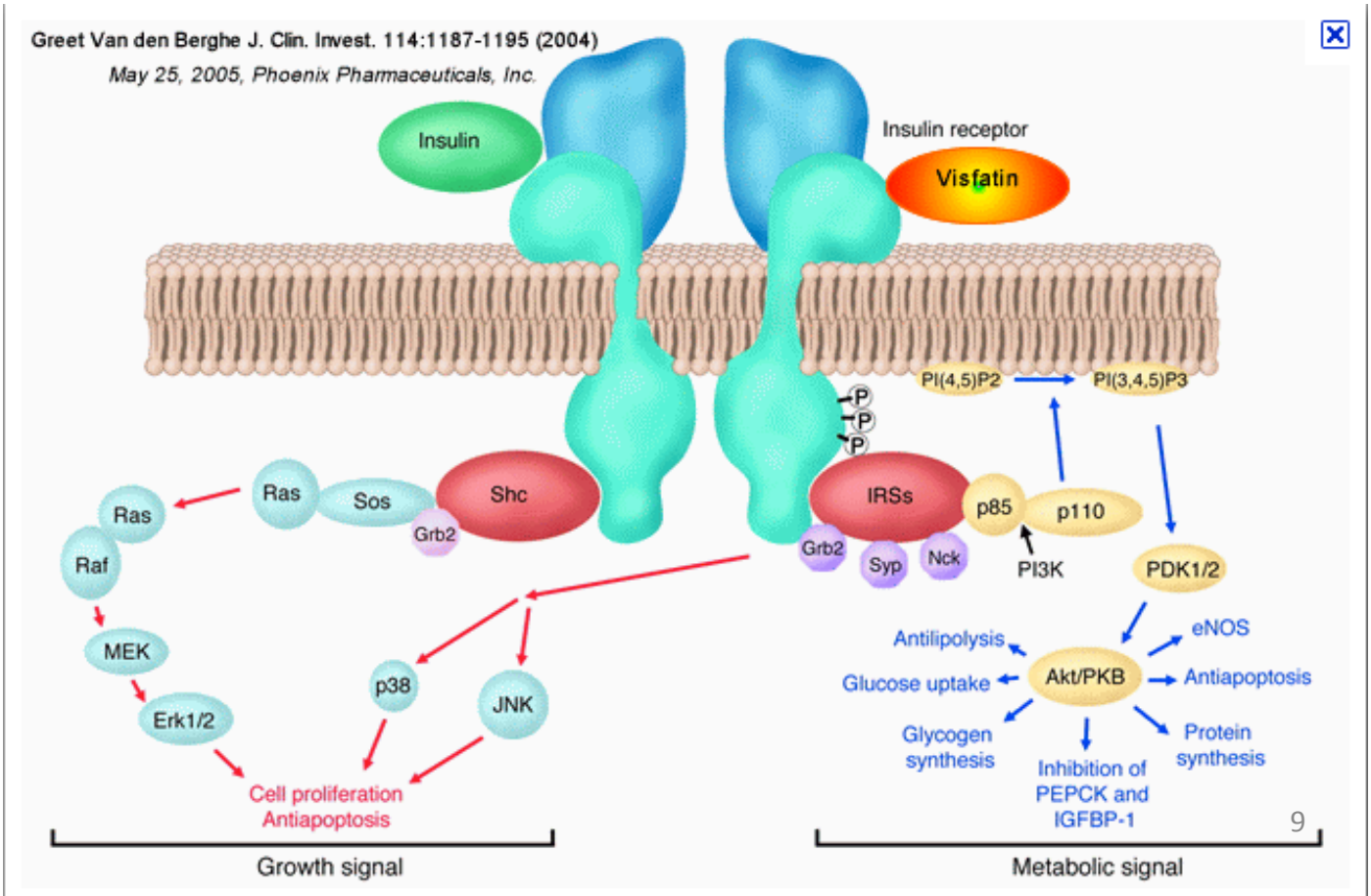
3. LIGAND-RECEPTOR BINDING AND DOWNSTREAM EVENTS

- **1/ Insulin binds insulin receptor**
  - Insulin binds to the alpha (extracellular) subunits of the insulin receptor.

- **2/ Insulin receptor tyrosine kinase activity is activated**
  - Binding of insulin to the alpha subunits, produces a conformational change in the beta subunits, which stimulates the kinase activity of the insulin receptor.

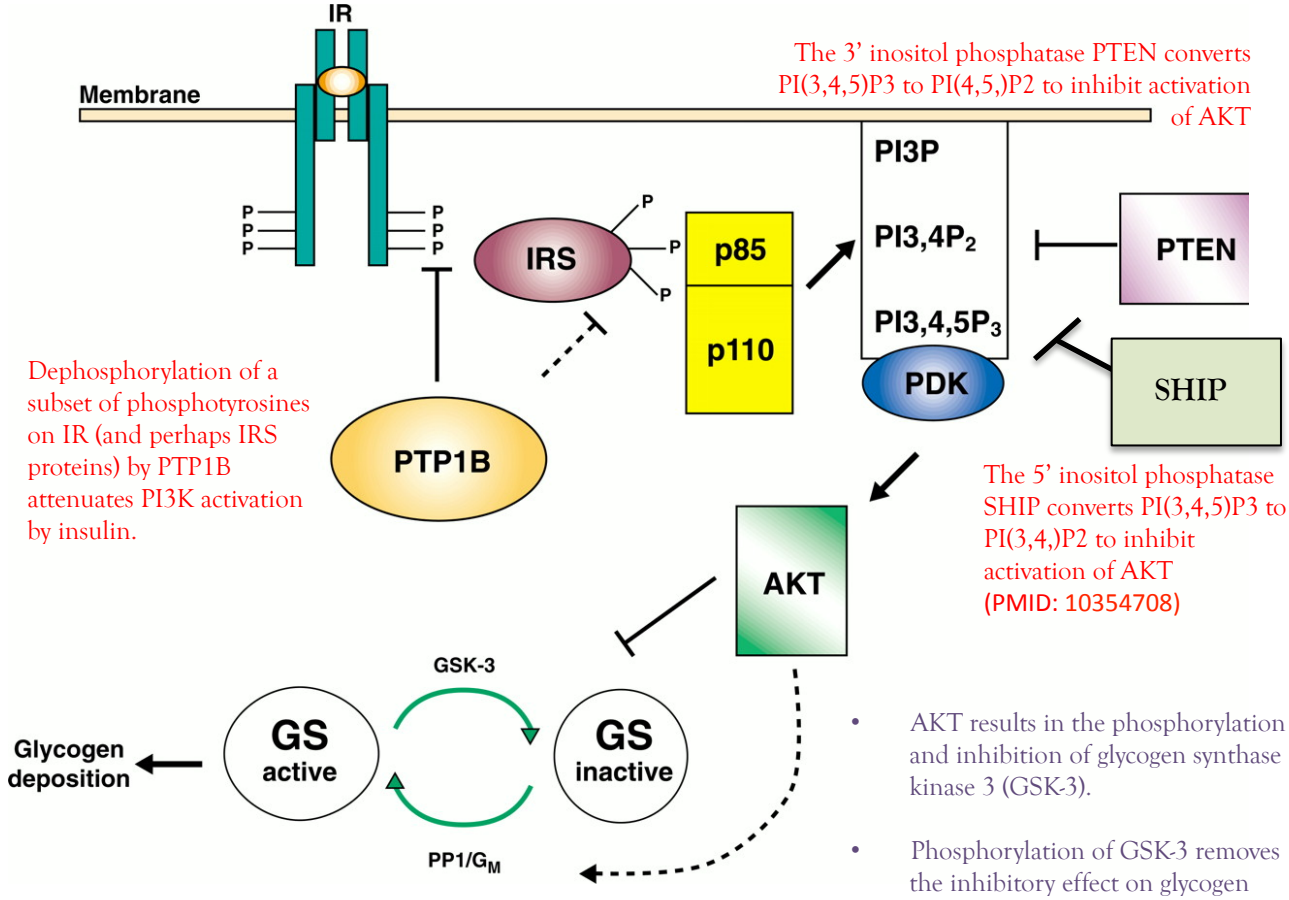
- **3/ Insulin receptor autophosphorylates**
  - The insulin receptor phosphorylates its own tyrosine residues at multiple sites in a trans-phosphorylation event (one beta-subunit of the receptor phosphorylates the other, rather than itself).
  - Autophosphorylation activates the insulin receptor.

The activated insulin receptor can signal via IRS1 OR AKT:



3.1 INSULIN RECEPTOR SIGNALING VIA IRS

- The activated insulin receptor binds IRS
- The activated insulin receptor phosphorylates tyrosine residues on IRS
- Phosphorylated IRS binds to SH2-domains of p85 (the regulatory subunit of PI3K), to activate PI3K.
- PI3K phosphorylates PI(4,5)P2 to produce PI(3,4,5)P3 (PIP2 to PIP3 conversion)
- PI(3,4,5)P3 and PI(3,4)P2 bind the PH subunit of AKT, causing it to translocate to the plasma membrane.
- Activation of AKT is a multistep process and involves Thr phosphorylation by PDK1 and Serine phosphorylation by PDK2.



- AKT results in the phosphorylation and inhibition of glycogen synthase kinase 3 (GSK-3).
- Phosphorylation of GSK-3 removes the inhibitory effect on glycogen synthase (GS).

PMID:11752618. Meeting report: targeting protein phosphatases-medicines for the new millenium.
http://stke.sciencemag.org/cgi/content-nw/full/sigtrans;2000/57/pe1/F2

3.1.1 DOWNSTREAM EFFECTORS OF AKT/PKB

- AKT (aka PKB/protein kinase B) is a protein Ser/Thr kinase.

- AKT phosphorylates FOXO transcription factor
- Phosphorylation of FOXO retains FOXO in the cytoplasm so it is unable to enter the nucleus to activate transcription.
- Active FOXO can stimulate expression of the insulin receptor gene in a feedback loop.

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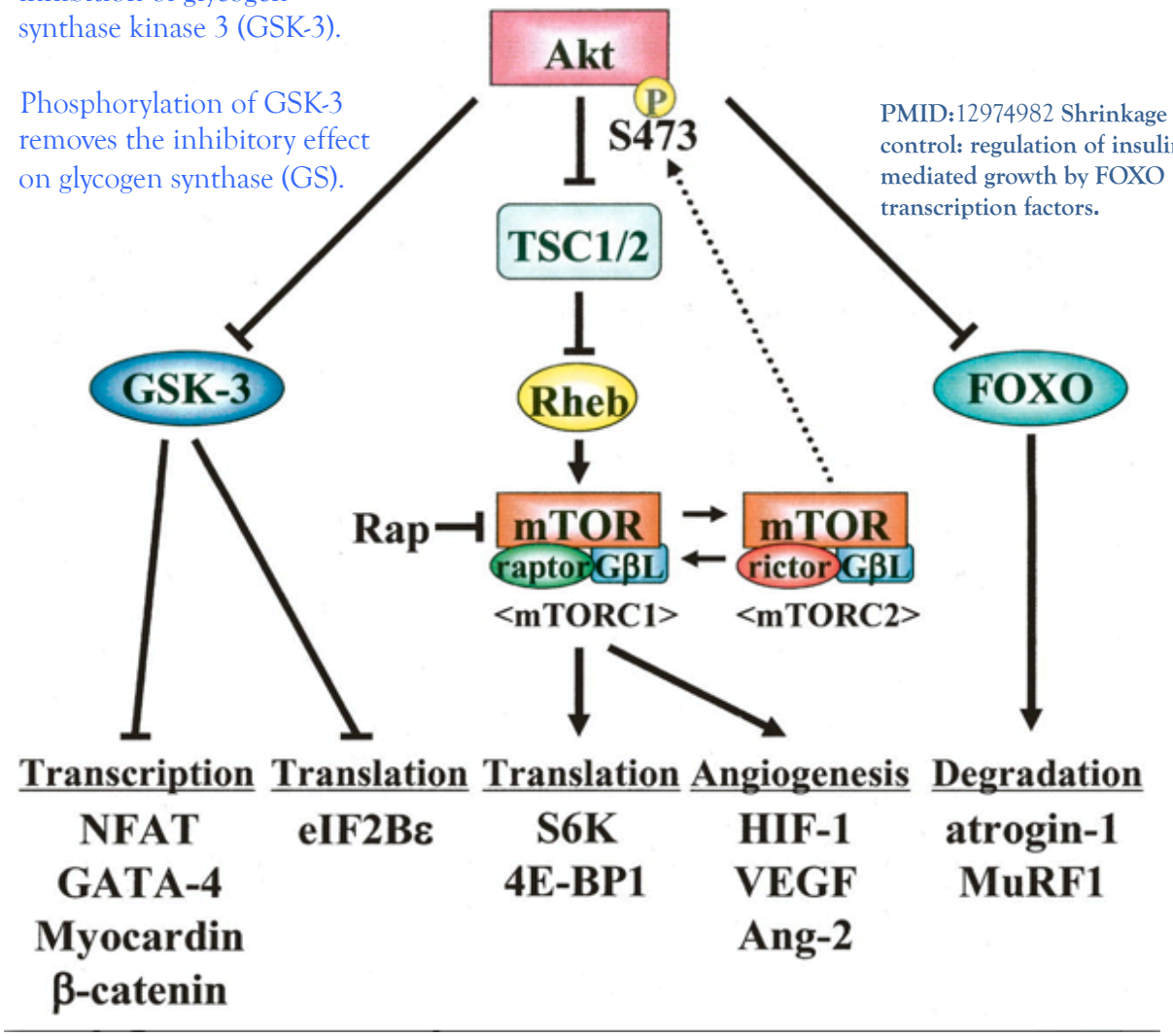
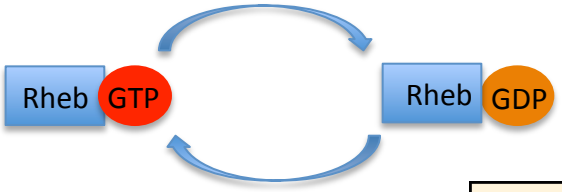


PMID:12974982 **Shrinkage control: regulation of insulin-mediated growth by FOXO transcription factors.**

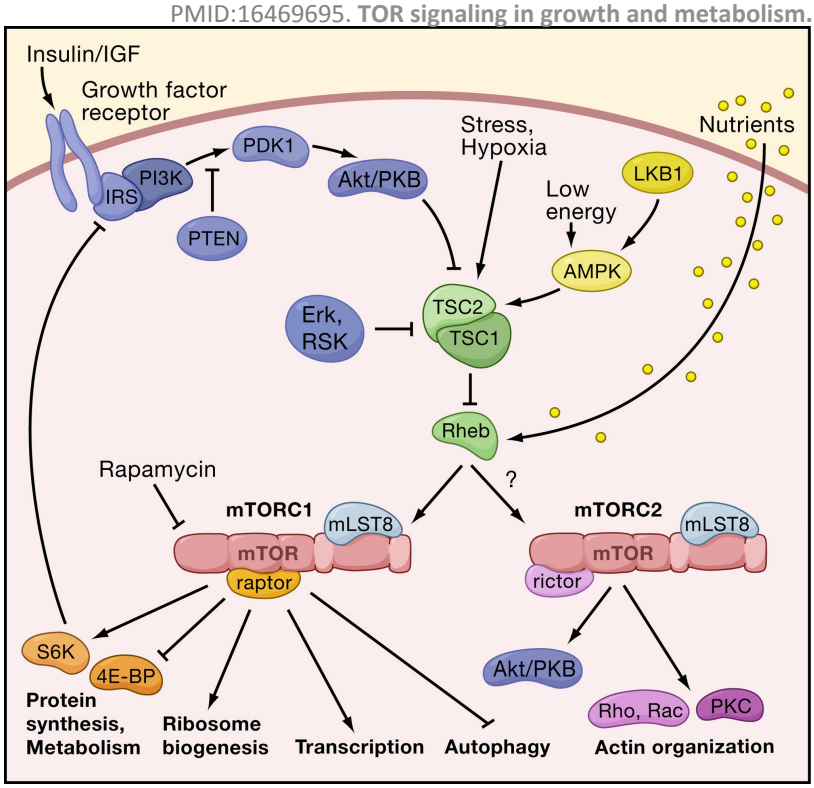
3.1.1.1 TOR SIGNALING DOWNSTREAM OF AKT

TSC1-TSC2 complex
(Rheb GAP)/(GTPase activating protein)



- 1/ The TSC1-TSC2 complex has GTPase-activating activity (GAP) for Rheb.

PMID:16469695. **TOR signaling in growth and metabolism.**



- 2/ AKT phosphorylates TSC2, and decreases the Rheb-GAP function of the TSC1-TSC2 complex. This leads to accumulation of the GTP-bound Rheb, which can activate TOR.

- 3/ TOR is a serine/threonine kinase which has multiple downstream targets, including 4EBP1 and S6K1:

S6K1:

- mTORC1 phosphorylates S6K1 on at least two residues.
- Phosphorylation by TOR promotes subsequent stimulation of S6K1 by PDK1.
- Activate S6K1 can stimulate the initiation of protein synthesis through activation of S6 ribosomal protein and other members of the translational machinery.
- In a positive feedback loop. S6K1 can phosphorylate TOR to stimulate TOR activity

4E-BP1:

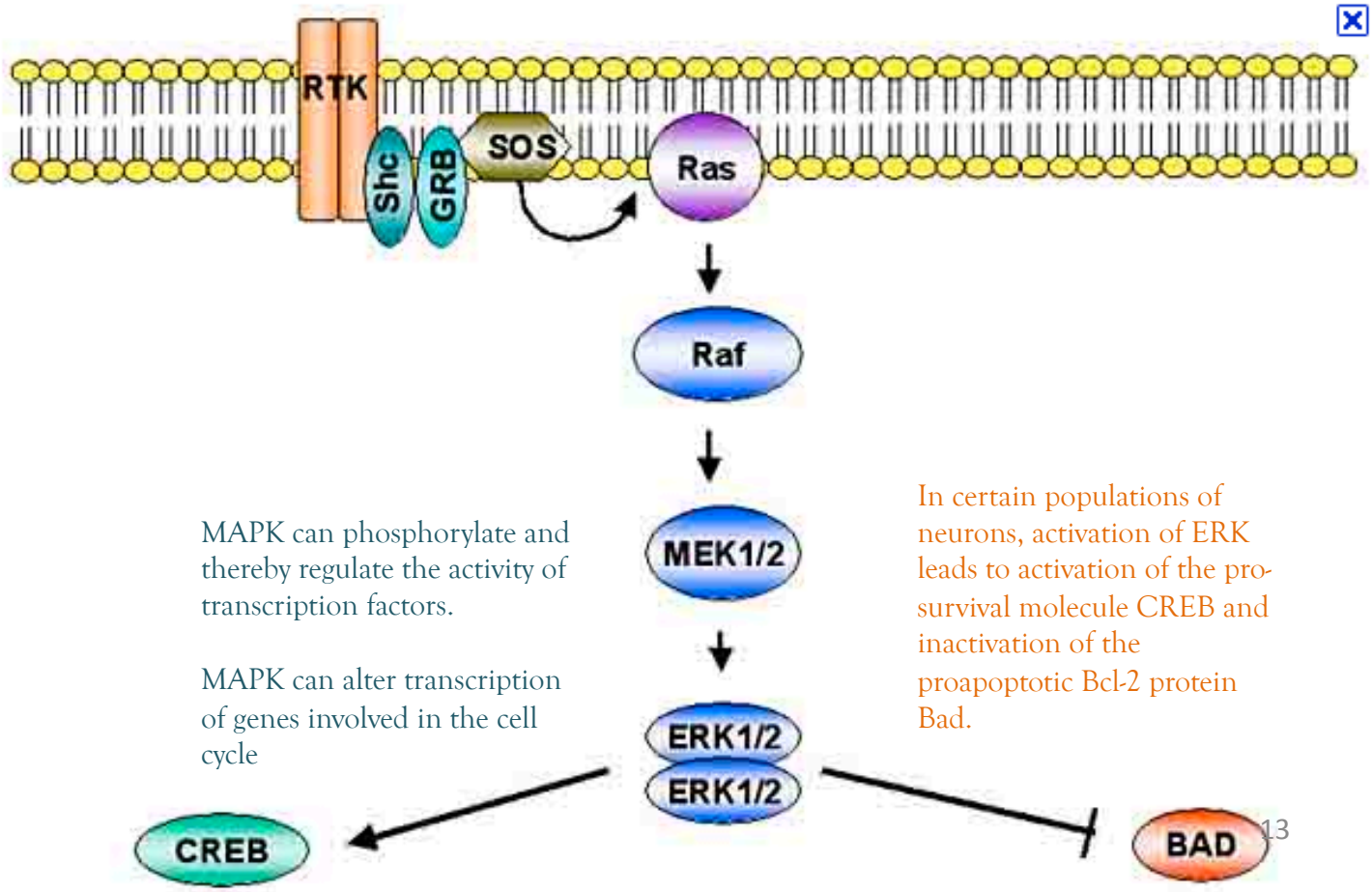
- Non-phosphorylated 4E-BP1 binds to the translation initiation factor eIF4E, preventing eIF4E from binding RNAs to recruit them for translation.
- mTORC1 phosphorylates 4E-BP1, leading to release of eIF4E, allowing it initiate translation.

PMID: 18992839: Insulin/TOR signaling in growth and homeostasis: a view from the fly world.
PMID: 20849947: Ageing in Drosophila: The role of the insulin/Igf and TOR signalling network.
PMID: 17041621. Upstream of the mammalian target of rapamycin: do all roads pass through mTOR?
PMID: 14668532. TOR signaling.
PMID: 14668532. TOR signaling. Harris and Lawrence, 2003. Review.

3.2 INSULIN RECEPTOR SIGNALING VIA SHC

- The activated insulin receptor binds and phosphorylates SHC
- Phosphorylated SHC binds to SH2-domains of GRB2.
- The SH3 domain of SOS binds GRB2.
- SOS acts as a RAS-GEF (GTP exchange factor), activating RAS by catalyzing the exchange of GDP for GTP on RAS.
- RAS activation leads to activation of RAF via series of phopshorylation events on multiple residues.
- RAF is a serine/threonine kinase that can phosphorylate MEK (MAPKK) to activate MEK.
- MEK phosphorylates the MAP kinase ERK on two residues, resulting in activation.

In an alternative route, (RAS can also activate PI3K)

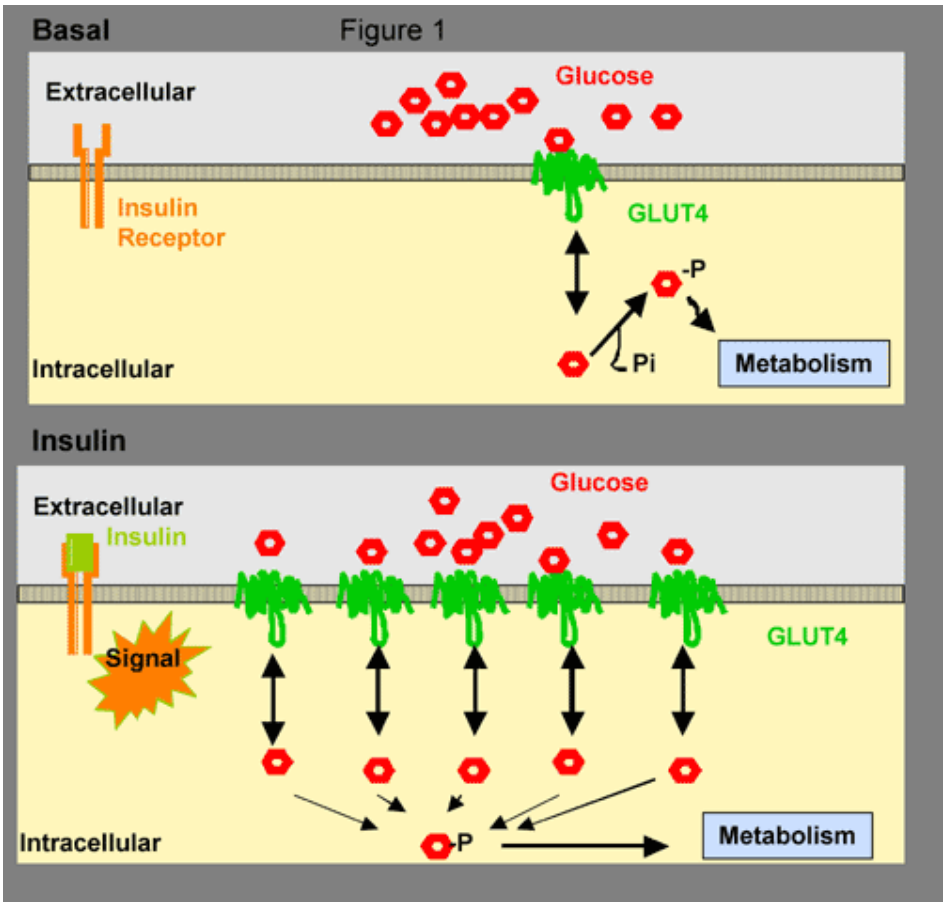


4 DOWNSTREAM CELLULAR PROCESSES

- The following have been covered elsewhere in the summary:
  - increased glycogen synthesis (via inhibition of GSK-3)
  - increased protein synthesis (via S6K1 and 4E-BP1)
  - anti-apoptosis (via inhibition of BAD, and indirectly via inhibition of apoptotic gene transcription)
  - regulation of transcription (inhibition of forkhead TFs/activation of MAPK)

4.1 REGULATION OF GLUCOSE UPTAKE INTO CELLS

- Insulin regulates glucose uptake by recruiting membrane vesicles containing the **GLUT4 glucose transporters** from the interior of cells to the cell surface.
- This allows glucose to enter the cell by passive diffusion
- Once in the cytoplasm, the glucose is phosphorylated and thereby trapped inside cells.



http://www.med.cornell.edu/biochem/mcgraw/insulin-regulated.html

5. REGULATORY EVENTS

5.1 RECEPTOR MEDIATED ENDOCYTOSIS OF LIGAND-RECEPTOR COMPLEXES

- Phosphorylation of the tyrosine in the NPEY sequence of the insulin receptor is also a signal for **endocytosis** to occur.
  - Whilst invagination of the plasma membrane commences, the receptor tyrosine kinase activity continues unabated as does substrate phosphorylation.

- Endocytosis of activated receptors has the dual effect of:
  - Concentrating receptors within endosomes
  - Allowing the insulin receptor tyrosine kinase to phosphorylate substrates that are spatially distinct from those accessible at the plasma membrane.

- In conditions in the endosome, **insulin dissociates from the receptor**

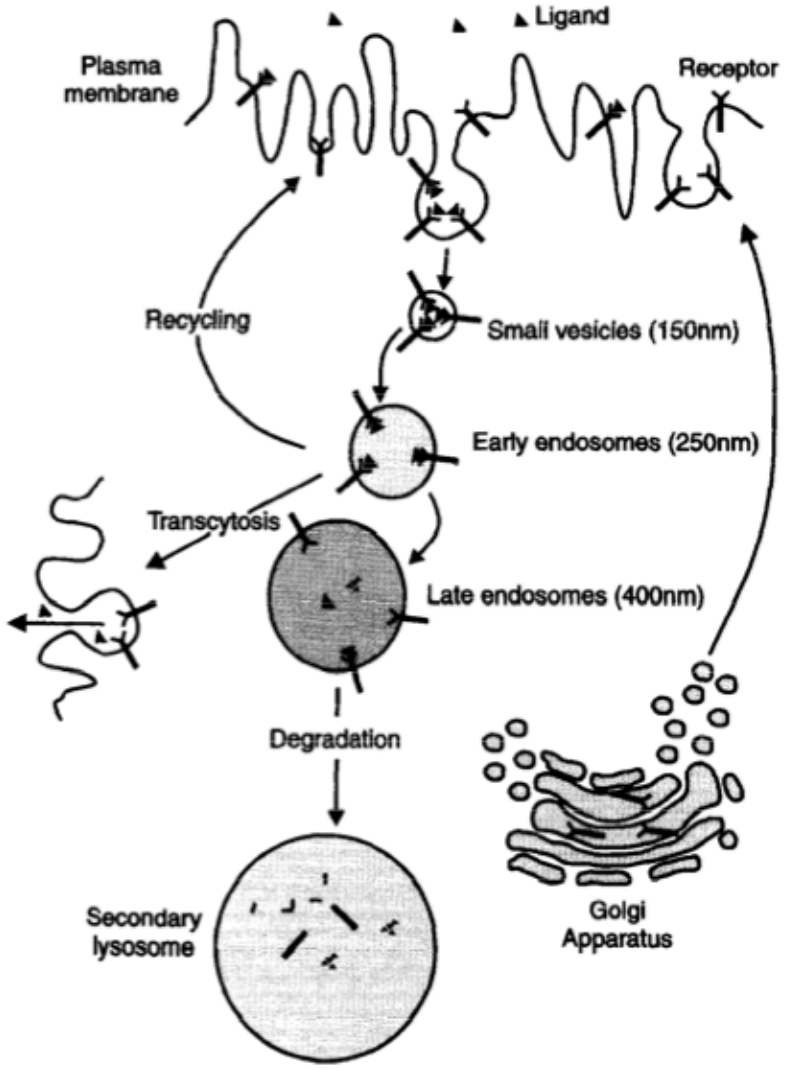
- **Free insulin is degraded** by 'insulin degrading activity (IDA)' present in the endosome.

PMID:3061785. Insulin degradation: mechanisms, products, and significance.

- **Insulin receptor dephosphorylation**
  - With insulin dissociated from its receptor, the signal to sustain the receptor kinase's activity is also removed.
  - Endosomally-associated protein tyrosine phosphatases (PTPs) dephosphorylate the receptor.

PMID:9609117. Insulin receptor-associated protein tyrosine phosphatase(s): role in insulin action.

PMID:9609114. Insulin receptor internalization and signalling.
PMID:18406720. Intracellular signal transduction: The role of endosomes.
PMID: 17545147: Insulin-like growth factor type-I receptor internalization and recycling mediate the sustained phosphorylation of Akt.



UPON LIGAND DISSOCIATION, THE RECEPTOR CAN BE EITHER BE:

- **5.1.1 RECYCLED TO THE PLASMA MEMBRANE**
  - The endosome fuses with the PM, allowing the insulin receptor to reintegrate at the cell surface.
  - Receptor recycling is complete when the dephosphorylated receptor is back in the plasma membrane available to bind the next insulin molecule presented to it.

- **5.1.2 DELIVERED TO THE LYSOSOME FOR DEGRADATION**

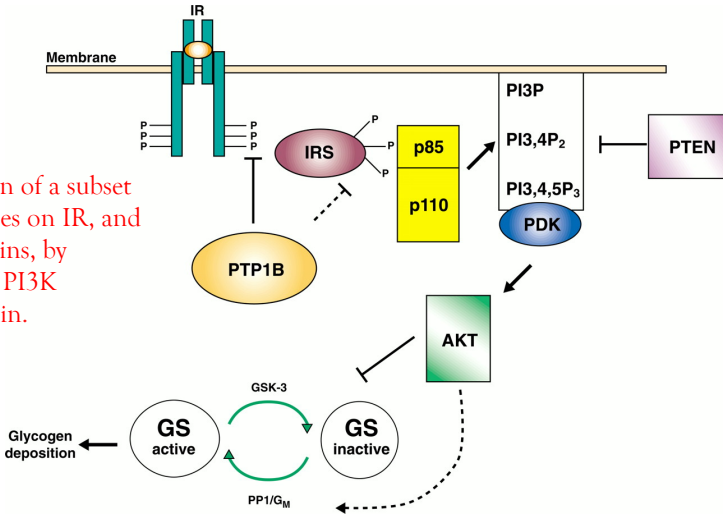
5. REGULATORY EVENTS (cont)

5.2 SIGNAL ATTENUATION

- Downstream signaling molecules are also dephosphorylated leading to the collapse of the signaling complexes and signal attenuation
  - IRS1 tyrosine dephosphorylation
  - SHC dephosphorylation
  - SOS phosphorylation and dissociation from SHC, IRS, GRB2

PMID:7739560. Insulin-stimulated disassociation of the SOS-Grb2 complex.

Dephosphorylation of a subset of phosphotyrosines on IR, and perhaps IRS proteins, by PTP1B attenuates PI3K activation by insulin.



- The inositol 3-phosphatase activity of PTEN catalyses the conversion: PI(3,4,5)P3 -> PI(4,5,)P2.
- PTEN antagonises the action of PI3K.

PMID: 15632081: Coordinated regulation of insulin signaling by the protein tyrosine phosphatases PTP1B and TCPTP.

5.2.1 SERINE/THREONINE PHOSPHORYLATION OF IRS

- Serine/threonine phosphorylation of IRS proteins inhibits insulin receptor signaling
  - Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation.

PMID:15671481. Ser/Thr Phosphorylation of IRS Proteins: A Molecular Basis for Insulin Resistance. http://stke.sciencemag.org/cgi/reprint/sigtrans;2005/268/pe4.pdf

PMID:9368067. A molecular basis for insulin resistance. http://www.jbc.org/content/272/47/29911.full.pdf+html

6. RECEPTOR ANTAGONISM

- *in vitro*, amyloidβ is a competitive inhibitor of insulin binding to its receptor.

PMID:12006603. **Alzheimer's beta-amyloid peptides compete for insulin binding to the insulin receptor.**

PMID:18782558. **A novel high-affinity peptide antagonist to the insulin receptor.**

(S661)