INTRACELLULAR SIGNALLING

R. Benacka, MD, PhD
Department of Pathophysiology
Medical faculty, Safarik University

General section
Fundamentals of signaling between cells

Introduction

- Drugs act by modifying the extracellular environment, influencing intracellular structures or metabolism, or influencing signaling cascades
- The concept of humoral signaling – the unification of various signal transmissions at the cellular level
- Hormone, mediator, transmitter - the first messenger
- Second, third, (fourth) messenger inside the cell
- Receptor theory - agonists, antagonists
- Development of selective drugs
- Pathogenesis of diseases and pathological conditions

Mozog, PNS, ANS
β-Endorfín (31), Dopamín (DA), Serotonín, Noradrenalín, Acetylcholín, LEK (5), MEK(5)

Epifýza Melatonín, Serotonín

Hypotalamus
TRH(3), GnRH(41), SS (28), GH-RH (40), CRH (41), PRF(20), DA

Adenohypofýza
ACTH (31), TSH (204), GnRH, FSH (209), LH (213), Prolaktín (199), STH (191), β-Endorfín (31), αMSH (13), LEK, MEK

Neurohypofýza
Vazopresín (9), Oxytocín (9)

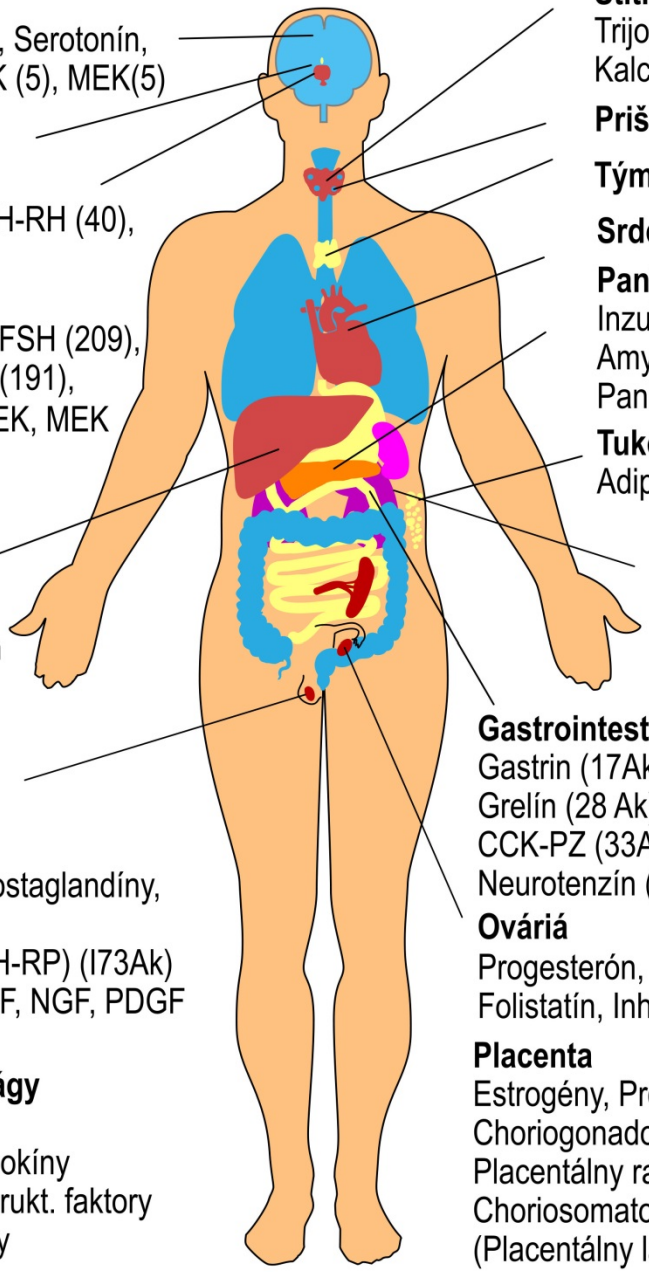
Pečeň IGF1(70), IGF2 (67)

Nadoblička
Aldosterón, 17-OH-progesterón
Kortizol, DHEA, DHEAS,
Androstendión, Katecholamíny

Testes Testosterón, Inhibín B

Mnohé typy buniek
Estrogény, Galanín (30 Ak), Prostaglandíny, Leukotriény, Tromboxány,
Parathormónu-podobný h. (PTH-RP) (I73Ak)
Rastové faktory: EGF, FGF, TGF, NGF, PDGF HRF, VEGF

Monocyty/ lymfocyty/makrofágy
Cytokíny
- interleukíny, interferóny, chemokíny
- tumor nekrotizujúci faktor, inštrukt. faktory
- monikíny, lymfokíny, neurokíny



Štítna žľaza
Trijodotyronín (T3), Tyroxín (T4), Kalcitonín (32)

Prištítne žľazy Parathormón (84)

Týmus Tymozín (28), Tymopoetín (49)

Srdce ANP (28), BNP)(32)

Pankreas
Inzulín (51 Ak), Glukagón (29 Ak), Amylin (37 Ak), Somatostatín (14 Ak)
Pankreatický polypeptid (39 Ak)

Tukové tkanivo
Adiponektín (224), Leptín (167), Rezistín (94)

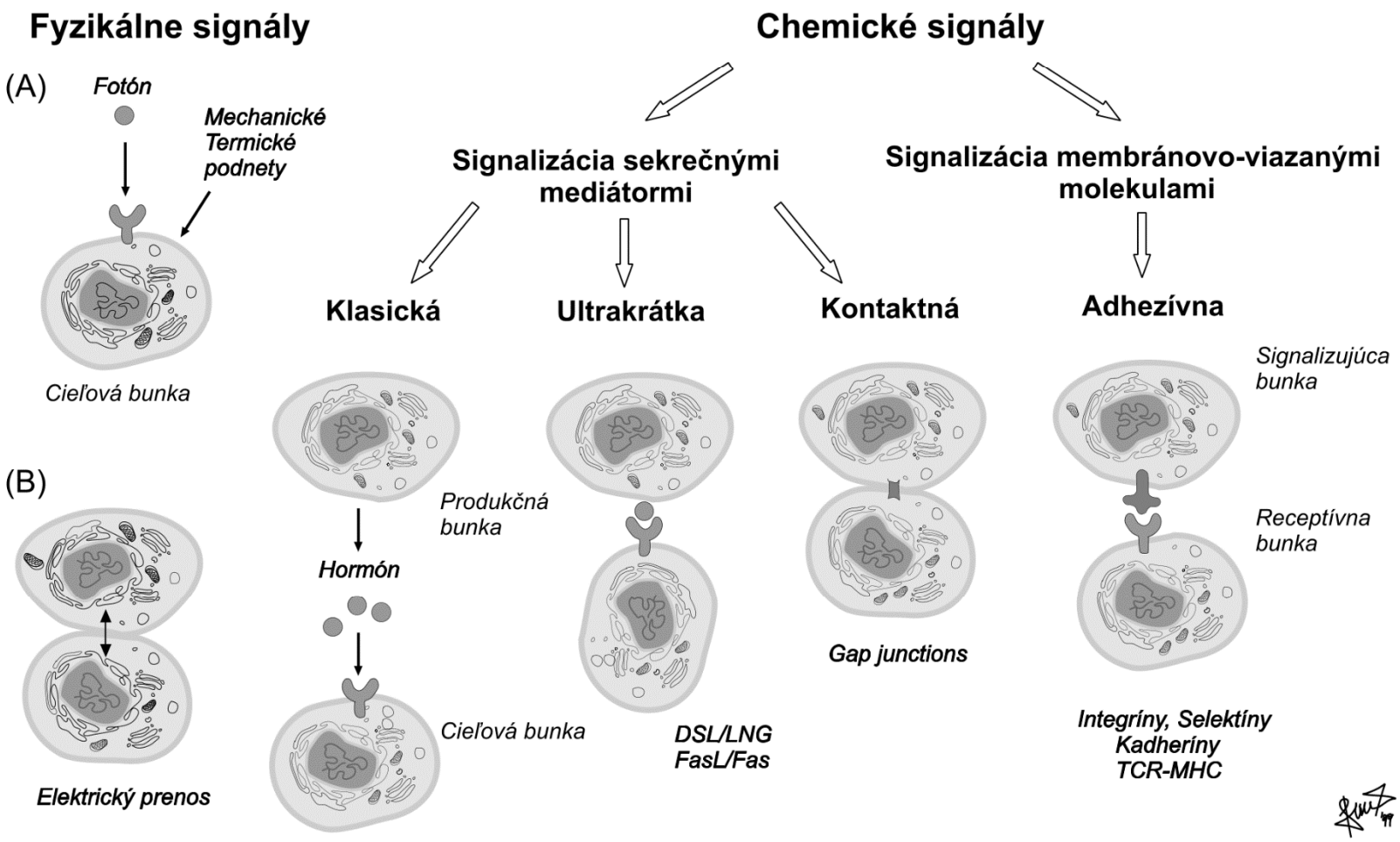
Obličky
1,25-$(OH)_2$ cholecalciferol
Erytropoetín (165), Renín (297) → Ang1 (10) → Ang2 (8)

Gastrointestinálny trakt
Gastrin (17Ak, 34 Ak), Motilín (22Ak), VIP (28 Ak), Grelín (28 Ak), Sekretín (27 Ak), Bombezín (14Ak), CCK-PZ (33Ak), GIP (42 Ak), GLP (31 Ak), Neurotenzín (13 Ak), SP (látka P) 11Ak)

Ovária
Progesterón, Estriadiol, DHEA, Relaxín
Folistatín, Inhibín A a B, Aktivín A a B

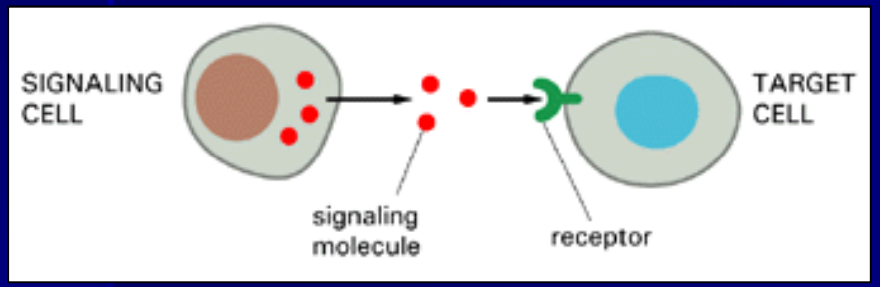
Placenta
Estrogény, Progesterón, Relaxín
Choriogonadotropín (CG)
Placentálny rastový hormón (GH-V)
Choriosomatomamotropín (CS) (191 Ak)
(Placentálny laktogén (PL))

Formy extracelulárnych signálov



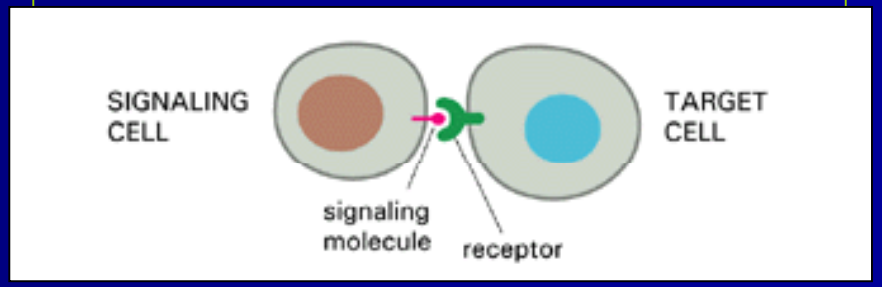
Humoral signaling

Signaling by secretory molecules

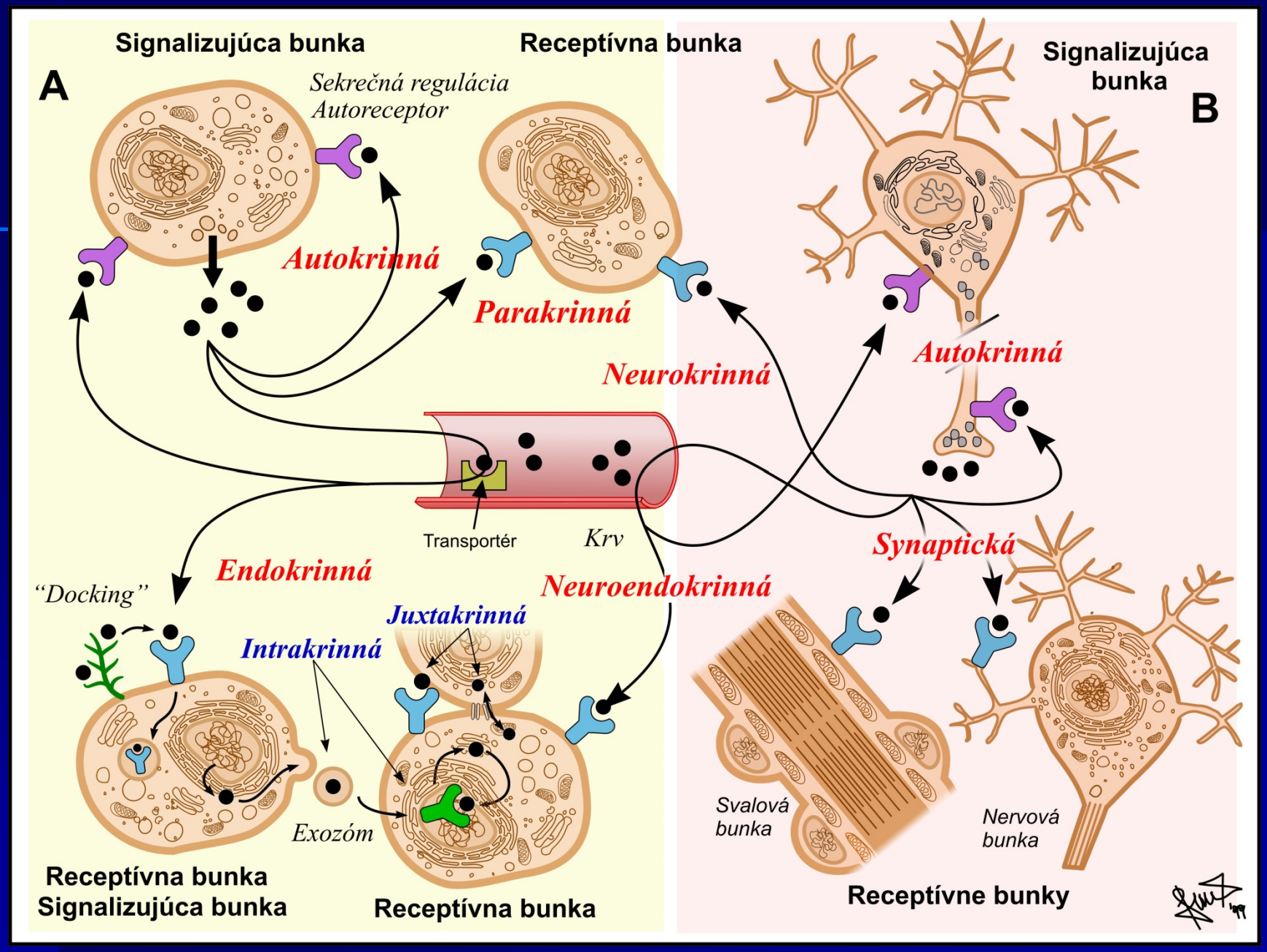


- The mediator is secreted by the producing cell into the environment
- The mediator travels different distances and through different environments to the target cell (blood, interstitium, axon)
- The target cell has a recognition receptor

Signaling by membrane-bound molecules (contact)

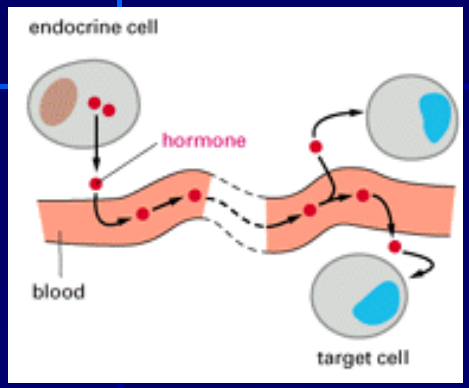


- The mediator is fixed directly/indirectly to the cytoplasmatic membrane
- The mediator reaches the target cell when approaching the product. cells
- The target cell has a recognition receptor


A
Signalizujúca bunka
Receptívna bunka
Sekrečná regulácia
Autoreceptor
Autokrinná
Parakrinná
Neurokrinná
Transportér
Krv
Endokrinná
"Docking"
Neuroendokrinná
Juxtakrinná
Intrakrinná
Exozóm
Receptívna bunka
Signalizujúca bunka
Receptívna bunka
B
Signalizujúca bunka
Autokrinná
Synaptická
Svalová bunka
Nervová bunka
Receptívne bunky

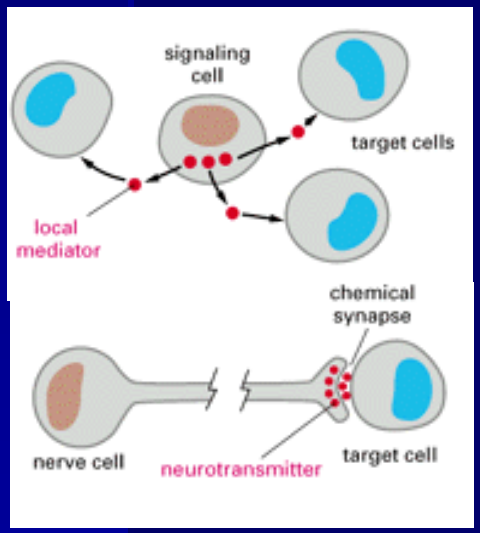
Signaling by secretory moleculesmi

Endokrine



- The substance secreted by the production cell penetrates into the circulation, acts on distant or nearby targets
  - Examples: insulin, glucagon, sexual hormones, corticoids
- **Neuroendocrine signaling** - production cells are neurons; the mediator is excreted into the circulation
  - Examples: Vasopressin, oxytocin, hypothalamic effects - statins, liberins

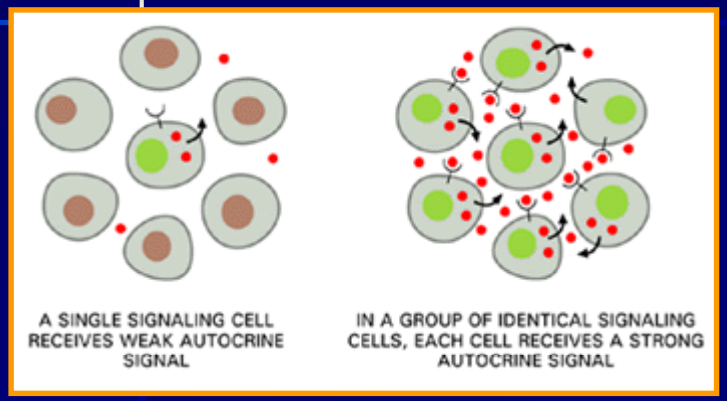
Paracrine



- The substance secreted by the producing cell penetrates to the target cell by diffusion in the interstitial fluid (other than blood)
  - Example: secretin, cholecytokinin,
- In a broader sense – any short-distance signaling (within tissue, organ) (including blood)
  - Example: cytokines, prostanoids in inflammatory response
- **Synaptic** – ultra short distance (neuron - neuron; neuron - muscle)
- **Neurocrine signaling** (neuron – cell; sympathetic, parasympathetic)

Signaling by secretory molecules

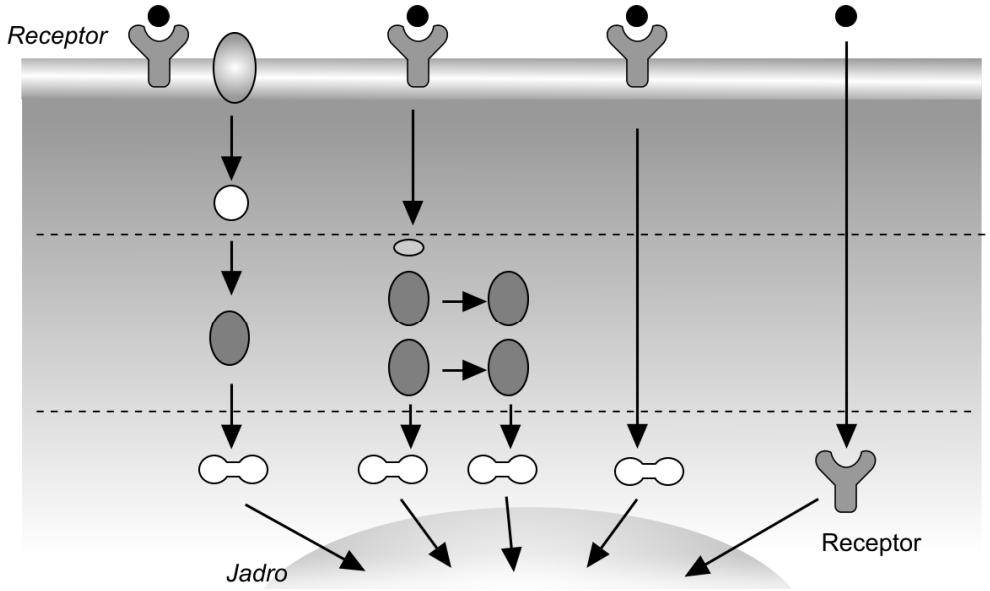
Autocrinne



- The cell has a receptor for the mediator that it secretes
- It is essentially a feedback loop – for a short distance - if the concentration of the mediator exceeds a certain level
- over a long distance - theoretically, the mediator can return to the cell after circulating in the blood

- Mediators (hormone, transmitter) do not have a calling card to which location they should travel and which cell they should act on
- Method of signaling or the path by which the mediator reaches the target cell is not predetermined. They are a matter of the mutual topology of the production and target cells. For example the same insulin secreted from b-cells has a paracrine effect on the neighboring a-cell, on other endocrines

The main elements of signal transmission



Prvý posol

Hormón, transmiter, cytokín

Druhý posol

cAMP, cGMP, Ca^{2+}, IP3, IP2, DG, NO, KA, KLP

Tretí posol

cytoplazmatické kinázy, nízkomolekulové G-proteíny, adaptérové proteíny

Štvrtý posol

transkripčné faktory, enhancery

- Regardless of the method of "transportation" of chem. signal to the cell (nervous, endocrine) intracellular signaling pathways seem to be universal
- Similar if not identical signaling pathways exist across the phylogenetic spectrum of animals

Pathophysiology Deficiency of hormonal effect

- **Decreased hormone production (reduced blood concentration)**
  - Destruction of the gland, horm dystrophy. product cells
  - Disorder of hormone production in the cell (hereditary dysgenesis; enzymatic defects;
  - Attenuation of production (excessive feedback; other hormonal effects; release disorder)
- **Disorder of hormone transport (concentration in the blood)**
  - Transport disorder (insufficiency of the transporter in the blood)
- **Hormone insensitivity (sensitivity) syndromes (hormone resistance syndromes) blood concentration normal or increased)**
  - receptor defects (sy. of androgen insensitivity AGR, Laron's sy. GHR; DM type 2)
  - postreceptor defects (DM type 2); IGH1 insensitivity syndrome
  - disorders of feedback regulation in the cell
  - disorders of feedback regulation outside the cell (feedback) (DM type 2)

Receptors

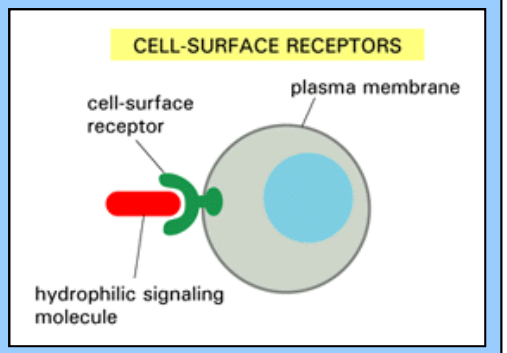
Surface receptors

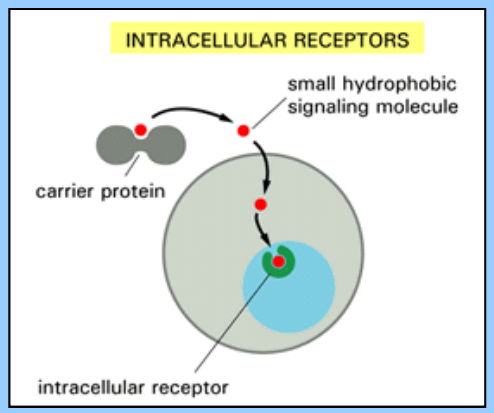
without enzymatic activity
- Ionotropic receptors
- GPCRs

with enzymatic activity
- Guanyl cyclase
- Tyr-kinase
- Ser/Thr - kinase
- Ser/Thr - phosphatase

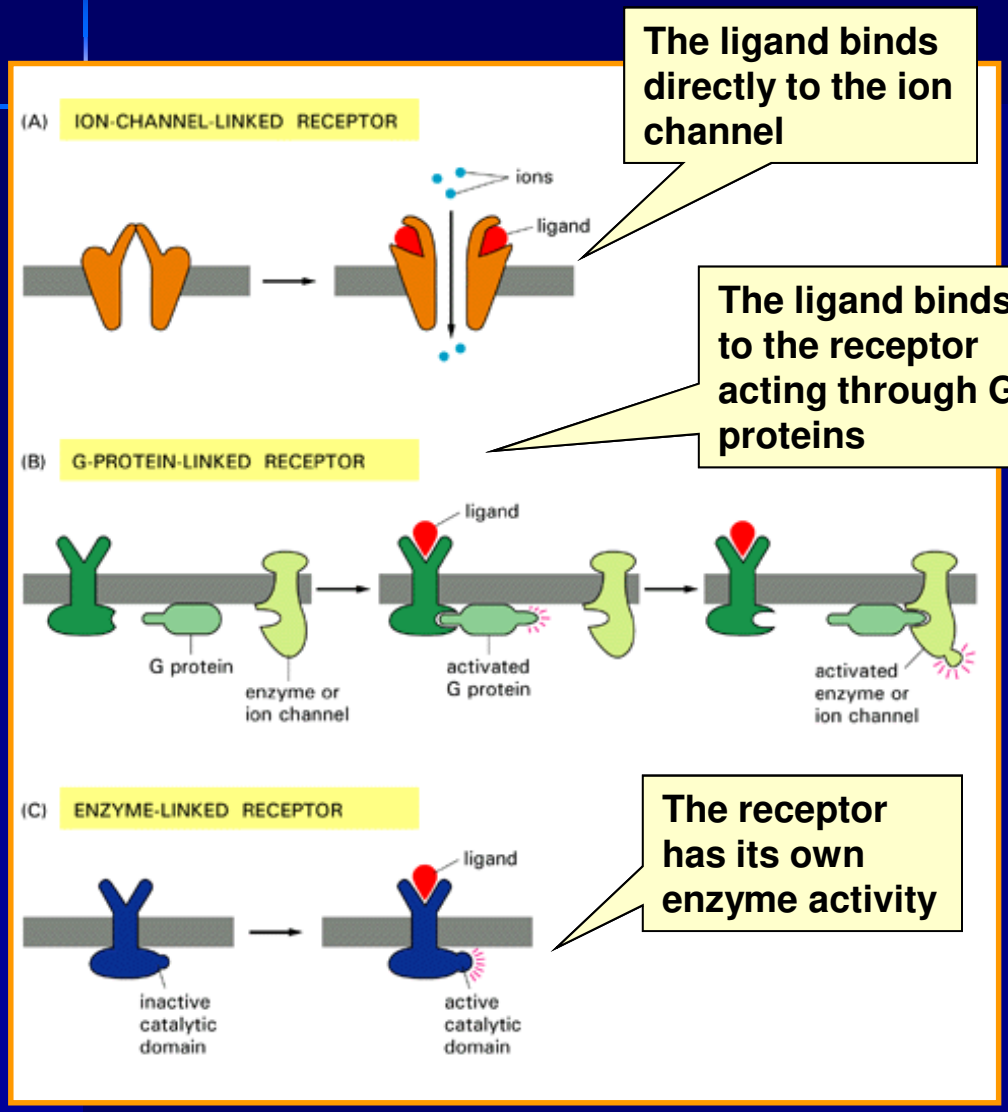
Intracellular receptors

- cytoplasmic receptors
- nuclear receptors





Surface (cytoplasmic) receptors



The ligand binds directly to the ion channel

Acetylcholine (N), GABA (A), Glutamate (NMDA, AMPA, Serotonin(3), Purines,

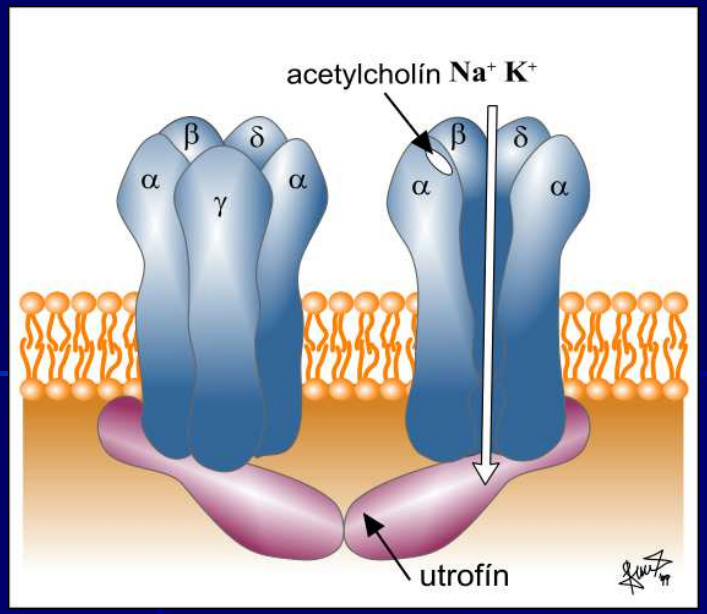
The ligand binds to the receptor acting through G proteins

Catecholamines (a/b), Acetylcholine (M), GABA (B), Glutamate (M) Serotonin (1,2), Interleukins, Prostaglandins,

The receptor has its own enzyme activity

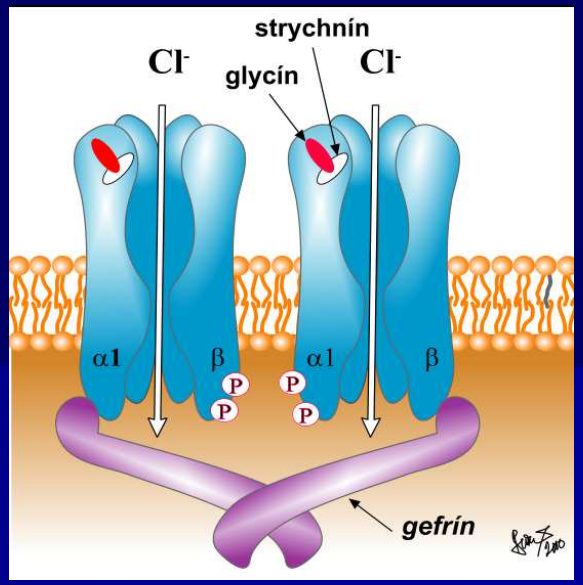
Growth factors (FGF, EGF, NGF, PDGF), Insulin

Ionotropic receptors



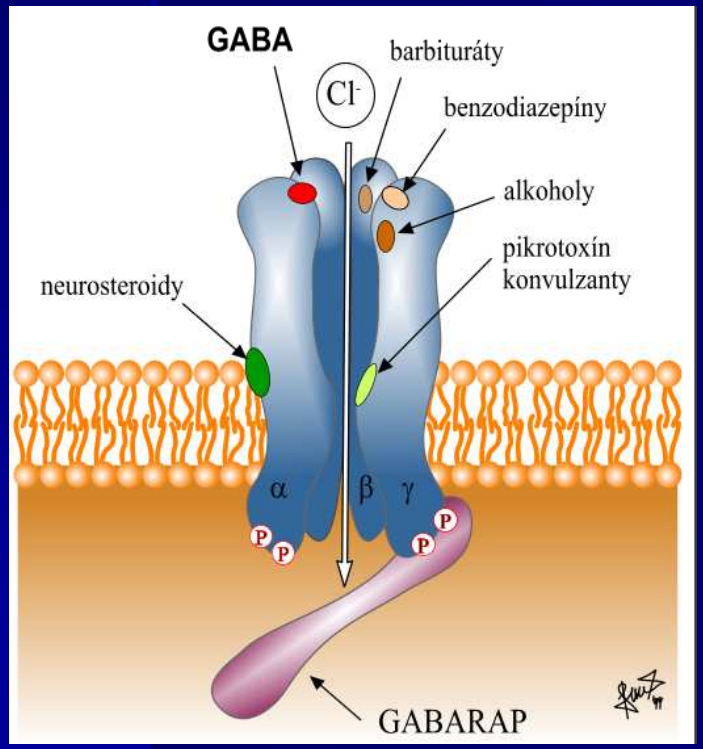
Nicotinic receptor

Neuromuskulárna platnička, Mozog



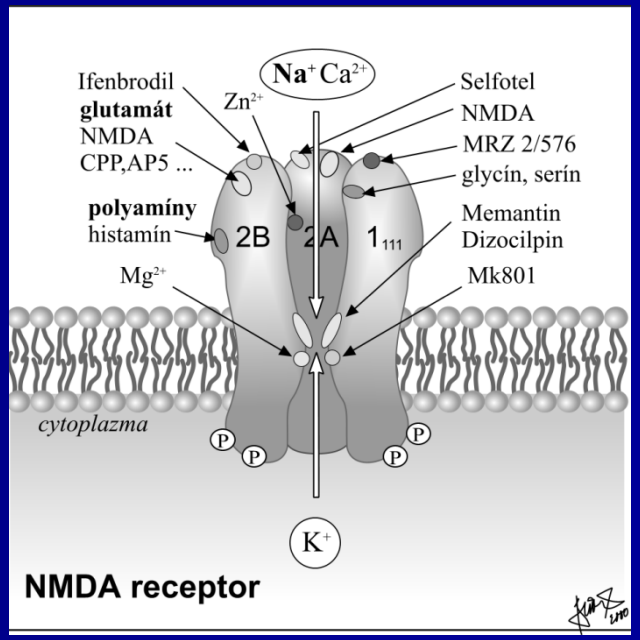
Glycine receptor

Spinal cord – the main inhibitory mediator



GABA (A) receptor

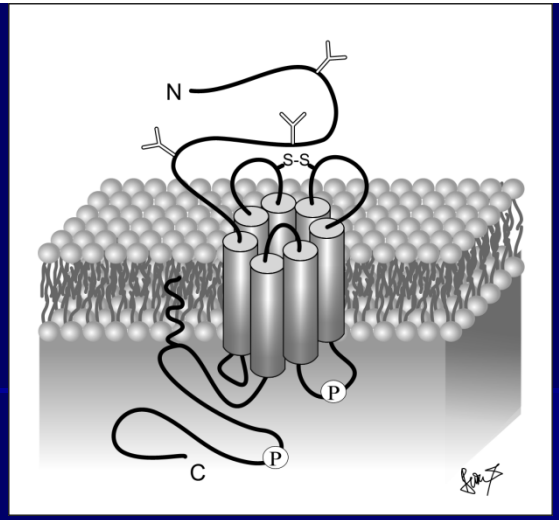
Brain (main inhibitory signal transduction)
Spinal cord (less)



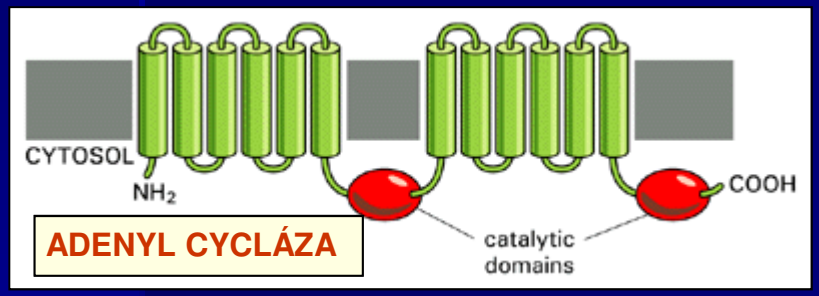
Glutamate receptor NMDA

Brain (one of the main excitatory transmissions)

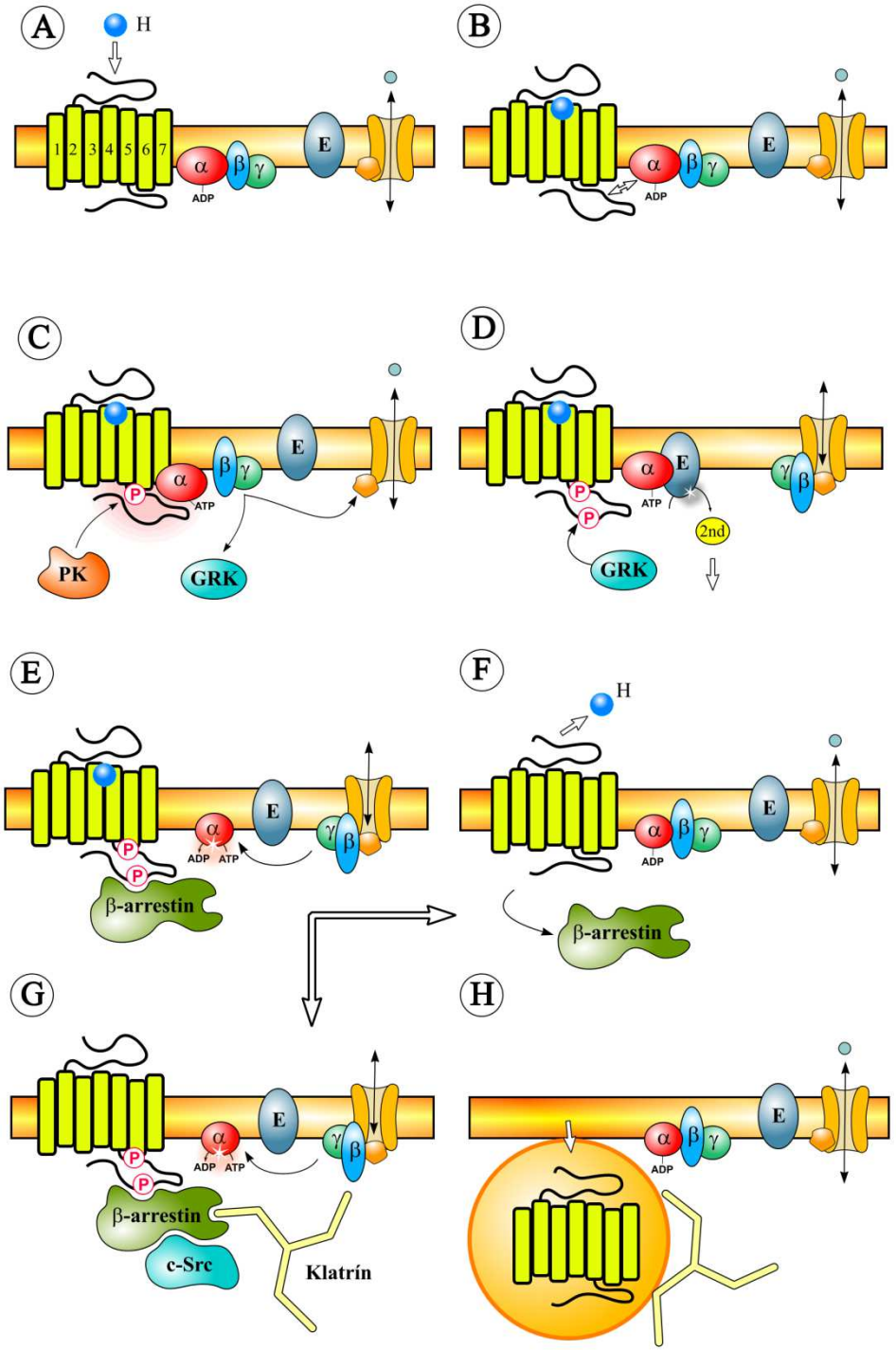
GPCR



The receptor contains 7 transmembrane alpha-helical segments intracellular and extracellular domains



The enzyme contains about 1100 amino acids, 6 transmembrane domains and 2 cytoplasmic domains. There are 6 types of adenylyl cyclase

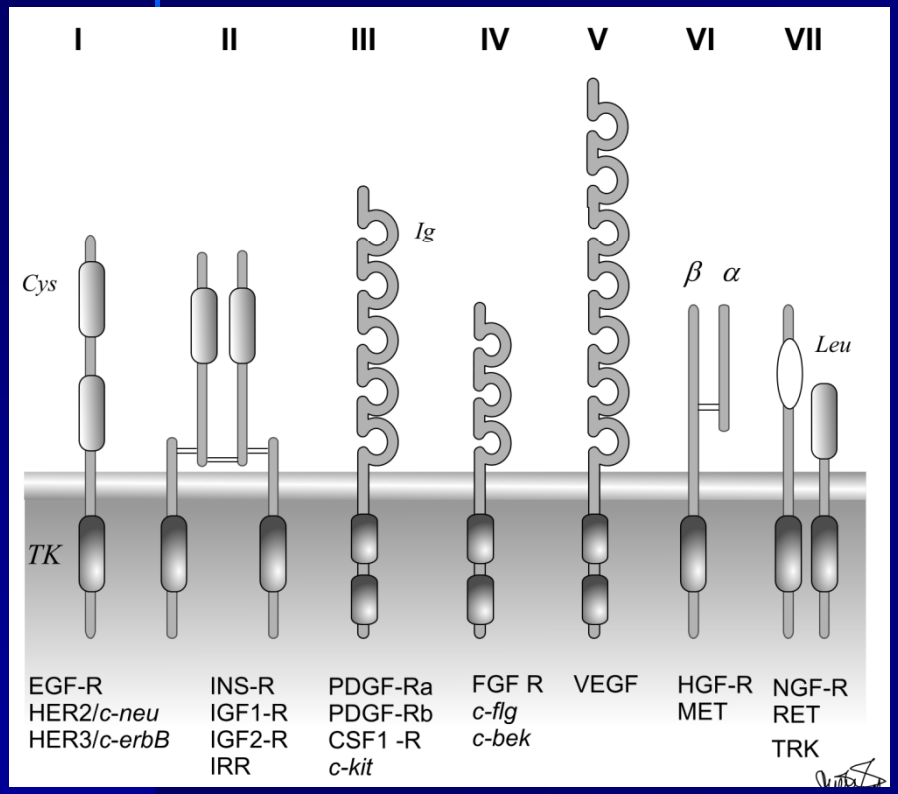


Endocrine diseases caused by GPCR mutations

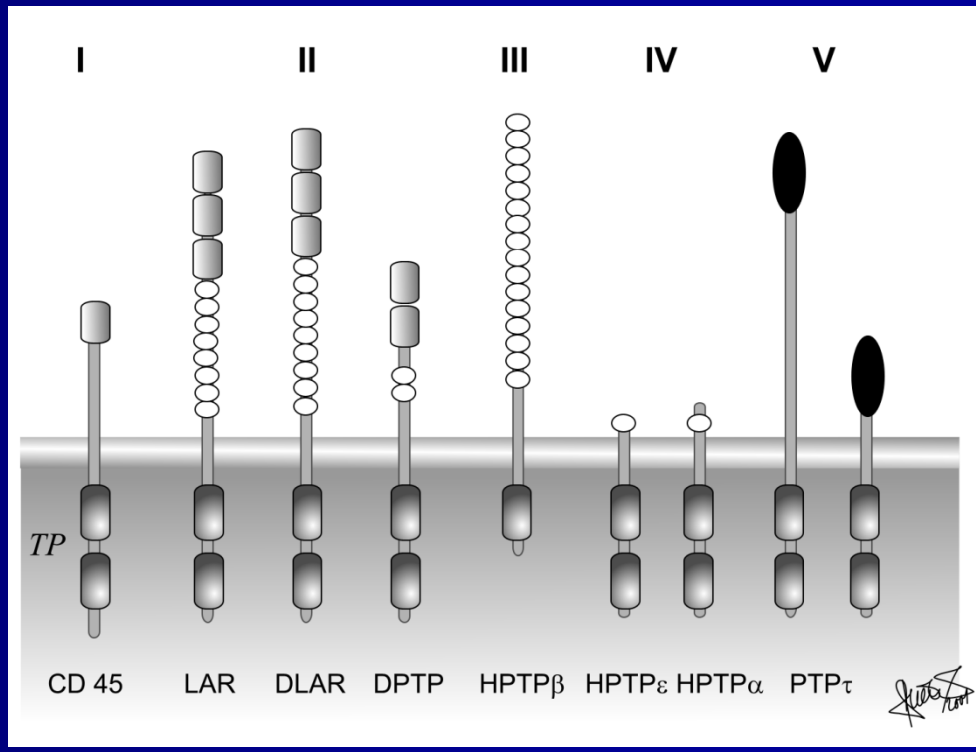
Endocrine disorders associated with mutation of serpentine receptors		
Mutated protein	**Disorder**	**Type of mutation**
Gain of function		
LH receptors	Familiar male prococious puberty	Autosomal dominant
TSH receptor	Non-autoimmune hereditary hyperthyroidism	Autosomal dominant
	Hyperfuncioning thyroid adenoma	Somatic
PTH receptor	Jansen metaphysal chondrodysplasia	Autosomal dominant
Calcium receptor	Hypoparathyroidism	Autosomal dominant
Loss of function		
LH receptor	Male pseudohermafroditism	Autosomal recessive
FSH rceptor	Hypergonadotrophic ovarian dysgenesis	Autosomal recessive
GHRH	Laron dwarfism	Autosomal recessive
TSH receptor	Congenital hypothyroidism	Autosomal recessive
TRH receptor	Congenital hypothyroidism	Autosomal recessive
ACTH	Familial ACTH resistance	Autosomal recessive
Vasopressin	Nephrogenic diabetes insipidus	X-linked
Calcium receptor	Familial hypocalciuric hypercalcaemia	Autosomal dominant
	Neonatal severe hyperparathyroidism	Autosomal recessive

Receptors with enzyme activity in mitogenic pathways

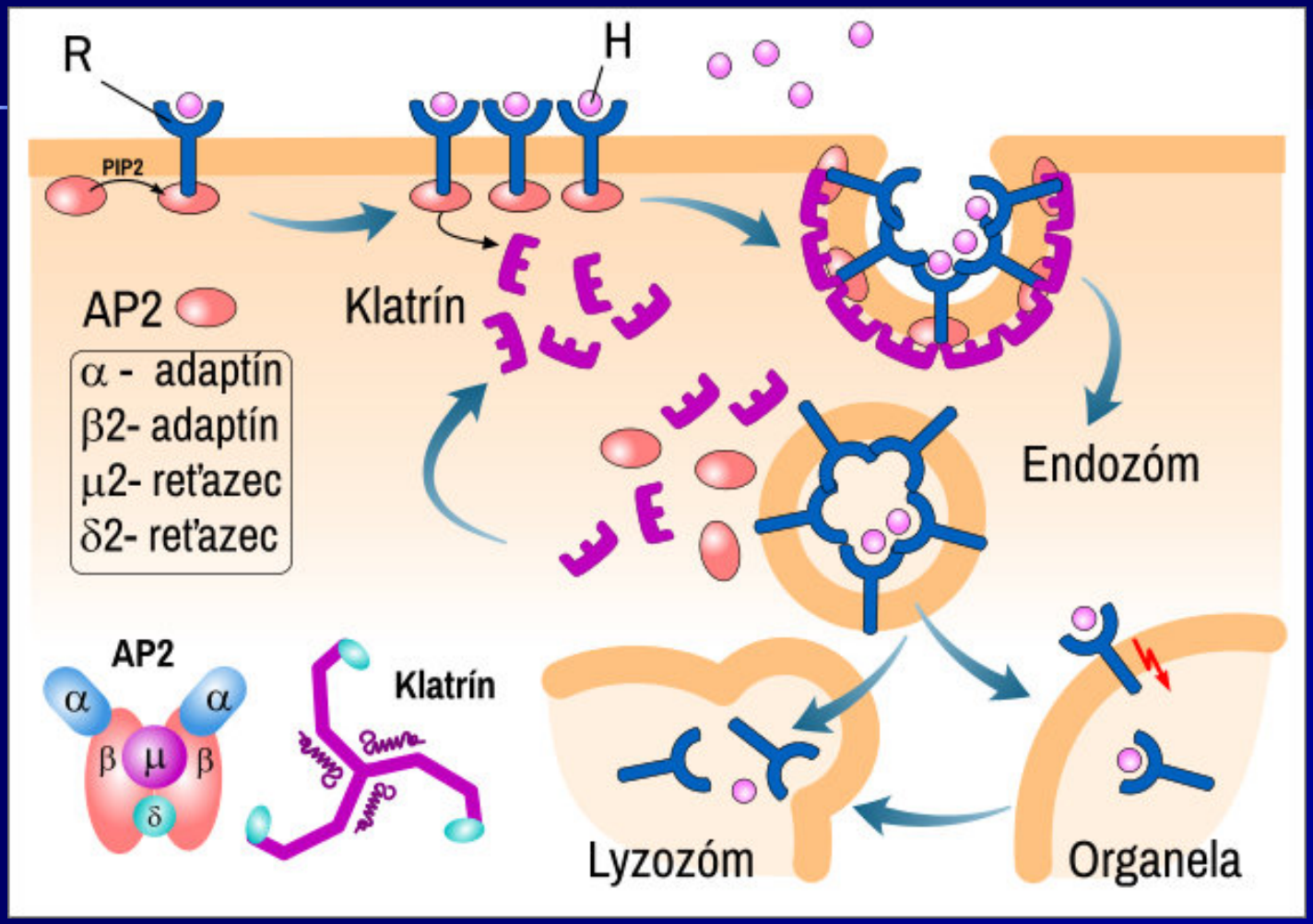
Receptors with Tyr-kinase activity



Receptors with Tyr-phosphatase activity

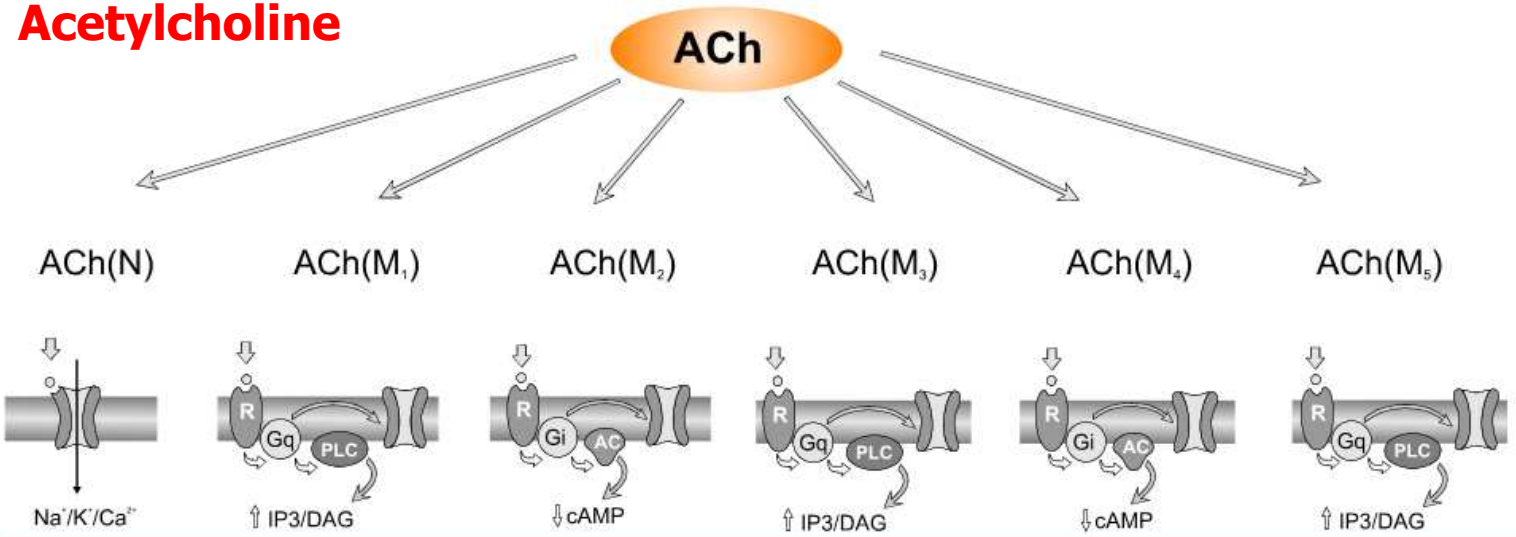


Receptor internalisation



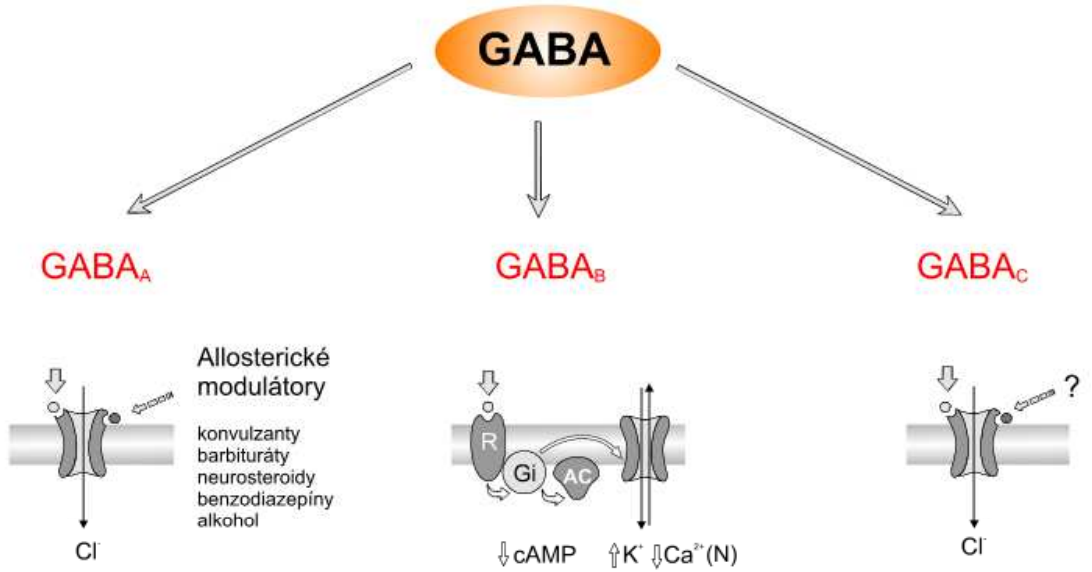
1 The same mediator usually acts through several specific receptors

Acetylcholine



Agonisti					
Nikotin a - Anatoxín Epibatín Epiboxidín	Oxotremorín-M McN-A-343	Oxotremorín-M	Oxotremorín-M	Oxotremorín-M	Oxotremorín-M
Tubokurarín a - Bungarotoxín a - Kobratoxín a - Konotoxín Neosurugatoxín Dekametónium Hexametónium Metyllykatónium Gallamín	Atropín Skopolamín Pirenzepín Telenzepín QNX hemioxalát	Atropín Gallamín Skopolamín Himbacín Metoktramín Imperialín	Atropín Skopolamín 4-DAMP Hexahydrozyl Odifenidol	Atropín Skopolamín Himbacín Tropicamid	Atropín Pirenzepín 4-DAMP AF-DX 384

Ach binds to ionotropic nicotinic receptor (4 subtypes) and metabotropic muscarinic receptor (5 subtypes)



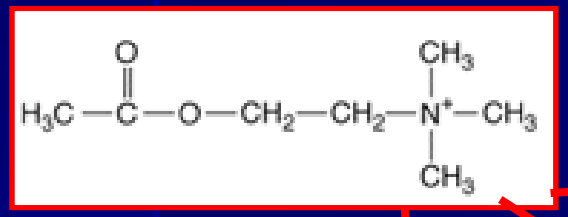
Modulátory

GABA	Diazepam	GABA	GABA
ZAPA	Flunitrazepam	Baklofen	CACA
TACA	Zolpidem, Zopiclon	3APPA	TACA
Muscimol	DHEA, 3APMA	TACA	Muscimol
Izoguvacin	RO194603		I4AA
Abecamil	Alprazolam, TBPS		
THIP	Chlordiazepoxid		
DMCM	Carbamazepin		
	Allopregnenolón		

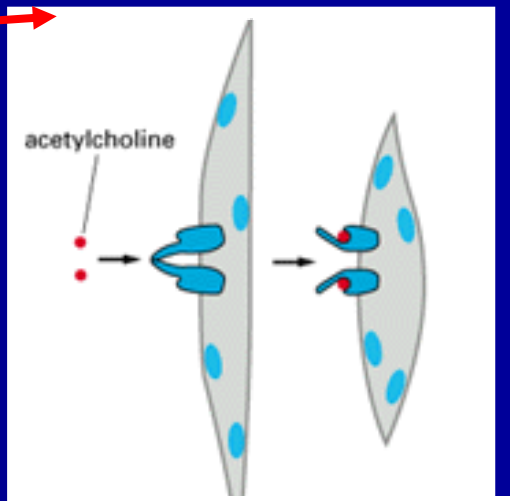
Bikukulín	Flumazenil	Saklofen	ZAPA
Pikrotoxín	ZK 93426	2-OH saklofen	3APPA
Gabazín		Faklofen	TPMPA
SR 95531		CGP 55845	THIP
Hydrastín			SKF 97541

2 Different cells can respond differently to the same mediator (specifically for them)

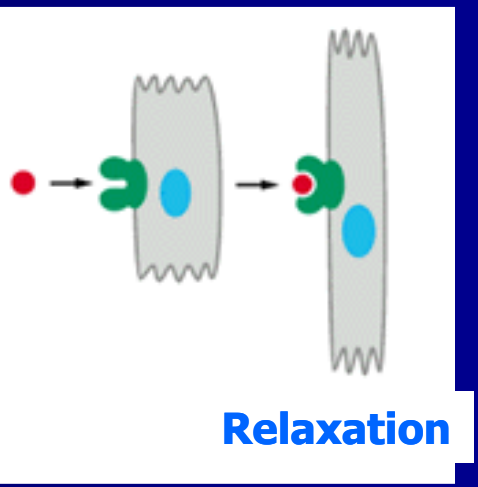
Acetylcholine



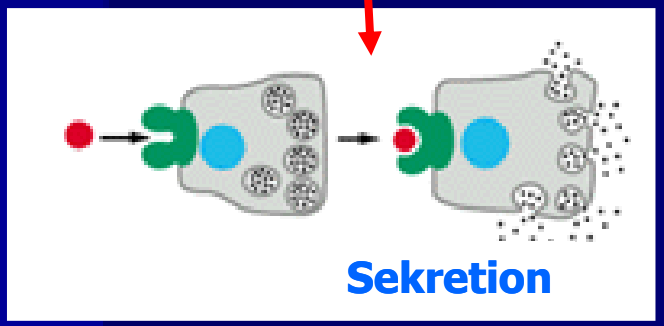
Skeletal muscle



Heart muscle

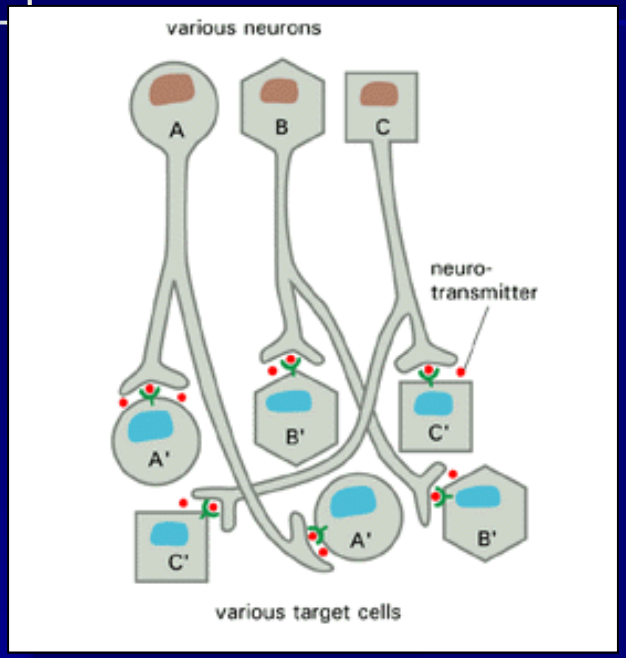


Gland



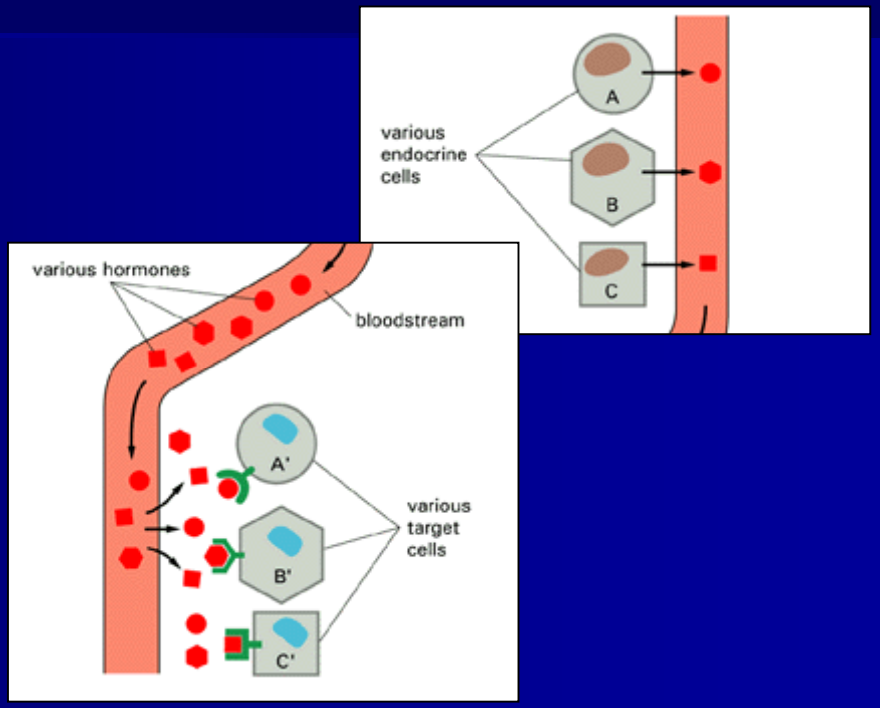
3 Špecifická nervového a endokrinného sy.

Nervous signaling system



- Most synapses are chemical
- A neuron affects specific types of cells according to specific patterns
- The contact is given by the target cells, so they usually have the necessary receptors

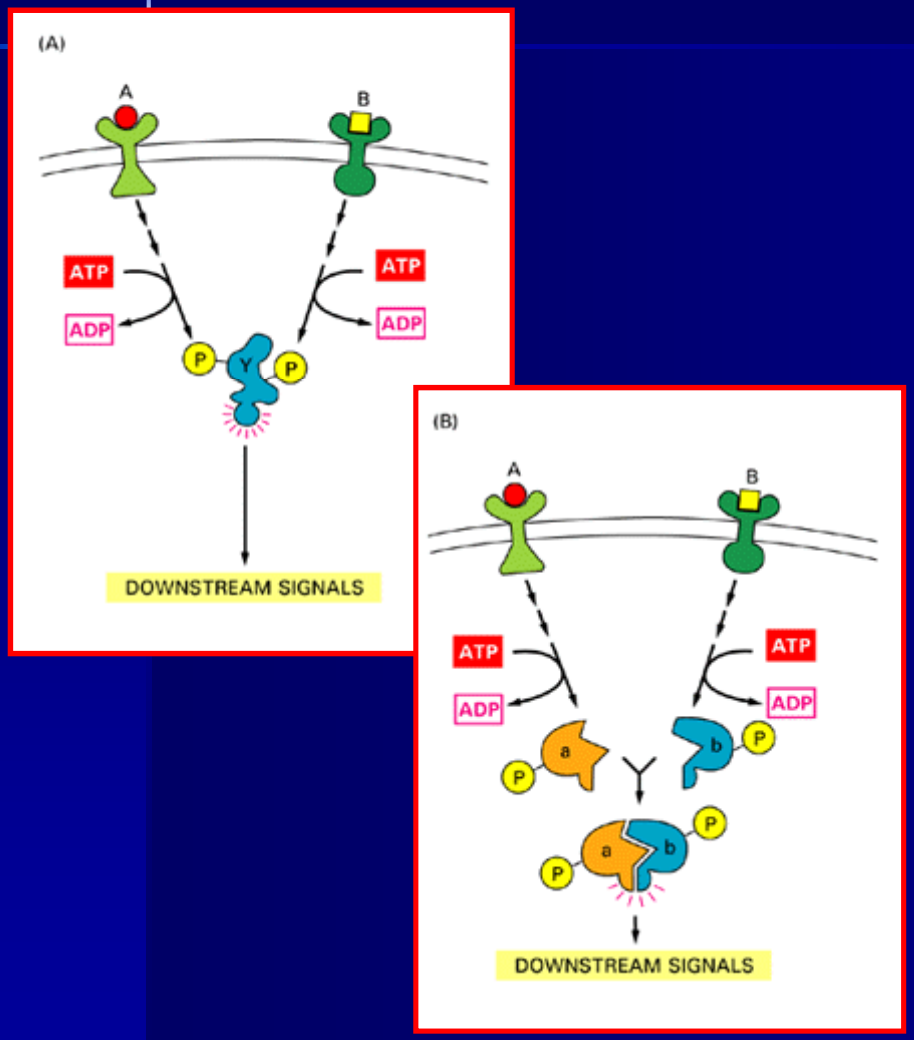
Endocrine signaling system



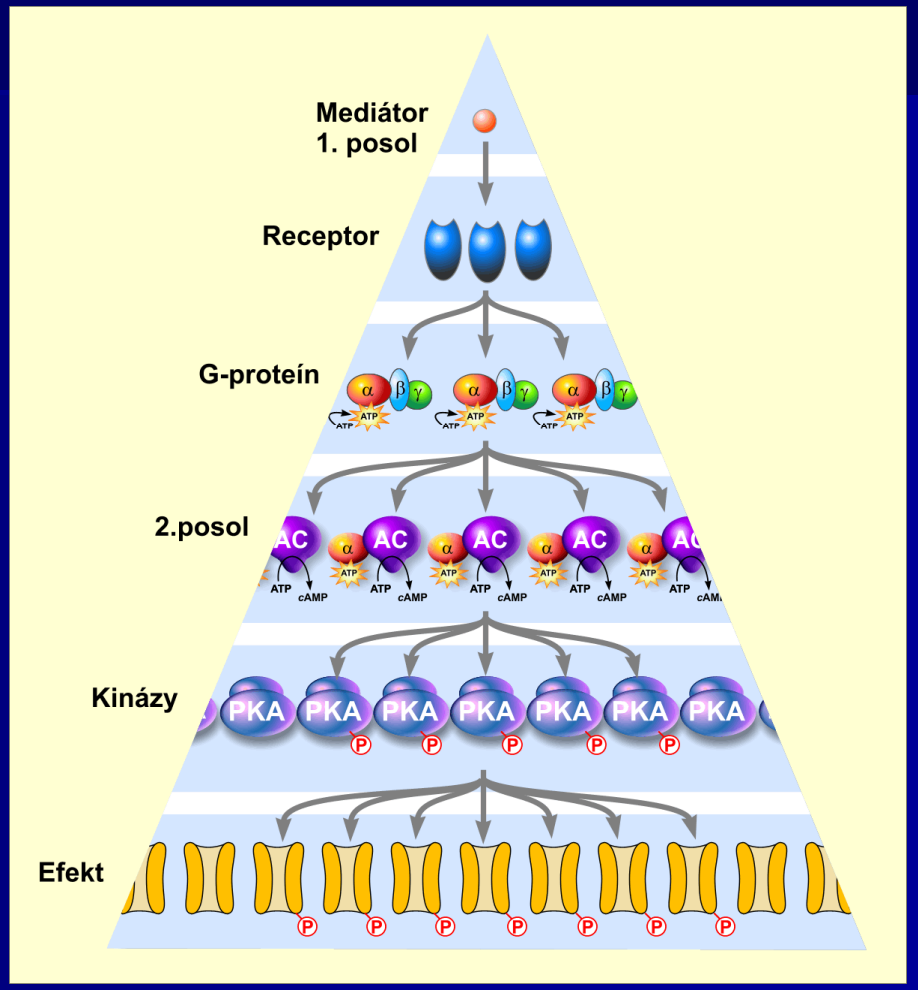
- The target cells are not predetermined
- Signal guidance to the cells is partial
- Cells that have receptors for the corresponding signal respond

4

Integration of downstream signaling pathways



Signal amplification



Special part
Signaling cascades

1. Long distance chemosignalling

A. Receptors without enzymatic activity

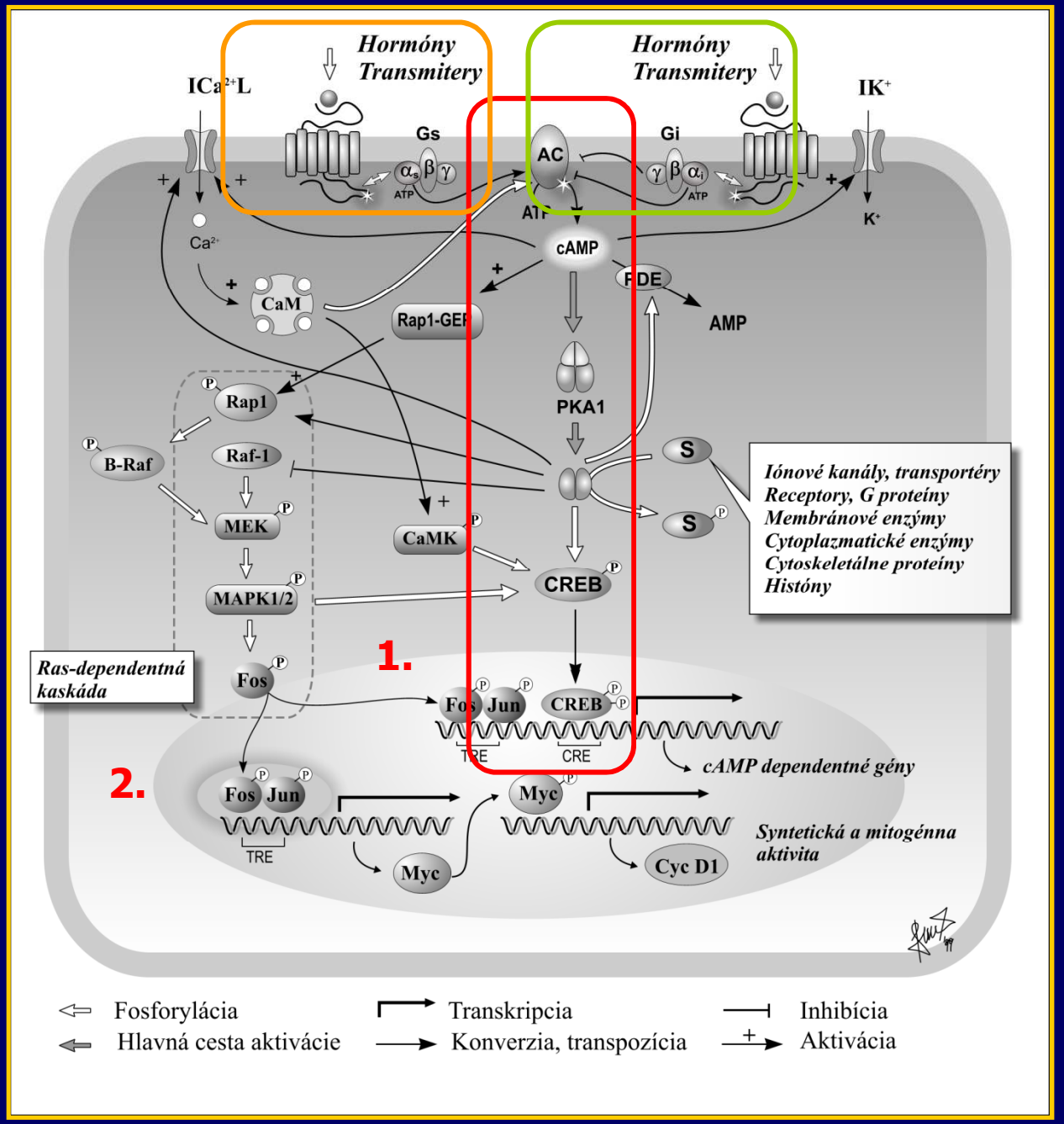
- c-AMP signalling
- IP3- dependent signalling
- c-GMP/NO – signalling
- PLA2 –dependent signalling
- Ca^{2+}- dependent signalling

- **Earl Wilbur Sutherland Jr.** (1915 –1974) American pharmacologist, biochemist Nobel Prize for Physiology and Medicine 1971: "discoveries concerning the mechanisms of the action of hormones"
- Metabolic effects of noradrenaline, glucagon associated with an increase in cAMP *T.W Rall, E.W Sutherland: Formation of a cyclic adenine ribonucleotide by tissue particles J. Biol. Chem., 232 (1958), pp. 1065–1076*
  Hardman, G.A Robison, E.W Sutherland: Cyclic nucleotides . Rev. Physiol., 33 (1971), pp. 311–336

- **Eric Richard Kandel** (1929) neuropsychiatrist; Nobel Prize for Physiology and Medicine 2000: "physiological basis of memory storage in neurons"
- Mechanism of long-term memory associated with cytoskeletal remodeling and axon growth
- CREB – cAMP responsive genes; gene effects of cAMP signaling

c –AMP-dependent signalling

- Trigger: many hormons, transmitters, cytokines
- Membrane G.protein coupled receptors
- Stimulatory & inhibitory transfer (Gs or Gi)
- Effectors: Adenyl cyclase – cAMP - PKA
- Effects: widespread
  - Immediate: phosphorylation of proteins
  - Late: gene expression



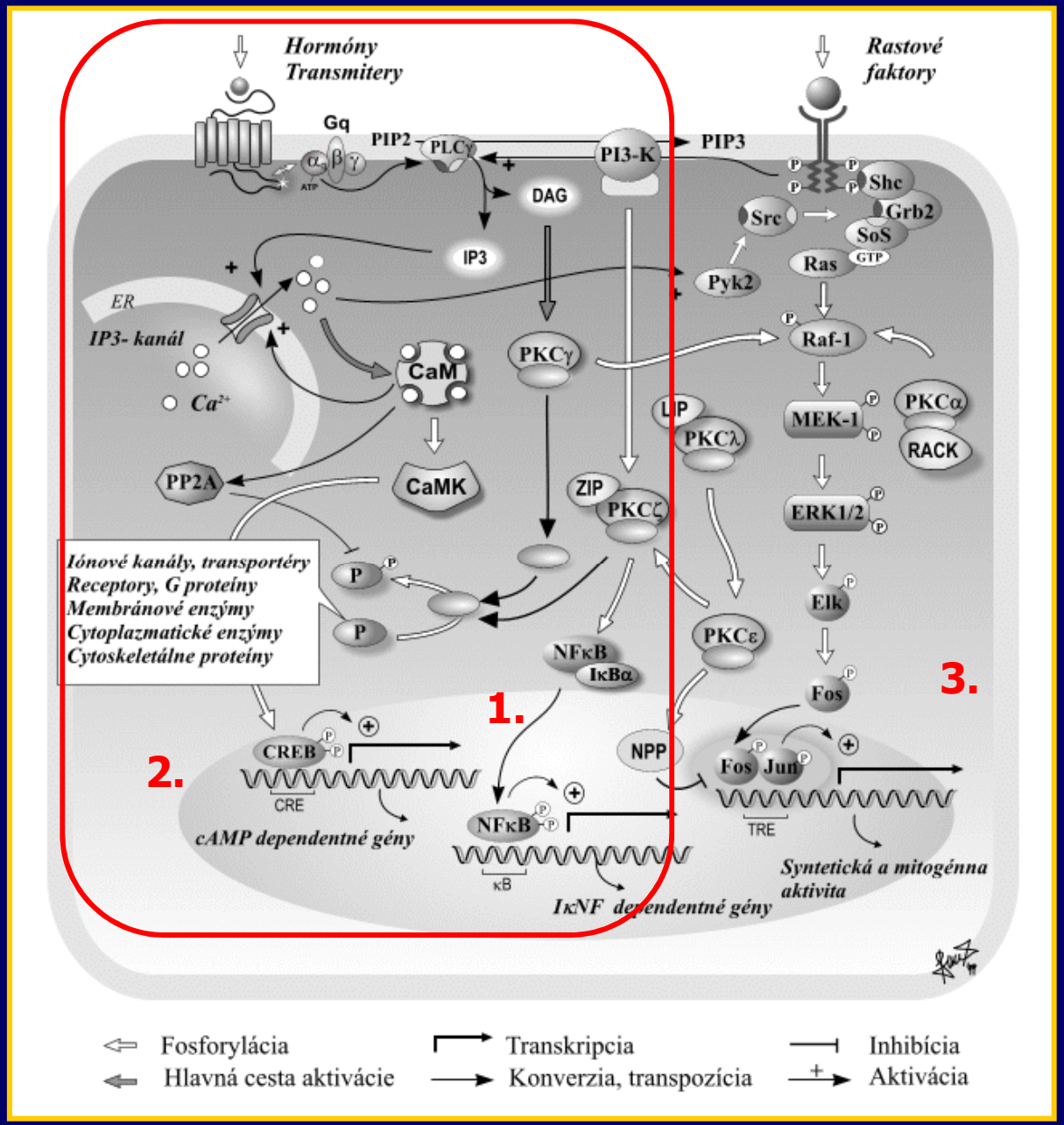
cAMP/IP3 dependent signaling

Some Hormone-induced Cellular Responses Mediated by Cyclic AMP		
Hormone	**Target Tissue**	**Major Response**
Thyroid-stimulating hormone (TSH)	Thyroid gland	thyroid hormone synthesis and secretion
Adrenocorticotropic hormone (ACTH)	Adrenal cortex	cortisol secretion
Luteinizing hormone (LH)	Ovary	progesterone secretion
Adrenaline	Muscle	glycogen breakdown
Noradrenalin, Adrenaline	Heart	increase in heart rate and force of contraction
Parathormone	Bone	bone resorption
Glucagon	Liver	glycogen breakdown
Vasopressin	Kidney	water resorption
Adrenaline, ACTH, glucagon, TSH	Fat	triglyceride breakdown

Some Cellular Responses Mediated by G-Protein-linked Receptors Coupled to the Inositol-Phospholipid Signaling Pathway		
Signaling Molecule	**Target Tissue**	**Major Response**
Vasopressin	Liver	glycogen breakdown
Acetylcholine	Pancreas	amylase secretion
Acetylcholine	Smooth muscle	contraction
Antigen	Mast cells	histamine secretion
Thrombin	Blood platelets	thrombin

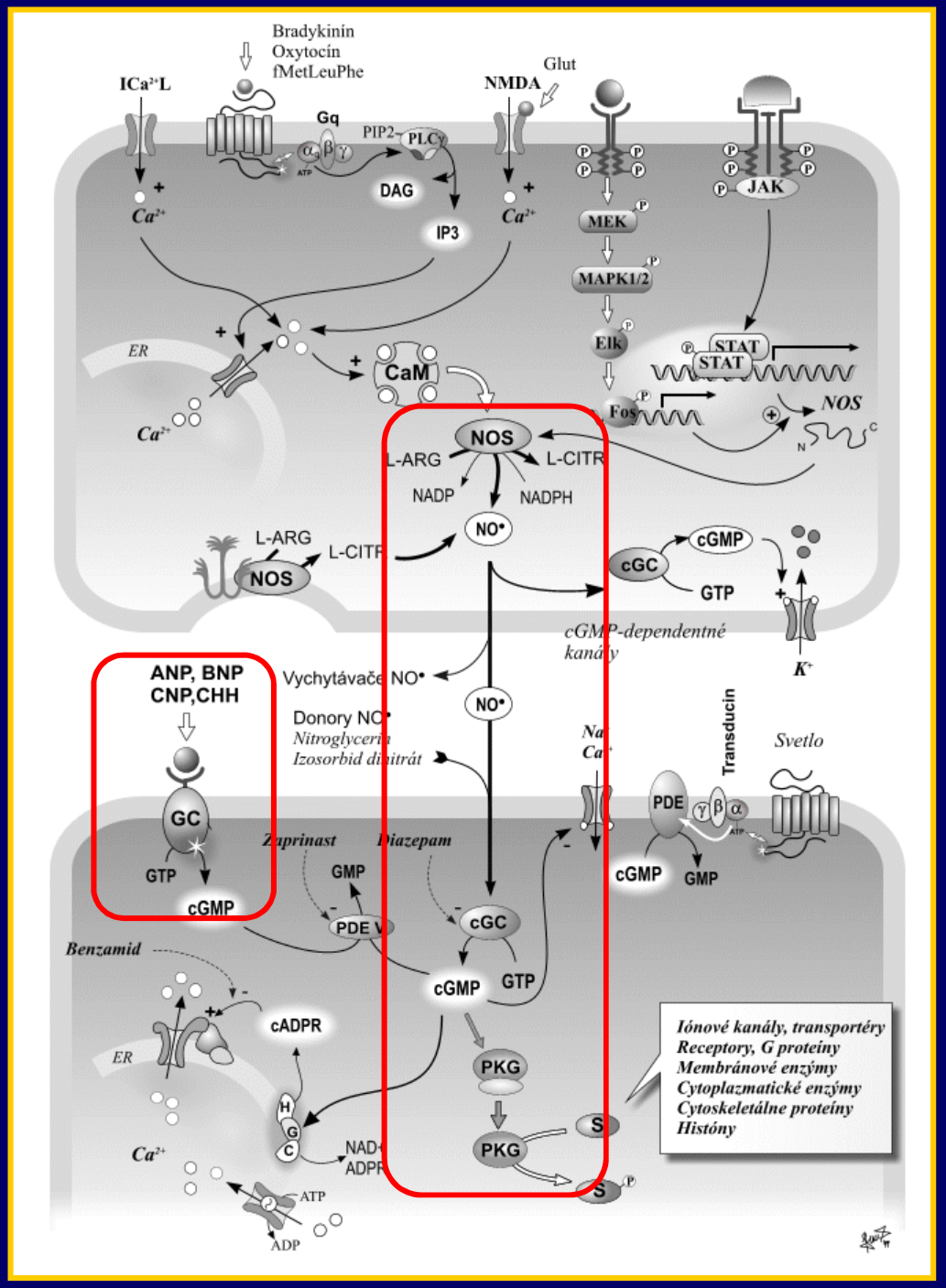
IP3 / DAG – signalling

- Trigger: many hormons, transmitters, cytokines
- Membrane G.protein coupled receptors (Go)
- Both stimulatory & inhibitory influence
- Effectors: Phospholipase C (PLC) – DAG, IP3 - PKC
- Effects: widespread
  - Immediate: phosphorylation of proteins
  - Late: gene expression



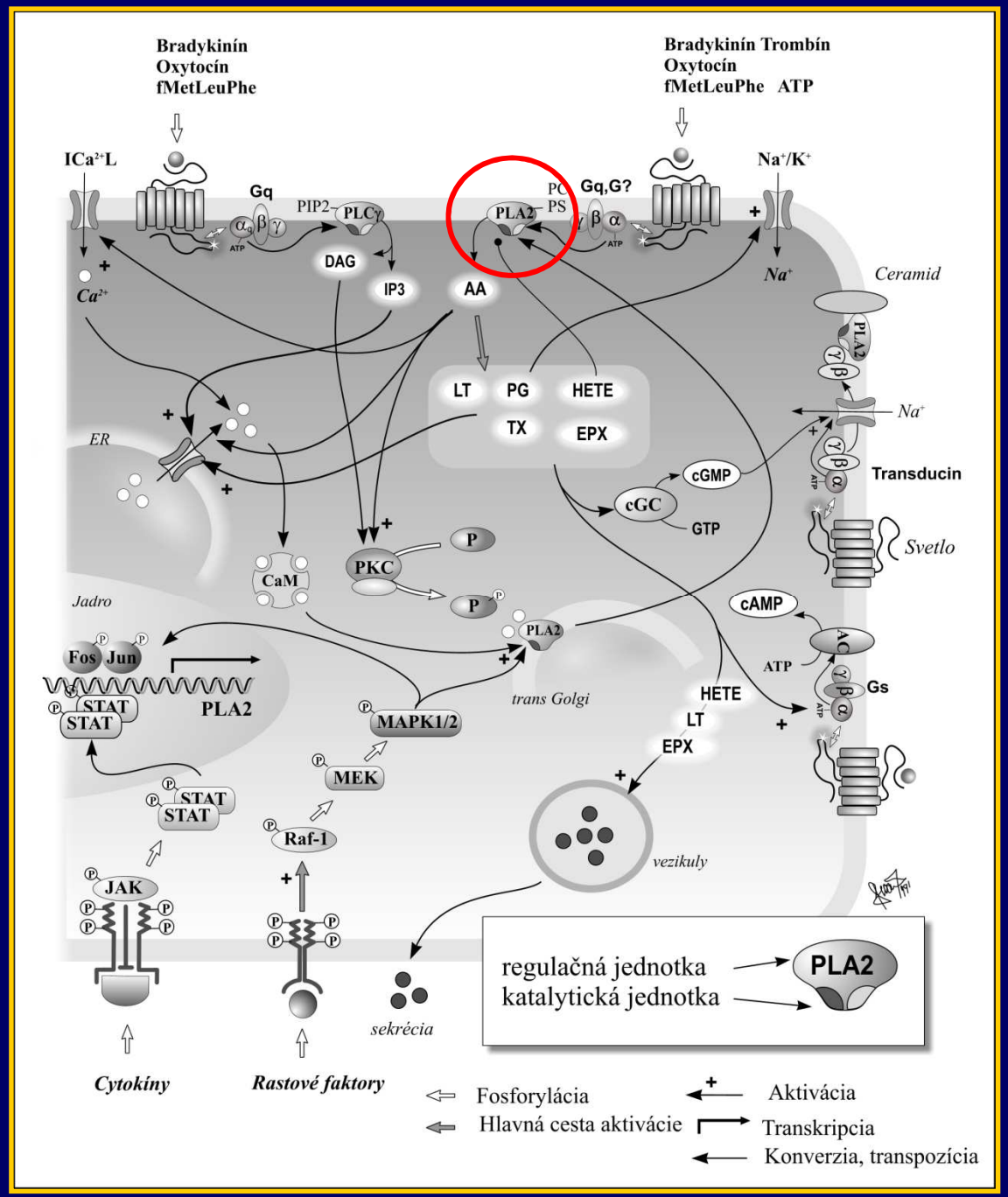
cGMP/NO – signalling

- Triggers:
  A. Ca2+ - signalling
  B. ANP,BNP
  C. NO - donors
- Membrane G-protein coupled receptors + Membrane receptors with GC activity
- Effectors: NO, cGMP - PKG
- Effects: phosphorylation of proteins
- Use: - vascular smooth muscle dilation
  - vision, etc.



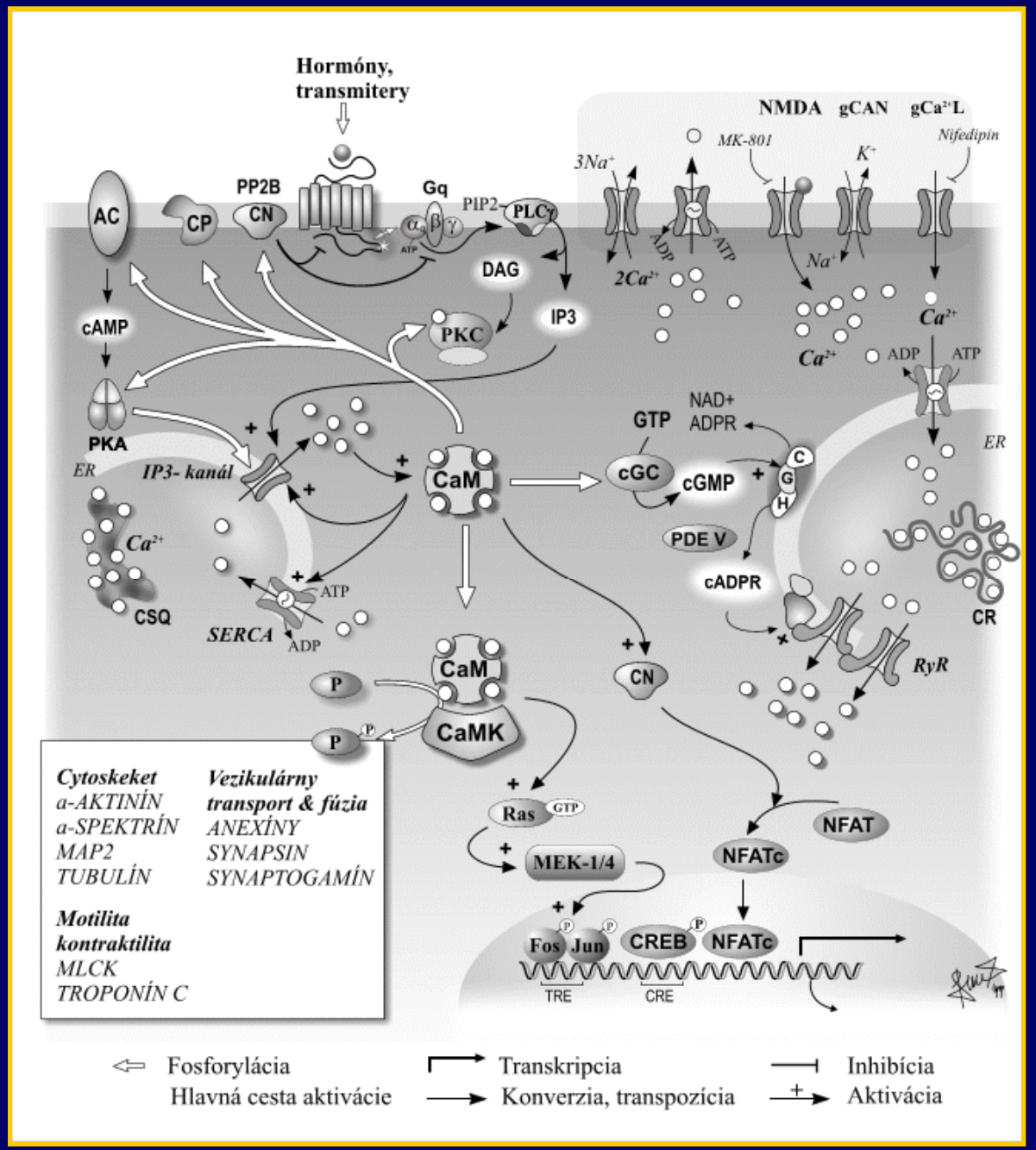
PLA2 – dependent signalling

- G-protein coupled receptors
- Use: supplementar to IP3-dependent signalling
- Phospholipase A2 (PLA2)
- Arachidonic acid (AA)
- 2nd messeng.: AA derivatives
  - Leucotriens (LT)
  - Prostaglandins (PG)
  - Tromboxans (TX)
  - Epoxides (EPX)
  - HETE
- Interactions with other signallings: cAMP, cGMP, JAK/STAT, MEK



Ca^{2+}-dependent signalling

- Ubiquitous, of particular importance in skeletal & smooth muscles, nervous sy.
- Trigger: any rise of cytosolic $[Ca^{2+}]i$
- Effectors: calmodulin (CaM) – Ser/Thr kinase
- Effects:
  - Immediate: phosphorylation/ dephos-phorylation
  - Late: gene transcription
- Use: trasmission of excitatory processes



1. Long distance chemosignalling

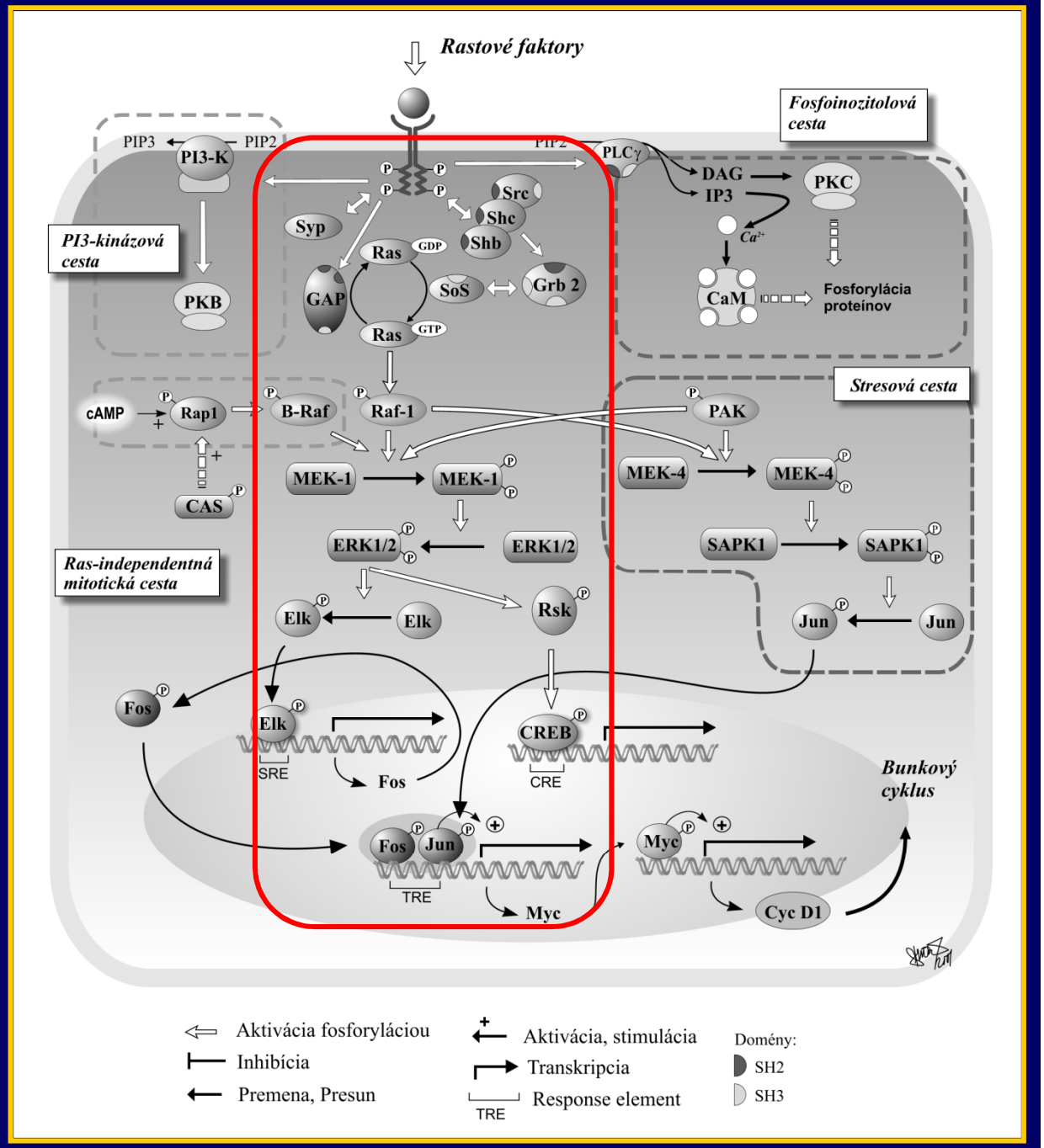
A. Receptors without enzymatic activity

B. Receptors with enzymatic activity

- Classical growth signalling
- Stress signalling
- Signalling via PI-3K
- Signalling through JAK/STAT
- Signalling via SMAD
- Signalling via Toll
- Signalling via NFkB

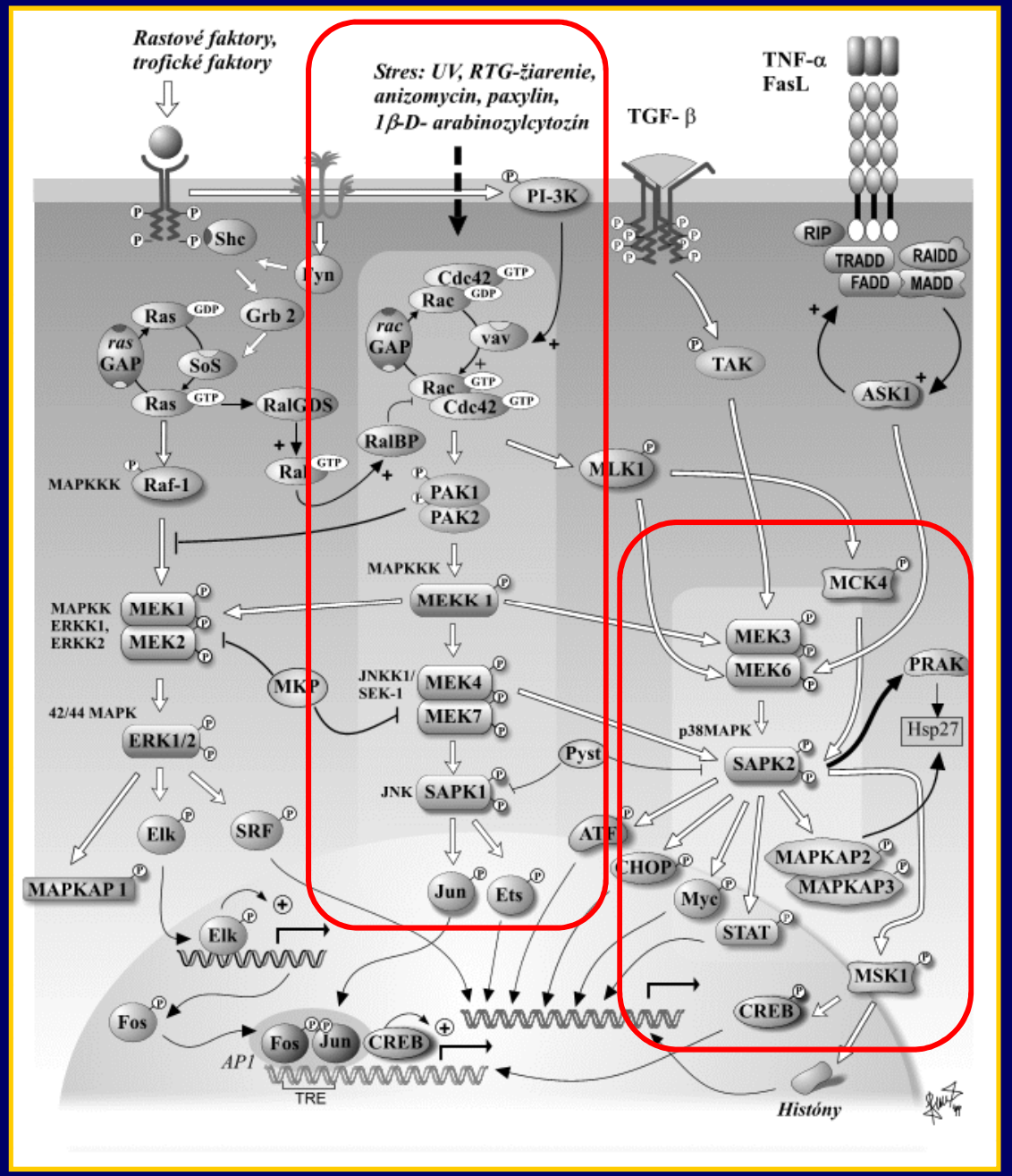
Classical growth signalling

- **Trigger:** growth factor familes
- **Receptors** with enzymatic activity
- **Effectors:**
  - **Ras – switch**
  - **MAPK cascade**
- **Effect:**
  - Gene transcription
- **Use:**
  - proliferative & growth process
  - mitosis



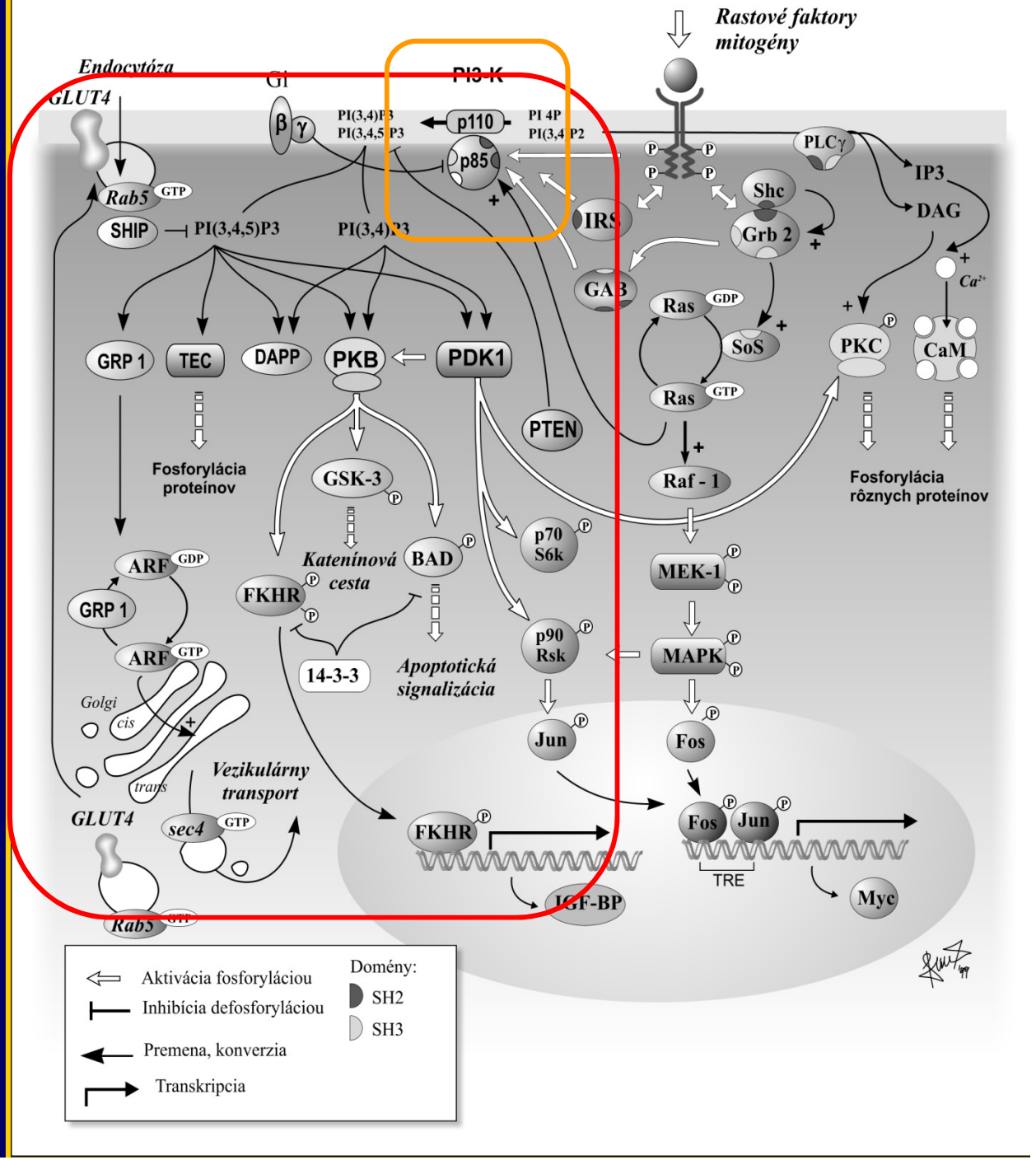
Stress signalling

- **Trigger**:
  - growth factor familes
  - physical : UV, X-rays, temp.
  - chemicals
- **Receptors with enzymatic activity**
- **Effectors**:
  - **Ras – switch**
  - **MAPK cascade**
- **Effect**:
  - Gene transcription
- **Usage:**
  - proliferative & growth process (mitosis)
  - adaptations



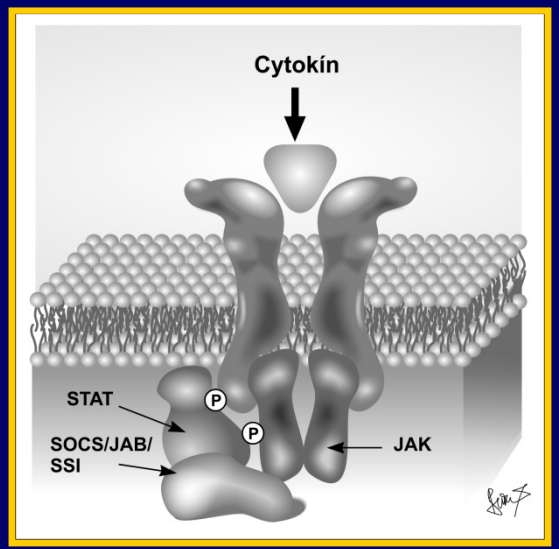
Signalling via PI-3K

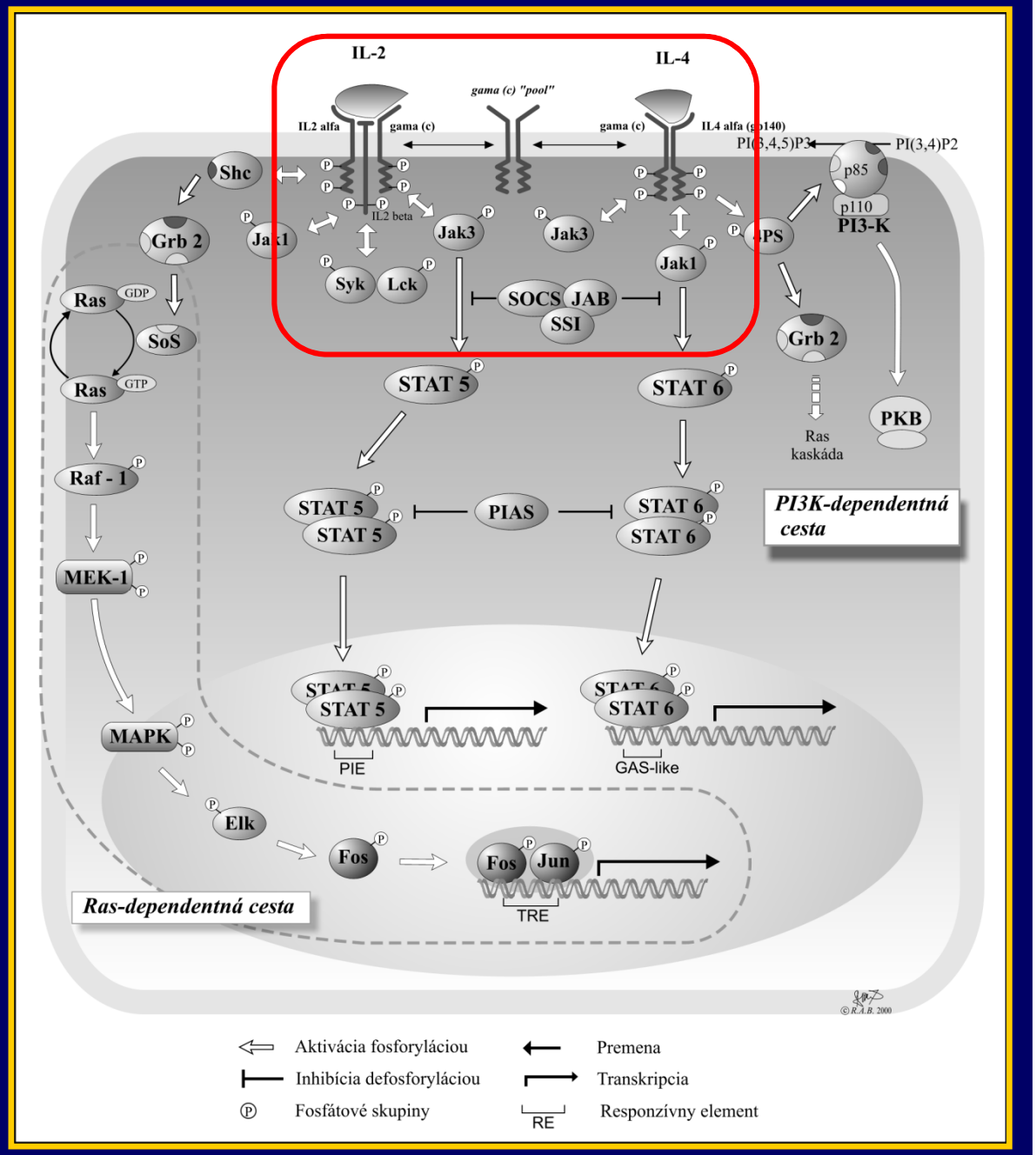
- **Trigger:** growth factor familes
- **Receptors** with enzymatic activity
- **Effectors:**
  - **PI3K –PKB, PDK**
- **Effect:**
  - Gene transcription
  - Vesicular transportation
  - Apoptotic machinery
  - Fosforylation of proteins
- **Use:** proliferative & growth process control



Signalling through JAK/STAT

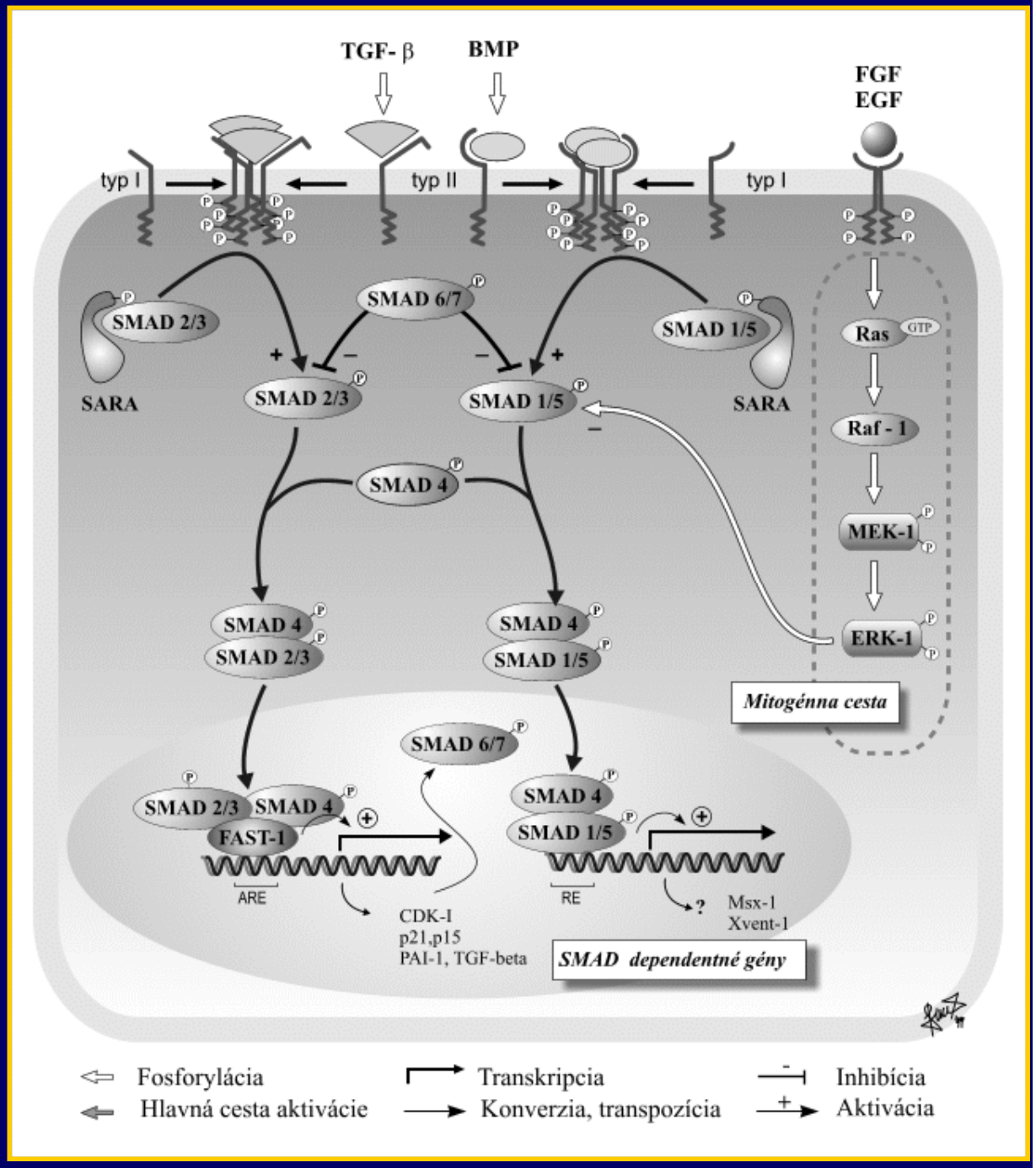
- Membrane receptors with associated enzymatic activity
- Trigger: IL-2, IL.4, IL-6 family, GH, PRL EPO
- Effectors: SMAD family
- Effect: early responses gene expression
- Use: growth & differentiation processes





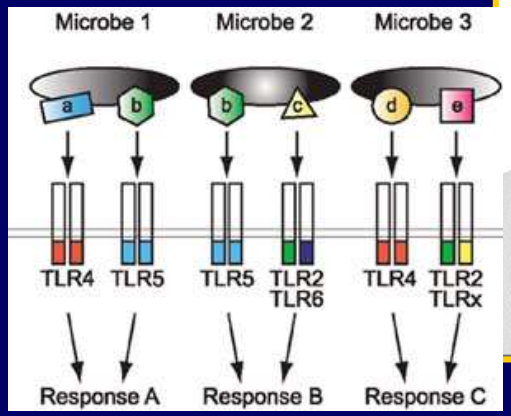
Signalling via SMAD

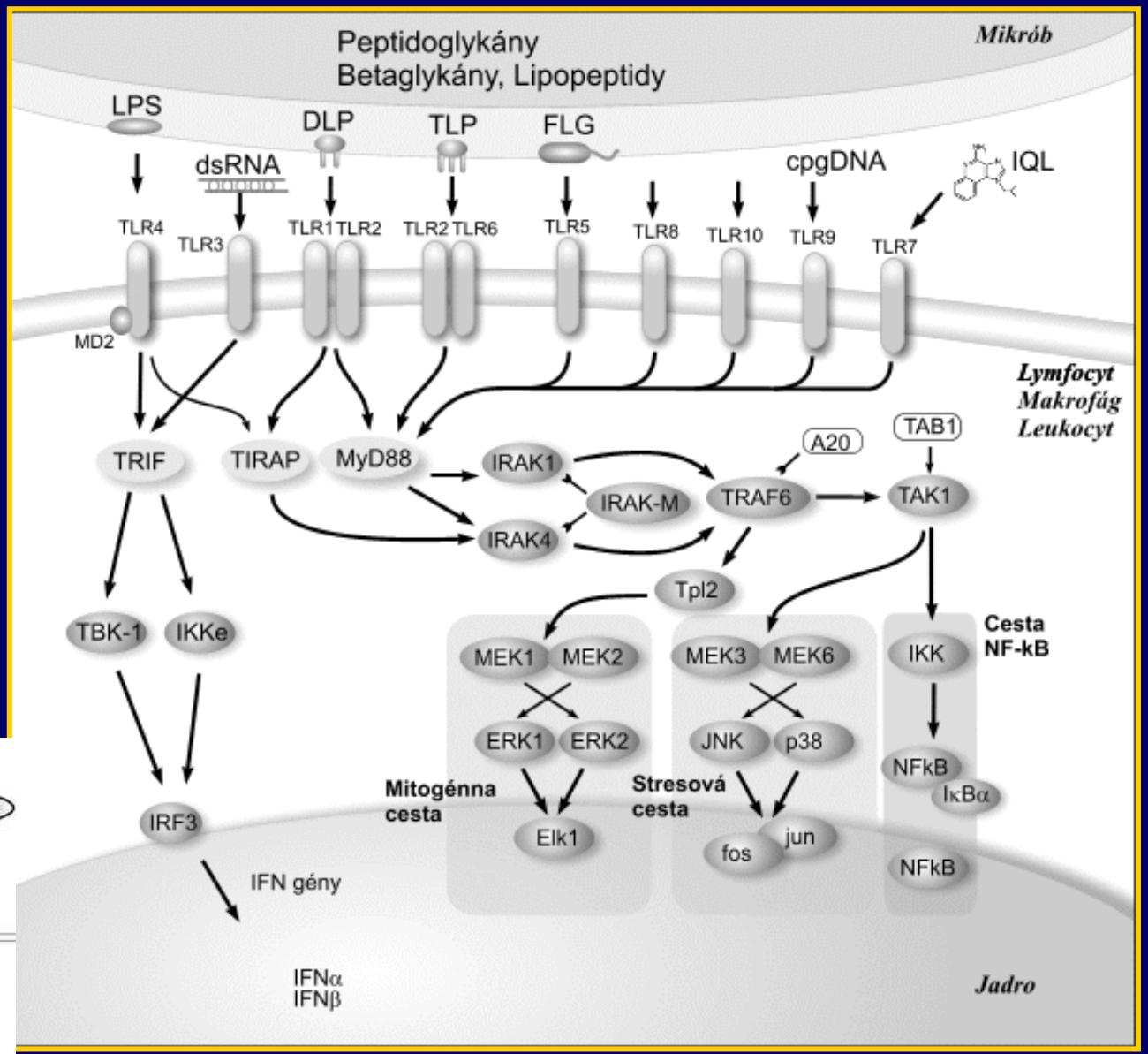
- Mutiunite membrane receptors with enzymatic activity
- Trigger: TGF & BMP factors
- Effectors: SMAD family
- Effect: delayed - gene expression
- Use: growth & differentiation processes



Signalling via Toll receptors

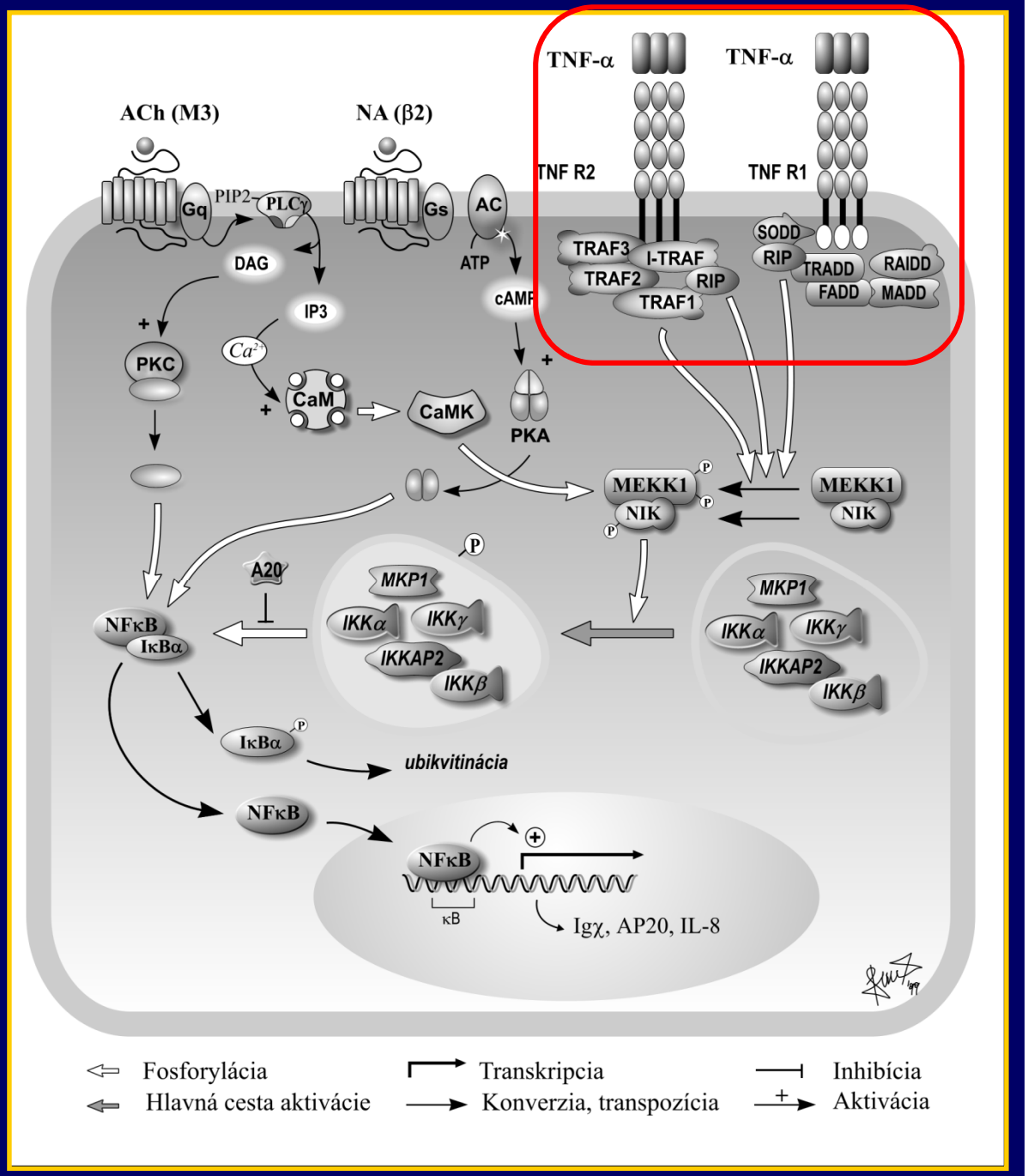
- **Trigger:** components of bacterial body, chemicals
- **Effectors:** IRAK, TRIF, TIRAP
- **Effect:** modifing other signalling pathways
- **Use:** Component of innate immune response
- Inflammation – „specific" response to antigen





Signalling via NFkB

- **Trigger:** TNF.alpha family
- **Effectors:** TRAF, TRADD, RIP
- **Effects:** Gene expression
- **Use:** wide spead
  immune reactions



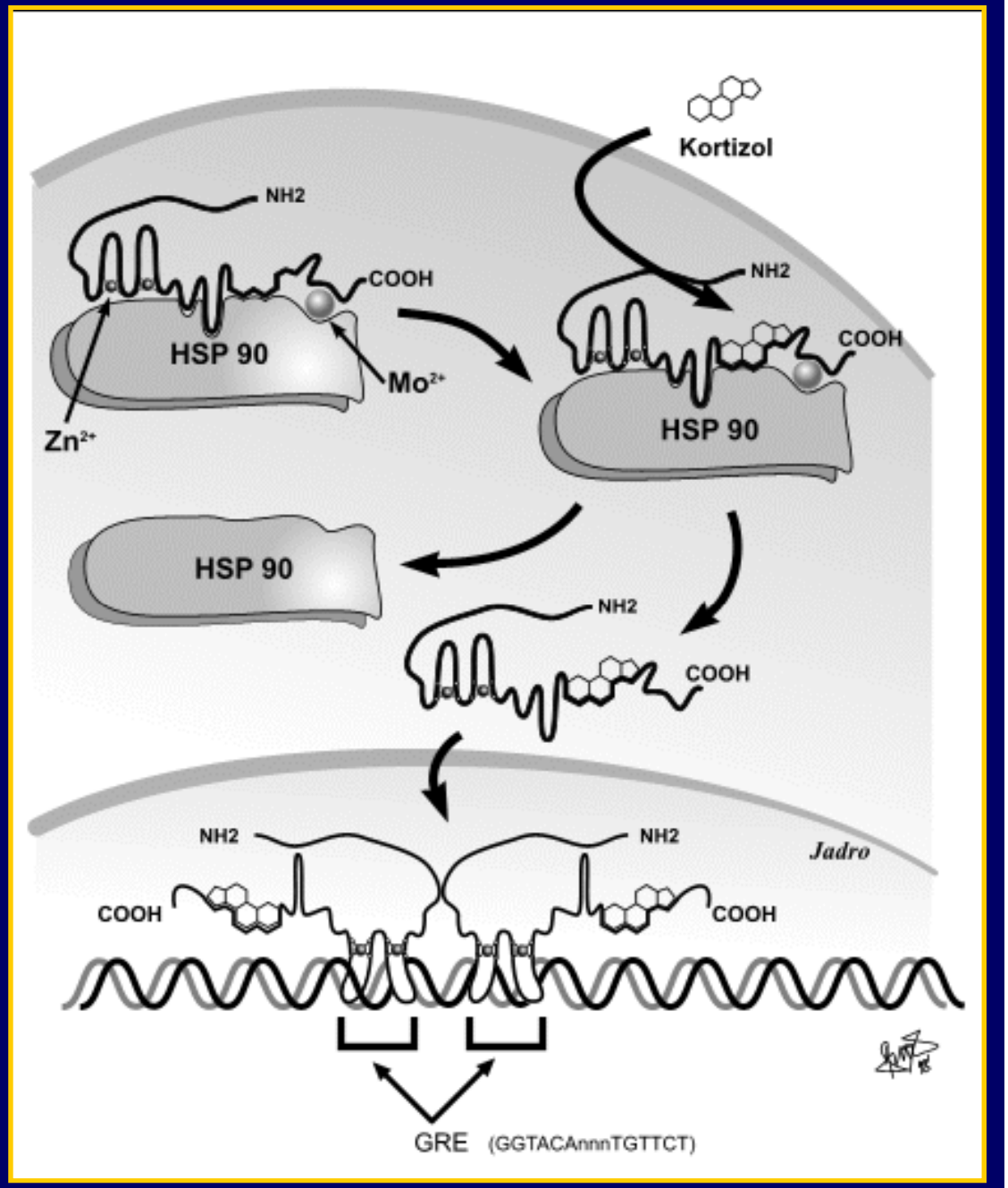
1. Long distance chemosignalling

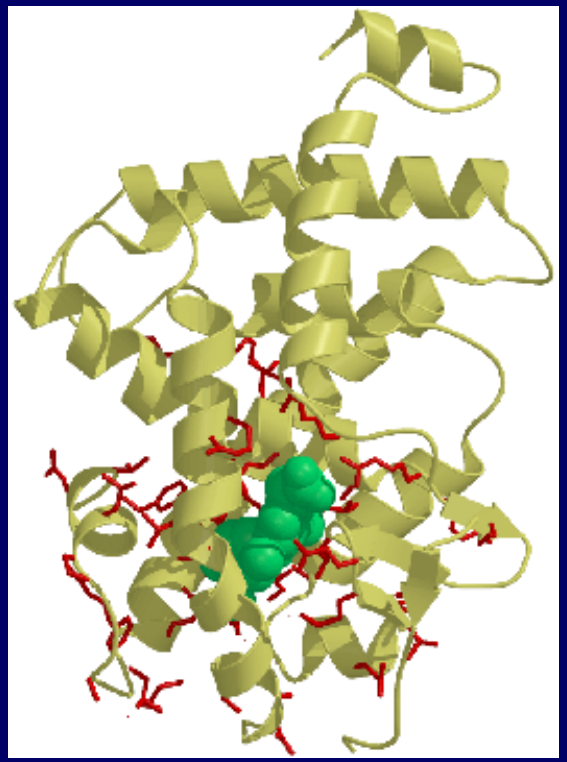
A. Receptors w/o enzymatic activity
B. Receptors with enzymatic activity
C. Intracellular receptors

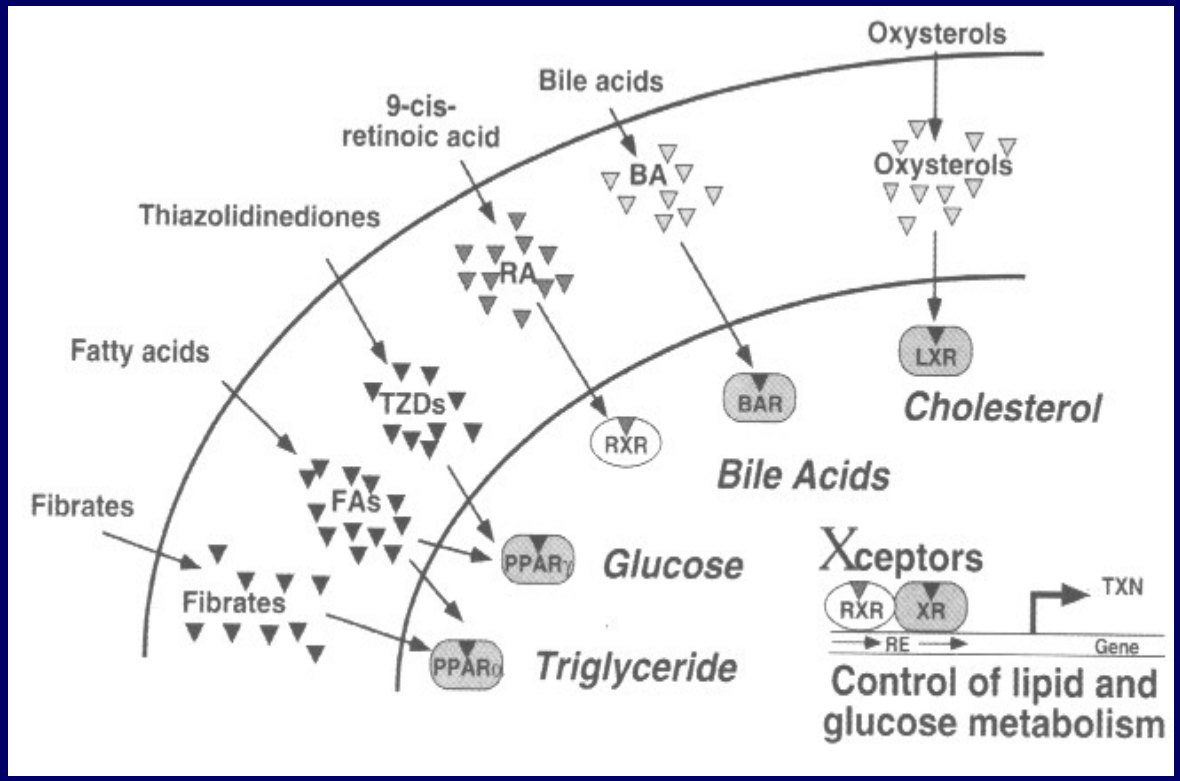
- Cytoplasmatic
- Nuclear receptors

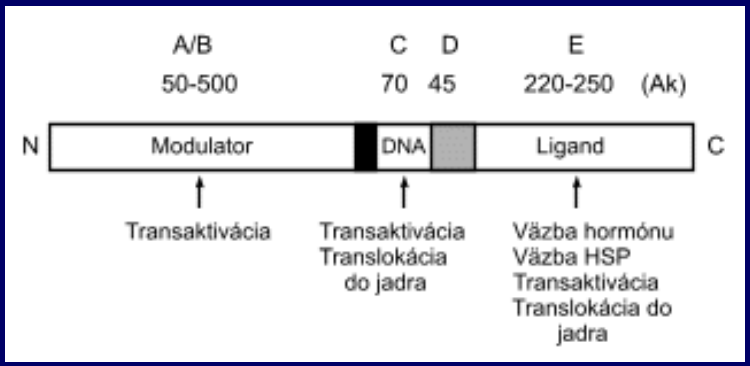
Signalling via nuclear receptors

- **Trigger:** gonadosteroids, retinoids adrenocorticosteroids, T4/T3, vitD3, deoxycholesterol, PGJ2, LTB4, fatty acids?, bile acids
- **Effect:** gene transription





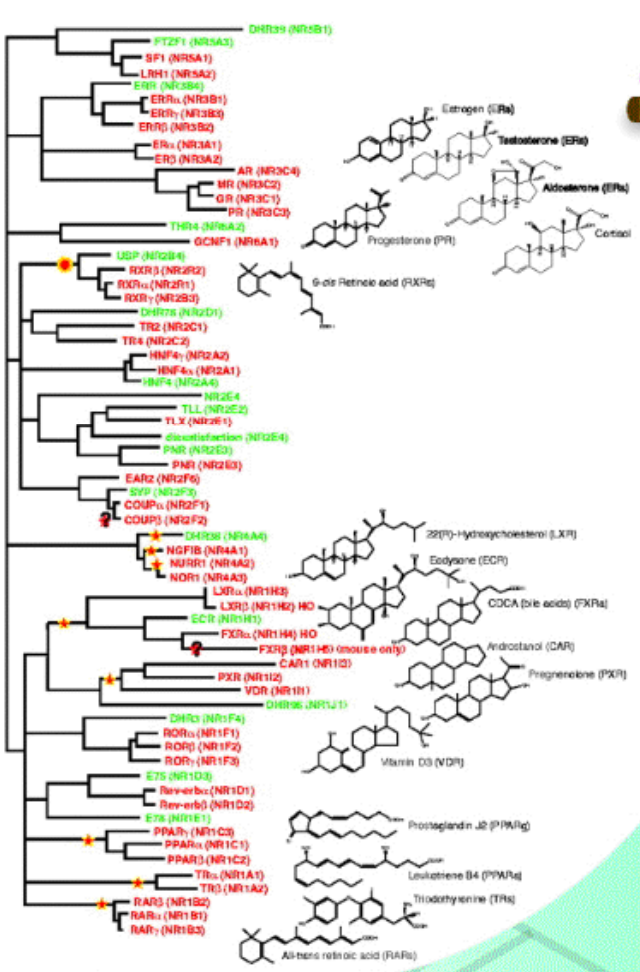

Oxysterols
Bile acids
9-cis-
retinoic acid
BA
Oxysterols
Thiazolidinediones
RA
Fatty acids
TZDs
LXR
BAR
Cholesterol
RXR
Bile Acids
Fibrates
FAs
PPARγ
Glucose
Xceptors
RXR
XR
TXN
RE
Gene
Fibrates
PPARα
Triglyceride
Control of lipid and
glucose metabolism

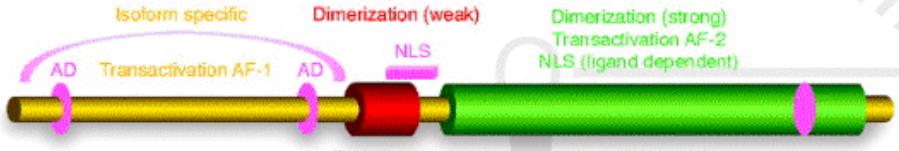

A/B
C
D
E
50-500
70
45
220-250
(Ak)
N
Modulator
DNA
Ligand
C
Transaktivácia
Transaktivácia
Translokácia
do jadra
Väzba hormónu
Väzba HSP
Transaktivácia
Translokácia do
jadra

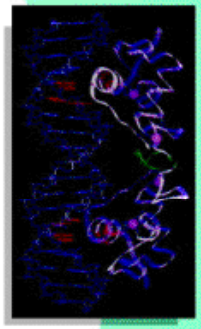
Journal of Cell Science

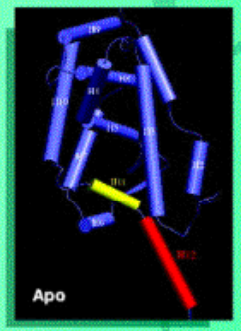
The Nuclear Receptor Superfamily

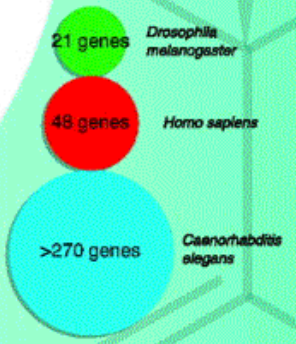
Marc Robinson-Rechavi, Hector Escriva Garcia and Vincent Laudet

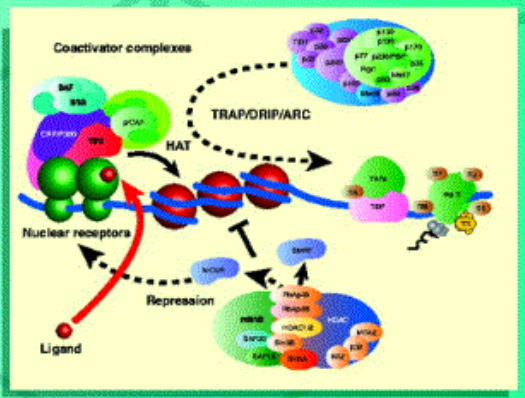


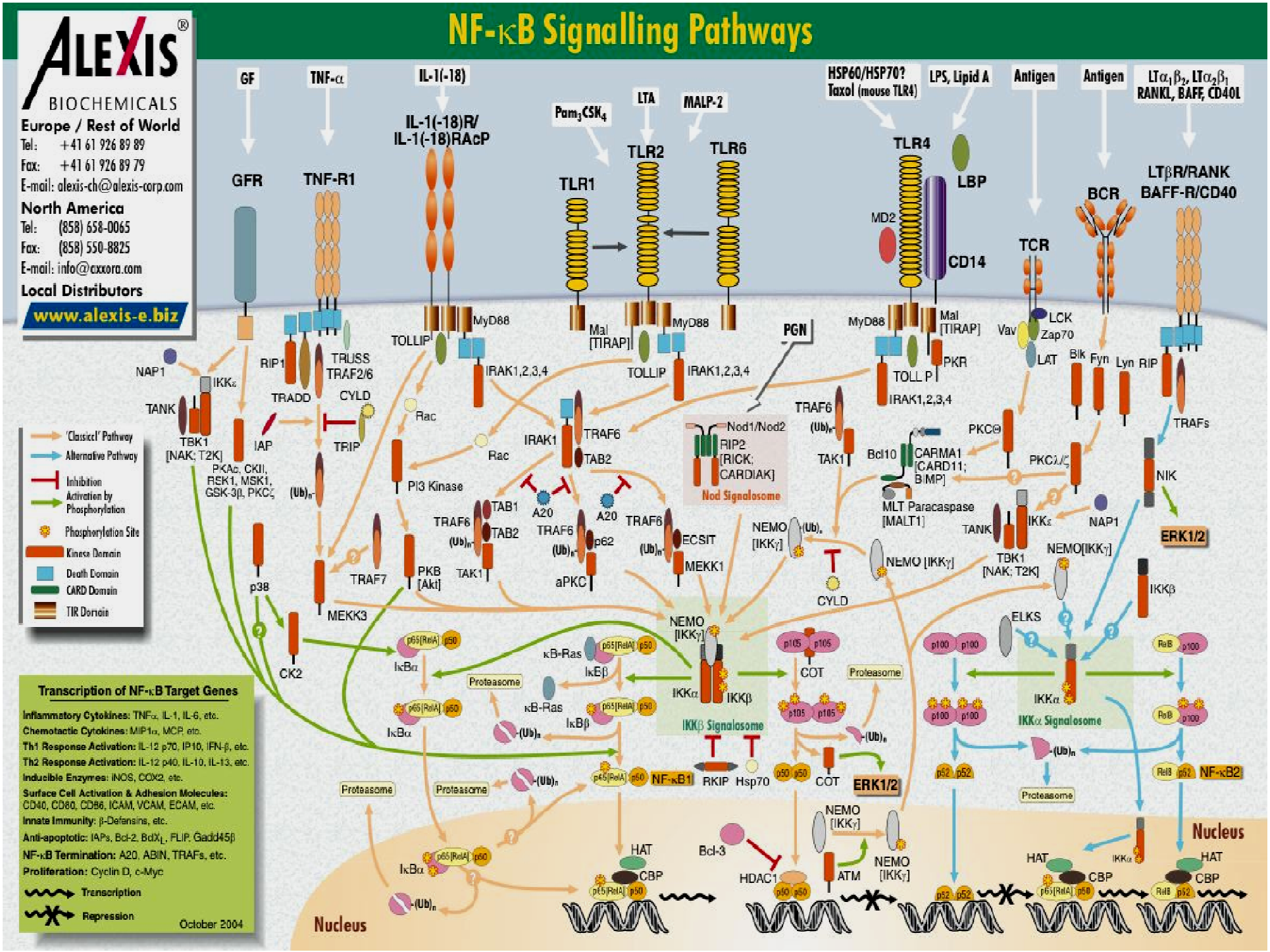










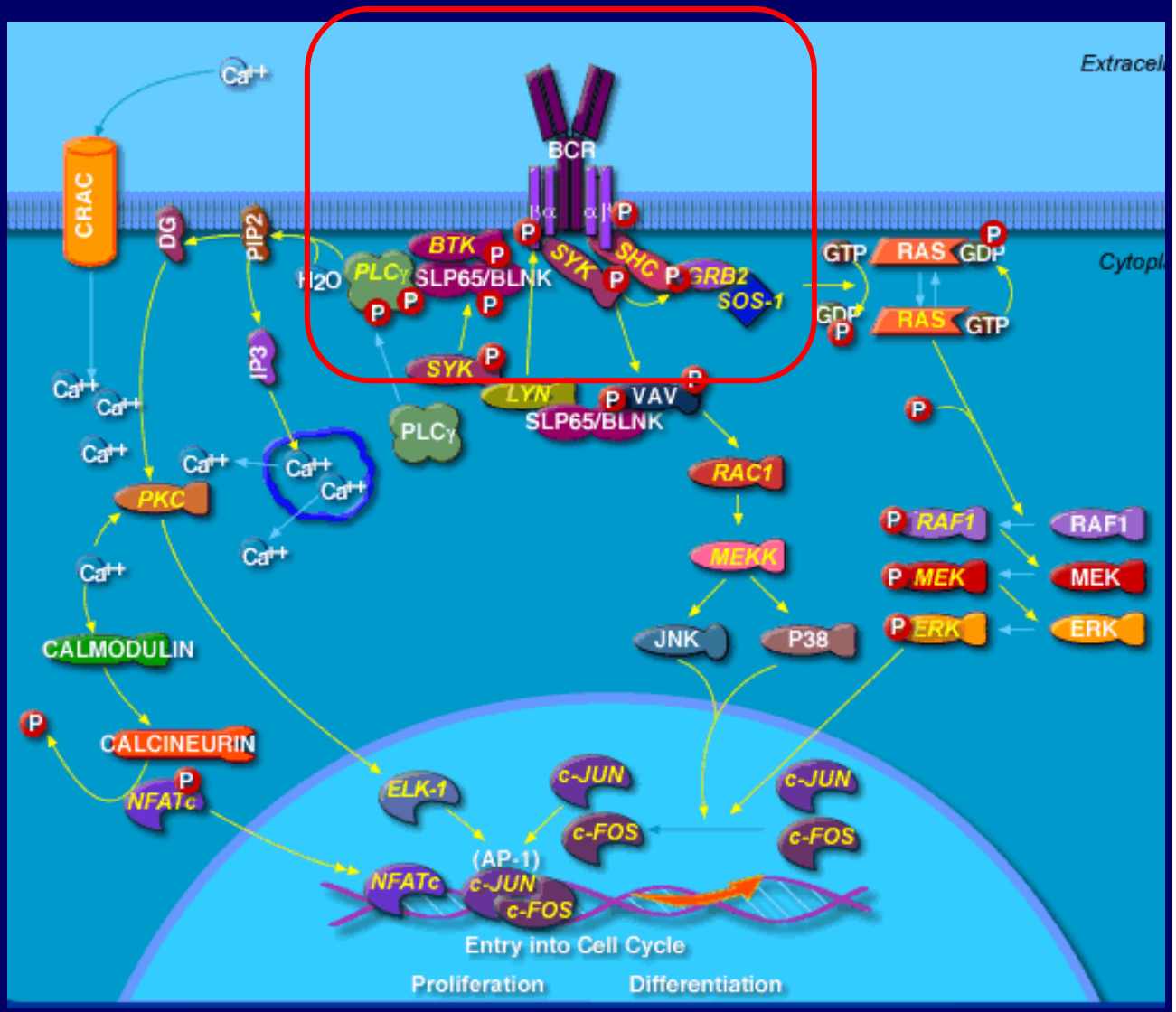

NF-κB Signalling Pathways
ALEXIS®
BIOCHEMICALS
Europe / Rest of World
Tel: +41 61 926 89 89
Fax: +41 61 926 89 79
E-mail: alexis-ch@alexis-corp.com
North America
Tel: (858) 658-0065
Fax: (858) 550-8825
E-mail: info@cxxora.com
Local Distributors
www.alexis-e.biz
GF
TNF-α
IL-1(-18)
HSP60/HSP70? Taxol (mouse TLR4)
LPS, Lipid A
Antigen
Antigen
LTα1β2, LTα2β1 RANKL, BAFF, CD40L
GFR
TNF-R1
IL-1(-18)R/ IL-1(-18)RAcP
Pam3CSK4
LTA
MALP-2
TLR1
TLR2
TLR6
TLR4
LBP
MD2
CD14
BCR
LTβR/RANK BAFF-R/CD40
TCR
PGN
NAP1
IKKε
TANK
TBK1 [NAK; T2K]
RIP1
TRADD
TRUSS TRAF2/6
CYLD
TRIP
IAP
PKAc, CKII, RSK1, MSK1, GSK-3β, PKCζ
TOLLIP
MyD88
IRAK1,2,3,4
Mal [TIRAP]
Rac
PI3 Kinase
IRAK1
TRAF6
TAB2
TAB1
A20
p62
aPKC
TAK1
ECSIT
MEKK1
MEKK3
TRAF7
PKB [Akt]
p38
CK2
Nod1/Nod2
RIP2 [RICK; CARDIAK]
Nod Signalosome
TOLL P
PKR
Vav
LCK
Zap70
LAT
Blk
Fyn
Lyn
RIP
PKCΘ
PKCλ/ζ
Bcl10
CARMA1 [CARD11; BIMP]
MLT Paracaspase [MALT1]
TRAFs
NIK
ERK1/2
NAP1
IKKε
TBK1 [NAK; T2K]
NEMO [IKKγ]
CYLD
ELKS
IKKβ
IKKα
IKKβ Signalosome
IKKα Signalosome
COT
IκBα
IκBβ
κB-Ras
Proteasome
RKIP
Hsp70
NF-κB1
NF-κB2
ERK1/2
HAT
CBP
Bcl-3
HDAC1
ATM
Nucleus
'Classical' Pathway
Alternative Pathway
Inhibition
Activation by Phosphorylation
Phosphorylation Site
Kinase Domain
Death Domain
CARD Domain
TIR Domain
Transcription of NF-κB Target Genes
Inflammatory Cytokines: TNFα, IL-1, IL-6, etc.
Chemotactic Cytokines: MIP1α, MCP, etc.
Th1 Response Activation: IL-12 p70, IP10, IFN-β, etc.
Th2 Response Activation: IL-12 p40, IL-10, IL-13, etc.
Inducible Enzymes: iNOS, COX2, etc.
Surface Cell Activation & Adhesion Molecules: CD40, CD80, CD86, ICAM, VCAM, ECAM, etc.
Innate Immunity: β-Defensins, etc.
Anti-apoptotic: IAPs, Bcl-2, BclXL, FLIP, Gadd45β
NF-κB Termination: A20, ABIN, TRAFs, etc.
Proliferation: Cyclin D, c-Myc
Transcription
Repression
October 2004

2. Close contact chemosignalling

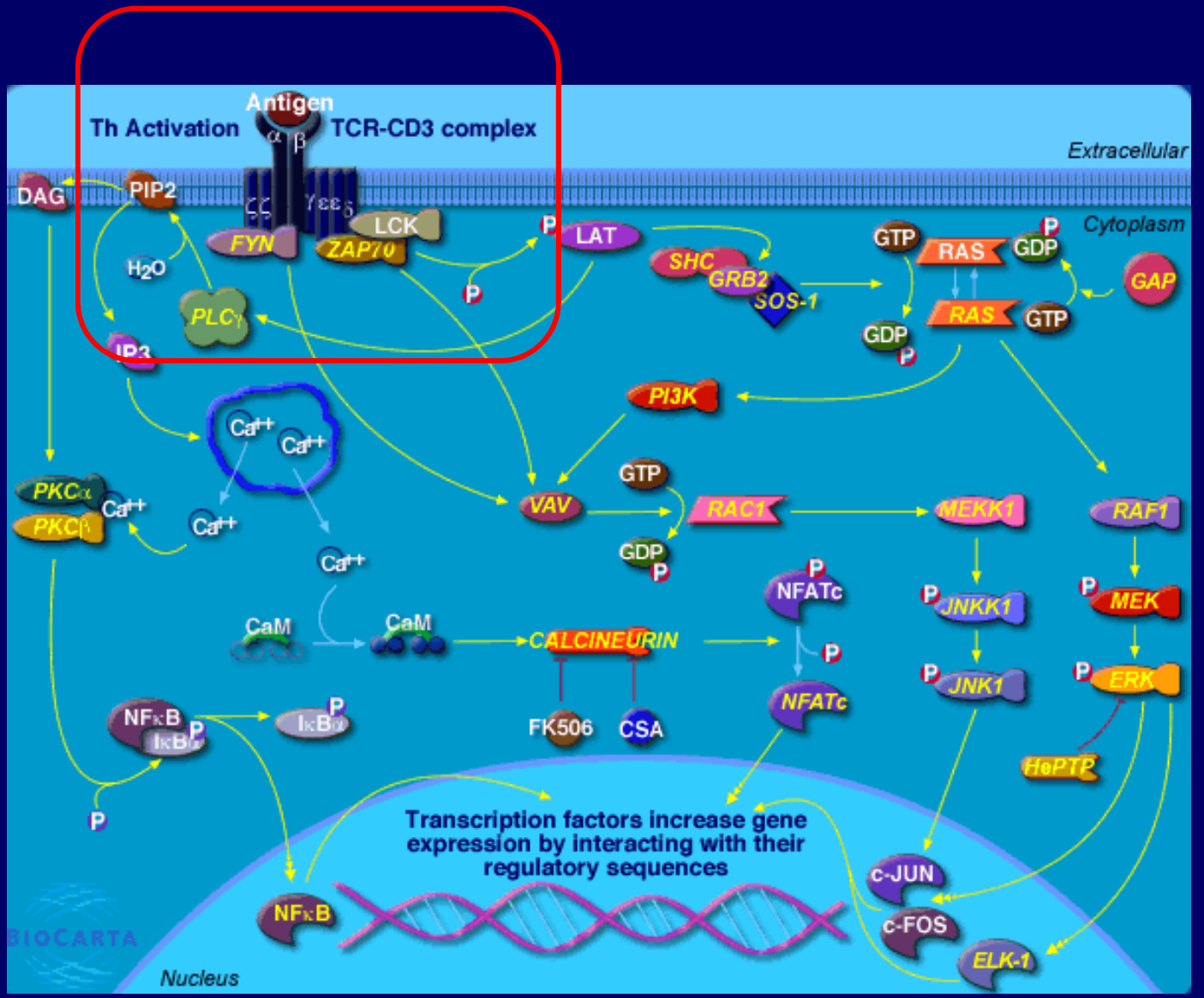
- BCR signalling
- TCR signalling
- Wnt + β-catenin
- DSL-Notch
- Hedgehog signalling

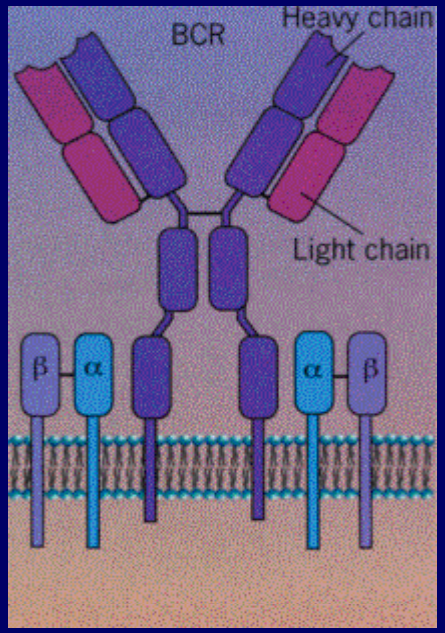
BCR signalling

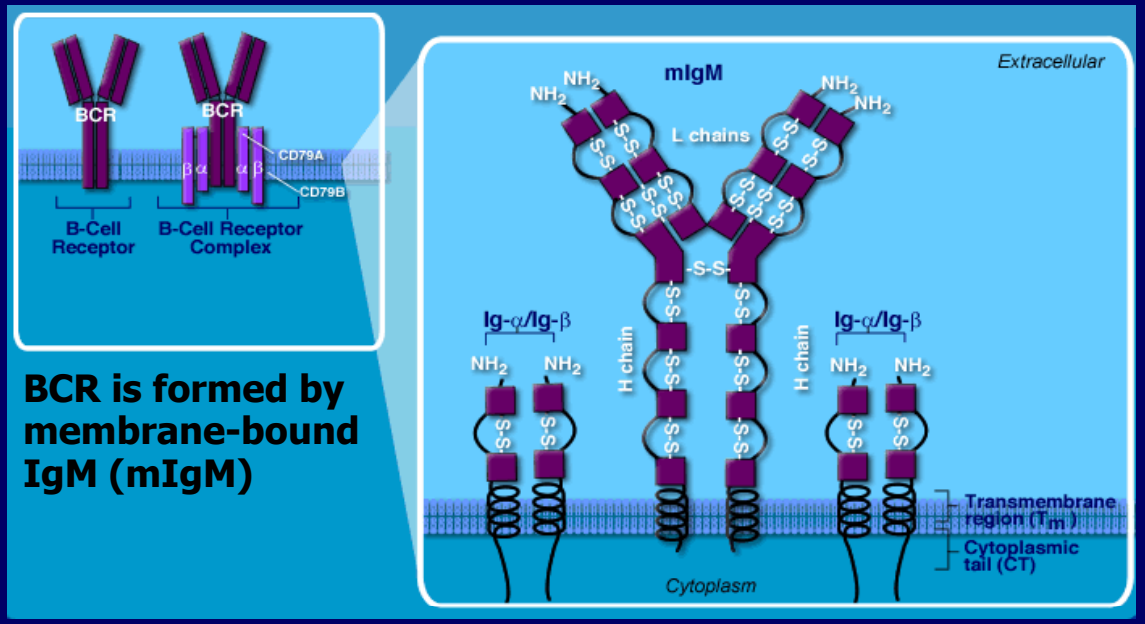
- **Trigger:** antigen
- **Receptors** – multicomponent Ig-family
- **Effectors:**
  - Growth pathway (Ras-Raf-MEK-ERK)
  - Stress pathway (Rac-MEKK-Jnk,P38)
  - Ca^{2+}-CaM pathway
- **Effect:**
  - Gene transcription
- **Use:**
  - proliferation (mitosis)
  - synthesis of new proteins



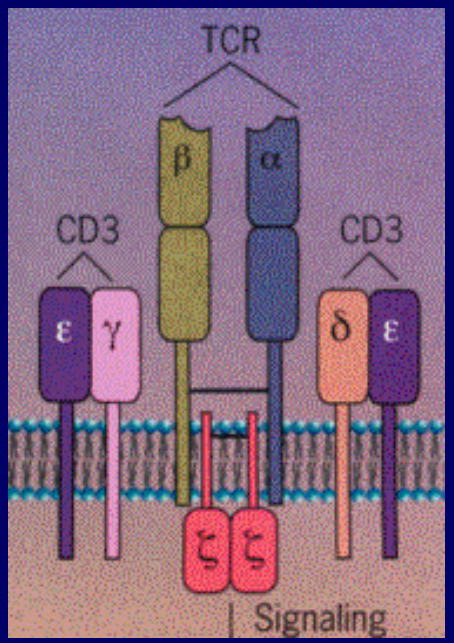
TCR signalling

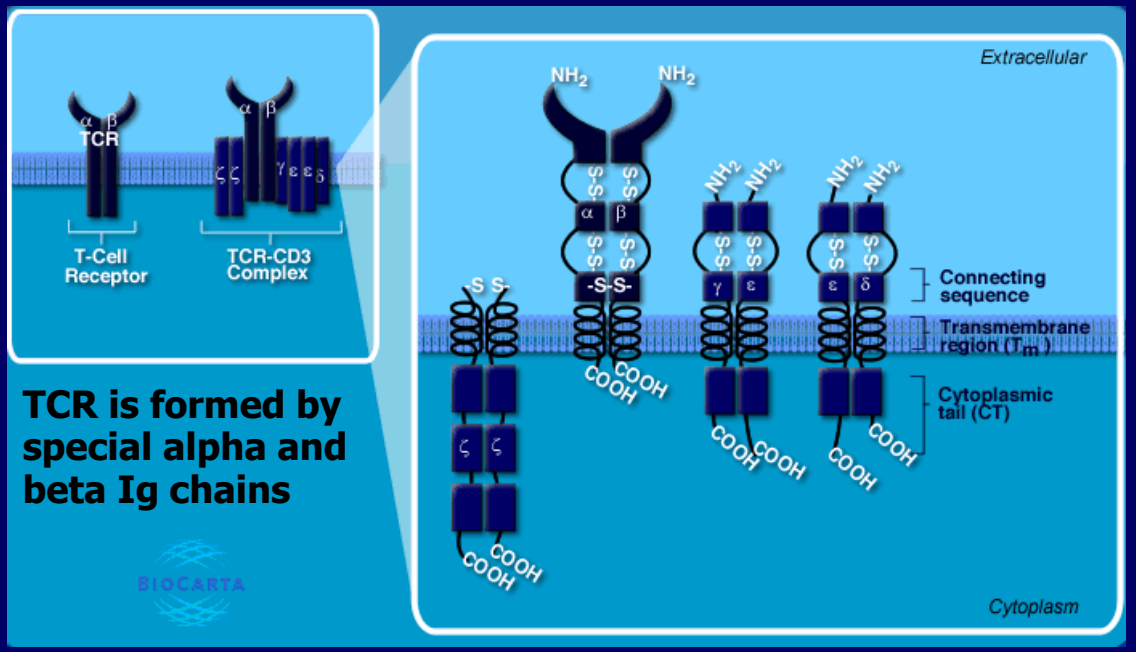






BCR is formed by membrane-bound IgM (mIgM)

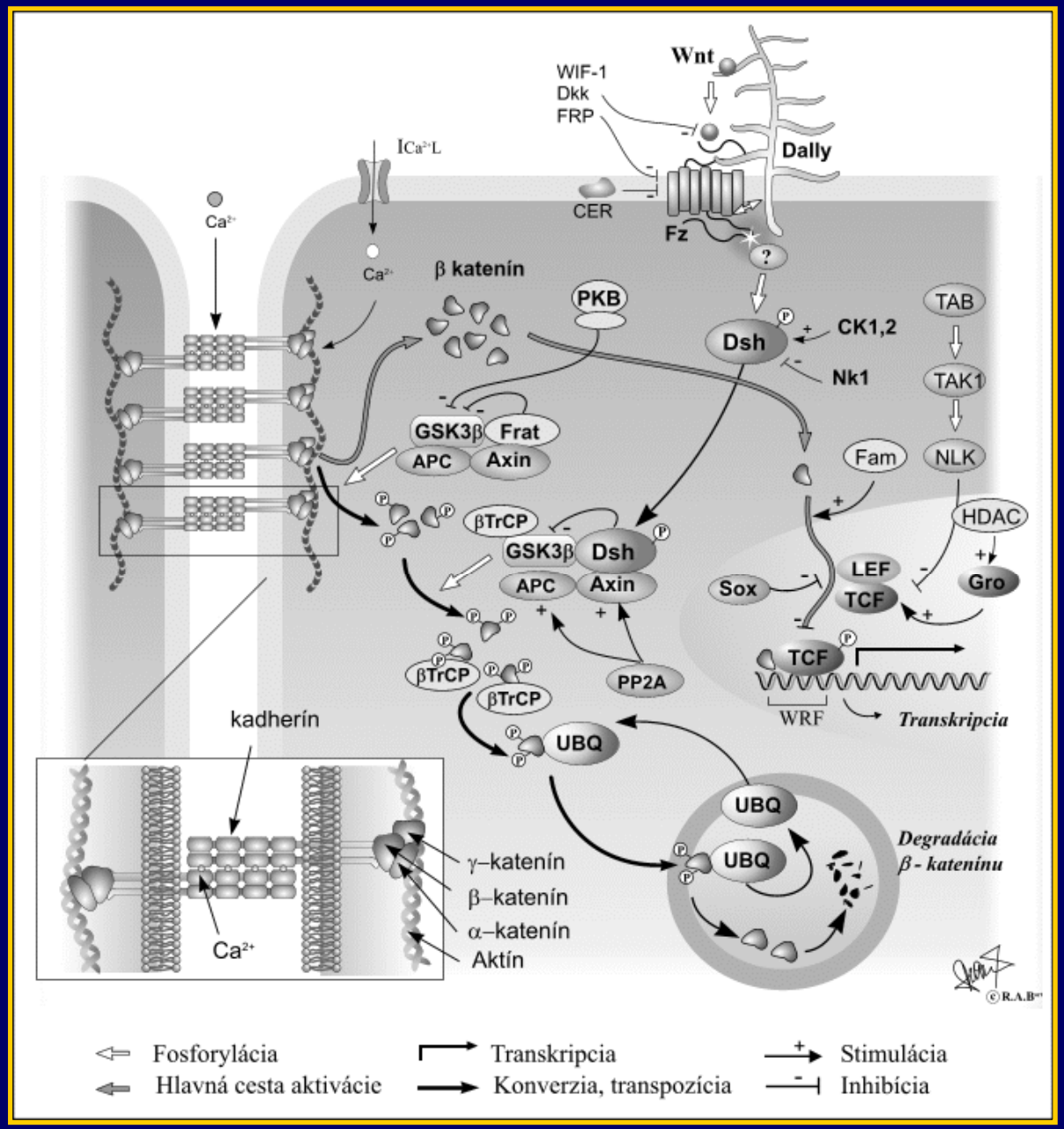




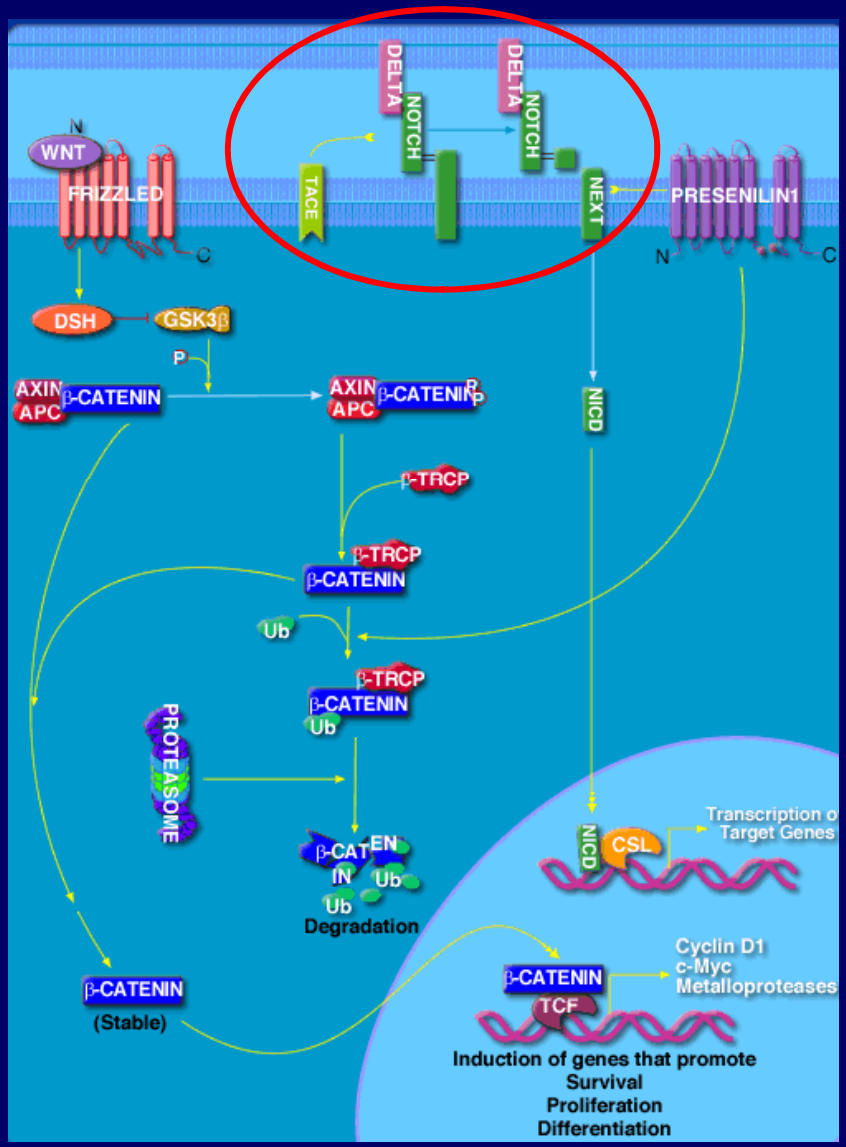
TCR is formed by special alpha and beta Ig chains

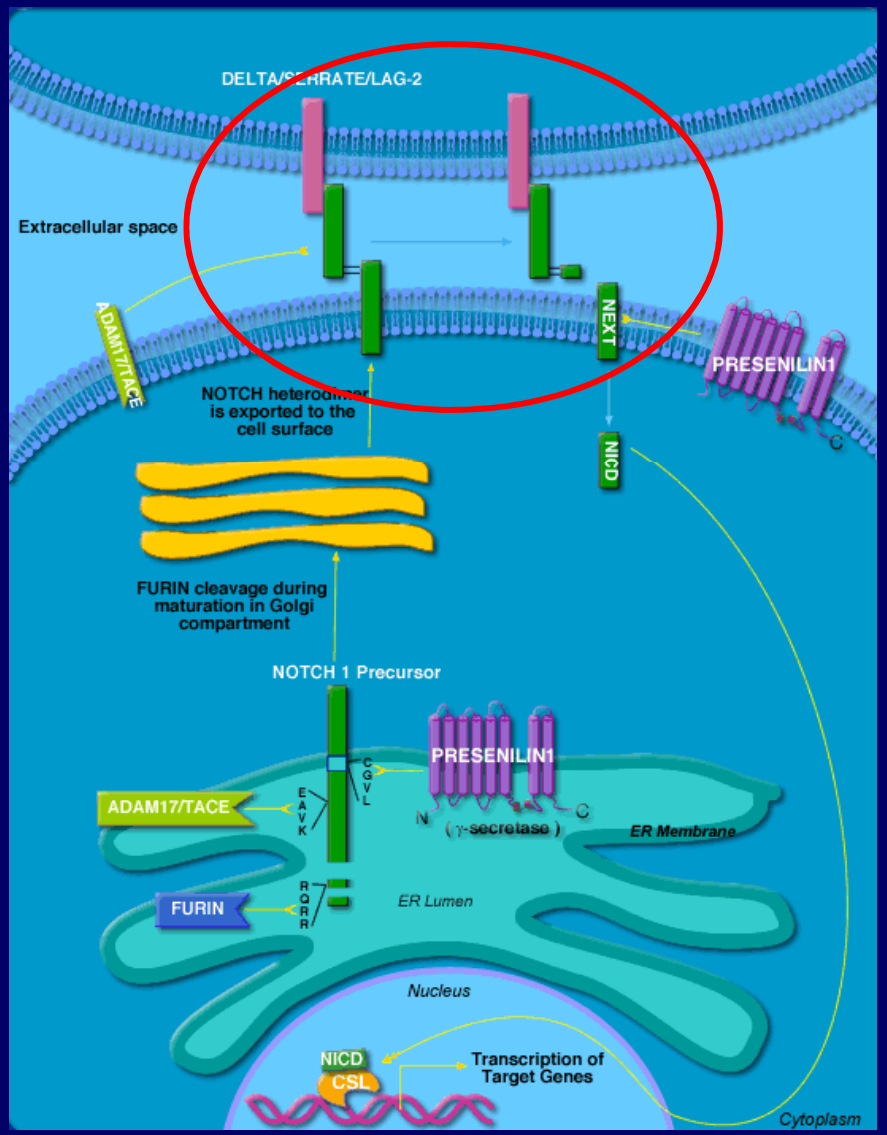
BIOCARTA

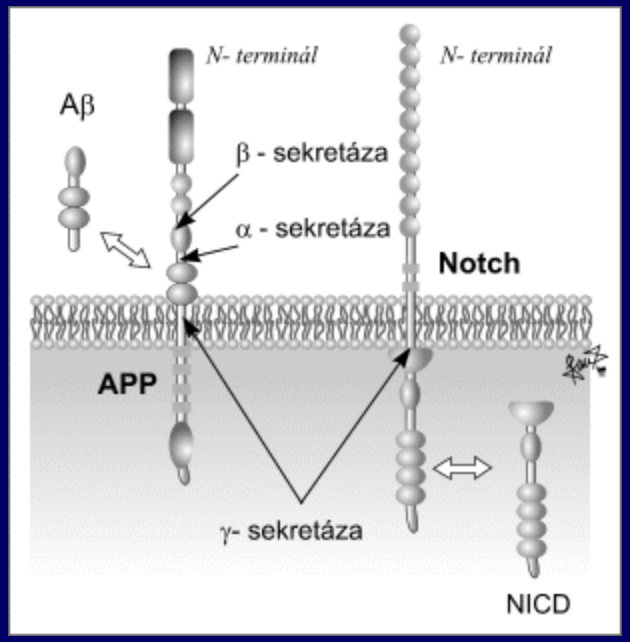
Wnt + β - catenin

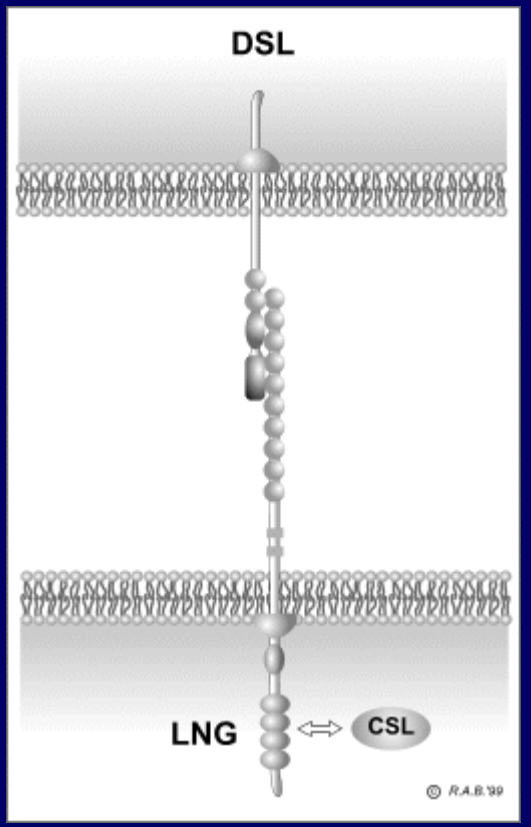


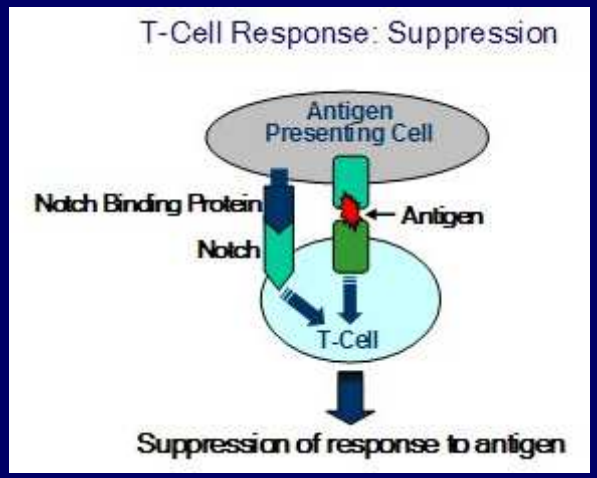
DSL-Notch signalling

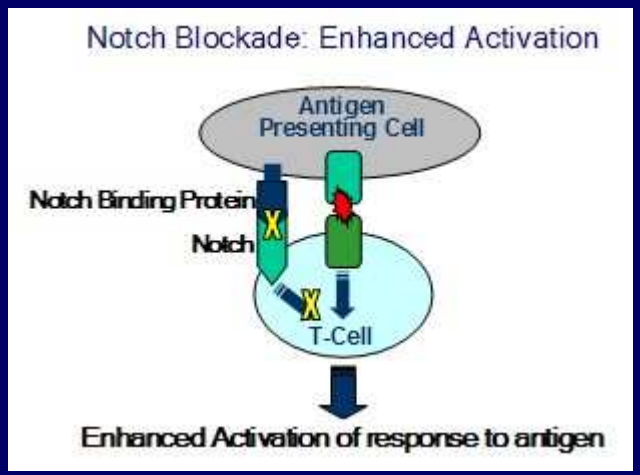





N- terminál
N- terminál
Aβ
β - sekretáza
α - sekretáza
Notch
APP
γ- sekretáza
NICD


DSL
LNG
CSL
© R.A.B.'99


T-Cell Response: Suppression
Antigen Presenting Cell
Notch Binding Protein
Antigen
Notch
T-Cell
Suppression of response to antigen


Notch Blockade: Enhanced Activation
Antigen Presenting Cell
Notch Binding Protein
X
Notch
X
T-Cell
Enhanced Activation of response to antigen

Hedgehog signalling

- **Trigger:** Hh family
- **Receptors:**
  - Ptc – patched
  - Smo –smoothed
- **Effect:**
  - Gene expression
  - Other signallings
- **Use:**
  - Control over prolifertion & differentiation