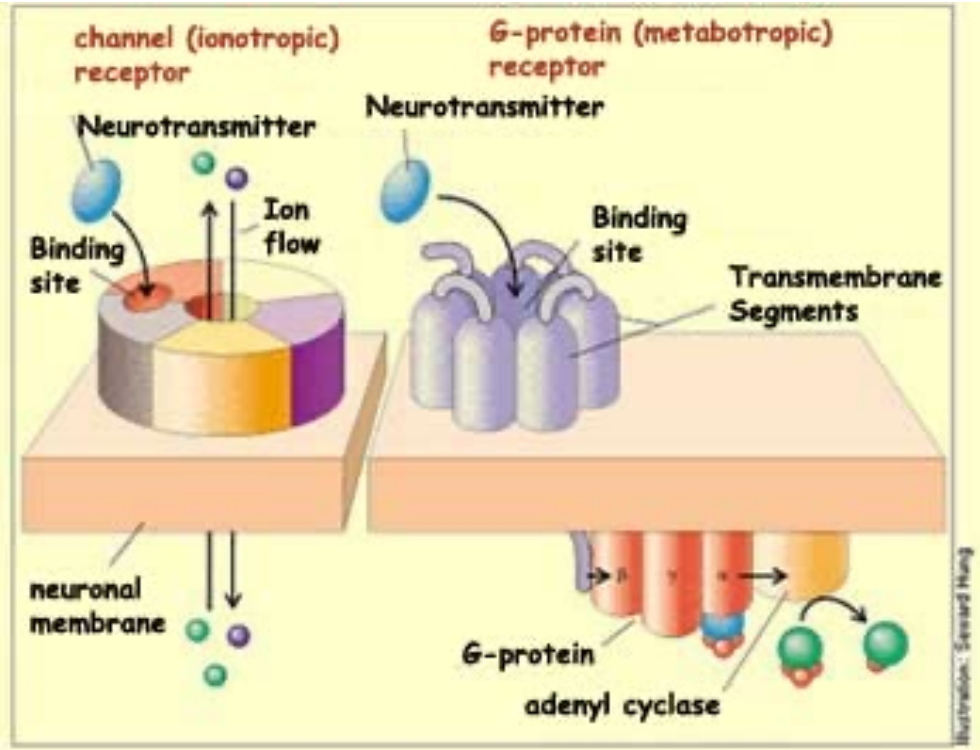
Sender = secretory cell

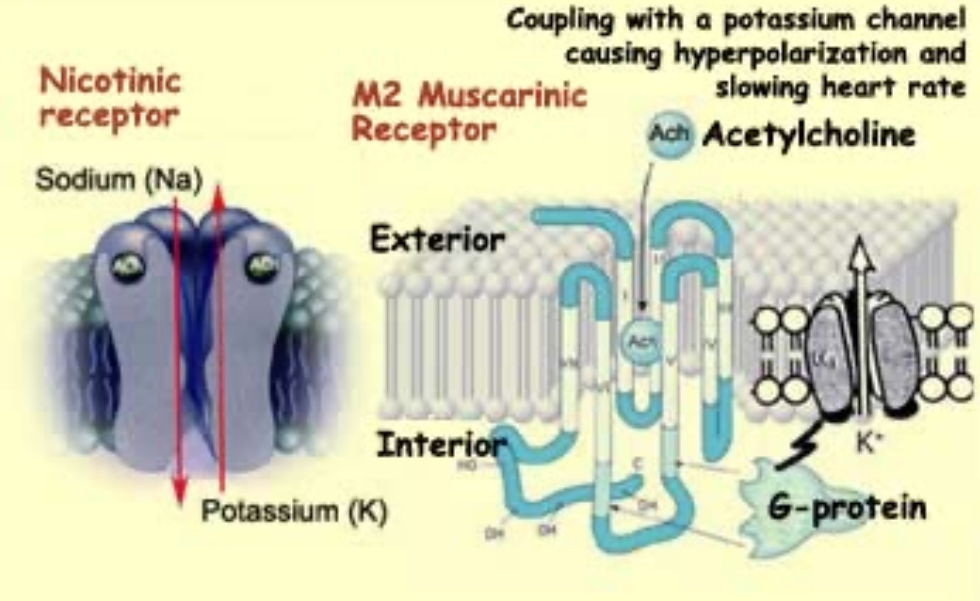
Receiver = target cell

<u>Receptor</u>
protein that selectively binds
the signalling molecule
(specificity of response)

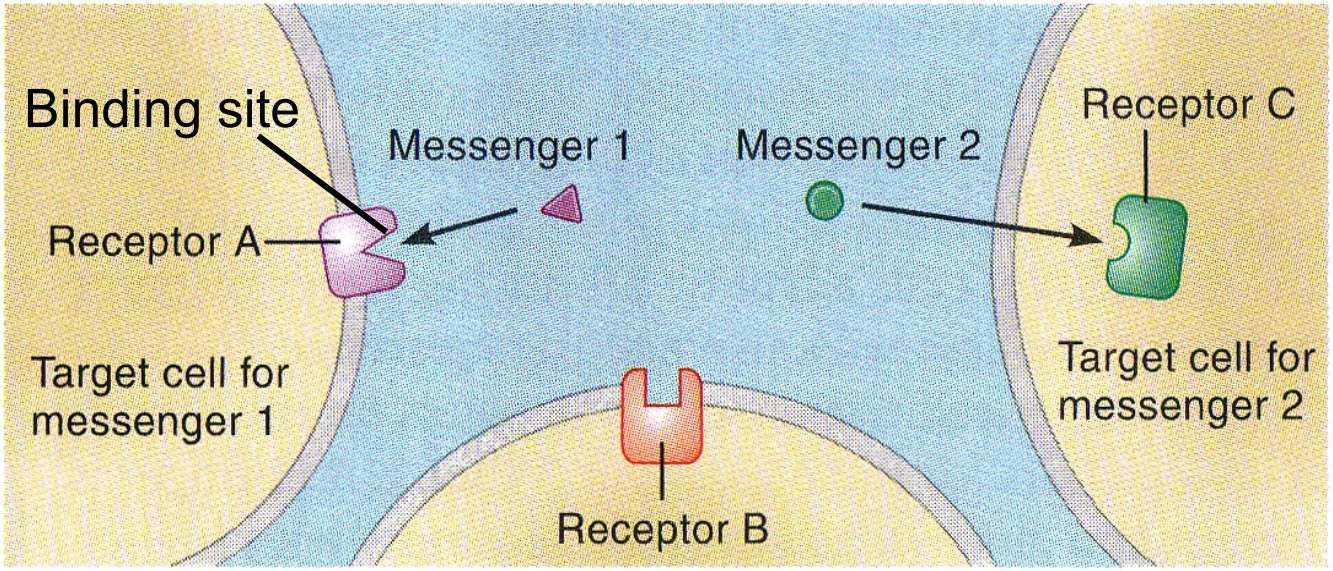
Endpoint = signal transduction:
time-limited process whereby the first messenger is converted (transduced) into an intracellular signal

Ionotropic receptors and metabotropic receptors





Signal transduction requires receptors



- **Specificity** (binding site; lock and key mechanism)
- **Receptor affinity** (time-limited activation of the target cell, reversibility of the signal transduction)
- **Localisation** (predictive for the chemical properties of the messenger)
- **Structure** (predictive for the signal transduction mechanism)

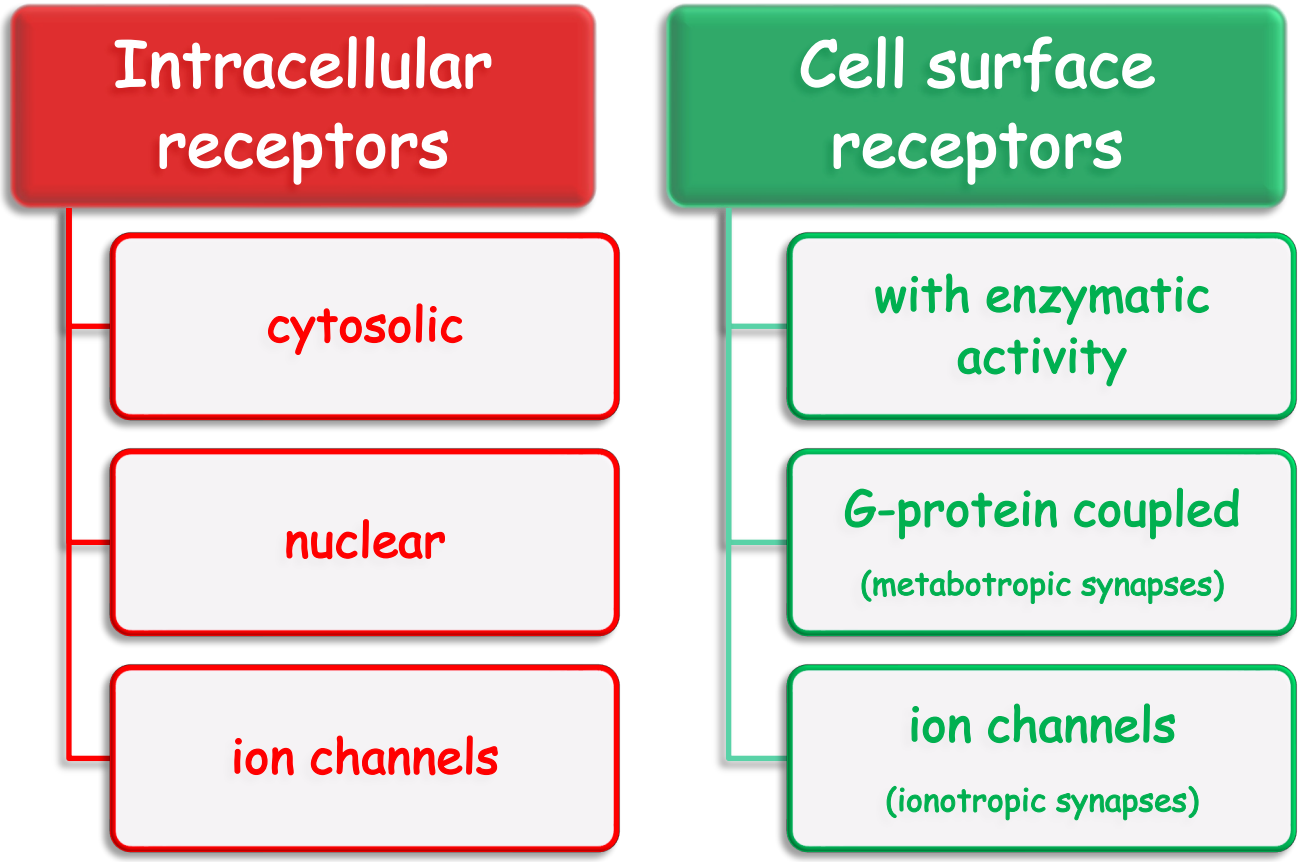
ligand = physiological messenger (e.g. ACh)
agonist = non-physiological messenger, activates the receptor to produce the biological response (e.g. Nic)
antagonist = non-physiological messenger, blocks or inhibits the biological response (e.g. α-BuTX)

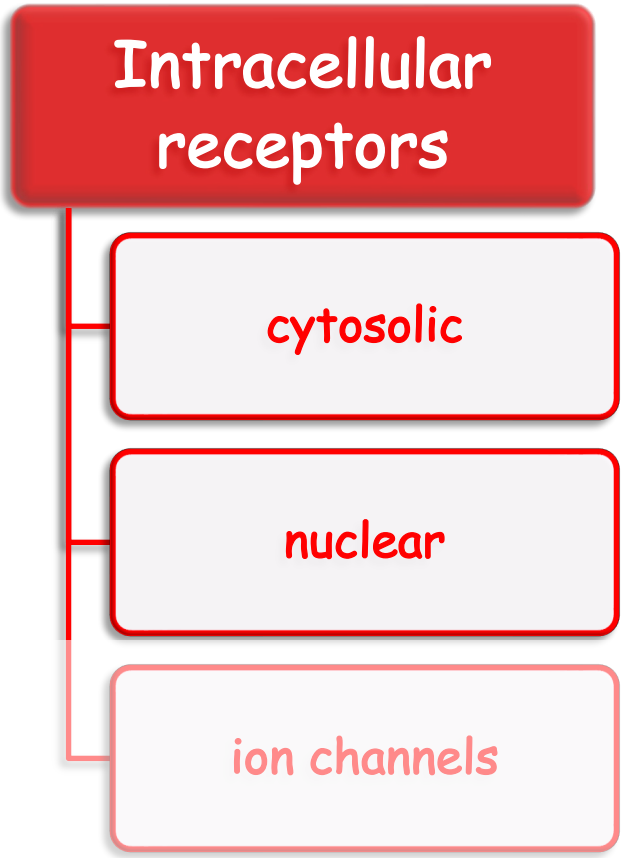
The localisation of the receptors depends on the chemical properties of the soluble factor

TABLE 5.2 CHEMICAL CLASSIFICATION OF MESSENGERS

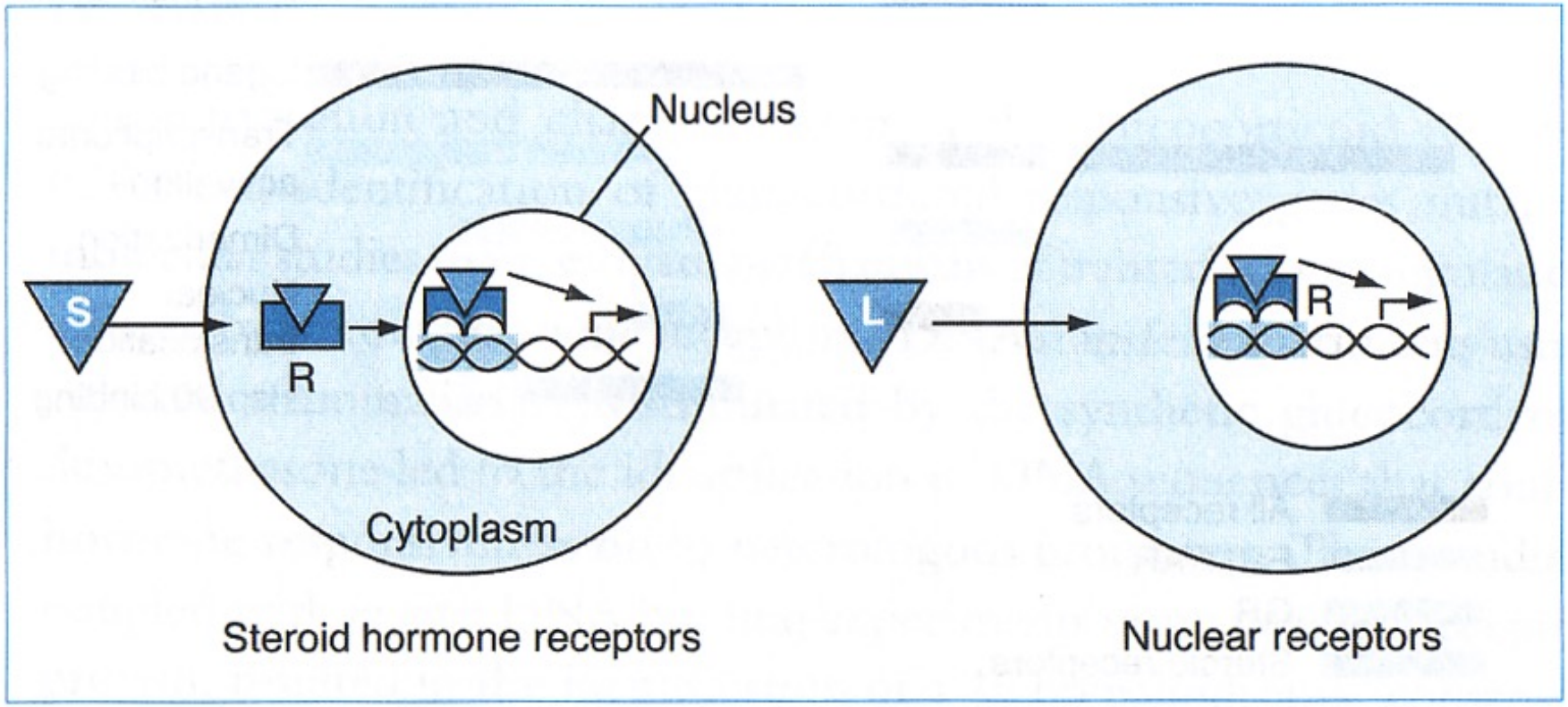
CLASS	CHEMICAL PROPERTY	LOCATION OF RECEPTORS ON TARGET CELL	FUNCTIONAL CLASSIFICATION
Amino acids	Lipophobic	Plasma membrane	Neurotransmitters
Amines*	Lipophobic	Plasma membrane	Paracrines, autocrines, neurotransmitters, hormones
Peptides	Lipophobic	Plasma membrane	Paracrines, autocrines, cytokines, neurotransmitters, hormones
Steroids	Lipophilic	Cytosol†	Hormones
Eicosanoids	Lipophilic	Cytosol	Paracrines

*One exception is the thyroid hormones, which, although amines, are lipophilic and have receptors in the nucleus of target cells.
†A few steroid hormones have receptors on the plasma membrane.


Intracellular receptors
cytosolic
nuclear
ion channels
Cell surface receptors
with enzymatic activity
G-protein coupled
(metabotropic synapses)
ion channels
(ionotropic synapses)


Intracellular receptors
cytosolic
nuclear
ion channels

Intracellular receptors
are located in the cytoplasm or in the nucleus

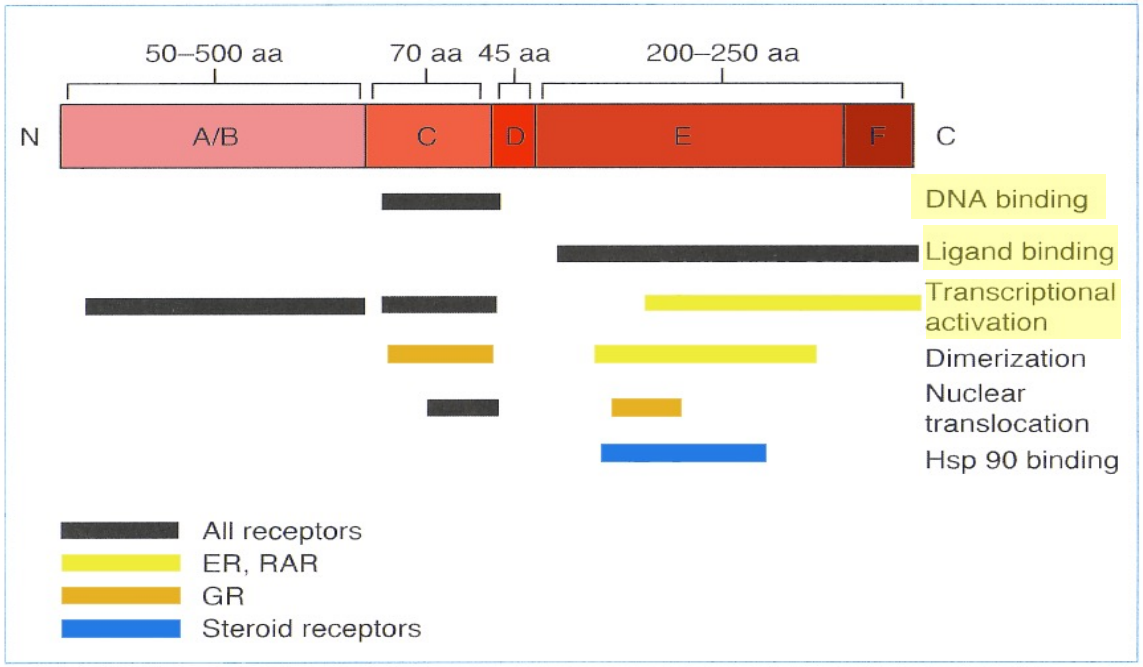


Endpoint

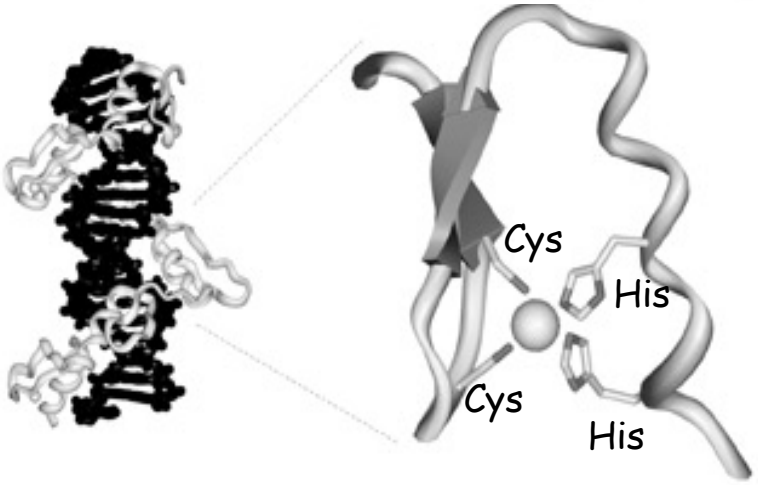
Regulation of gene expression in the target cell

Structure of the intracellular receptors

Figure 20.2 Domain structure of steroid and nuclear receptor family members. Functions associated with each domain for various classes of receptors are indicated by bars (see key). The estrogen receptor contains a weak dimerization function in the C domain.[17]



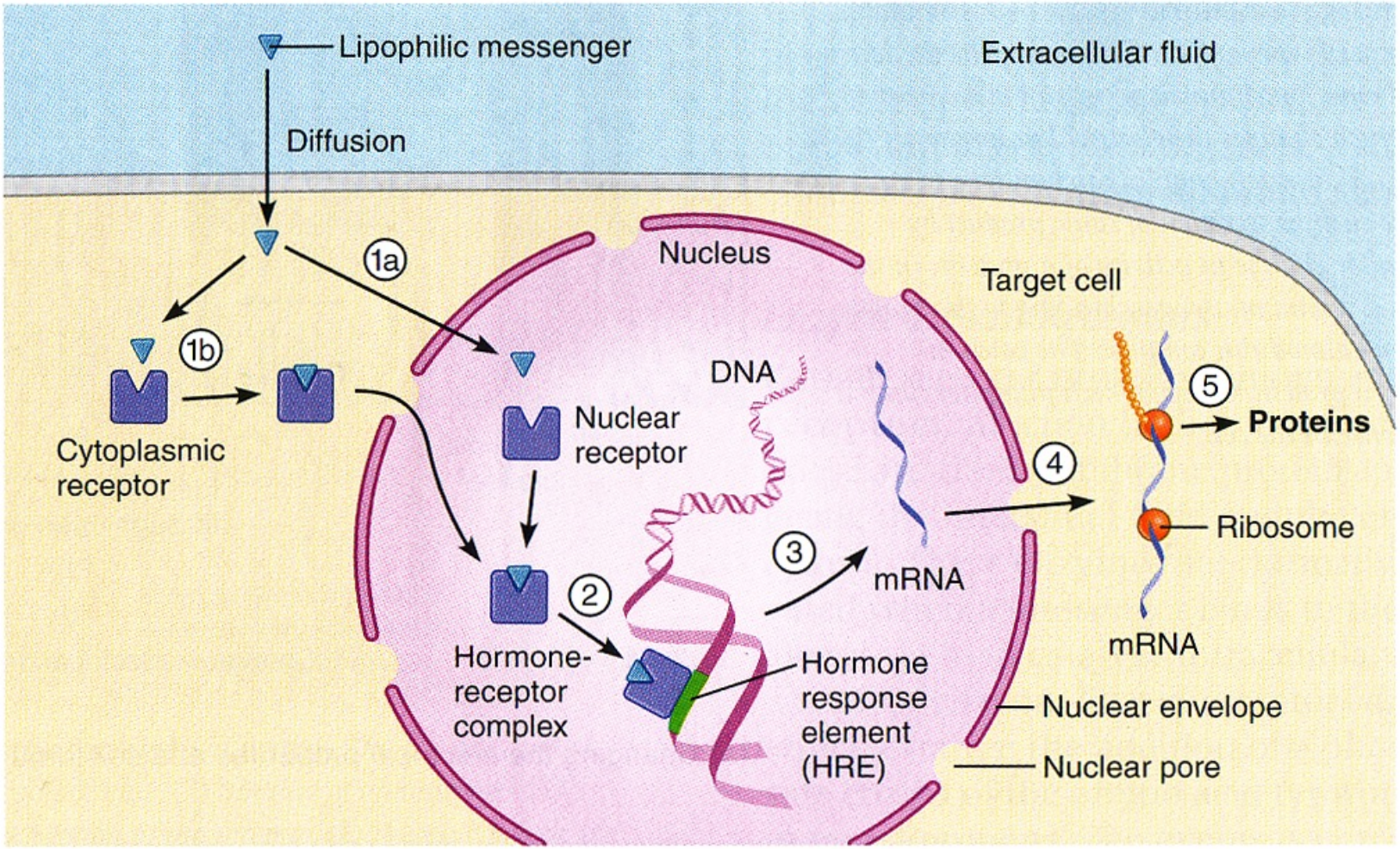
α-helix contacts
the major groove of the DNA



zinc finger domain (DNA binding domain)

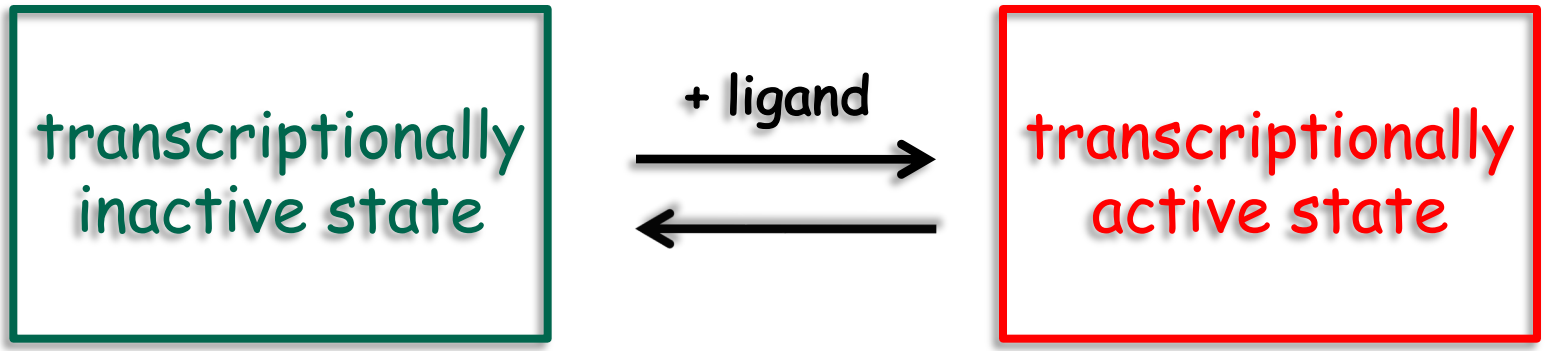
N = amino-terminal
C = carboxy-terminal

Hormone-receptor complex binds to specific DNA sequence (hormone-response elements, HREs)



HRE: palindromic sequence in the promotor region

Why the receptors do not bind to HREs in the absence of the ligand?



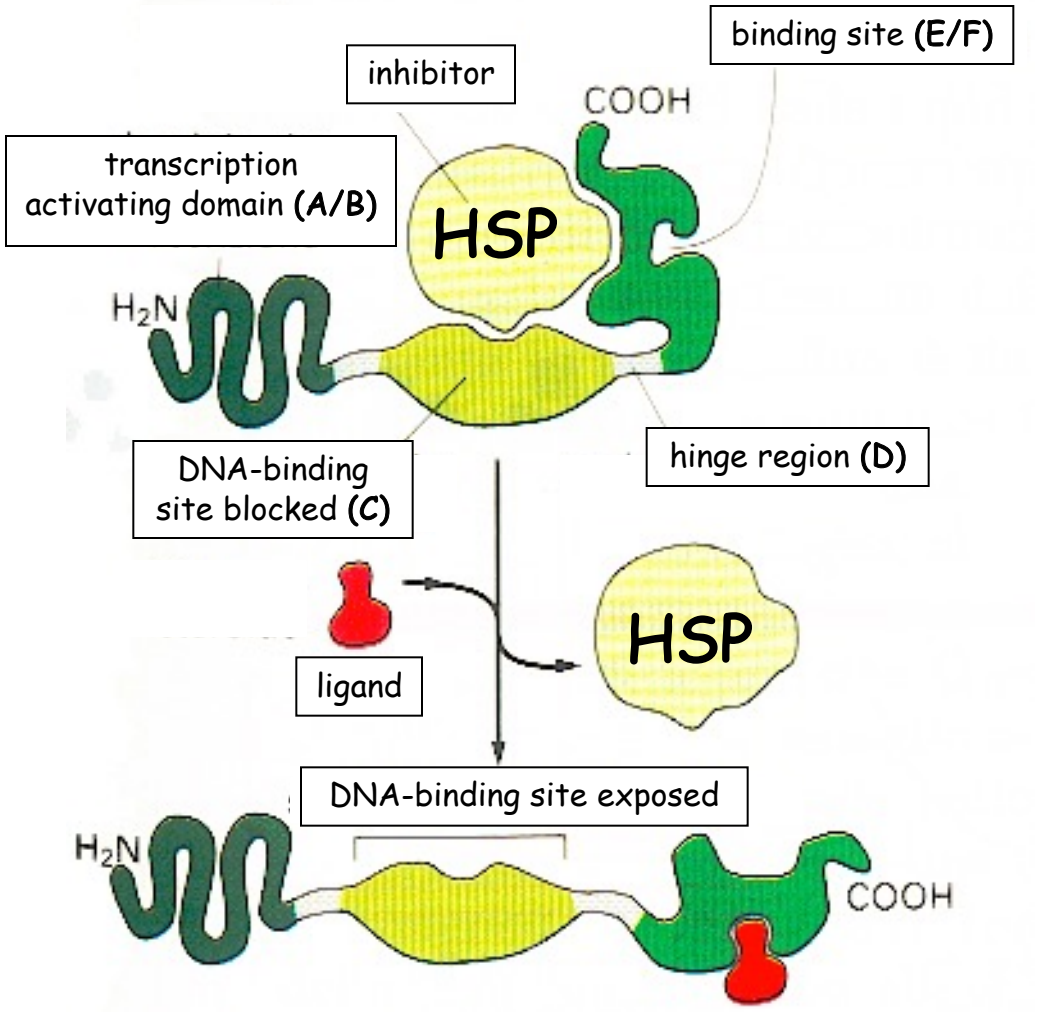
An activation model of cytoplasmic receptors ("transformation")

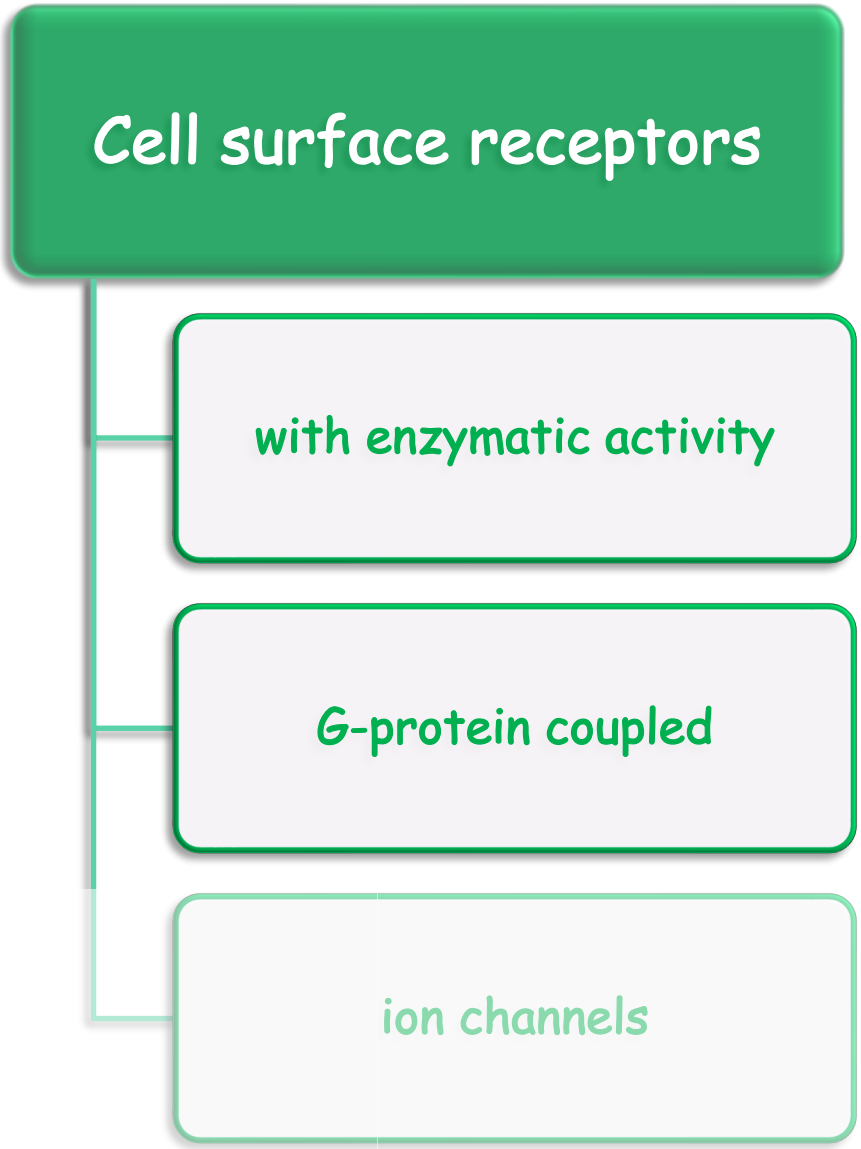
Heteromeric complexes:

inactive state = receptor + HSP

active state = receptor + ligand

HSP = heat shock protein
(HSP90; HSP70, HSP56)




Cell surface receptors
with enzymatic activity
G-protein coupled
ion channels

Cell surface receptors:
receptors with enzymatic activity (*protein kinase domain*)

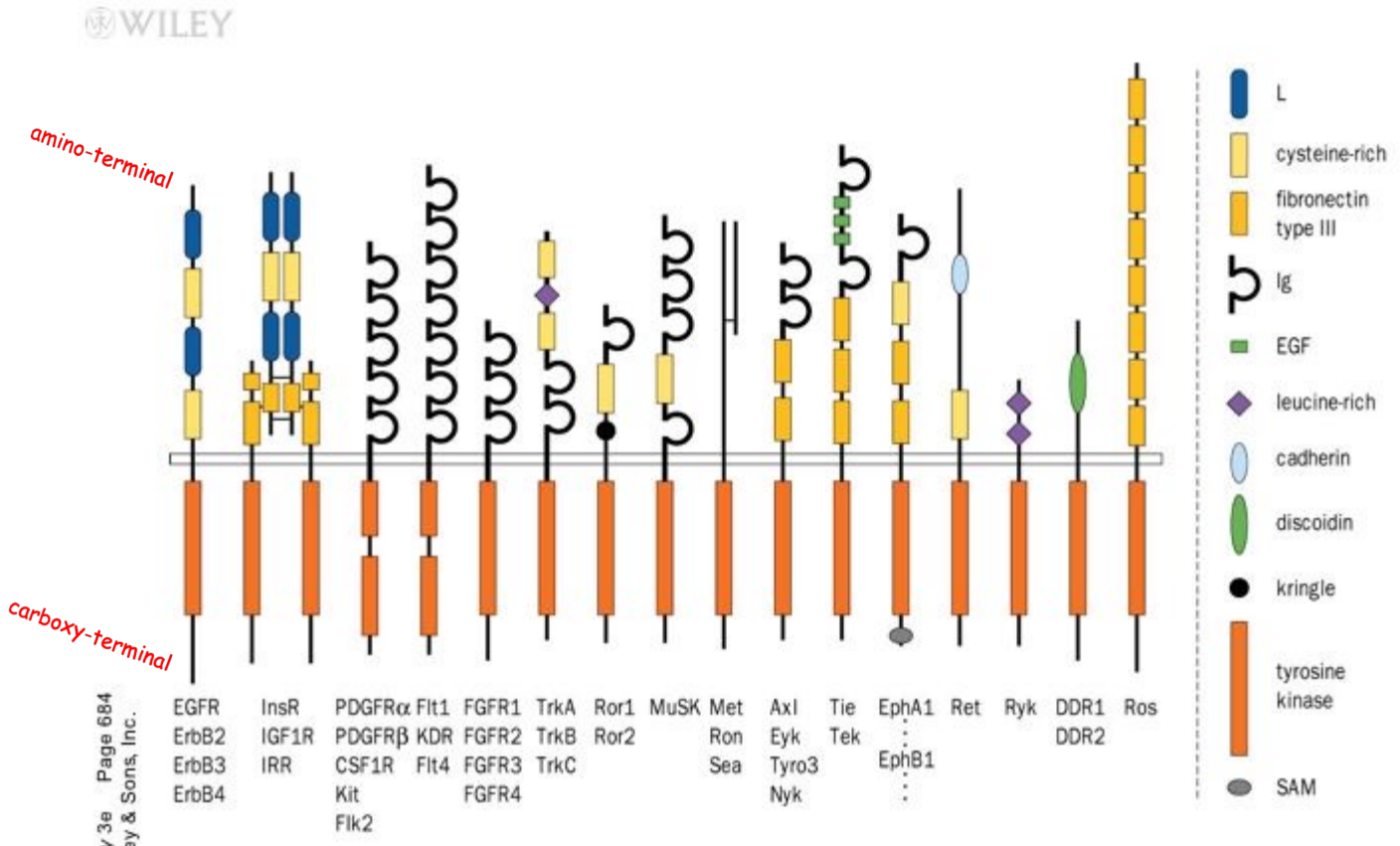
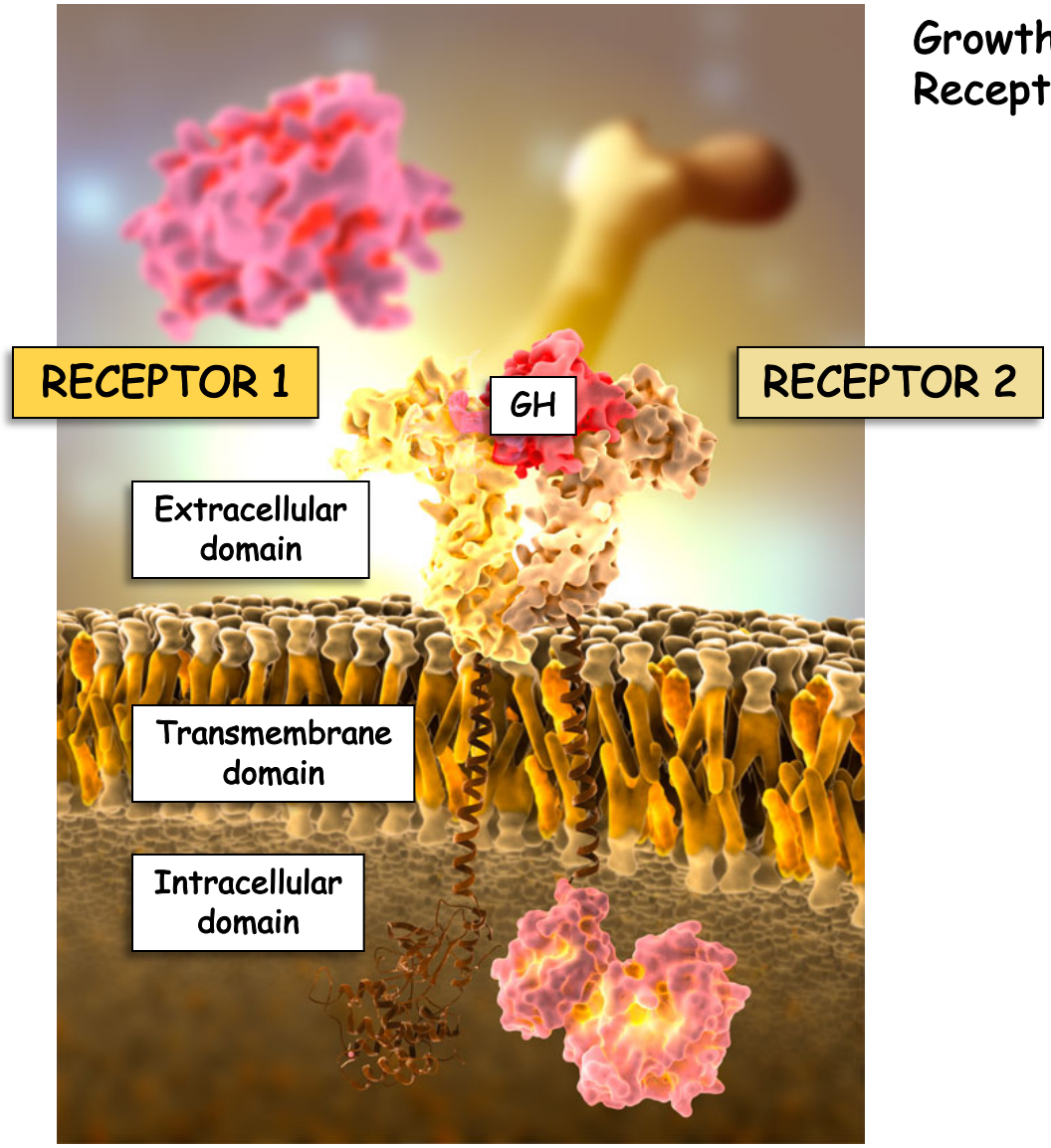


Figure 19-23 Domain organization in a variety of receptor tyrosine kinase (RTK) subfamilies.

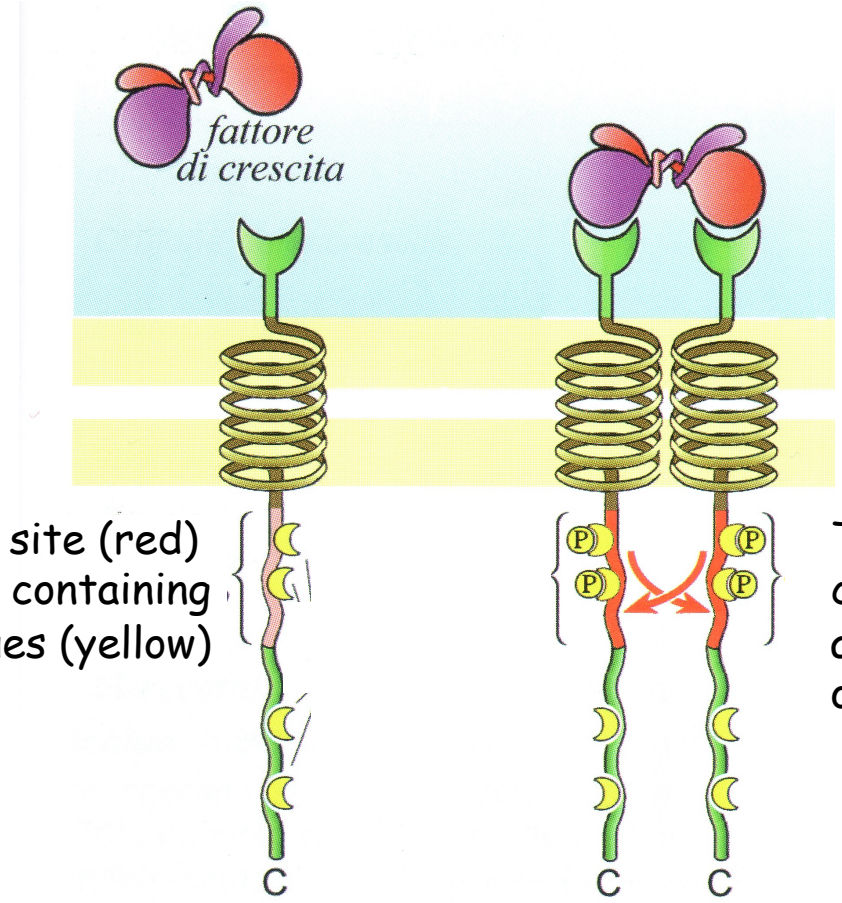
The importance of the transmembrane domain



Growth hormone Receptor (GHR)

Dimerization allows trans-phosphorylation and triggers signal transduction

Transphosphorylation first occurs at juxtamembrane region



Catalitic site (red) containing tyrosine residues (yellow)

Transphorylation causes a conformational change allowing fully activation of the catalitic site

Fully transphosphorylation and docking site formation

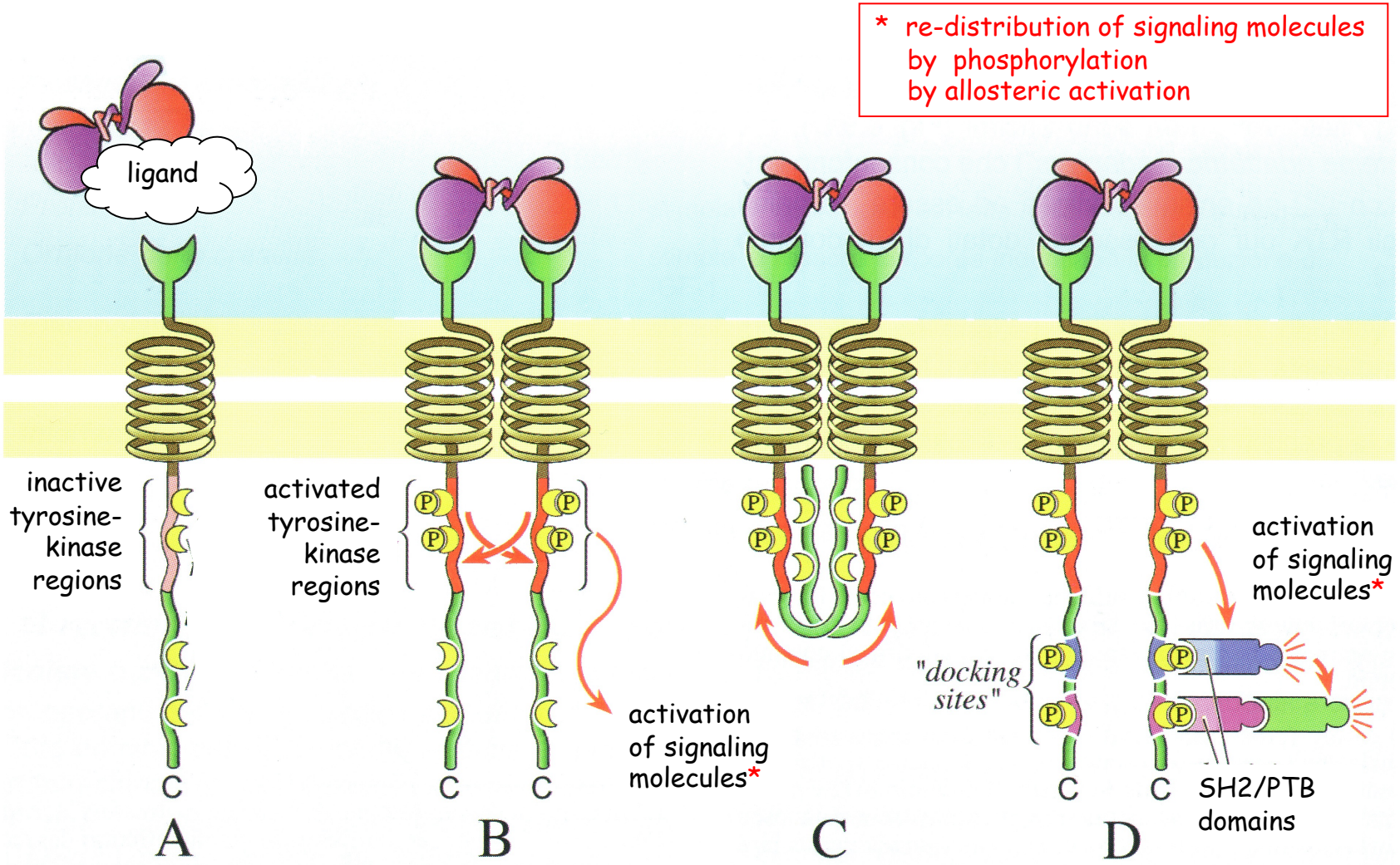


Fig. 6.9 – A: recettore tirosin-chinasi (s'immagina che si tratti di un recettore per un fattore di crescita, ad esempio l'EGF), rappresentato in forma monomerica; B: il legame col fattore di crescita determina la dimerizzazione e l'autofosforilazione crociata dei domini tirosin-chinasici; C: fosforilazioni di residui tirosinici situati al di fuori dei domini tirosin-chinasici; D: attracco ai "docking sites" di proteine-segnale dotate di un dominio SH2 e loro fosforilazione in cascata.

SH2 and PTB domains

SH2: Src homology 2 domain

PTB: phosphotyrosine binding domain

Table 1.2 Comparison of SH2 and PTB domains

Parameter	SH2	PTB
Size	~100 aa	~186 aa
Distribution	>100 proteins	Shc, Sck IRS-1, others
FLVRES sequence*	Yes	No
Domain structure	Central β-sheet; flanking helixes	Similar to PH domains
Target binding site:		
Length	Five residues	~12 residues
p-Tyr specific	Yes	Yes
Residues recognized	Carboxy-terminal	Amino-terminal
Structure	Extended	β turn

*Phe-Leu-Val-Arg-Glu-Ser

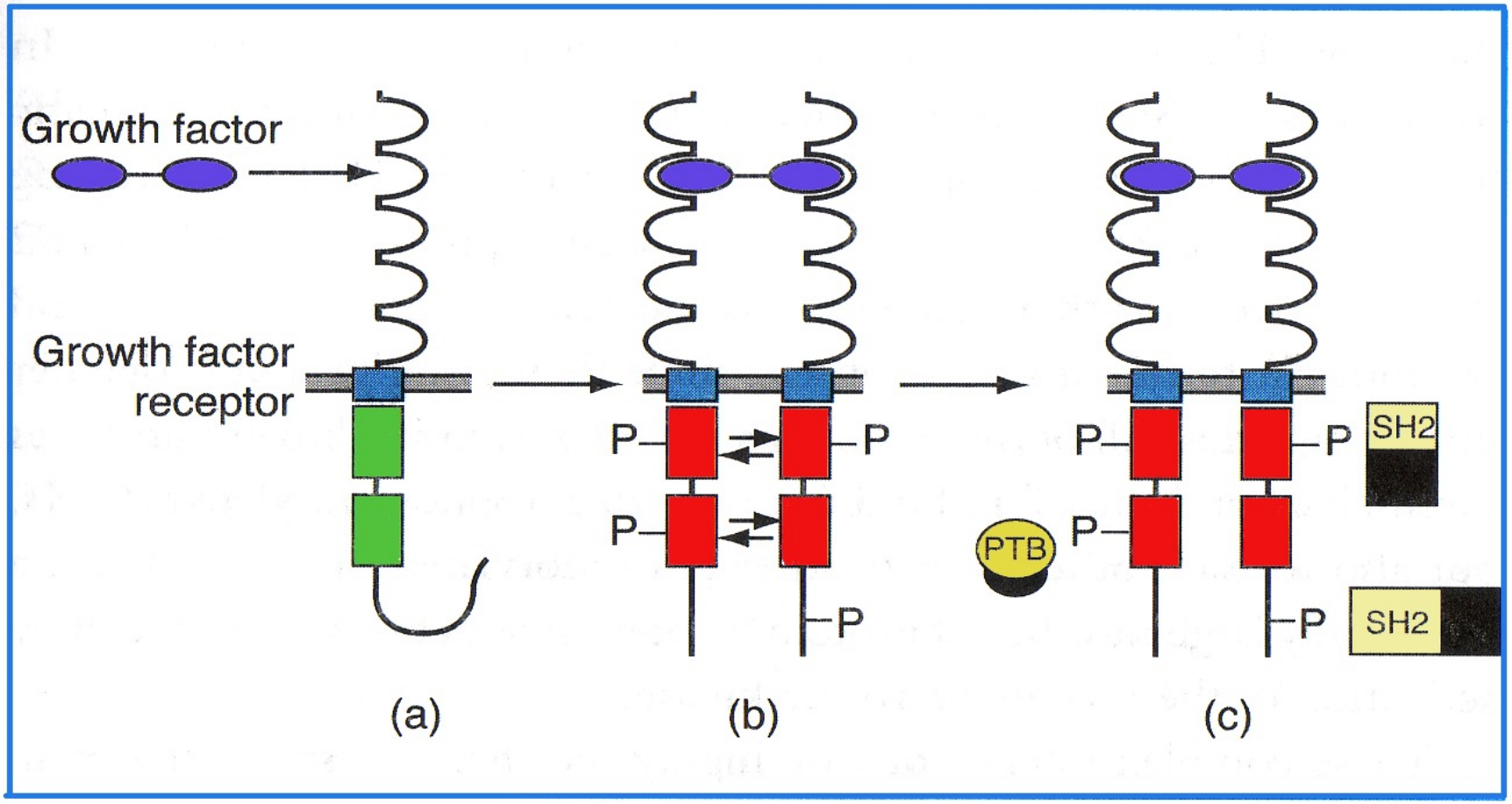
Many proteins have SH2 or PTB domains

Table 1.1 Examples of Signaling Proteins Containing Modular Binding Domains

Protein	Structural organization[a]	Activity[a]
I. Proteins with known activities		
PLC-γ	PH–SH2–SH2–SH3–PH–catalytic domain	PtdIns(4,5)P_2 hydrolysis
GAP	SH2–SH3–SH2–PH–catalytic domain	Ras GTPase activator
SH-PTP1 and 2	SH2–SH2–catalytic domain	Tyrosine phosphatase
Src	SH3–SH2–catalytic domain	Tyrosine kinase
Fps	SH–2–catalytic domain	Tyrosine kinase
Syk	SH2–SH2–catalytic domain	Tyrosine kinase
VAV	PH–SH3–SH2–SH3	Ras GNEF
STAT proteins	Leucine repeats–SH3–SH2	Transcription factor
II. Proteins with no apparent intrinsic activity (adapters)		
p85	SH3–SH2–SH2	(Bound to PI3 kinase)
Grb2/Sem5	SH3–SH2–SH3	
Snc	PTB–SH2	
Nck	SH3–SH3–SH3–SH2	
Crk	SH2–SH3	
CrkII	SH2–SH3–SH3	

[a] Abbreviations: PH, pleckstrin homology domain; PtdIns(4,5)P2, phosphoinositol bisphosphate; GNEF, guanine nucleotide exchange factor; PTB, phosphotyrosine binding domain.

Dimerization allows trans-phosphorylation and triggers signal transduction

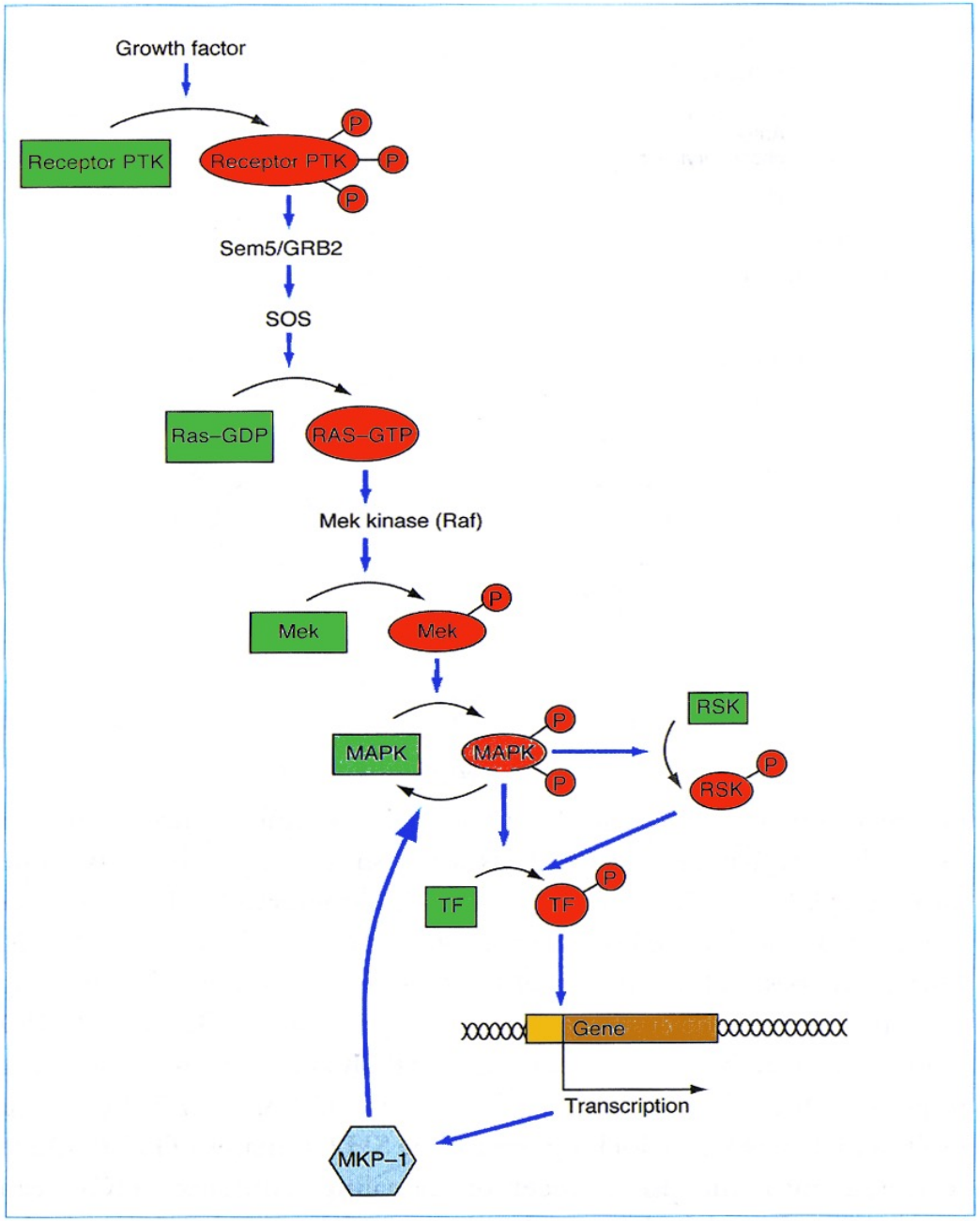


Main endpoint

Regulation of gene expression in the target cell

An example

Figure 17.2 A signaling pathway triggered by growth factor receptor PTKs. Following activation of a growth factor receptor PTK by binding to its cognate ligand, autophosphorylation of the receptor creates docking sites that recruit the adaptor protein Grb2, leading to activation of Sos, the GDP–GTP exchanger for Ras. Then a cascade of phosphorylation is triggered, leading to activation of mitogen-activated protein kinase (MAPK). MAPK in turn phosphorylates transcription factors (TF), either directly or through activation of another Ser/Thr kinase, Rsk, thus promoting the transcription of genes required for the growth response. One of the genes induced by growth factor stimulation, presumably through the MAPK pathway, encodes a MAPK phosphatase (MKP-1). As denoted by the filled arrow, MKP-1 may feed back on the pathway by dephosphorylating and inactivating MAPK, thus attenuating the signaling response. [Reproduced with permission from Sun, H. and Tonks, N.K. (1994) *Trends Biochem. Sci.*, 19, 480–5.]



An example of TKR: Muscle-Specific Kinase (MuSK)

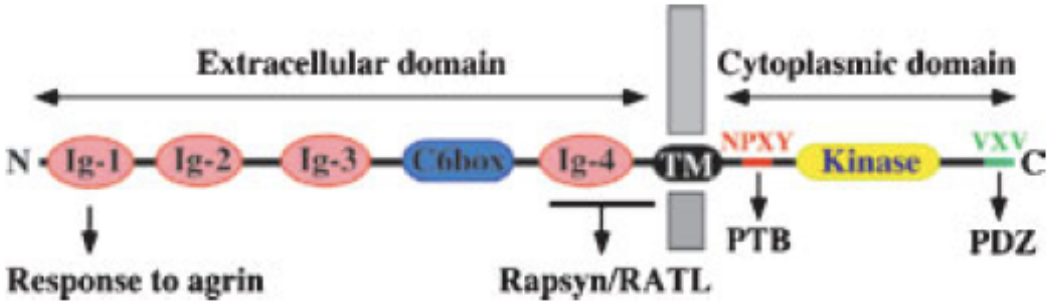
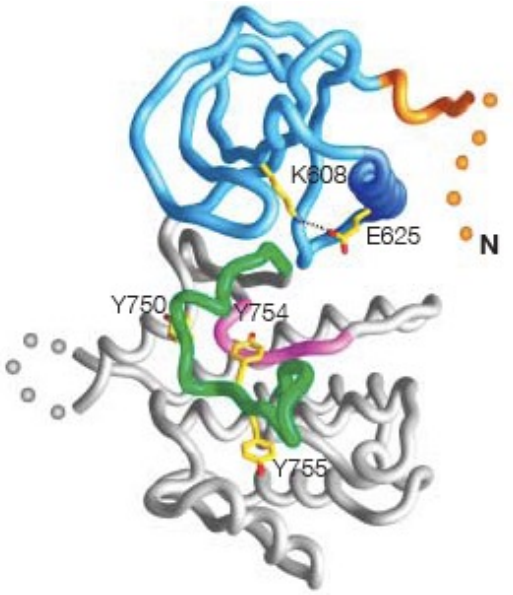


Figure 1. Domain organization of the receptor tyrosine kinase MuSK. The ectodomain of MuSK contained four immunoglobulin-like domains (Ig-1 to Ig-4) and a region containing six phylogenetically conserved cysteine residues (C6 box). The first Ig-like domain is required for agrin responsiveness (interaction with agrin/MASC) whereas the fourth Ig-like domain and adjacent sequences are necessary for rapsyn–RATL interaction (see also Fig. 3). In the cytoplasmic region of MuSK, a recognition site NPXY for phosphotyrosine binding (PTB) domain-containing proteins as well as the ATP-binding/kinase domain are both essential for activity. The C-terminal VXV consensus recognition site for PSD-95/Dlg/ZO-1-like (PDZ) domain-containing proteins is engaged in the binding of MAGI-1c. TM, transmembrane domain.



juxtamembrane region

(Strochlic *et al*, 2005, *BioEssays* **27**: 1129-1135)

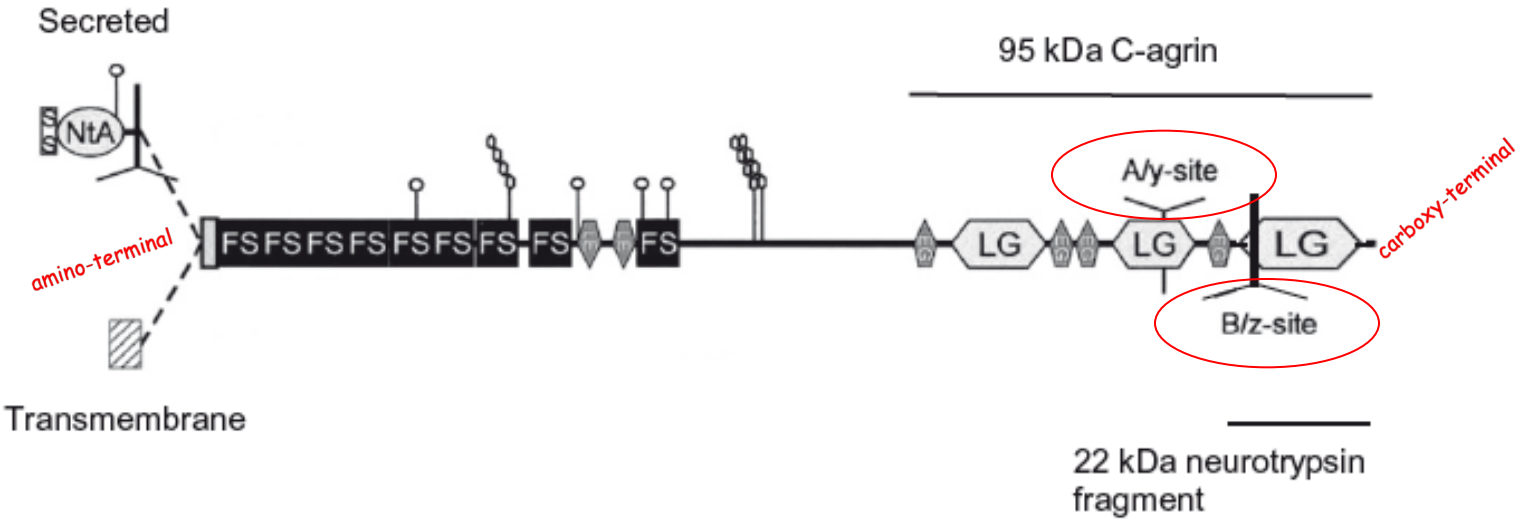
The soluble ligand: neural agrin

Agrin is a large heparansulfate proteoglycan.

The deduced protein from full-length cDNAs has calculated MW of more than 200 KDal.

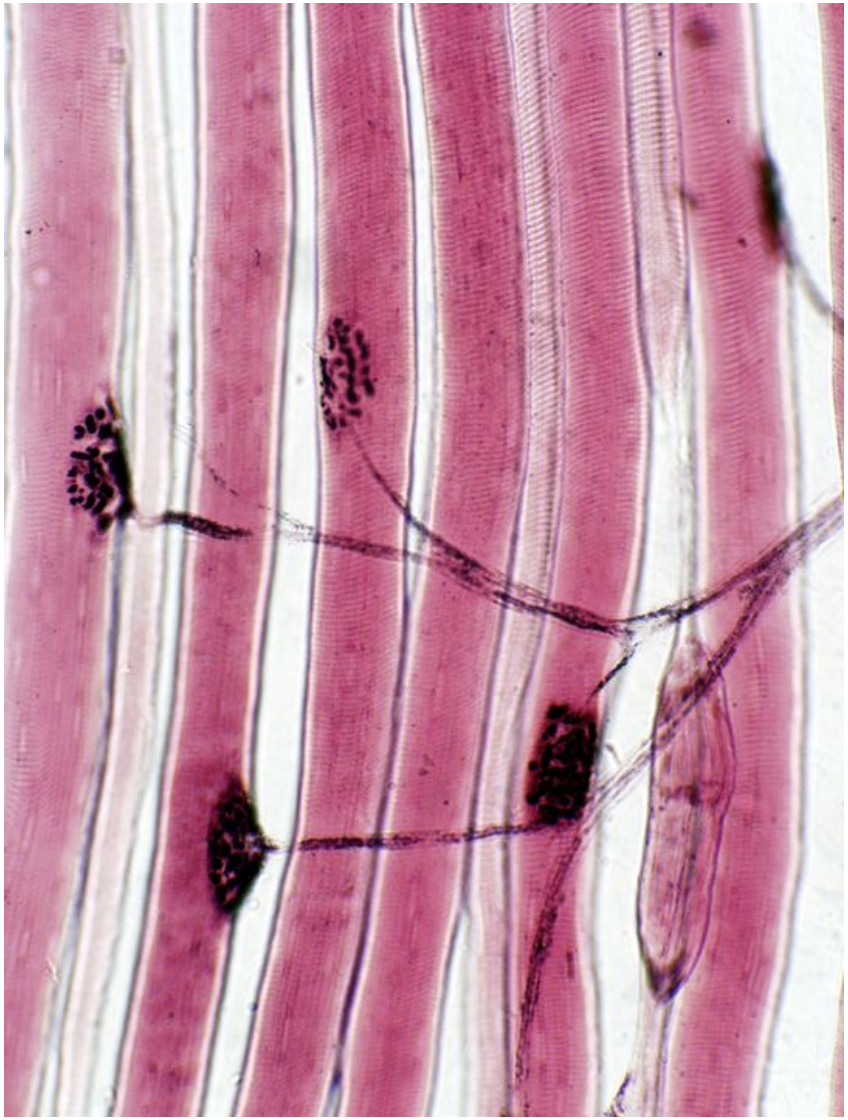
In Mammals, site Y encodes 4 amino acids and site Z for either 8, 11 or 19 (8+11). In chick, the sites Y and Z are referred to as site A and B.

The prevalent neural isoform is A4B8.

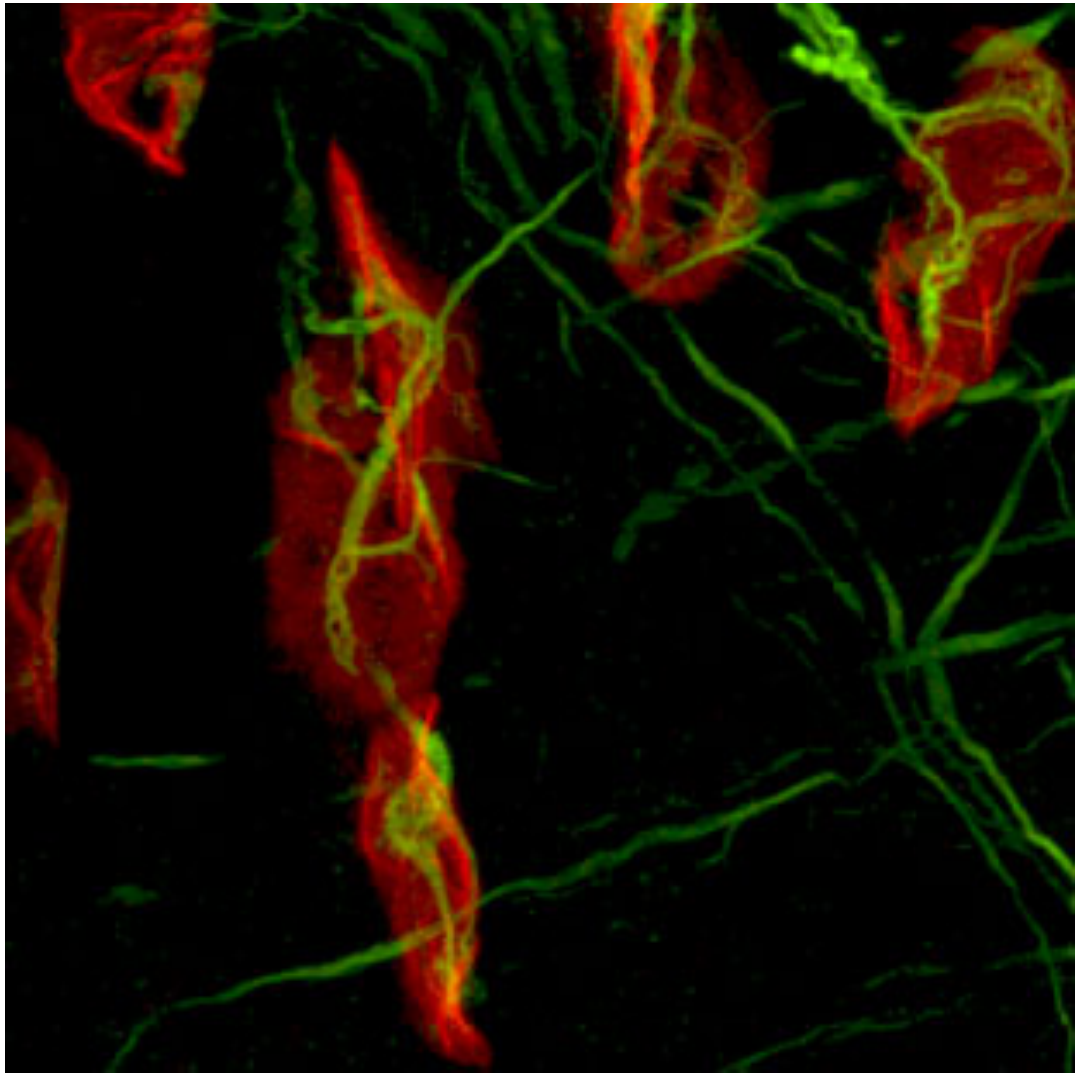


Non neural and muscle cells express only mRNA encoding agrin A4B0 and agrin A0B0.

The neuromuscular junction (NMJ)



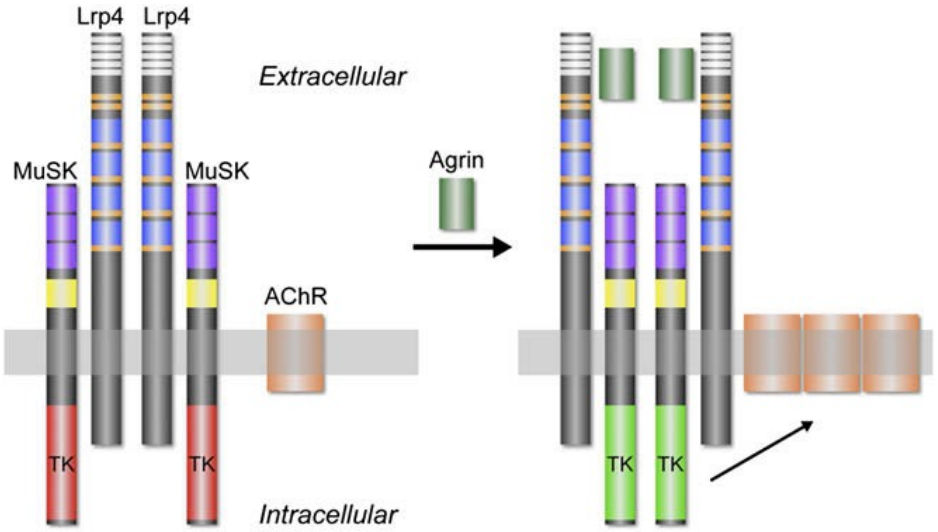
The neuromuscular junction (NMJ)



Point 1. Lrp4 serves as a coreceptor of agrin forming a complex with MuSK

MASC:
Muscle-Associated Specific Component
(Glass *et al*, 1996, Cell **85**: 513-523)

Lrp4:
Low-density lipoprotein receptor-related protein 4
(Kim *et al*, 2008, *Cell* 135: 334-34; Zhang *et al*, 2008, *Neuron* **60**: 285-297)

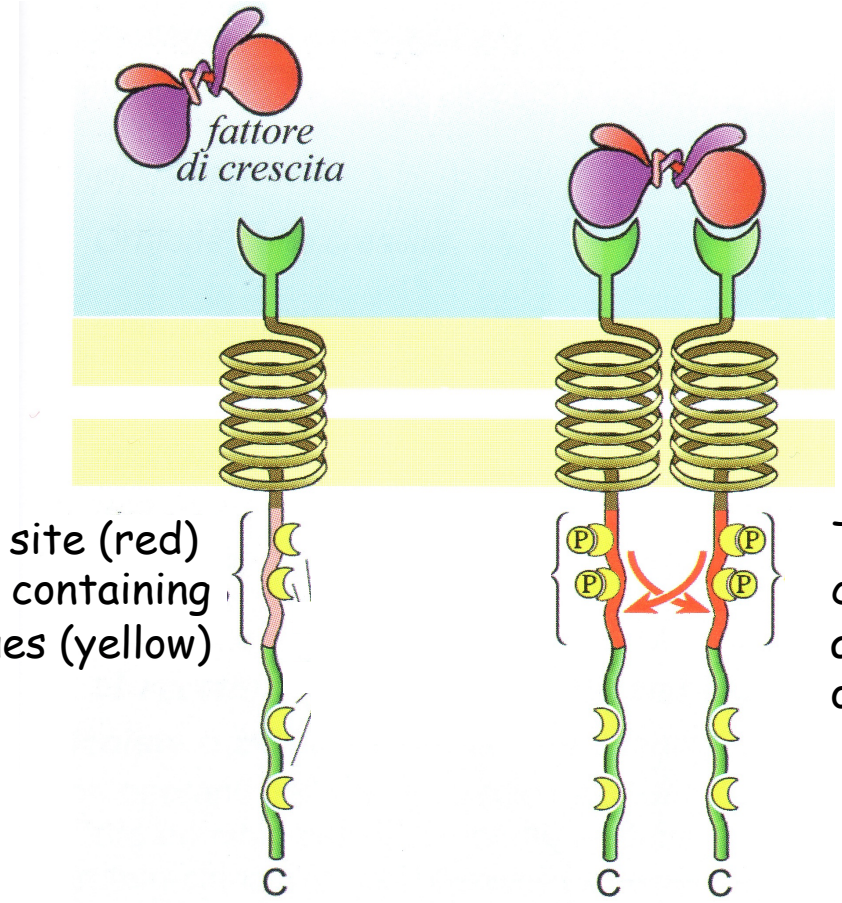


(from Kim *et al*, 2008, *Cell* **135**: 334-342)

(reviewed Hubbard & Gnanasambandan 2013, *Biochim Biophys Acta* **1834**: 2166-2169)

Dimerization allows trans-phosphorylation and enhances the catalytic receptor activity

Transphosphorylation first occurs at juxtamembrane region



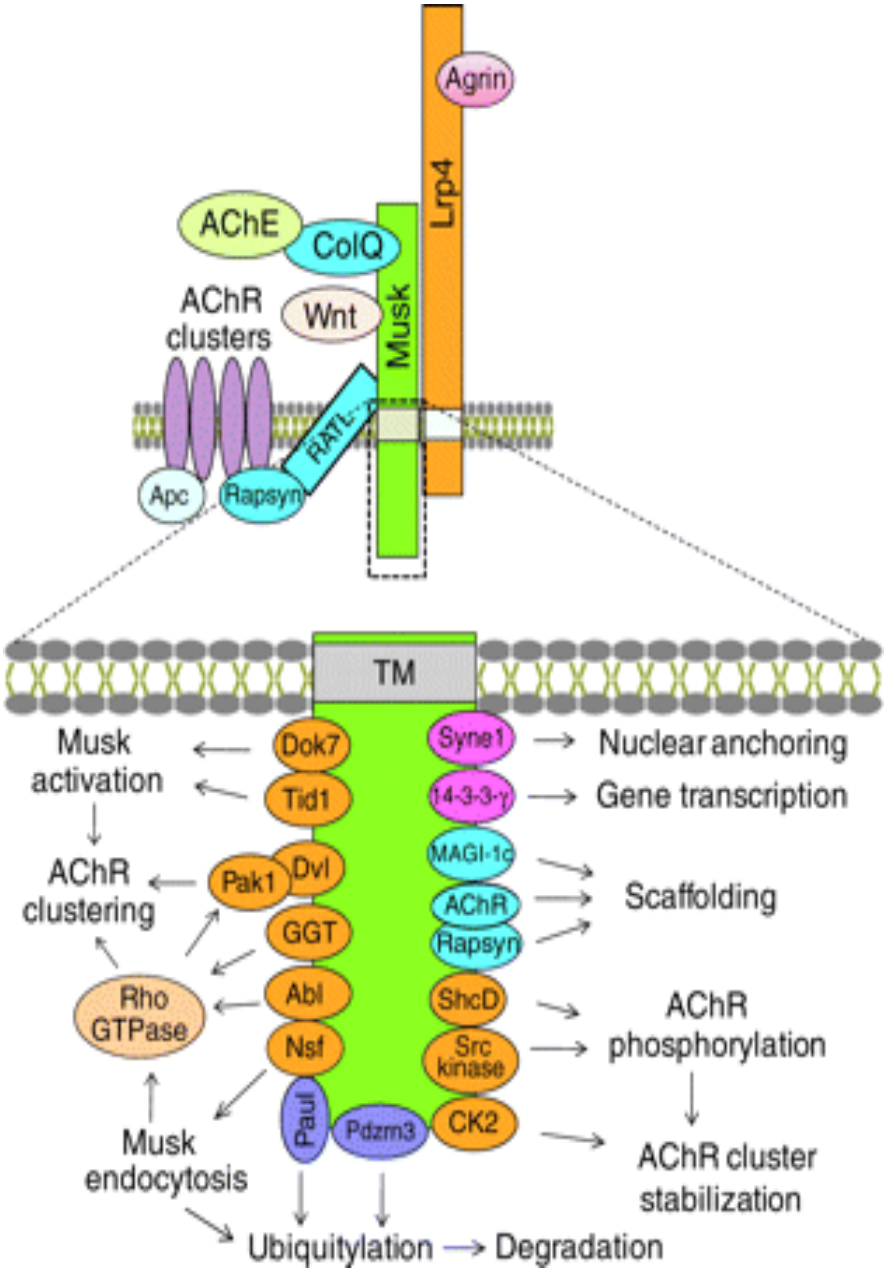
Catalitic site (red) containing tyrosine residues (yellow)

Transphorylation causes a conformational change allowing fully activation of the catalitic site

Point 2.

How many docking sites?!

One receptor, different cascades



MuSK-mediated effects

1) gene expression

2) AChR clusterization

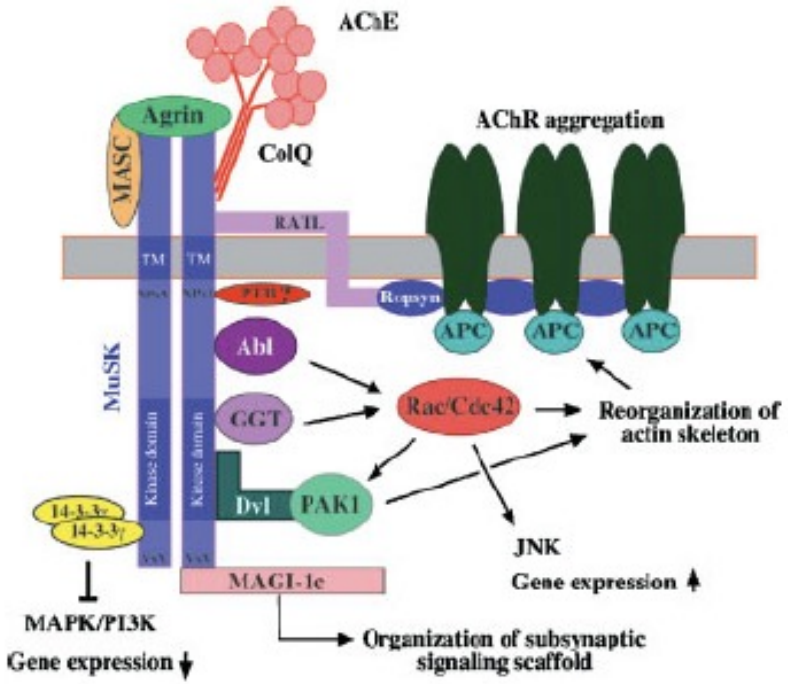


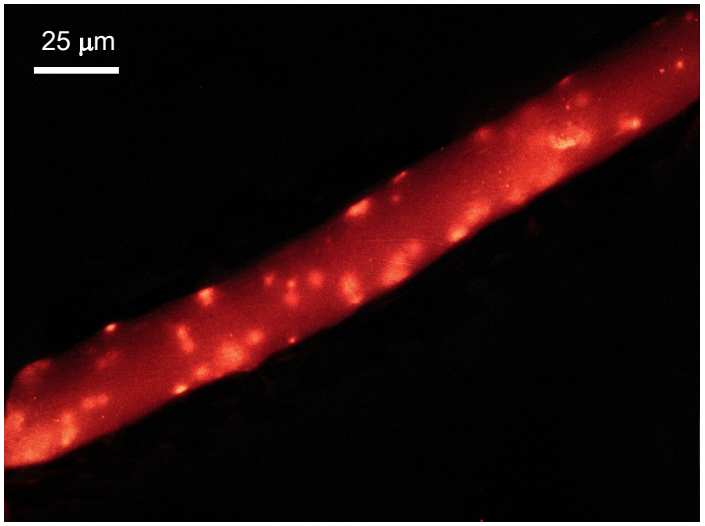
Figure 2. Model of the MuSK complex and downstream signaling pathways. The extracellular domain of MuSK accommodates binding sites for agrin (with the help of the putative muscle-associated specific component, MASC), RATL and ColQ. Cytoplasmic effectors Abl, GGT and Dvl interacting with MuSK activate Rac/Cdc42–PAK1 leading to actin cytoskeleton reorganization and AChR aggregation, likely through APC. The juxtamembrane NPXY motif is required for MuSK signaling through putative PTB domain-containing proteins. The scaffolding protein MAGI-1c which binds to the C-terminal consensus PDZ binding site of MuSK (VXV), potentially recruits multiple, yet unidentified, subsynaptic signaling molecules. Finally, the adaptor protein 14-3-3 γ regulates synaptic gene expression via inhibition of MAPK–PI3K signaling pathways (see Fig. 3). The various molecules are not represented at scale. TM, transmembrane domain.

(Strochlic *et al*, 2005, *BioEssays* **27**: 1129-1135)

Point 3. Ligand-independent activation of MuSK (and TKRs?)

(see Tezuka *et al*, 2014, *PNAS* **111**: 16556-16561)

Before innervation



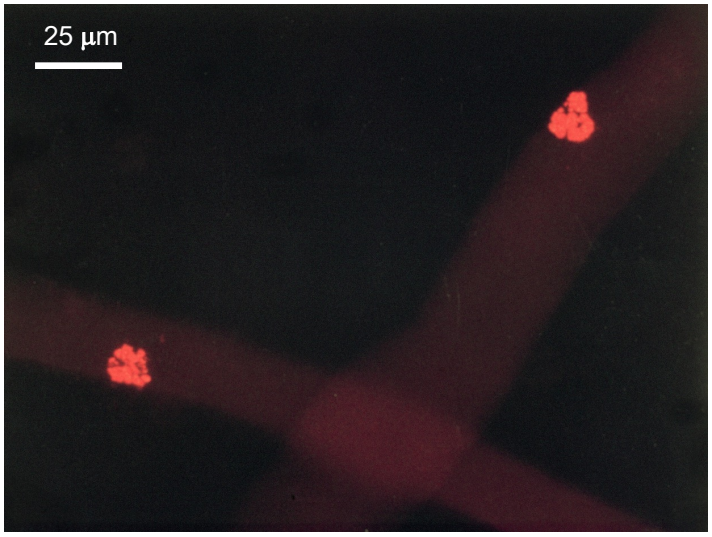
nAChR distribution (prepatterned)

controlled

via a ligand-independent activation of MuSK

(MuSK/Lrp4/**Dok7**)

After innervation

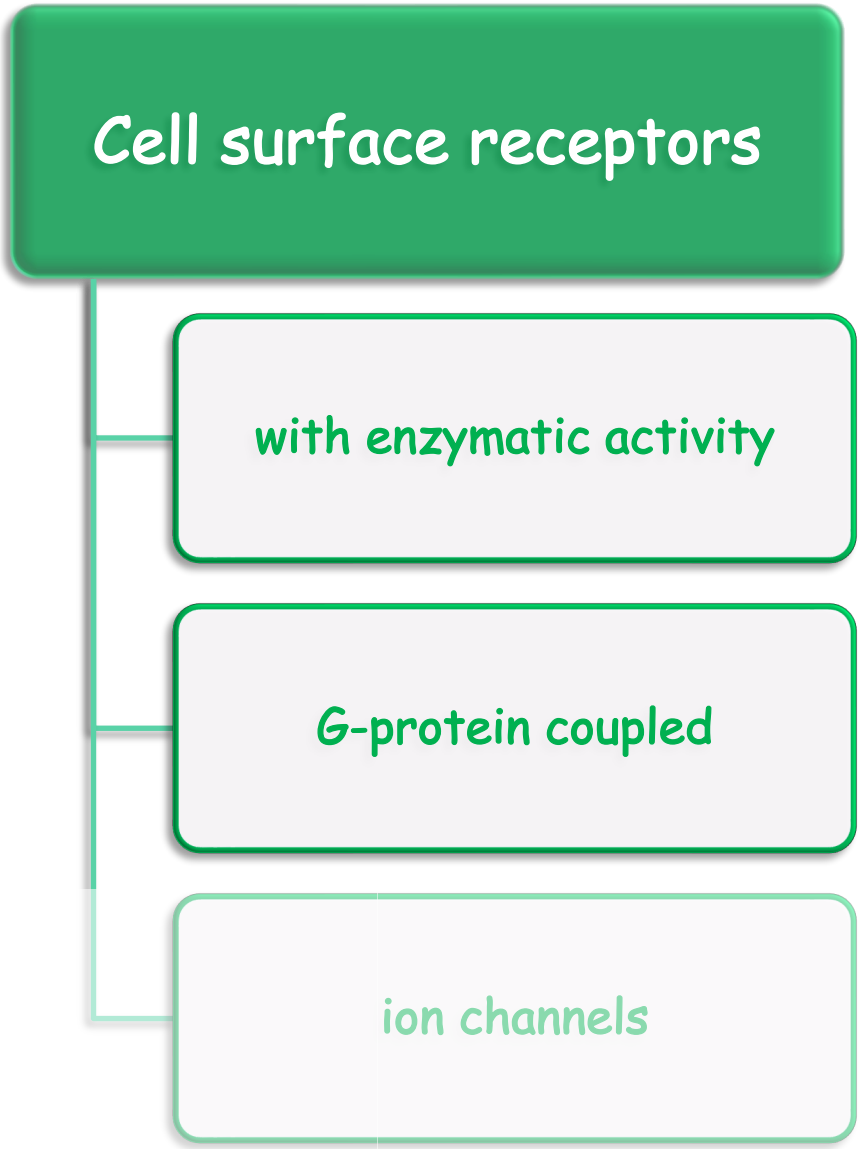


nAChR distribution (endplate region)

controlled

via a ligand-dependent activation of MuSK

(**Agrin**/MuSK/Lrp4/Dok7)


Cell surface receptors
with enzymatic activity
G-protein coupled
ion channels

Cell surface receptors: G-protein-coupled receptors

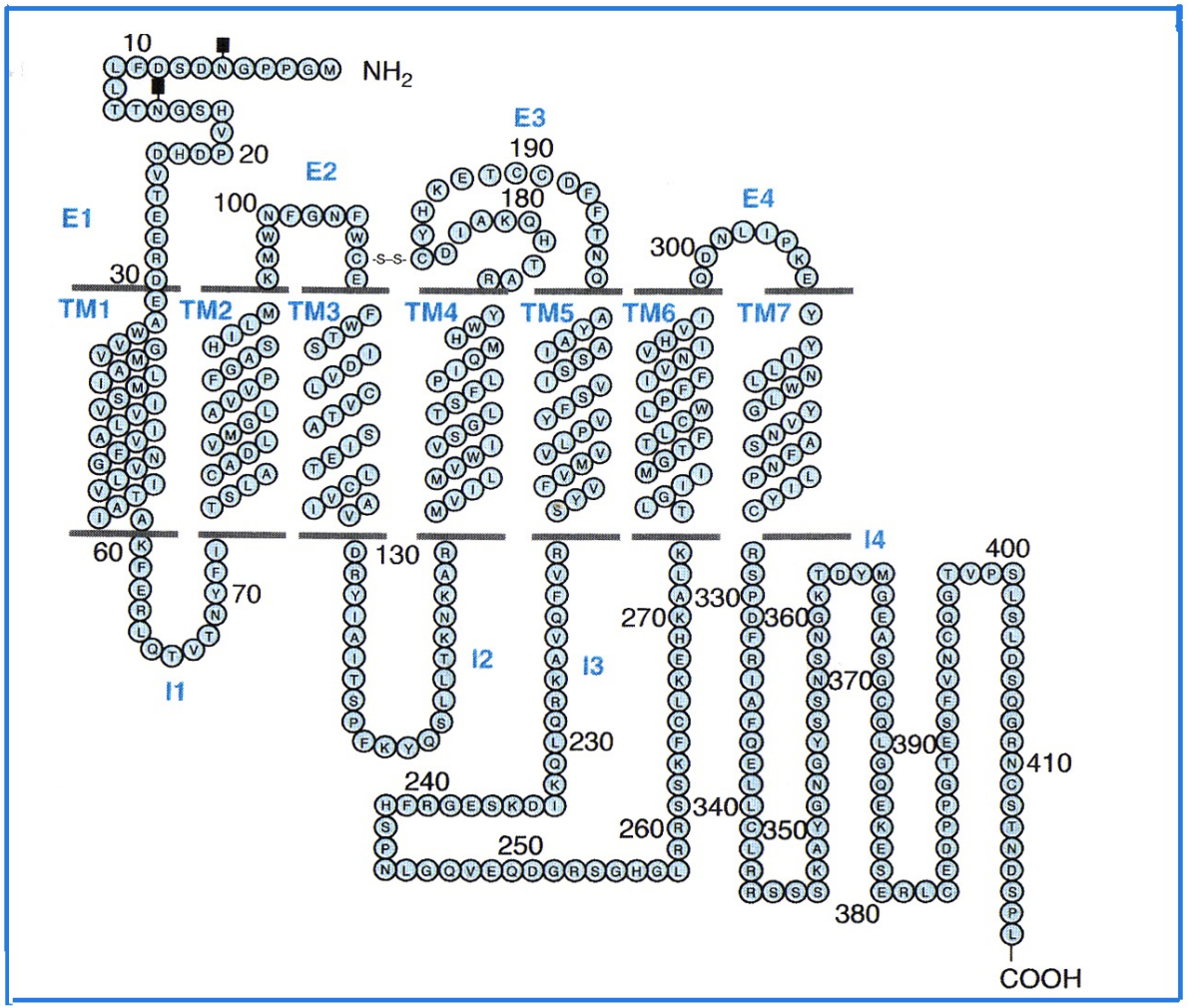


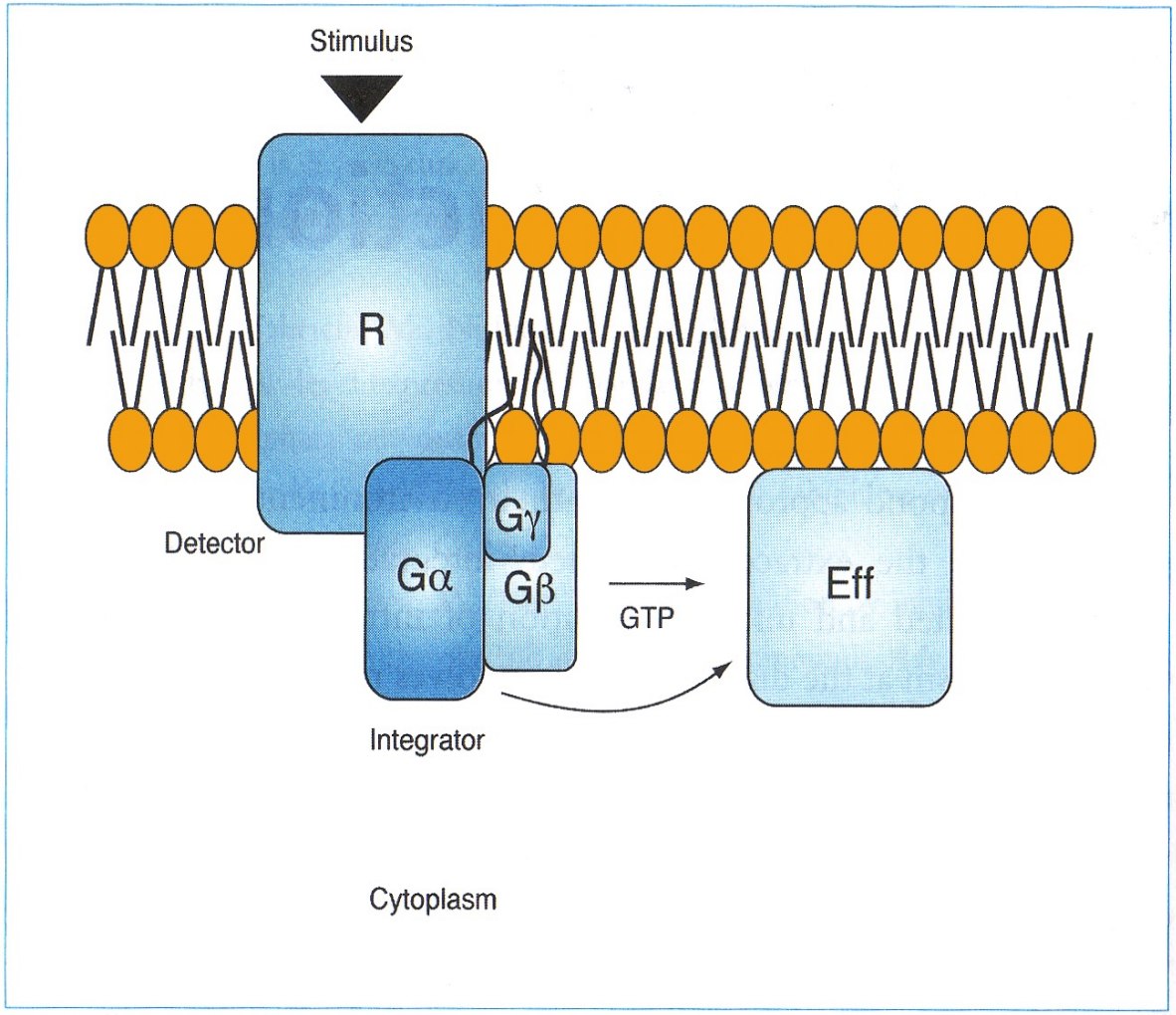
Figure 7.1 Model depicting the transmembrane topology of the β_2-adrenergic receptor. The positioning of the seven transmembrane domains was accomplished by hydropathy analysis of the primary amino acid sequence. Extended domains of hydrophobic amino acids were assigned as putative transmembrane spanning regions. Further examination of these sequences by Chou–Fasman analysis suggests that the transmembrane domains exist in an α-helical conformation. The putative transmembrane domains are labeled TM1–TM7, the extracellular domains E1–E4 and the intracellular domains I1–I4.

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

The actors

Figure 19.1 Basic pathway for G-protein-dependent signal transduction. Stimulation of receptors initiates a vectorial signaling cascade that results in the production of an amplified intracellular signal. See text for details. Abbreviations: R, receptor; G, G protein; Eff, effector.



When effectors are ion channels

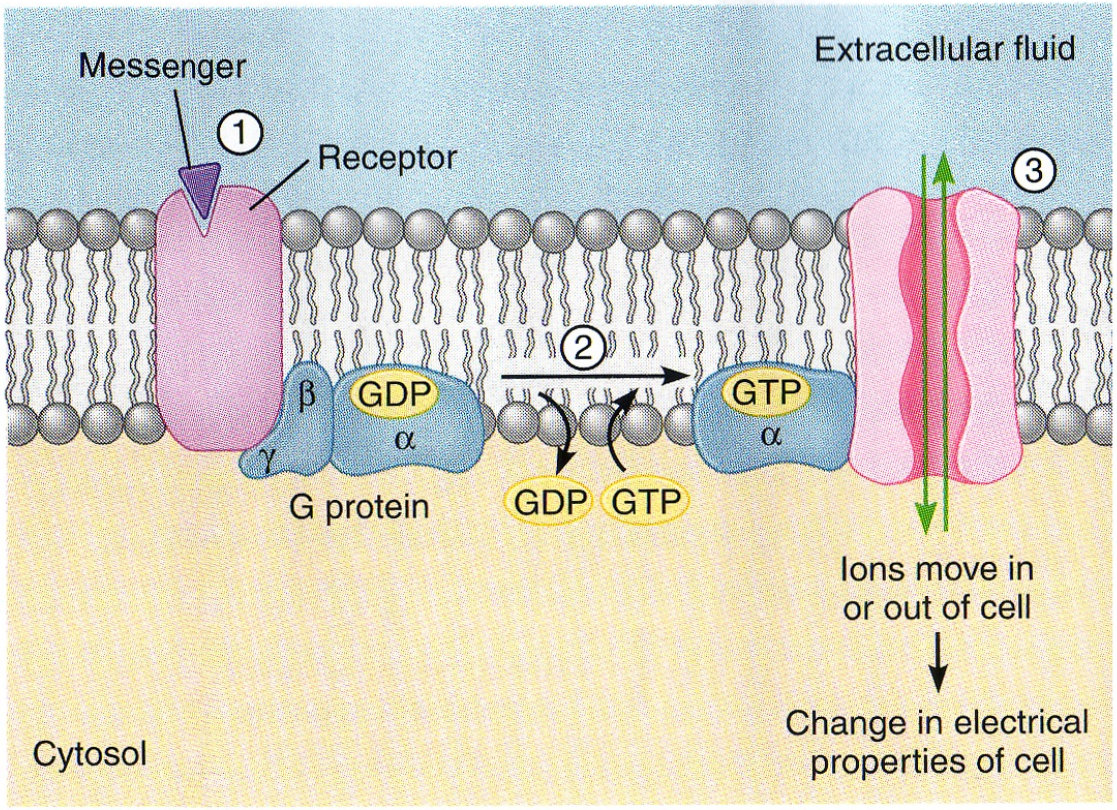


FIGURE 5.15 Action of a G protein on a slow ligand-gated ion channel. ① *Binding of the messenger to the receptor activates the G protein.* ② *The alpha subunit moves to an ion channel in the membrane.* ③ *The ion channel opens or closes, changing the permeability of the membrane to a specific ion. Ions move across the plasma membrane, changing the electrical properties of the cell.*

Endpoint

Change in excitability

When effectors are enzymes

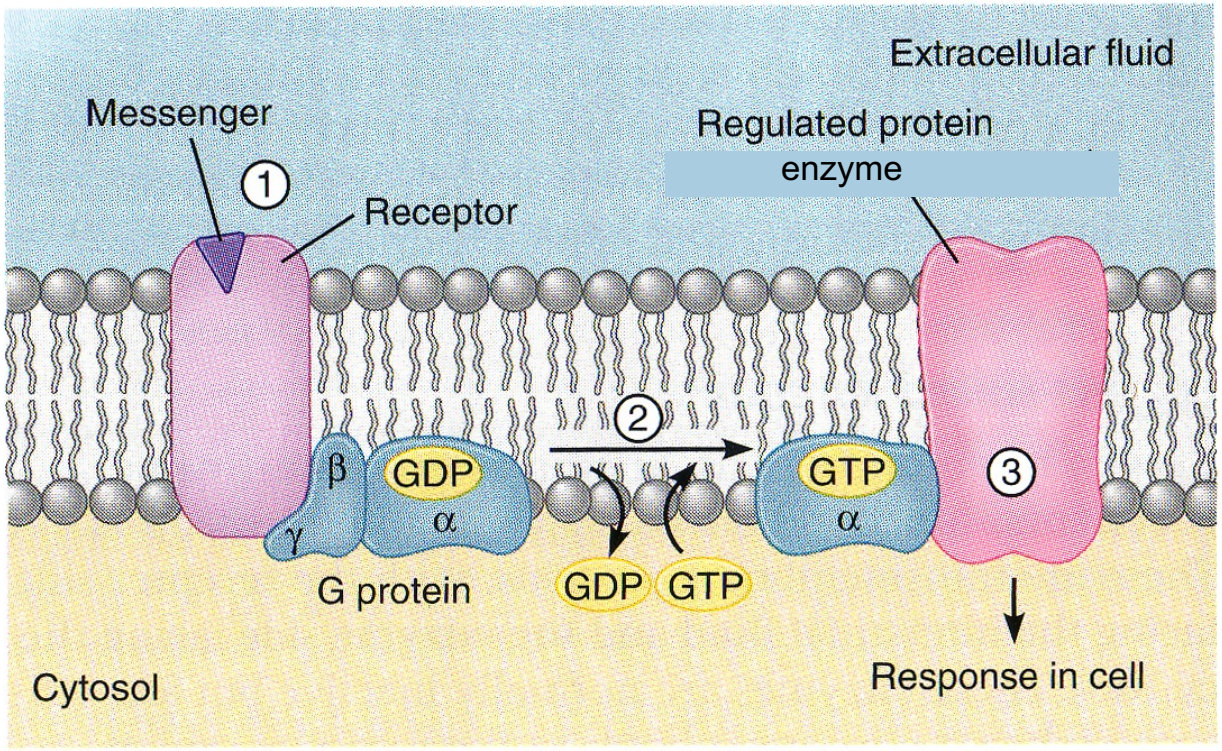
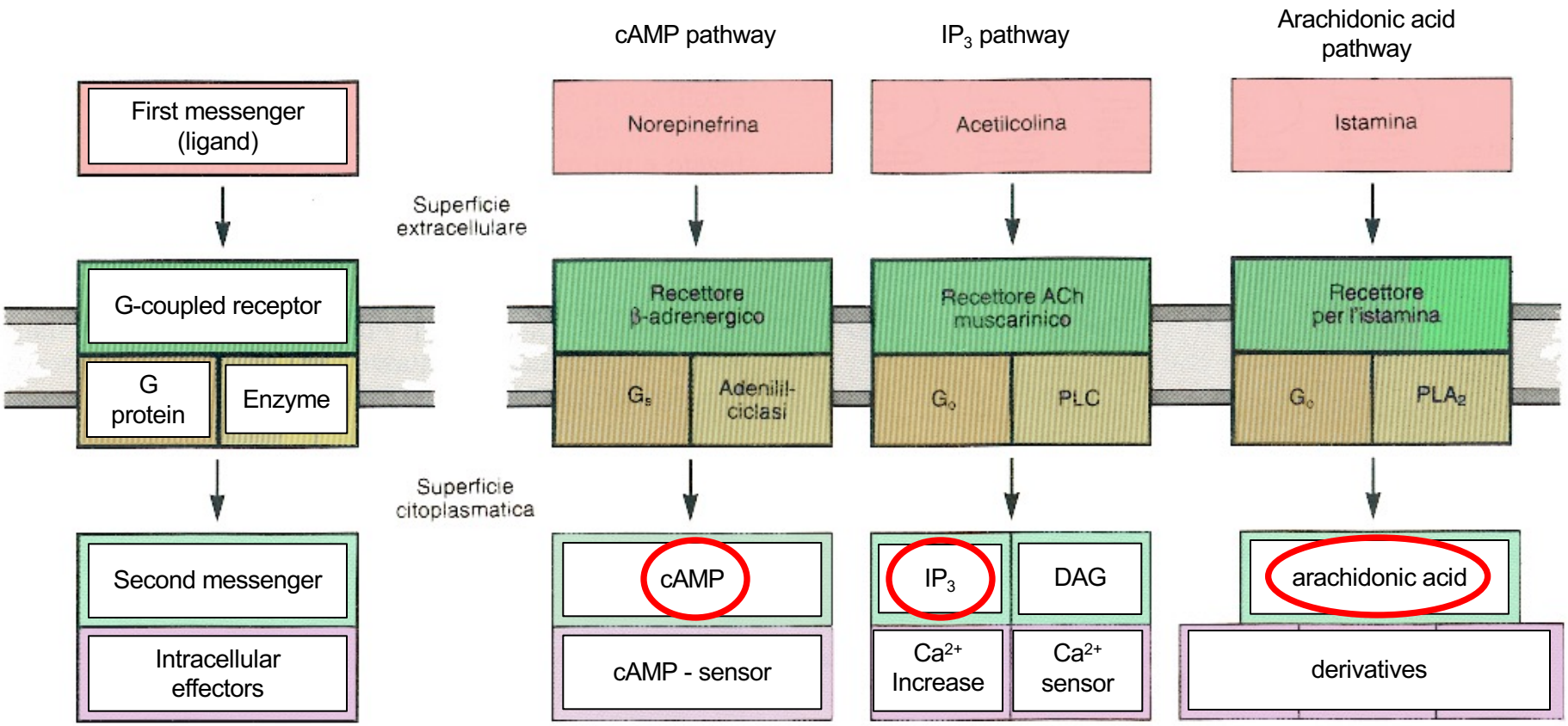


FIGURE 5.14 Actions of G proteins. *G proteins have three subunits: alpha, beta, and gamma. The alpha subunit has binding sites for guanosine nucleotides. In the inactive state, GDP is bound to the alpha subunit. (1) Binding of a messenger to a G-protein-linked receptor activates the G protein. (2) The GDP is released as the alpha subunit moves laterally within the membrane and binds a GTP. (3) The alpha unit then activates another membrane protein, producing a response in the cell.*

Endpoint

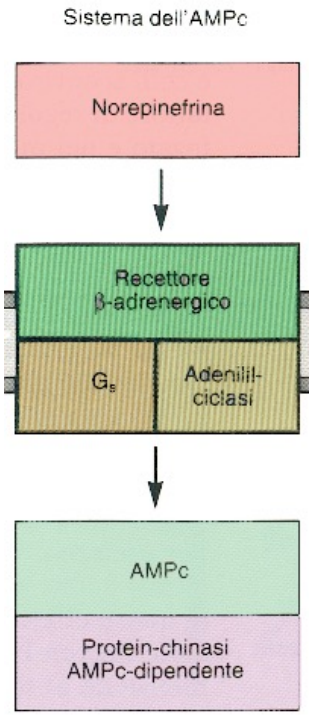
Metabolic processes
(via PK activity)

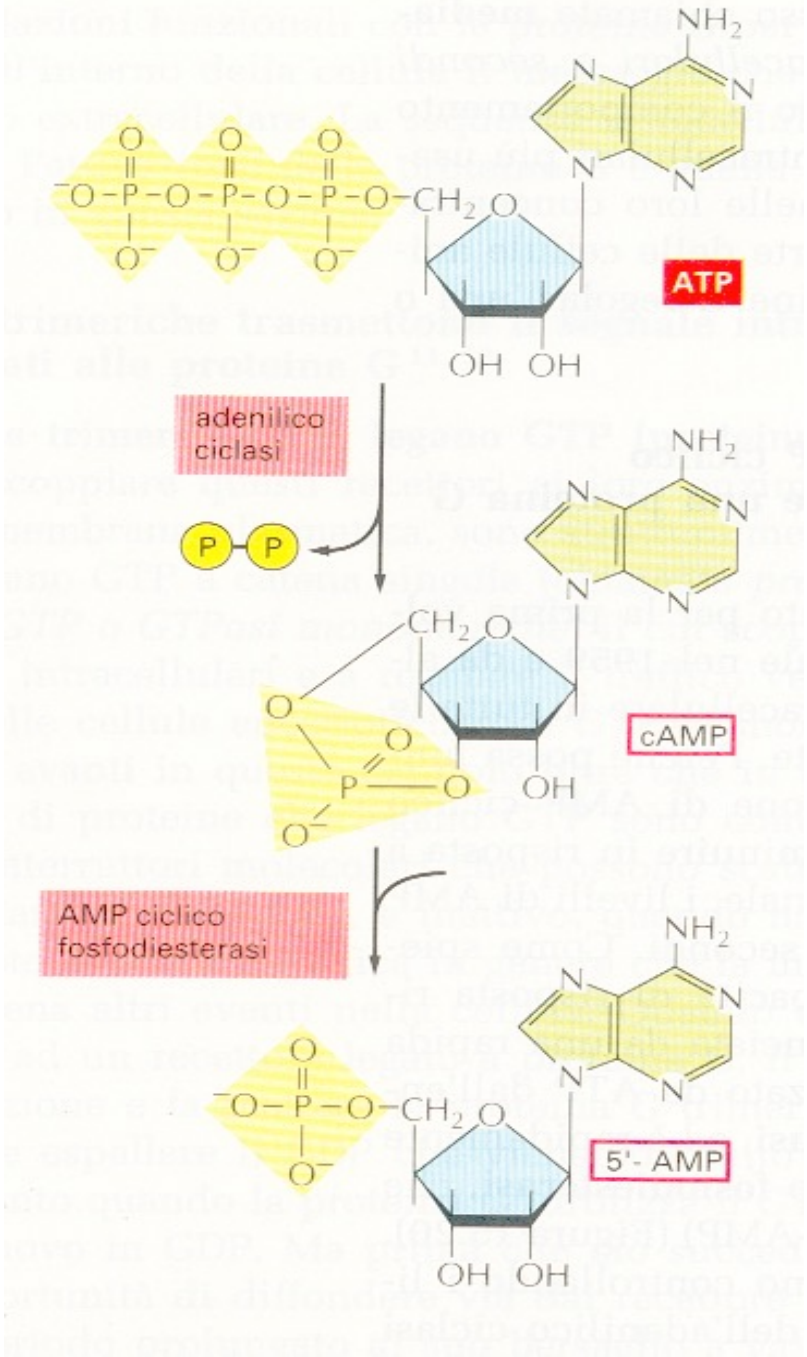
When effectors are enzymes



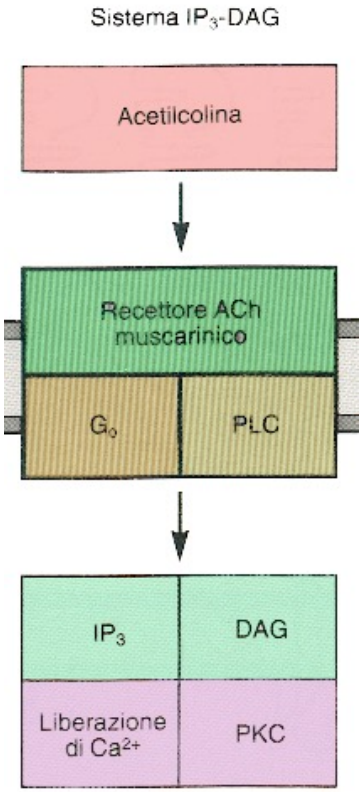
Intermediate step

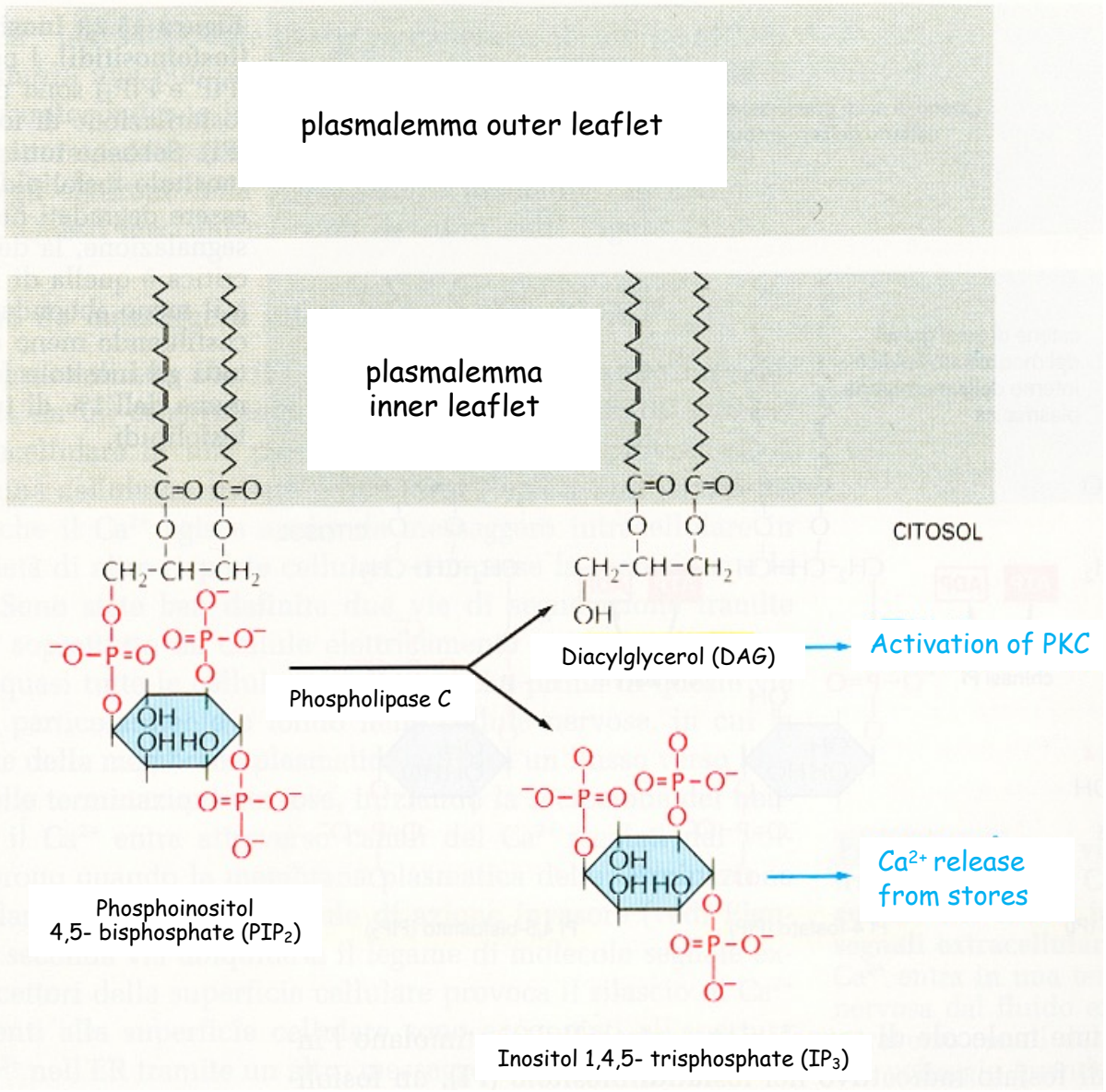
Change in the concentration of a second messenger



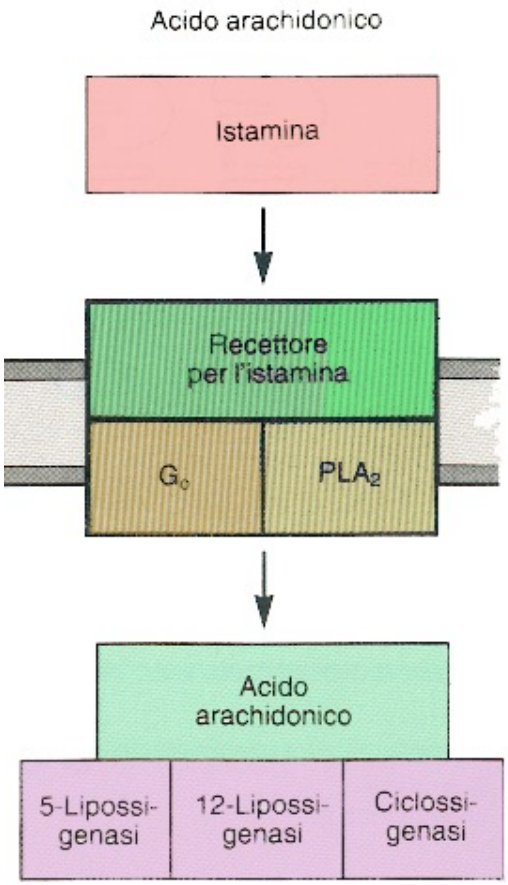


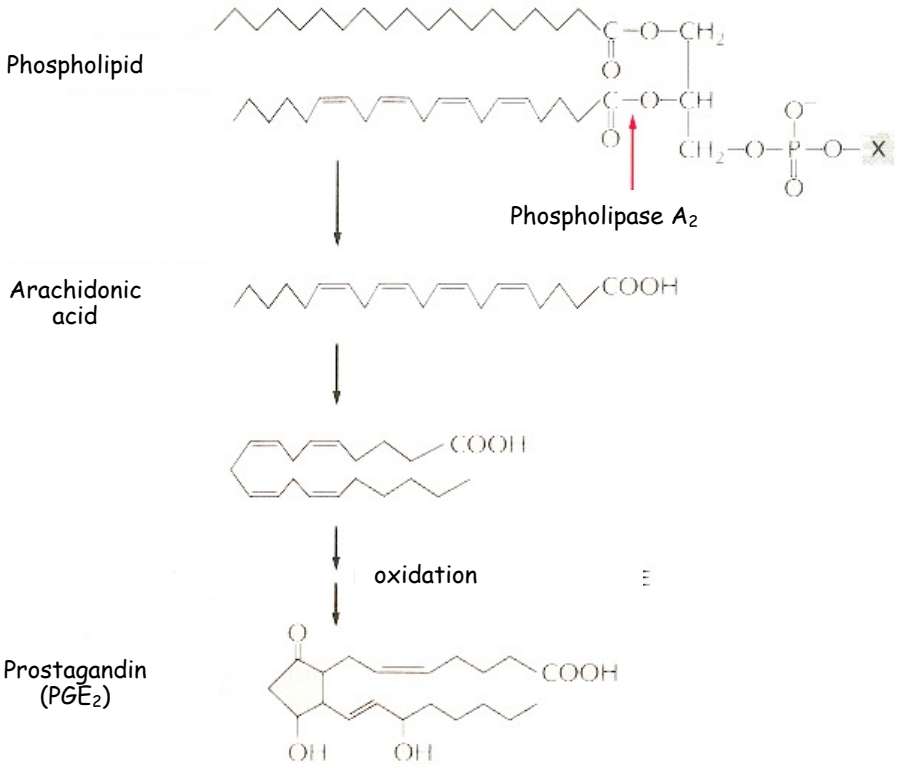
Synthesis of cAMP





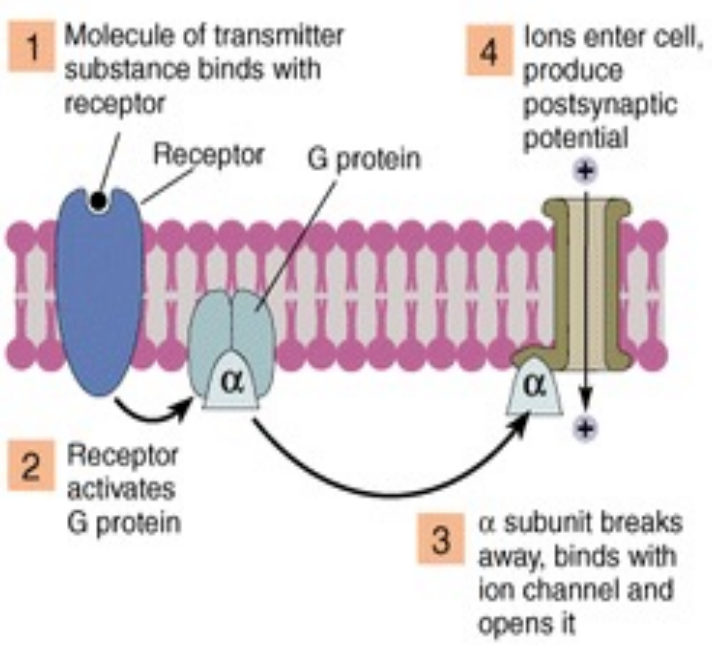
Synthesis of IP_3

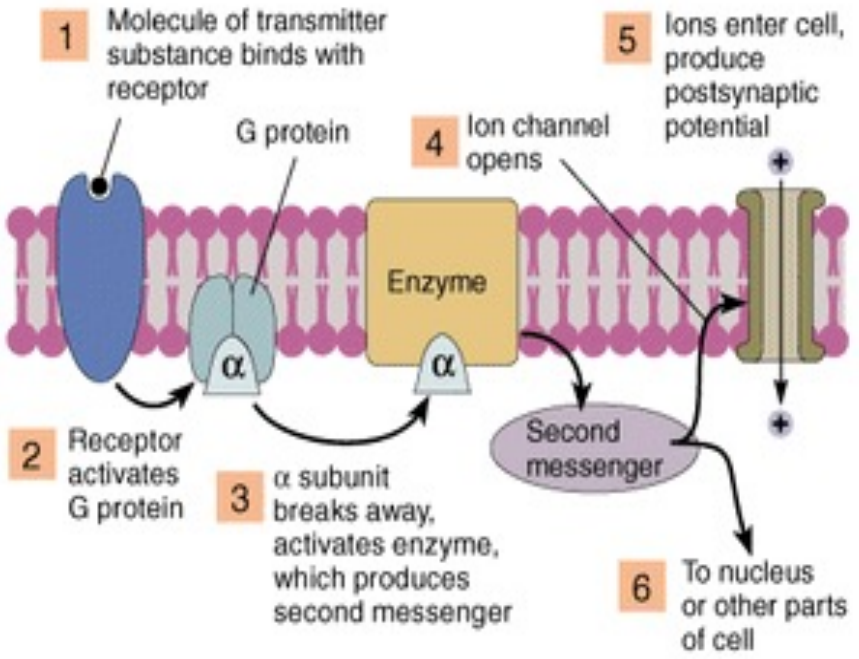




Synthesis of arachidonic acid

Metabotropic receptors in neurobiology: two activation modes

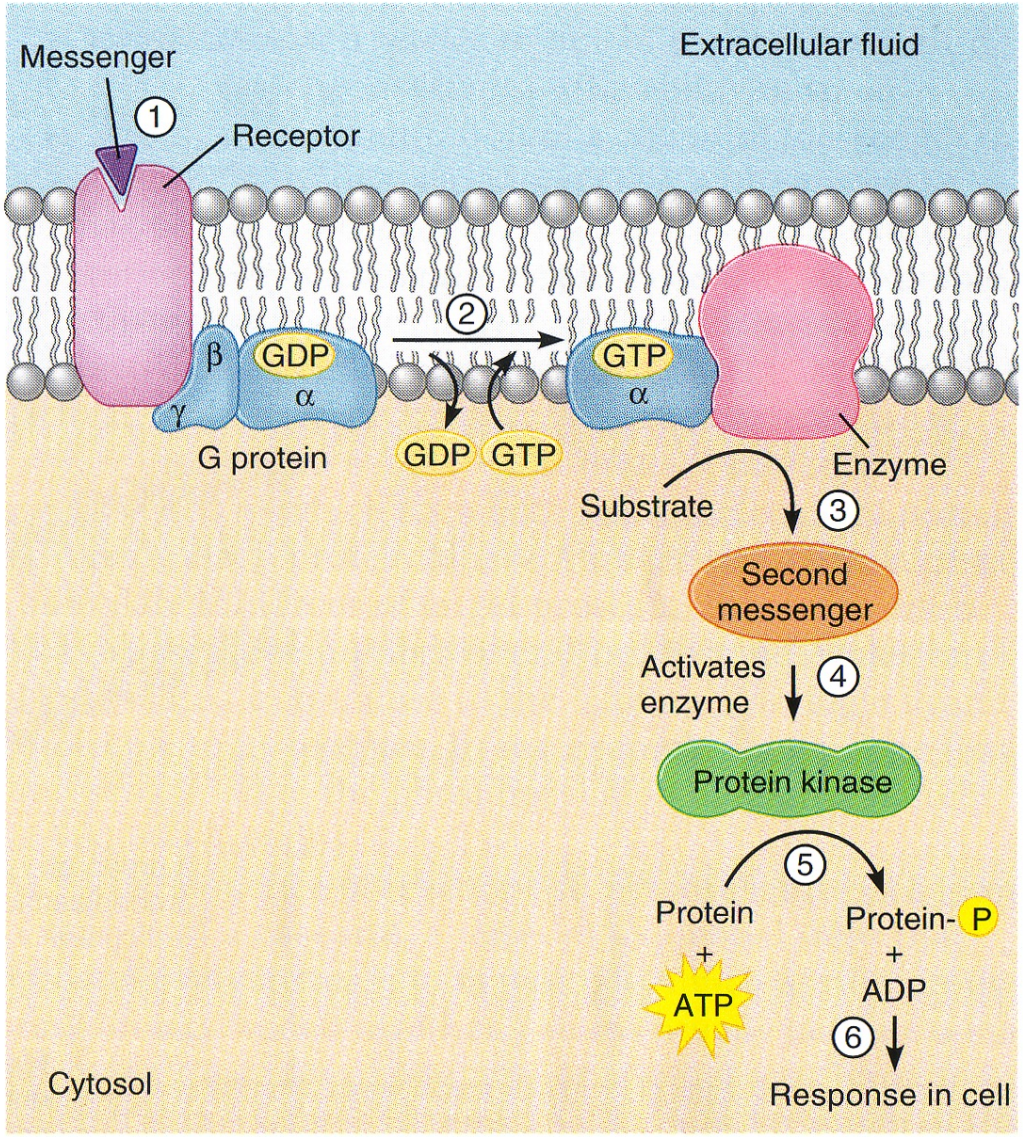




via G – protein

via second messenger

Second messengers modulate protein kinases



Endpoint

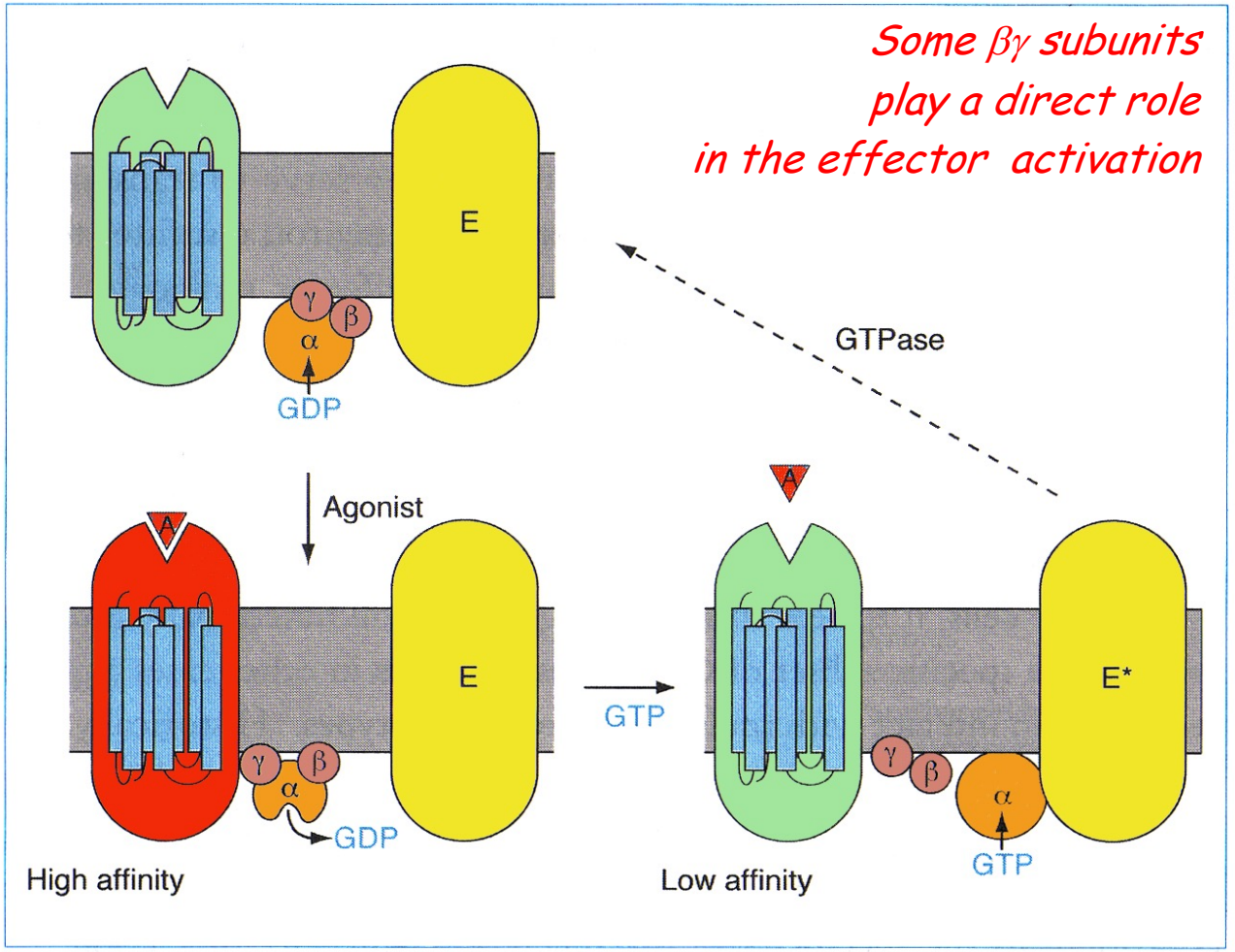
Modulation of pre-existing cell activity

(rapid cell response)

FIGURE 5.16 G-protein-regulated enzymes and second messengers. *In this example the G protein is coupled to an enzyme in the plasma membrane. ① Binding of the messenger to its receptor activates the G protein. ② The alpha subunit moves to and activates an enzyme in the membrane. ③ The activated enzyme catalyzes formation of a second messenger in the cytosol. ④ The second messenger activates a protein kinase, which ⑤ catalyzes phosphorylation of a protein, which ⑥ initiates a response in the cell.*

G-proteins modulate the affinity of receptors

Figure 7.2 Model for the activation of G protein by GPCR. Agonist binding is hypothesized to catalyze the formation of a ternary complex between agonist, receptor and G protein that promotes the replacement of GDP with GTP in the nucleotide binding site of the α subunit. Upon GTP binding, the α subunit dissociates from the βγ subunits and from the receptor, resulting in the shift of the receptor conformation into the state with low affinity for agonist and in the activation of the effector system by the α or the βγ subunits. The GTPase activity of the α subunit returns the system to its basal state.



Guanine nucleotide binding protein family

Different α, β and γ isoforms have been identified

Table 19.1 G protein α subunits[a]

Class	Members	Toxin[b]	Localization	Effector regulation[c d]	Signal[d]
G_s	α_s	CT	Ubiquitous	↑ AC, ↑ Ca^{2+} channels	↑ cAMP, ↑ Ca^{2+}
	α_{olf}	CT	Olfactory epithelium	↑ AC	↑ cAMP
G_i	α_{i1}	PT	Limited	↓ AC	↓ cAMP
	α_{i2}, α_{i3}	PT	Ubiquitous	↑ *K^+ channels*	Δ Voltage
	α_o	PT	Neuronally enriched	↓ *Ca^{2+} channels*	↓ Ca^{2+}
	α_{t1}, α_{t2}	PT	Retina	↑ cGMP-PDE	↓ cGMP
	α_{gust}	PT	Taste buds	?	
	α_z	–	Limited	?	
G_q	α_q, α_{11}	–	Ubiquitous	↑ PLC	↑ IP_3, DAG
	α_{14}	–	Limited	↑ PLC	↑ IP_3, DAG
	α_{15}, α_{16}	–	Hematopoetic cells	↑ PLC	↑ IP_3, DAG
G_{12}	α_{12}	–	Ubiquitous	*Na^+/H^+ exchange*	
	α_{13}	–	Ubiquitous	↓ *Ca^{2+} currents*	

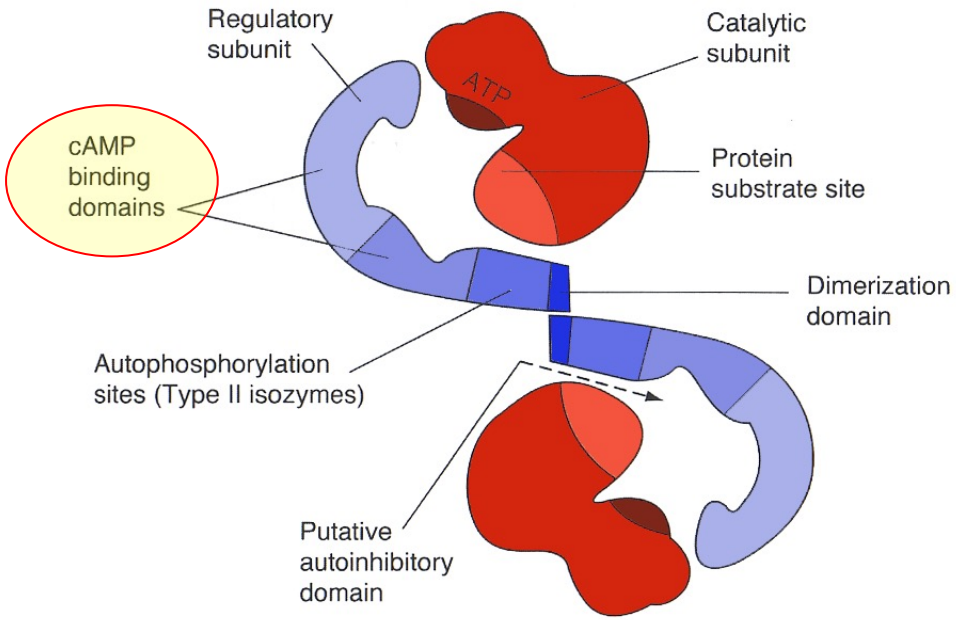
[a] The α subunits contain the binding site for association with and hydrolysis of GTP. The subunits listed represent unique mammalian genes. Several of the proteins also exist in multiple forms due to splice variation.

[b] Several of the α subunits can be modified by ADP ribosylation; cholera toxin (CT) enhances α_s activity while pertussis toxin (PT) attenuates the action of its substrates.

[c] Activities in italics are examples of regulated activities that may not evolve from direct interaction of the subunit with the effector.

[d] Abbreviations: AC, adenylyl cyclase; cGMP-PDE, cGMP-dependent phosphodiesterase; PLC, phosphatidylinositol-specific phospholipase C; DAG, diacylglycerol.

Sensors for second messengers: protein kinases



cAMP-dependent protein kinases

(stoichiometry 1:4)

Second messengers can bind to non-kinase molecules

Table 1. "Non-kinase" second messenger effector proteins and their functions

Protein	Second Messenger	GTPase target	Functions
Epac1/cAMP-GEF I	cAMP	Rap1A,Rap2B	PLC and Ca^{++} signaling[46] M3-muscarinic AChR signaling[47] Activates H,K-ATPase[48]
Epac2/cAMP-GEF II	cAMP	Rap1A	Insulin secretion[11], PTH signaling[49]
RasGRP1/CalDAG-GEF II	DAG, Ca^{++}	H-Ras, M-Ras TC21, R-Ras	Thymocyte development[24], Autoimmunity, transformation in vitro[7,15,50], ERK activation
RasGRP2/CalDAG-GEF I	DAG, Ca^{++}	Ki-Ras, N-Ras Rap1,Rap2, TC21	M1-muscarinic AChR signaling[51] Leukemogenic in mice[32] Myeloblast and platelet adhesion[31]
RasGRP3/CalDAG-GEF III	DAG,	H-Ras, R-Ras Rap1	PC12 differentiation[18], B-cell receptor signaling[52,53] transformation in vitro[18] MAPK/JNK activation
RasGRP4/CalDAG-GEF IV	DAG, Ca^{++}	H-Ras	Transformation in vitro[19] Mast cell differentiation, Systemic mastocytosis Mast cell leukemia, Bronchial airway hypersensitivity/ asthma predisposition[38]
α1-Chimaerin	DAG	Rac	Fibroblast adhesion, Lamellipodia formation[54]
α2-Chimaerin	DAG	Rac	Neuronal differentiation[54]
β1-Chimaerin	DAG	Rac	Spermatogenesis[54]
β2-Chimaerin	DAG	Rac	Astrocytoma progression[54] Cerebellar granule cell differentiation
Munc-13[a]	DAG		Neurotransmitter release, Diabetic nephropathy[54,55]
PDZ-GEF1/RA-GEF1	cGMP,cAMP[b]	Rap1,Rap2	B-Raf activation[40,41,43,56,57]
PDZ-GEF2/RA-GEF2	cGMP,cAMP[b]	Rap1	Associates with M-Ras[42,58]

[a]Munc-13 proteins do not have GEF or GAP activity.
[b]Binding of cyclic nucleotides has not been observed by all groups. PTH, Parathyroid Hormone; PLC,Phospholipase C.

***Epac**: Exchange protein directly activated by cAMP; **CAMP-GEF**: cAMP regulated Guanine nucleotide Exchange Factor; **CalDAG-GEF**: calcium and diacylglycerol regulated Guanine nucleotide Exchange Factor; **RasGRP**: Ras Guanine nucleotide Releaseing Protein; **Munc**: Mammalian Unc; **PDZ**: PSD-95/Discs-large/ZO-1 domain.*

Why does cAMP act on different target molecules?

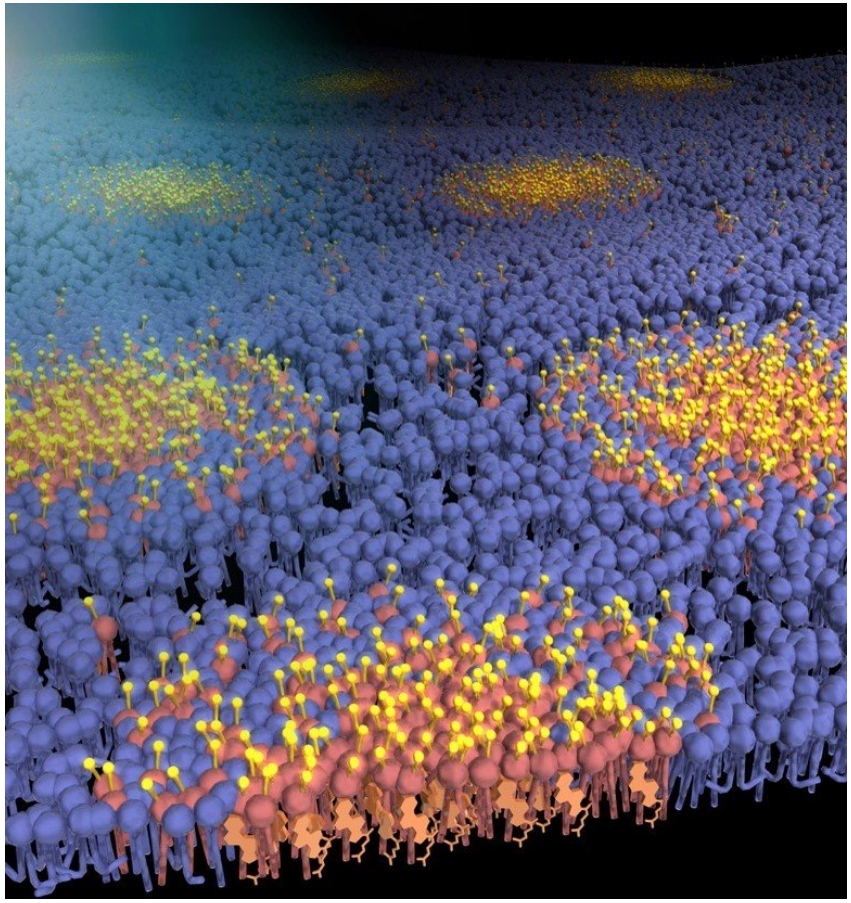
A larger teleologic question raised by the identification of EPAC is: why evolve and conserve another family of cAMP effectors? Inferences can be made based on differences in the way EPAC and PKA bind cAMP. PKA binds cAMP with a K_d in the range of 100 nM. In contrast, the affinity constant of EPAC for cAMP is in the range of 10 μM (100 times greater). This appears to be a consequence of differences in key amino amino acids in the cAMP-binding pocket of EPAC (Fig. 3). This implies that EPAC can "sense" changes in cAMP concentration in a range where PKA is already saturated. Hence the dynamic range of cAMP signaling is extended. Of course, this begs the question: why not just use a modified PKA isoform for this purpose? In this regard, it may be that EPAC engages in a very different set of protein–protein interactions and is therefore sensitive to completely different inputs and outputs.

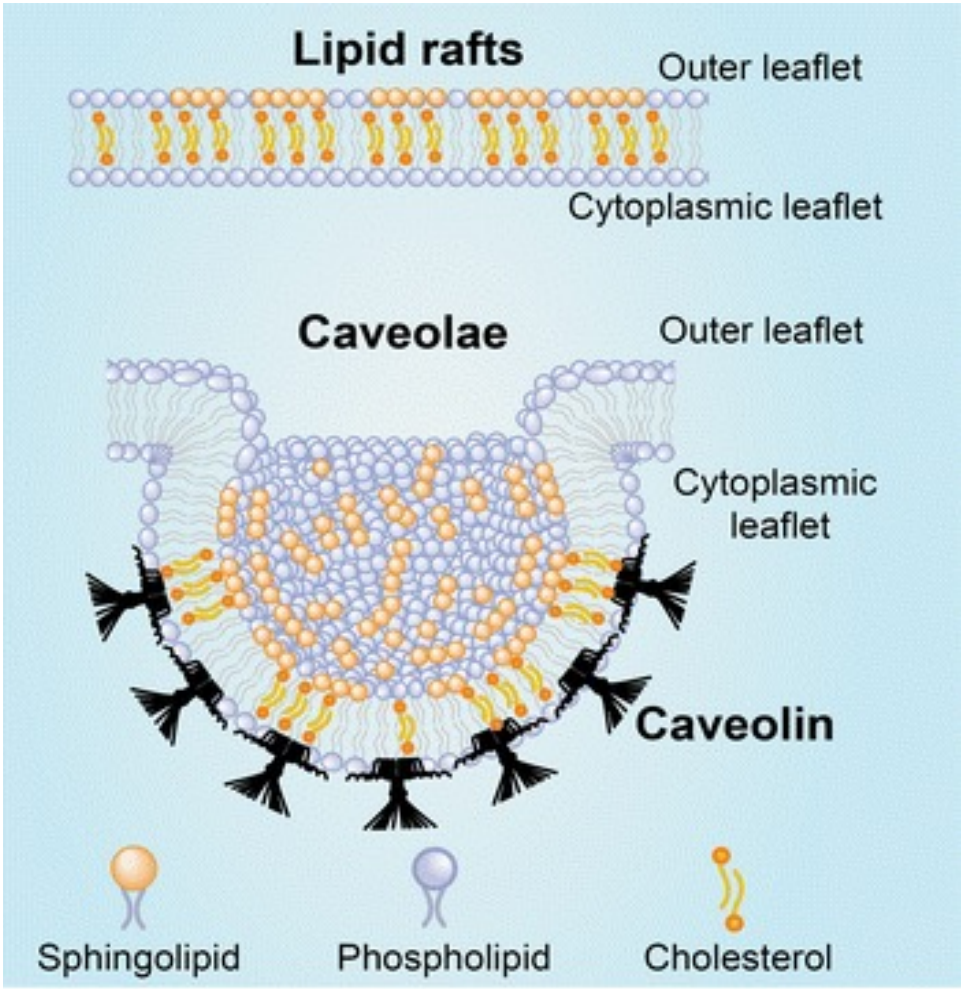
EPAC = Exchange protein directly activated by cAMP

BioEssays 26:730–738, © 2004

To increase the degrees of freedom of the signalling cascade

The specificity of signalling depends also on the localisation of transduction elements





Advantage of the multi-step signalling cascade

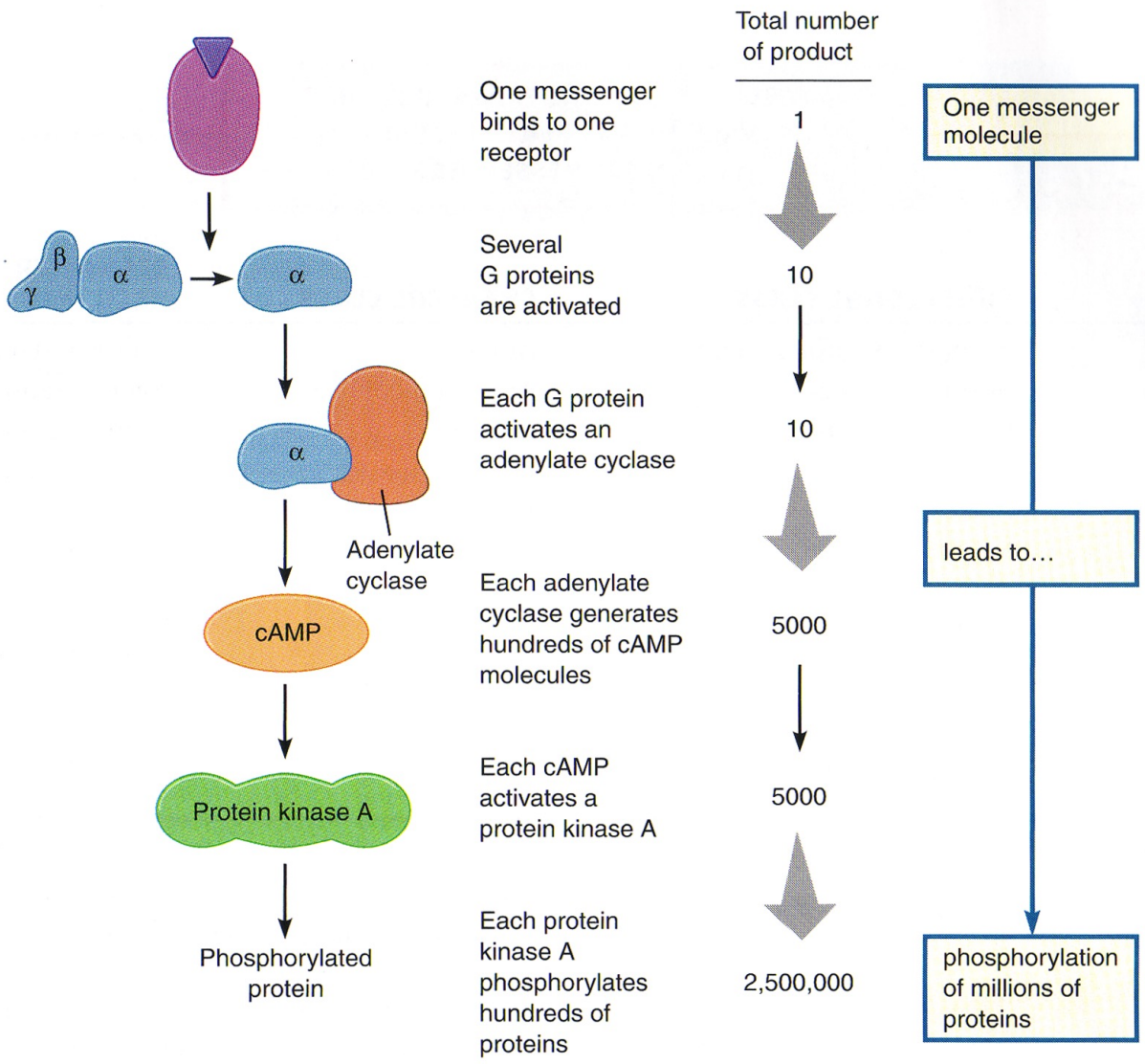


FIGURE 5.18 **Signal amplification, in this case by the second messenger cAMP.**

Control and reversibility of signalling cascades

1) Concentration of the first messenger
   - diffusion (after secretion arrest)
   - re-uptake
   - enzymatic degradation (hydrolysis)

2) Number of the available receptors
   - rapid desensitisation (receptor phosphorylation)
   - slow desensitisation (receptor internalisation)

3) Timed activation of G-proteins
   - GTPase activity

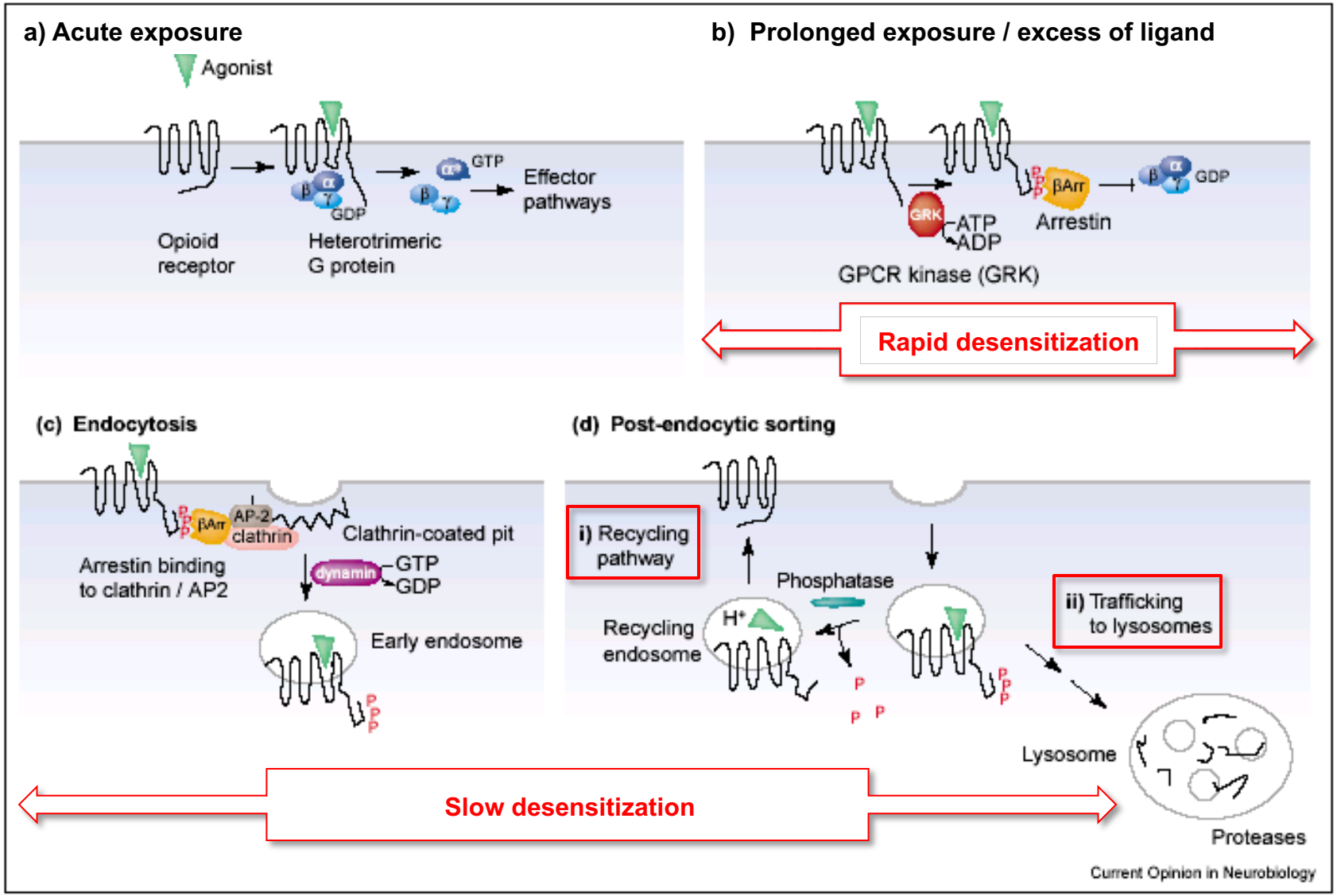
4) Timed activation of effectors
   - inactivation of the enzymes

5) Half life of second messengers
   - enzymatic degradation

6) Half life of phosphorylated proteins
   - phosphatase activity

to protect from overstimulation in presence of ligand

prevailing when the ligand disappears



Series of events occurring after agonist-induced activation of opioid receptors. **(a)** Acute signaling: receptor-mediated activation of heterotrimeric G proteins. The triangular agonist could be either an opioid peptide or a drug. The heterotrimeric G protein could be either Gi or Go. The effector pathways could be ion channels, adenylyl cyclase or kinase cascades. **(b)** Rapid desensitization: phosphorylation and arrestin binding prevent activation of G proteins. **(c)** Endocytosis: arrestin promoted concentration of receptors in a clathrin coated pit and dynamin dependent formation of endocytic vesicles. **(d)** Post-endocytic sorting (i) Recycling to the plasma membrane termed 'resensitization'. (ii) Trafficking to lysosomes termed 'down-regulation'.

Down-regulation of the number of receptors

Figure 8.1 Down-regulation of EGF receptors by EGF. Mouse NR6 cells that express transfected human EGF receptors (10^5 per cell) were incubated with an excess of EGF at 37 °C. The total amount of EGF receptor protein (a), and the number of EGF binding sites on the cell surface (b) were measured at various time intervals using Western blotting and an ^{125}I-EGF binding assay, respectively, as described in ref. 25. Quantitation of the data shown in (a) revealed that the total cellular pool of EGF receptors was decreased by 70% after a 4 h incubation with EGF.