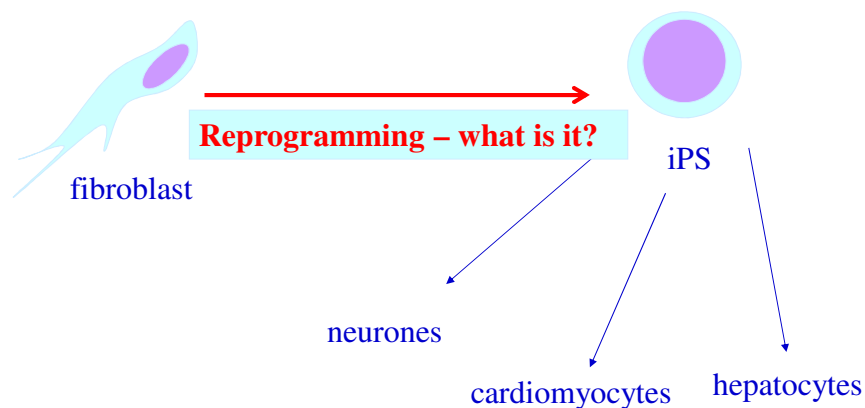


# General Mechanisms of Cell Signaling

## Signaling to Cell Nucleus

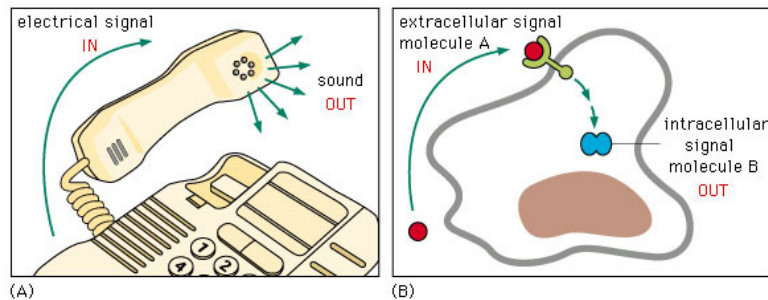
MUDr. Jan Pláteník, PhD.

Somatic cells can be reprogrammed to pluripotent stem cells !



Takahashi K & Yamanaka S. Cell 126, 2006, 663-676

# General mechanisms of cell signaling

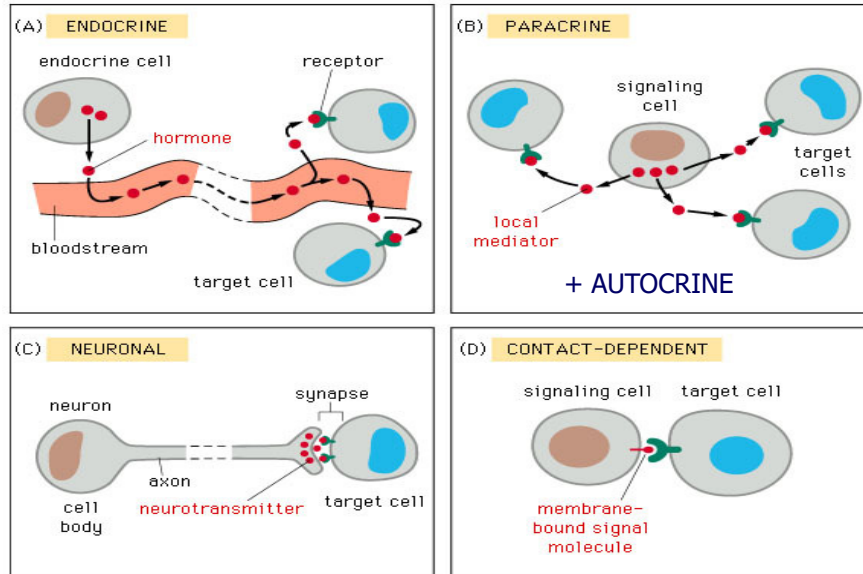


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## Cell-to-cell communication:

1. Synthesis and
2. Release of the signaling molecule by the signaling cell
3. Transport of the signal to the target cell
4. Detection of signal by specific receptor protein
5. Signal-receptor complex triggers a change in cell metabolism and/or gene expression of the target cell
6. Removal/Termination of the signal

## Signals operate over various distances :



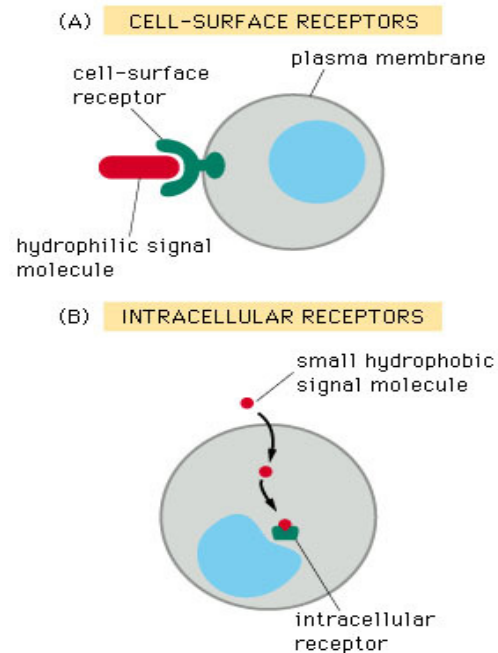
## Signaling molecule (ligand):

- **Peptides/proteins**
  - ACTH, insulin, glucagon, growth factors, cytokines, and many others
- **Small lipophilic molecules:**
  - steroids, thyroid hormones, prostaglandins
- **Small hydrophilic molecules:**
  - AA or derivatives of AA - epinephrine, norepinephrine, histamine, serotonin, glutamate, GABA, glycine etc.
- **Gases: NO, CO, H<sub>2</sub>S**

# Receptor

- Always a protein
- Ligand binding + Conformation change  
→ signal transduction
- Binding specificity
- Effector specificity

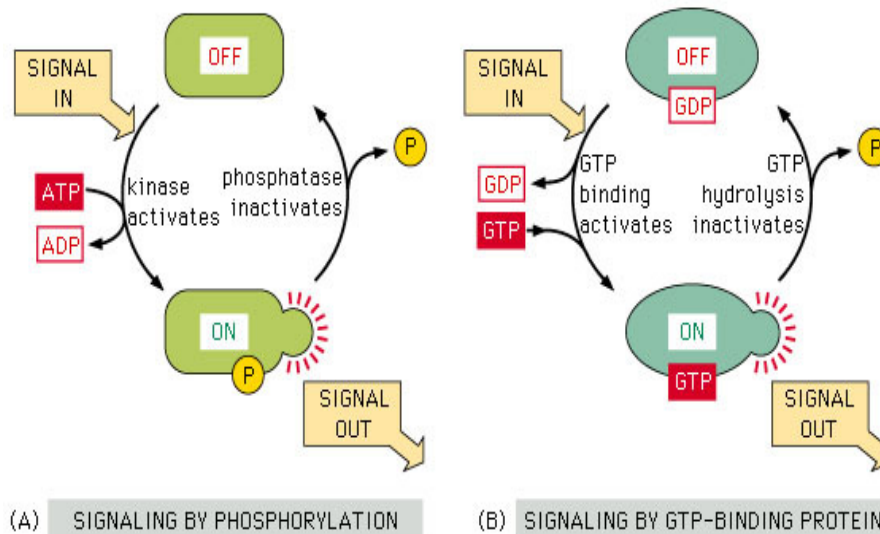
## Two classes of signaling molecules



## Second messengers:

- Cyclic adenosine monophosphate (cAMP)
- Cyclic guanosine monophosphate (cGMP)
- Inositol-1,4,5-tris-phosphate
- 1,2-diacylglycerol
- Calcium
- (NO, oxygen radicals)

## Molecular switches:



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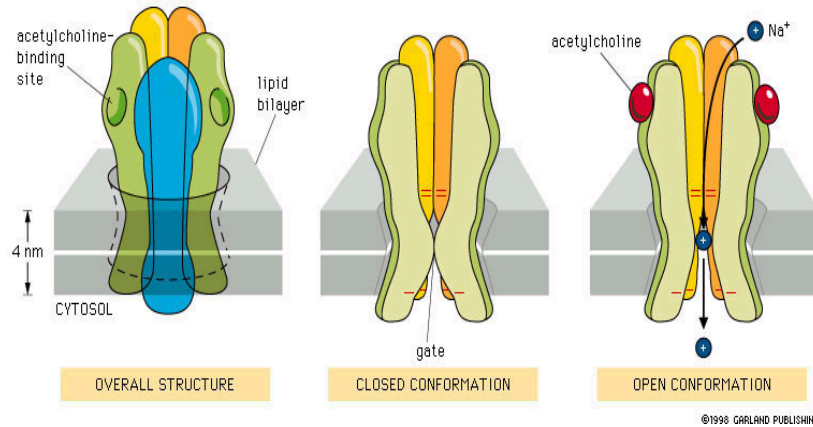
## Signal termination:

- **Receptor desensitization**
  - endocytosis & degradation
  - phosphorylation
- **Degradation or removal of signalling molecule**
  - cAMP: phosphodiesterase
  - Calcium: Ca<sup>2+</sup> pumps
- **Dephosphorylation by protein phosphatases**

## Cell-surface receptors for signaling molecules:

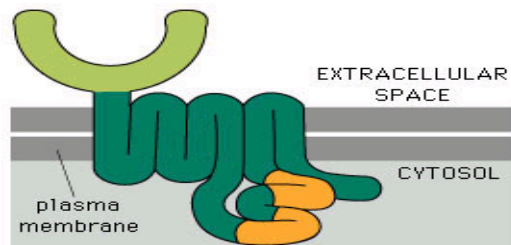
- **Ion channels**
  - for Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>
  - e.g. receptor for acetylcholine, GABA, glutamate, glycine
- **Seven-spanning G protein-linked**
- **Receptors associated with an enzymic activity**

## Muscle receptor for acetylcholine

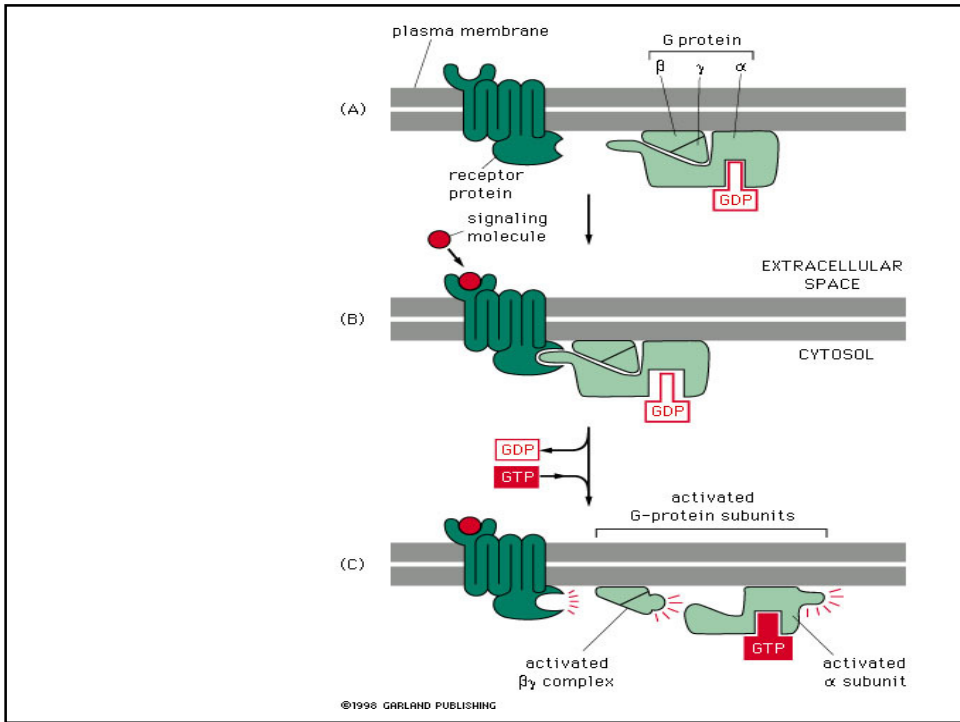


## Cell-surface receptors for signaling molecules:

- Ion channels
- Seven-spanning G protein-linked

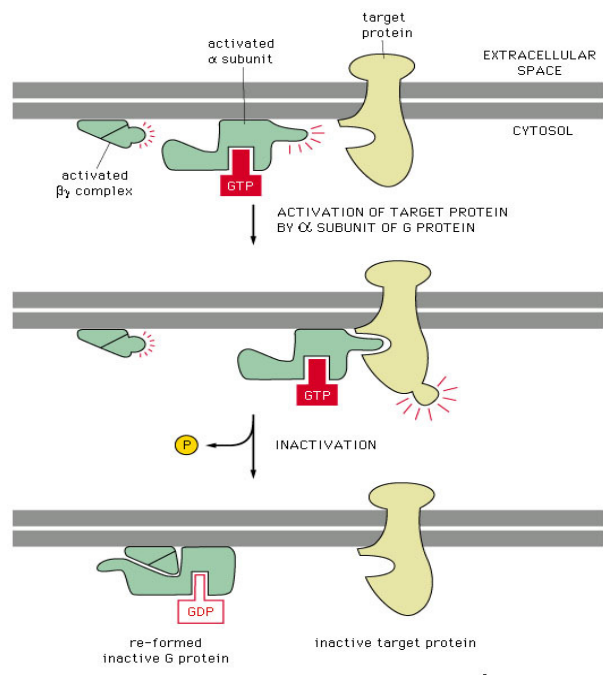


- Receptors associated with an enzymic activity

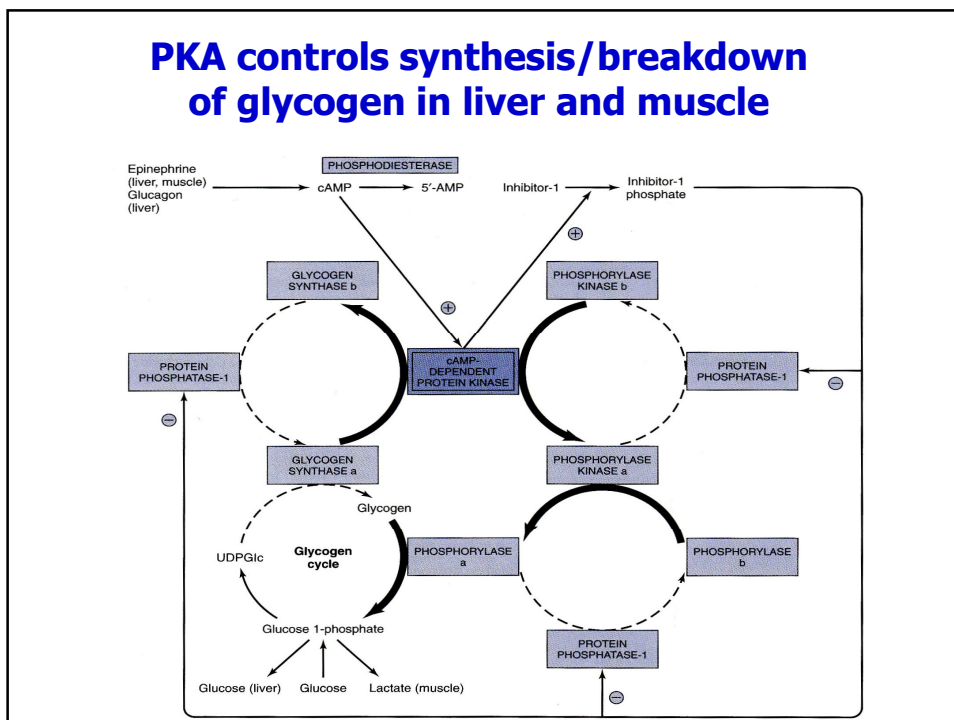
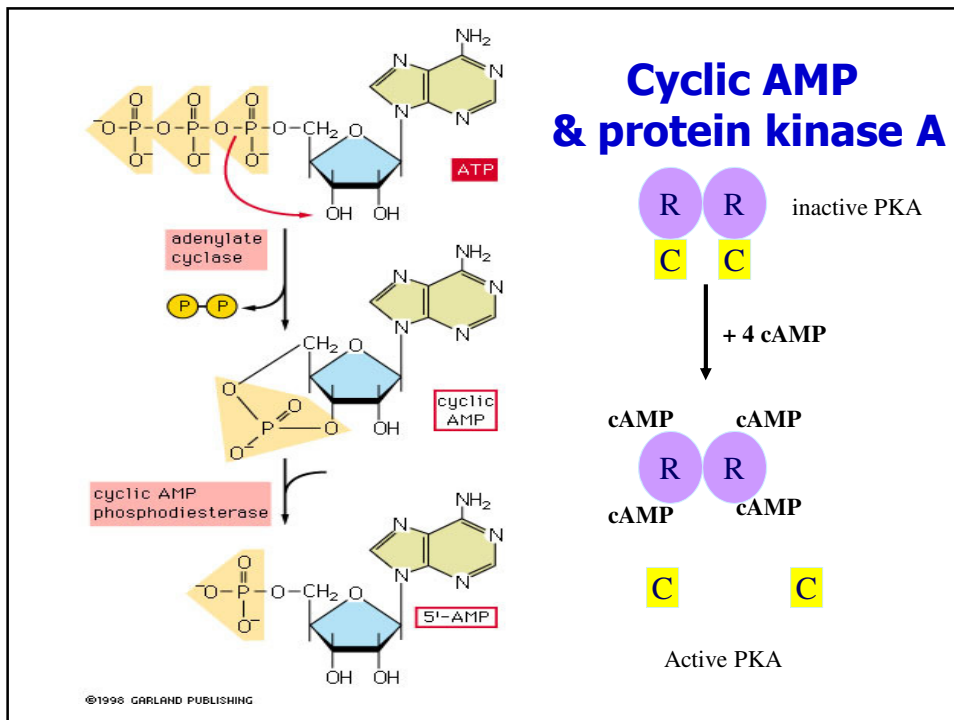


**G-protein targets:**

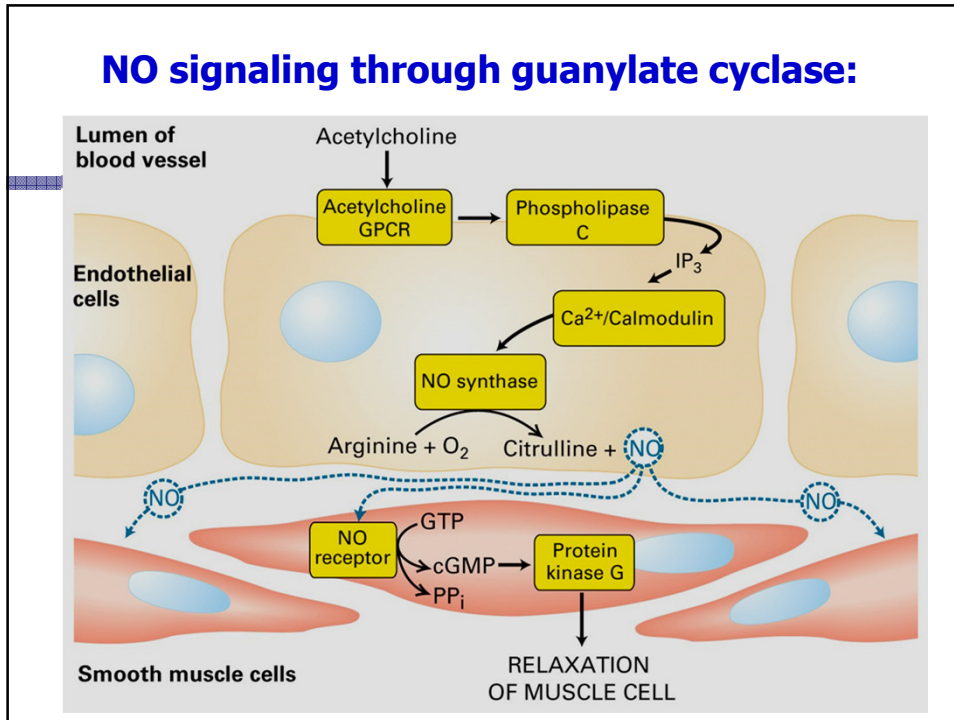
- membrane enzymes
- ion channels



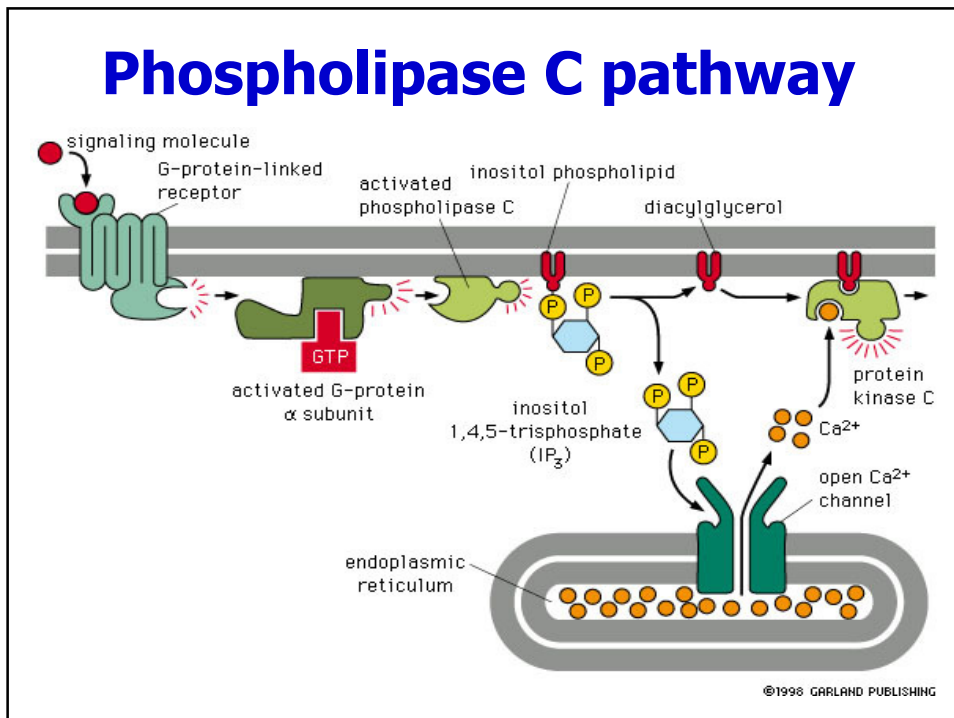




## NO signaling through guanylate cyclase:



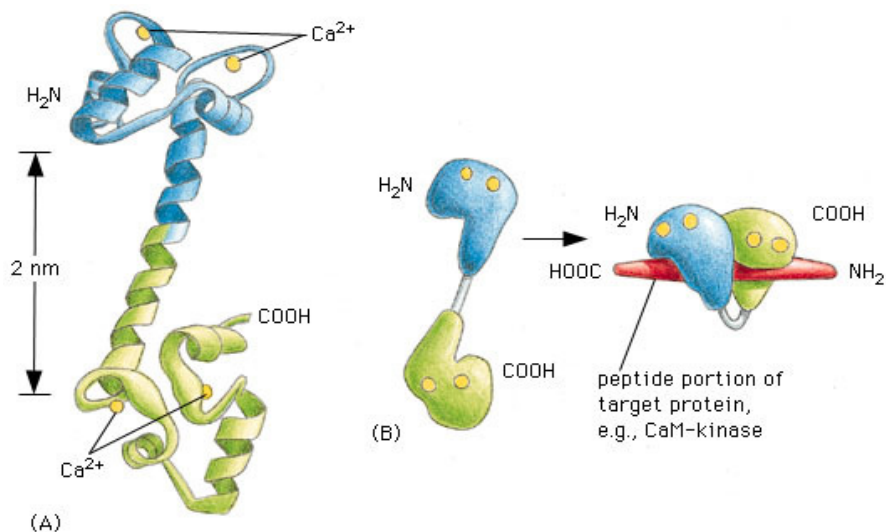
## Phospholipase C pathway

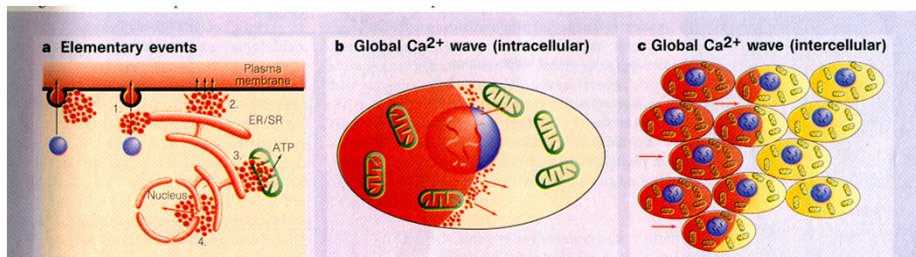


## Calcium in the cell:

- In cytosol only 0.1-0.2  $\mu\text{M}$ , about 1  $\mu\text{M}$  is a signal
- Source of the signal is:
  - outside:
    - ligand-operated  $\text{Ca}^{2+}$  channels
    - voltage-operated  $\text{Ca}^{2+}$  channels
  - ER stores:
    - PI3 receptor/channel
    - ryanodine receptor/channel
      - cell membrane potential-dependent (striated muscle)
      - $\text{Ca}^{2+}$  -dependent (heart, CNS)

## Calmodulin (148 AMK)





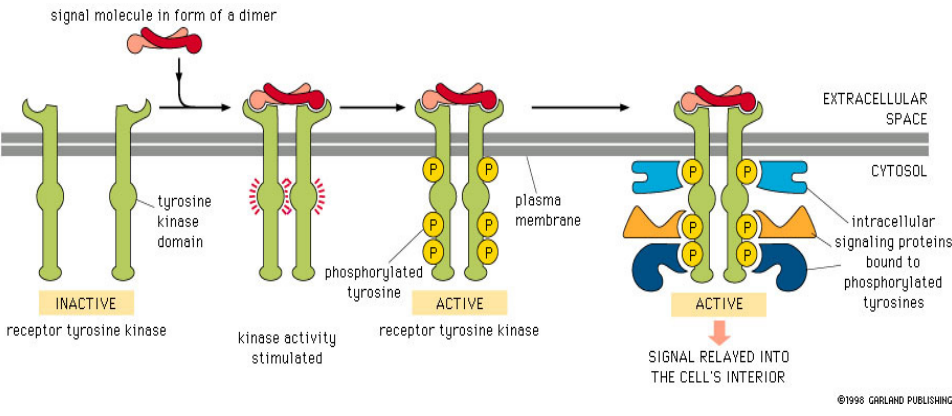
(Berridge et al., Nature 1998, 395: 645-648)

- **Information in  $\text{Ca}^{2+}$  signal is encoded by its**
  - **LOCALISATION**
  - **FREQUENCY**
  - **AMPLITUDE**

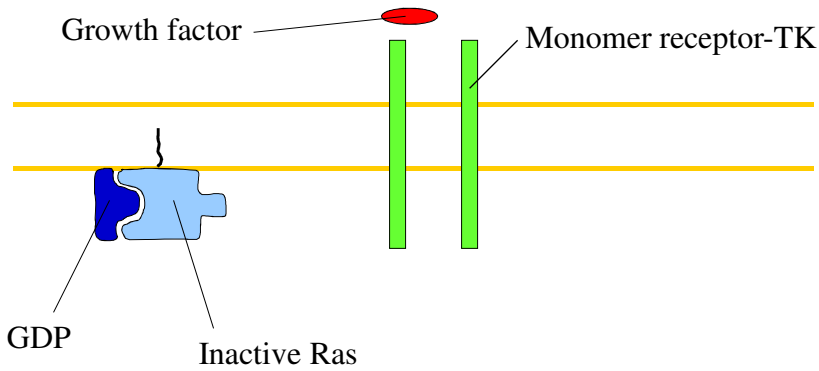
## Cell-surface receptors for signaling molecules:

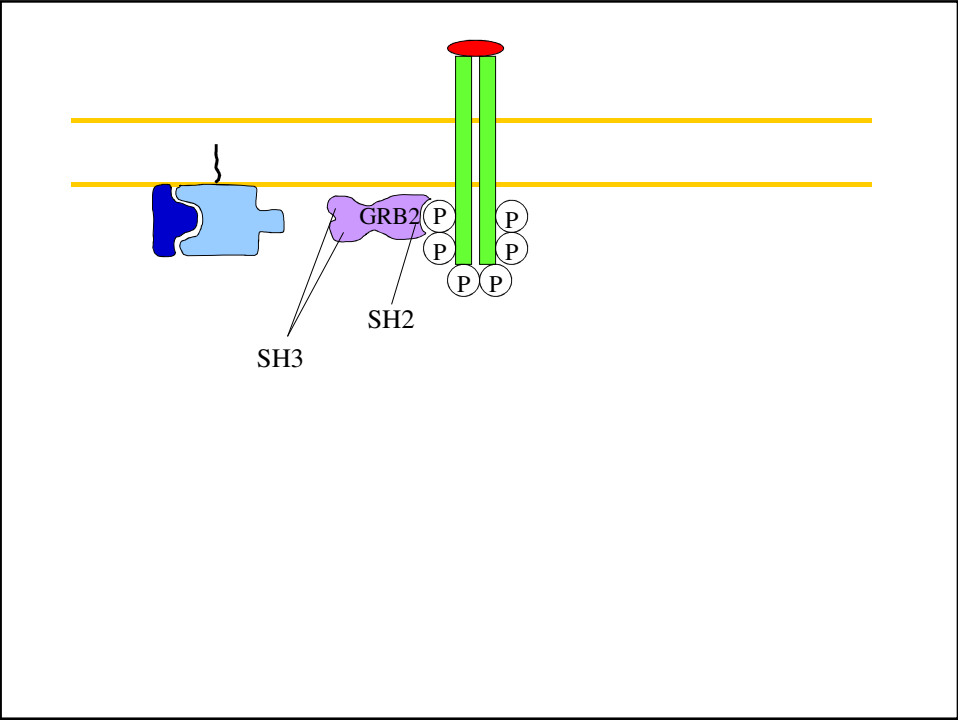
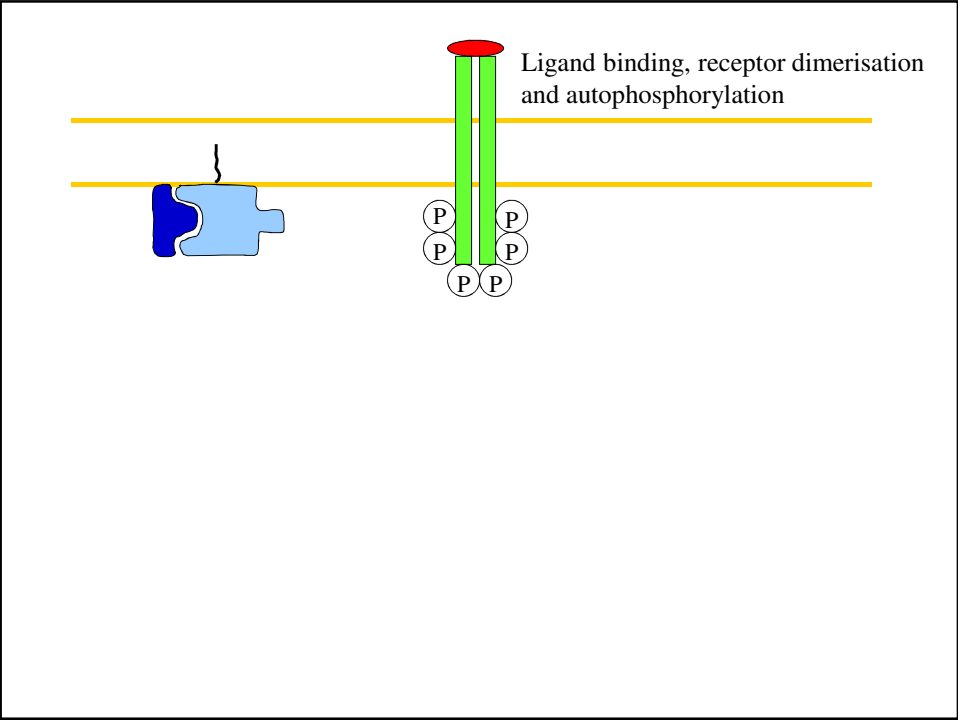
- **Ion channels**
- **Seven-spanning G protein-linked**
- **Receptors associated with an enzymic activity**
  - **intrinsic catalytic activity: receptor tyrosine kinases (RTKs, e.g. rec. for EGF, insulin)**
  - **associated with soluble tyrosine kinases (e.g. rec. for cytokines, interferons)**

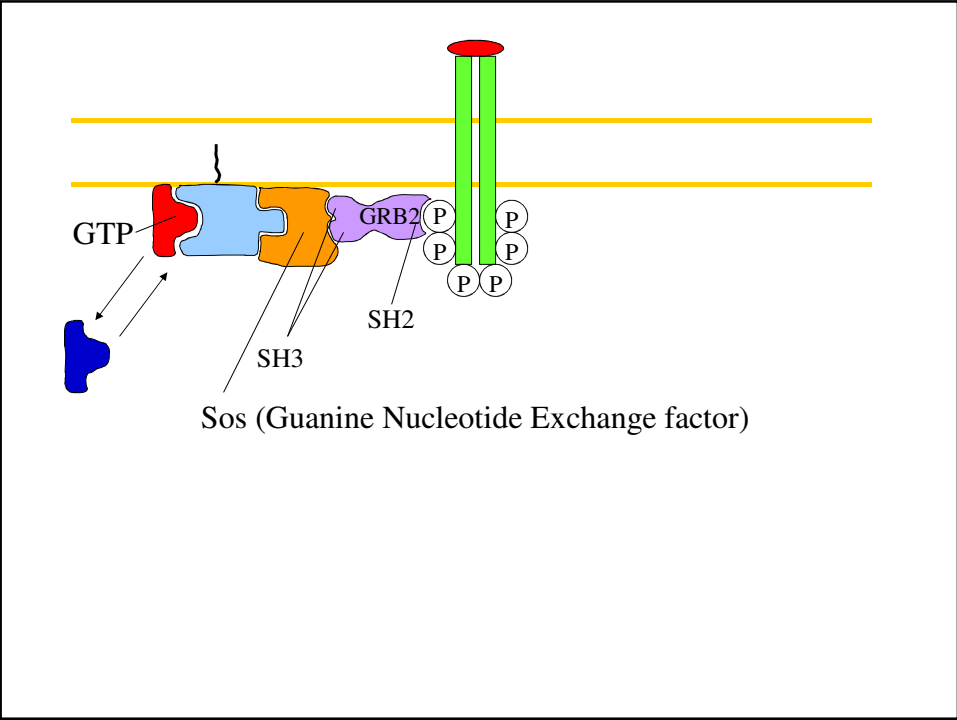
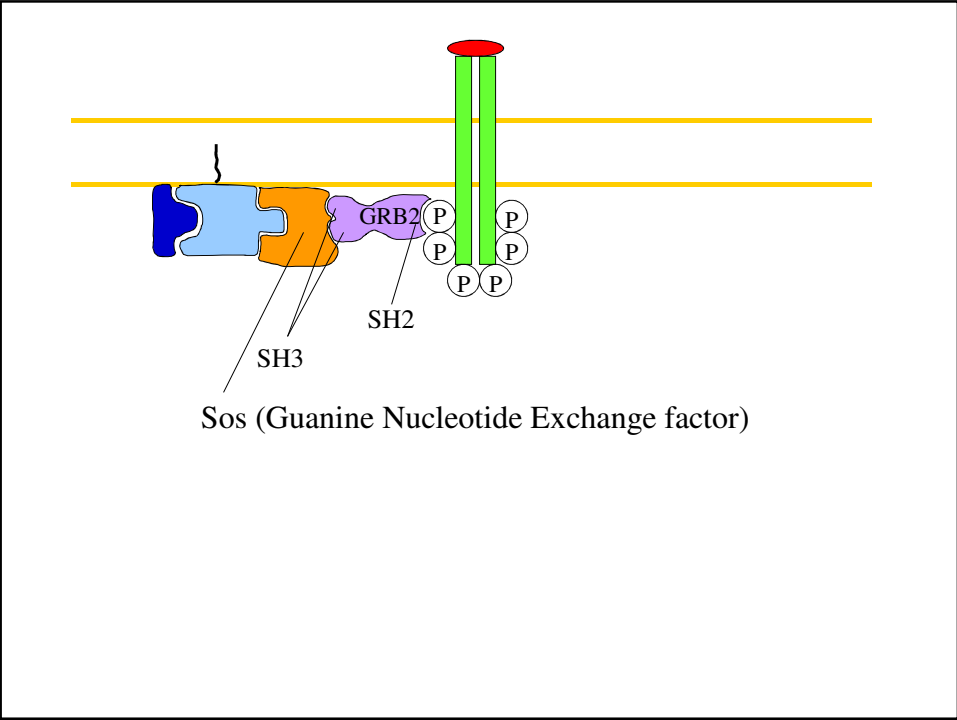
# Receptor tyrosine kinases

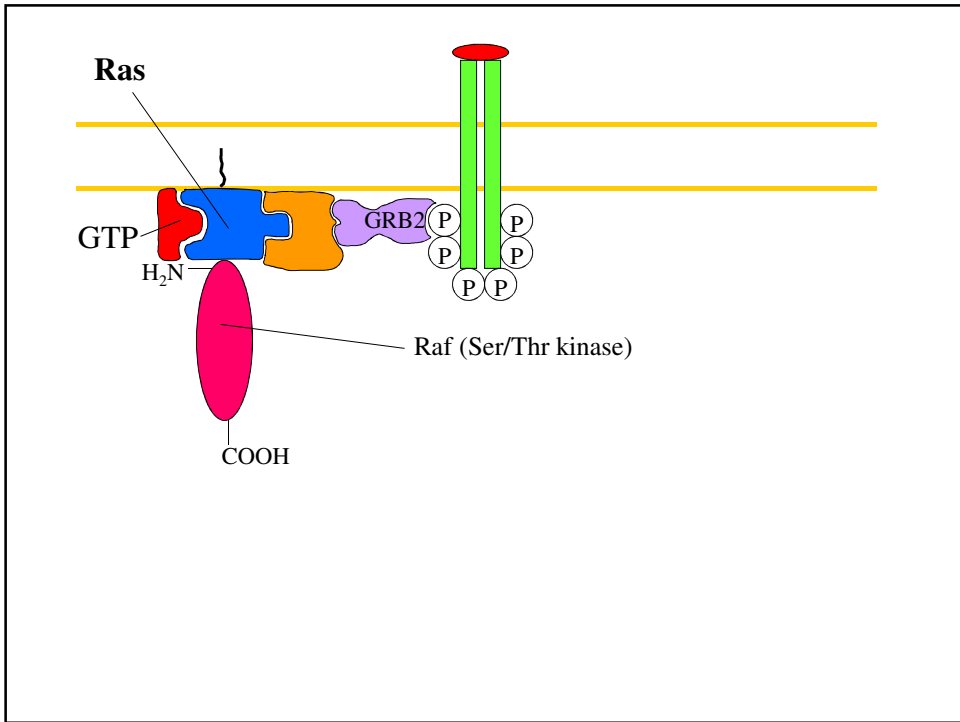
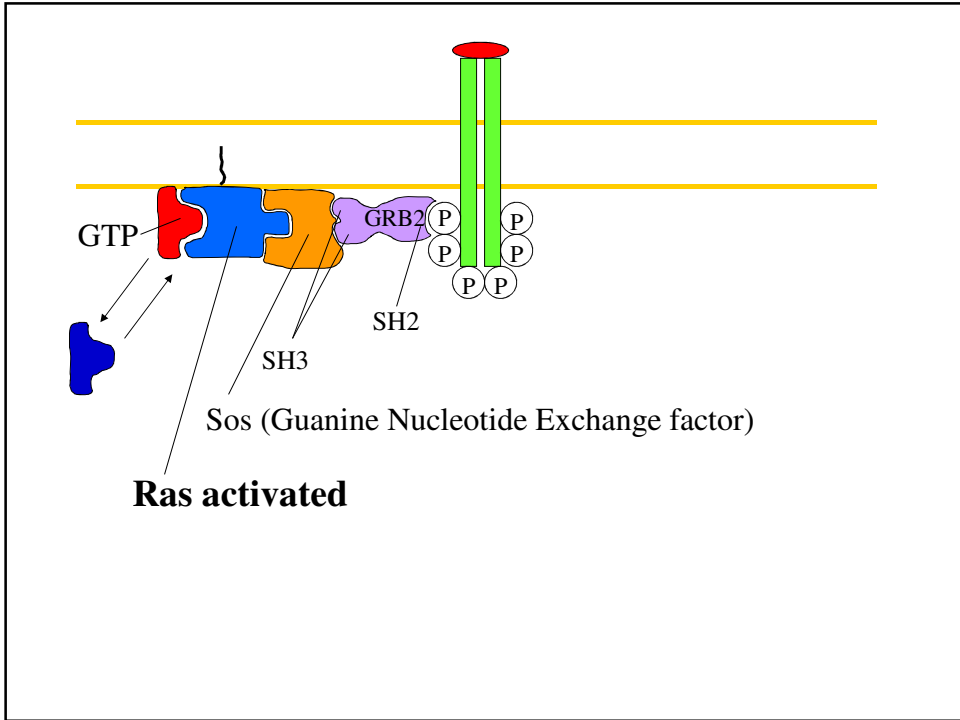


e.g.: Ras/MAPK

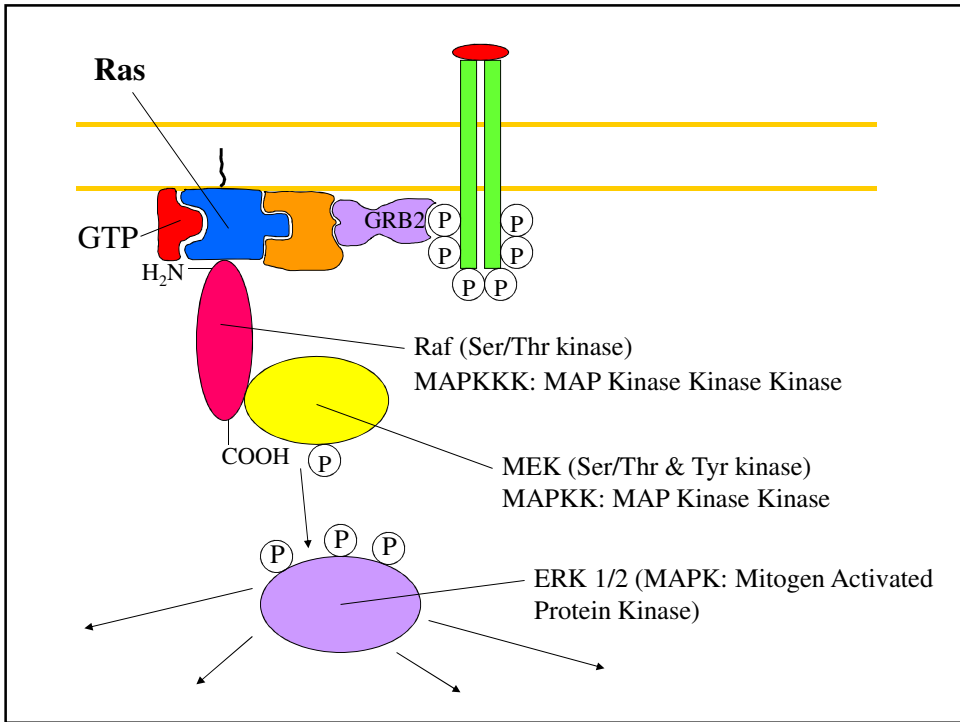
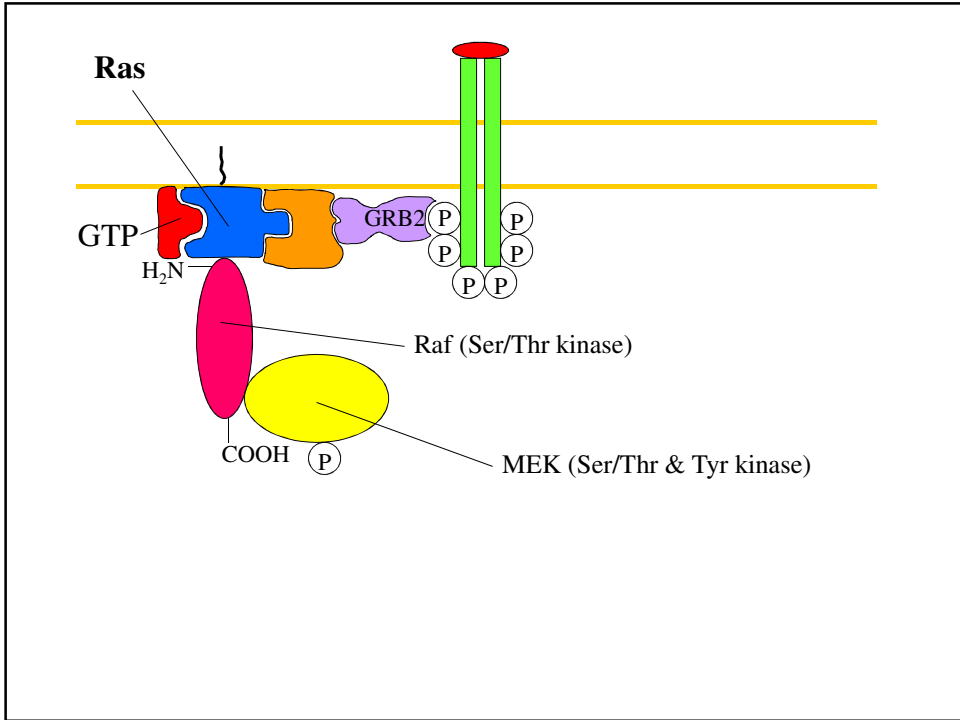


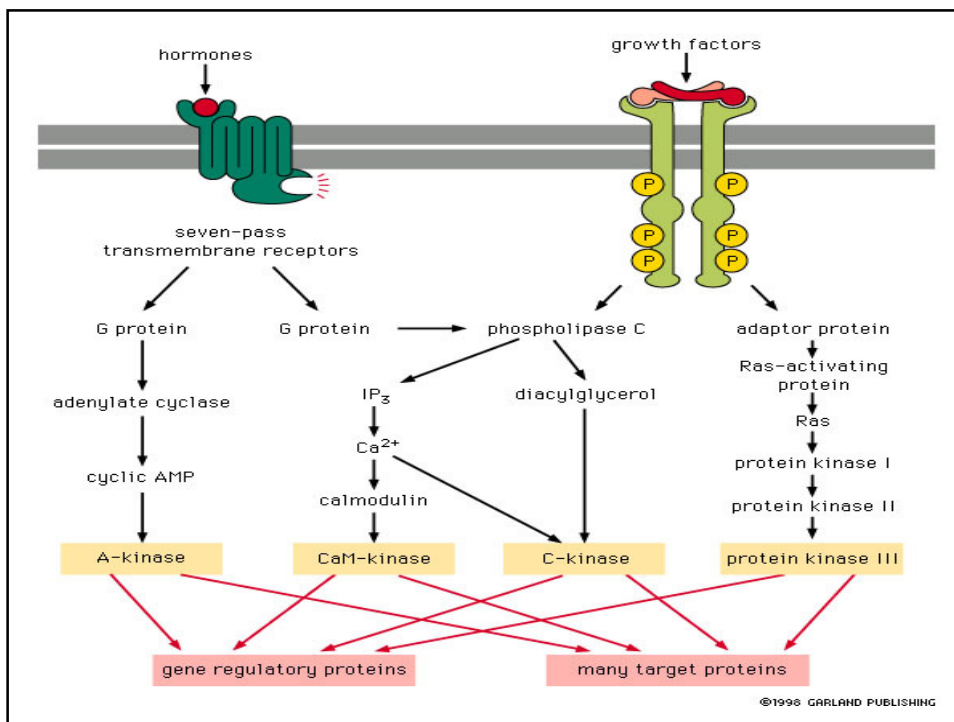
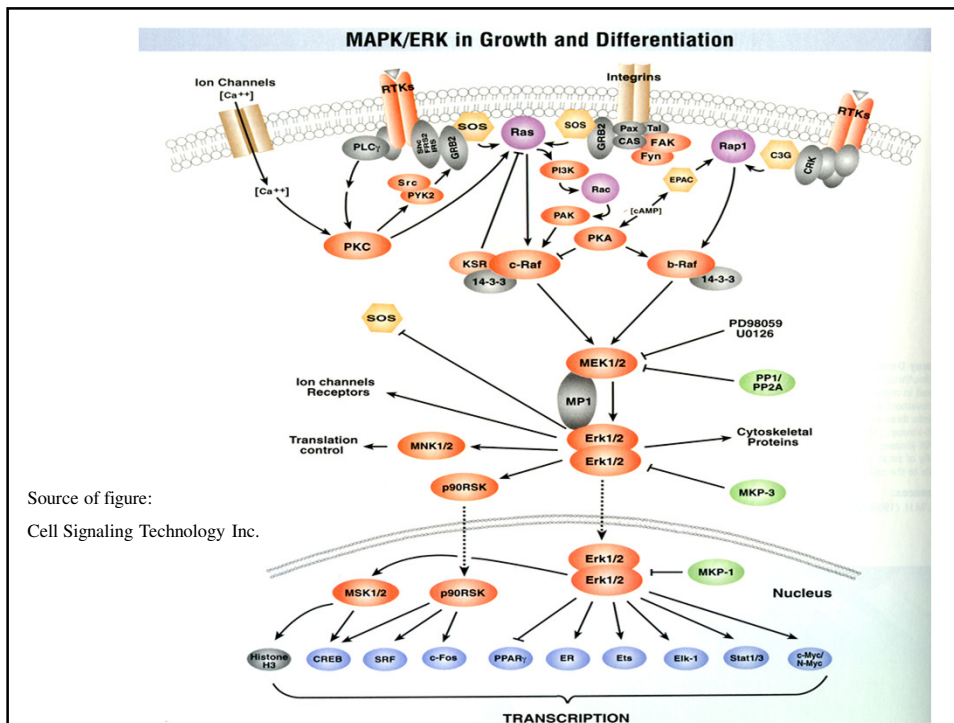










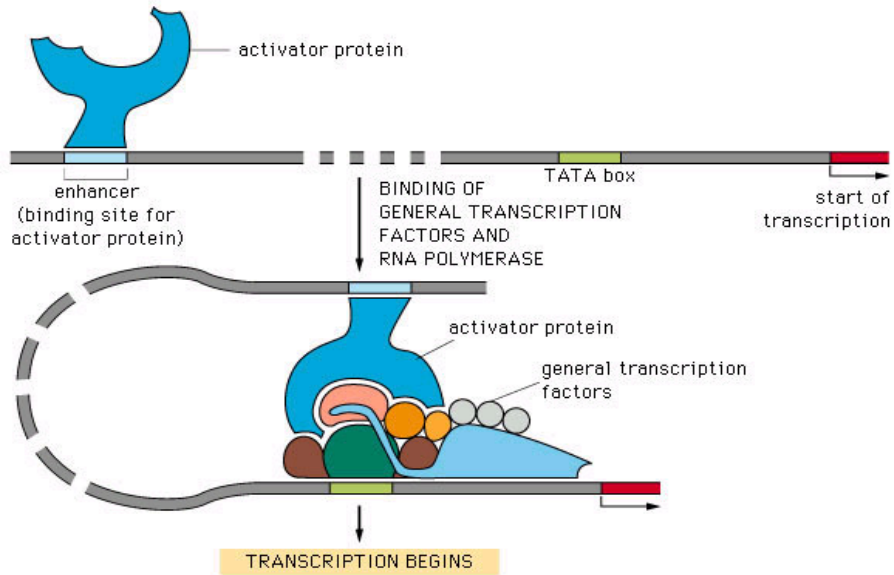


## **Signaling to the Cell Nucleus**

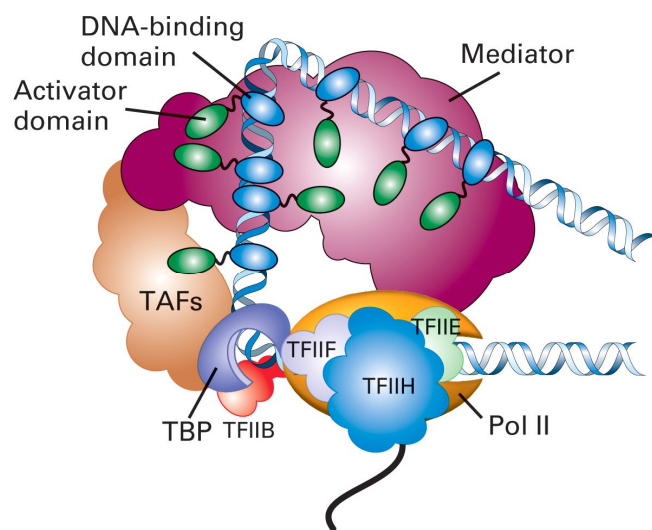
### **How signal affects protein function**

- **Modification of structure/ function of proteins present in the cell**
- **Change in spectrum /amount of proteins in the cell**
  - .... regulation of gene expression

## Regulation of eukaryotic gene transcription



## Model of interaction of several transcription activators with the mediator complex



Lodish et al.: Molecular Cell Biology, W.H. Freeman & Co, 5th ed., 2004

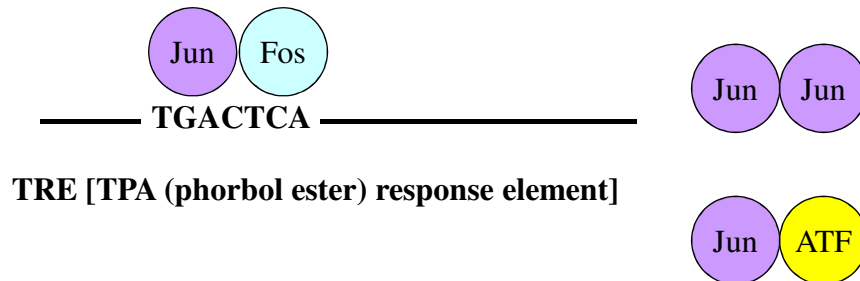
## **Eukaryotic transcription factors:**

- **Classification according to structural motifs:**
  - homeodomains
  - „zinc fingers“
  - „leucine zippers“
  - bHLH (basic Helix-Loop-Helix) proteins

## **Eukaryotic transcription factors:**

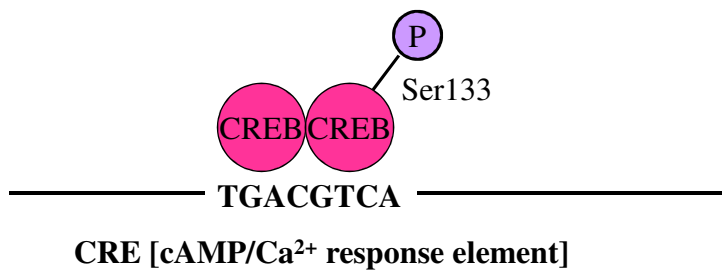
- **Classification according to way of expression/regulation:**
  - inducible
  - constitutive
  - ligand-activated

## Inducible TFs: e.g. AP1 (Activator Protein 1)

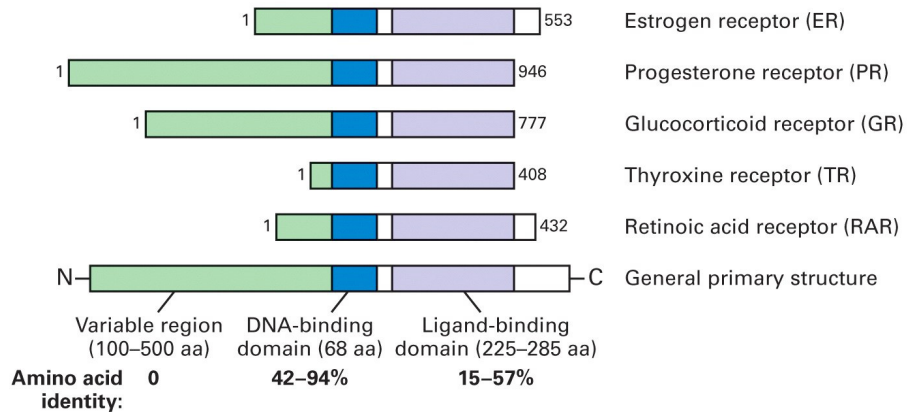


- Jun family: c-Jun, JunB, JunD
- Fos family: c-Fos, FosB, Fra-1, Fra-2

## Constitutive TFs: e.g. CREB (cAMP/Ca<sup>2+</sup> response element binding protein)



## Ligand-activated TFs: Superfamily of nuclear receptors



Lodish et al.: Molecular Cell Biology, W.H.Freeman & Co, 5th ed., 2004

## Signaling to the cell nucleus

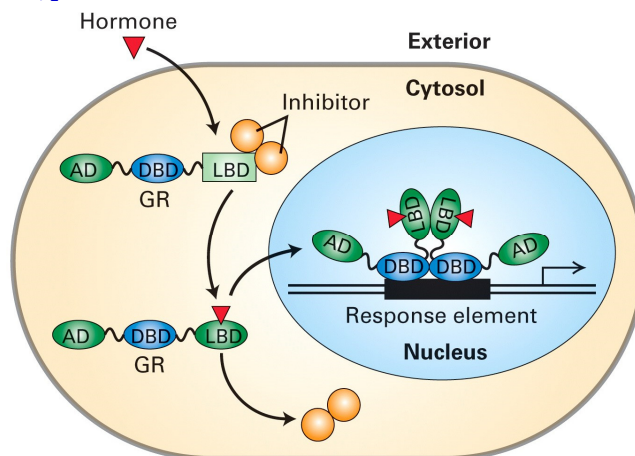
- what goes into nucleus:
  - ligand
  - receptor
  - transcription factor
  - another signalling molecule (kinase, second messenger)

**Ex.1: Ligand for nuclear receptor/ligand-activated transcription factor translocates to the nucleus**

- Receptors for vitamin D3, thyroxin, retinoic acid

**Ex.2: Nuclear receptor/ligand-activated transcription factor translocates to the nucleus**

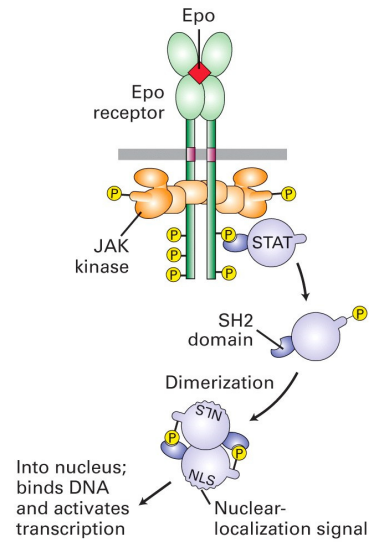
- Receptors for glucocorticoids and estrogens



Lodish et al.: Molecular Cell Biology, W.H.Freeman & Co, 5th ed., 2004



### Ex.3: Cytoplasmatic receptor activates transcription factor, which translocates to nucleus

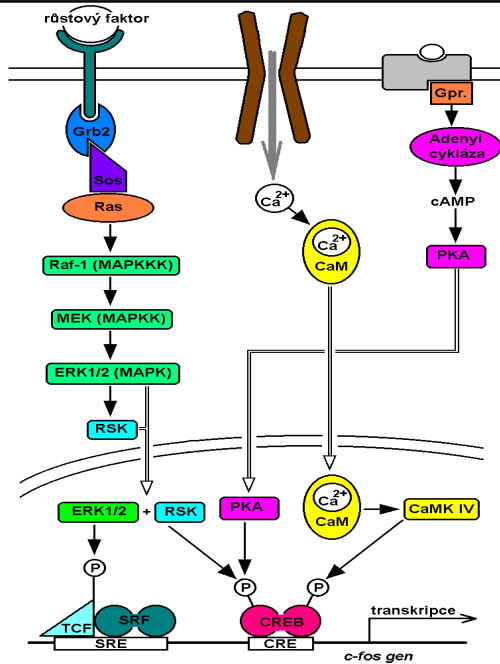


■ Signaling from cytokine receptors:  
**JAK-STAT system**

**STAT:**  
Signal Transducer & Activator of Transcription

**JAK:**  
Just Another Kinase

Lodish et al.: Molecular Cell Biology, W.H. Freeman & Co, 5th ed., 2004



Ex.4: Constitutive TF CREB in the nucleus activated by various kinases, nuclear translocation of:

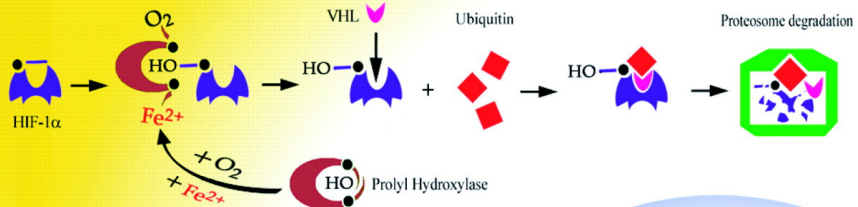
- complex MAPK/RSK
- Ca/calmodulin
- PKA

→ aktivace, tvorba

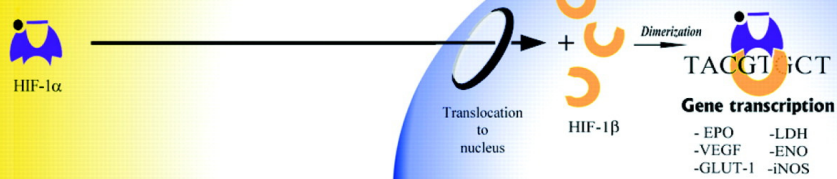
⇒ translokace

## Ex.5: Hypoxia-inducible factor:

### Normoxia



### Hypoxia



Joseph C. LaManna et al. J Exp Biol 2004;207:3163-3169

## Cascade of transcription response

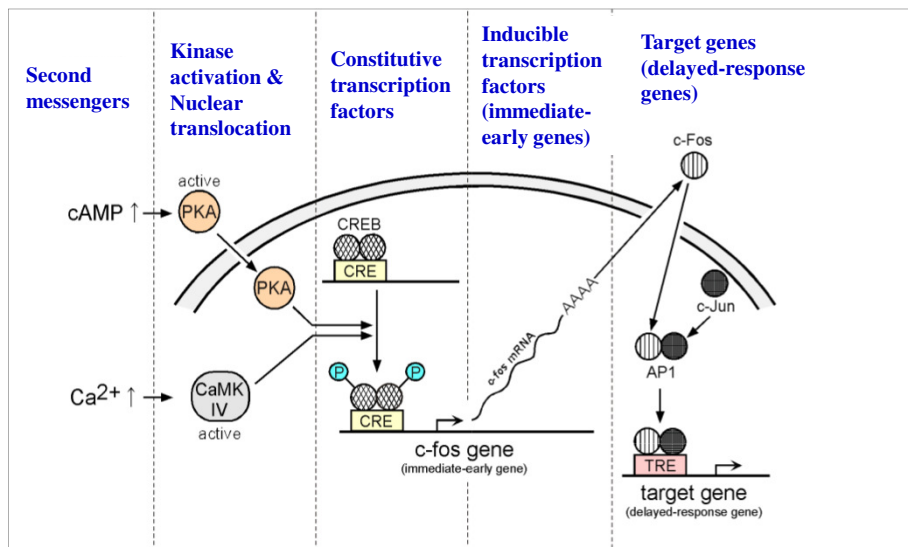
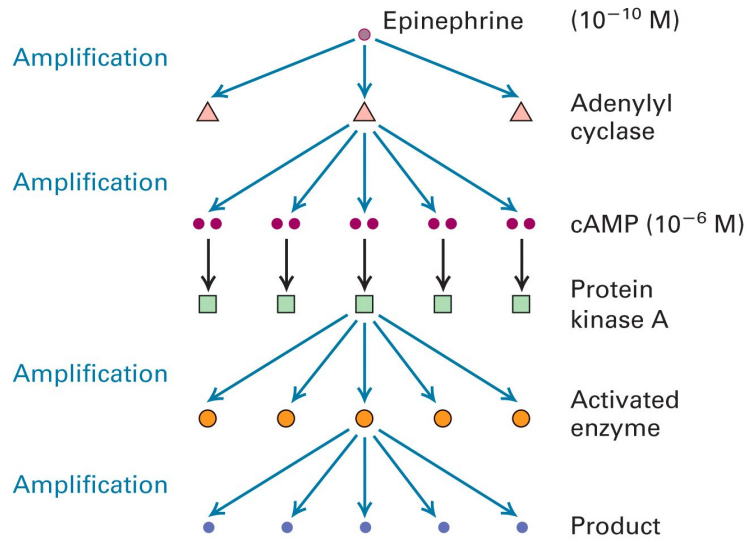


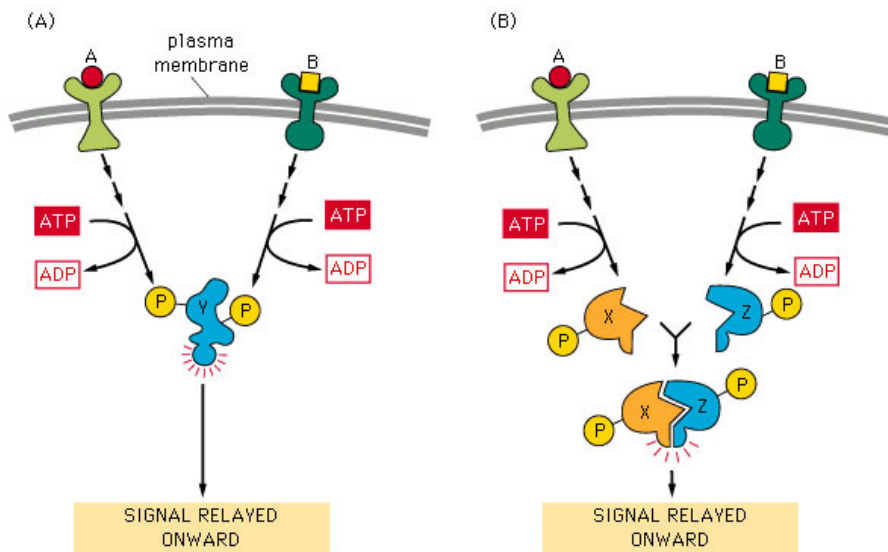
Fig.: Pláteník et al., Life Sci. 2000: 67, 335-364.

# Signal amplification

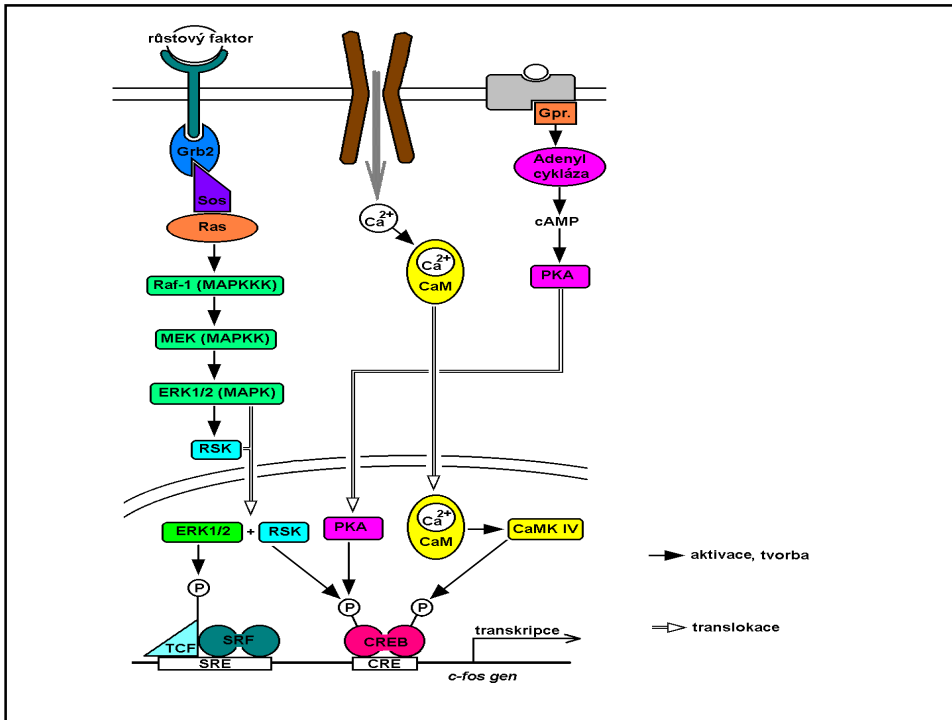


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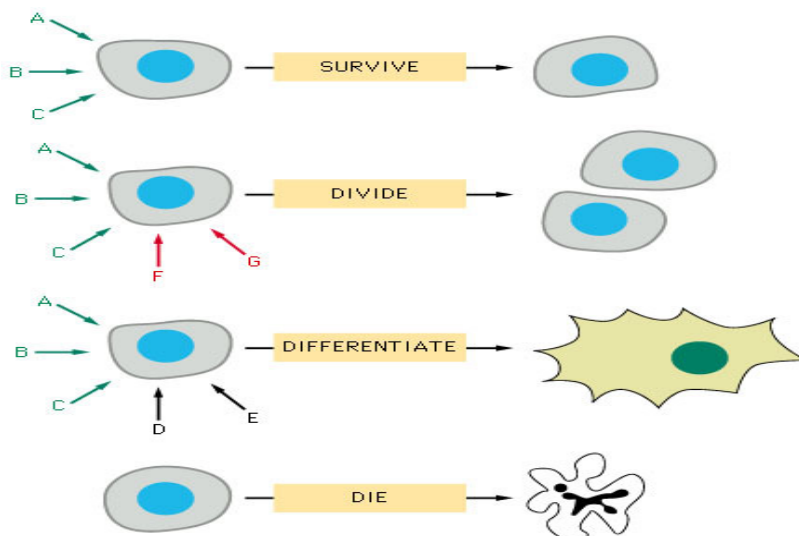
# Signal integration



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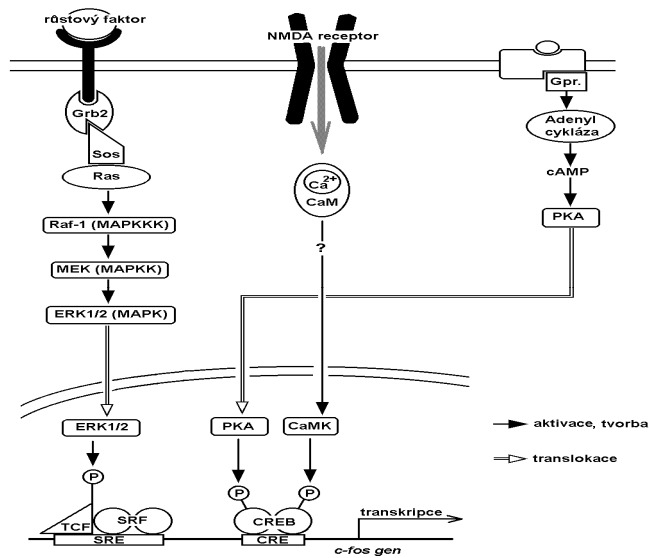


## Cell always integrates and responds to many signals:

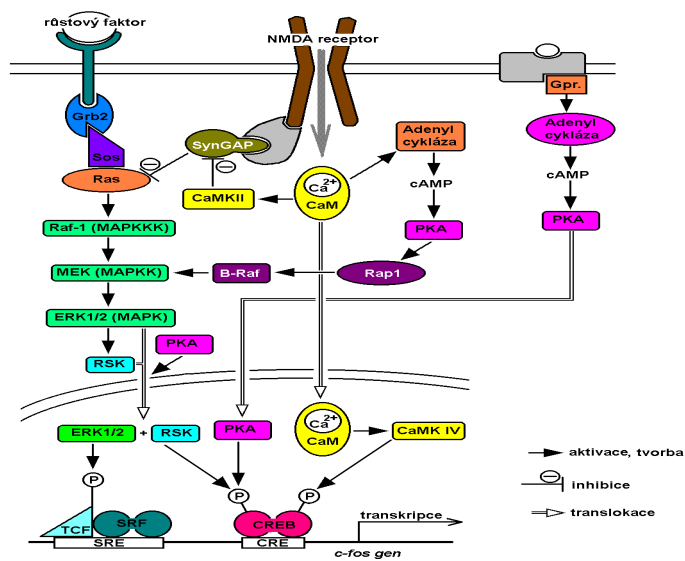


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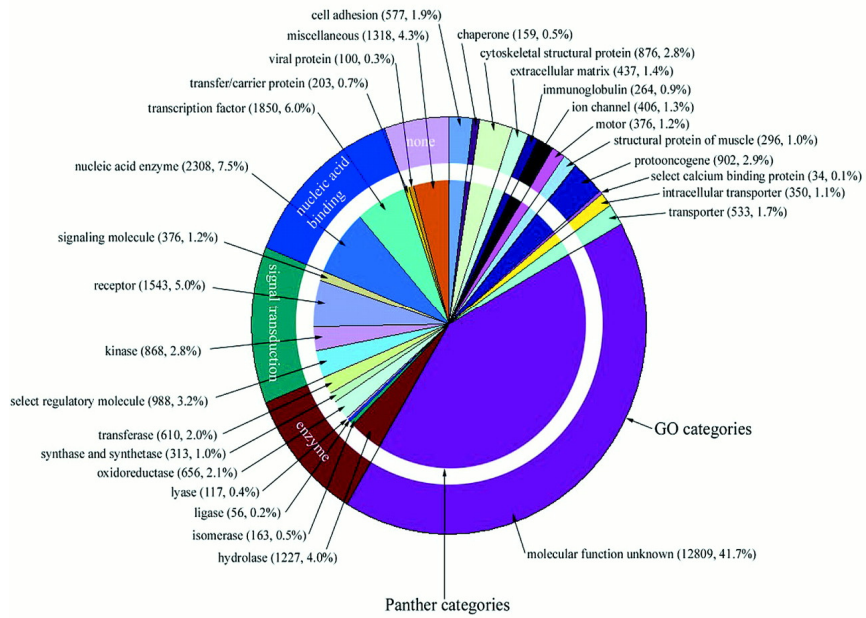
## Cross-talk of signaling pathways



## Cross-talk of signaling pathways



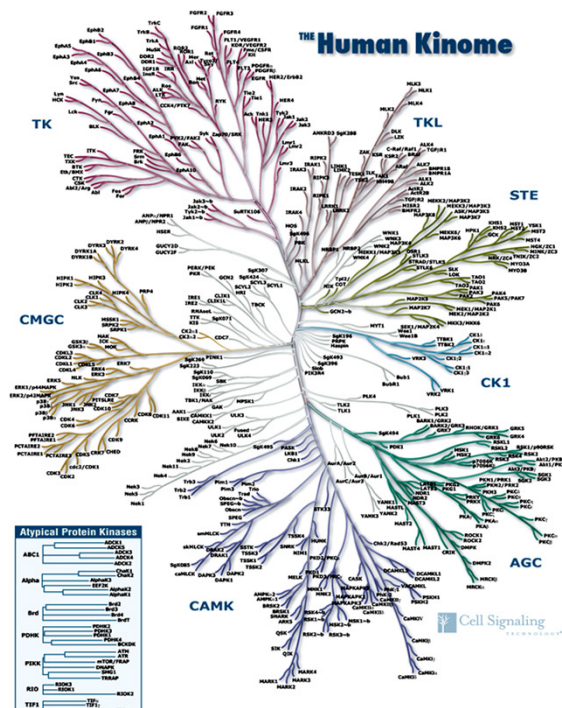
## Human genome analyses (Science 291, 2001):



## The human kinome: 518 kinases

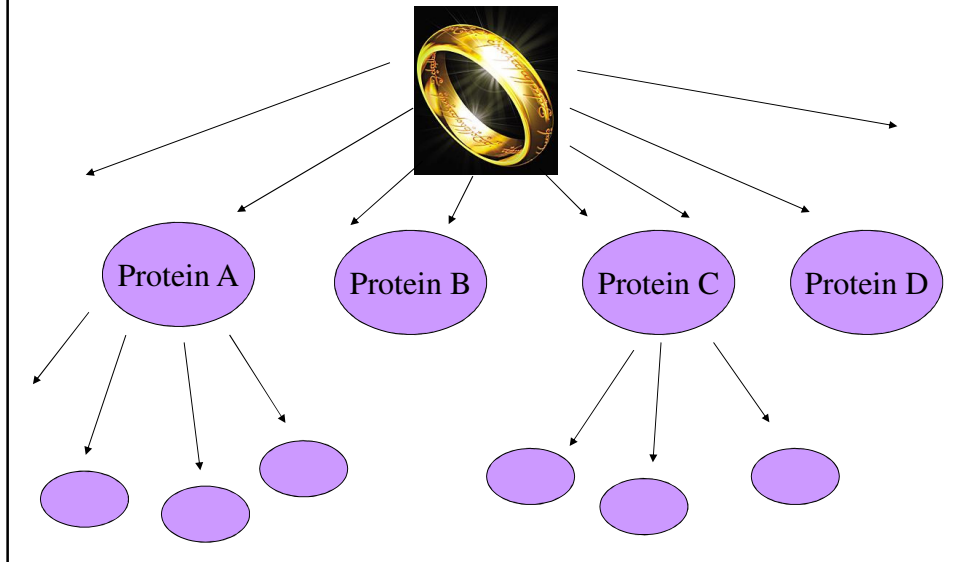
(Science 298, 2002)

Source of figure:  
Cell Signaling Technology Inc.



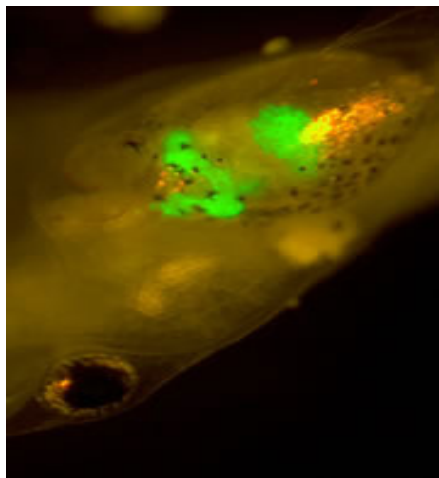
## "MASTER SWITCH":

One gene/protein controls all ...



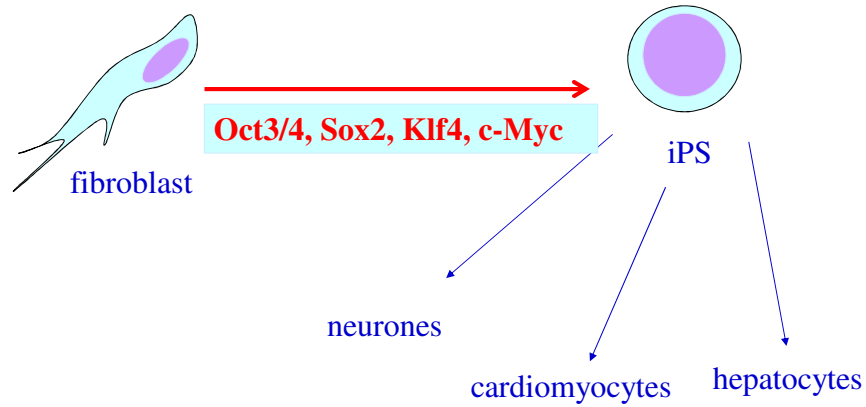
**Horb, M.E., et al.:**

**Experimental conversion of liver to pancreas.**  
***Current Biology*, 13, 105 - 115, (2003).**

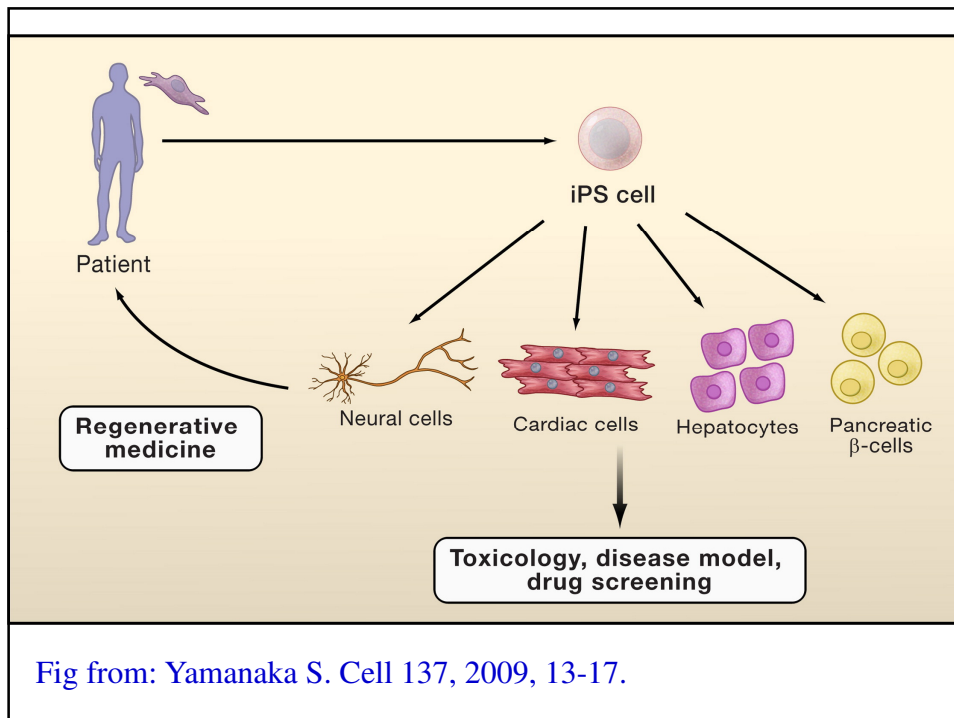


- Transient expression of single gene construct, coding modified key TF *Pdx1*, causes permanent change of liver cell to pancreatic cell, producing insulin, glucagon and amylase...

# Somatic cells can be reprogrammed to pluripotent stem cells !



**Takahashi K & Yamanaka S. Cell 126, 2006, 663-676**



**Fig from: Yamanaka S. Cell 137, 2009, 13-17.**