CONCEPT: INSULIN SIGNALING AS A GROWTH FACTOR

●Recall: In addition to regulating glucose metabolism, insulin can also act as a _____ *factor*.

□ Insulin growth factor RTK signaling triggers the following pathway: Grb2→ Sos → Ras → MAPK.

Insulin Growth Factor Signaling via Ras Pathway

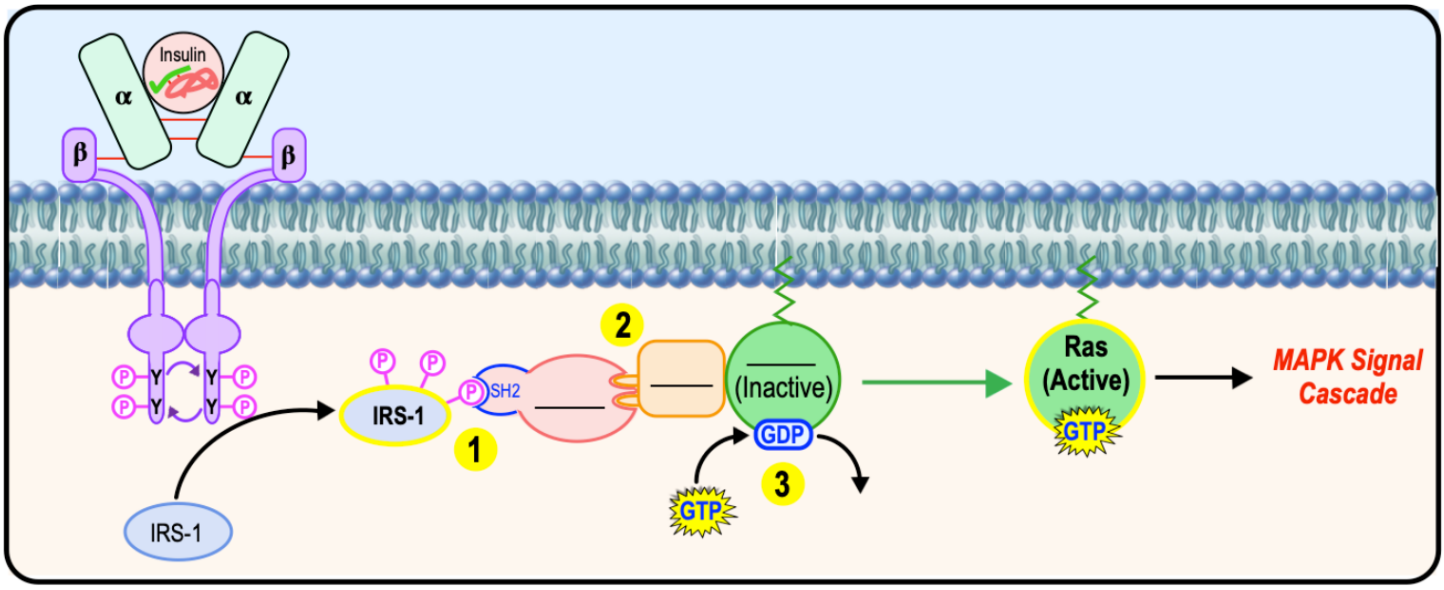
●Recall: Insulin results in active IRS-1, which acts as a _____ leading to many pathways (ex. *Ras Pathway*).

●_____: a lipid-linked monomeric G-protein existing in *two* forms: GDP-bound (*inactive*) & _____-bound (_____).

●A _____-step cascade of protein interactions *activates* Ras during insulin signaling.

1. Active IRS-1 serves as binding site for the SH2 domain of the *adaptor protein* _____.
2. Grb2's SH3 domain serves as binding site for the protein _____, forming an *IRS1-Grb2-Sos complex*.
3. Bound Sos is a ***G****uanine* ***E****xchange* ***F****actor* (_____): catalyzes replacement of GDP with GTP on Ras.

□ *Active Ras* regulates cell growth via activation of a _____ *signaling cascade*.



□ Similar pathways can also be initiated by a _____ of other *growth factors* (ex. EGF & PDGF).

EXAMPLE: G proteins such as Ras are activated when:

a) Bound to GDP.
b) Bound to GTP.
c) Phosphorylated at a serine or threonine residue.
d) Phosphorylated at a tyrosine residue.

PRACTICE: In the insulin signaling pathway to activate the protein Ras, activated IRS-1 binds to _____.

a) Adenylate cyclase. b) SH2 domain of Grb2. c) PLC. d) GTP-bound Ras. e) Insulin.

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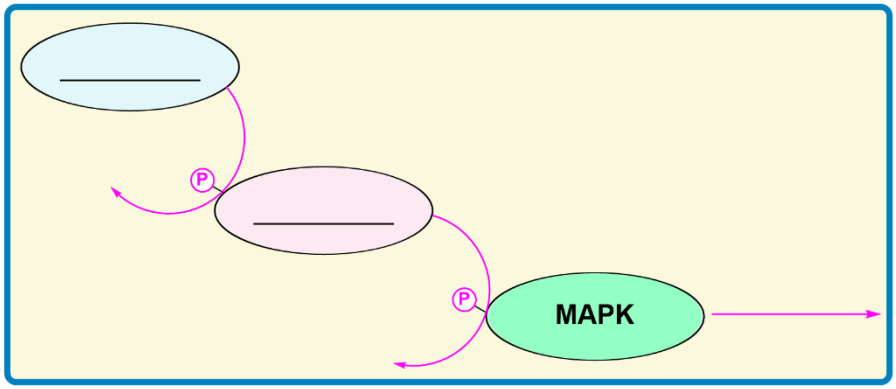
MAPK

●***M****itogen-****A****ctivated-****P****rotein-****K****inases* (_____): enzymes that phosphorylate their substrates, leading to cell growth.

□ _____: type of growth factor that induces *mitosis* & *cell division*.

●MAPK enzymes can be regulated & phosphorylated by other _____.

EXAMPLE: MAPK Kinases.



Ras Pathway Activates the MAPK Signaling Pathway

●Recall: Ras pathway results in an active, GTP-bound Ras that initiates a _____-step MAPK signaling cascade.

4 Active Ras-GTP directly *activates* _____ (a MAPKKK).

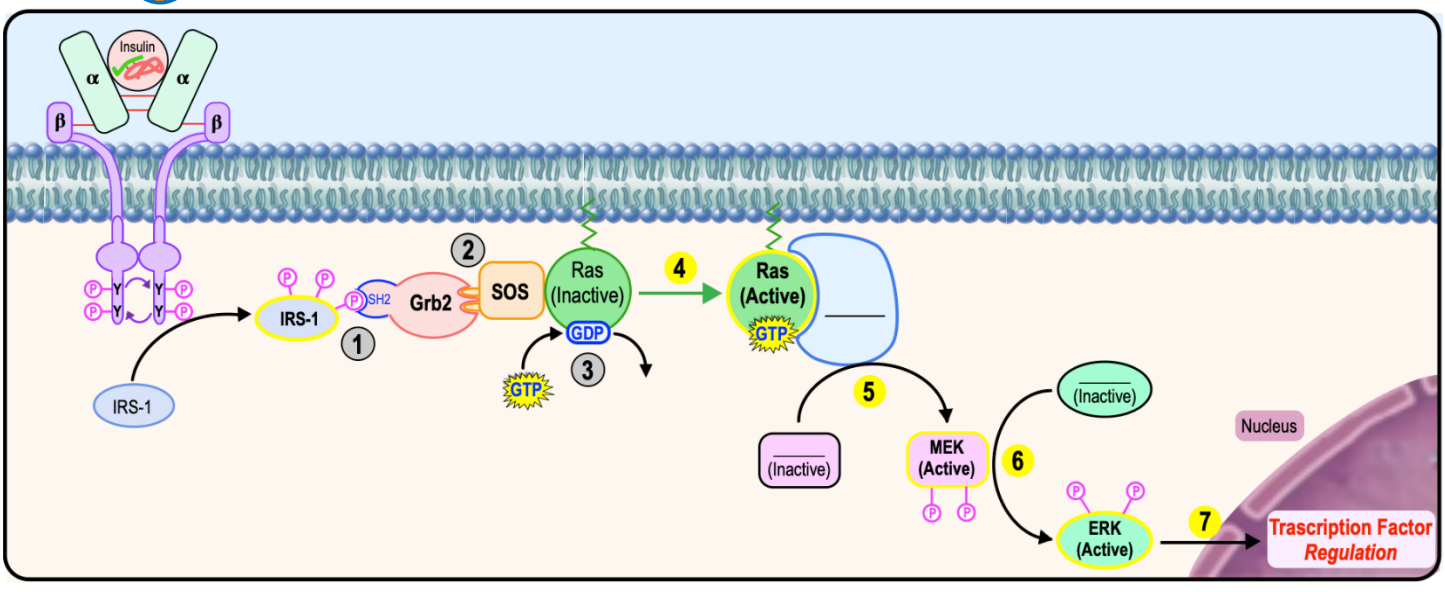
5 Active Raf-1 phosphorylates two Ser residues, *activating* _____ (a MAPKK).

6 Active MEK phosphorylates a Ser & Tyr, *activating* _____ (a MAPK).

7 Active ERK enters _____ & regulates activity of *transcription* _____ related to cell growth.

□ *Transcription Factors*: proteins that induce _____ (DNA → RNA) of specific target genes.

□ ERK transcription factor targets include Elk-1, SRF, Jun, Fos, Myc.



CONCEPT: INSULIN SIGNALING AS A GROWTH FACTOR

EXAMPLE: All of the following are true in the Ras and MAPK pathways EXCEPT:

a) Grb2 changes conformation and binds to the next protein (Sos) in the signal transduction pathway.

b) Sos acts as a GAP in the activation of the protein Ras.

c) Ras exchanges GDP for GTP and activates Raf-1, the first kinase in the MAPK pathway.

d) The MAPK pathway regulates transcription factors that regulate specific genes.

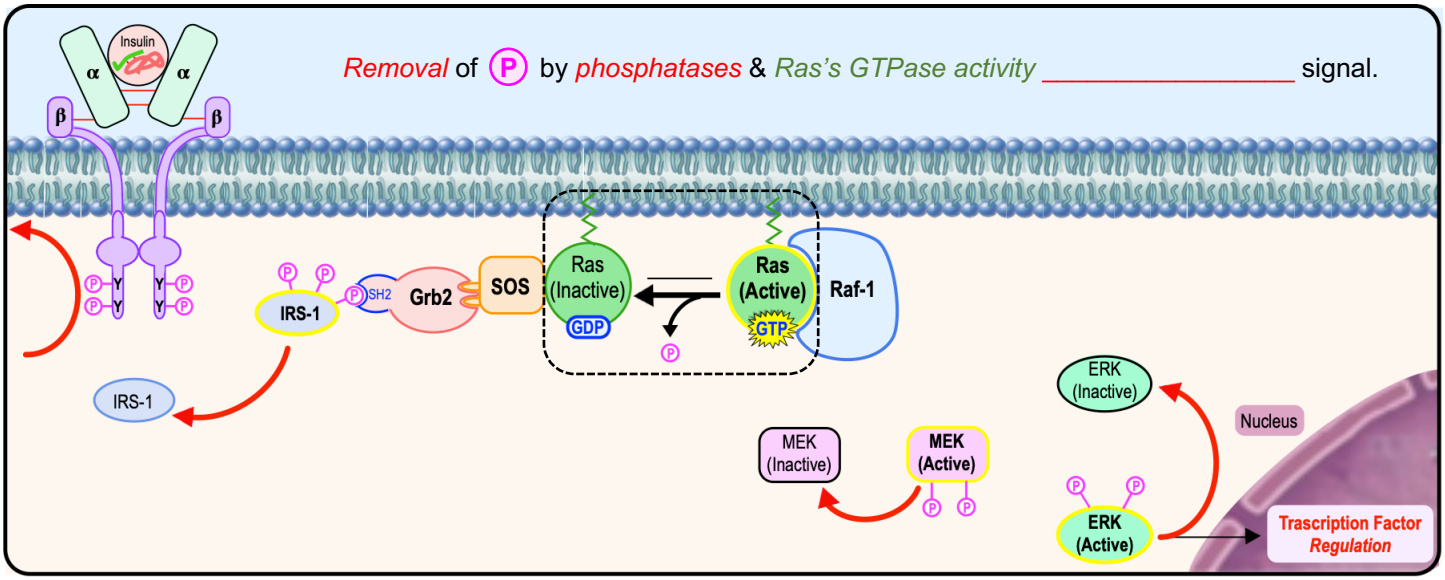
Termination of Insulin Signaling as a Growth Factor

●Ras possesses *GTPase activity* which serves to _____ the signal & reset the pathway to the inactive state.

●Ras's *GTPase* activity is very _____; HOWEVER, it can be *sped up* with help from other proteins.

□ ***G****TPase-****A****ctivating* ***P****rotein* (_____): increases rate of Ras's GTP hydrolysis (thus *inactivating* Ras).

●*Phosphatases* remove phosphate groups, reversing kinase activity & helping to _____ the pathway.



PRACTICE: The monomeric G-protein Ras is activated by _____, while _____ helps turn Ras off.

a) Grb2 ; MAPK.

b) Sos ; GAP.

c) IRS-1 ; GAP.

d) MAPK ; Raf-1.

e) GAP ; Sos.

f) GEF ; Sos.

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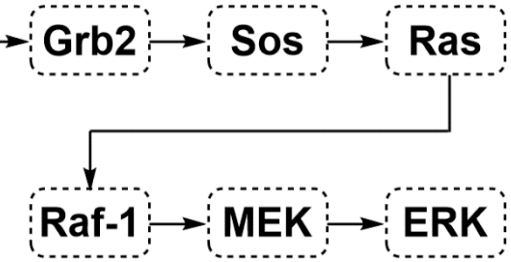
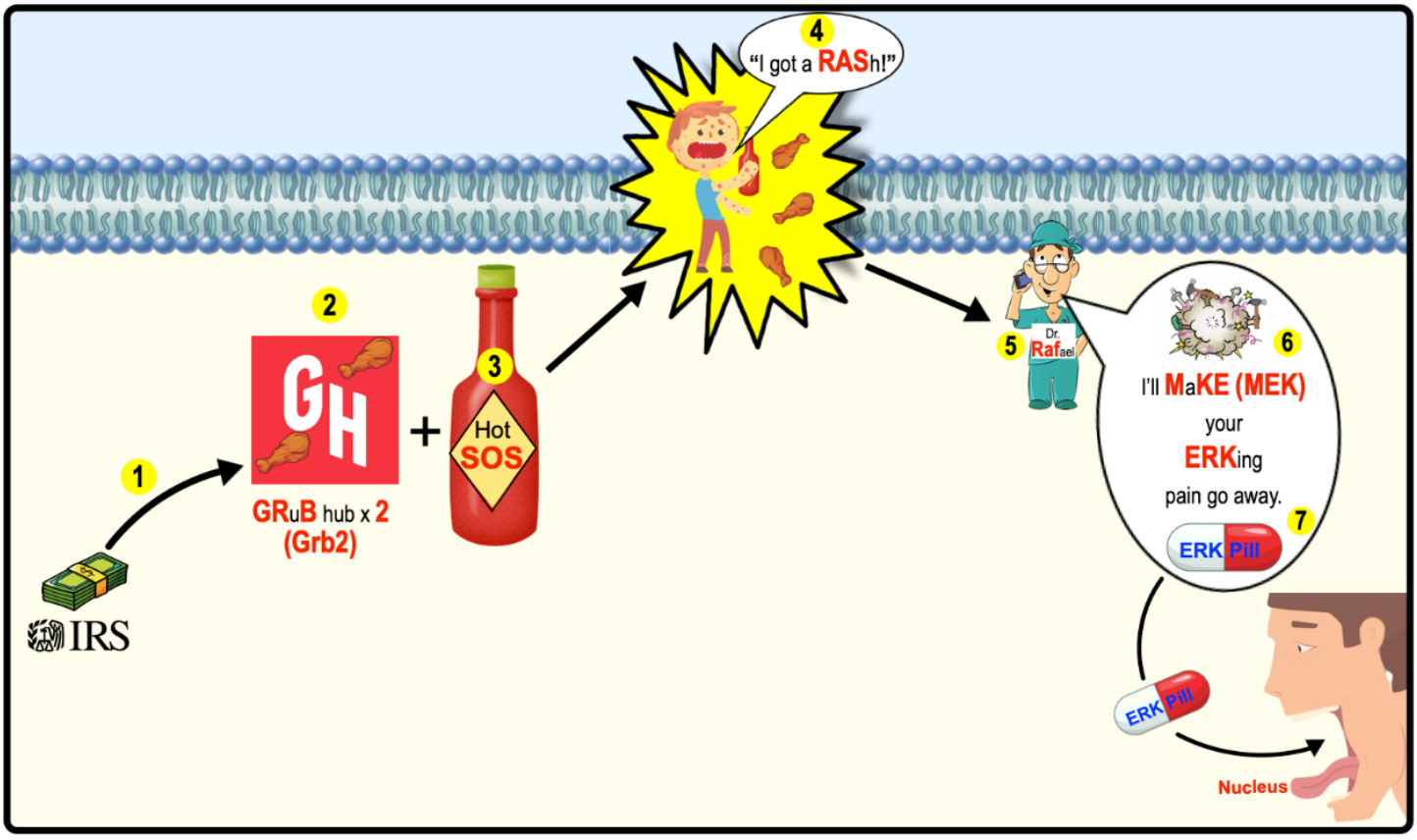
How to Remember Insulin RTK Signaling as a Growth Factor?

1. ***IRS*** gives a tax refund (***IRS-1***).
2. Cell buys ***2*** orders on ***Gr**u**b**-hub*** (***Grb2***).
3. When food arrives, cell adds hot ***S**auce*** (***Sos***).
4. Hot Sos *activates* a ***Ras**h*** (***Ras***).
5. Cell calls Dr. ***Raf**ael*** (***Raf-1***)….
6. To ***M**a**KE*** (***MEK***)….
7. ***ERK***ing-pain (***ERK***) go-away.





PRACTICE: Place the insulin RTK signaling events in order from 1-7:

a) Ligand binding results in autophosphorylation of the receptor tyrosine kinase _____.

b) MAPKK (Mek) phosphorylates MAPK (Erk) _____.

c) Sos binds then activates Ras _____.

d) Receptor tyrosine kinase phosphorylate IRS-1, which binds the SH2 domain of Grb2 _____.

e) Ras activates MAPKKK (Raf-1) _____.

f) MAPK (Erk) activates transcription factors to regulate mRNA synthesis _____.

g) MAPKKK (Raf-1) phosphorylates MAPKK (Mek) _____.

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PRACTICE: Ras is estimated to be responsible for 30% of human cancers. Most mutations in Ras prevent the protein from binding GTPase-activation proteins (GAPs). What is the effect of this mutation on the signal transduction?

a) The GAP protein will activate too many Ras proteins.
b) The Ras protein cannot be turned "off" by GTP hydrolysis, so the signaling pathways remains activated.
c) The Ras protein cannot be turned "on" so the signaling pathway cannot be activated.
d) The Ras protein is turned "on" by the action of a protein kinase rather than GTP exchange.

PRACTICE: Insulin is one of the hormones/growth factors that will activate the Ras and MAP kinase pathway. Concerning this system, all of the following are true EXCEPT

a) Grb2 binds to the phosphorylated IRS-1.
b) Grb2 binds to the phosphorylated IRS using its SH2 domain.
c) Grb2 uses its SH3 domain to bind the GEF, Sos.
d) Grb2 binds directly to the serine residues of the insulin receptor's alpha-subunits.
e) The beta-subunits of the insulin receptor are phosphorylated on tyrosyl residues.

PRACTICE: All of the following are true in the Ras and MAPK pathway EXCEPT:

a) The occupied growth factor receptor autophosphorylates serine residues.
b) Grb2 uses its Src homology 2 domain to bind to the phosphorylated IRS-1.
c) Grb2 binds Sos in the Ras signal transduction pathway.
d) In the MAPK pathway, Raf-1 phosphorylates MEK.
e) In the MAPK pathway, ERK regulates transcription factors that regulate mRNA synthesis.

PRACTICE: Guanine Exchange Factors (GEFs) bind Ras-GDP and promote dissociation of bound GDP to be replaced by GTP. GTPase Activating Proteins (GAPs) bind Ras-GTP and stimulates GTPase activity of Ras. How is the downstream signal affected by the presence of GEFs and GAPs?

a) Signaling pathway increases in the presence of GAPs and decreases in the presence of GEFs.
b) Signaling pathway increases in the presence of GEFs and decreases in the presence of GAPs.
c) GAPs and GEFs both decrease the signaling pathway.
d) GAPs and GEFs both increase the signaling pathway.
e) GAPs and GEFs have no downstream effect on the Ras signaling pathway.