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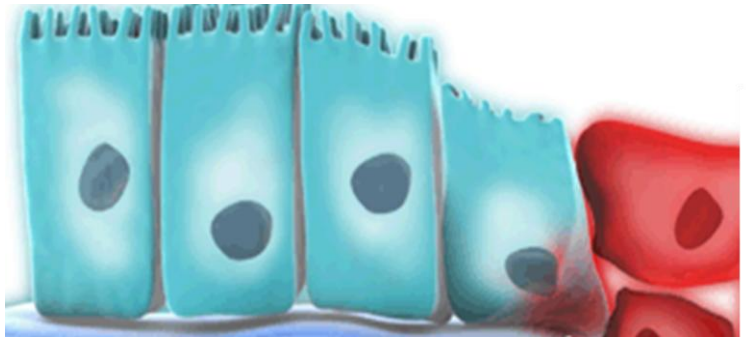
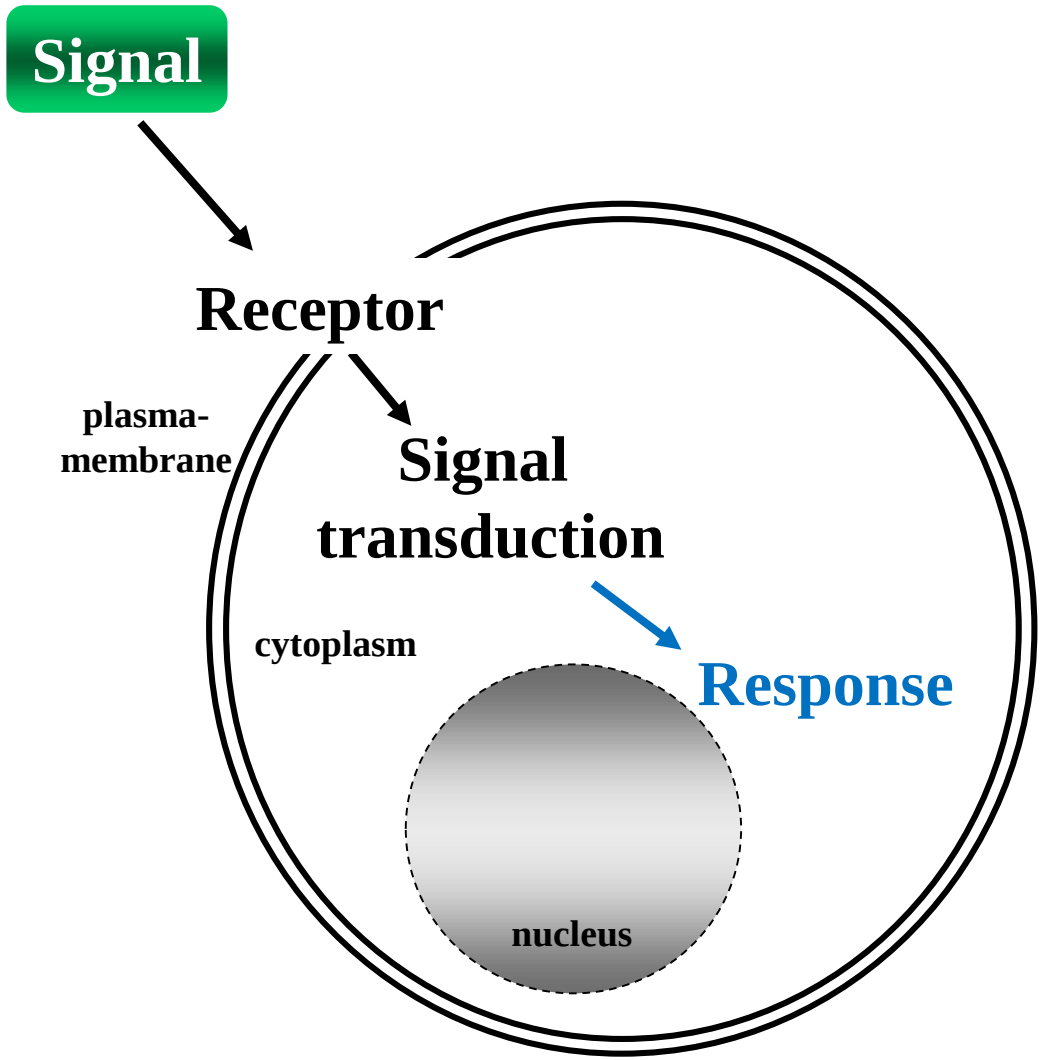


Oncobiology

Margarida Gama-Carvalho (DQB/FCUL) and Peter Jordan (INSA)

Cell Signalling – part 1

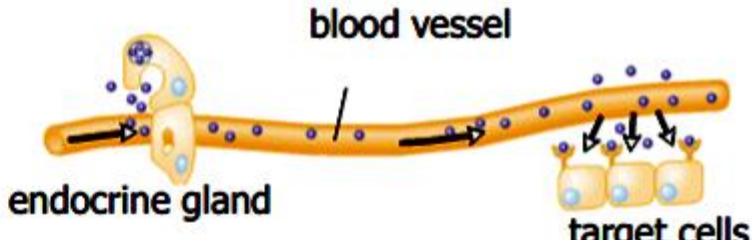
What is signal transduction?



Types of the Signal

Signal

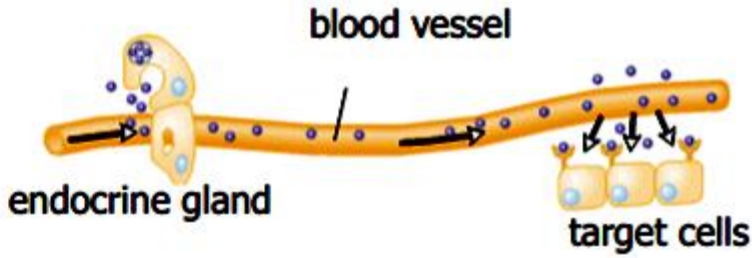
- endocrine:



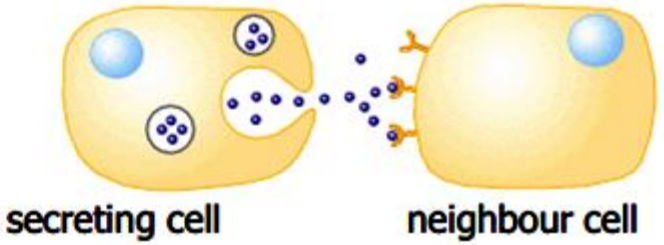
Signal

Types of the Signal

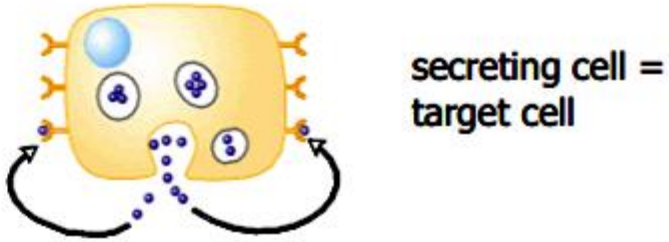
- endocrine:



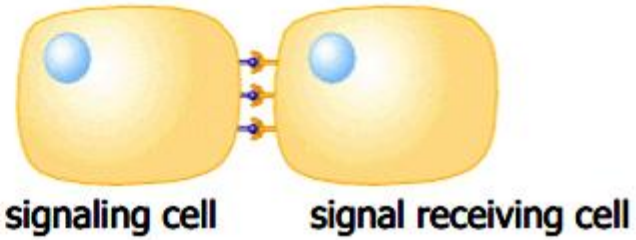
- paracrine:



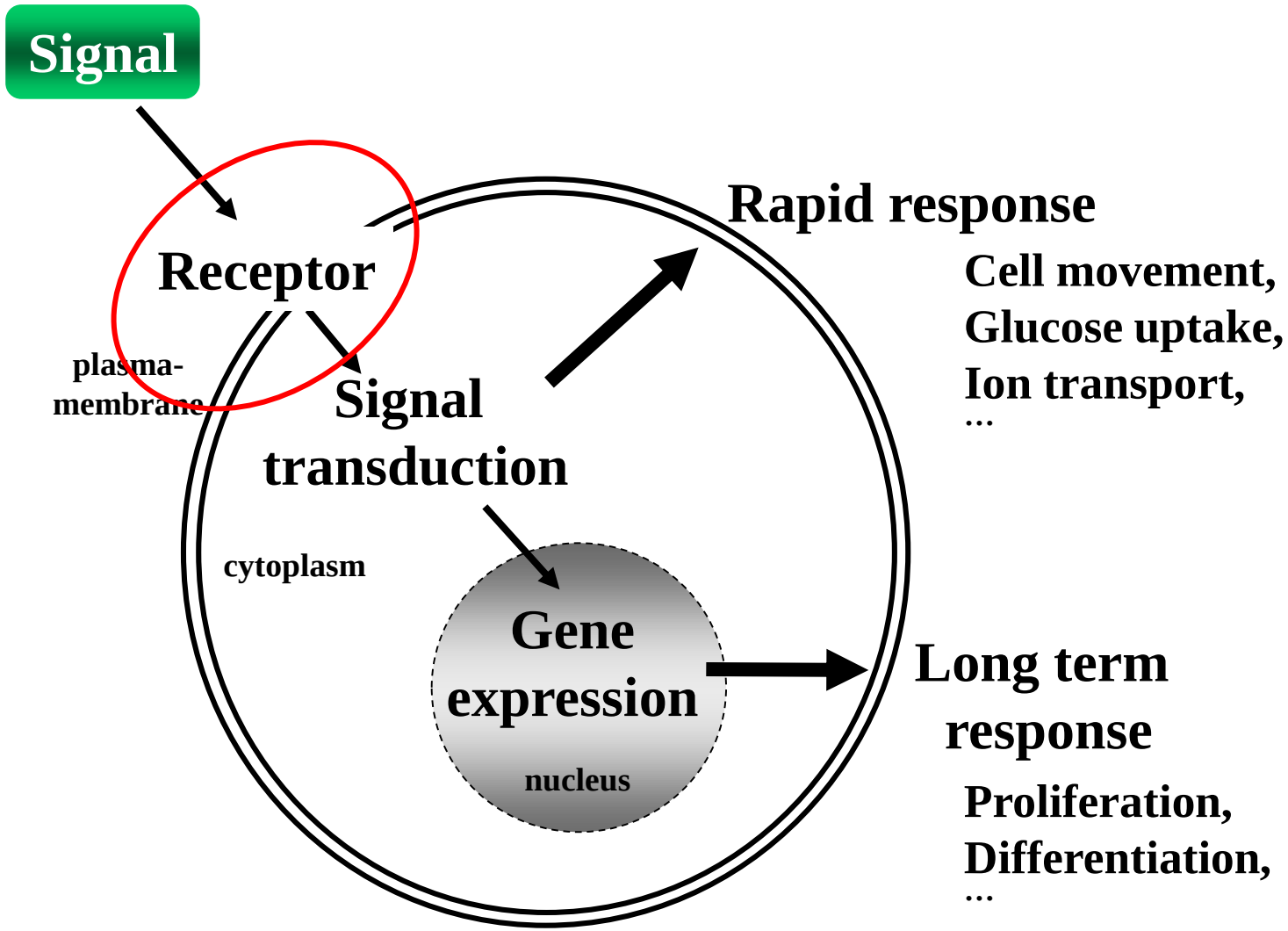
- autocrine:



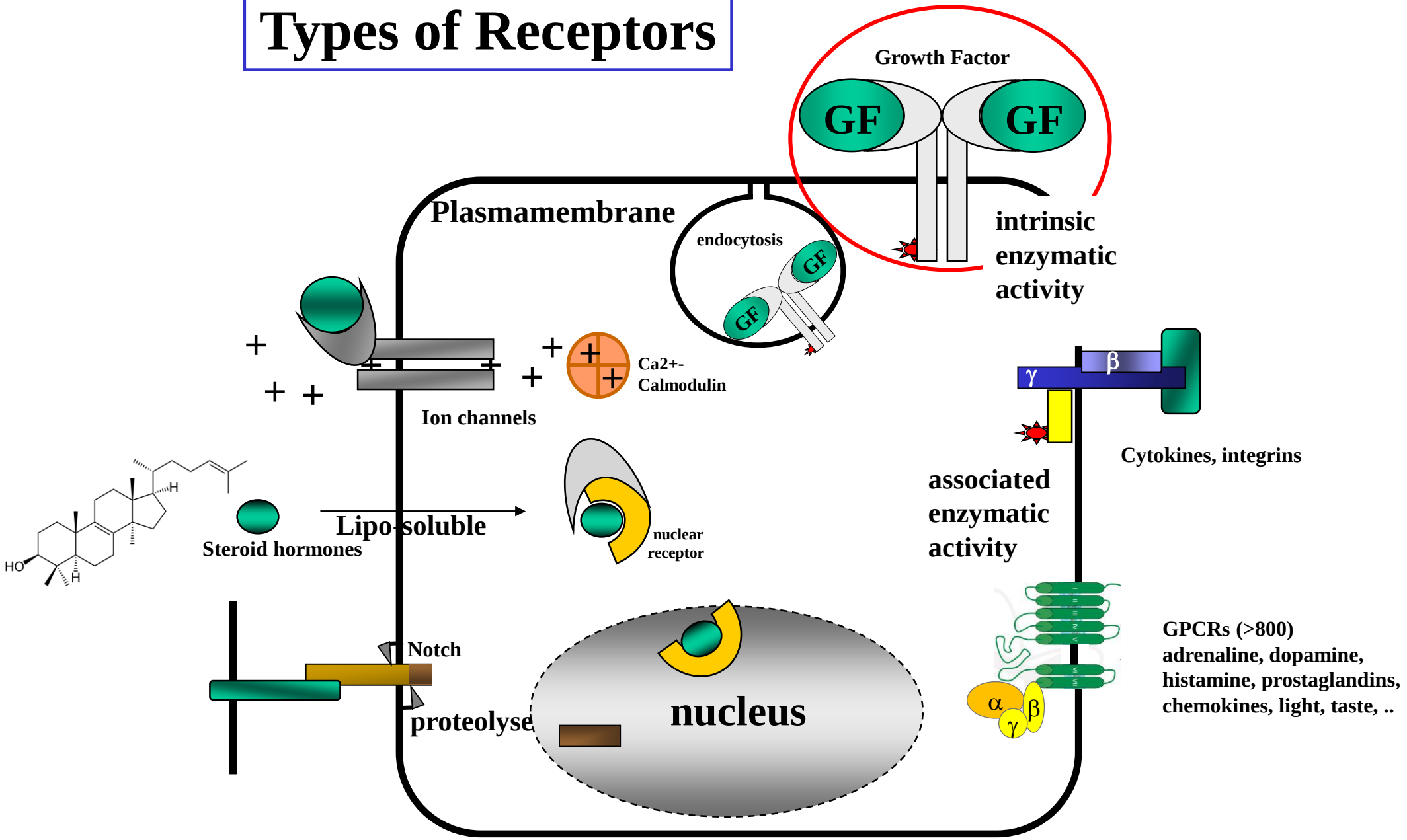
- direct cell contact:



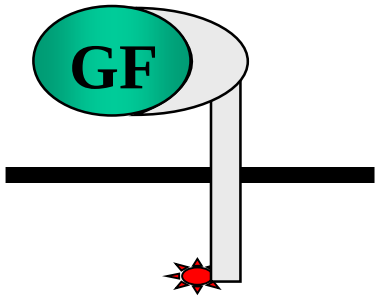
Extracellular signals and cellular response



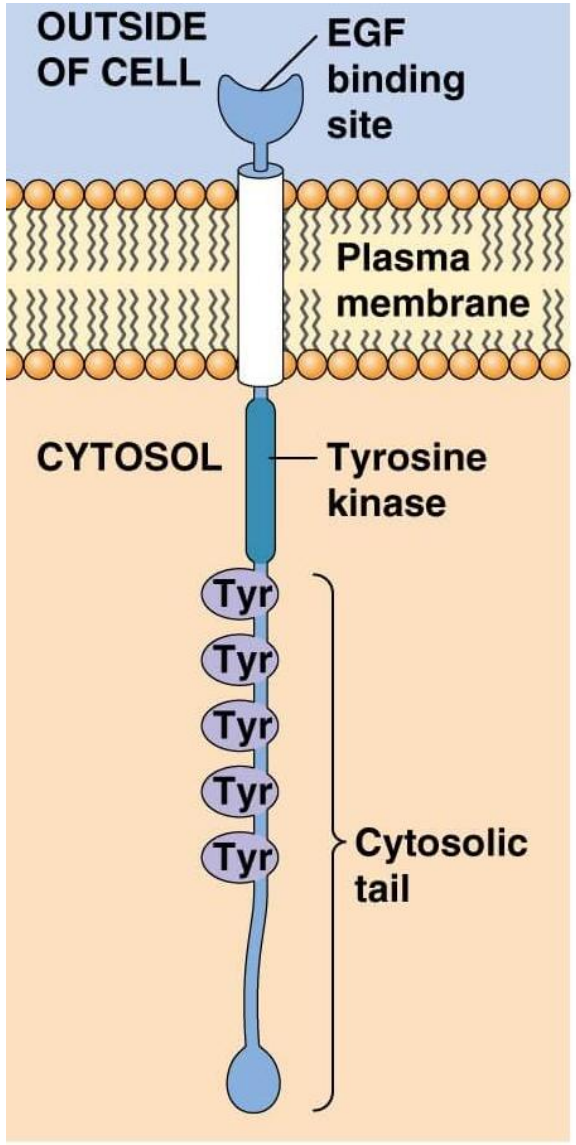
Types of Receptors



Growth factor receptor activation

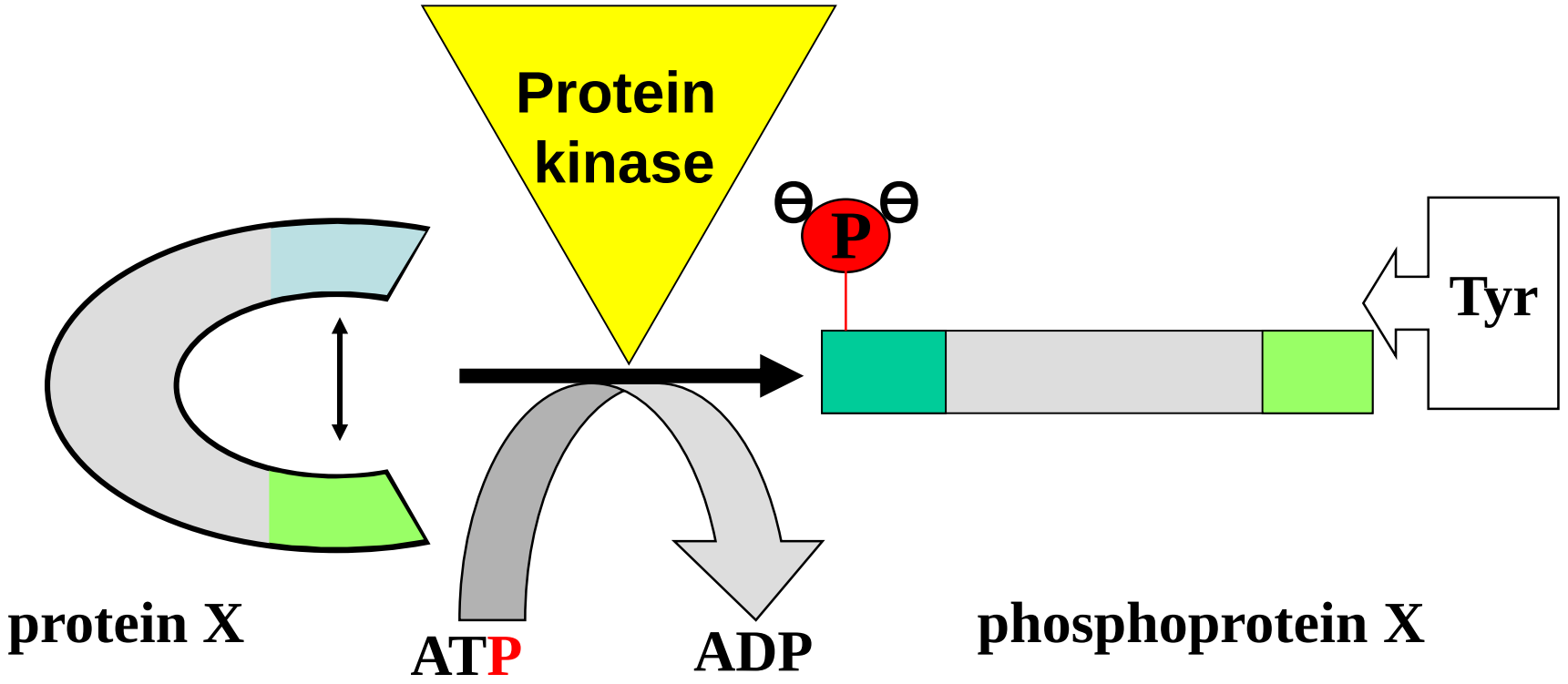


Receptor tyrosine kinase



a) Structure of the epidermal growth factor (EGF) receptor

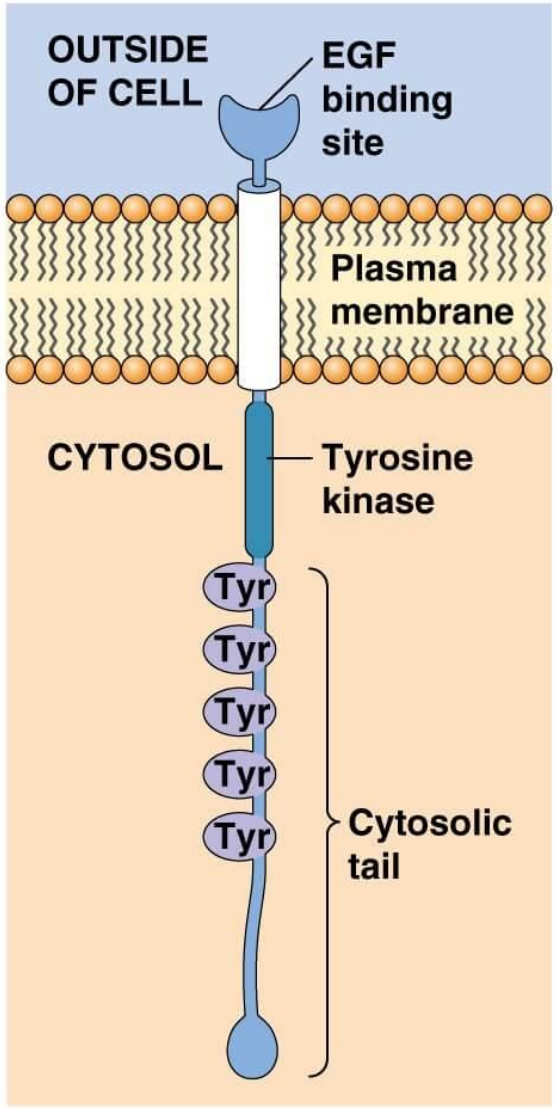
Image:
https://www.creative-biostructure.com/custom-mempro%E2%84%A2-receptor-type-kinases-services-55.htm, or
https://www.slideserve.com/lisle/lecture-9-july-13-2001-cell-signaling-receptor-tyrosine-kinases-no-and-nyc-chapter-15



inactive ‘Molecular switch’ **active**

Growth factor receptor activation

Step 1



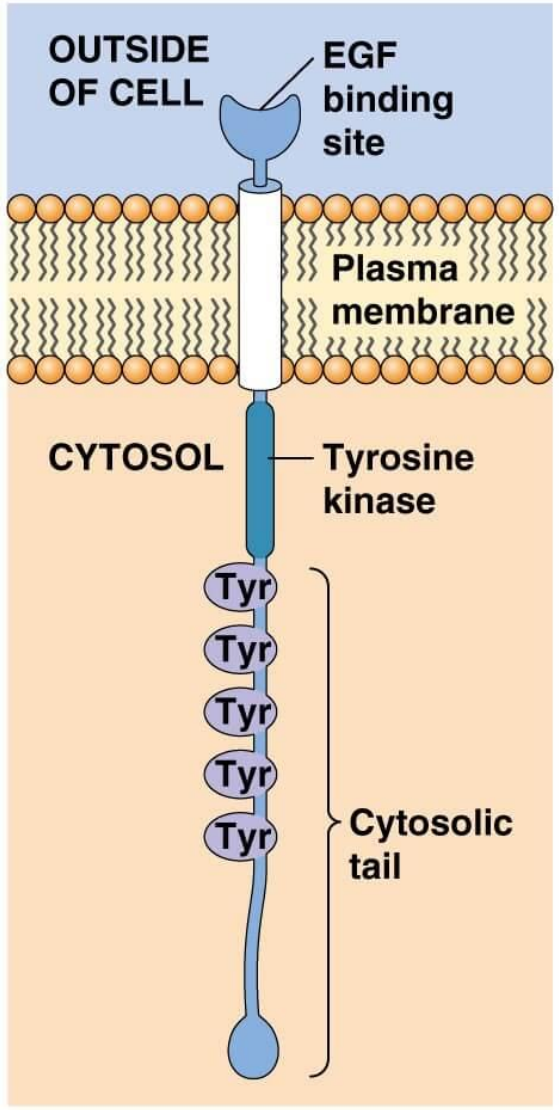
(a) Structure of the epidermal growth factor (EGF) receptor

...after ligand binding to its extracellular domain, the cytoplasmic tail of the receptor becomes phosphorylated;

but how??

Growth factor receptor activation

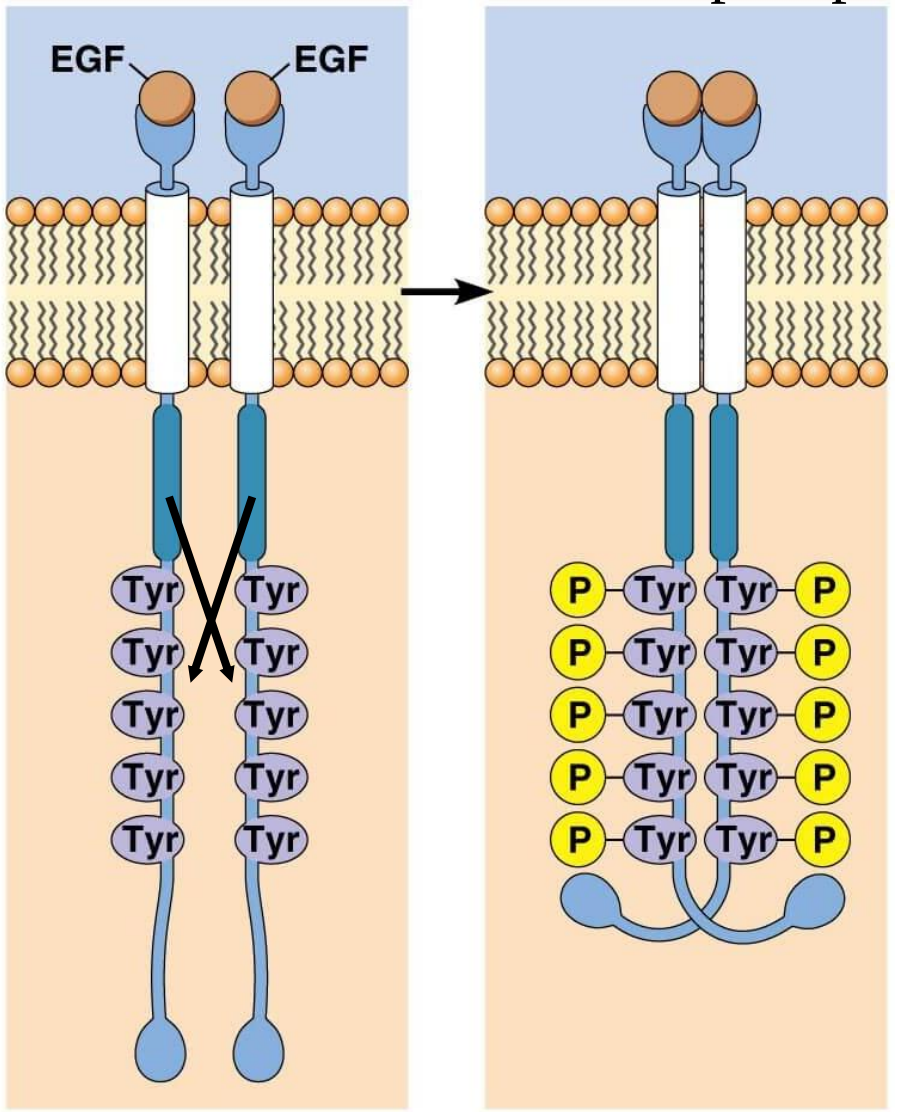
Step 1 +2



(a) Structure of the epidermal growth factor (EGF) receptor

Dimerization

Tyrosine kinase activation and cross-phosphorylation



(b) Activation of the EGF receptor

Examples:

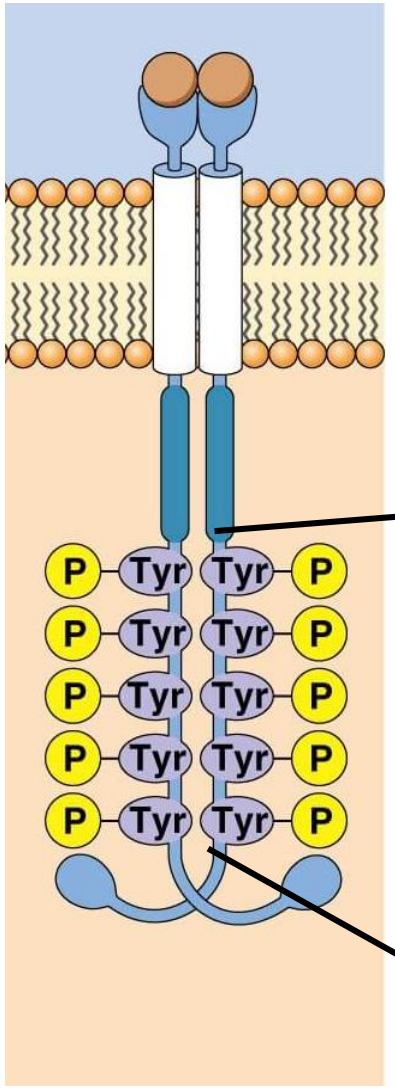
EGF-R

PDGF-R

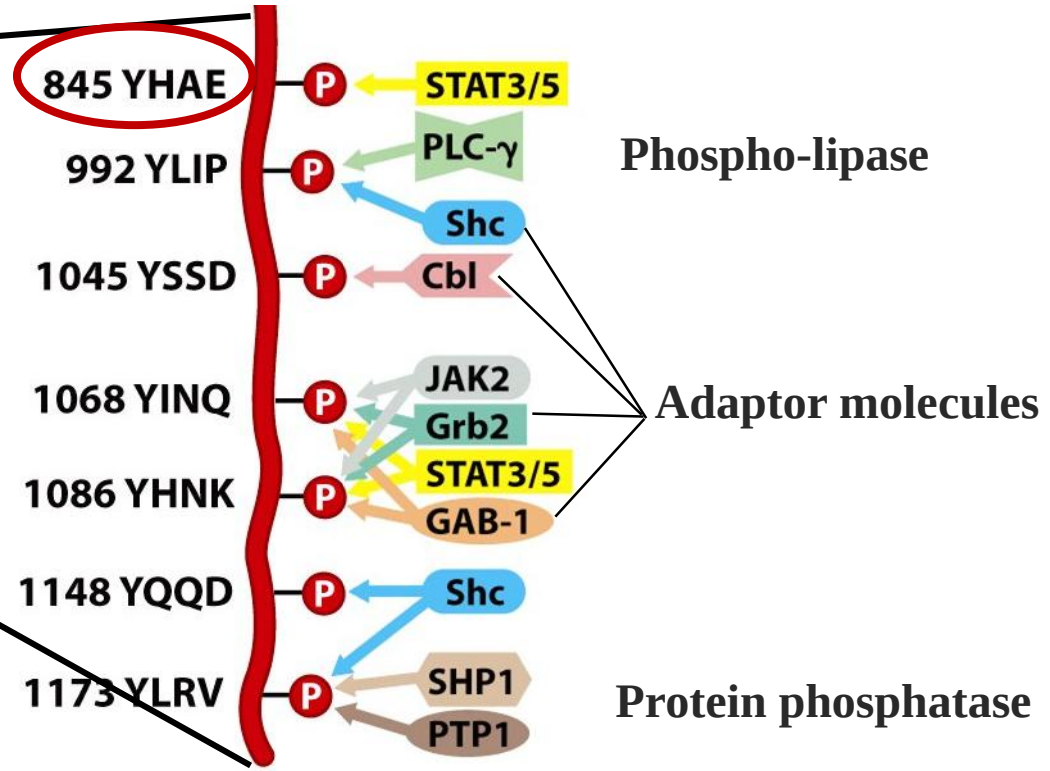
Insulin-R

Signalling by activated growth factor receptor

Step 2



- Each type of receptor is phosphorylated at specific tyrosine residues
- Each phosphorylated tyrosine residue is recognized by cytoplasmic proteins containing an **SH2 domain**
- Each SH2 domain recognizes a specific peptide sequence following the phospho-tyrosine residue



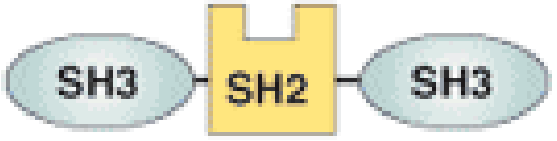
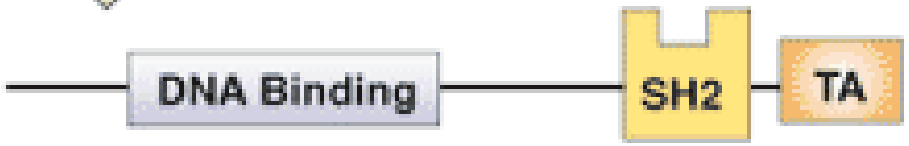
Signalling by activated growth factor receptor

Proteins containing the SH2 domain

can “read” the activation of the receptor

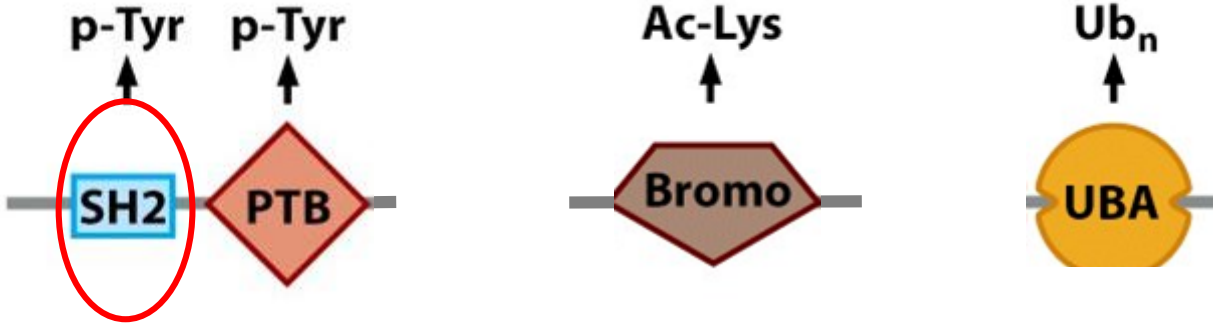
Examples of SH2 domain-containing proteins

https://www.mdpi.com/1422-0067/23/24/15944

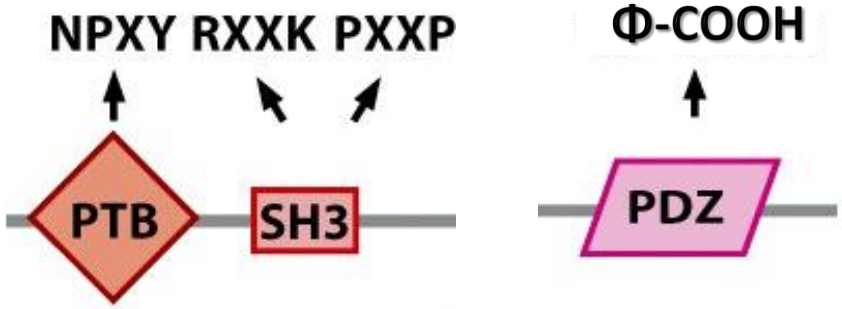
Enzymes	Btk	
Adaptors	Grb2	
Docking proteins	Shc	
Transcription factor	Stat	

SH2 domain is one example of a protein interaction domain; these can 'read' molecular changes tiggered in cellular molecules

Post-translational modifications of a protein



Peptide motifs within a protein



Phospholipid modifications

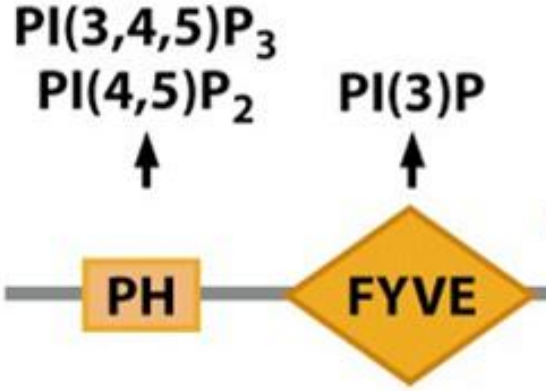
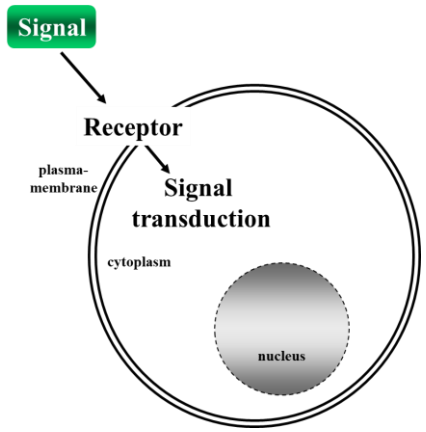
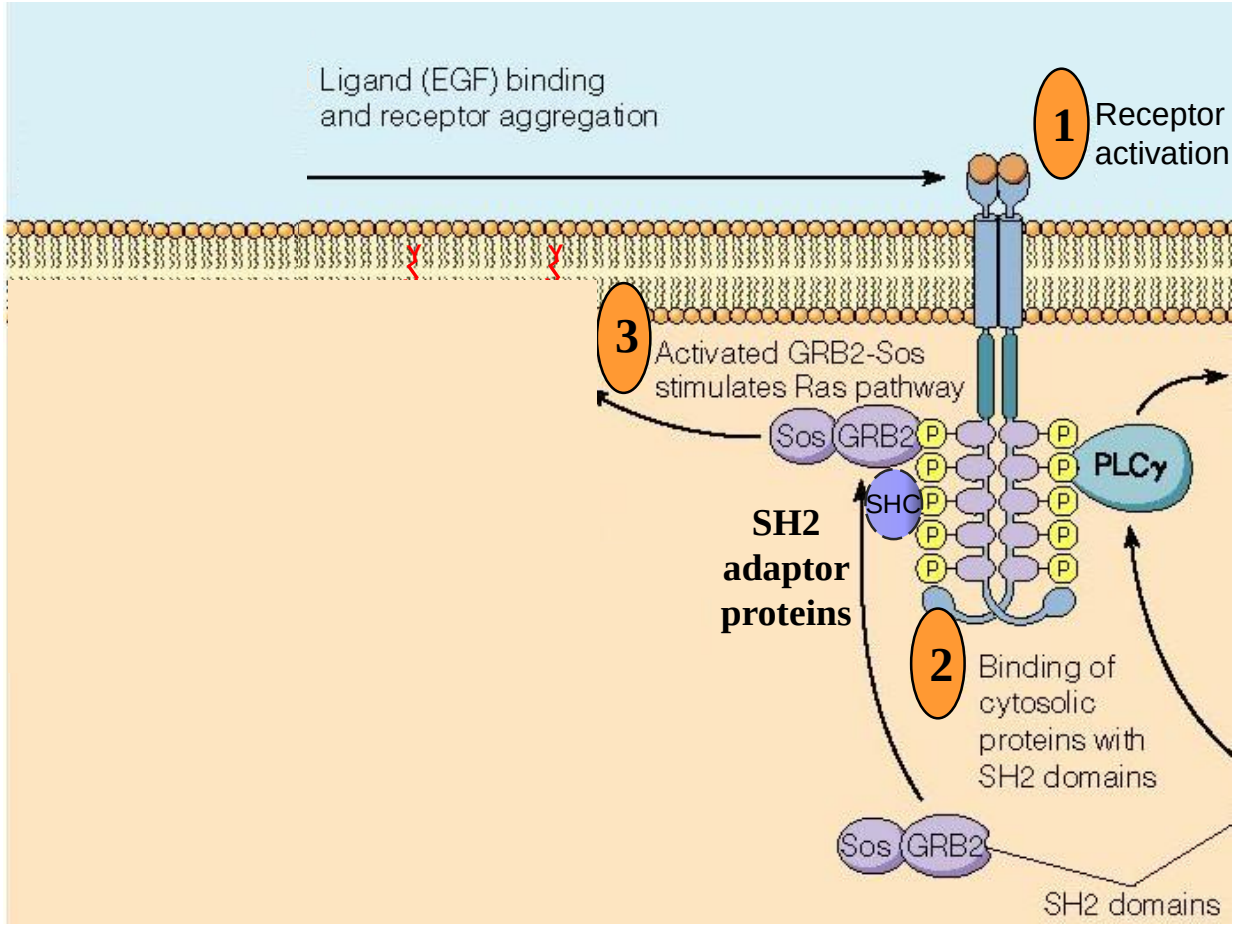


Figure 6.10b *The Biology of Cancer* (© Garland Science 2007)

Signalling by activated growth factor receptor

Step 3



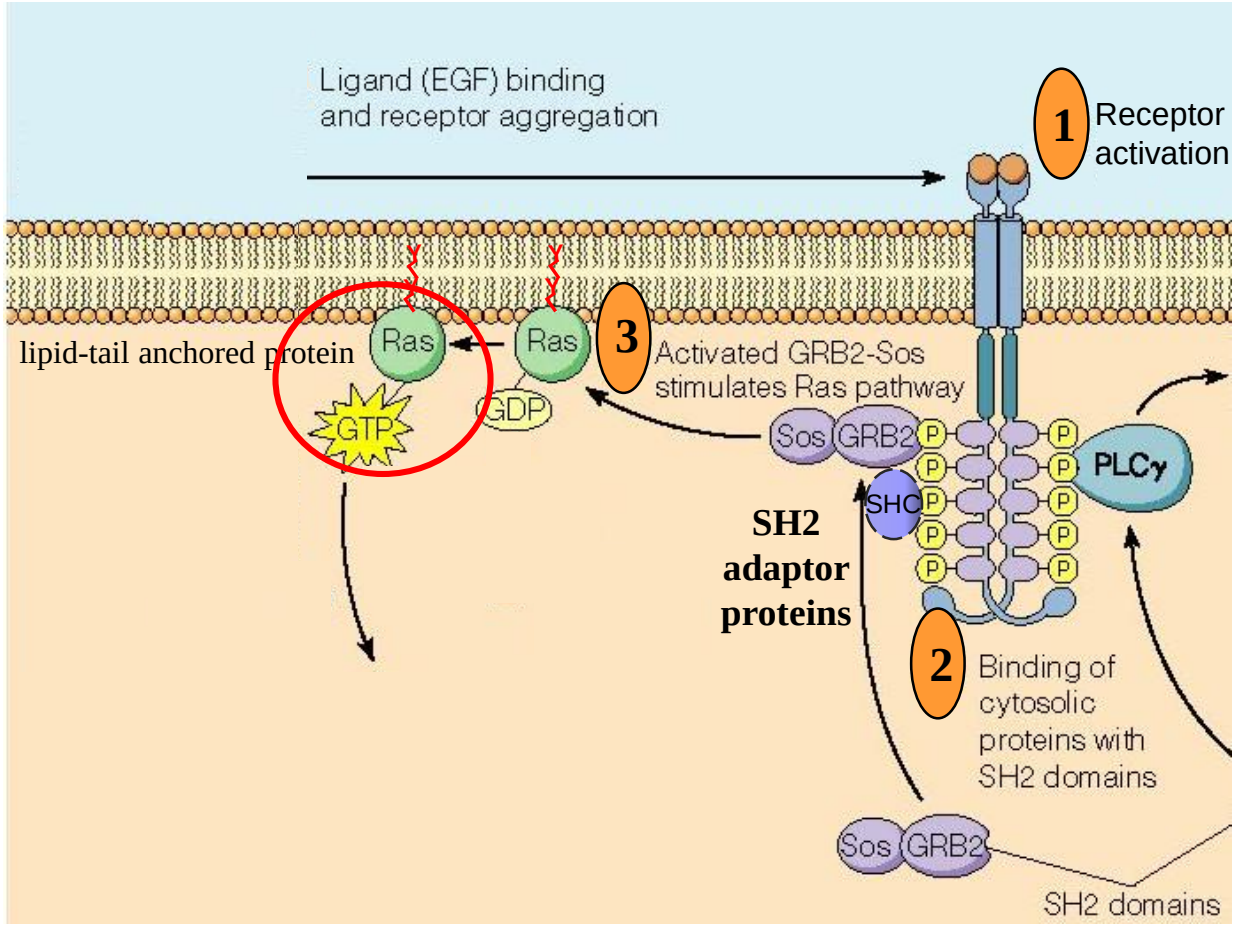


https://www.slideserve.com/lisle/lecture-9-july-13-2001-cell-signaling-receptor-tyrosine-kinases-no-and-nyc-chapter-15

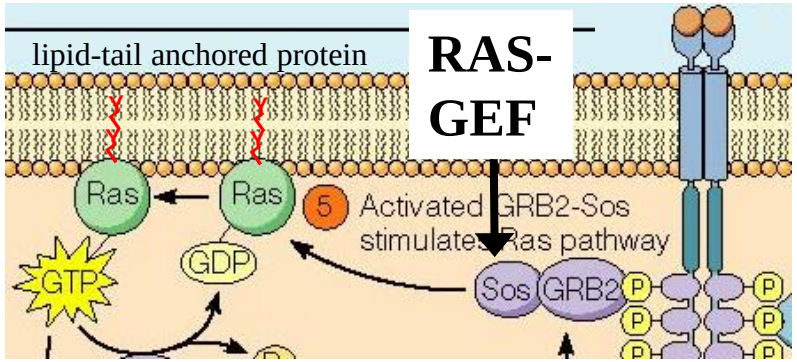
Signalling by activated growth factor receptor

Step 3

RAS activation



The activation cycle of small GTPases



GEFs

GDP

GTP

inactive conformation

active conformation

Signal propagation via effector proteins

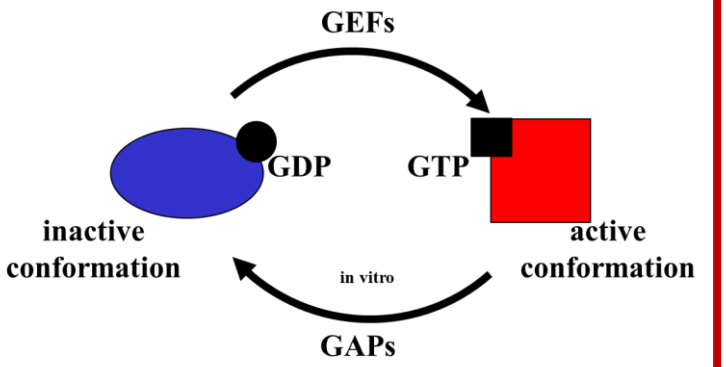
Effector protein

in vitro

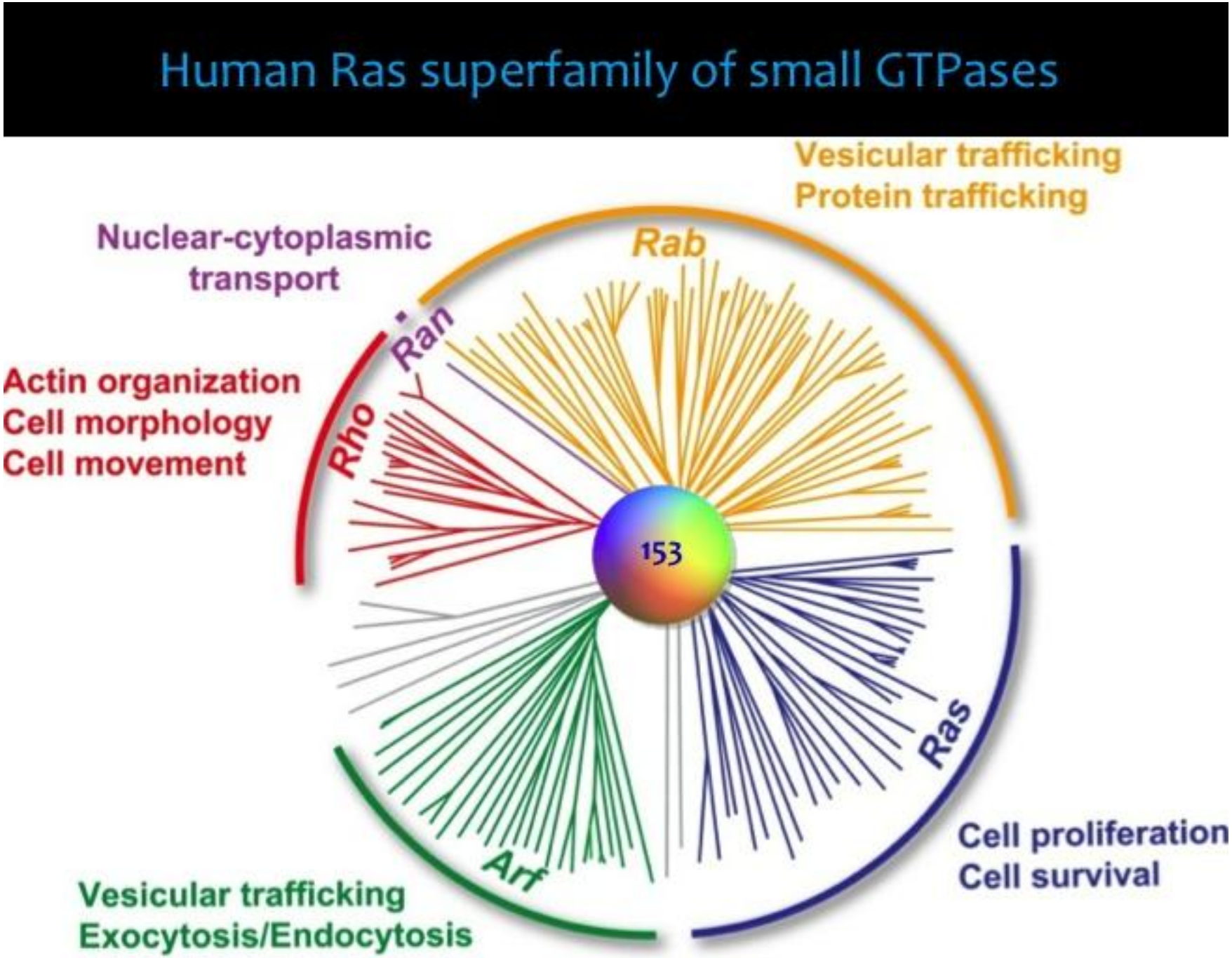
GAPs

Cellular response

small GTPases

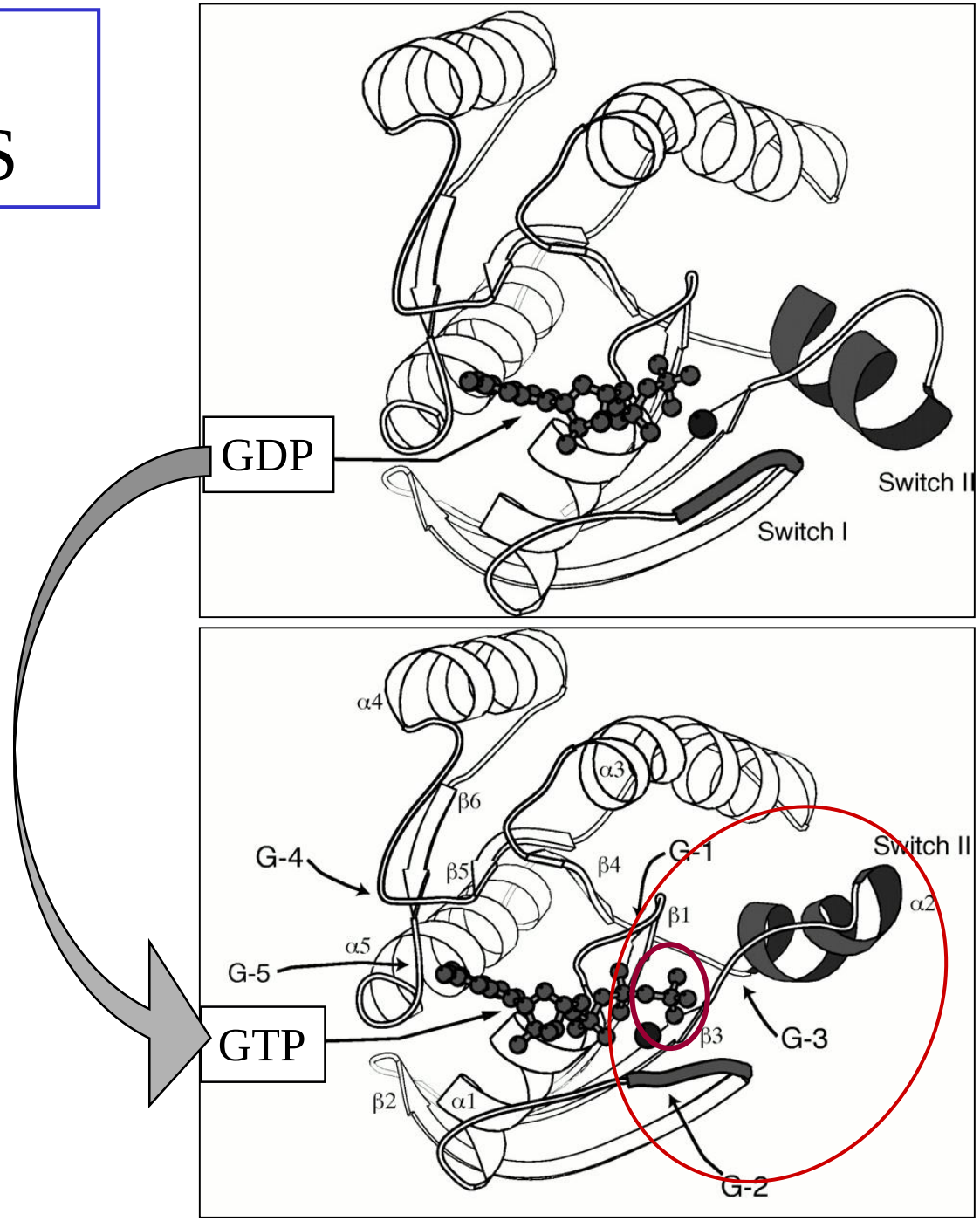


A fundamental molecular switch mechanism used in cell signalling

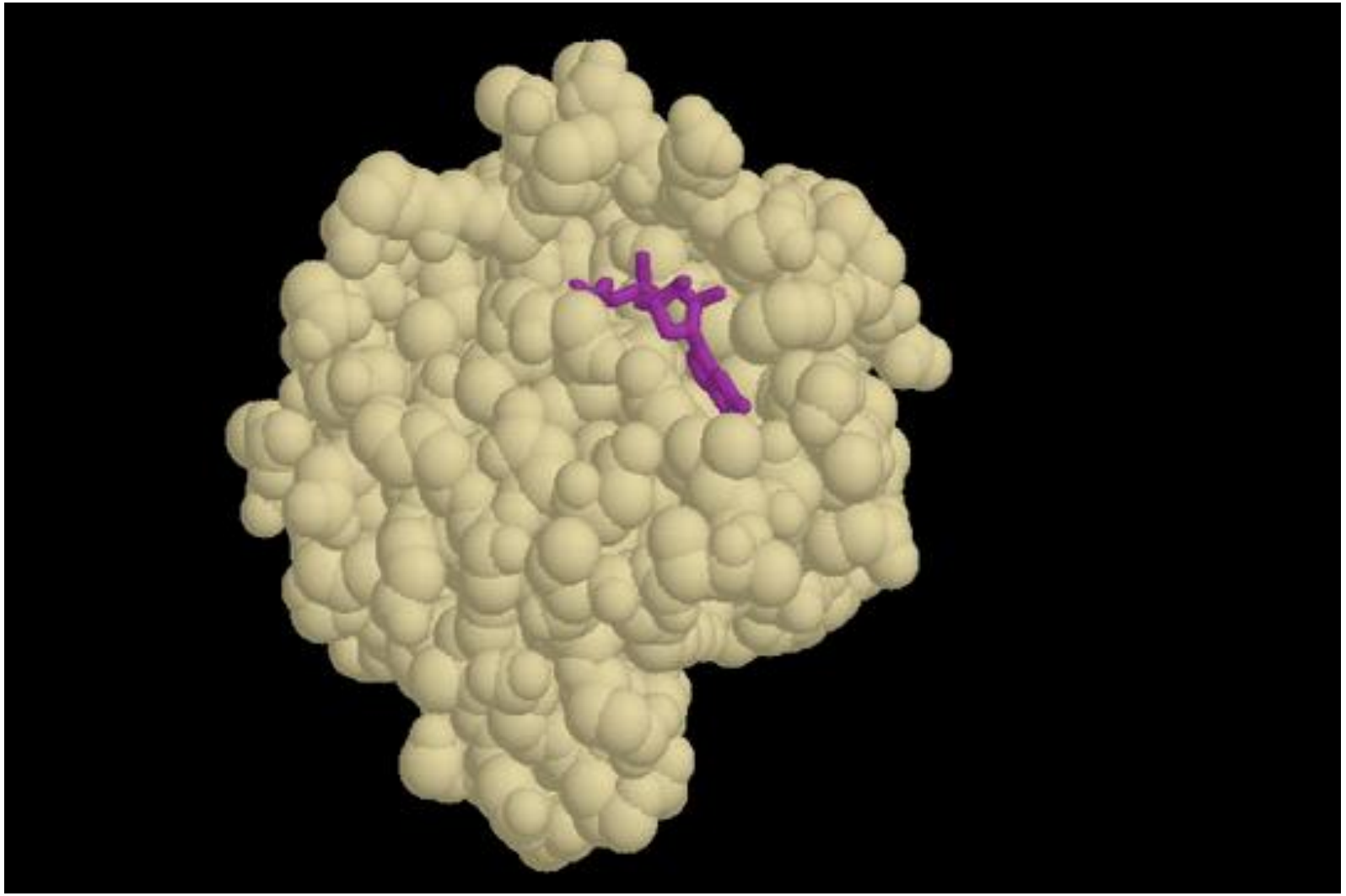


Structure of the GTPase RAS

GTP-binding changes RAS conformation, which is then recognizd by binding partners (‘effectors’)

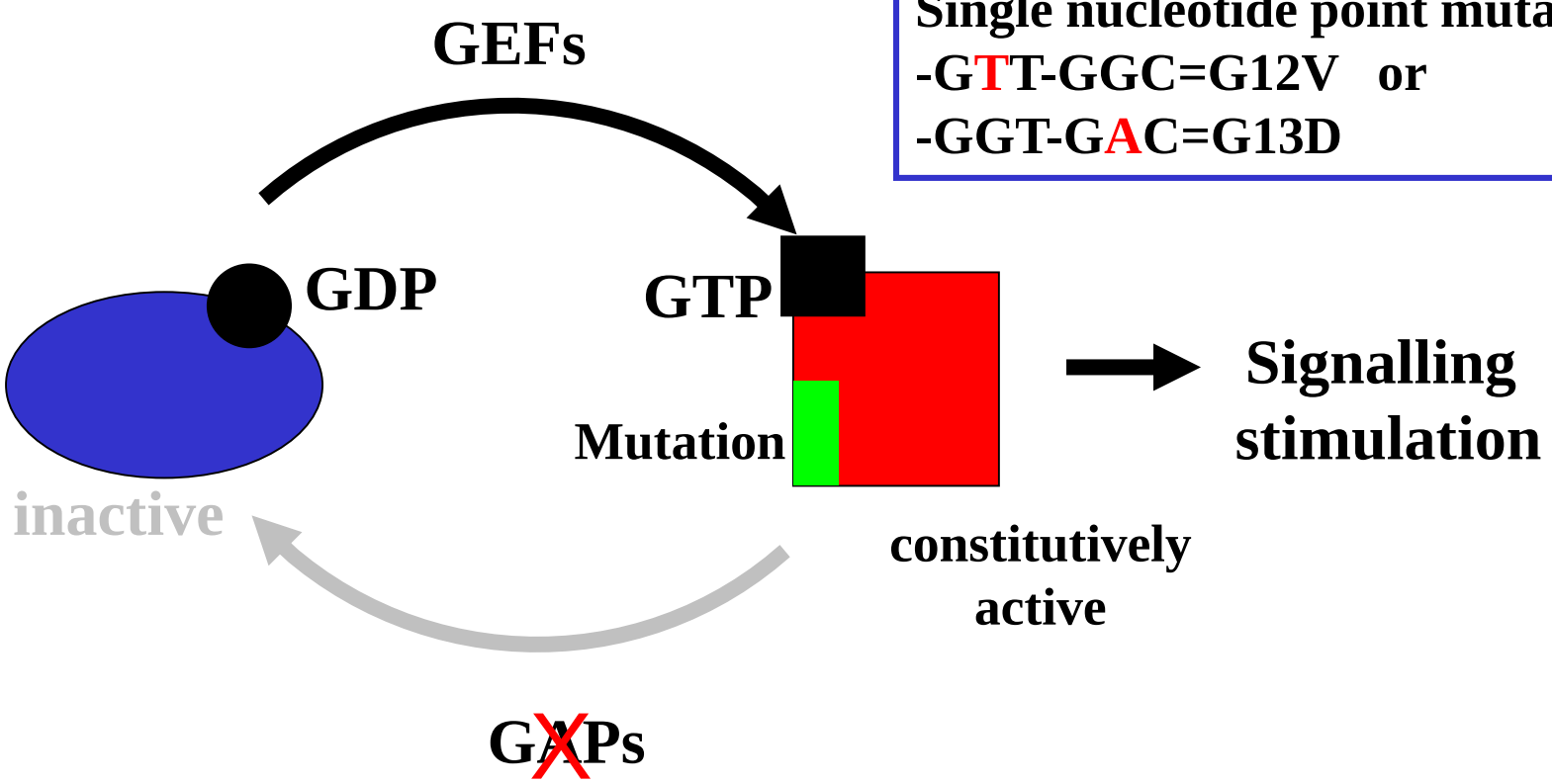


Video animation of the GTP cycle of RAS

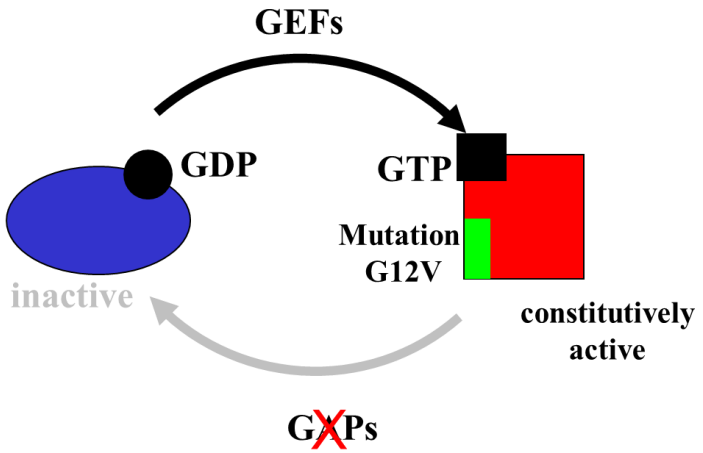


RAS is a proto-oncogene !

Mutation in codons 12 or 13= -GGT-GGC-
Single nucleotide point mutations:
-GTT-GGC=G12V or
-GGT-GAC=G13D

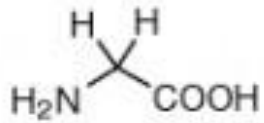


Mutated in around 30% of epithelial tumours
(especially colon, lung and pancreatic cancer)



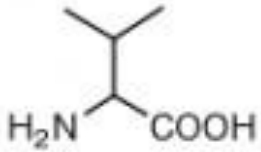
Why is the mutation RAS-Gly12Val oncogenic?

Small

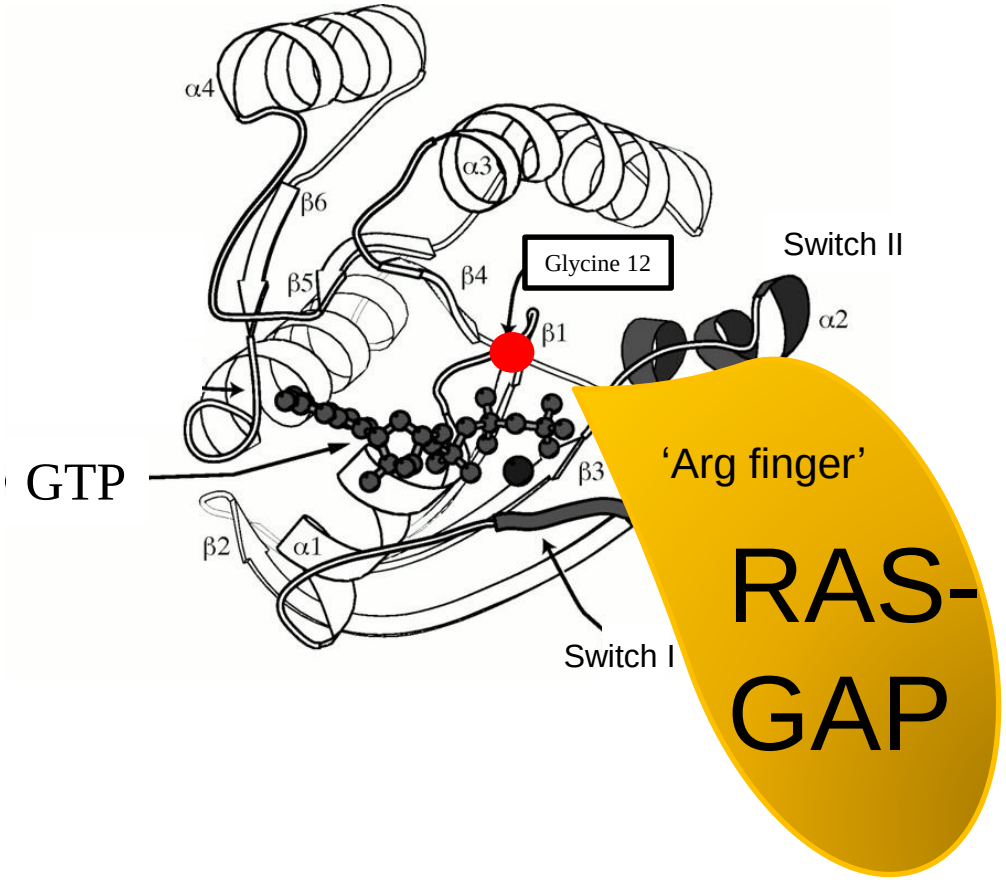


Glycine (Gly, G)
MW: 57.05

Hydrophobic

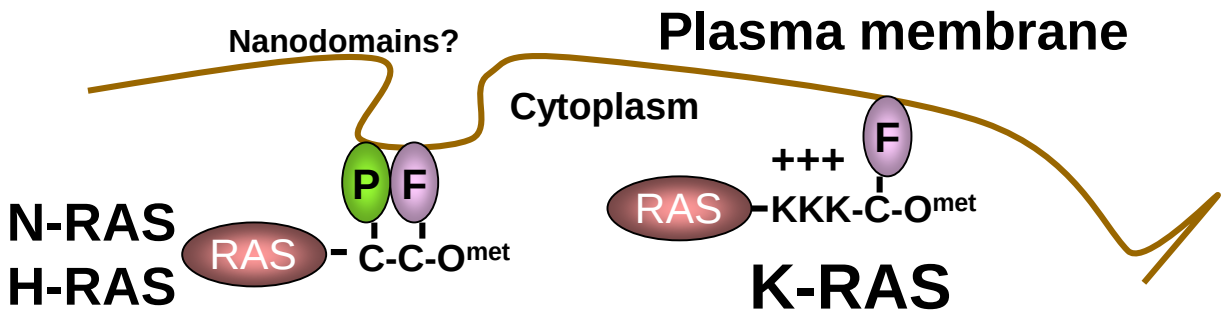


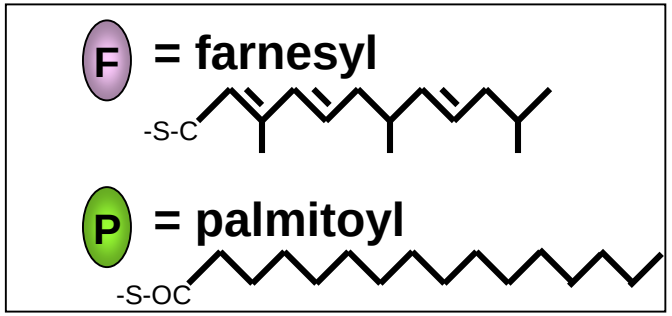
Valine (Val, V)
MW: 99.14



Three human *RAS* genes exist with tissue-specific expression

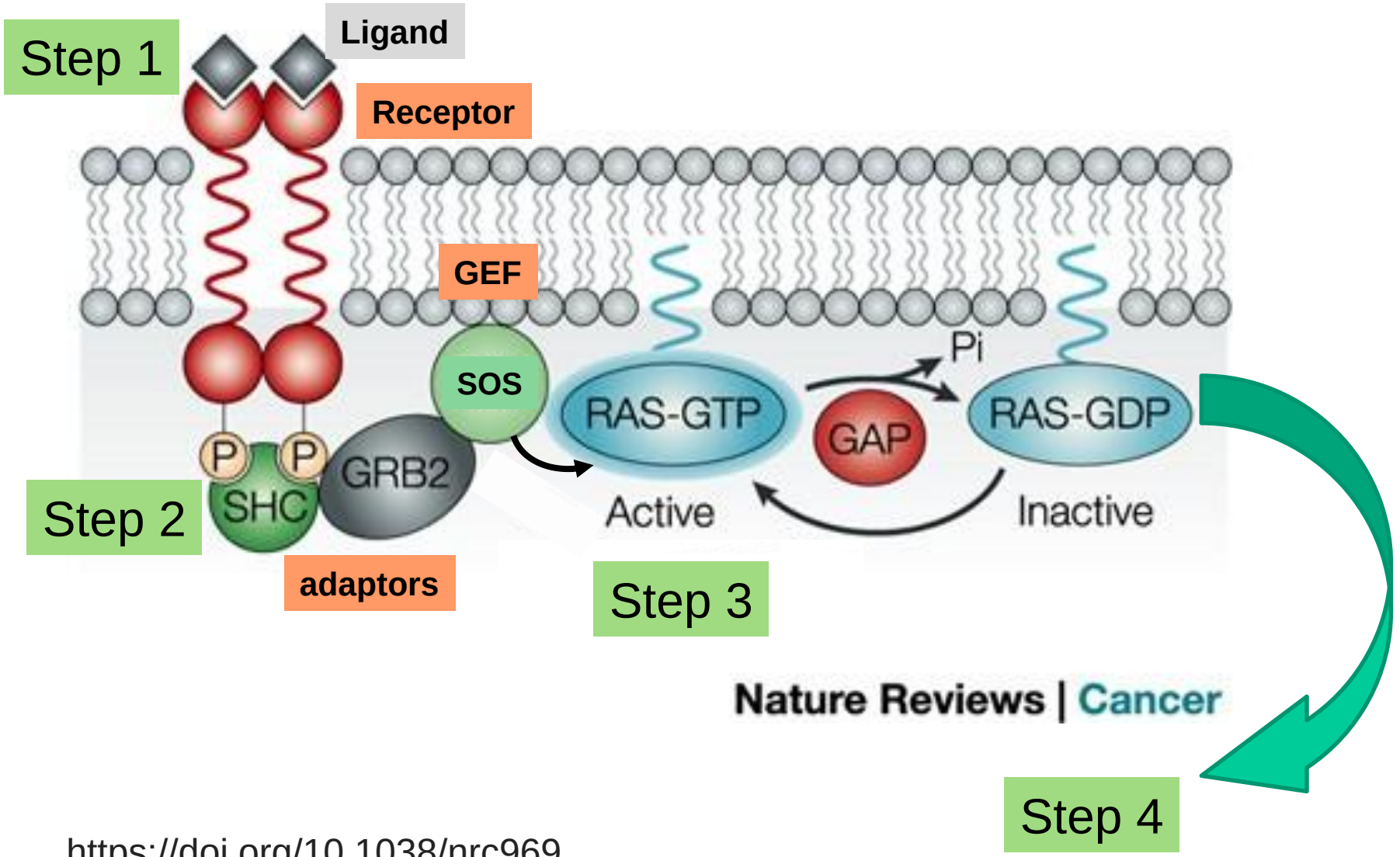
isoform	type of malignancy	knock-out mice phenotype
H-RAS	mutated in 1% of all human cancers bladder cancer squamous head-and-neck tumours	viable
N-RAS	mutated in 4% of all human cancers haematopoietic malignancies, skin cancer, some colon cancers	viable
K-RAS	mutated in 30% of all human cancers lung, colon and pancreatic carcinomas	embryonic lethal





Adapted from Shield et al, Trends Cell Biol, 2000

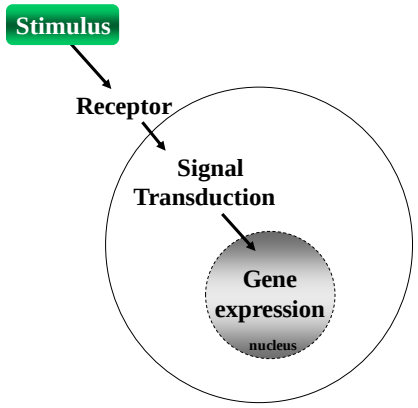
Summary: steps in growth factor receptor signalling

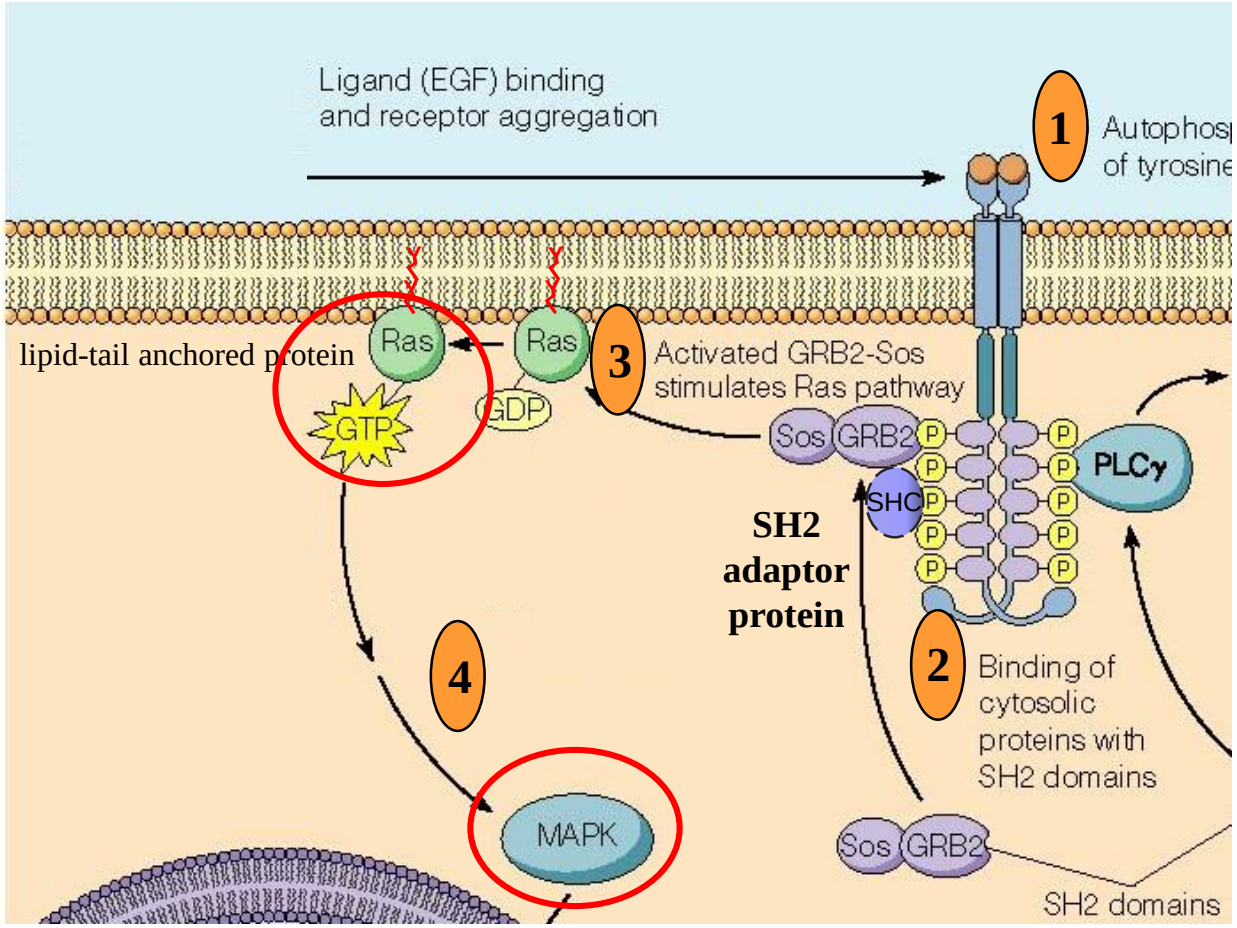


https://doi.org/10.1038/nrc969

Signalling by activated growth factor receptor

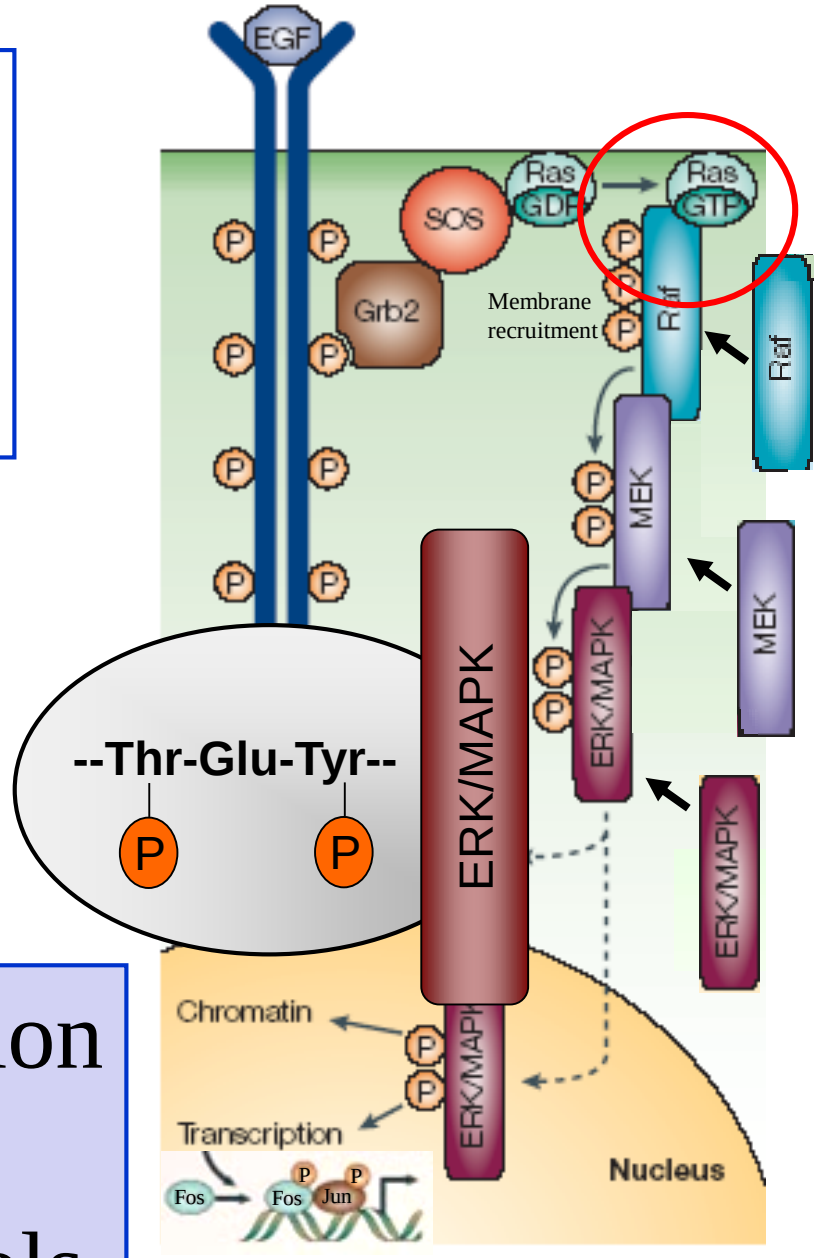
Step 4





The MAP kinase cascade

The MAP kinase cascade



Characteristic double phosphorylation

RAF= effector protein that recognizes RAS-GTP conformation

Receptor

GTPase, MAP KKK

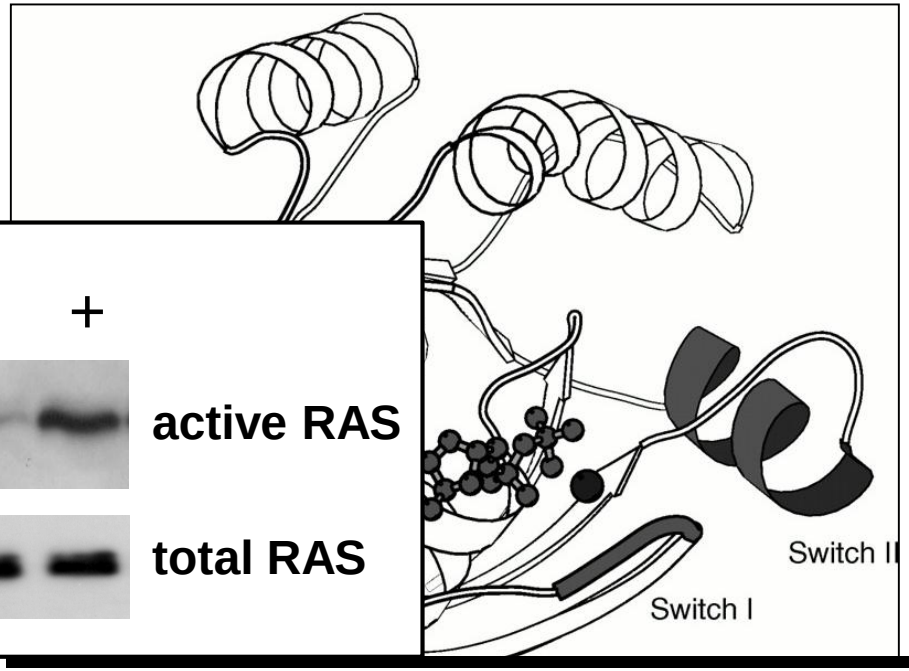
MAP KK

MAPK

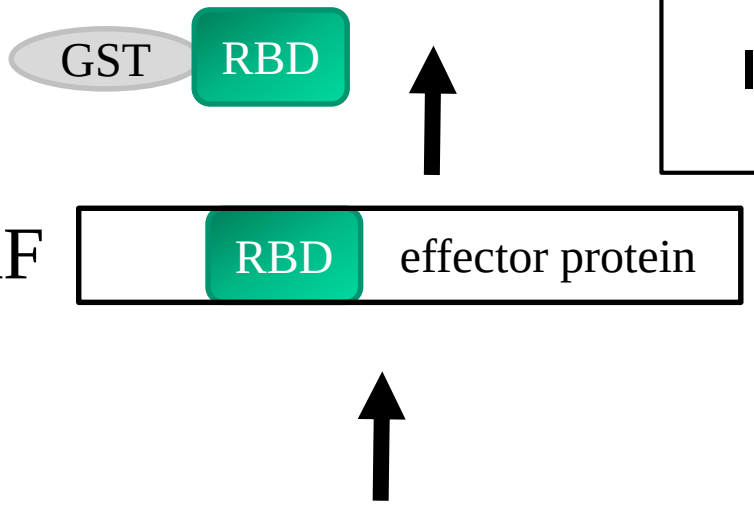
Transcription factor

Pathway characterization allowed developing important research tools

Tools to study activation of the GTPase RAS

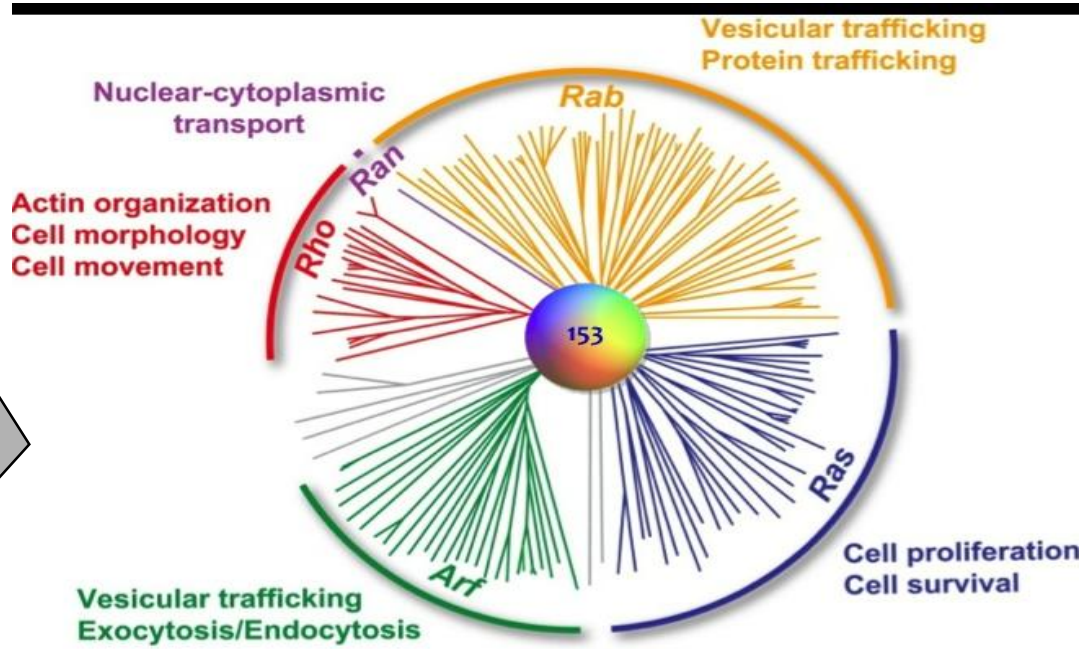


Pull-down assays

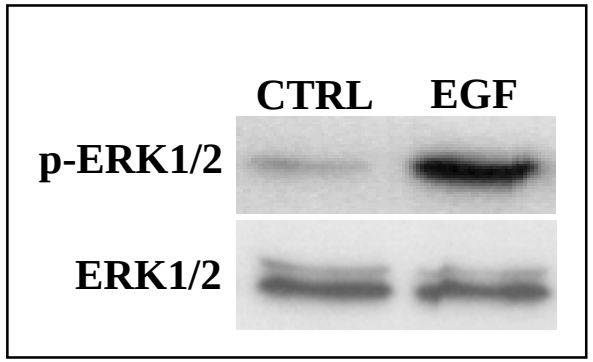


GTP-binding changes RAS conformation, which is then recognizd by binding partners (‘effectors’)

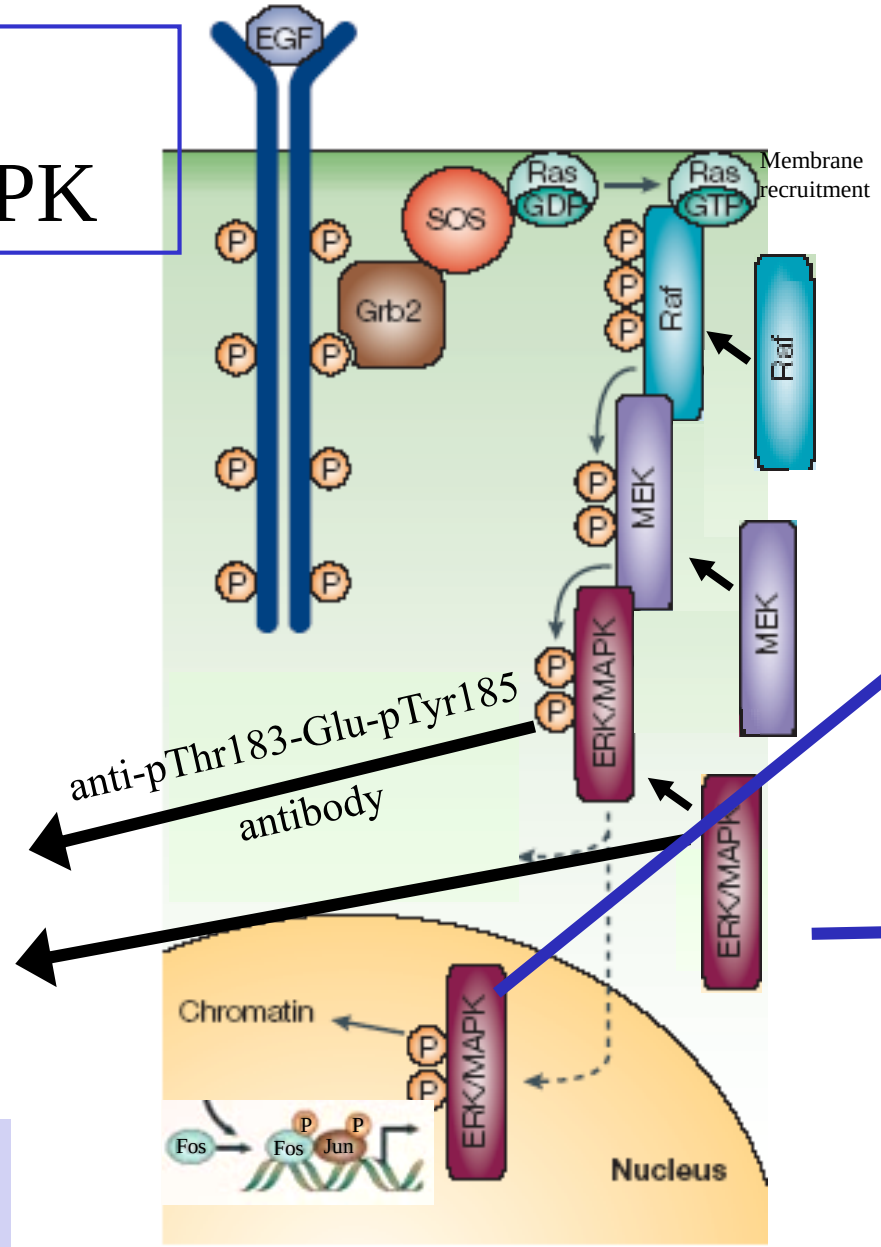
Pull-down assays also for other GTPases



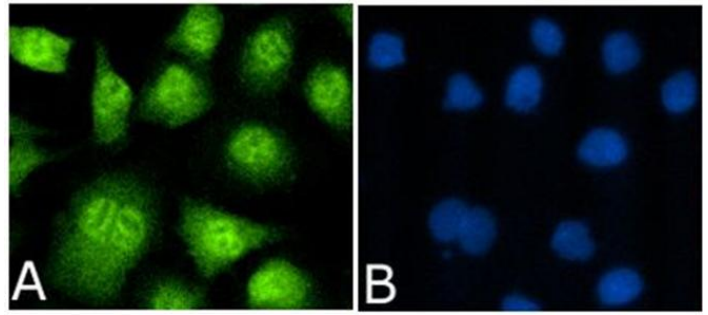
Tools to study activation of MAPK

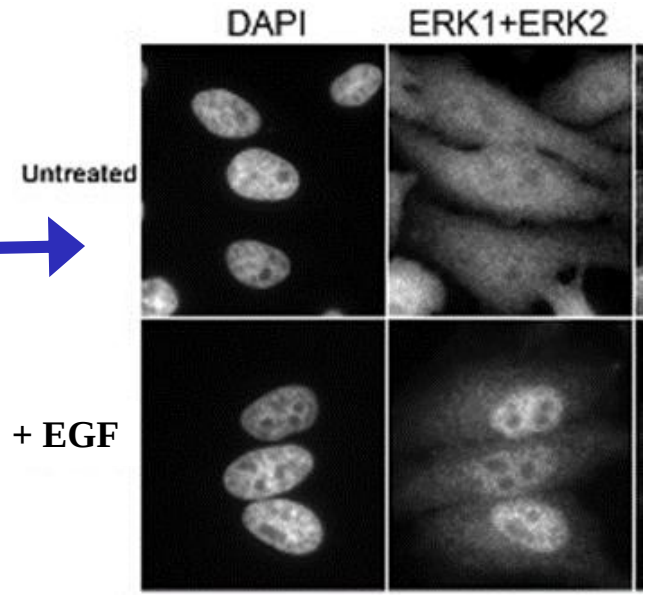


Anti-phospho antibodies as important research tools

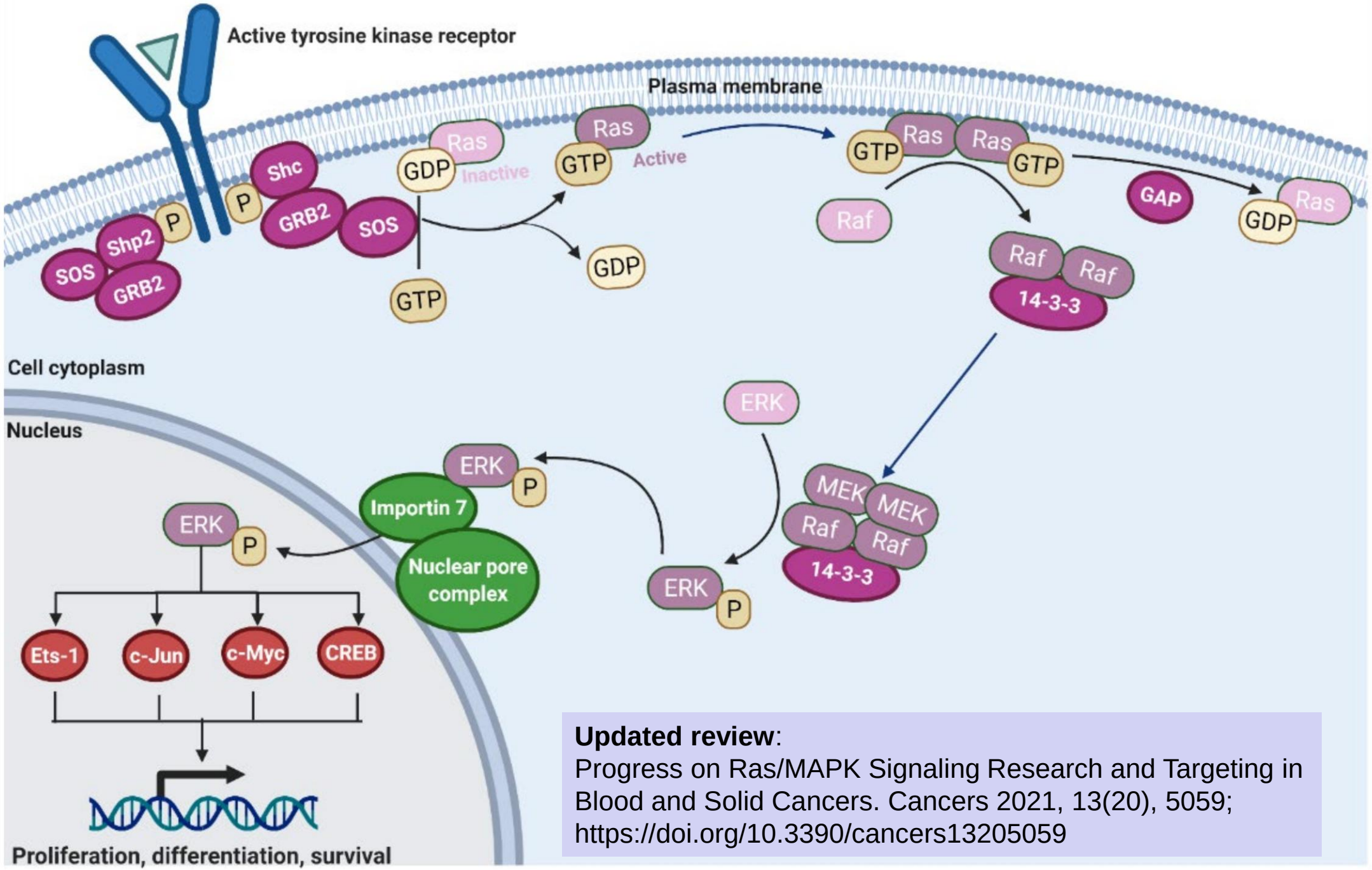


anti-phospho ERK

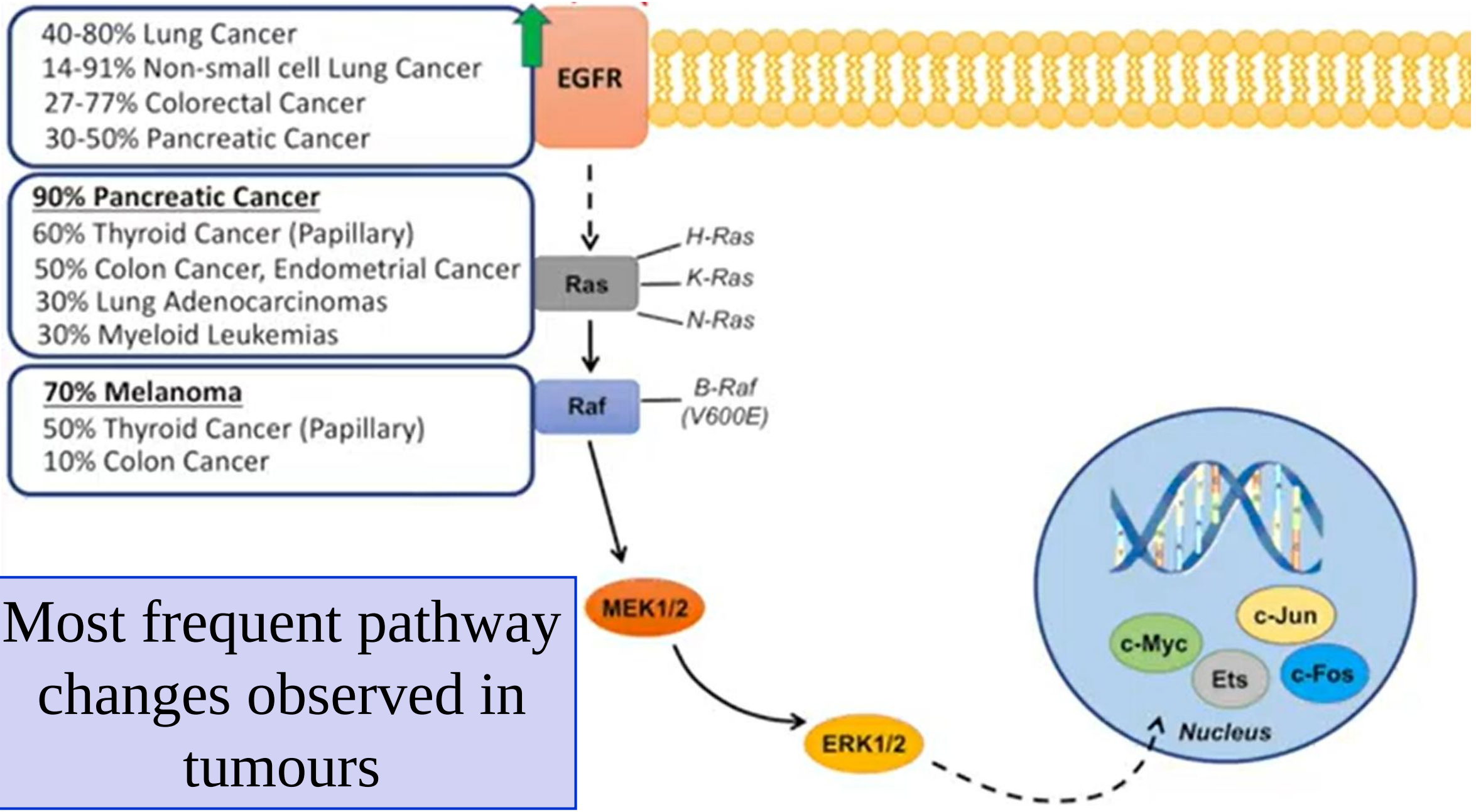




anti-total ERK



Updated review:
Progress on Ras/MAPK Signaling Research and Targeting in Blood and Solid Cancers. Cancers 2021, 13(20), 5059; https://doi.org/10.3390/cancers13205059

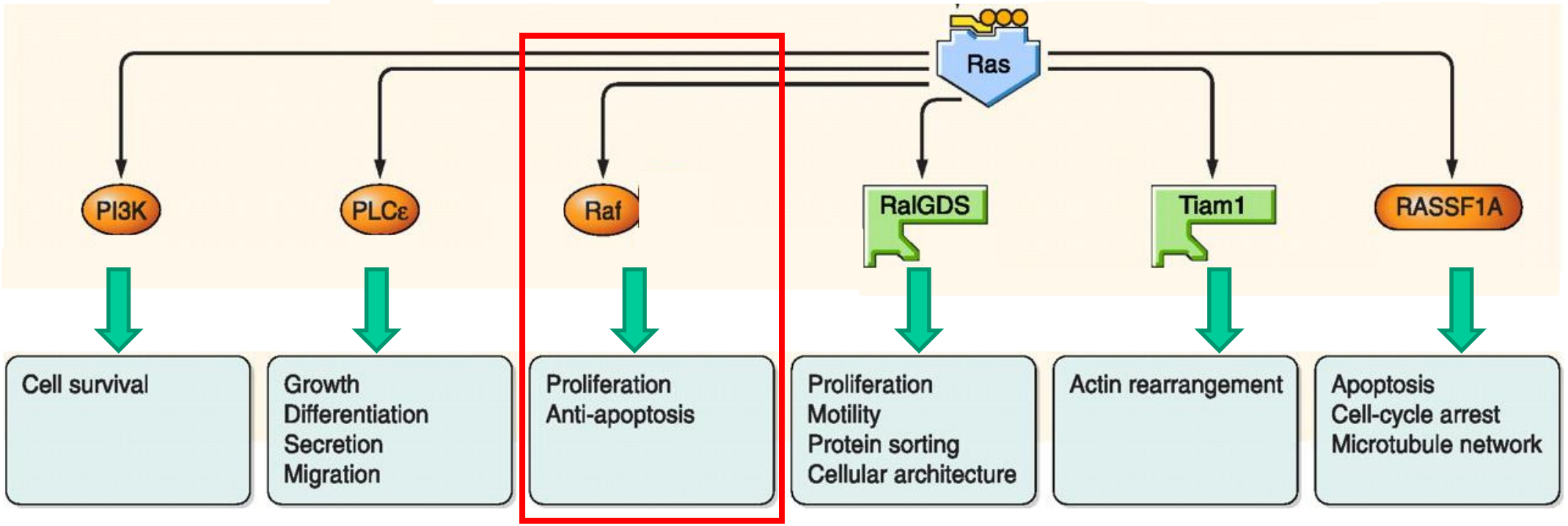


Most frequent pathway changes observed in tumours

9-min vídeo; https://www.youtube.com/watch?v=NlFDea3ig7A

Beyond the MAPK pathway..

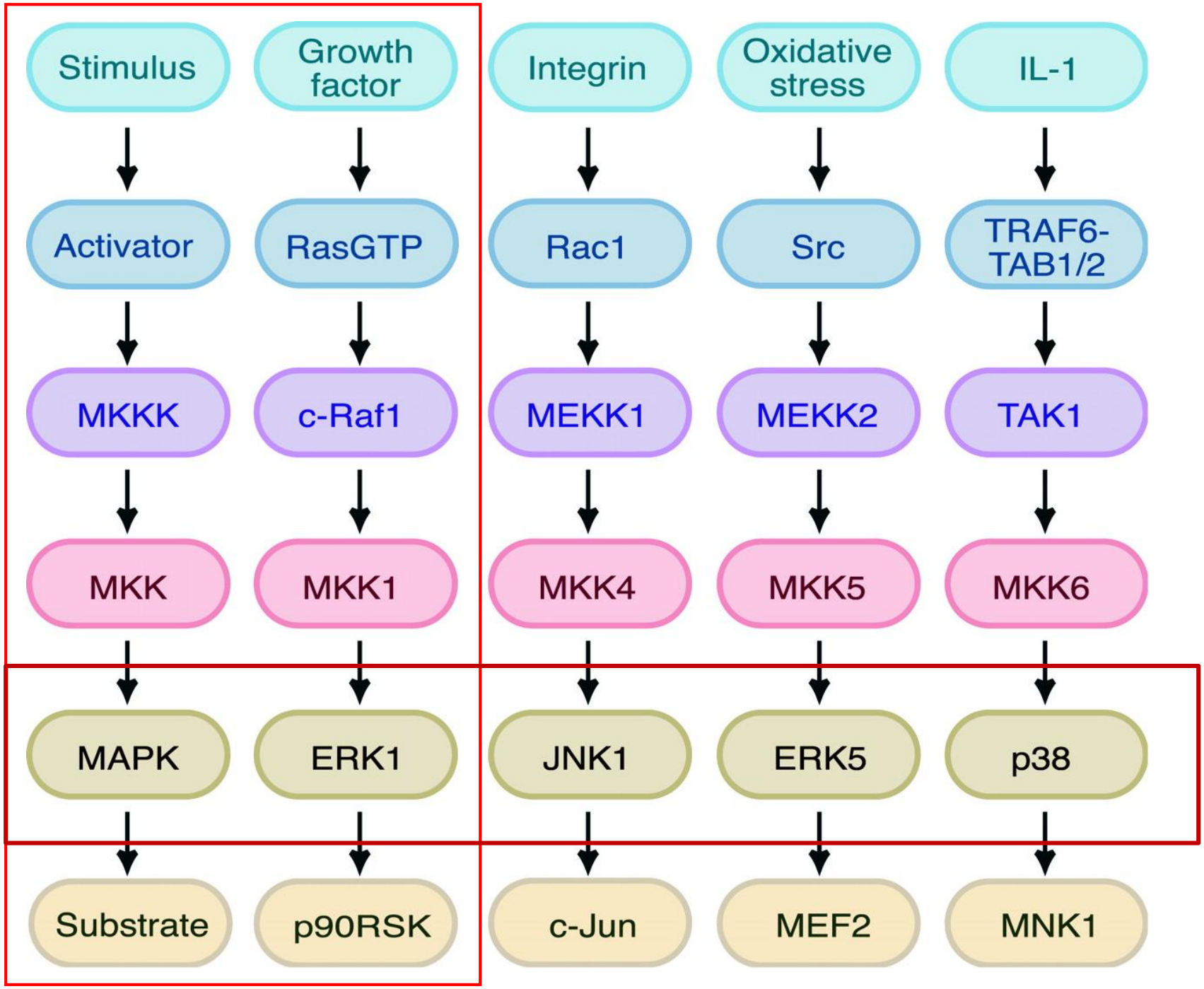
Besides Raf, other RAS effectors exist that activate other pathways



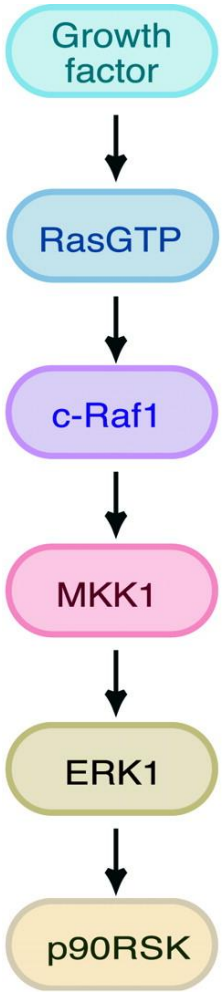
Erzsébet Ligeti et al. Physiol Rev 2012;92:237-272

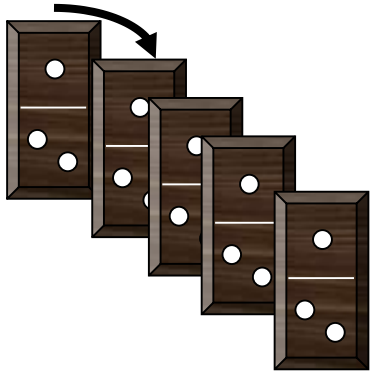
…shows that RAS operates as a signalling hub;

…explains why mutant RAS is such a potent oncogene



Four MAP kinase cascades exist

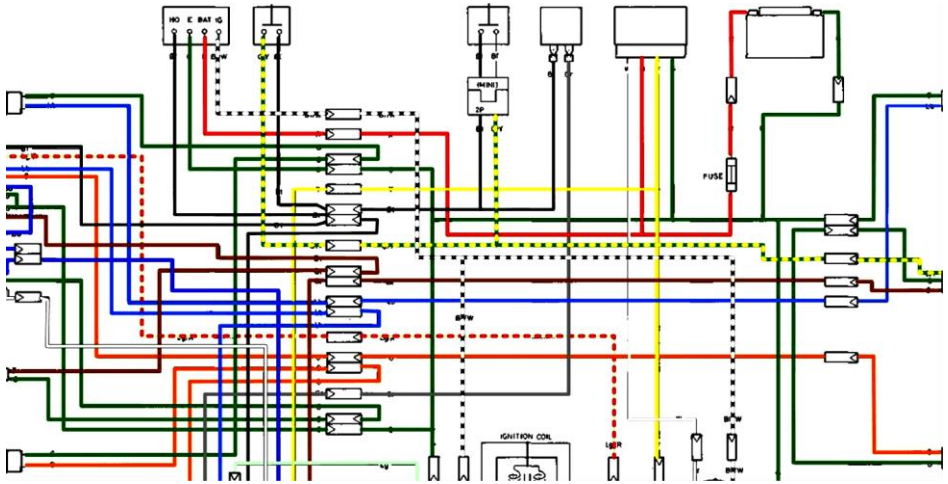




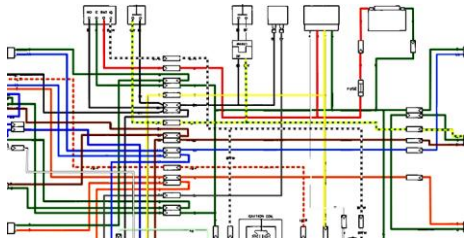
Simplified ‘domino’ model

versus

Electric circuit model



Signalling dynamics through ***Cross-talk*** – integrating complex signals



Growth factor=
Signal 1

Cell adhesion=
Signal 2

Ras

Rac

Raf

PAK

MEKK1

MEKK3

S218
S222
S298

MEK1/2

MKK4 / MKK7

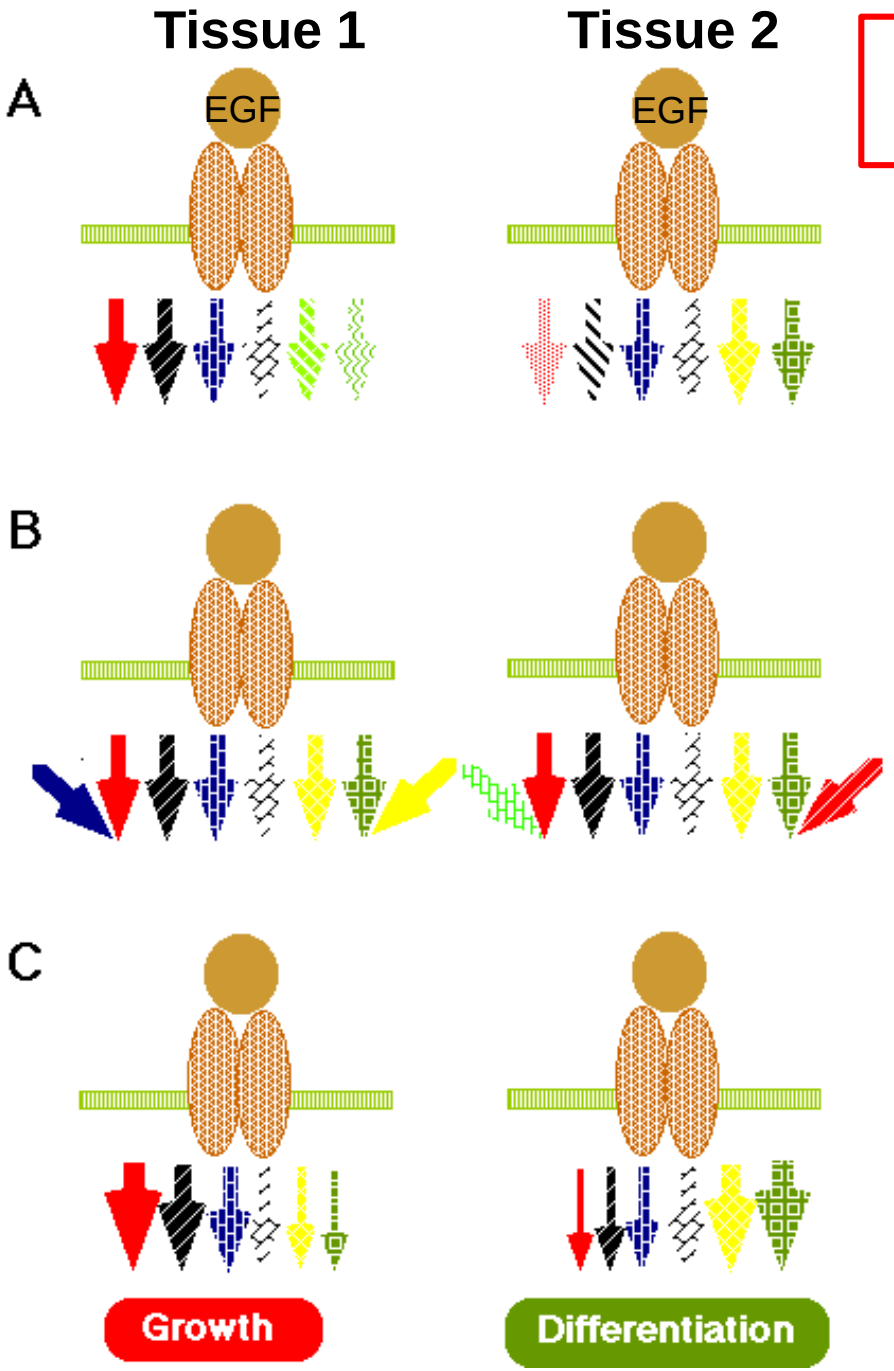
Increased
affinity

ERK

JNK

Ets/Tcf

AP-1(c-Jun)



The same signal (eg EGF) can have different outcomes in different tissues

Cells of different tissues may differ:

A) in the downstream signalling molecules that are expressed;

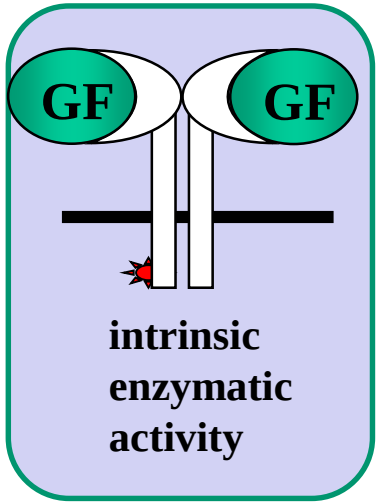
B) in the inhibitory or synergistic ***cross-talk*** from other receptors

C) in the amplitude of pathway activation

➡ **Tumors from different tissue types have different signalling networks**

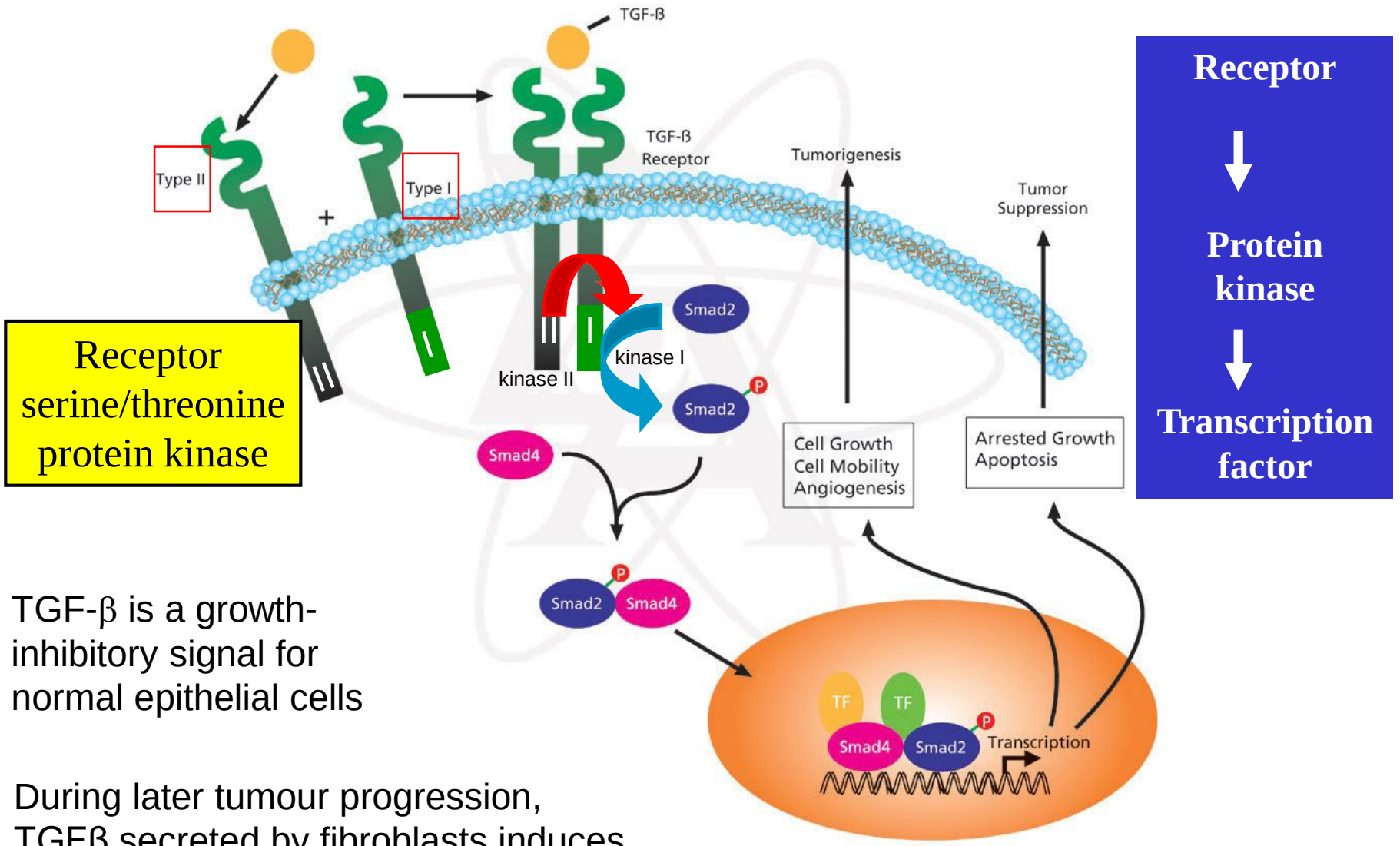
Examples of
other receptor types
and
other signalling pathways

…to recognize similar logic and principles



Signalling Pathway of TGF-β

SIGMA-ALDRICH
modified



Receptor serine/threonine protein kinase

Receptor

↓

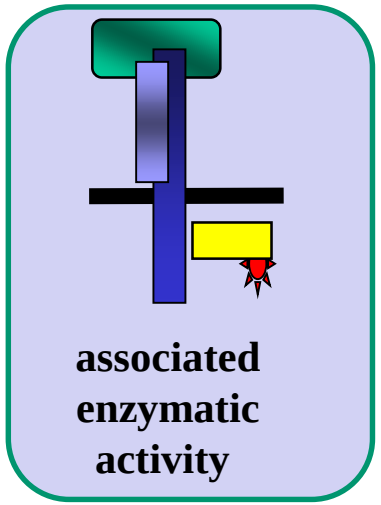
Protein kinase

↓

Transcription factor

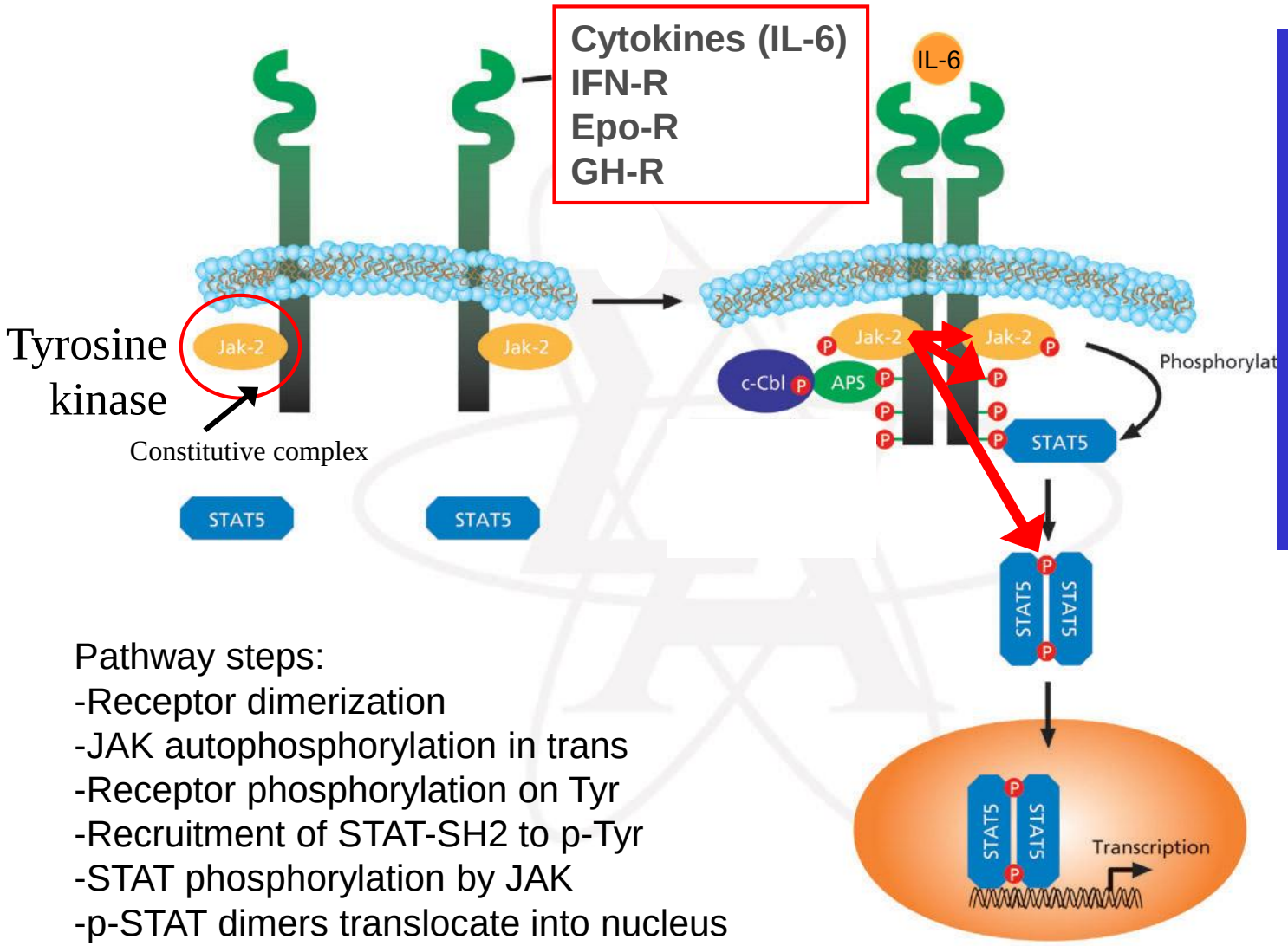
TGF-β is a growth-inhibitory signal for normal epithelial cells

During later tumour progression, TGFβ secreted by fibroblasts induces an invasive tumour cell phenotype



The JAK/STAT Signalling Pathway

SIGMA-ALDRICH
modified



Receptor

↓

Protein kinase

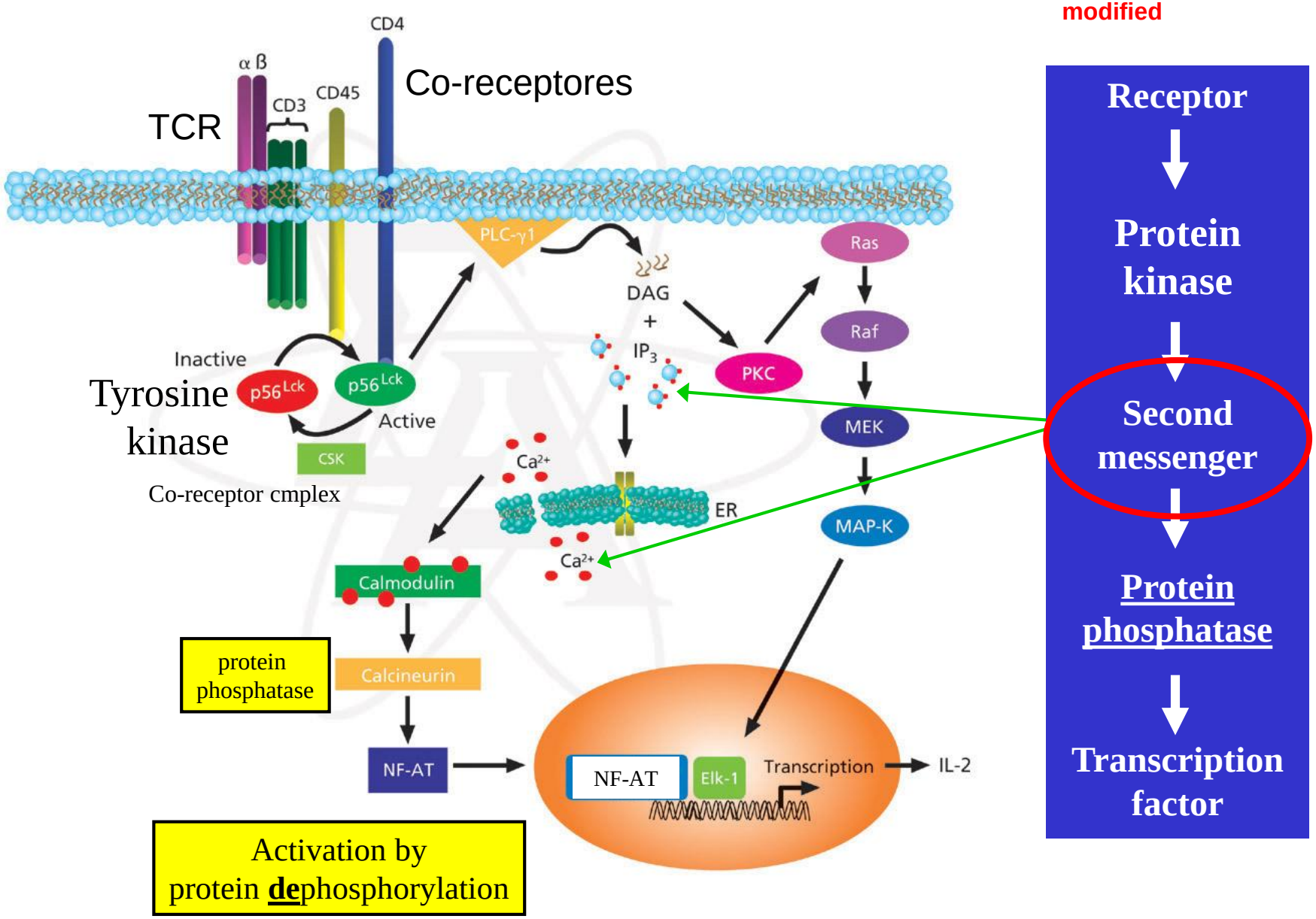
↓

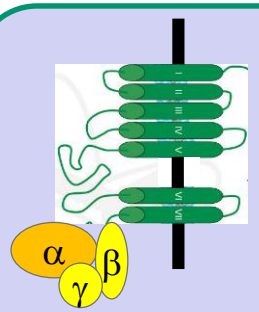
Transcription factor

Pathway steps:
-Receptor dimerization
-JAK autophosphorylation in trans
-Receptor phosphorylation on Tyr
-Recruitment of STAT-SH2 to p-Tyr
-STAT phosphorylation by JAK
-p-STAT dimers translocate into nucleus

T-Cell Receptor (TCR) Signalling

SIGMA-ALDRICH
modified





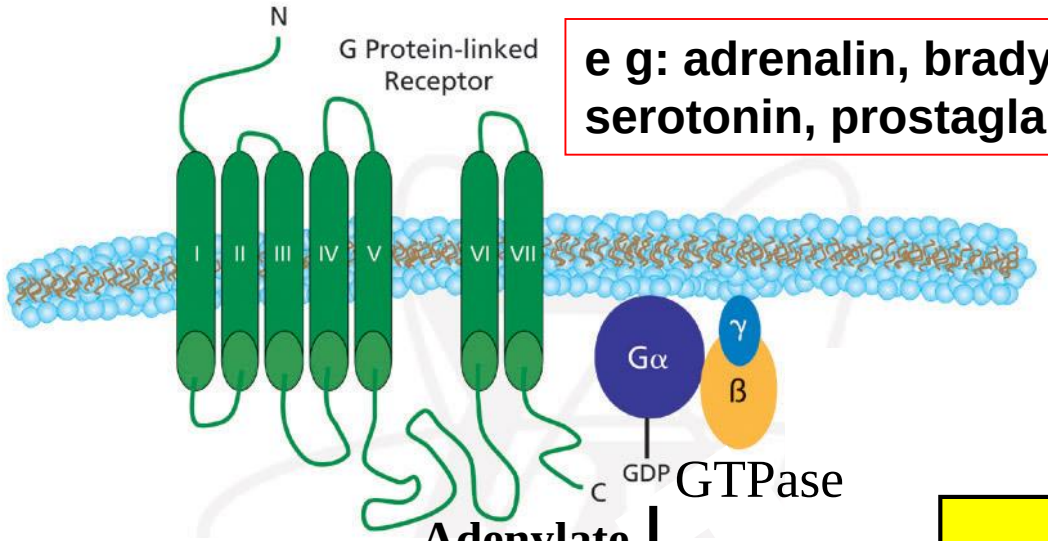
G-protein coupled receptor

G protein-coupled receptors and cyclic AMP

SIGMA-ALDRICH

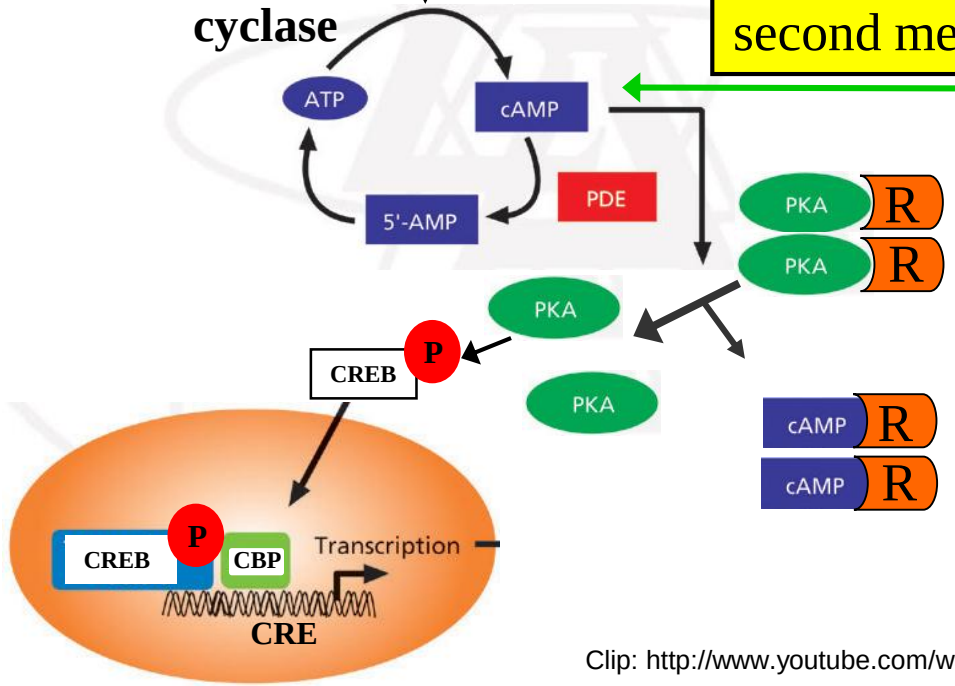
modified

e g: adrenalin, bradykinin, serotonin, prostaglandins, chemokines



Adenylate cyclase

Soluble second messenger



Receptor

↓

GTPase

↓

Second messenger

↓

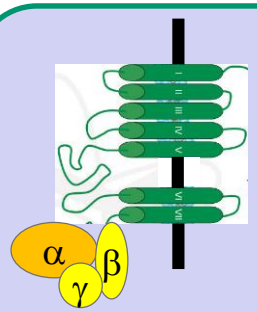
Protein Kinase

↓

Transcription factor

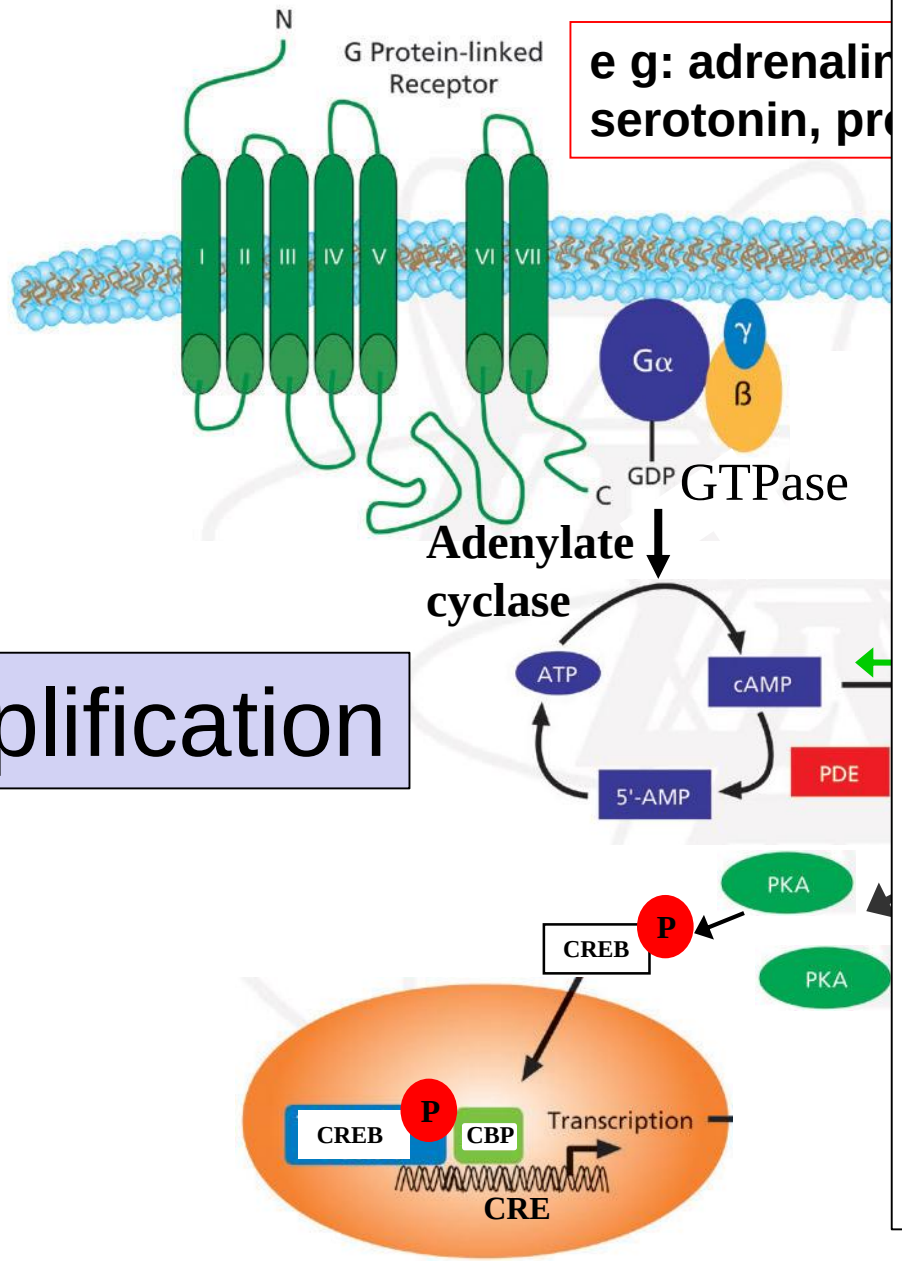
Clip: http://www.youtube.com/watch?v=ZF2_ItzzVbs&feature=related

G protein-coupled receptors and cyclic AMP



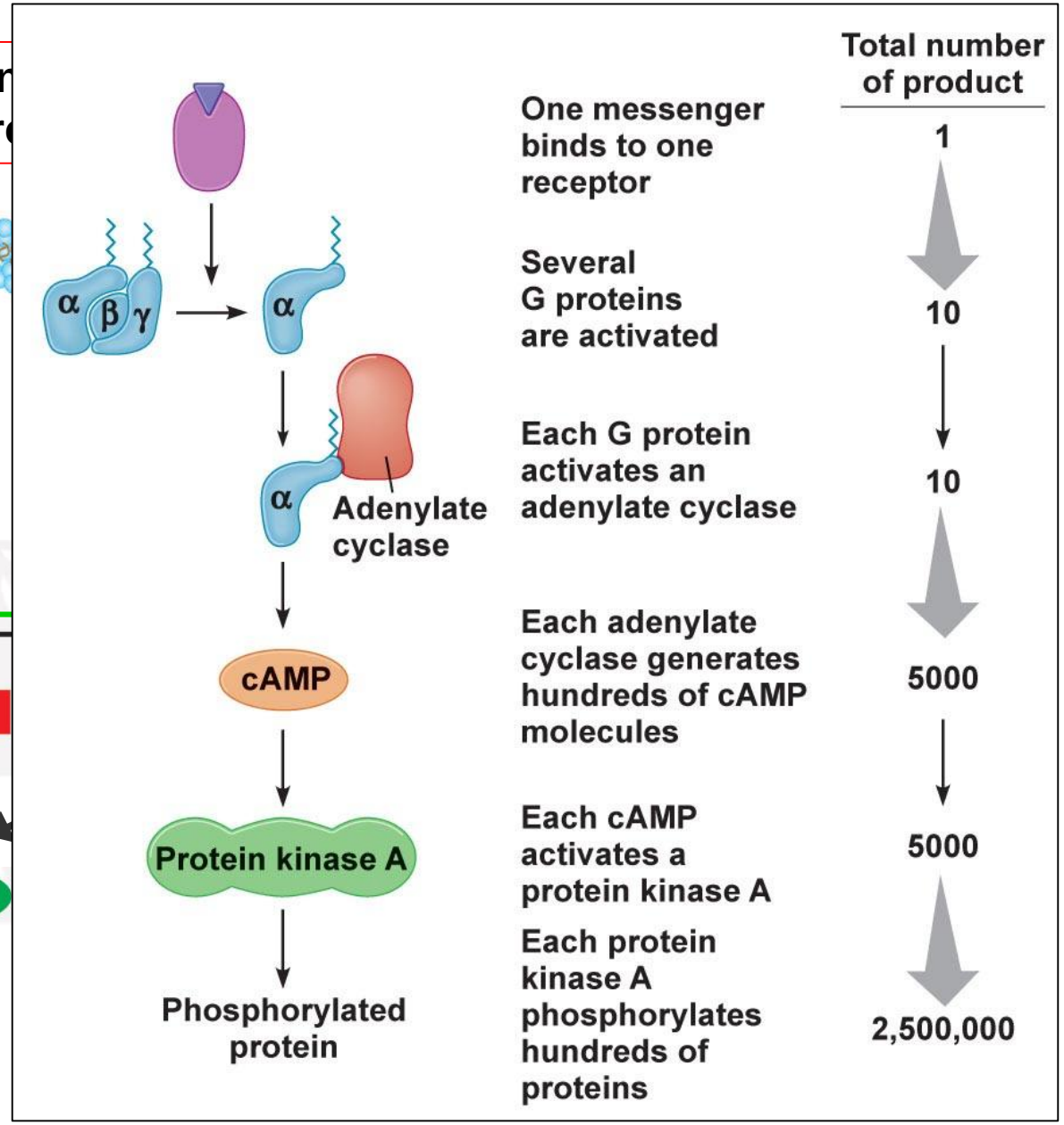
G-protein coupled receptor

signal amplification

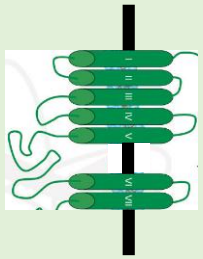


e g: adrenalin
serotonin, pr

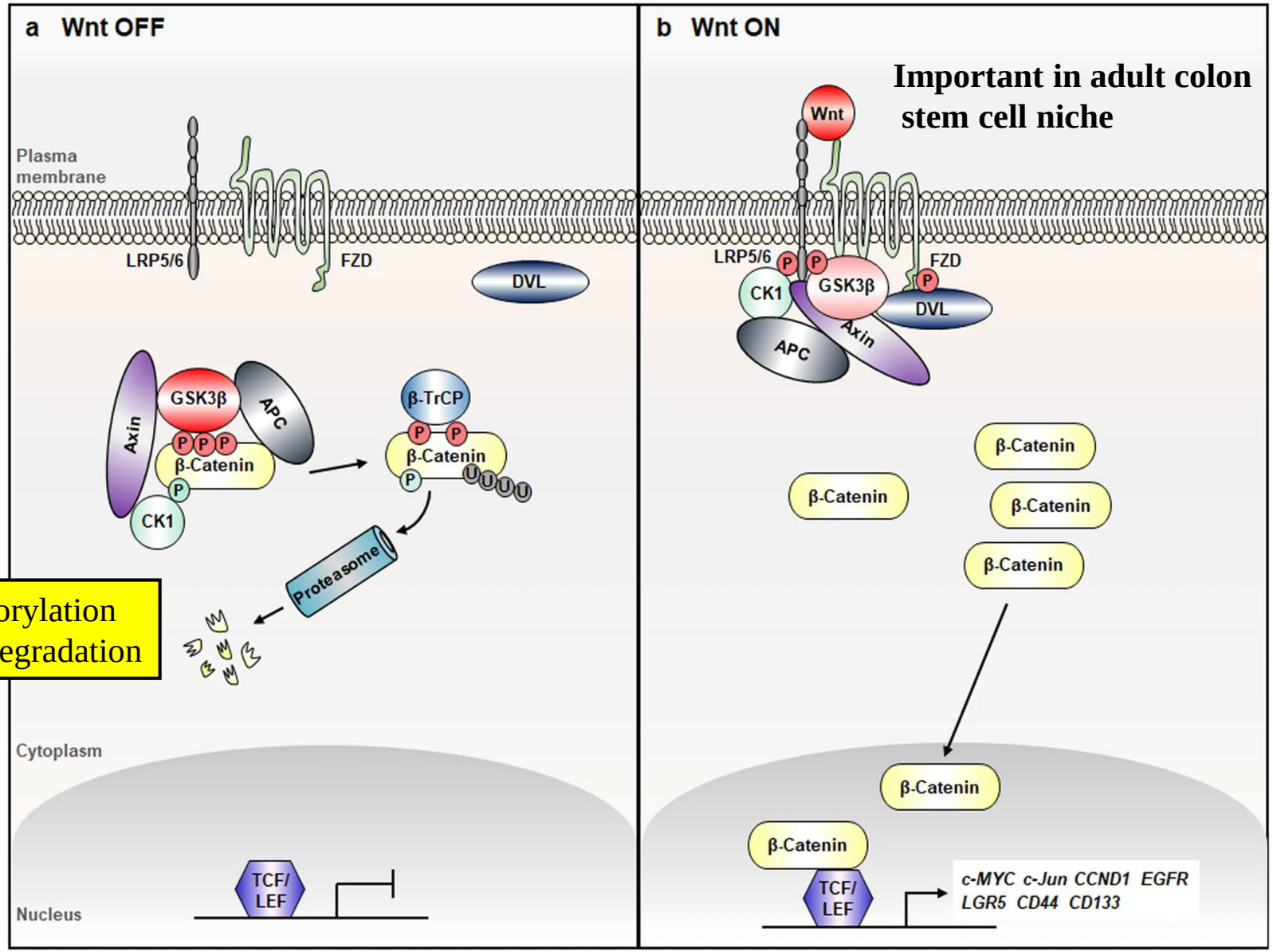
SIGMA-ALDRICH



Wnt pathway controls the transcriptional cofactor β-catenin



Atypical G-protein coupled receptor



Default phosphorylation and proteolytic degradation

Receptor

↓

Protein kinase inhibition

↓

Transcription factor stabilization

Lecture 3- Some take-home concepts

- **Secreted growth factors are recognized at the plasma membrane by receptor tyrosine kinases**
- **Receptor activation leads to autophosphorylation at cytosolic tyrosines that recruit SH2 domain proteins**

- **Receptor-recruited factors promote activation of the small GTPase RAS**
- **GTPases cycle between inactive GDP-bound and active GTP-bound conformation**
- **Activation of MAP kinase cascade stimulates proliferation-inducing transcription factors**

- **Signalling proteins undergo cycles of activation and inactivation**
- **Other receptor types may use different cytosolic effector proteins but follow a common logic**
- ***Cross-talk* is the molecular integration of signals from different receptors by modulating pathway activity**