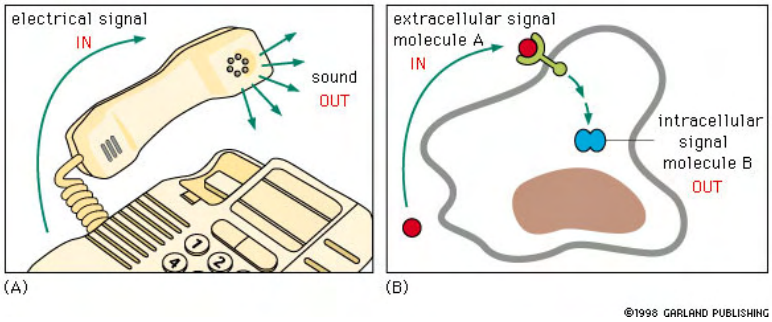
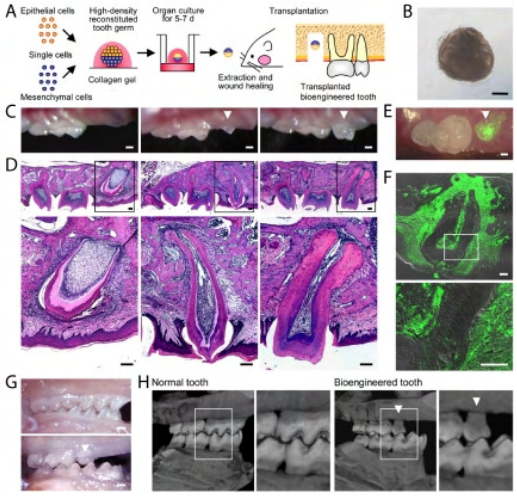
Essentials of Cell Signaling

MUDr. Jan Plášeník, PhD.



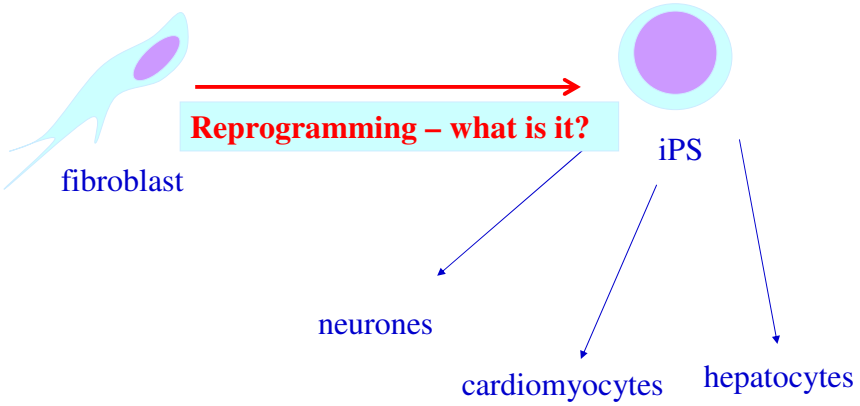
Bioengineered tooth in mice



Ikeda e. et al.: Fully functional bioengineered tooth replacement as an organ replacement therapy, PNAS 106, 2009, 13475-13480.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2720406/figure/F1/

Somatic cells can be reprogrammed to pluripotent stem cells !

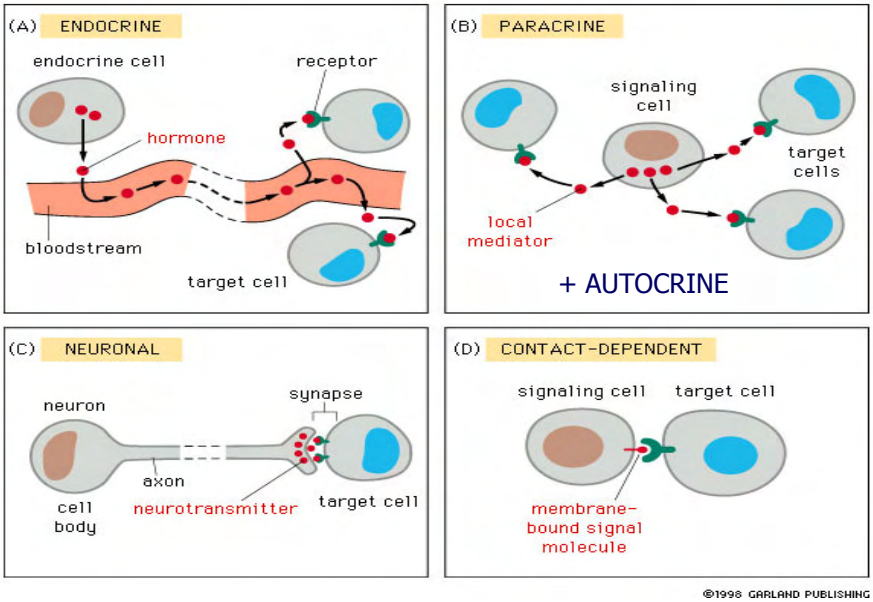


Takahashi K & Yamanaka S. Cell 126, 2006, 663-676

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 :



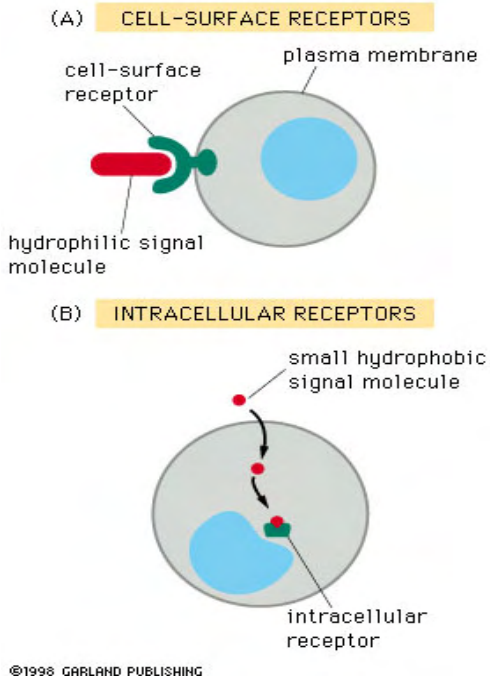
Signaling molecule (ligand):

- 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, serotonine, glutamate, GABA, glycine etc.
- Gases: NO

Receptor

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

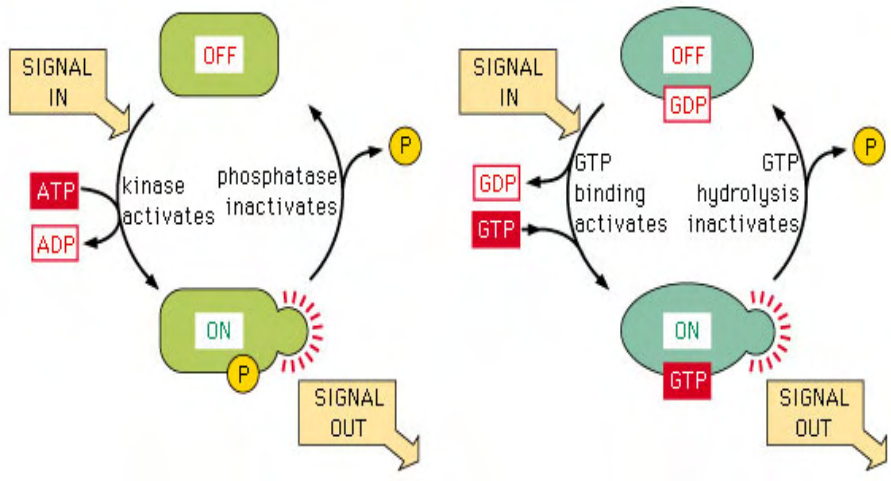
Two classes of signaling molecules



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:



Signal termination:

- Receptor desensitization
  - endocytosis & degradation
  - phosphorylation
- Degradation or removal of signalling molecule
  - cAMP: phosphodiesterase
  - Calcium: Ca^{2+} pumps
- Dephosphorylation by protein phosphatases

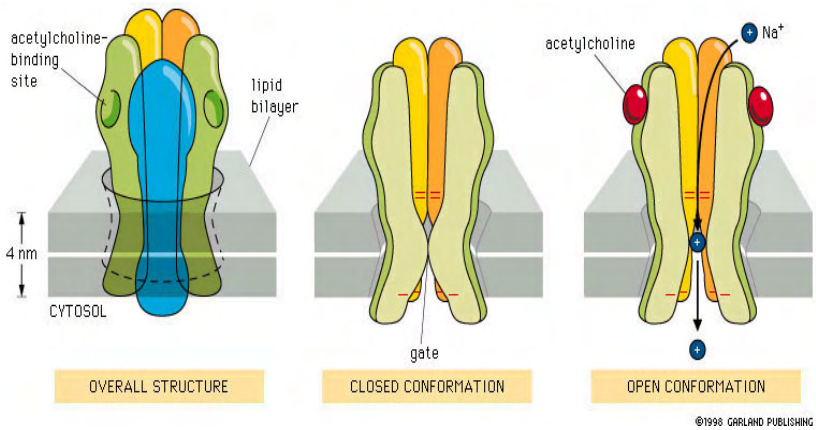
Cell-surface receptors for signaling molecules:

- Ion channels
- Seven-spanning G protein-linked
- Receptors associated with an enzymic activity

Cell-surface receptors for signaling molecules:

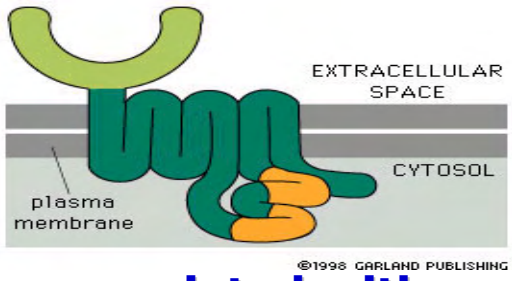
- **Ion channels**
  - for Na^+, K^+, Ca^{2+}, Cl^-
  - e.g. receptor for acetylcholine, GABA, glutamate, glycine
- **Seven-spanning G protein-linked**
- **Receptors associated with an enzymic activity**

Muscle receptor for acetylcholine

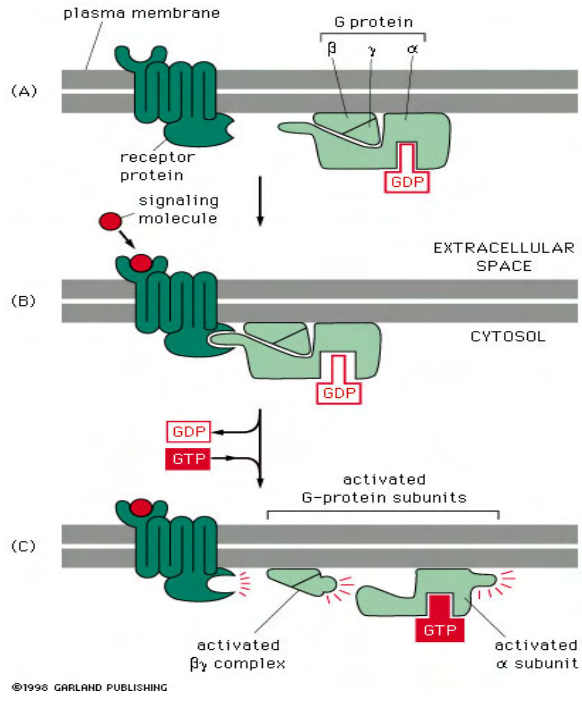


Cell-surface receptors for signaling molecules:

- Ion channels
- Seven-spanning G protein-linked

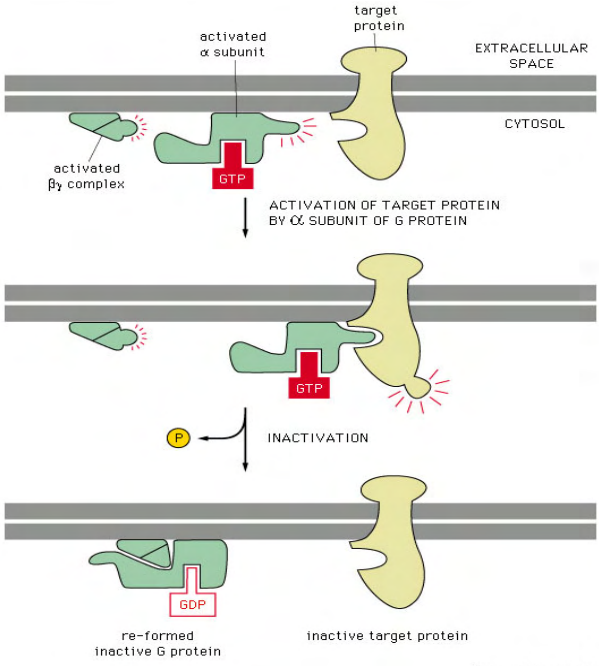


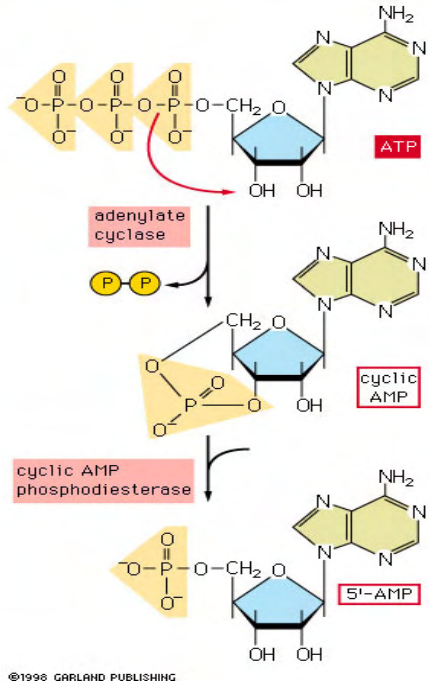
- Receptors associated with an enzymic activity



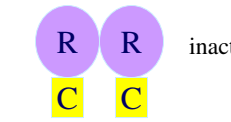
G-protein targets:

- membrane enzymes
- ion channels



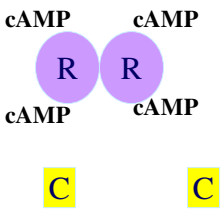


Cyclic AMP & protein kinase A



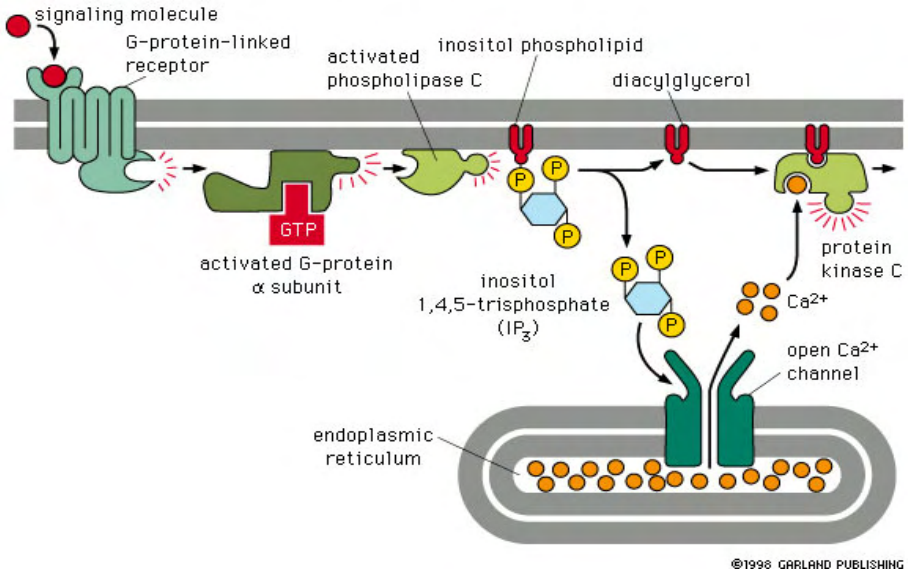
inactive PKA

+ 4 cAMP



Active PKA

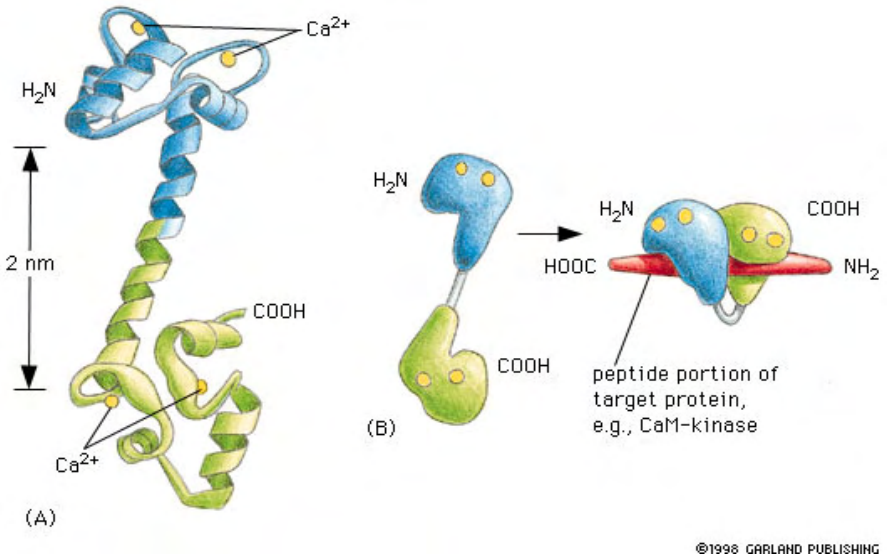
Phospholipase C pathway

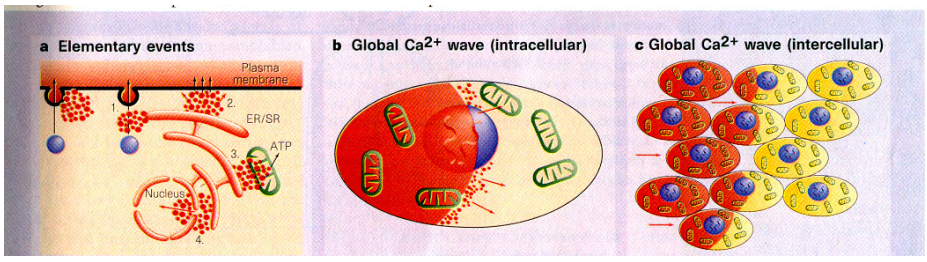


Calcium in the cell:

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

Calmodulin (148 AMK)





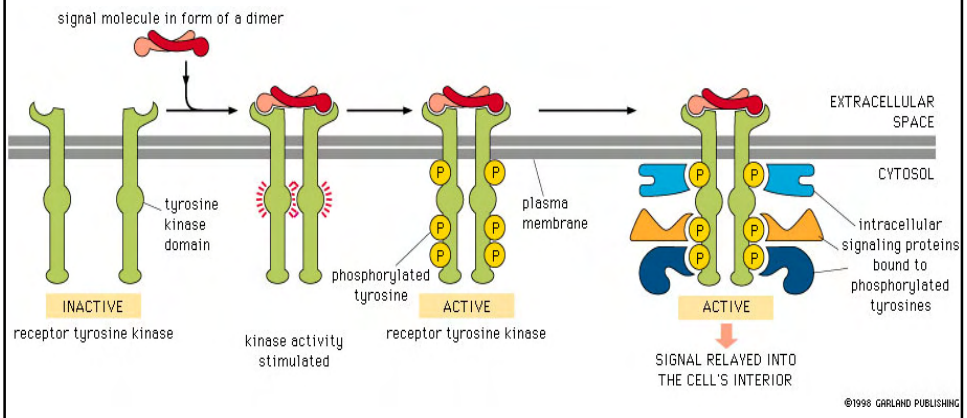
(Berridge et al., Nature 1998, 395: 645-648)

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

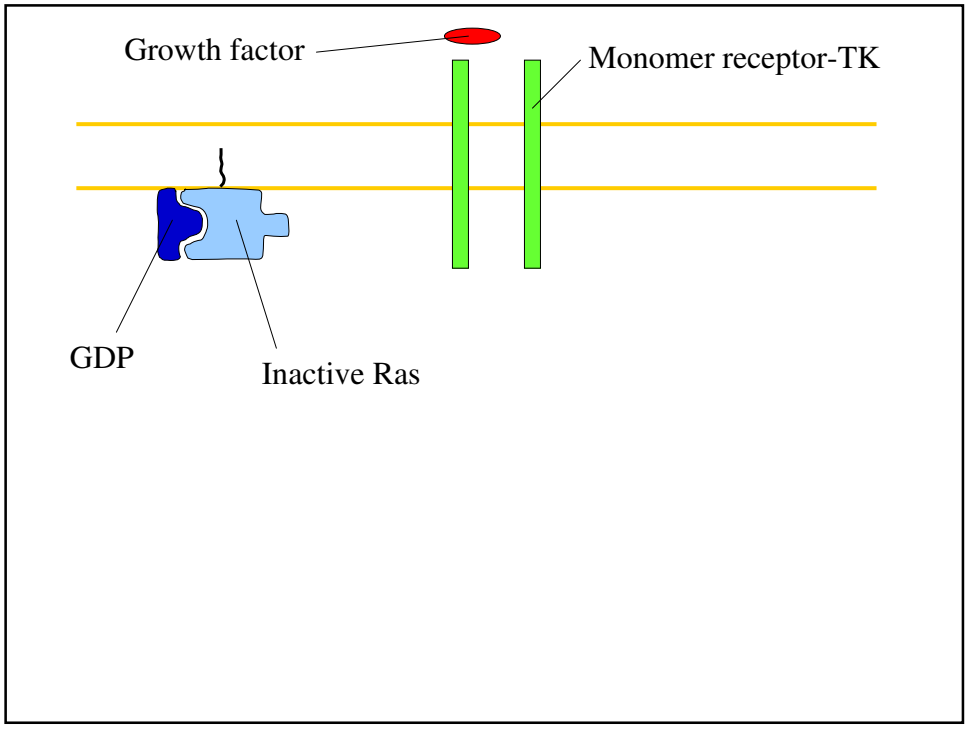
Cell-surface receptors for signaling molecules:

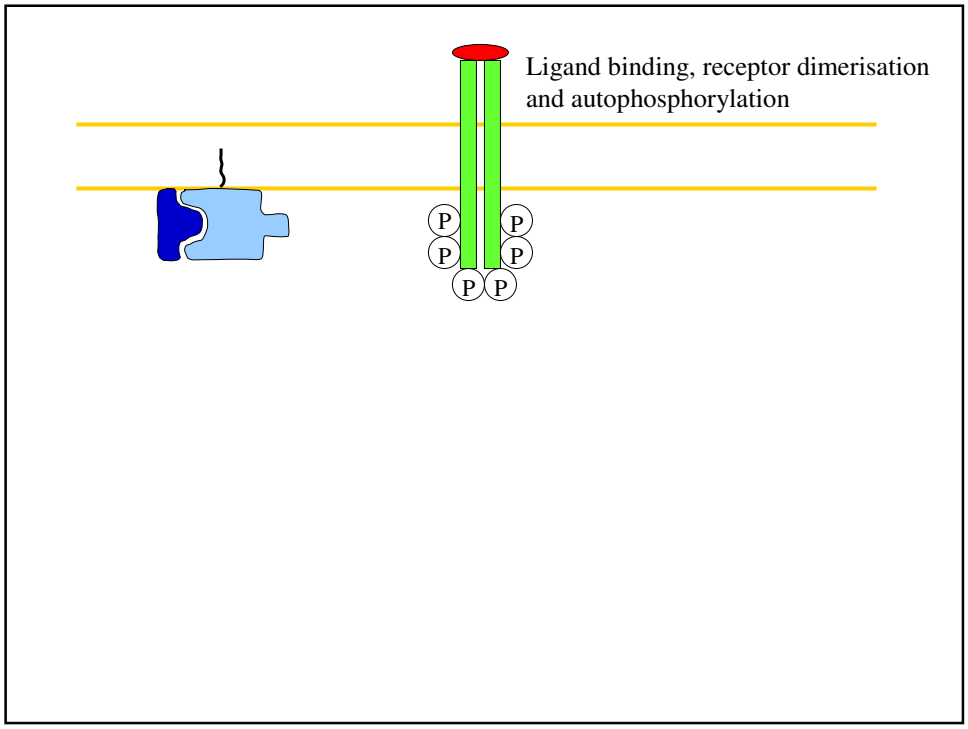
- Ion channels
- Seven-spanning G protein-linked
- Receptors associated with an enzymic activity
  - intrinsic catalytic activity: receptor tyrosine kinases (RTKs, e.g. rec. for EGF, insulin)
  - associated with soluble tyrosine kinases (e.g. rec. for cytokines, interferons)

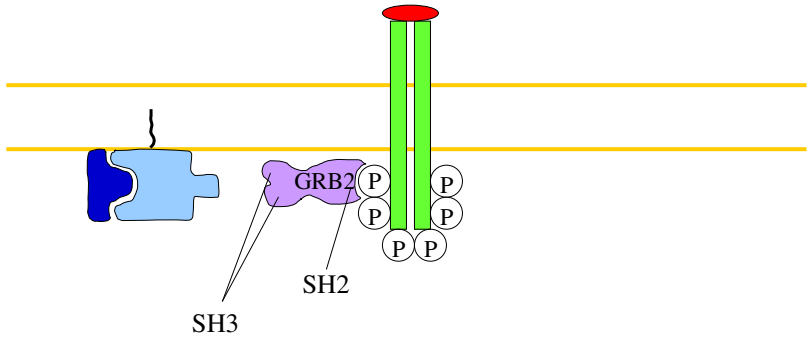
Receptor tyrosine kinases

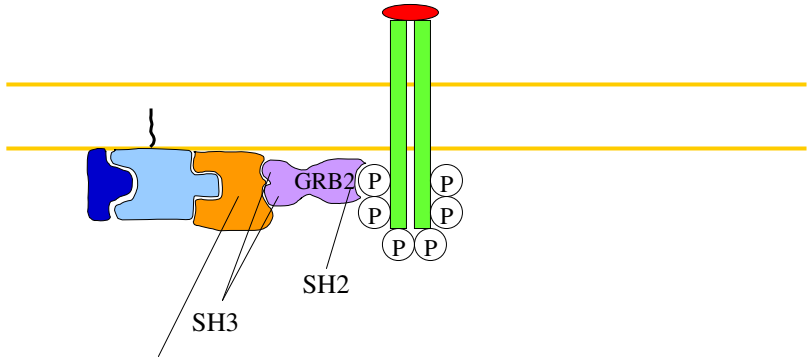


e.g.: Ras/MAPK

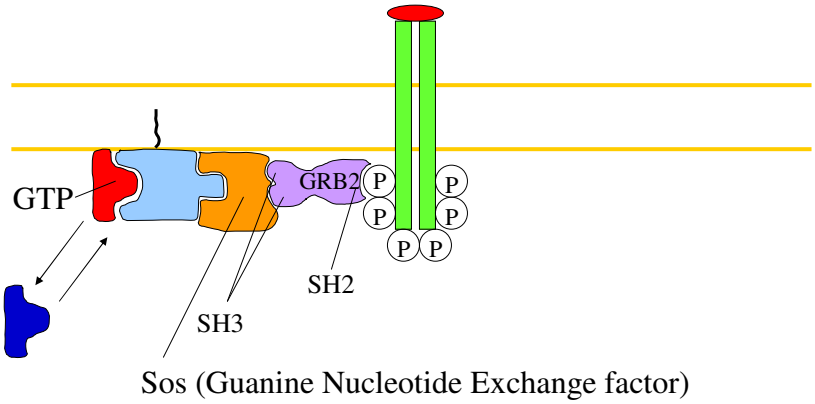

Growth factor
Monomer receptor-TK
GDP
Inactive Ras


Ligand binding, receptor dimerisation
and autophosphorylation
P
P
P
P
P
P

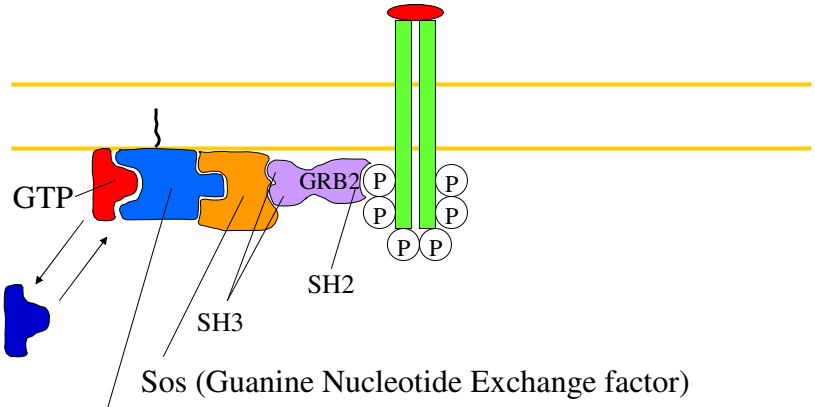




Sos (Guanine Nucleotide Exchange factor)

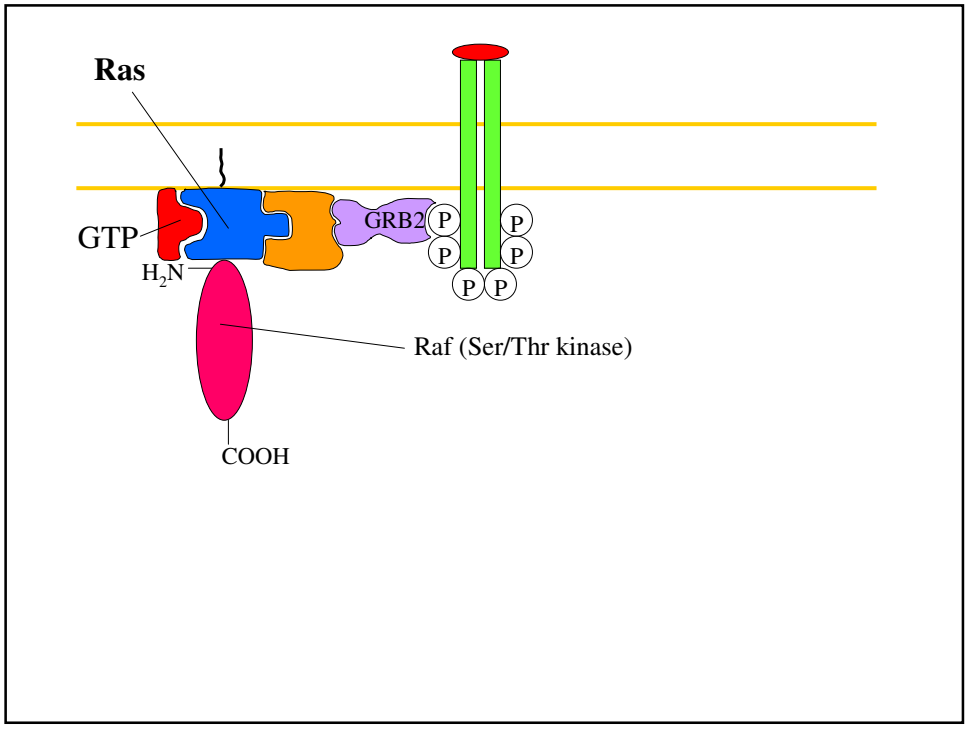


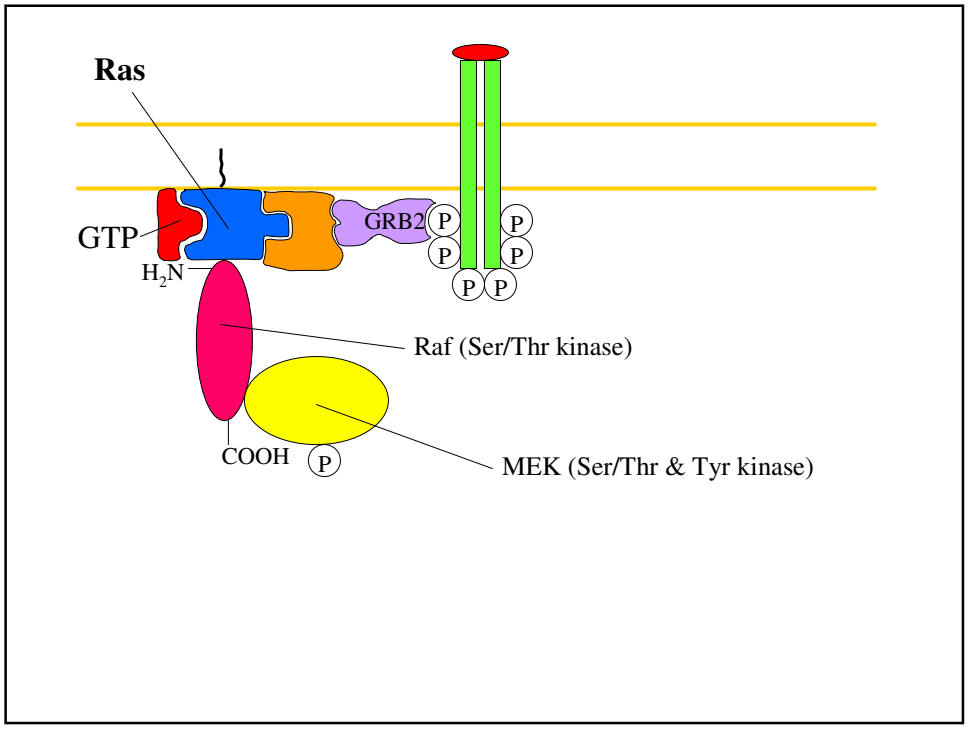
Sos (Guanine Nucleotide Exchange factor)

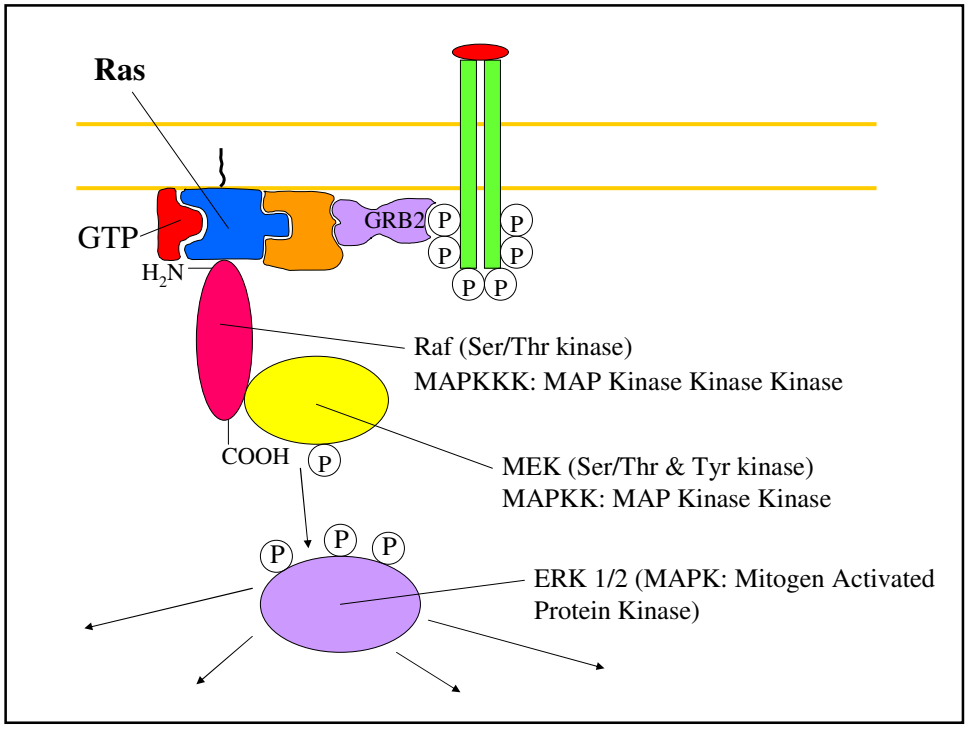


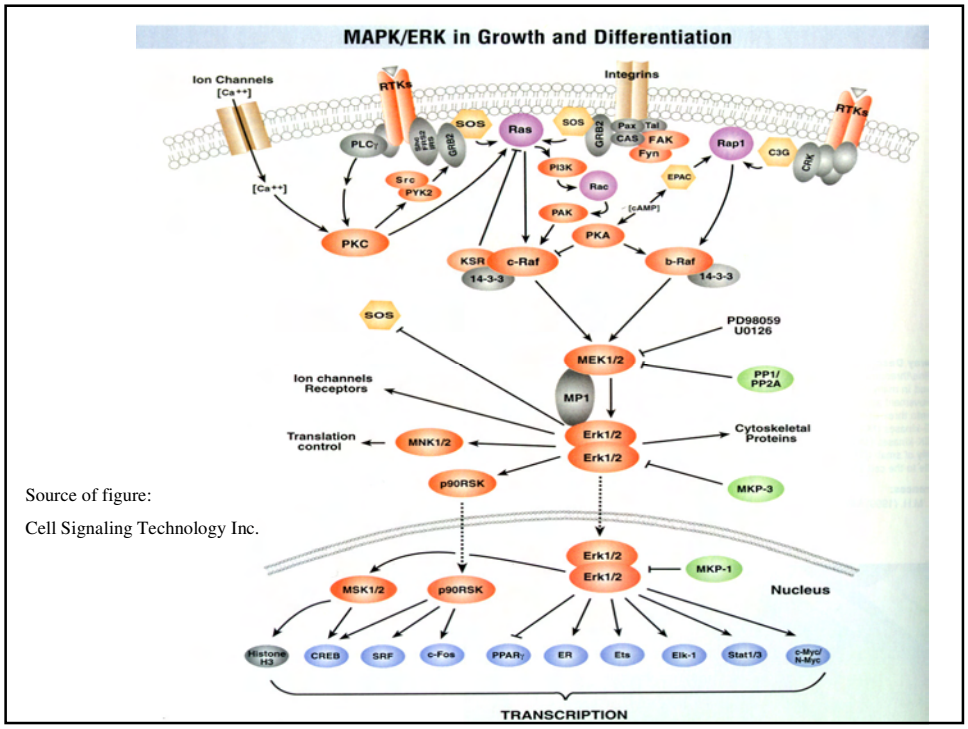
Sos (Guanine Nucleotide Exchange factor)

Ras activated


Ras
GTP
H_2N
GRB2
P
P
P
P
P
P
Raf (Ser/Thr kinase)
COOH

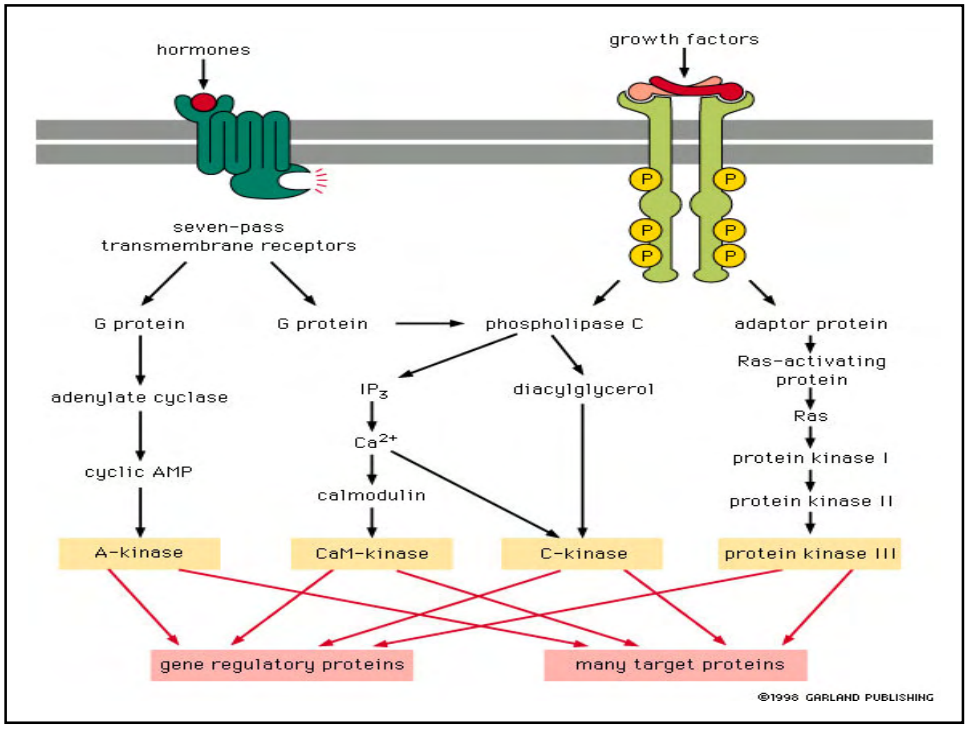

Ras
GTP
H_2N
GRB2
P
P
P
P
P
P
Raf (Ser/Thr kinase)
COOH
P
MEK (Ser/Thr & Tyr kinase)





Source of figure:

Cell Signaling Technology Inc.

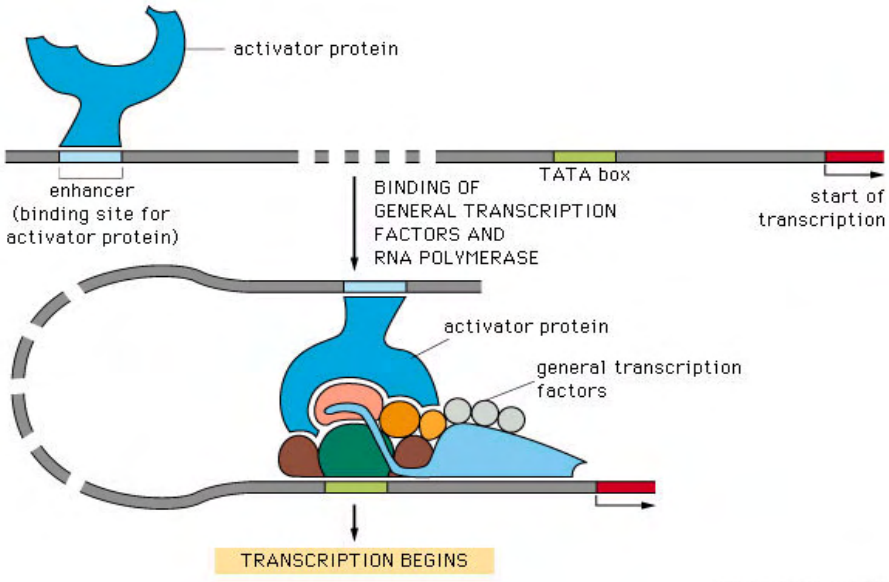


Signaling to the Cell Nucleus

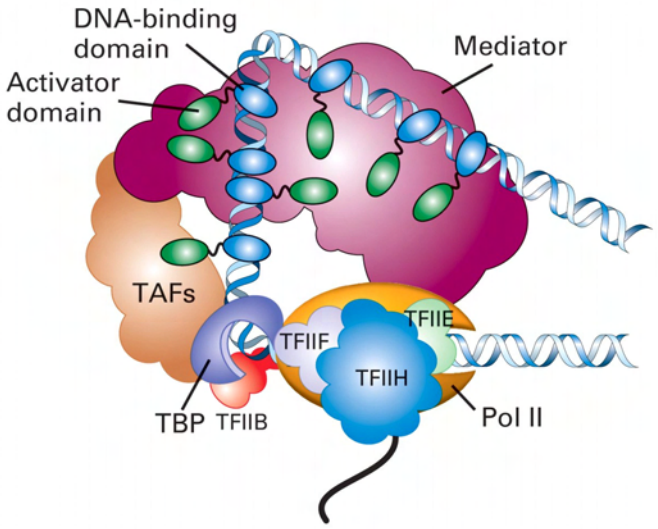
How signal affects protein function

- 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

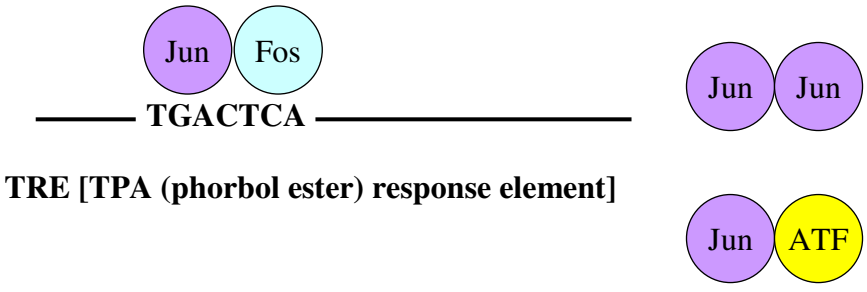
Eukaryotic transcription factors:

- 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)

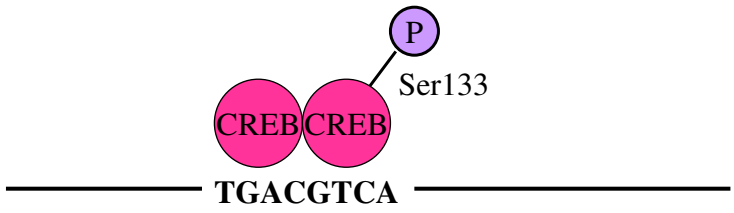


TRE [TPA (phorbol ester) response element]

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

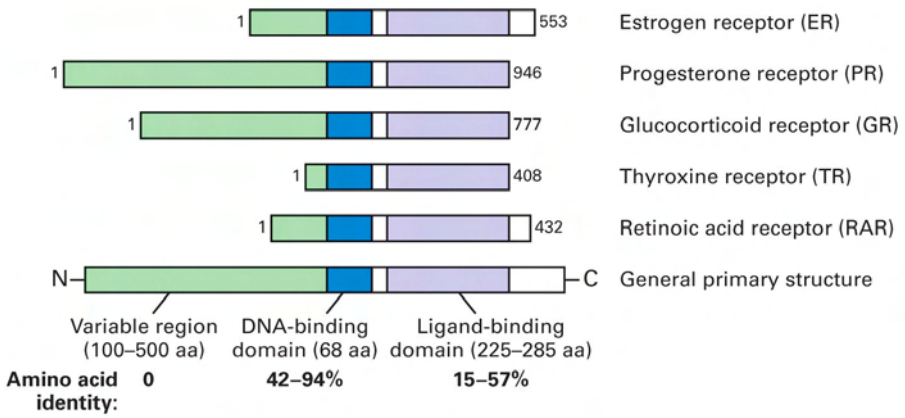
Constitutive TFs: e.g. CREB

(cAMP/Ca^{2+} response element binding protein)



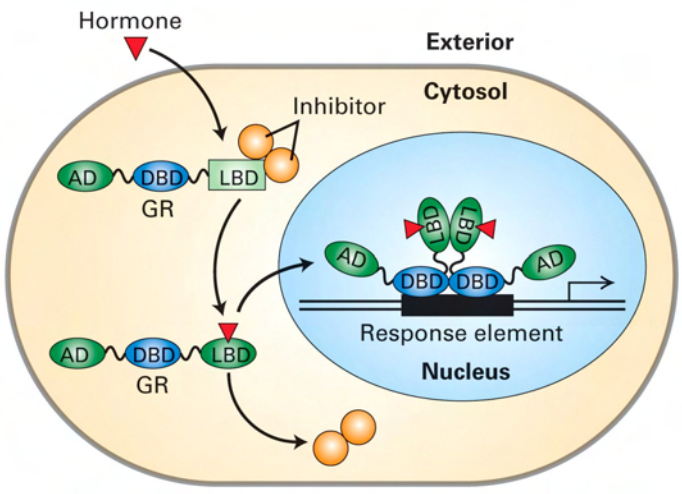
CRE [cAMP/Ca^{2+} response element]

Ligand-activated TFs: Superfamily of nuclear receptors



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

Receptors for glucocorticoids and estrogens are ligand-activated transcription factors that translocate to the nucleus

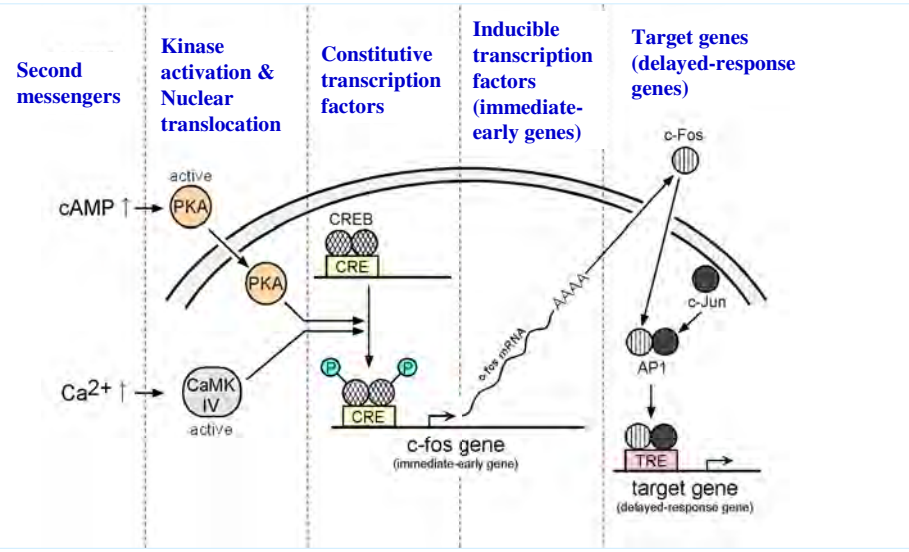


Lodish et al.: Molecular Cell Biology, W.H.Freeman & Co, 5th ed., 2004

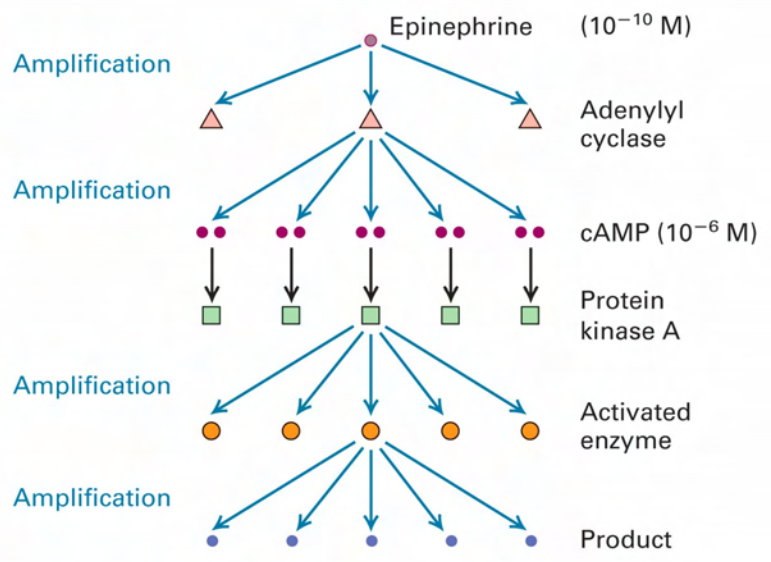
Signaling to the cell nucleus

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

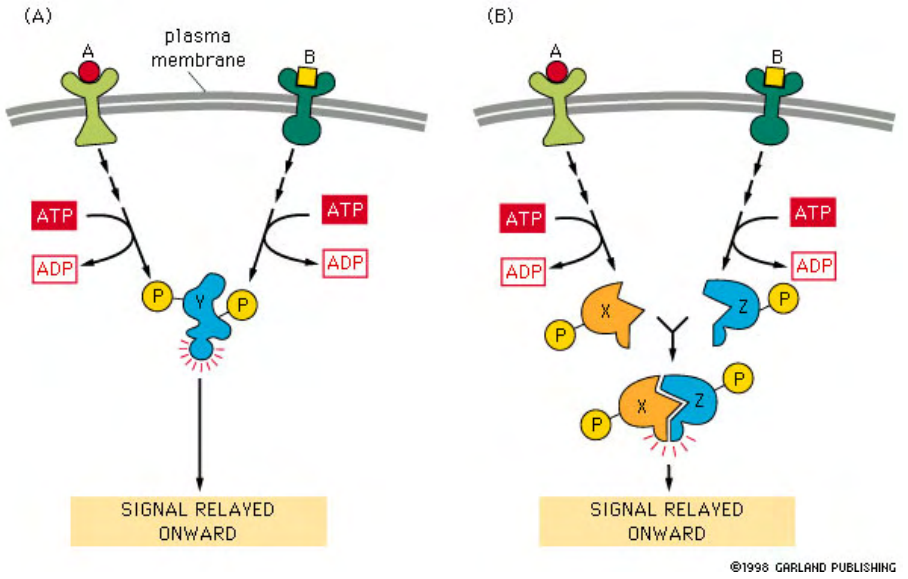
Cascade of transcription response



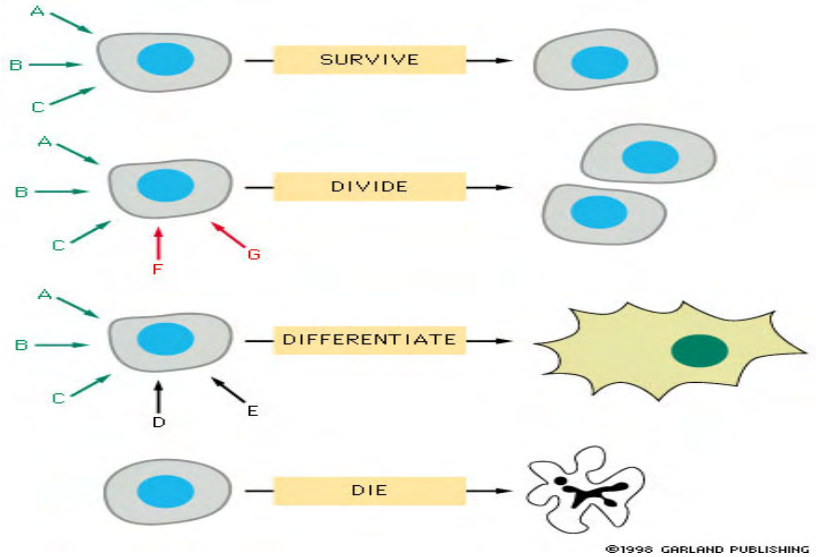
Signal amplification



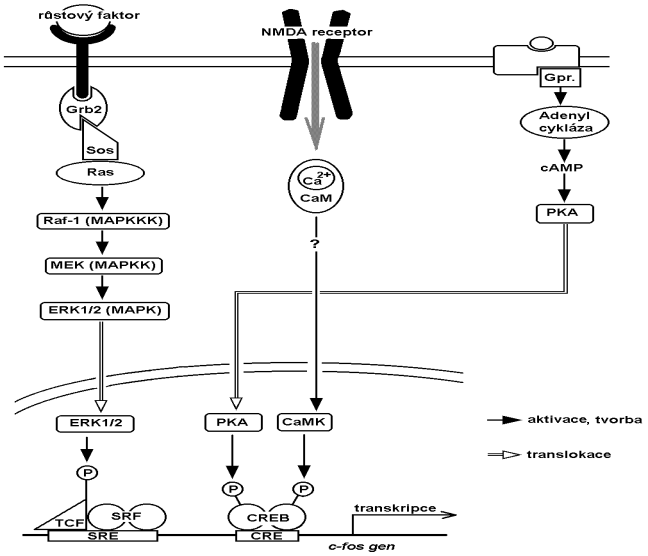
Signal integration



Cell allways integrates and responds to many signals:



Cross-talk of signaling pathways



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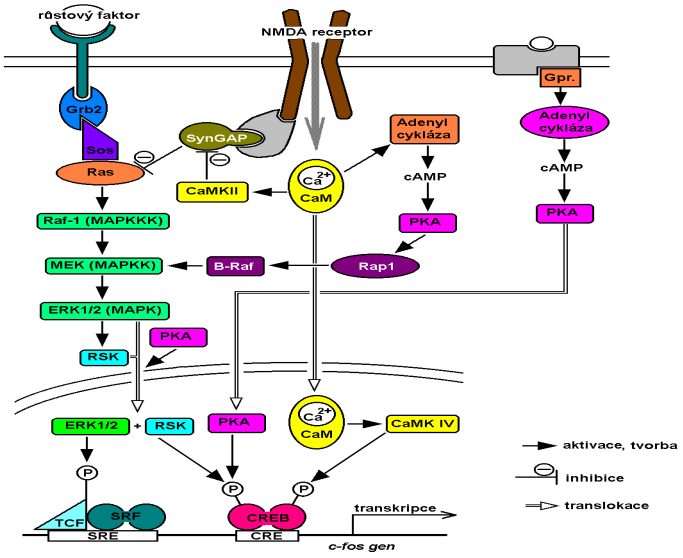
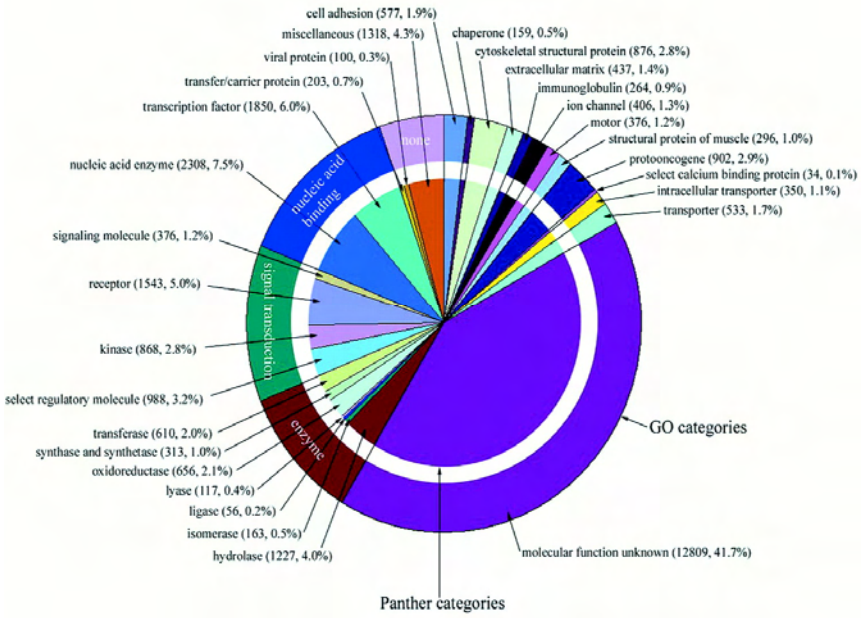
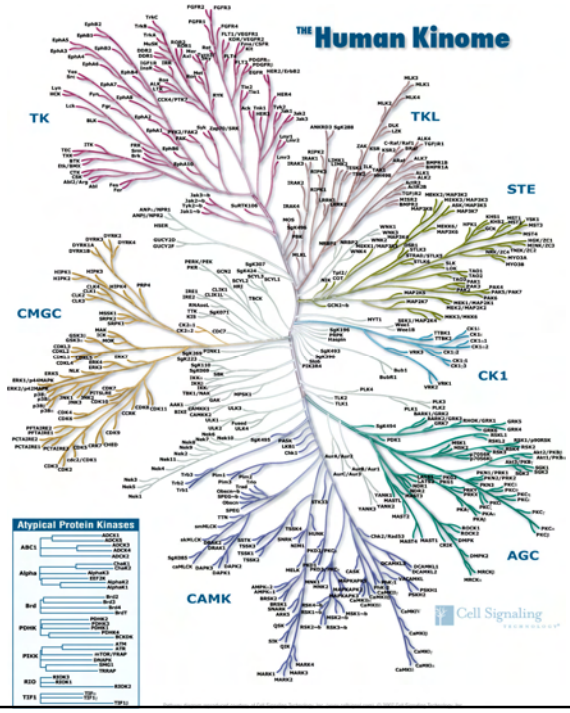


Human genome analyses (Science 291, 2001):



The human kinome: 518 kinases

(Science 298, 2002)

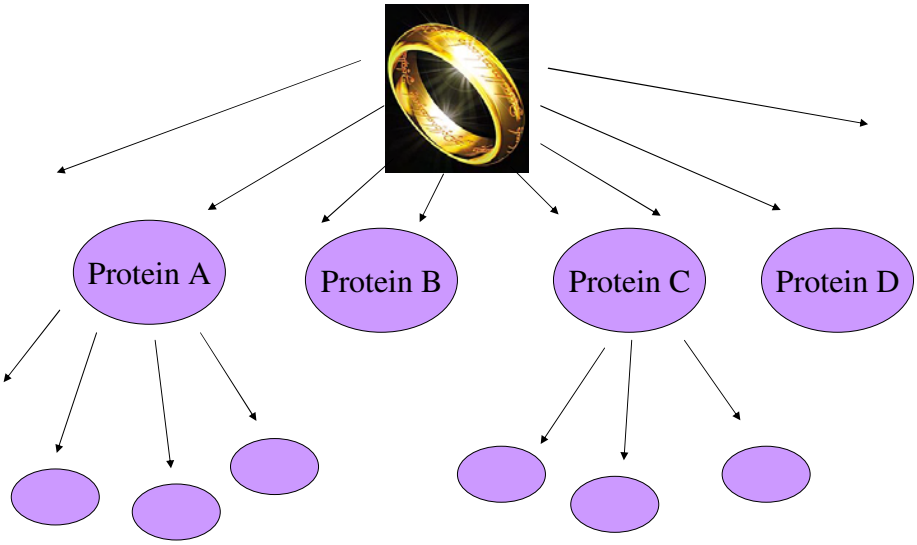


Source of figure:

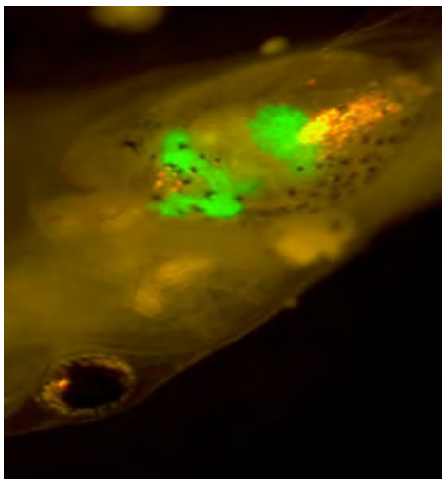
Cell Signaling Technology Inc.

"MASTER SWITCH":

One gene/protein controls all ...

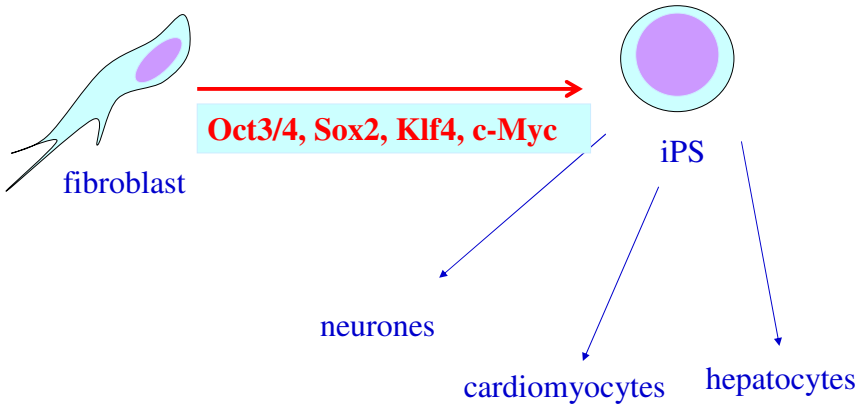


Horb, M.E., et al.:
Experimental conversion of liver to pancreas.
***Current Biology*, 13, 105 - 115, (2003).**



- 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

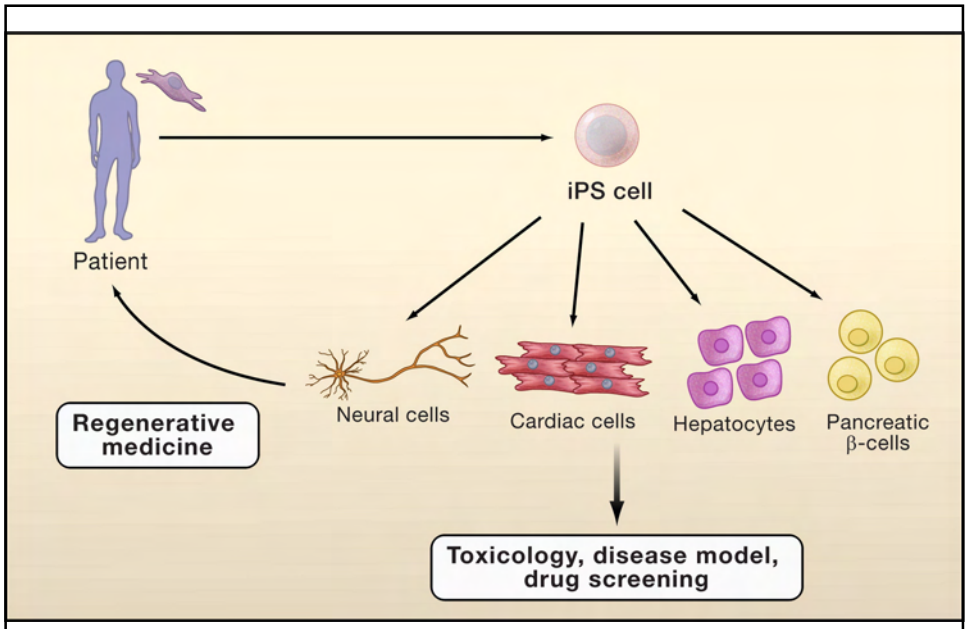
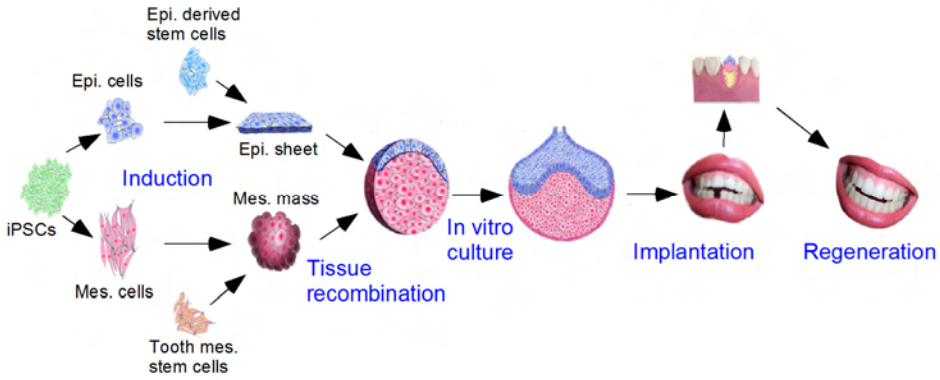
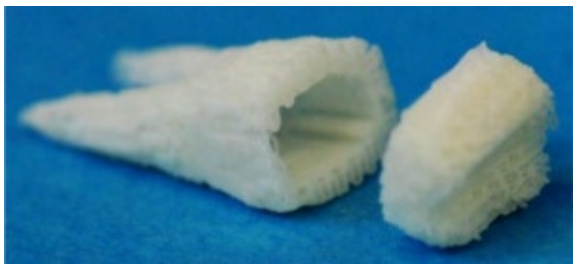


Fig from: Yamanaka S. Cell 137, 2009, 13-17.



A blueprint of stem cell-based tooth regeneration with a scaffold-free approach. Schematic procedures of stem cell-based scaffold-free tooth regeneration in humans. The procedures include induction of iPSCs or epithelial derived stem cells into epithelial (epi.) sheets and induction of iPSCs or dental mesenchymal (mes.) stem cells into mesenchymal masses with odontogenic potential, tissue recombination, in vitro organ culture of the recombinants to the late bud or early cap stage, implantation of bioengineered tooth germs into the lost tooth sites of patients, and regeneration of functional replacement teeth.
Zhang and Chen *Cell Regeneration* 2014 3:8 doi:10.1186/2045-9769-3-8

"When dental stem cell therapies become routine it will be historic, and the most fantastic time to practice as a dentist."



http://singularityhub.com/2012/05/10/toothless-no-more-researchers-using-stem-cells-to-grow-new-teeth/

Quote by prof. Peter Murray, College of Dental Medicine, Nova Southeastern University