ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Medicine and Surgery

A.Y. 2025/2026 - Programme Code: 6734

84284-Signaling pathways in health and disease I.C.

Module A – 84285 **Cell signaling** 4 CFU, 34 hrs – BIOS-07/A

+ asynchronous online activities (16 hrs)

Lecture A.05
Signaling mechanisms regulated by receptor tyrosine kinases – part 1
(insulin/EGF & the MAPK cascade)

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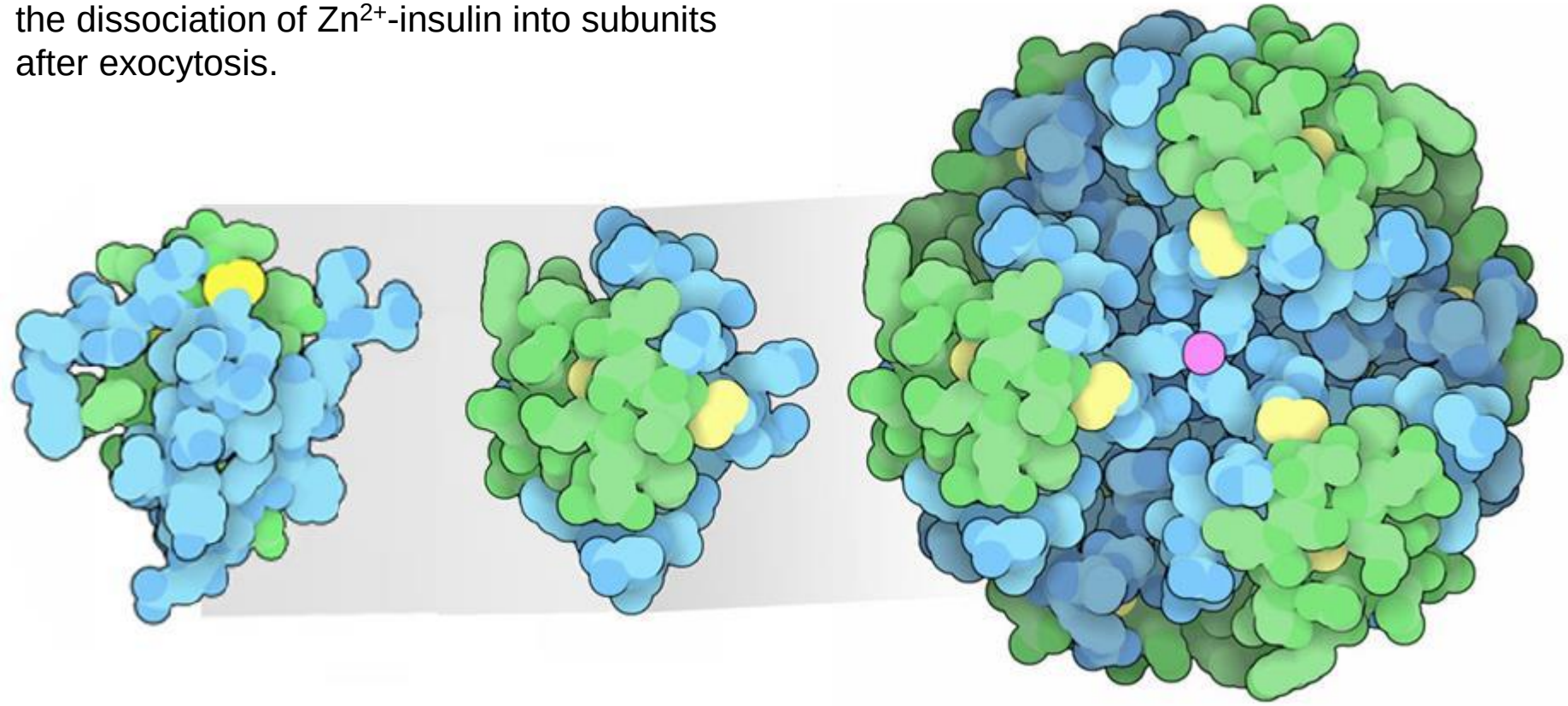
INSULIN

(quaternary structure)

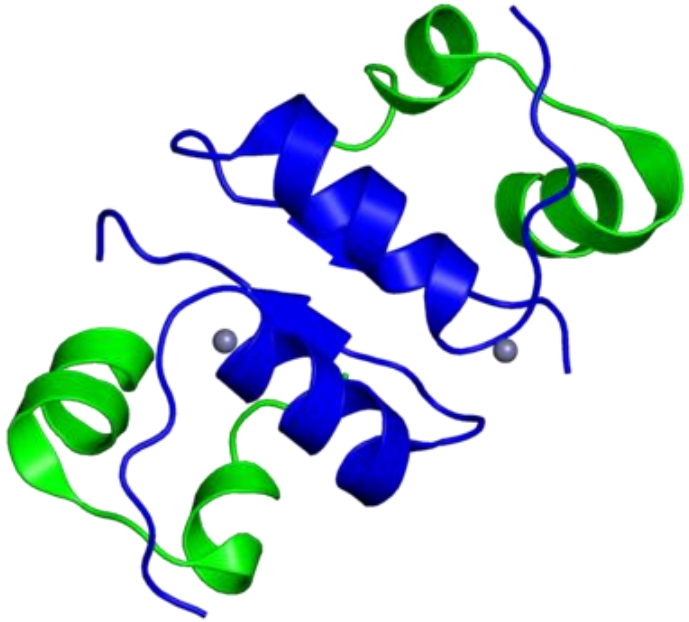
β-cells release hexameric Zn^{2+}-insulin into the extracellular space, but monomeric Zn^{2+}-free insulin appears to be the only biologically active form. The mechanisms implicated in dissociation of the hexamer remain unclear, but they seem to be Zn^{2+} concentration-dependent.

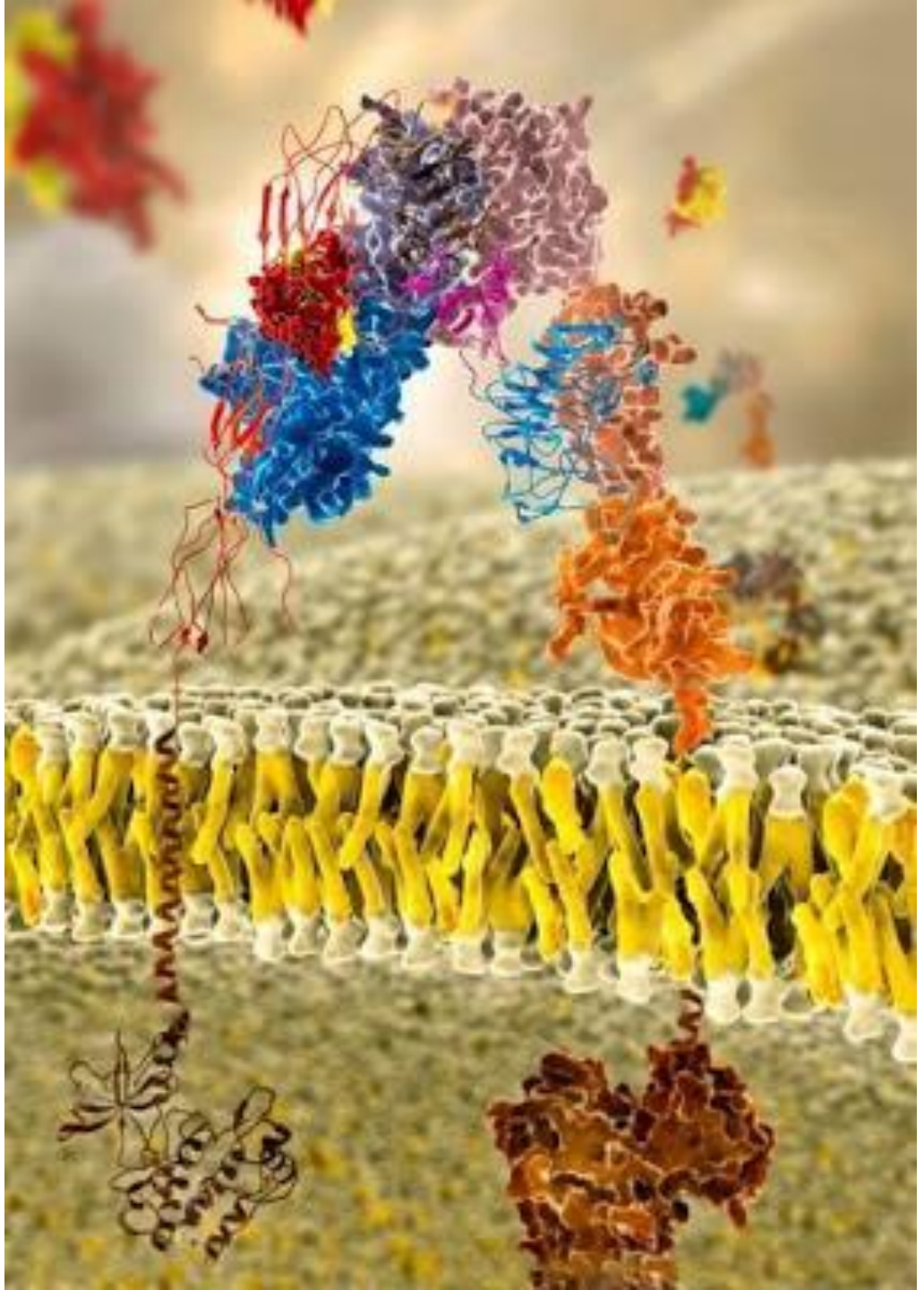
The Zn^{2+}-binding properties of albumin improve the dissociation of Zn^{2+}-insulin into subunits after exocytosis.



monomer **→ dimer** **→ hexamer**

(secretory vesicles)



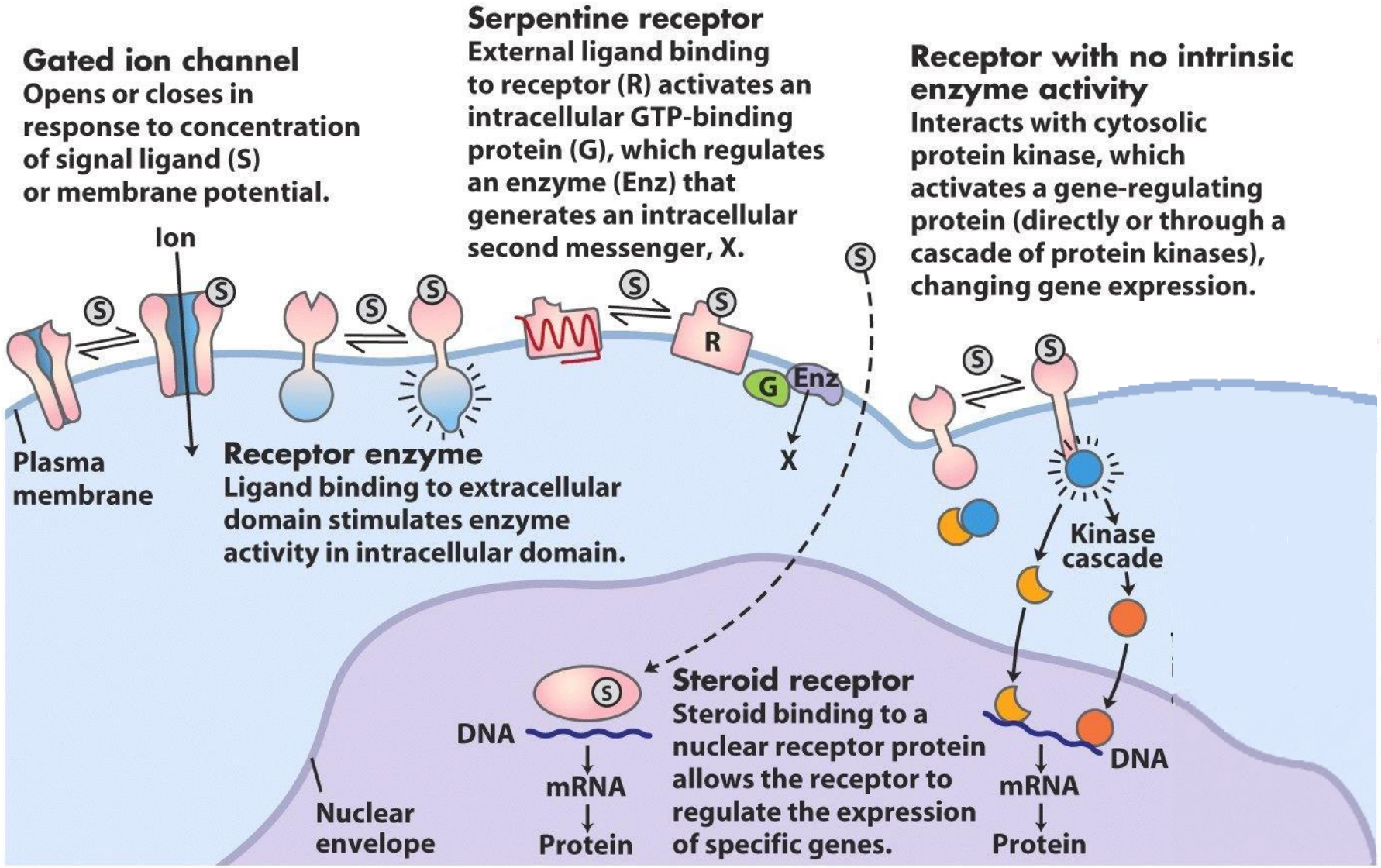


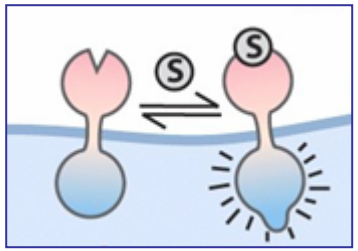
Dimer dissociation (loss of the interfacial β sheet and solvation of the hydrophobic core) is a prerequisite for insulin to bind to its cellular receptor.

The insulin receptor is a large protein that binds to insulin and carries its message inside the cell.

https://pdb101.rcsb.org/motm/182

RECEPTORS

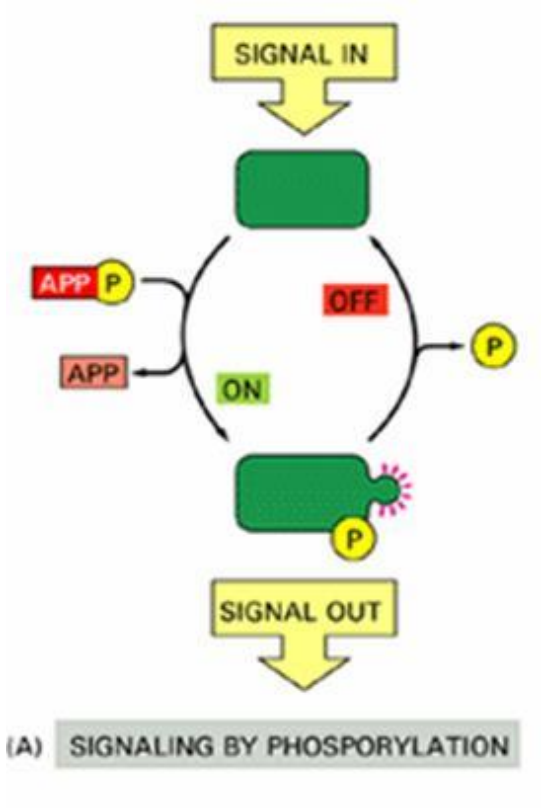

Gated ion channel
Opens or closes in response to concentration of signal ligand (S) or membrane potential.
Ion
Plasma membrane
Receptor enzyme
Ligand binding to extracellular domain stimulates enzyme activity in intracellular domain.
Serpentine receptor
External ligand binding to receptor (R) activates an intracellular GTP-binding protein (G), which regulates an enzyme (Enz) that generates an intracellular second messenger, X.
R
G
Enz
X
Receptor with no intrinsic enzyme activity
Interacts with cytosolic protein kinase, which activates a gene-regulating protein (directly or through a cascade of protein kinases), changing gene expression.
Kinase cascade
DNA
mRNA
Protein
Steroid receptor
Steroid binding to a nuclear receptor protein allows the receptor to regulate the expression of specific genes.
DNA
mRNA
Protein
Nuclear envelope



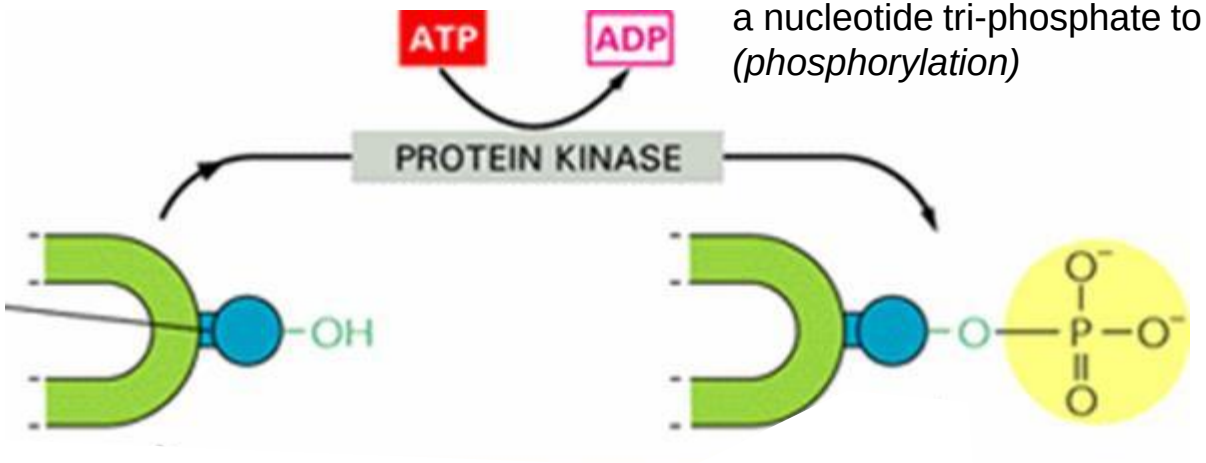
A family of plasma membrane receptors with intrinsic enzyme activity:

- receptor tyrosine kinases (RTK)
- receptor guanylyl cyclases

Tyr kinase activity:
enzymatic phosphorylation of Tyrosine residues (-OH)
by ATP

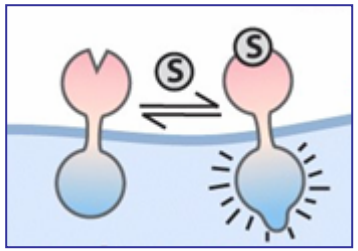


KINASE: enzyme that transfers a phosphoryl group from a nucleotide tri-phosphate to an acceptor molecule *(phosphorylation)*



Tyrosine side chain

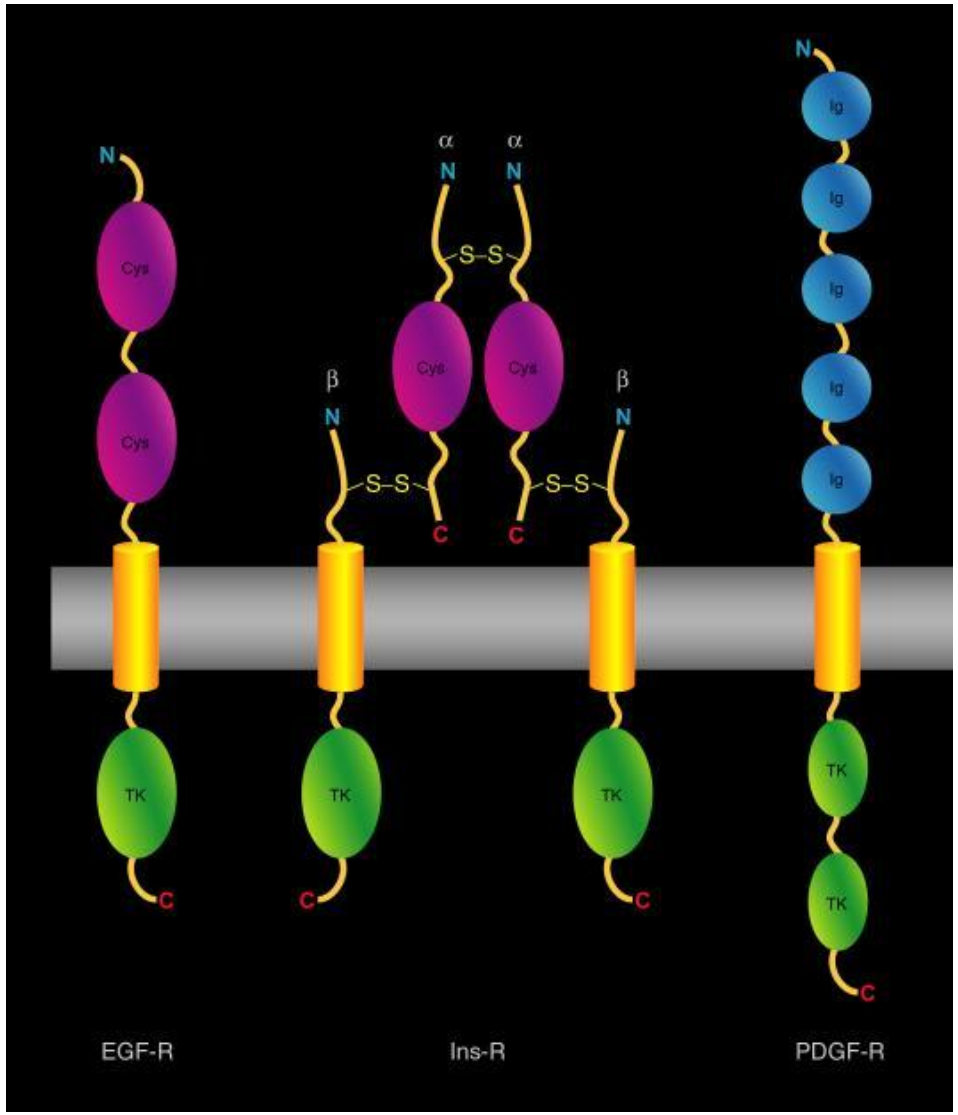
The phosphate group adds negative charges to the individual amino acid residue and to the whole polypeptide



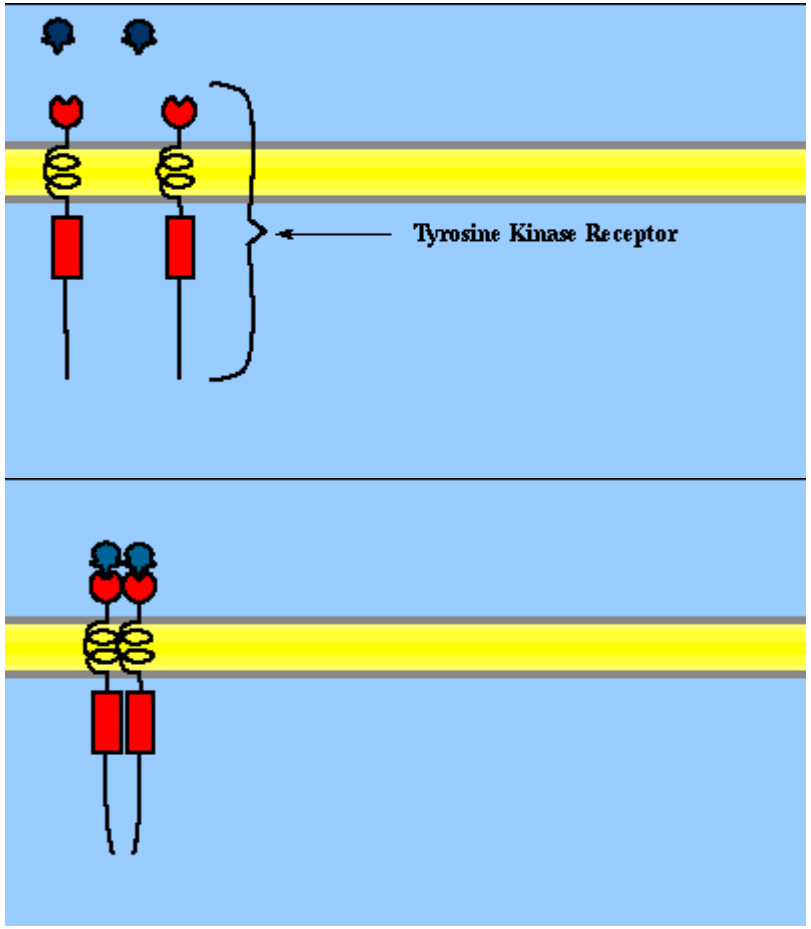
Receptor Tyrosine Kinases (RTK)

In general, RTKs are single **transmembrane** polypeptide chains that **cross the cell membrane once** (α-helix). The intracellular part of the receptor contains a **tyrosine kinase domain, inactive in the absence of ligand**. This C-terminal segment is rich in Tyr (multiple copies).

EGF (epidermal growth factor)
Insulin
PDGF (platelet-derived growth factor)



What structural changes occur in the ectodomain upon hormone binding and how are these structural changes transmitted to the TK domain, temporally and spatially?



Signal transduction is mediated by receptor activation due to hormone-induced dimerization

In the presence of ligand(s) bound to the extracellular domain, the RTK becomes an **activated dimer**.

The kinase domains come in contact and are activated by **trans/auto-phosphorylation**, resulting in phosphorylation of specific Tyr residues in the intracellular part of the receptor next to the kinase domain

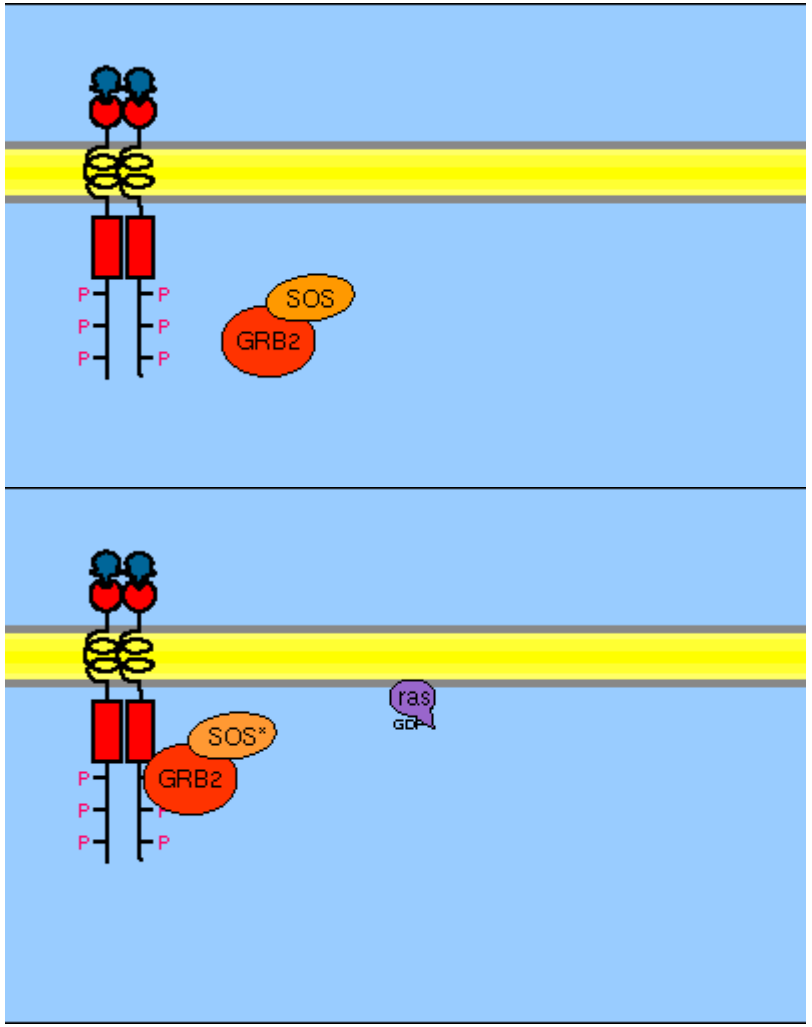
Covalent trans/auto-phosphorylation of specific Tyr residues in the intracellular part of the receptor allows signal activation even after hormone dissociation.

Signaling is disattivated by:

1) Dephosphorylation of the Tyr residues
(*PTP, phosphatase*)

2) Additional phosphorylation of intracellular residues of Ser and Thr
(by *cAMP-processes*)

Intracellular transmission



The phosphorylated residues become binding sites for signaling partner proteins (**transducers**) that contain SH2 (Src homology 2) domains; the transducers also become phosphorylated by the kinase or are activated by conformational changes, and start the intracellular signal transduction.

Grb2 is an adapter protein that exists in a complex with SOS, a guanyl nucleotide exchange factor that promotes GDP/GTP exchange on the small G protein **Ras**

GTP-Ras = active form

Types of docking proteins (transducers)

without catalytic activity		enzymes	
recruitment of other signal proteins	GRB2	PLC-γ	phospholipase
	Shc	c-Src	Tyr-protein kinase
		GAP	Ras-GTPase activator
		SH-PTP1 e PTP2	P-protein phosphatase
		PI3K	phosphoinositide kinase

Growth-factor-receptor-binding protein 2 **(GRB2)**

Src Homologous and Collagen Protein **(Shc)**

Son of sevenless **(Sos)**

Cellular SARC **(c-Src)**

Sarcoplasmic calcium-binding protein **(SARC)**

GTPase activating protein **(GAP)**

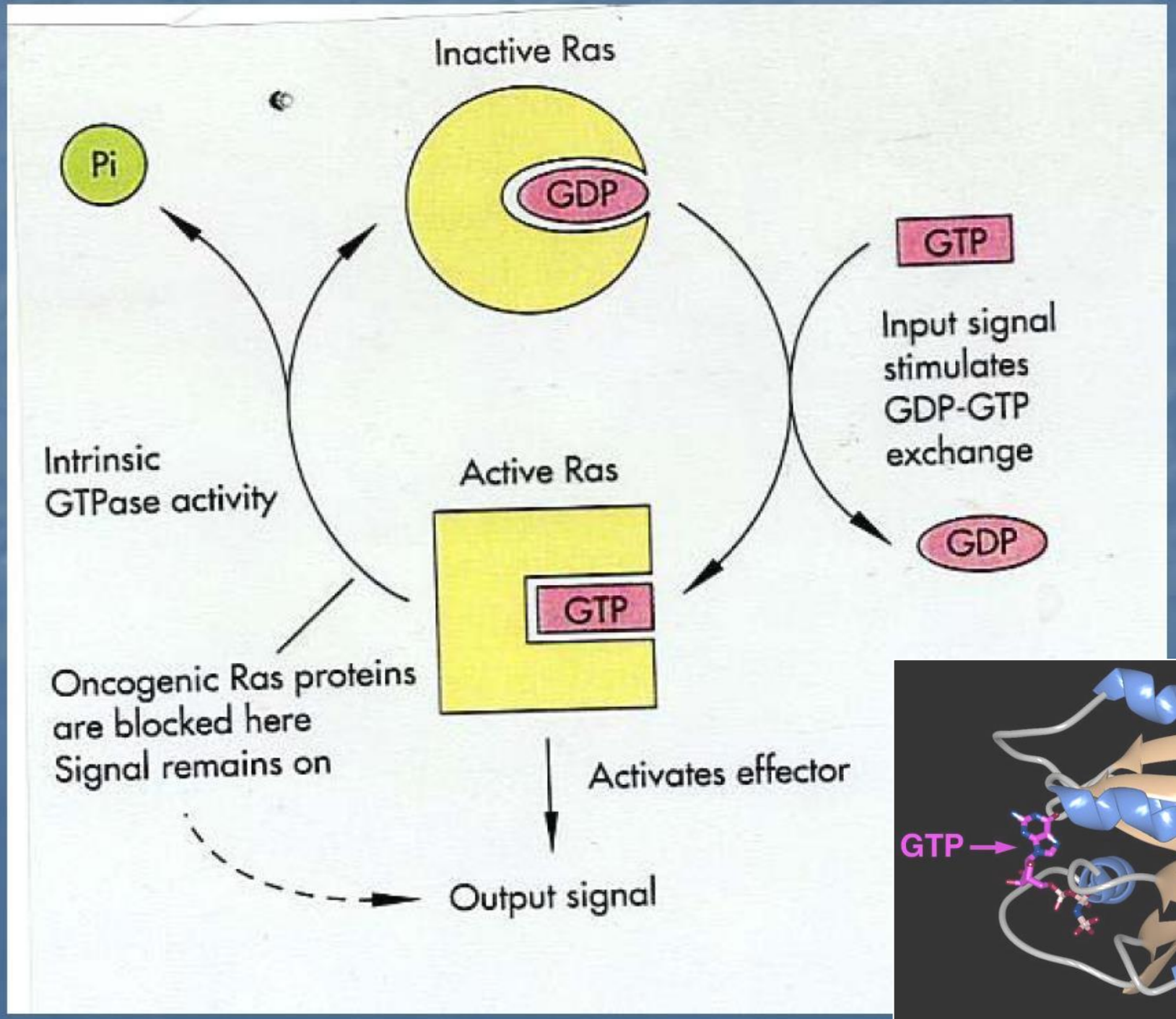
Rat Sarcoma Protein **(Ras)**

Serum Response Factor **(SRF)**

Kinase Suppressor of Ras **(KSR)**

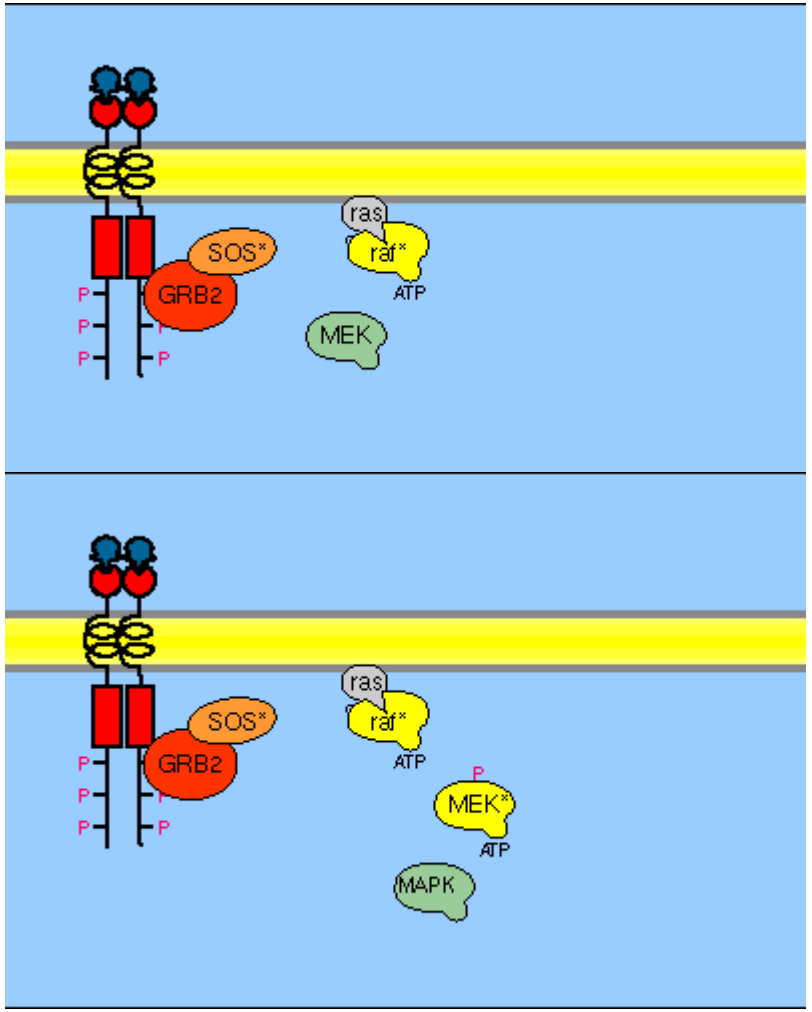
"while Kinase Suppressor of Ras may be its name,
phosphorylation may not be its game"

(inactivating mutations in KSR suppress
the phenotypic effects induced by activated Ras)


Inactive Ras
Pi
GDP
GTP
Input signal
stimulates
GDP-GTP
exchange
Intrinsic
GTPase activity
Active Ras
GDP
GTP
Oncogenic Ras proteins
are blocked here
Signal remains on
Activates effector
Output signal

GTP

Intracellular transmission



MAPKKK

The membrane bound GTP-Ras can activate the cytosolic Ser/Thr protein kinase **Raf**

A CASCADE IN WHICH EACH KINASE ACTIVATES THE NEXT BY PHOSPHORYLATION

MAPKK

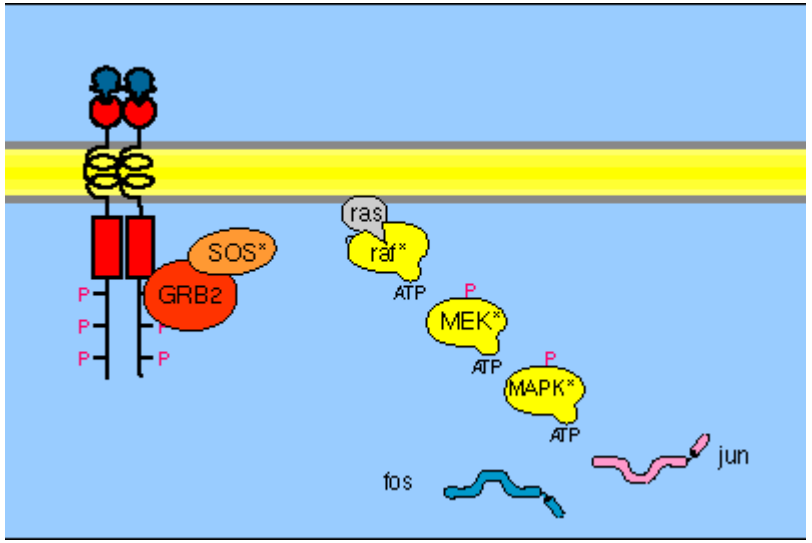
Raf activates **MEK** by phosphorylation

MAPK

P- MEK is also a Ser/Thr, Tyr protein kinase which activates the next: a protein kinase (e.g. ERK, *extracellular-signal-regulated kinase*) of the **MAPK protein family** = Mitogen Activated Protein Kinases

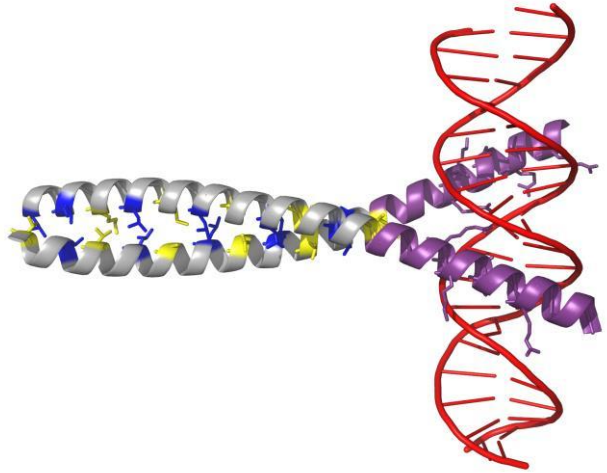
MAPK CASCADE

Intracellular transmission

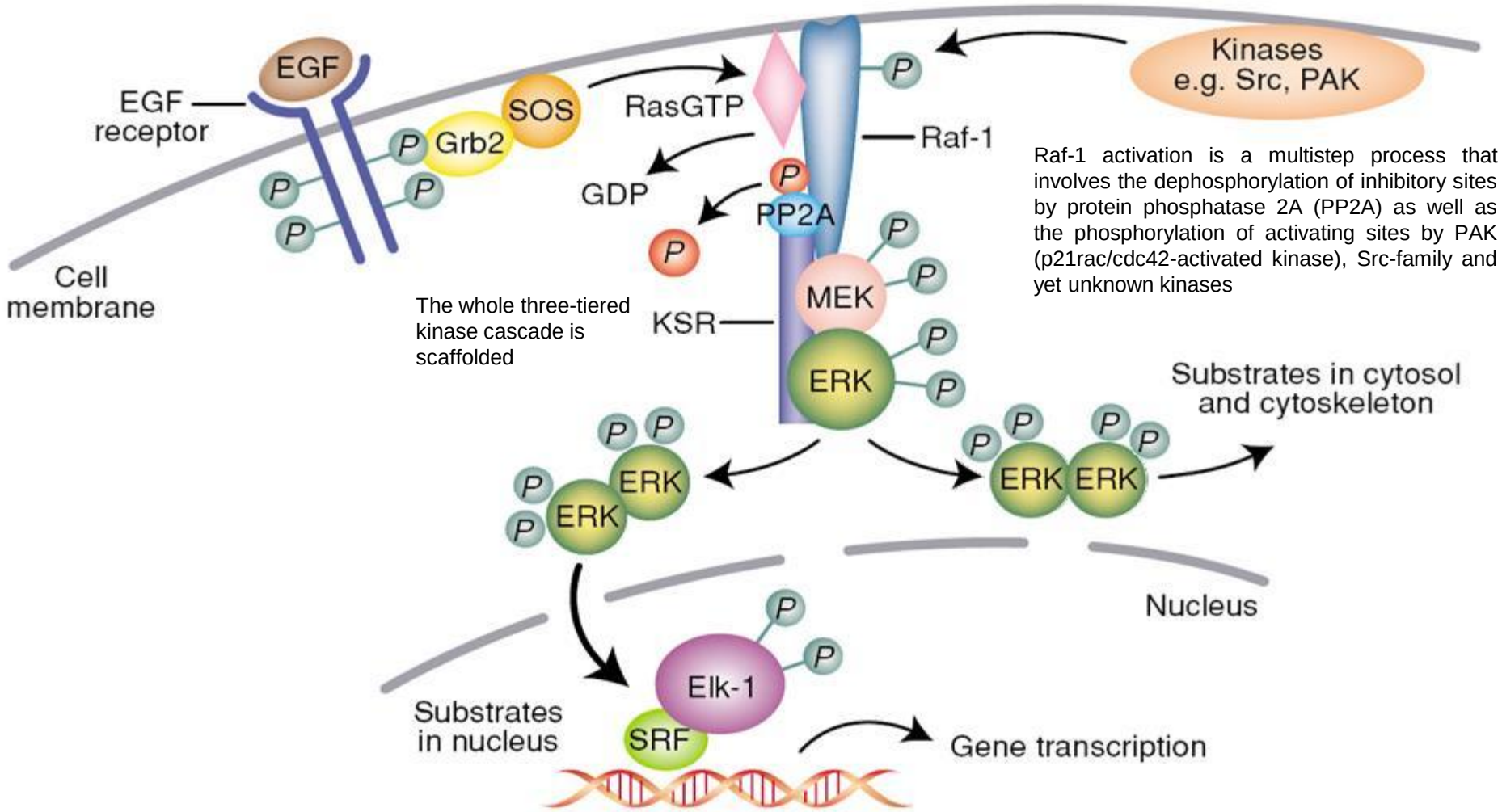


P-MAPKs like ERK **move into the nucleus and phosphorylate several *nuclear transcription factors*** (e.g. Fos, Jun, Myc, Elk1), activating them

Stimulate the transcription and translation of a set of genes (needed for cell division)



Crystal structure of c-Fos:c-Jun heterodimer and DNA complex
In the "Leucine zipper" domain (gray), the hydrophobic residues of c-Fos and the hydrophobic residues of c-Jun pack together at the interface of the coiled-coil (leucines are colored in blue, and the other hydrophobic residues are colored in yellow).
Residues from the "basic region" (purple) directly interact with the DNA (red).



Raf-1 activation is a multistep process that involves the dephosphorylation of inhibitory sites by protein phosphatase 2A (PP2A) as well as the phosphorylation of activating sites by PAK (p21rac/cdc42-activated kinase), Src-family and yet unknown kinases

The whole three-tiered kinase cascade is scaffolded

The organisation and function of the Ras-Raf-MEK-ERK pathway

My suggested ref. papers about KSR are:

1) Terrell and Morrison (2019)
Ras-Mediated Activation of the Raf Family Kinases
[soon available on Virtuale>]

Members of the KSR family are close relatives of the Raf kinases

However, the KSRs do not have an RBD (Ras-binding domain)

Although it was initially thought that the KSRs would function as typical protein kinases, the mammalian KSR proteins were found to lack a lysine residue that is normally required for the phosphotransferreaction , raising the question of whether the KSRs indeed possess intrinsic kinase activity—a question that is still debated.

Subsequently, reports emerged indicating that the KSR proteins may have scaffolding activities,

1 In quiescent cells, KSR is found in a multiprotein complex containing MEK, sequesters the inactive KSR complex in the cytosol

2a On signal activation, KSR rapidly translocates to the plasma membrane to increase the local pool of MEK, thus facilitating MEK activation.

Moreover, KSR proteins can also form side-to-side dimers with the Rafs it can function as an allosteric activator of Raf

2b Importantly, the KSR proteins also contain FxFP docking sites for activated ERK

Serine or Threonine-Proline core consensus for phosphorylation

facilitates the phosphorylation of KSR and the Rafs on S/TP sites these feedback sites disrupt the signaling complexes and promote the release of KSR and the Rafs from the cell surface

Thus, KSR can modulate the dynamics of Ras pathway signaling by both potentiating and attenuating Raf activity and signal transmission to MEK and ERK.

and

2) McKay et al. (2009)
Signaling dynamics of the KSR1 scaffold complex
[LINK]

KSR (Kinase Suppressor of Ras)

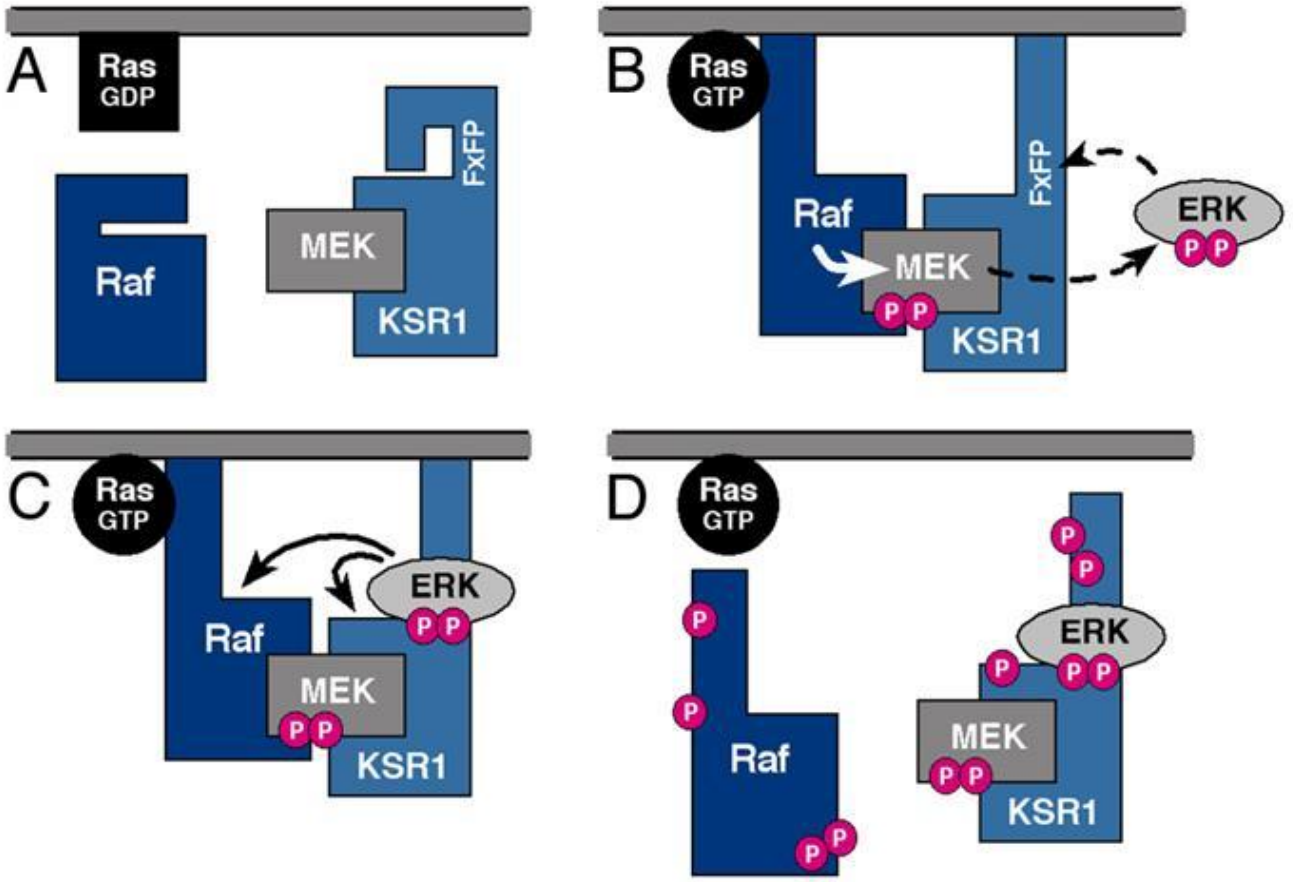


Fig. 5. KSR1 regulates the intensity and duration of ERK cascade activation. In quiescent cells, KSR1 prevents improper ERK cascade activation by sequestering MEK away from Raf (*A*). In signaling cells, KSR1 first potentiates signal transmission from Raf to MEK by facilitating the Raf/MEK interaction (*B*) and then attenuates signaling by docking activated ERK, thus facilitating the disruption of the KSR1 scaffold complex via feedback phosphorylation (*C* and *D*).

Phosphorylation reactions and the Ras activation process stimulated by RTKs on the inner surface of cell membranes are very rapid events

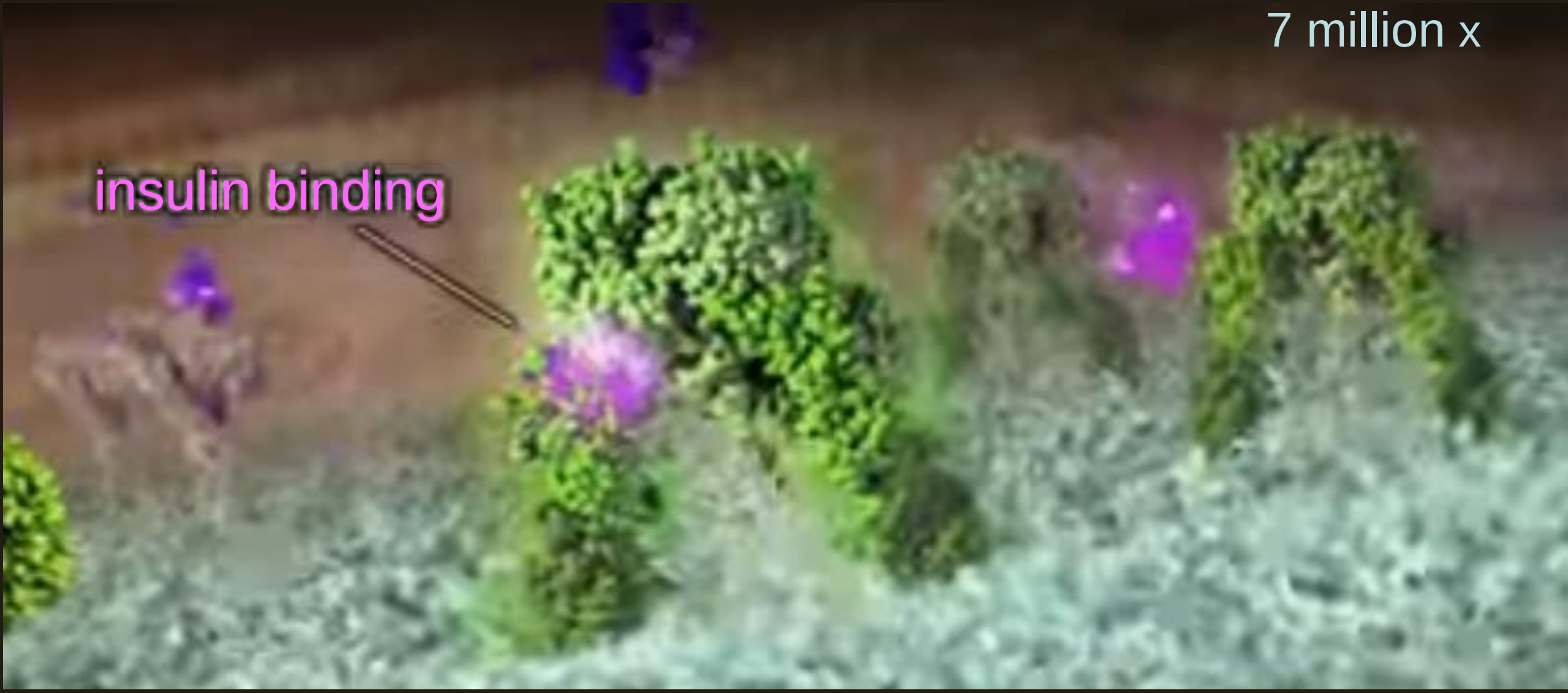
BECAUSE

specific tyrosine phosphatases and the intrinsic GTPase activity of Ras (further enhanced by GAP) re-establish the basic conditions.

Pathways emanating from Receptor Tyr-Kinases are involved in the regulation of gene expression and in the control of cell growth ("mitogenesis") and differentiation.

Insulin Receptor

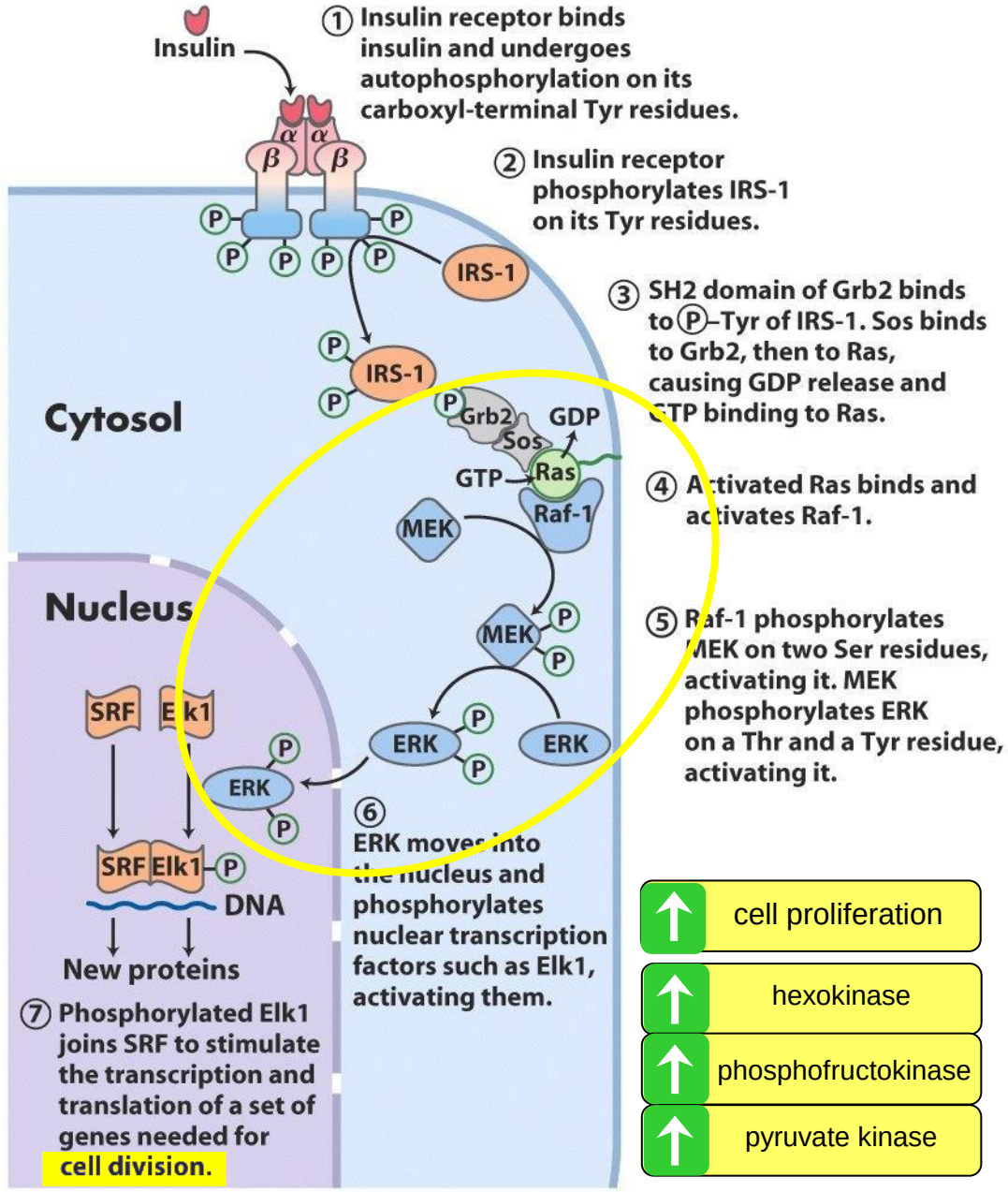
cell surface
7 million x



https://www.youtube.com/watch?v=VbwRYFMPZS4

Insulin Receptor (IR)

- Transmembrane glycoprotein of the RTK family
- Comprises 2 alpha- and 2 beta-subunits ($\alpha_2\beta_2$) intertwinned to form the insulin-binding site in the extra-cellular portion of the receptor
- X-ray crystallographic studies have identified that the disulfide-bonded ectodomain of IR has a **Λ-shaped** structure
- Insulin DOES NOT ENTER CELLS, but binds to the α–subunits and initiates a signal that travels a branched pathway* (to the nucleus and also to insulin-sensitive enzymes in the cytosol)



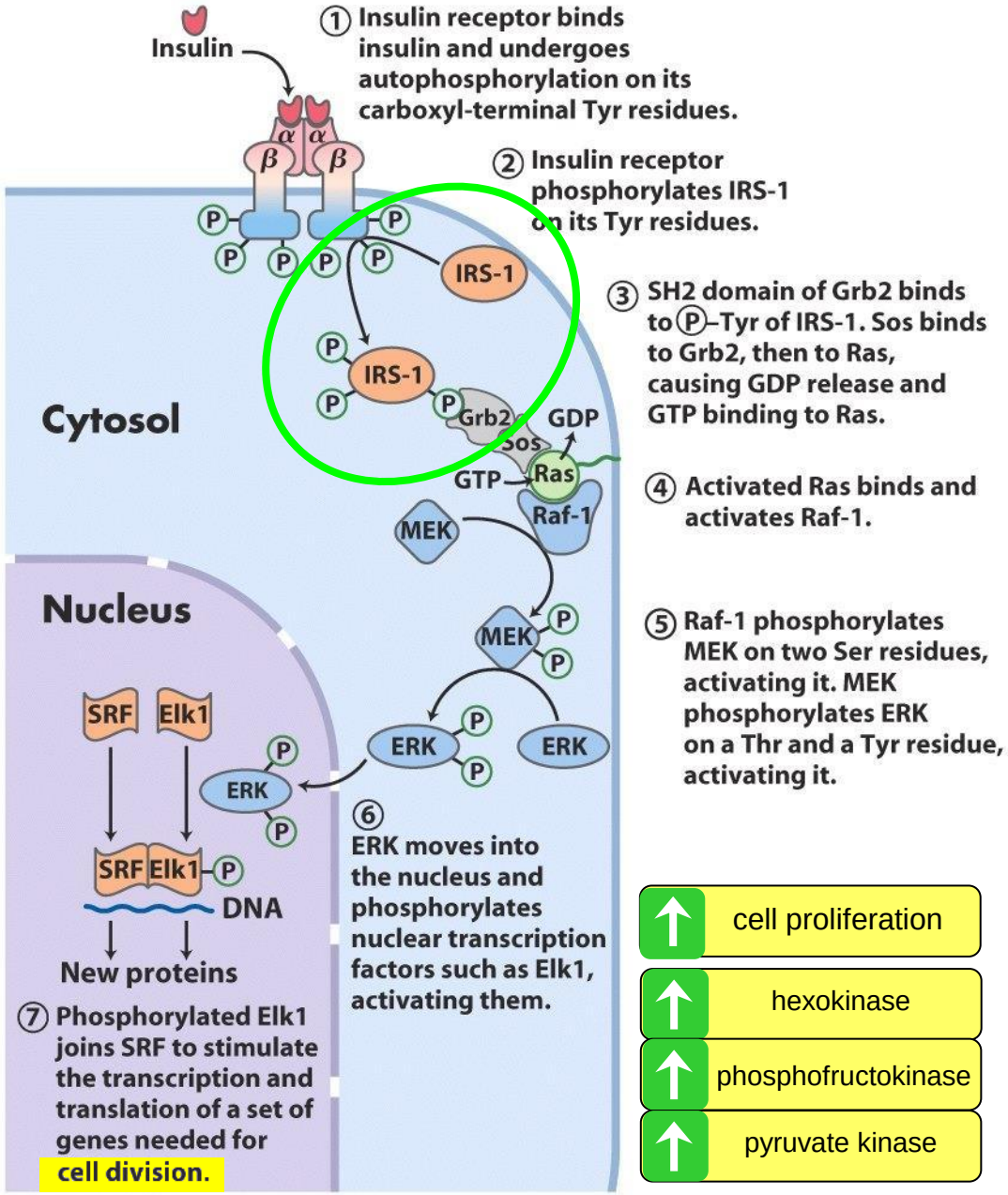
The three best studied nodes are:
- insulin receptor/IRS complex;
- PI3 kinase (PI3K);
- AKT/PKB.

Insulin Receptor (IR)

- trans-autophosphorylation of the receptor at multiple tyrosine residues (the two juxtaposed β-chains phosphorylate each other) is followed by **phosphorylation of intracellular IRS proteins** *(insulin receptor substrate)*
- Once phosphorylated on several of its Tyr residues, IRS-1 becomes the point of nucleation for a complex of proteins (GRB2-SOS-Ras-Raf; PI3K).

The insulin signaling machinery is central to cell growth and metabolism, since insulin affects mechanisms of gene transcription.

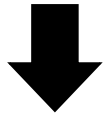
However, such transcriptional effects occur slowly and require several hours or days after initiation of insulin signaling.

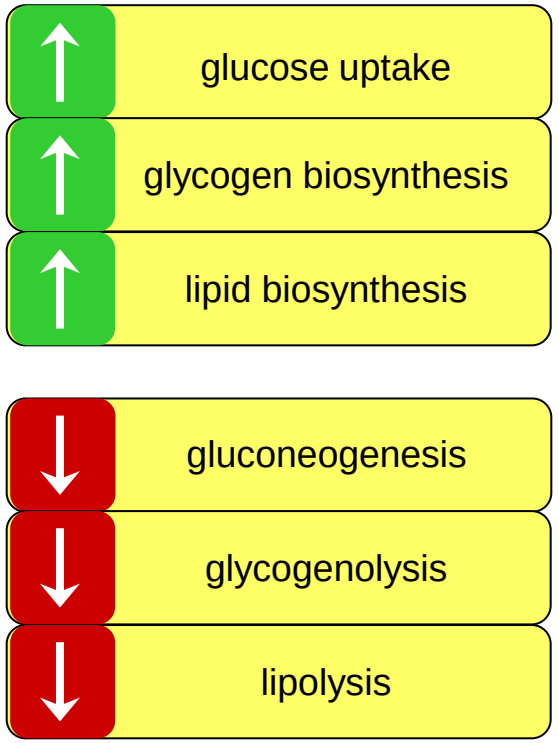


Biological effects of insulin

Immediate responses are observed since a few seconds/minutes after insulin has bound to its membrane receptor:

- increased glucose transport within adipocytes and skeletal muscle fibers;
- modification of the enzymatic activity and/or phosphorylation status of pre-existing proteins.





ACTIVATION OF ADDITIONAL SIGNALING PATHWAYS

Grb2 is not the only protein that associates with phosphorylated IRS-1