G Protein Coupled Receptor

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The Nobel Prize in Physiology or Medicine 1994

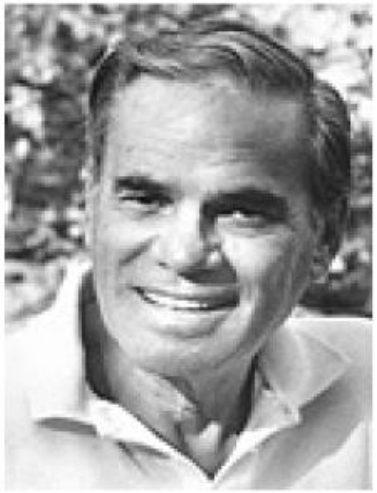
"for their discovery of G-proteins and the role of these proteins in signal transduction in cells"

Alfred G. Gilman

1/2 of the prize

USA

University of Texas
Southwestern Medical Center
Dallas, TX, USA



Martin Rodbell

1/2 of the prize

USA

National Institute of
Environmental Health Sciences

Research Triangle Park, NC,
USA

The Nobel Prize in Chemistry 2012
Robert J. Lefkowitz, Brian K. Kobilka

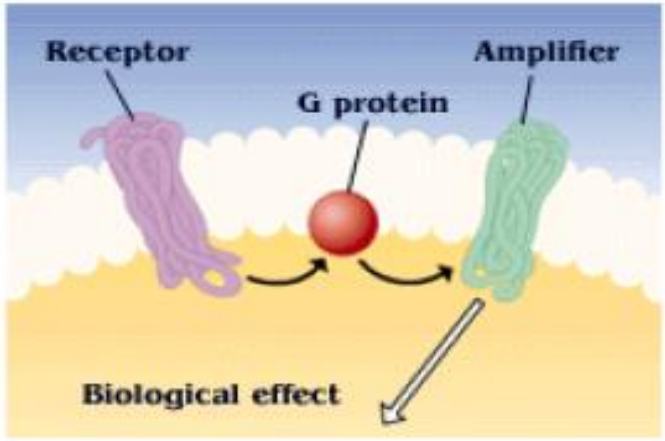
Robert J. Lefkowitz

Brian K. Kobilka

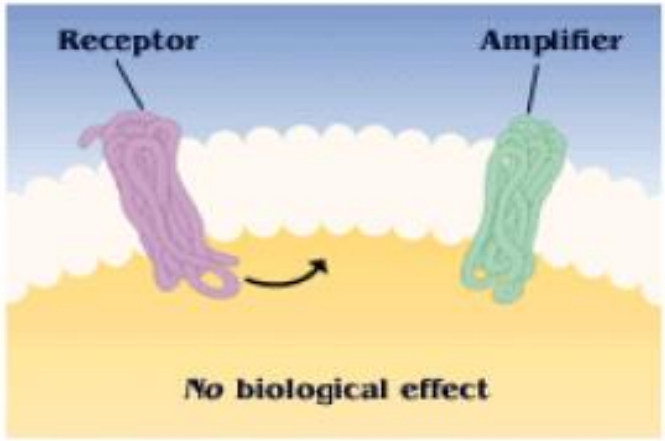
The Nobel Prize in Chemistry 2012 was awarded jointly to Robert J. Lefkowitz and Brian K. Kobilka *"for studies of G-protein-coupled receptors"*

By tracking the radiation emitted by the isotope, they succeeded in finding a receptor for adrenaline, which allowed them to build an understanding of how it functions.
It was later discovered that there is an entire family of receptors that look and act in similar ways - known as G-protein-coupled receptors.

The Discovery of G Proteins



Normal Lymphoma Cell

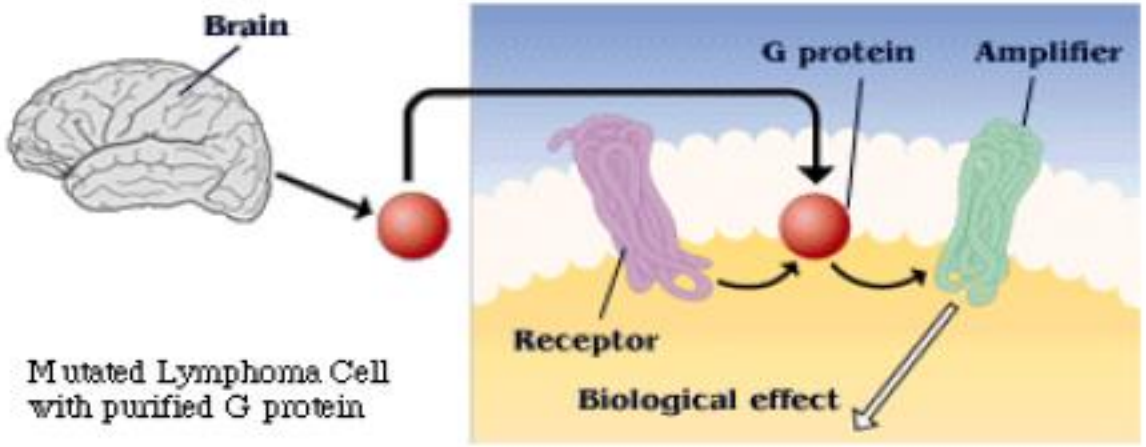


Martin Rodbell and his collaborators found that a transducer provided the link between the hormone receptor (the discriminator) and the amplifier. Alfred G. Gilman and his co-workers used genetic and biochemical techniques to identify and purify the G protein. They used lymphoma cells that normally can be activated by a receptor to form cyclic AMP.

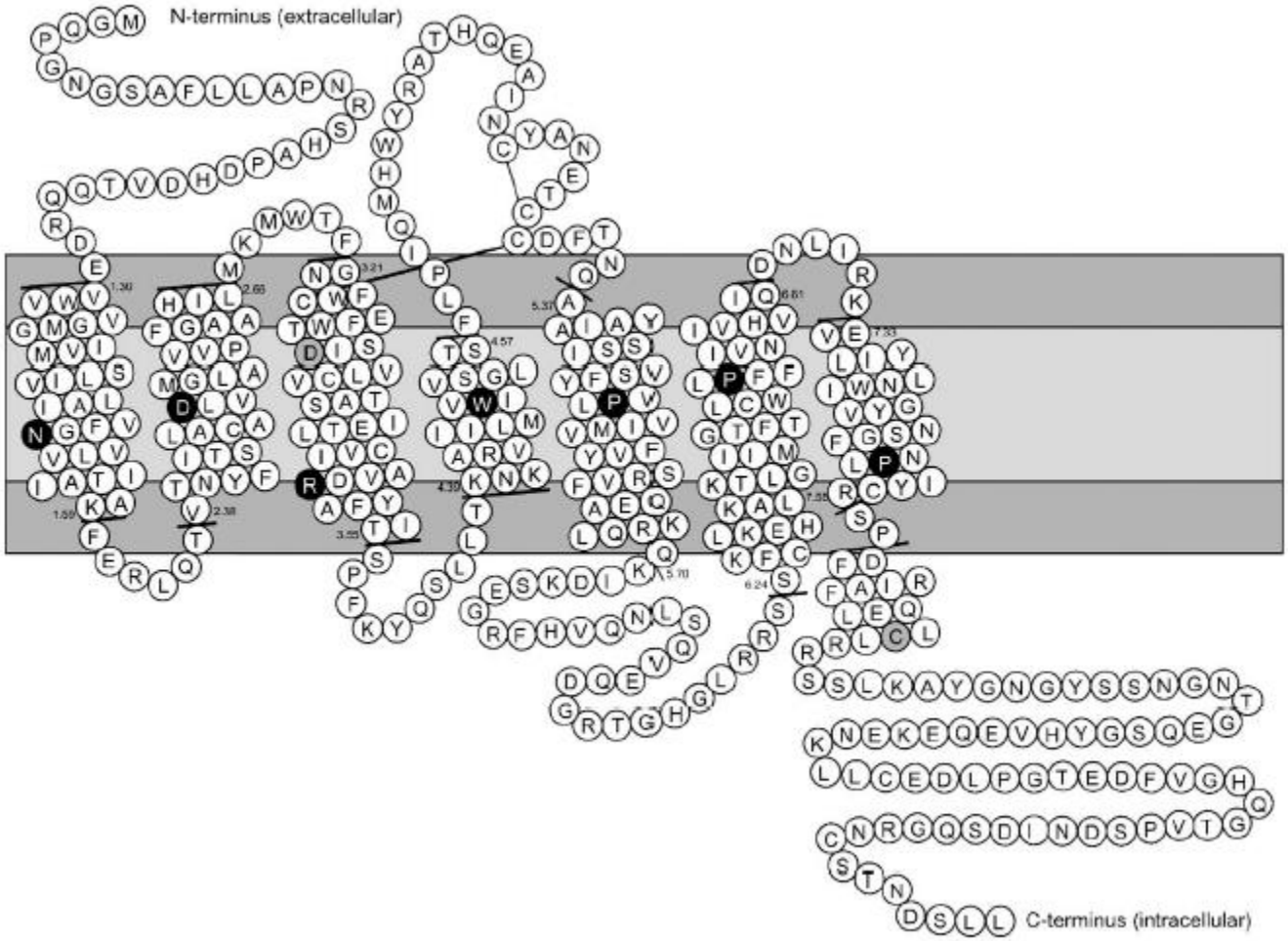
A mutated lymphoma cell was found to contain a normal receptor and a normal cyclic AMP-generating enzyme but was yet unable to respond because it lacked the transducer.
This was a good system to assay purified G proteins.

Mutated Lymphoma Cell

A G protein could be isolated from normal brain tissue and inserted in the mutated cell, thereby restoring its function.



What is GPCR? Definition?

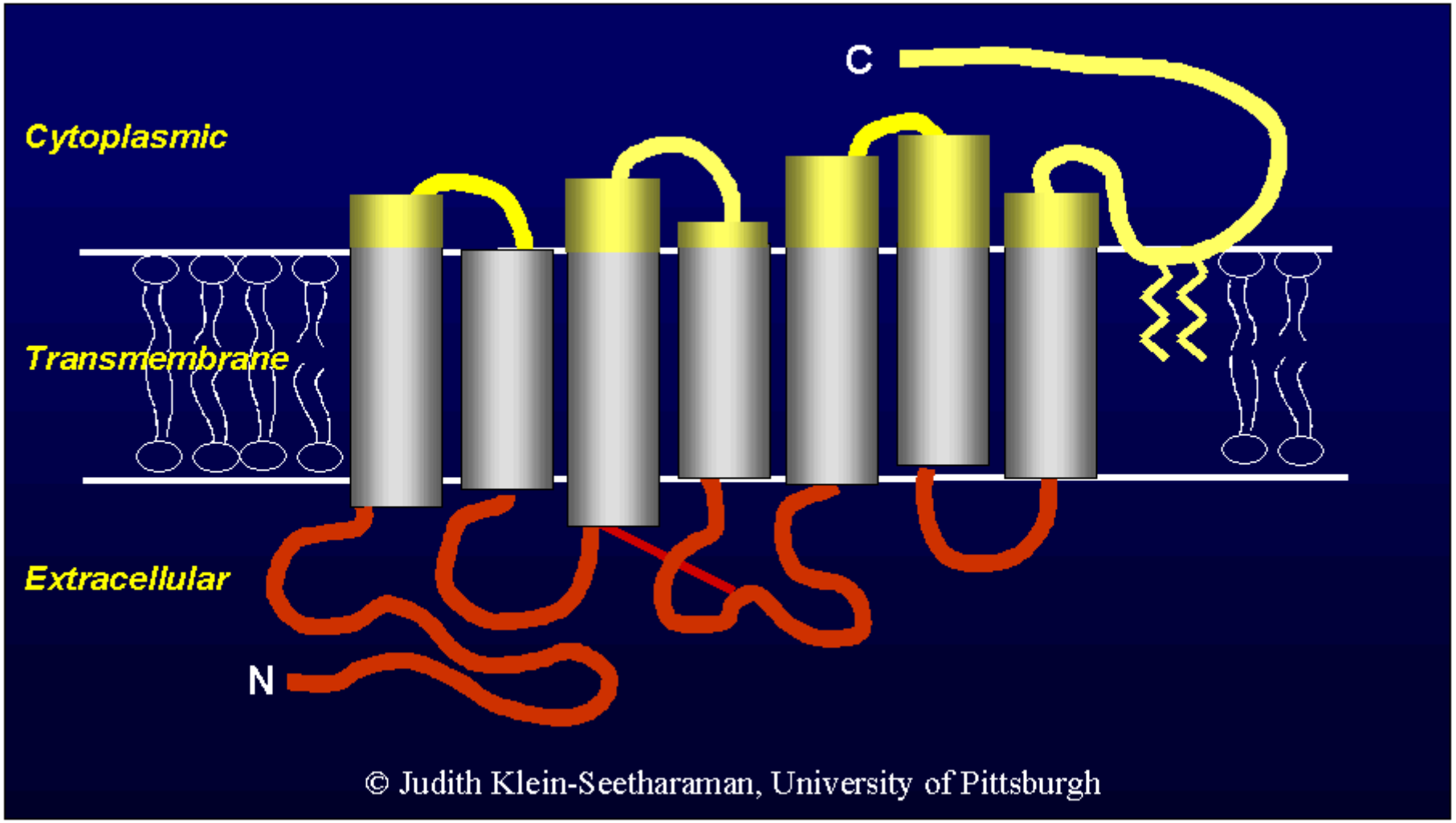


The largest family of plasma membrane–localized receptors is the superfamily of G protein–coupled receptors (GPCRs). GPCRs represent ≈1% of the human genome and are activated by an array of signals, from single photons to polypeptide hormones. Many receptors in the family are still "orphans," recognized in the genome by their characteristic serpentine, 7 transmembrane domain signature, but awaiting identification of their activating ligand.

Gerda E. Breitwieser

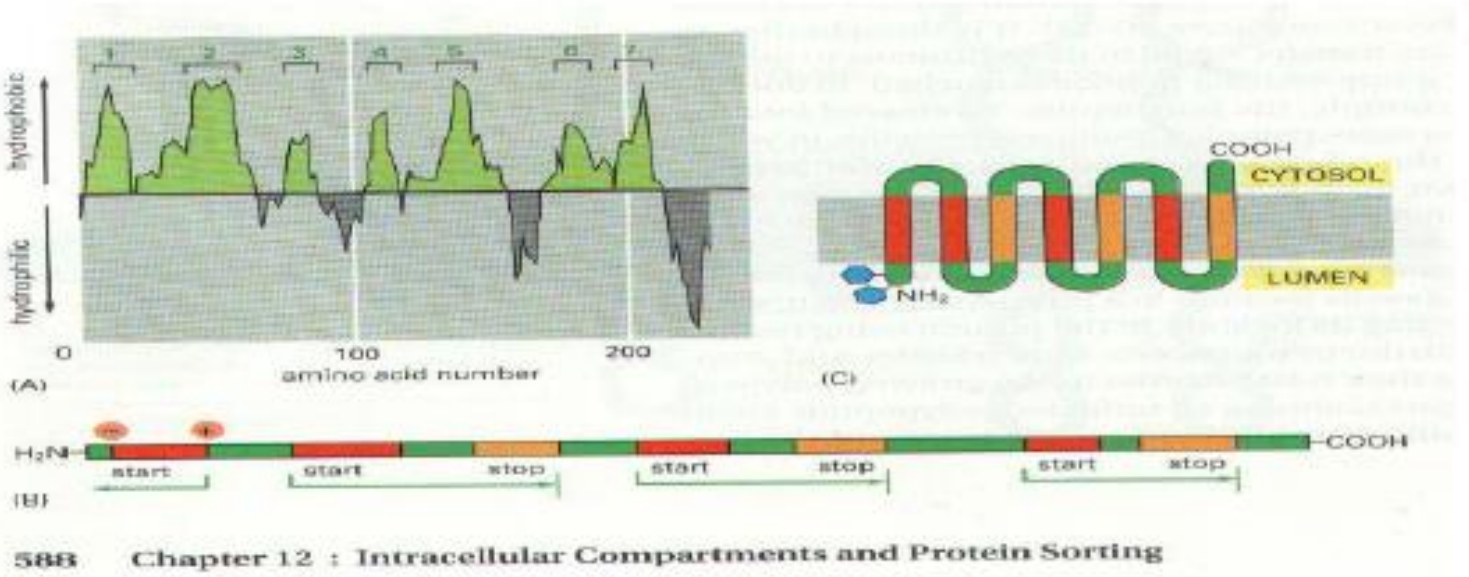
Circulation Research **January 9/23, 2004**

Why Seven Transmembranes?



Hydropathy Plot (profile) rhodopsin

(a 7-transmembrane protein)

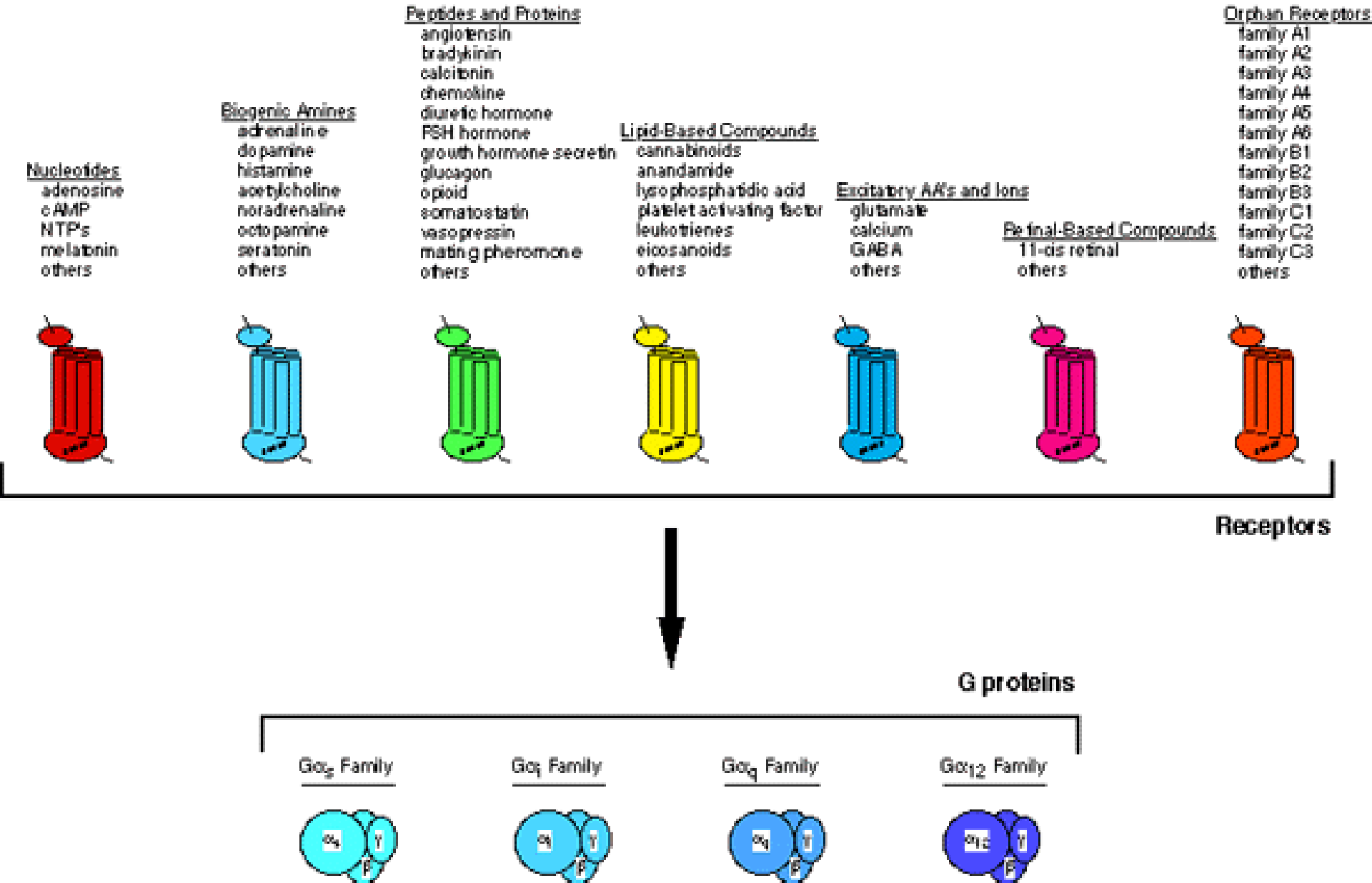


Hydropathy Index:

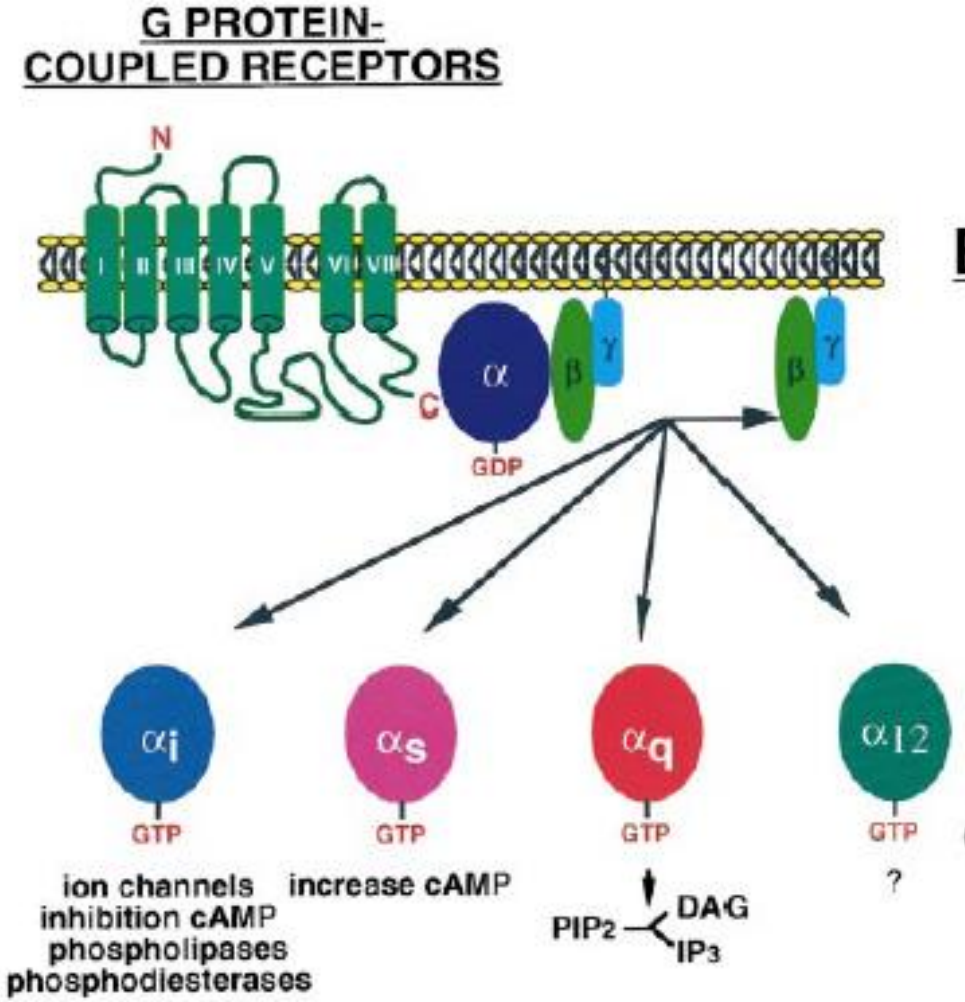
- Partition coeficient and others

Kyte and Doolittle (1982) J Mol Bio 157, 105-132.

Who are the ligands?



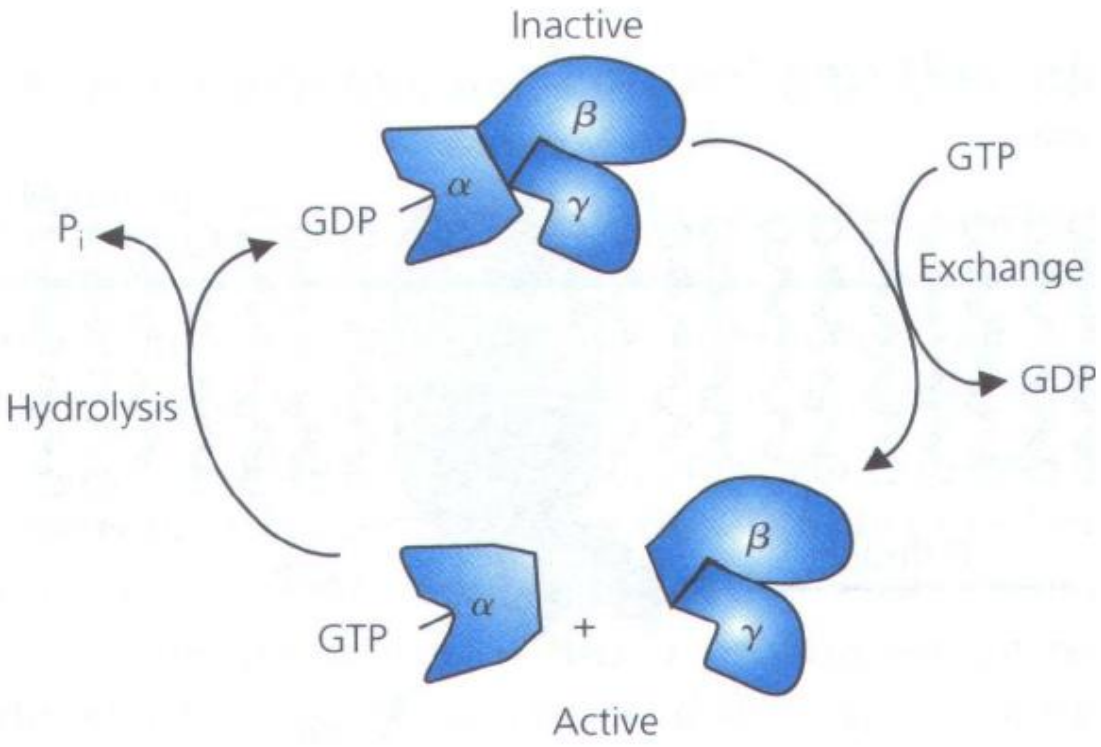
What are the downstream Signals ?



Biological functions

smell and taste
(~1000 types of receptors)
perception of light
neurotransmission
function of endocrine
and exocrine glands
chemotaxis
exocytosis
control of blood pressure
embryogenesis
development
cell growth and differentiation
HIV infection
oncogenesis

G-protein cycle

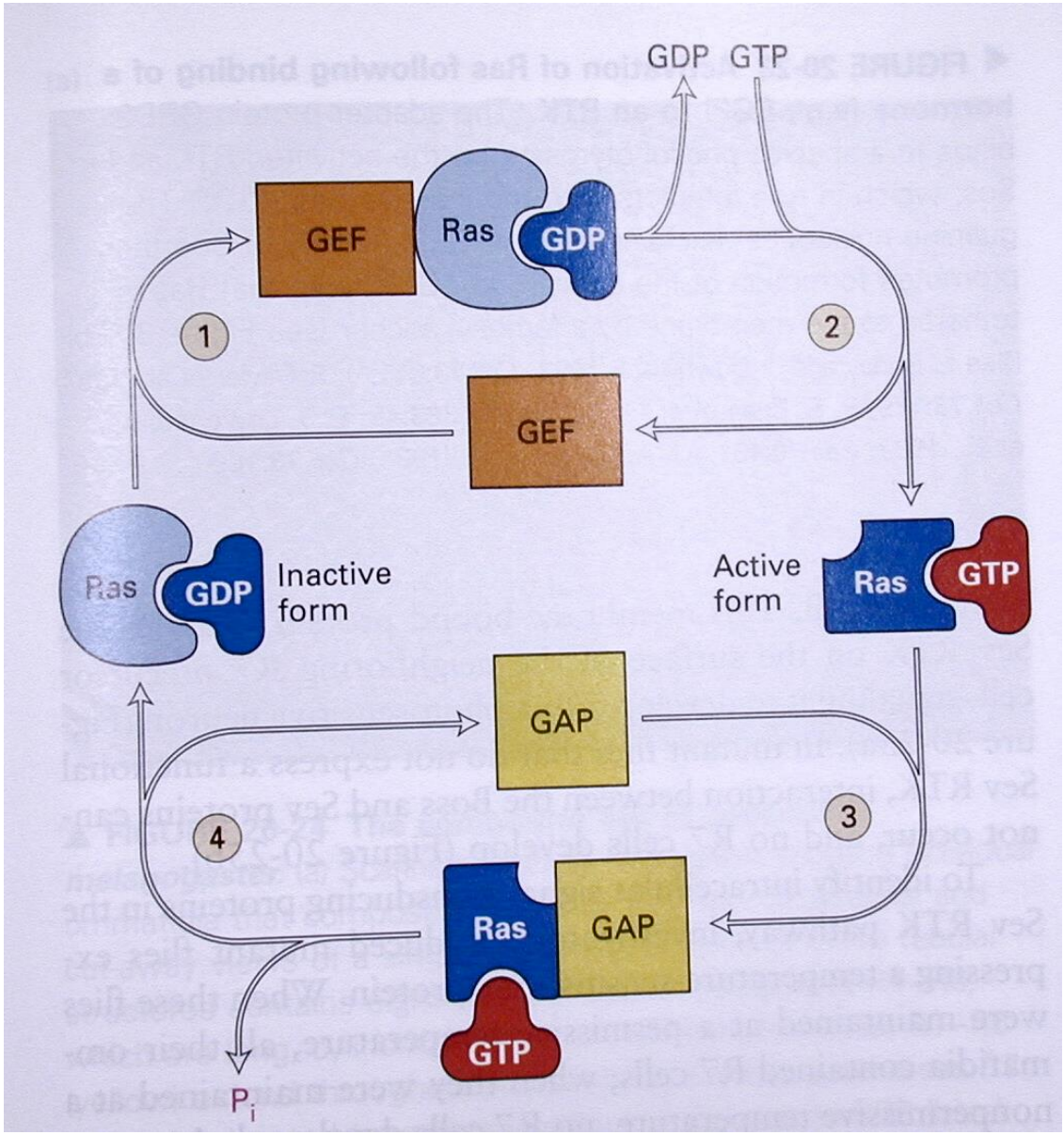


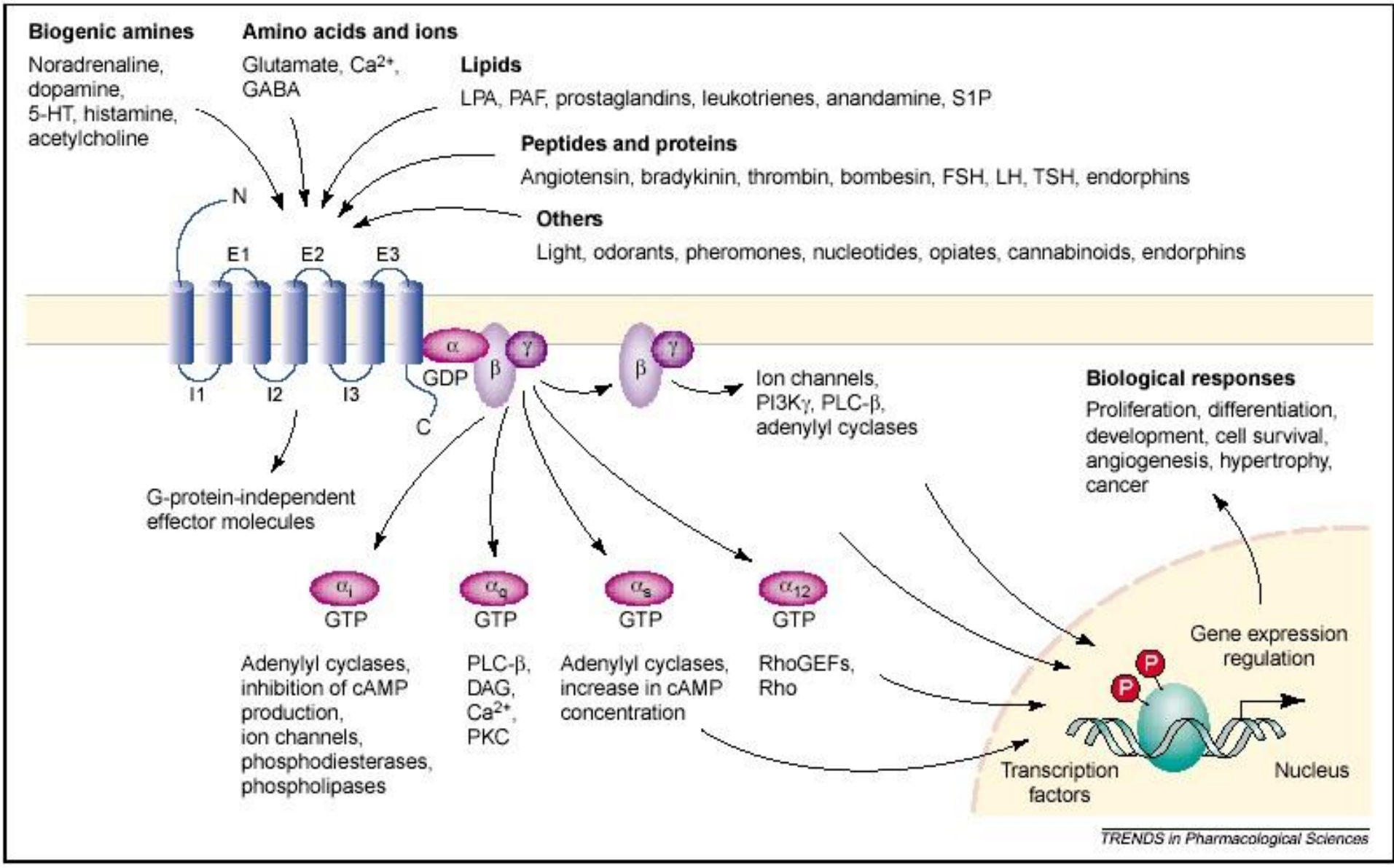
Discussion question 1

- Please compare the differences between **trimeric G proteins** with the **small G proteins (Ras)**.

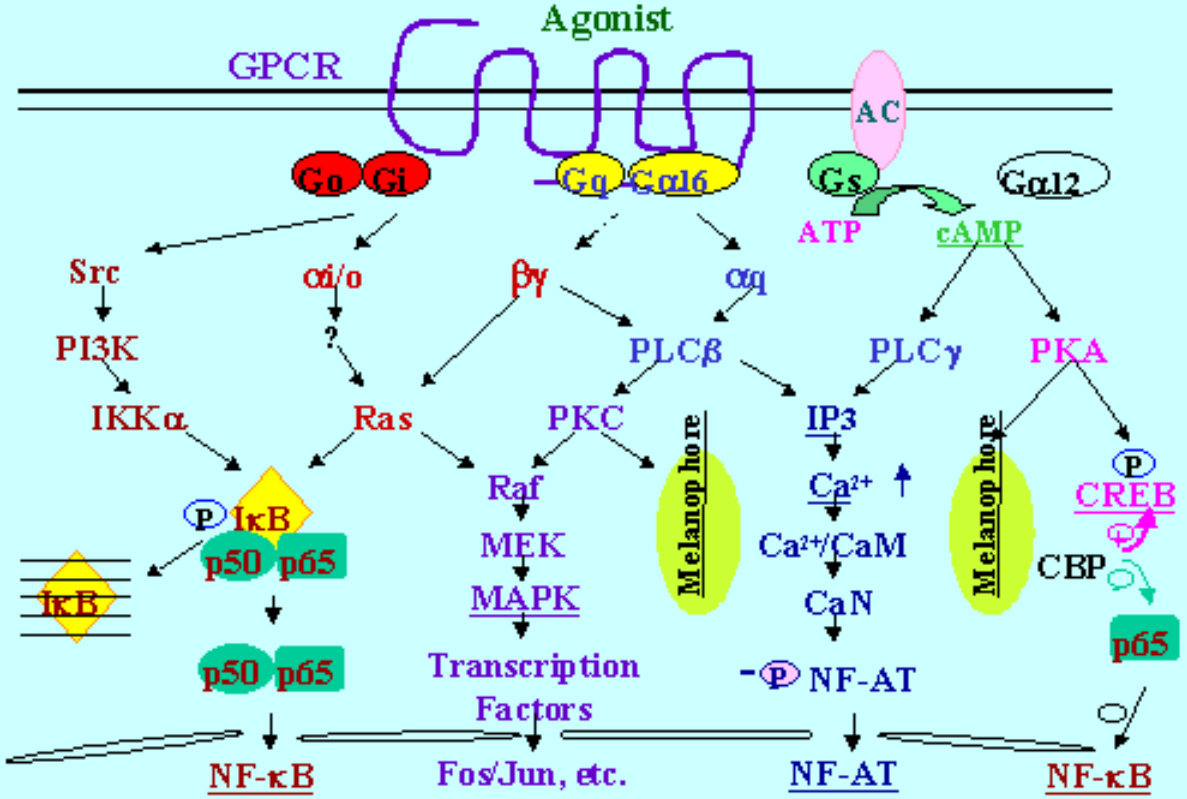
Answers

- Size
- Organization
- Termination signals: GAP vs internal activity
- Activation signals: GEF vs ligand bound receptor


GDP GTP
GEF
Ras
GDP
1
2
GEF
Ras
GDP
Inactive
form
Active
form
Ras
GTP
GAP
4
3
Ras
GAP
GTP
P_i


Biogenic amines
Noradrenaline, dopamine, 5-HT, histamine, acetylcholine
Amino acids and ions
Glutamate, Ca2+, GABA
Lipids
LPA, PAF, prostaglandins, leukotrienes, anandamine, S1P
Peptides and proteins
Angiotensin, bradykinin, thrombin, bombesin, FSH, LH, TSH, endorphins
Others
Light, odorants, pheromones, nucleotides, opiates, cannabinoids, endorphins
N
E1
E2
E3
I1
I2
I3
C
α
β
γ
GDP
Ion channels, PI3Kγ, PLC-β, adenylyl cyclases
Biological responses
Proliferation, differentiation, development, cell survival, angiogenesis, hypertrophy, cancer
G-protein-independent effector molecules
αi GTP
αq GTP
αs GTP
α12 GTP
Adenylyl cyclases, inhibition of cAMP production, ion channels, phosphodiesterases, phospholipases
PLC-β, DAG, Ca2+, PKC
Adenylyl cyclases, increase in cAMP concentration
RhoGEFs, Rho
Gene expression regulation
P
P
Transcription factors
Nucleus
TRENDS in Pharmacological Sciences

Proposed G Protein-Coupled Pathways for Chemokine Receptor Signaling



Nuclear Translocation and Regulation of Cytokine Gene Transcription

Reporter Gene Assays

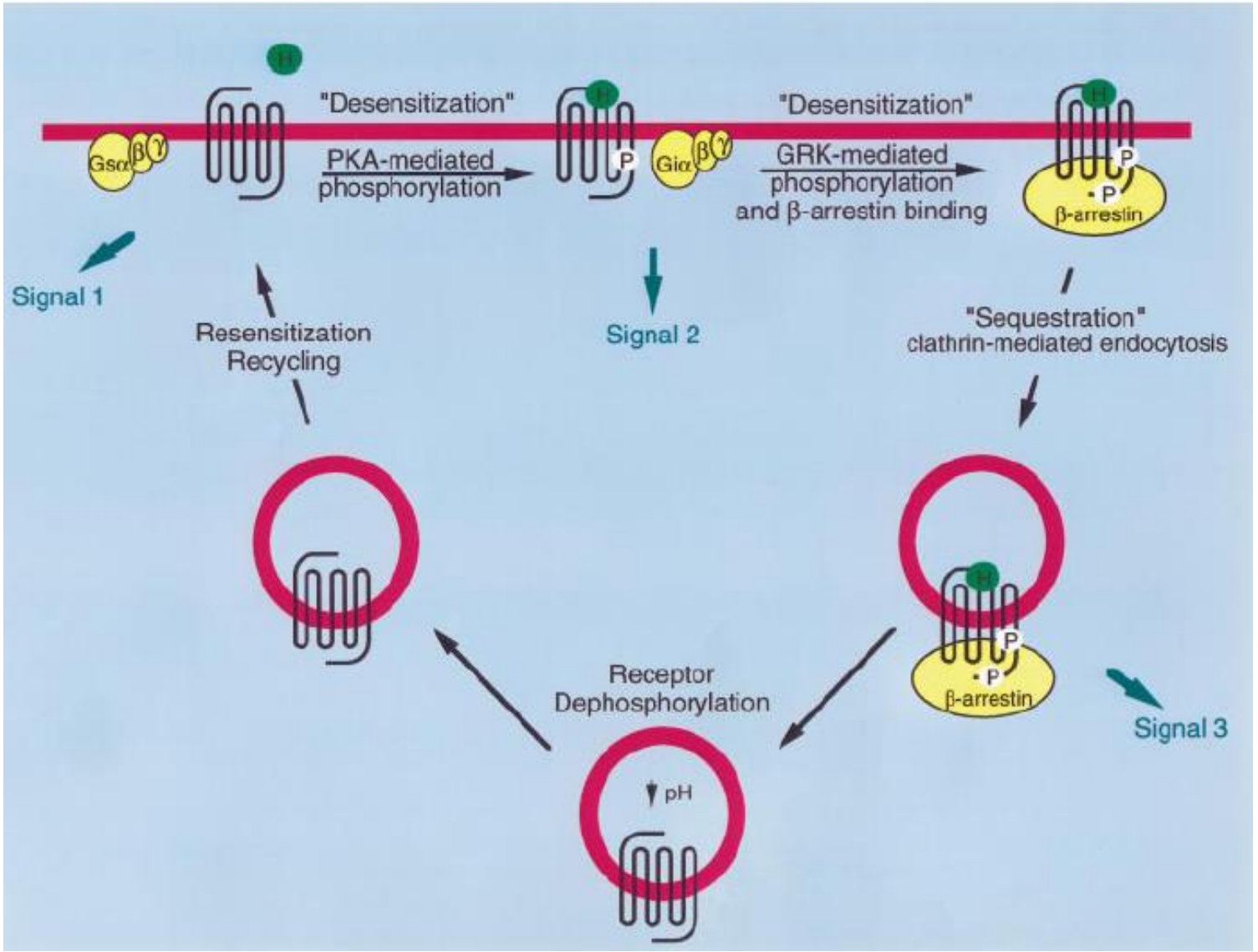
Matthew H. Hsu 10/5/98

Discussion question 2

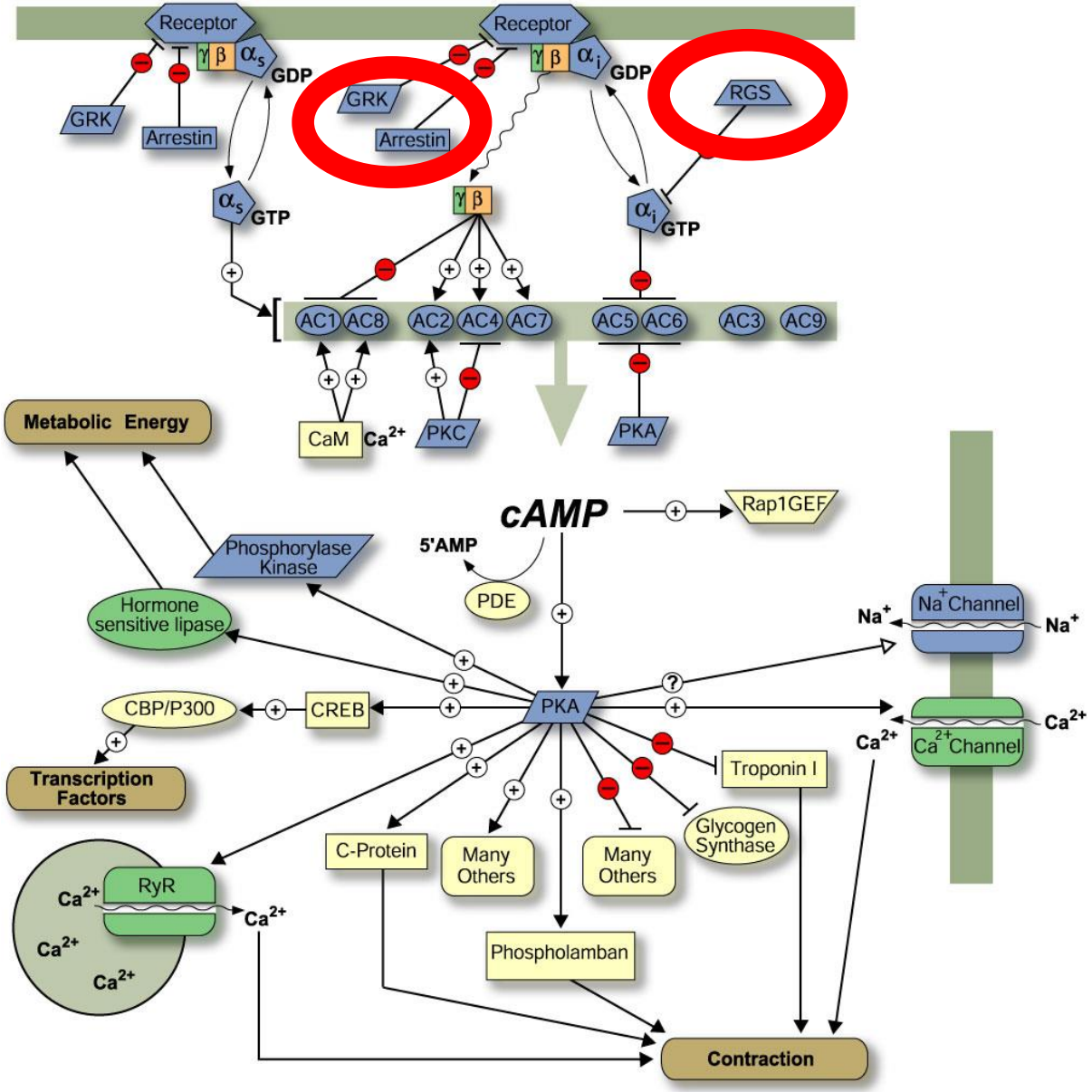
How to modulate the GPCR-coupled Signals ?

Walk or Stop

Multiple signaling roles of a GPCR illustrated for the β 2-adrenergic receptor



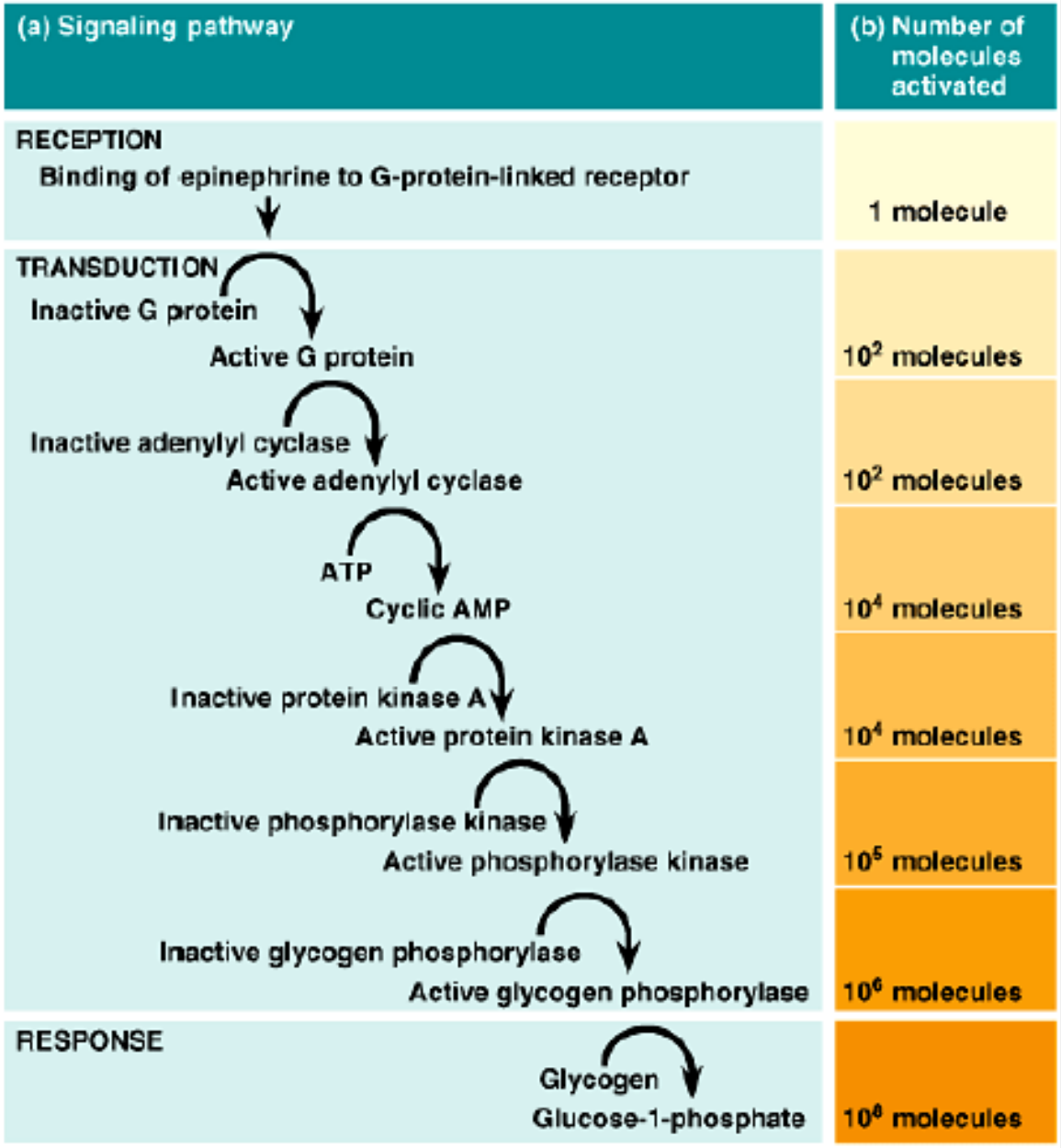
G Protein-Coupled cAMP Signaling Cascade



Discussion question 3

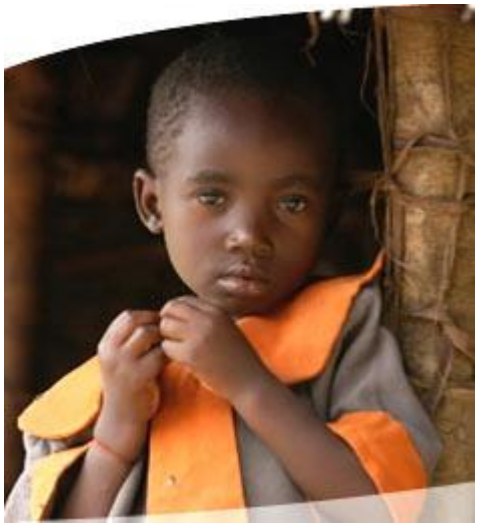
- Why second messenger system is necessary?

Amplification of the Signal



What is Orphan Receptor ?

→



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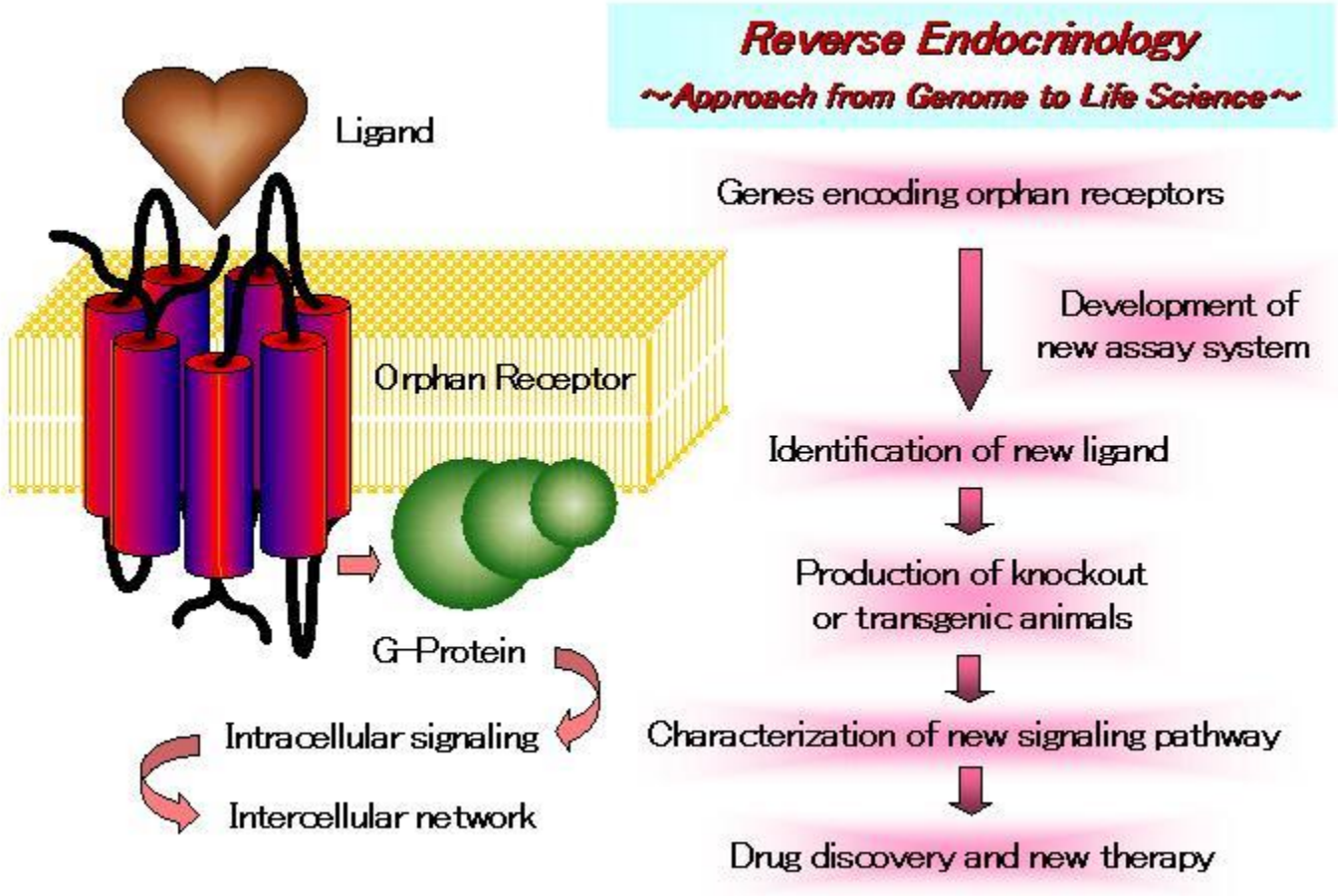
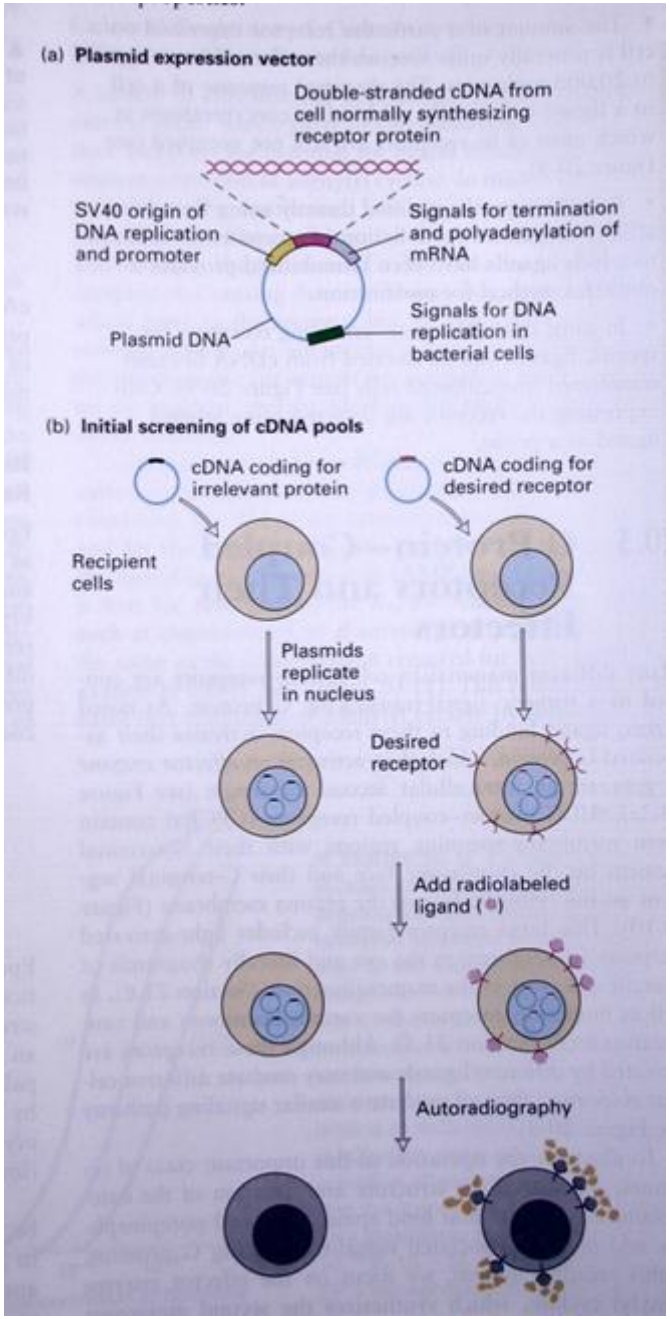
Human Genome Project **Orphan Receptor** **Try to Find out**

De-orphanization

Find their parents!!!!!

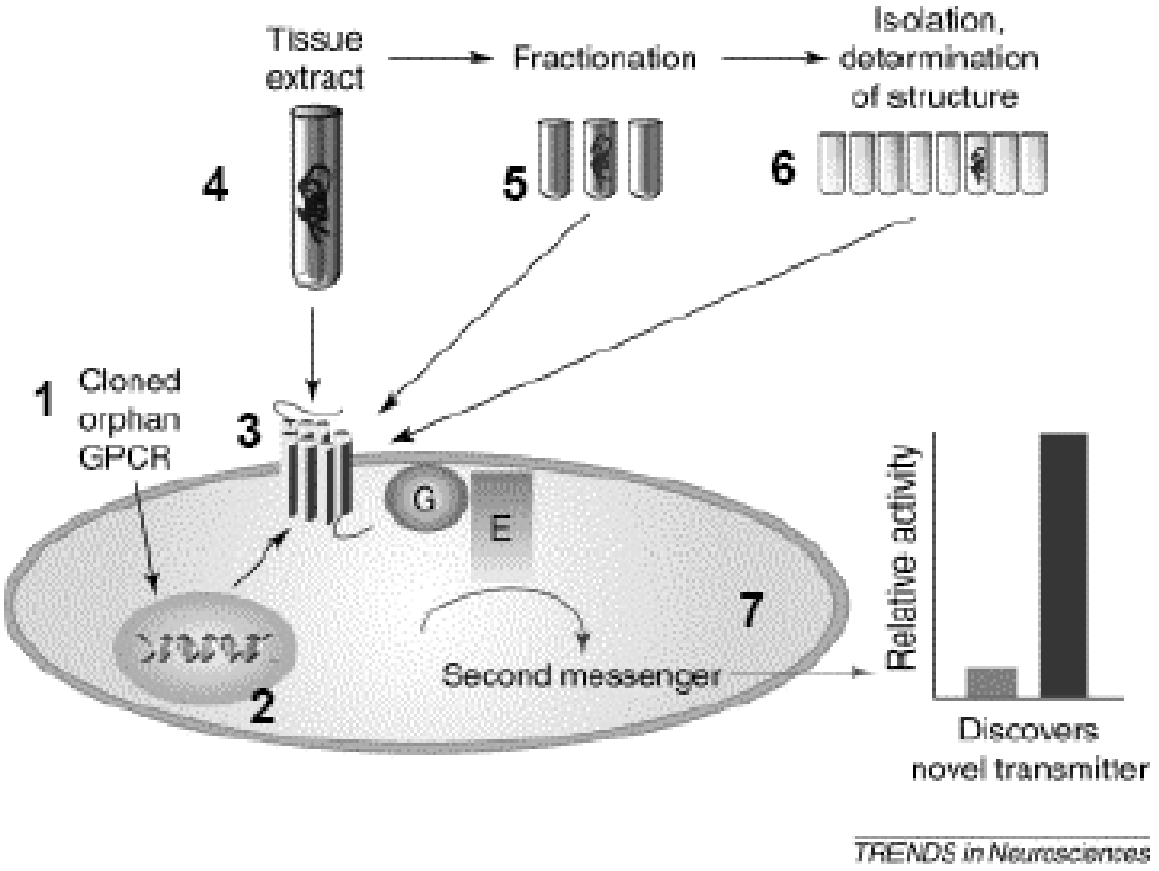


Expression cloning ~1990

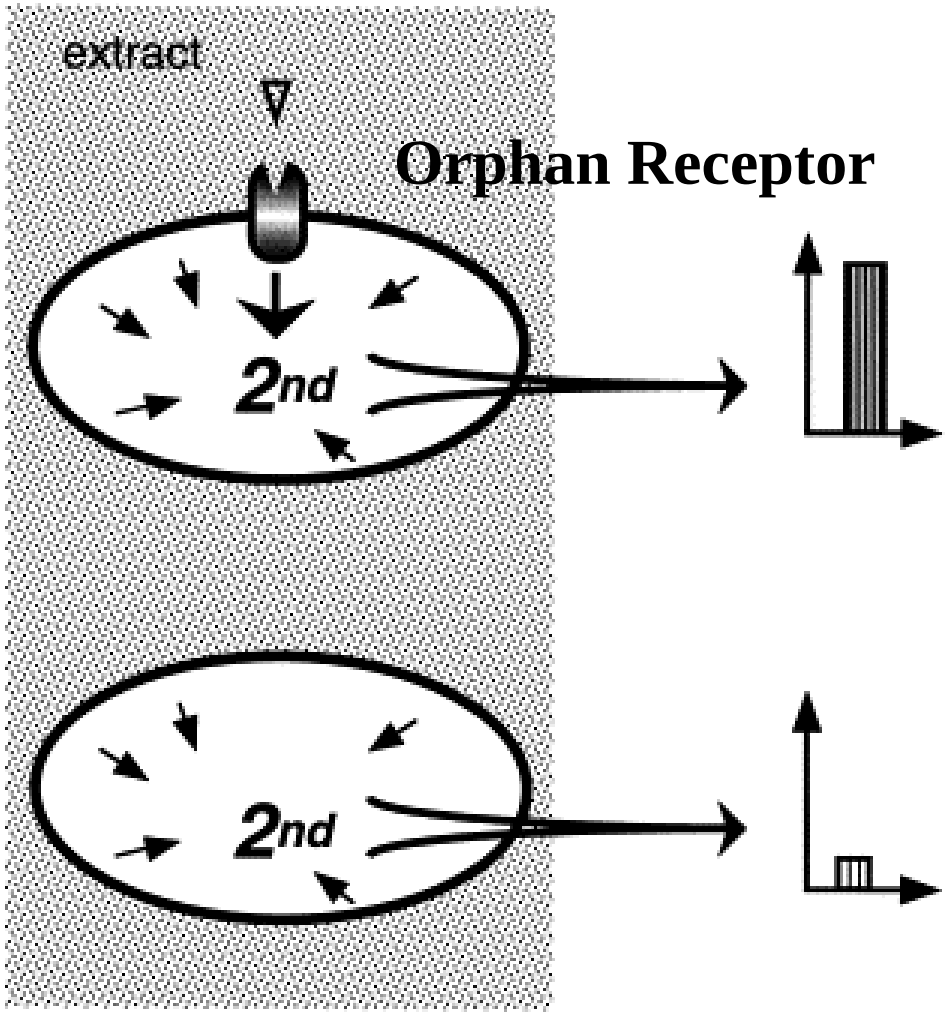


Reverse Pharmacology

~1995



GPCR deorphanizations



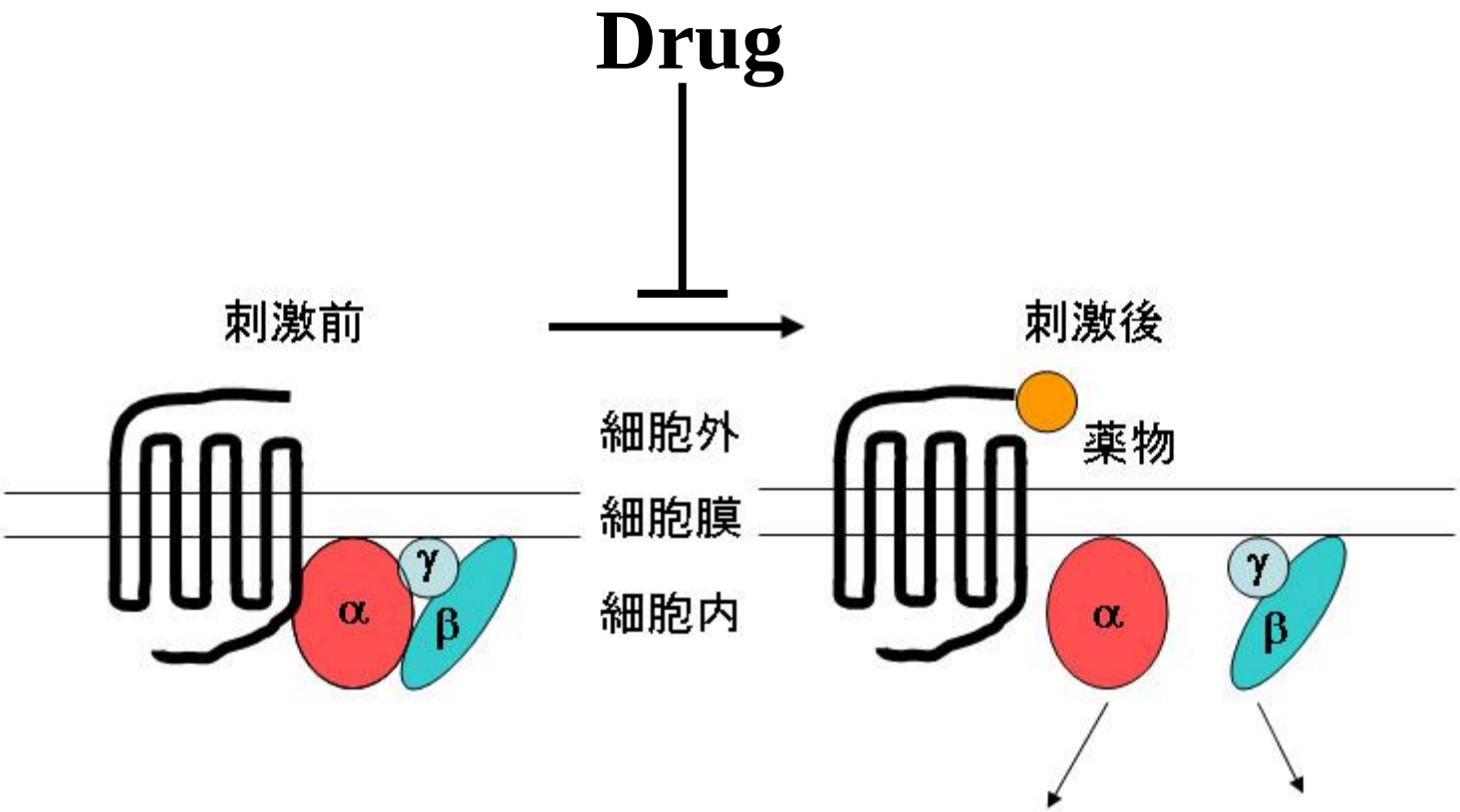
GPCR-based **Drug** Discovery

Table 1 Drugs targeting GPCRs

GPCR	*Generic*	*GPCR*	*Generic*
Acetylcholine	Bethanechol	Leukotriene	Pranlukast
	Dicyclomine		Zafirlukast
	Ipratropium		
		Opioid	Buprenorphin
Adrenoceptor	Atenolol		Butorphanol
	Clonidine		Alfentanil
	Propranolol		Morphine
	Terazosin		
	Albuterol	Prostaglandin	Epoprostenol
	Carvedilol		Misoprostol
Angiotensin II	Losartan	Somatostatin	Octreotide
	Eprosartan		
		Serotonin	Sumatriptan
Dopamine	Metoclopramine		Ritanserin
	Ropinirole		Cisapride
	Haloperidol		Trazodone
			Clozapine
Histamine	Dimenhydrinate		
	Terfenadine		
	Cimetidine		
	Ranitidine		

Table 2 Diseases associated with GPCR mutations

Receptor	*Disease*
Rhodospin	Retinitis pigmentosa
Thyroid stimulating hormone	Hyperfunctioning thyroid adenomas
Lutenizing hormone	Precocious puberty
Vasopressin V_2	X-linked nephrogenic diabetes
Calcium	Hyperparathyroidism, hypocalciuria, hypercalcemia
Parathyroid hormone	Short limbed dwarfism
β_3-Adrenoceptor	Obesity, NIDDM
Growth hormone releasing hormone	Dwarfism
Adrenocorticotropin	Glucocorticoid deficiency
Glucagon	Diabetes, hypertension


Drug
刺激前
刺激後
細胞外
細胞膜
細胞内
薬物
α
β
γ
α
β
γ

GPCRs / RTKs Communication

Why we choose this paper for discussion?

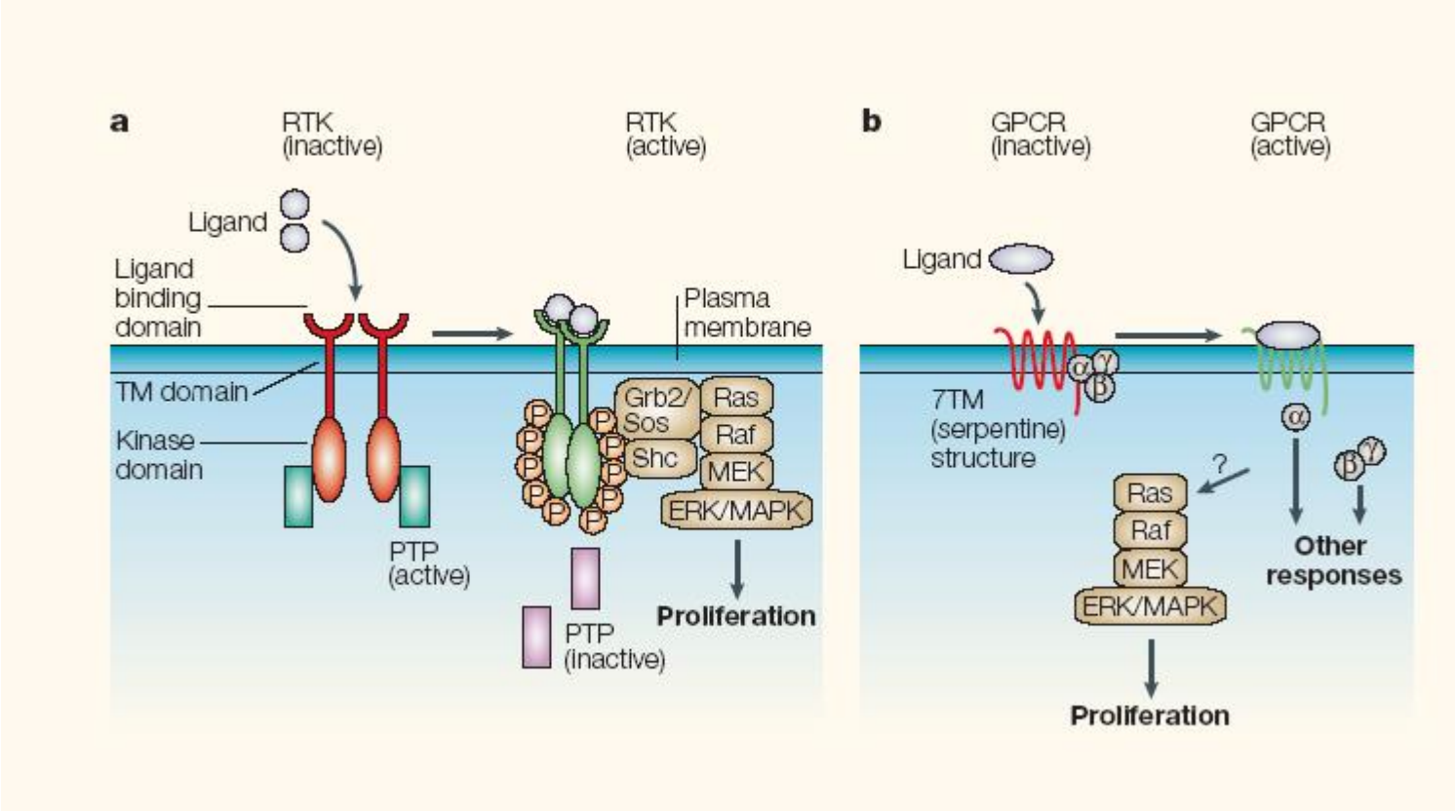
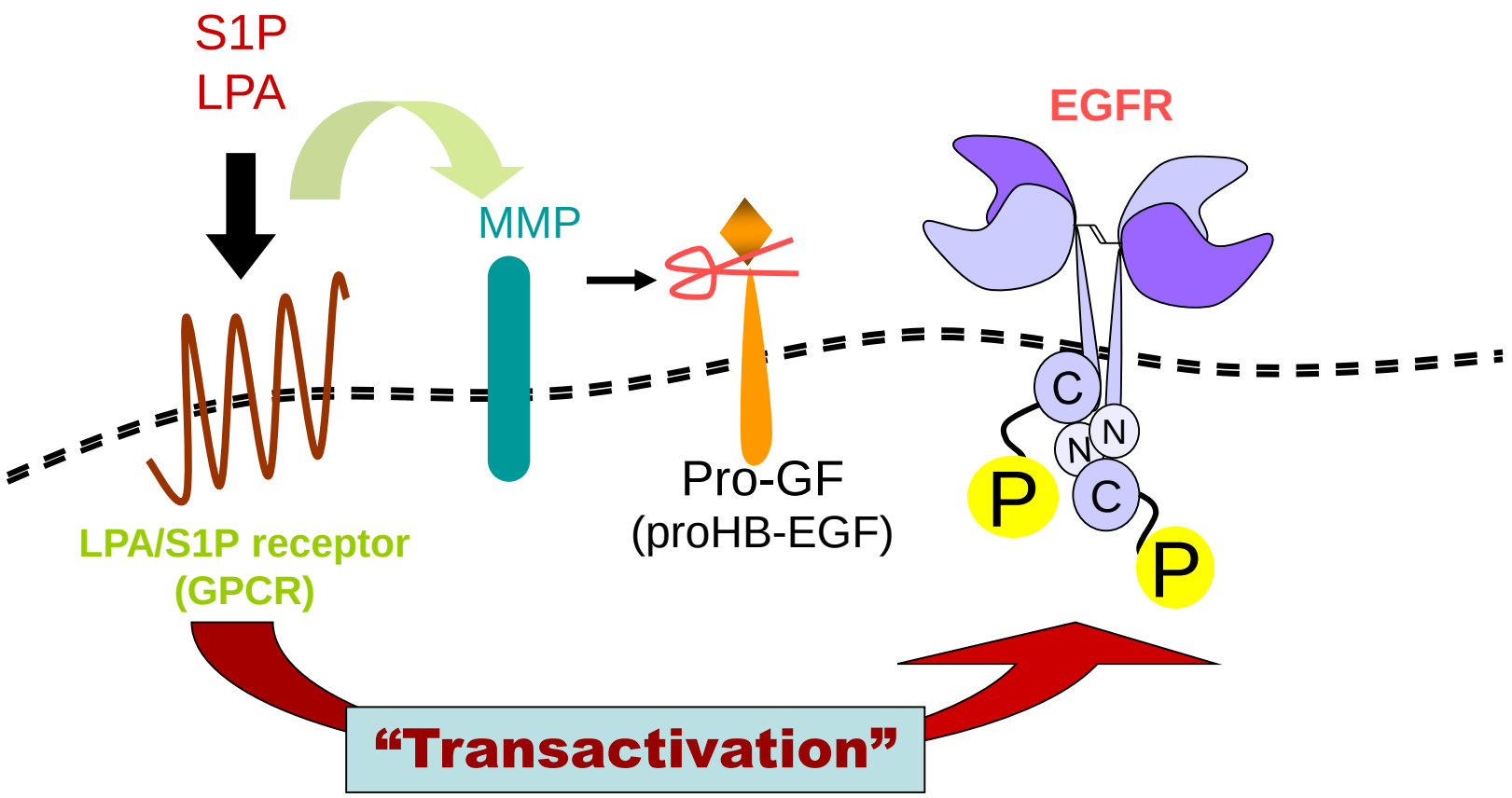


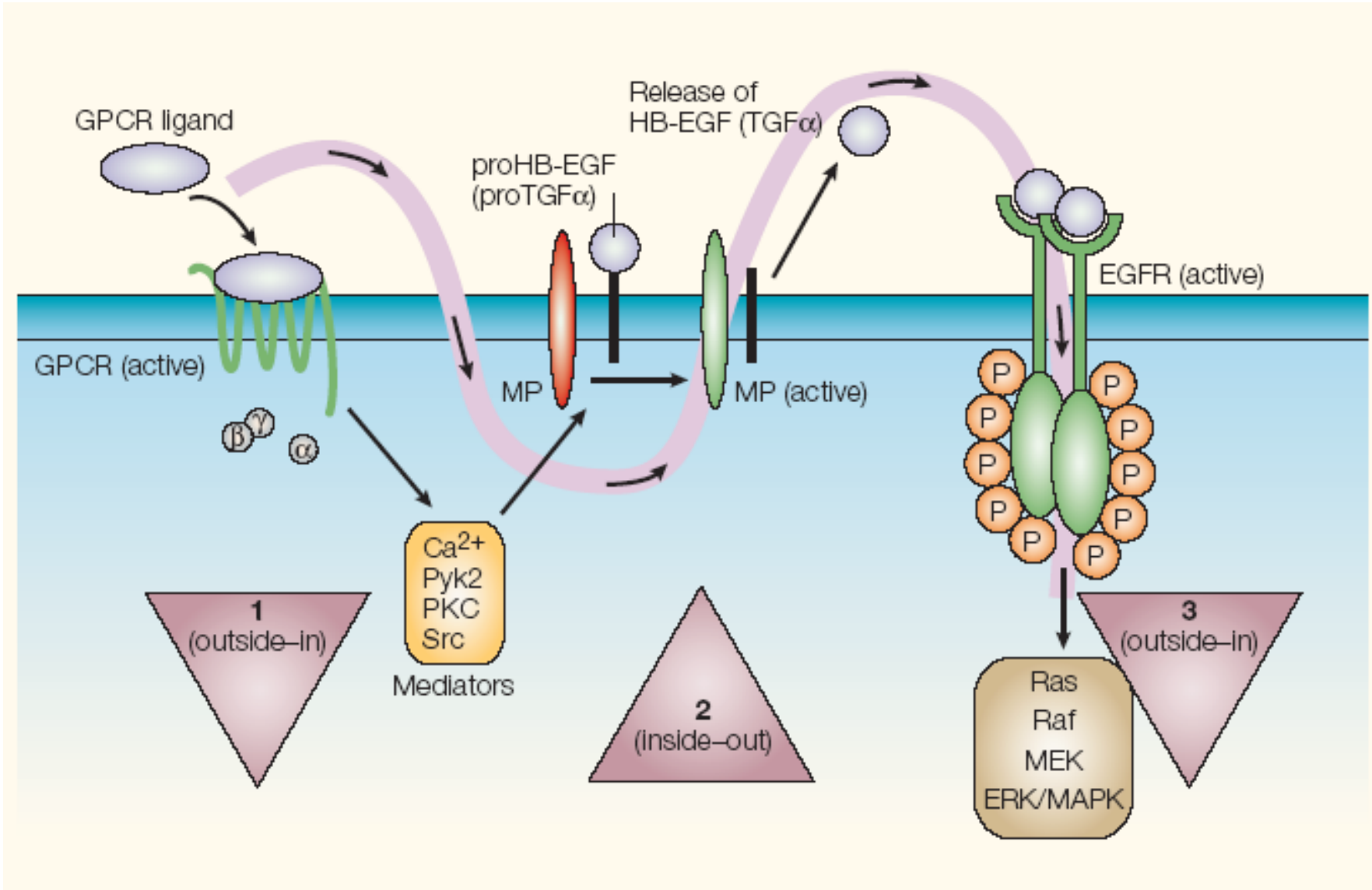
Table 1 | EGFR activation by different agents*

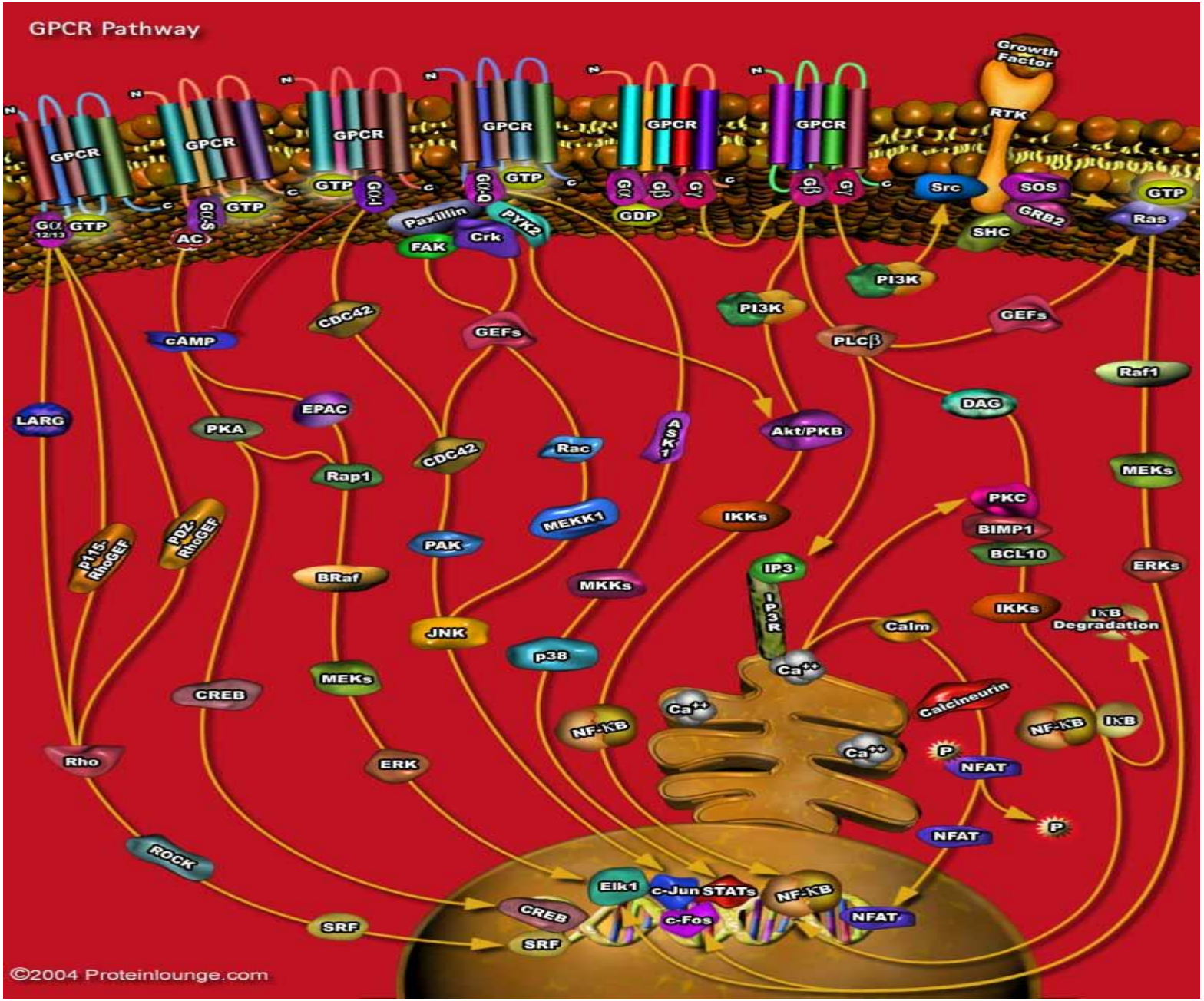
Agent	Cell type	References
Stress factors		
Ionizing radiation	A431 cells	5,20
Oxidants	EGFR-transfected B82 L-cells HeLa cells Rat-1 cells	4
UV radiation	EGFR-transfected B82 L-cells	4
G-protein-coupled receptor agonists		
Angiotensin II	Cardiomyocytes	14
ATP	Primary mouse astrocytes	13
Bombesin	Bombesin-receptor-transfected COS-7	13
Bradykinin	PC12 rat pheochromocytoma cells Bradykinin type-2-receptor transfected COS-7 cells	52 41
Carbachol	M1R- and M2R-transfected COS-7	13
Endothelin	Rat-1 fibroblasts Cardiomyocytes	6 14
Lysophosphatidic acid	Rat-1 fibroblasts Mouse embryo fibroblasts Squamous-cell carcinoma lines	6 38 15
Phenylephrine	Cardiomyocytes	14
Thrombin	Rat-1 fibroblasts Cardiac fibroblasts Cardiac myocytes	6 42 45
Other agents		
Helicobacter pylori	Gastric epithelial tumour cells	19
Integrin ligands	Human primary skin fibroblasts ECV304 endothelial cells	53
IGF-1	Mammary epithelial cells	51
Phorbol ester	JB6 P+ (1-1) cells Mouse embryo fibroblasts	54

*Direct ligands of the EGFR are not included. EGFR, epidermal growth factor receptor; IGF-1, insulin-like growth factor 1; M1R and M2R, muscarinic receptors 1 and 2; UV, ultraviolet. An extended version of this table can be found as a supplement to the online version of this article.

Transactivation




GPCR ligand
GPCR (active)
β
γ
α
proHB-EGF
(proTGFα)
MP
Release of
HB-EGF (TGFα)
MP (active)
EGFR (active)
P
Ca2+
Pyk2
PKC
Src
Mediators
1
(outside–in)
2
(inside–out)
3
(outside–in)
Ras
Raf
MEK
ERK/MAPK


GPCR Pathway
Growth Factor
RTK
GPCR
GTP
GDP
Gα 12/13
Gαs
AC
Gαi
Gαq
Gβ
Gγ
Src
SOS
GRB2
SHC
Ras
Paxillin
PYK2
FAK
Crk
PI3K
PLCβ
GEFs
cAMP
CDC42
LARG
EPAC
PKA
Rap1
Rac
ASK1
Akt/PKB
Raf1
DAG
MEKs
PKC
BIMP1
BCL10
ERKs
IKKs
IκB Degradation
IκB
NF-κB
MEKK1
PAK
BRaf
MKKs
JNK
p38
IP3
IP3R
Calm
Ca++
Calcineurin
P
NFAT
p115-RhoGEF
PDZ-RhoGEF
Rho
ROCK
CREB
ERK
SRF
Elk1
c-Jun
STATs
c-Fos
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References

- http://www.cs.cmu.edu/~blmt/SeminarMaterials/GPCR.html

GPCR deorphanizations: the novel, the known and the unexpected transmitters

TRENDS in Pharmacological Sciences Vol.26 No.1 January 2005

Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function

Pharmacology & Therapeutics 103 (2004) 21–80