News

13-02-2016
The 3rd edition of "Signal Transduction"; a book for students and teachers

30-03-2013
the updated cell biology resources are now hosted by UniSciel

07-12-2012
Education catching up with science; preparing students for 3D literacy in cell biology

Overview

Signal Transduction Education

Active Learning Projects - Obesity Project - Team 4

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general information

active learning, any time, any place and anywhere

4. Signalling by ghrelin, insulin and leptin in the hypothalamus

Summary:

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Introduction

Here we deal with receptors and downstream signalling pathways that affect expression of neurotransmitters and the firing rate of neurons. Receptors are "receptive substances" at the surface of the cell that bind to a specific messenger. Although very specific, often as not many receptors recognize more than one messenger (which naturally resemble each other) and some messengers are recognized by two different receptors (in short there is a low level of promiscuity). For instance, the membrane protein INSR is the receptor for insulin but also for insulin-like growth factor (IGF) and the neurotransmitter acetylcholine is recognized by the muscarinic receptor (a 7 TM protein) and by the nicotinic receptor (an multiprotein ion channel). Because most messengers do not cross the membrane, they bind to the outside of the cell. After binding to the receptor, the message is next translated into a signal that the cell can understand, often through the production of second (intracellular) messengers. The second messengers will set in train a cascade of events that eventually reach the nucleus, there to change expression of genes (plasticity of cell). The cascade may also have immediate effects, like secretion of substances, contraction of muscle or a change in the polarity of the membrane (in the case of electro-excitable cells). The process of transmission into the cell and translation of the signal is called "signal transduction" (the subject of this web page).

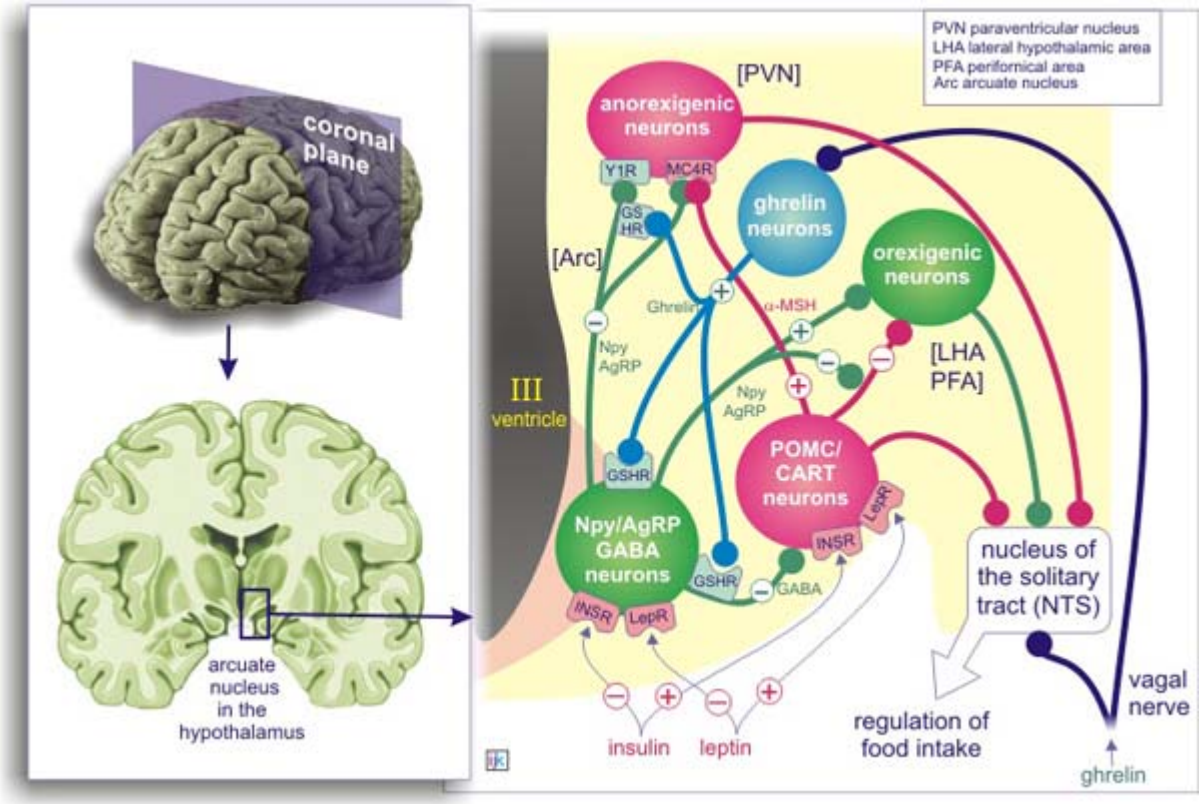


Figure 1 *Regulation of food intake at the level of the arcuate nucleus by ghrelin (orexigenic = inducing food-seeking behaviour), insulin and leptin (both anorexigenic = inducing fasting behaviour). For more information consult the web page of team 2. Red represents "stop eating" and green "go eating".*
[Arc], arcuate nucleus; [LHA/PFA], lateral hypothalamic area/perifornical area; [PVN], paraventricular nucleus.

In this page we focus on signal transduction in and around the arcuate nucleus (figure 1), an important brain region for the integration of hormonal messages, coming from different organs and dealing with the regulation of food intake. We limit our subject to the action of one appetite messenger, ghrelin, and two satiety messengers, insulin and leptin.

Ghrelin is produced by the stomach but also by neurons surrounding the arcuate nucleus. It acts on Npy/AgRP/GABA neurons (because they carry its receptor) and it augments expression of genes coding for the neurotransmitters Npy and AgRP. Concomittantly, Ghrelin increases membrane potential (hypopolarization), leading to an increased firing rate of these neurons. Through the intermediate of the neurotransmitter GABA, ghrelin reduces the firing rate of POMC/CART neurons (thus reducing anorexigenic signals).

Insulin (produced by the pancreas) and leptin (produced predominantly by adipose tissue) act on both Npy/AgRP/GABA and POMC/CART neurons (because both neuron types carry their receptors). They augment expression of the gene coding for POMC, a precursor protein in the production of α-MSH (an essential anorexigenic neurotransmitter). On the contrary, insulin and leptin, inhibit expression of AgRP, an anorexigenic neurotransmitter that acts as an agonist of α-MSH. We will detail the molecular events that link the occupied cell membrane receptor with changes in gene expression.

The source, the molecular composition, as well as the physiological importance of ghrelin, insulin and leptin are treated in the web page of team 2. For a global view of the action of these hormones see figure 1.

Signalling by gherlin

Ghrelin-mediated activation of PLCβ

Ghrelin is a peptide hormone (tiny protein) that requires the attachment of an octanoyl fatty acid chain in order to bind its receptor, the growth hormone secretagogue receptor or GHSR (figure 2). How the hormone interacts with its receptor is not know. GHSR belongs to the large family of seven trans-membrane spanning proteins (7 TM) also known as G-protein coupled receptors (GPCR). These are by far the most abundant type of receptors. Our vision, taste and smell (about 1035 different odorant receptors in humans) relies on G-protein coupled receptors and the majority of hormones act through them. Examples are given in figure 3 (click on image to get the full picture). G-protein coupled receptors are the targets of 50% of all medicaments: think of beta-blockers (propranolol, carvedilol) to keep heartbeat under control or salbutamol (ventolin) to facilitate breathing in case of asthma or morphine to ease pain (analgesic)).

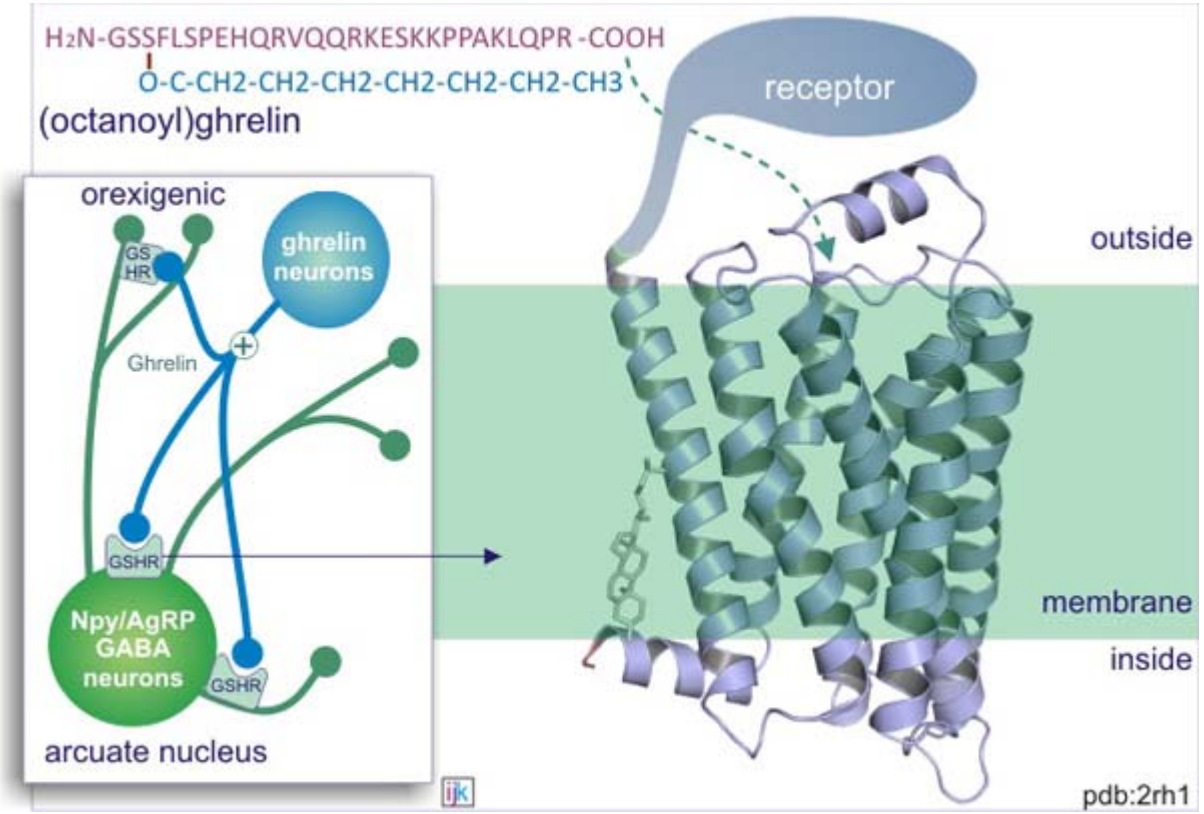


Figure 2 *Composition of octanoyl-ghrelin and structure of 7TM receptor (protein shown in figure is the β2-adrenergic receptor (pdb;2rh1)*

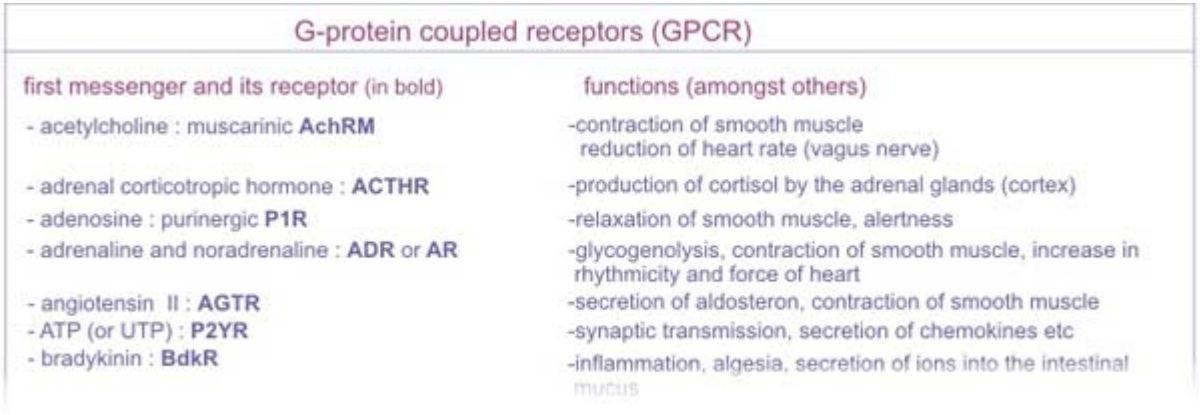


Figure 3a *Examples of GPCR receptors (click on image (3b) to get the full picture)*

The ghrelin receptor interacts with a heterotrimeric protein complex, composed of three different subunits α, β and γ, of which the α-subunit has a guanine nucleotide binding pocket (which either binds GDP or GTP). These protein-complexes are named heterotrimeric G-proteins. In the resting state they bind GDP. G-proteins are anchored to the membrane by short lipid tails (they are "integral" membrane proteins). The α-subunit is linked to a geranylgeranyl chain and the γ-subunit to a myristoyl chain. Upon arrival of ghrelin, the receptor changes the shape of the α-subunit. GDP now readily escapes and the nucleotide pocket is immediately occupied by GTP (an exchange reaction). This leads to a second conformational change (due to the presence of a 3rd phosphate in the guanine nucleotide) which causes the separation of Gα from Gβ/γ subunits (figure 4).

Like the 7TM receptors, the heterotrimeric G-proteins too are part of a family of proteins (see textbox below). Not all 7TM receptors interact with the same G-protein. In the case of ghrelin, its receptor (GHSR) interacts with the αq-subunit (Gαq). This particular subunit, once liberated from Gβγ, binds to an enzyme named phospholipase-C type-β (PLCβ). A lipase is a lipid-cleaving enzyme; a phospholipase is thus a phospholipid-cleaving enzyme. Phospholipids are in membranes and an important role of Gαq is to recruit PLCβ to the membrane, placing it next to its substrate. The preferred phospholipid is phosphatidylinositol-4,5-bisphosphate, also indicated as PI-4,5-P2 or PIP2. This is cleaved by PLCβ into diacylglycerol, a membrane bound second messenger, and inositol-1,4,5-trisphosphate (IP3), a soluble second messenger that diffuses into the cytoplasm (see figure 5 for details of the lipid composition and the phospholipase cleavage sites).

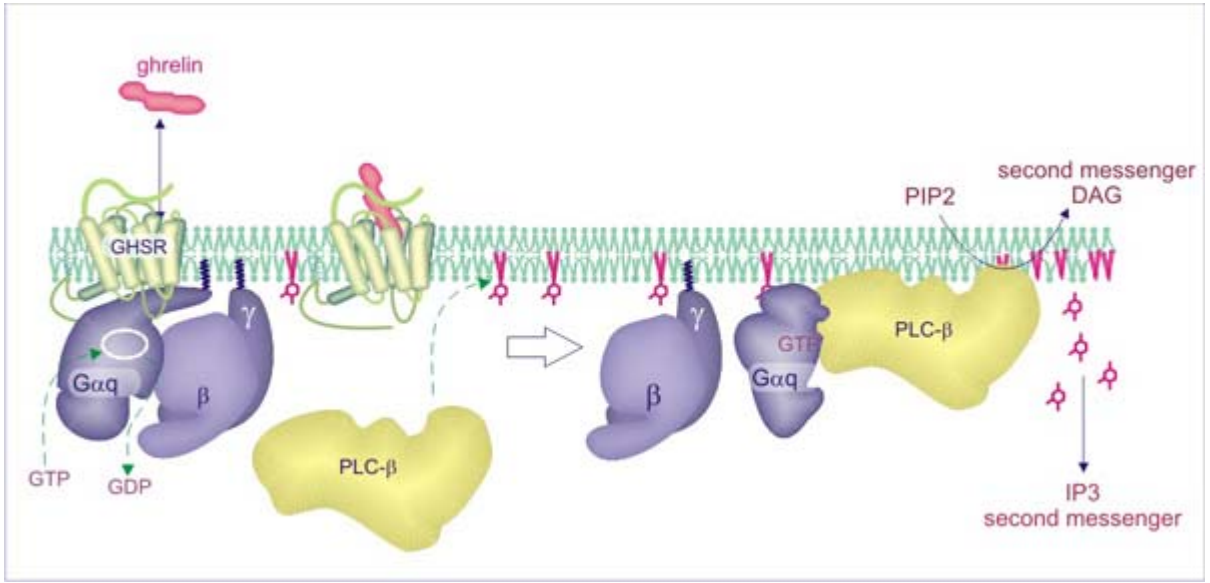


Figure 4 *binding of ghrelin to its receptor (GHSR) leads to an exchange of GDP for GTP in Gαq. The GTP-bound α-subunit separates from Gβγ and recruits PLCβ to the membrane, next to its substrate phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2 or PIP2). An enzymatic reaction ensues that lead to the formation of a soluble second messenger, inositol-1,4,5-trisphosphate (IP3) and a membrane bound second messenger, diacylglycerol (DAG).*

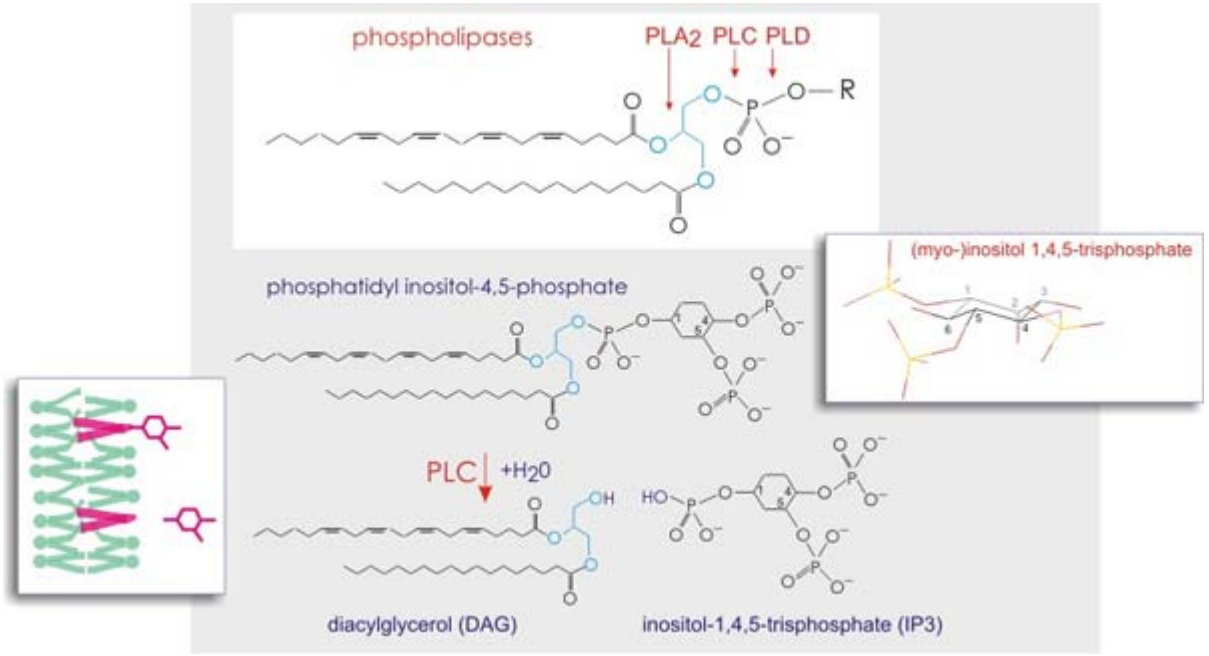


Figure 5 *Detail of the action of phospholipases; cleaving phospholipids in the membrane. PLC cleaves phosphatidyl inositol-4,5-bisphosphate (PIP2), giving rise to diacylglycerol and inositol-1,4,5-trisphosphate (shown is the "myo" configuration of the inositol phosphate).*

A family of heterotrimeric G-proteins

The structure of heterotrimeric G-proteins, shown below (with the subunits Giα1, Gβ1 and Gγ2), reveals the nucleotide binding pocket (occupied by GDP) and the β-propeller structure of Gβ1 interacting with Giα1. The β-propeller contains tryptophane-arginine (WD) repeats. This propeller configuration occurs in numerous proteins and it plays an important role in protein-protein interactions. How G-protein coupled receptors interact with the Gα subunit is not known, but somehow this interaction leads to a transient change in the nucleotide binding pocket, allowing GDP to escape. Because there is always ten times more GTP in the cell, the empty pocket will immediately be occupied by GTP (which, on top of that, also binds more firmly than GDP). A subsequent change in leads to the dissociation of Gα and Gβγ. Note the lipid anchors that keep the proteins attached to the phospholipid membrane.

Once dissociated, both Gα and Gβγ interact with effector proteins, i.e. proteins that propagate the message into the cell. Effector proteins that interact with Gα-subunits tend to promote the hydrolysis of GTP into GDP + Pi, thus terminating the response to the ligand (negative feedback). Because of their intrinsic capacity to hydrolyse GTP, G-proteins are therefore also referred to as GTPases. Stimulation of hydrolysis can also occur by specialized proteins, regulators of G-protein signalling (RGS). It must be clear that once GTP is hydrolysed, the whole system returns to a "resting state", the subunits re-assemble, the effectors proteins seize their activity and the signal is switched off (reset). A new series of events is initiated when the G-proteins encounter an occupied receptor.

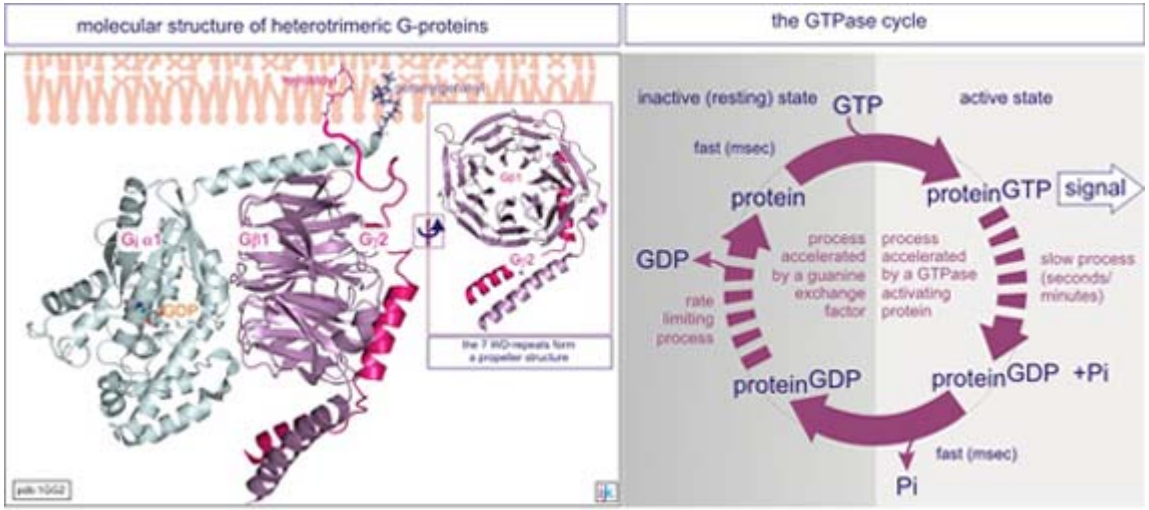


Figure 6 *structure of heterotrimeric GTP binding proteins. The Ga subunit caries the nucleotide binding pocket. The interaction between Ga and the Gb/Gg complex occurs through the beta propeller. This interaction is ruptured upon exchange of GDP for GTP. The GTP proteins behave as metastable switches, like the time-switches in buildings. You press the button to illuminate the hall and after a while the light switches off. The on signal is an exchange of nucleotides, GDP replaces GTP and this occurs with the help of a GTP exchange protein. The off signal occurs through hydrolysis of GTP into GDP and Pi (GTPase reaction). This process is accelerated by GTPase activating proteins (GAPs).*

Heterotrimeric G-proteins come in different combinations, it is not known how many. Below we show the different Gα subunits and the effector proteins they engage (leading to the production of different types of second messengers that propagate the signal into the cell) (figure 7). It should be noticed that different receptors can interact with the same Gα-subunits and thus, despite the fact that they are activated by different first messengers (hormones etc) may lead to exactly the same intracellular signal. The outcome of the signal will then depend on the cell type and its context i.e. what other messengers are bound to the cell surface receptors, what regions of the genome are accessible for regulation and what downstream effectors are expressed. Context also means that cells may not necessarily respond the same way to the same messenger. They may be desensitized or other signals may dominate, leading to a lack of response or to a quite different response.

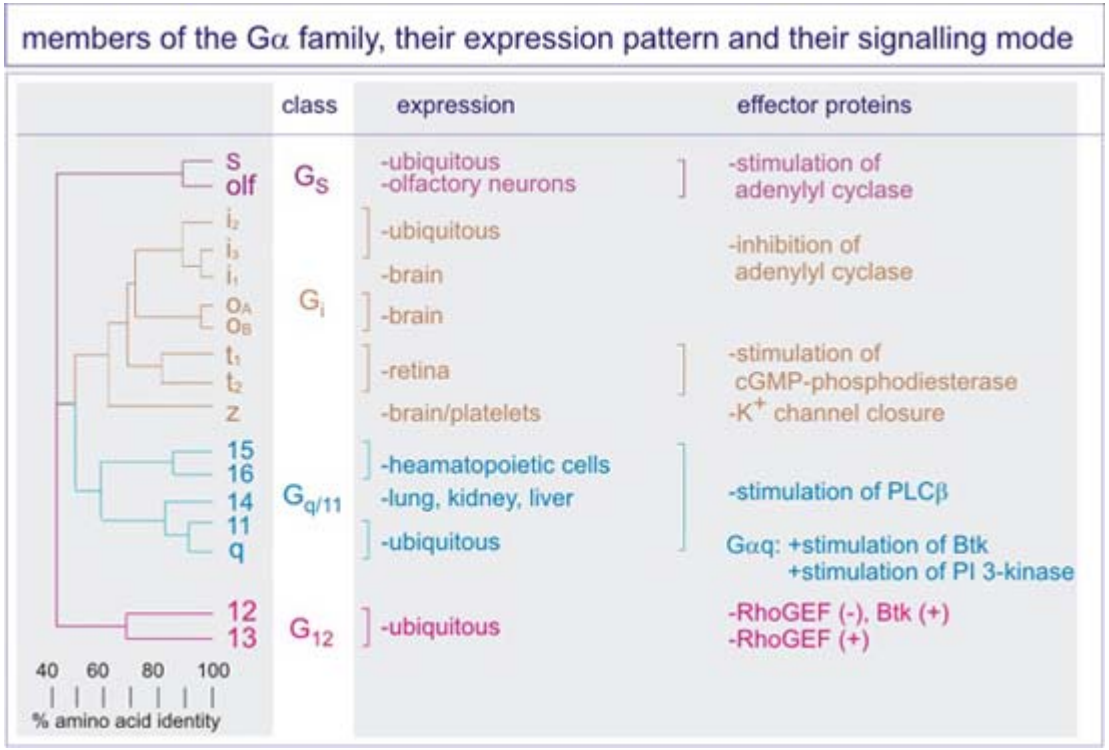


Figure 7 *(a) the various Gα subunits interacting with 7TM receptors, their site(s) of expression and their effectors*

IP3-mediated release of Ca^{2+} leads to inactivation of mTOR

- The liberated IP3 diffuses into the cell and binds its receptor which is situated on the smooth endoplasmic reticulum. The receptor is a multiprotein complex (of 1100 kDa) that forms a Ca^{2+}-channel which, upon opening, releases Ca^{2+} from its intracellular storage compartment. Depending on the IP3 concentration this results in very brief blips, more sustained puffs or big waves of Ca^{2+}, which last several seconds and traverse the entire cell (figure 8).

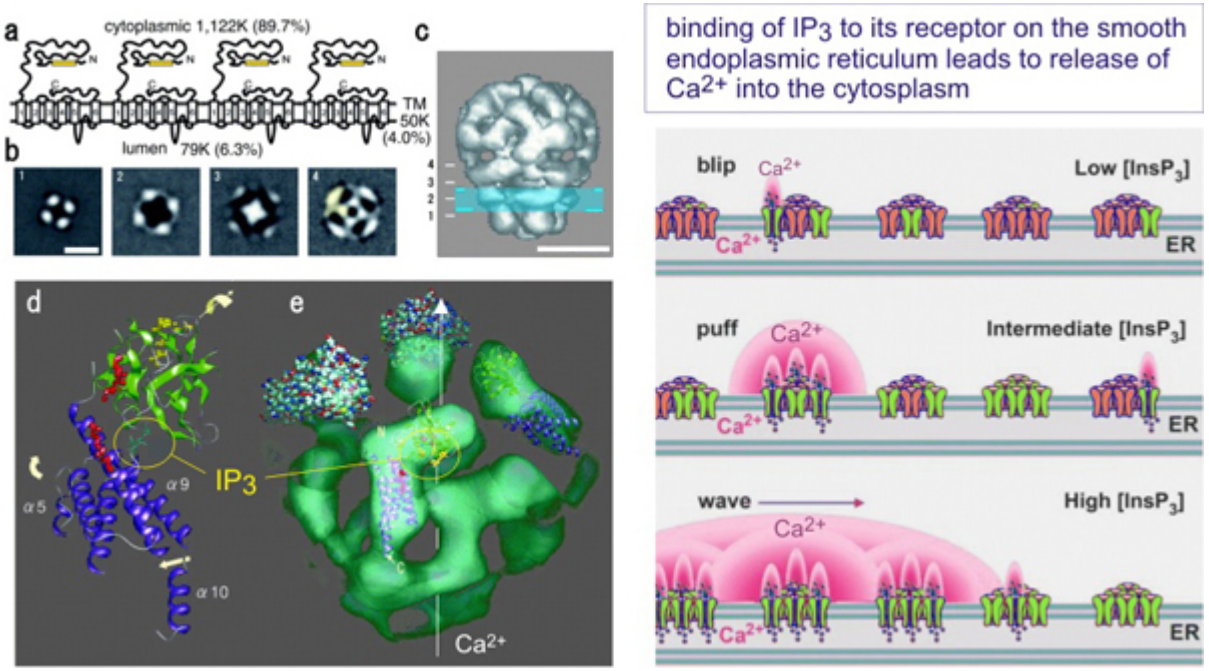


Figure 8 *Left panel: the IP3 receptor, a large multicomplex Ca^{2+} channel (1100 kDa) situated in the membrane of the smooth endoplasmic reticulum (in which Ca^{2+} is stored). Right panel: depending on the concentration of IP3, the Ca^{2+} is release in the form of blips, puffs or waves, leading to a local or more generalized increase in intracellular free Ca^{2+}.*
Left images adapted from Sato et al. J Mol Biol 2004;336:155-164.

The intracellular free Ca^{2+} will bind to Ca^{2+}-binding proteins of which calmodulin (CaM) is abundantly present in cells. The Ca^{2+}/Calmodulin complex (loaded with two Ca^{2+} ions) interacts with numerous proteins; calmodulin serves as an intermediate protein that transmits the Ca^{2+} signal to a broad set of effector proteins. One such effector protein is the Ca^{2+}/Calmodulin kinase kinase (CaMKK). Binding leads to its activation, meaning, rendering the protein kinase competent to transfer phosphate from ATP onto a substrate (phosphoryl transfer reaction or phosphorylation). In the context of ghrelin signalling, the substrate of CaMKK is the 5'-AMP-dependent protein kinase AMPK-α (figure 9). This protein kinase, in turn, phosphorylates and activates the GTPase complex TSC1/2. Because of this, the small G-protein Rheb is kept in a resting (GDP-bound) state and this causes inactivation of the mTOR protein kinase complex (yet another kinase in the cascade). From here on matters are not yet investigated but inactivation of mTOR leads to an orexictic signal.

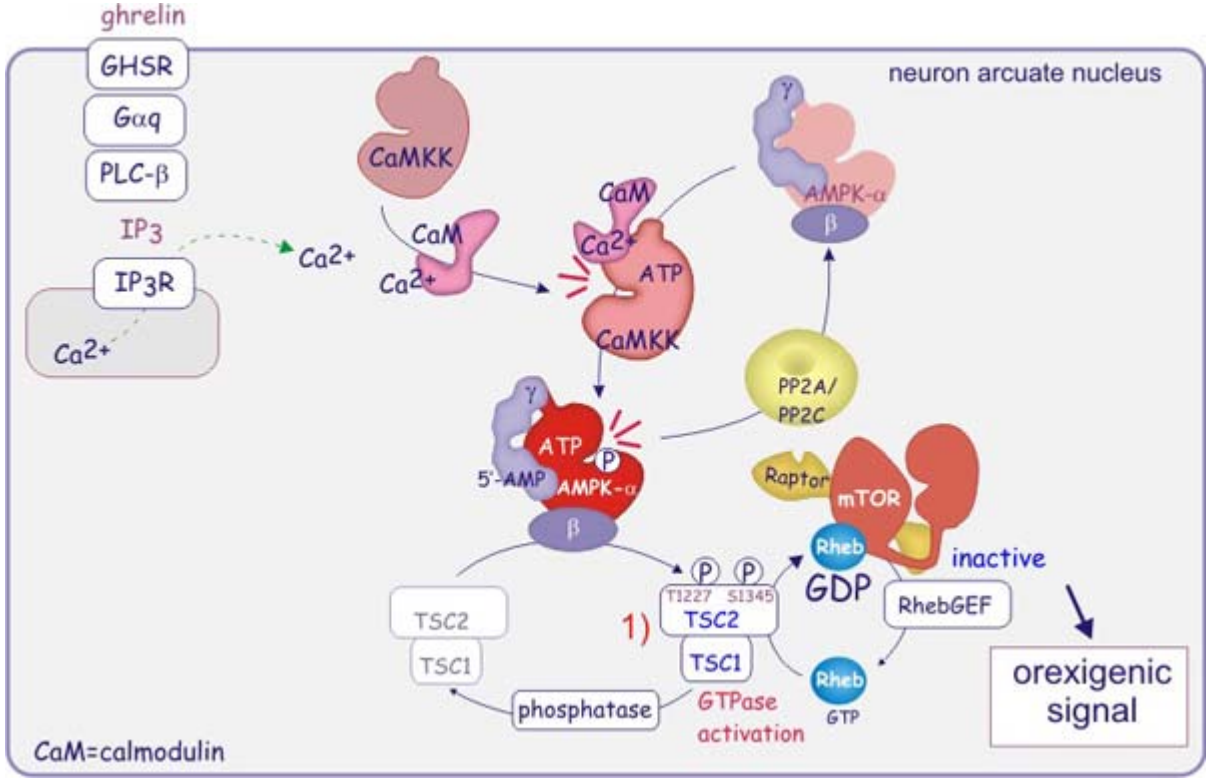


Figure 9 *Ca^{2+}-mediated inactivation of mTOR gives rise to an orectic (food-seeking) signal. What follows after inactivation of mTOR is not known.*

AMPK

The AMP-dependent protein kinase AMPK is also known as the "sensor of fuel and energy status" in skeletal muscle. AMPK senses the presence or lack of ATP through the intermediate of AMP. AMP is generated in an attempt to quickly restore ATP levels, during for instance the onset of exercise, in the reaction ADP + ADP -> ATP + 5'-AMP. This reaction, catalyzed by myokinase (or adenylate kinase), occurs in the inter-membrane space of the mitochondria. 5'-AMP (not to be confused with cyclic-AMP!) leaks out, enters the cytoplasm and binds the γ-subunit of AMPK. This exposes the catalytic subunit to other protein kinases, which render it competent through phosphorylation of the activation segment. The best known activating-protein kinase in skeletal muscle is LKB1 (figure 10). Recent studies have shown that CaMKK is another candidate in other cell types.

Elevated AMPK activity has three important consequences: 1) elevated basal uptake of glucose with excessive glycogen storage as a result; 2) stimulation of mitochondrial β-oxidation of fatty acids (into acetyl-CoA, which is used in the Krebs cycle) thus boosting ATP production and 3) inhibition of the protein kinase mTOR, thereby reducing protein synthesis (preventing increase in muscle mass). We will detail the mechanism by which AMPK stimulates β-oxidation in the paragraphs below. Somehow, in the arcuate nucleus, stimulation of this metabolic pathway leads to induction of food-seeking behaviour.

1. Review; Hardie DG, Sakamoto K. AMPK: a key sensor of fuel and energy status in skeletal muscle. Physiology (Bethesda). 2006;21:48-60.

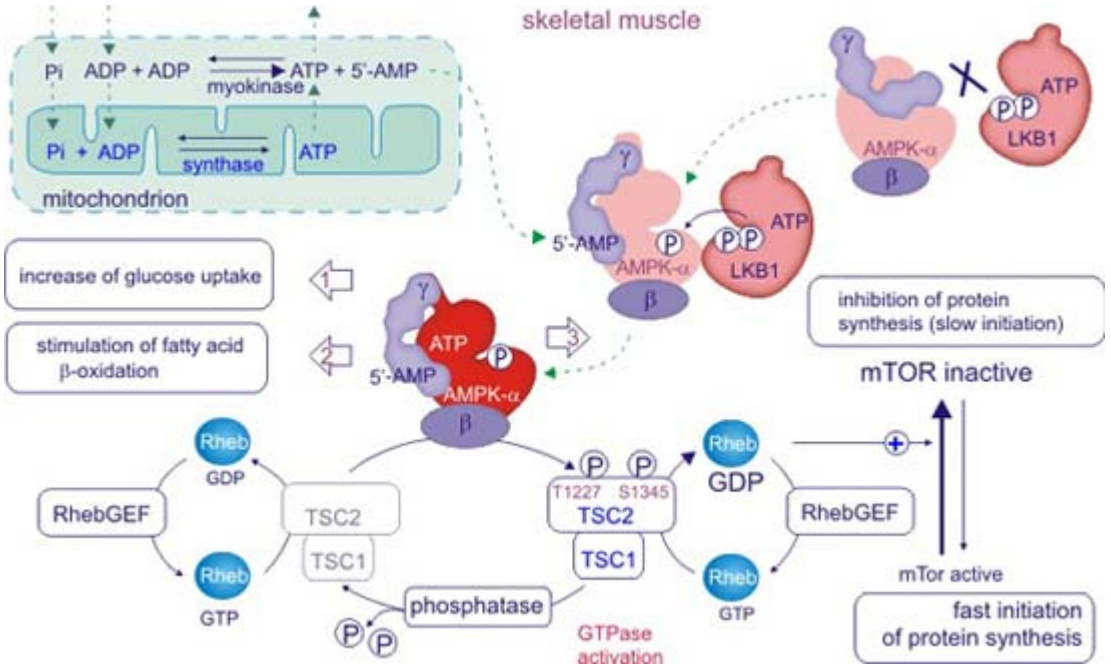


Figure 10 *AMP-dependent protein kinase (AMPK) as sensor of ATP content in skeletal muscle cells. Increased usage of ATP (by myosin) leads increased levels of 5'-AMP which binds to AMPK. The catalytic domain of the kinase becomes exposed to the upstream "activating-protein kinase" LKB1. Activation of AMPK causes; 1) increased uptake of glucose; 2) stimulation of β-oxidation (to boost ATP production) and; 3) inactivation of mTOR (which amongst many yet unknown events, leads to inhibition of protein synthesis).*

mTOR and Rapamycin

Mammalian Target Of Rapamycin (mTOR) has recently reached great public interest with the finding that its inhibition by Rapamycin increases the life span of ageing mice by up to 38% (news issues in the The New York Times, "Antibiotic delayed aging in experiments with mice", and in BBC News, "Tests raise life extension hopes", both released on july 8th, 2009. For more information see reference of Harrison et al. (below).

Rapamycin is a product of the bacterium streptomyces hygroscopicus (belongin to the actinomycetales, which are bacteria that grow in branching filamentous structures). The Streptomyces strain from which it was isolated was recovered from a soil sample from Easter Island (famous for its monumental statues), also known, in m�ori, as Rapa Nui (big island), hence the name Rapamycin. It is a macrolide and its formal name is Sirolimus. It has antifungal properties but is mainly employed as an immunosuppressant, used to prevent rejection after organ transplantation. It also prevents proliferation of body cells. Its cellular receptor is the protein FKBP12 (a 12kDa protein originally identified as the intracellular receptor for FK506, FK-binding protein, another immunosuppressant but with a different target (named calcineurin)). The complex Rapamycin/FKBP12 targets the protein kinase mTOR. The interaction occurs at a specific domain which has been named FKBP12/rapamycin binding domain (FRB) and this prevents activation of the protein

kinase (by inhibiting autophosphorylation).

mTOR is a protein kinase discovered in yeast. It is a big protein, with lots of different domains (figure 11), and form a kinase amino-acid sequence point of view it belongs to the phosphatidyl inositol kinases, like the enzyme PI 3-kinase. Unlike PI 3-kinase, mTOR happily interacts with protein substrates. Its alternative names are RAFT1 (rapamycin and FKBP12 target) or FRAP (FKBP12-rapamycin associated protein). It occurs in two different protein complexes, one sensitive to rapamycin (mTOR-complex1, comprising mTOR-Raptor-Lst8) and one insensitive (mTOR-complex2, comprising mTOR-Rictor-MAPKAP1-Lst8). Here we deal with the mTORcomplex1 (mTOR1). We have omitted Lst8 (also known as GβL) from the figures for the sake of clarity.

References:

1. *Sabatini DM et al. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 1994;78:35-43.*
2. *Harrison et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 2009;460:392-395.*
3. *Banaszynski LA et al. Characterization of the FKBP-rapamycin-FRB ternary complex. J Am Chem Soc 2005;127:4715-4721.*

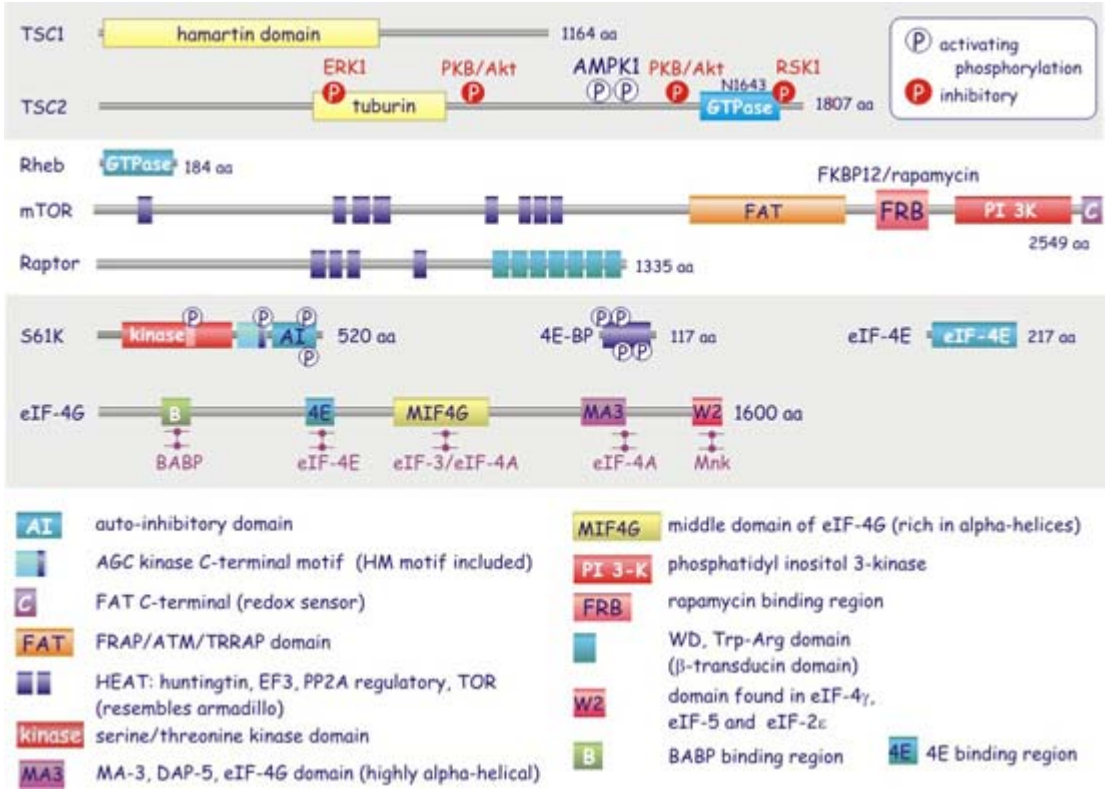


Figure 11 *domain structures of proteins involved in the regulation of the activity of mTOR and two of its downstream targets (S61K and eIF-4G), both proteins that regulate initiation of protein*

synthesis.

Tuberous sclerosis (TSC)
First described by Désiré-Magloire Bourneville in 1880 (and called Bourneville's disease in France). TSC is a rare (~50 cases per million) multi-system genetic disease characterized by the growth of benign tumours in the brain and other vital organs including the kidneys, heart, eyes, lungs, and skin. It is caused by mutations on either of two TSC genes, 1 or 2, which encode for the proteins hamartin and tuberin respectively. These act as tumour suppressors, holding cell proliferation in check and warranting cell differentiation.

NB: PTEN, which acts in the same signal transduction pathway (opposing the action of PI 3-kinase, see figure 18), is also a tumour suppressor but loss of its function is linked to Cowden disease, in which patients have a predisposition to malignancies such as prostate cancer, glioblastoma, endometrial tumours and small-cell lung carcinoma.

Reference:

1. *Anderson KA, Ribar TJ, Lin F, Noeldner PK, green MF et al. Hypothalamic CamKK2 contributes to the regulation of energy balance. Cell Metabolism 2008;7:377-388.*

Ghrelin-mediated activation of AMPK leads to phosphorylation and inactivation of acetyl-CoA carboxylase

- The application of ghrelin in the hypothalamus leads to β-oxidation of fatty acids (degradation into acetyl Co-A) and this, in turn, leads to food seeking behaviour. Below we will describe the signalling pathway involved in ghrelin-stimulated β-oxidation. It remains unclear to us how this leads to an orectic signal.

- First a description of how glucose and acetyl-CoA carboxylase (ACC) cause a shift in fatty acid metabolism; from a catabolic to an anabolic state. Intracellular glucose is converted into pyruvate (by the glycolysis pathway) which is then transported into the mitochondria. There is enters the citric-acid cycle (Kreb-cycle), contributing to the production of citrate (and of course NADPH and CO2). An excess of citrate leaves the mitochondria through the citrate/malate exchanger. Cytosolic citrate is next converted into acetyl-CoA by ATP-citrate lyase. Accumulation of cytosolic acetyl-CoA indicates a state of "plentitude" (an abundance of cytosolic glucose in relation to ATP requirements). Accumulating acetyl-CoA then acts as a substrate for the cytoplasmic acetyl-CoA carboxylase (ACC), giving rise to malonyl CoA. This acts in two ways: 1) it serves as a precursor for the synthesis of fatty acids (palmitate) and 2) it acts as an inhibitor of the carnitine-palmitoyl transferase enzyme and therefore inhibits import of fatty acids into the mitochondria, hence preventing β-oxidation (figure 12).

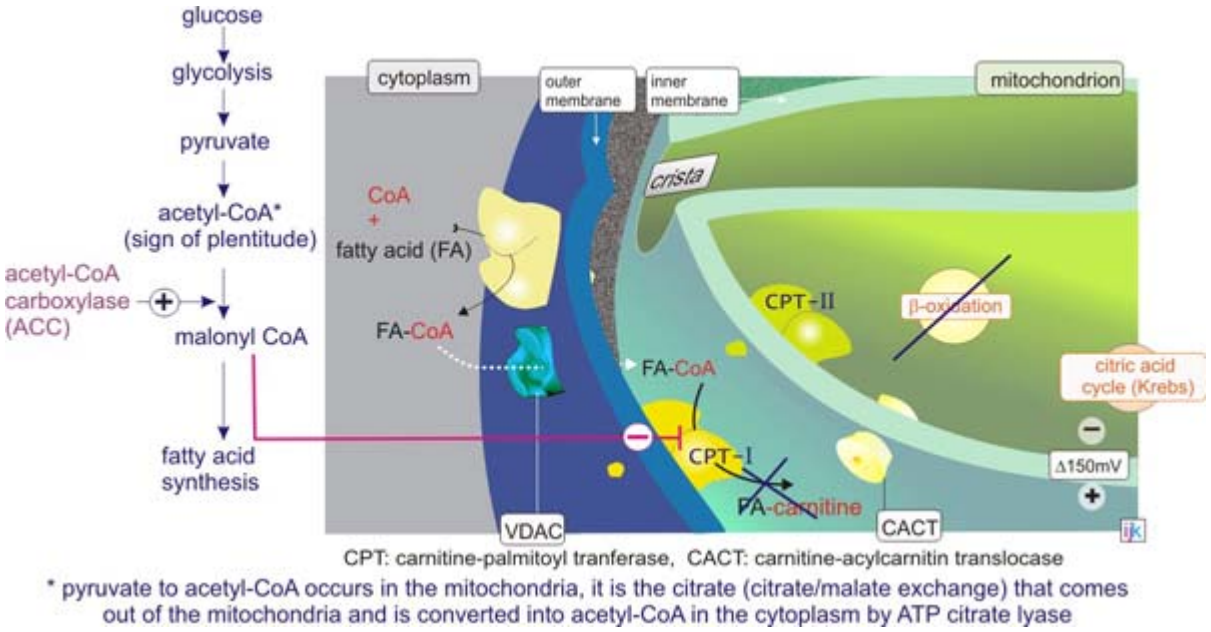


Figure 12 *High levels of glucose (and a low requirement for ATP) lead to high levels of acetyl malonyl CoA in the cytoplasm. This shifts fatty acid metabolism from a catabolic to anabolic state. Malonyl CoA is both a precursor in the synthesis pathway of fatty acids and a potent inhibitor of mitochondrial β-oxidation by inhibiting carnitine-palmitoyl transferase, thus prevening entry of fatty acids into the mitochondrial lumen.*

Ghrelin, a messenger that signals an empty stomach, reverses the situation by inhibiting acetyl-CoA carboxylase. This occurs through AMPK-mediated phosphorylation at two serine residues (S1200 and S1215). Cytoplasmic acetyl-CoA is no longer converted into malonyl-CoA. This prevents de novo synthesis and leads to a renewed uptake of fatty acids into the mitochondrial lumen, thus boosting β-oxidation (figure 13). Together, this conducts towards the emission of an orectic signal.

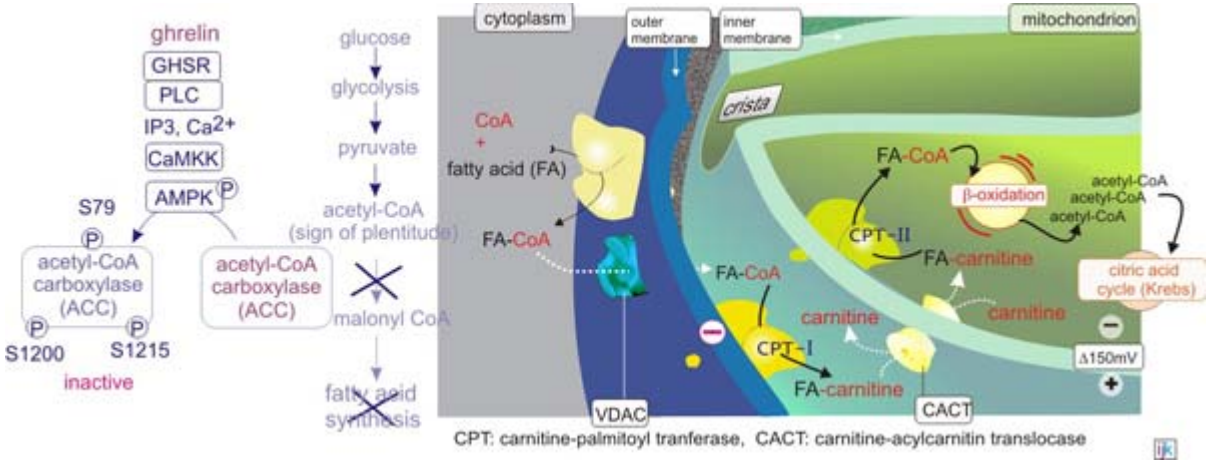


Figure 13 *ghrelin-mediated activation of AMPK leads to phosphorylation and inactivation of acetyl-CoA carboxylase. The ensuing inhibition of fatty acid synthesis and concomitant augmentation of mitochondrial β-oxidation plays a role in the generation of an orectic signal.*

References:

1. *Lopez M, Lage R, Saha AK, Perez-Tilve D, Vazquez MJ et al. Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. Cell Metabolism 2008;7:389-399.*
2. *Lam TKT, Schwartz GJ, Rossetti L. Hypothalamic sensing of fatty acids (review). Nature Neuroscience 2005;8:579-584.*

Ghrelin augments the firing rate of NPY/AgRP-containing neurons and inhibits the firing of POMC neurons (anorexigenic).

What matters in neuronal circuits is the release of neurotransmitters that pass-on or block electric signals. Neurotransmitter release is determined by 1) the cellular content of neurotransmitter, i.e. production and storage in secretory vesicles, and 2) by the firing rate of the neuron that carries it. Increased firing rate, in the presence of sufficient amounts of the neurotransmitter, will increase its release. One paper has reported that addition of ghrelin to hypothalamic slices (from mice and rats) augments the firing rate of Npy/AgRP-containing neurons (orexigenic) whereas it inhibits that of POMC neurons (anorexigenic) (figure 14). The inhibition of POMC neurons occurs most likely through the concomitant release of GABA from Npy/AgRP neurons. GABA acts through activation of Cl- permeant receptors, leading to membrane hyperpolarization (thus rendering the POMC neuron resistant to the induction of action potentials).

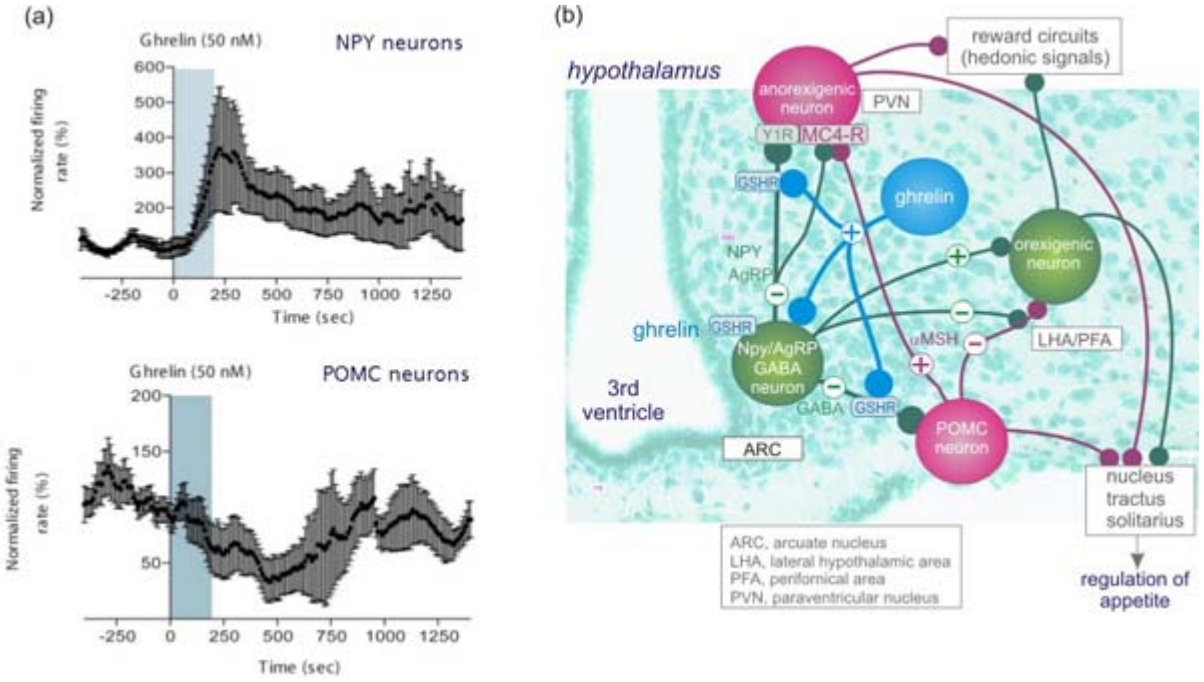


Figure 14 *(a) Ghrelin augments the firing rate of Npy/AgRP-containing neurons (orexigenic) and reduces firing of POMC neurons (anorexigenic). Image from Cowley et al. Neuron 2003;37:649-661.*
(b) A reminder of the neuronal circuit in the hypothalamus. Inhibition of POMC neurons is most like to occur through an increased release of GABA which, through its action on Cl- permeant channels, hyperpolarizes the membrane potential of POMC neurons (see also figure 13 of the web page of team 2)

Reference:

1. *Cowley MA, Smith RG, Diano S, Tschöp M, Pronchuk N, Grove KL et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron 2003;37:649-661.*

Signalling by insulin

Insulin mediated-receptor activation

Insulin is a small peptide of two amino-acid chains hold together by disulphide bridges. It binds to the insulin receptor, a dimeric transmembrane protein, composed of two subunits (α-chain and β-chain) (figure 15). It qualifies as a receptor protein tyrosine kinase (RPTK) because of the presence of a tyrosine protein kinase domain in the intracellular segment. Both the α- and β-subunits and the two monomers are kept together by disulfide bridges. Despite the presence of two binding sites, only one insulin molecule seems to fit the receptor dimer.

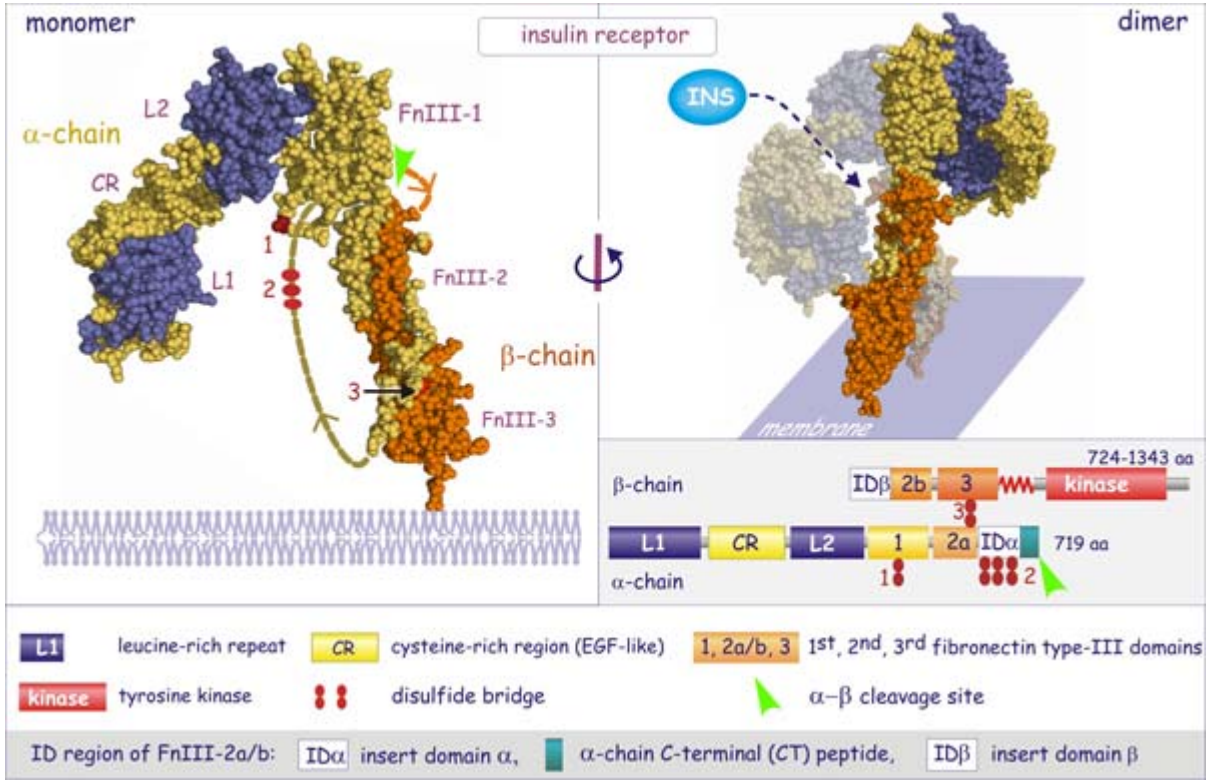


Figure 15 *Upper panels; structure of the extracellular domain of the insulin receptor. Each receptor monomer is made up of two chains, α and β (encoded by the same gene), which are hold together by extensive surface contact (yellow and orange domains) and a disulfide bridge. Two monomers constitute the functional receptor which provides a binding site for one insulin molecule. Lower panels: domain architecture of the insulin receptor monomer and explanation of the domain abbreviations. The interdomain region (ID) is composed of IDα, IDβ and the a-chain C-terminal peptide (which contains the chain cleavage site).*

The cytosolic segment of the insulin receptor comprises a tyrosine protein kinase. Binding of insulin leads to phosphorylation and activation of the tyrosine protein kinases. The phosphorylated protein acts as a docking site for numerous adaptor and docking proteins. These serve to attract yet other proteins, forming a receptor signalling complex, which, collectively, will assure the transmission of the insulin message into the cytoplasm. It is not clear yet how insulin brings about mutual phosphorylation of the two tyrosine protein kinases. Perhaps the protein kinase domains are kept apart in the non-occupied receptor (see figure 16).

Formation of a receptor signalling complex

Importantly, the phosphorylated receptor recruits a docking protein (Insulin receptor substrate-1 or IRS1) which, in turn, gets phosphorylated on numerous tyrosine residues. One of these phosphotyrosines binds the regulatory subunit (p85) of an enzyme named phosphatidylinositol 3-kinase (PI 3-kinase) (figure 16a). The elaborate cascade of phosphorylations serves to: 1) recruit a lipid kinase to the membrane (next to its substrate the inositol lipids) and 2) to undo the inhibitory action of its regulatory subunit (p85).

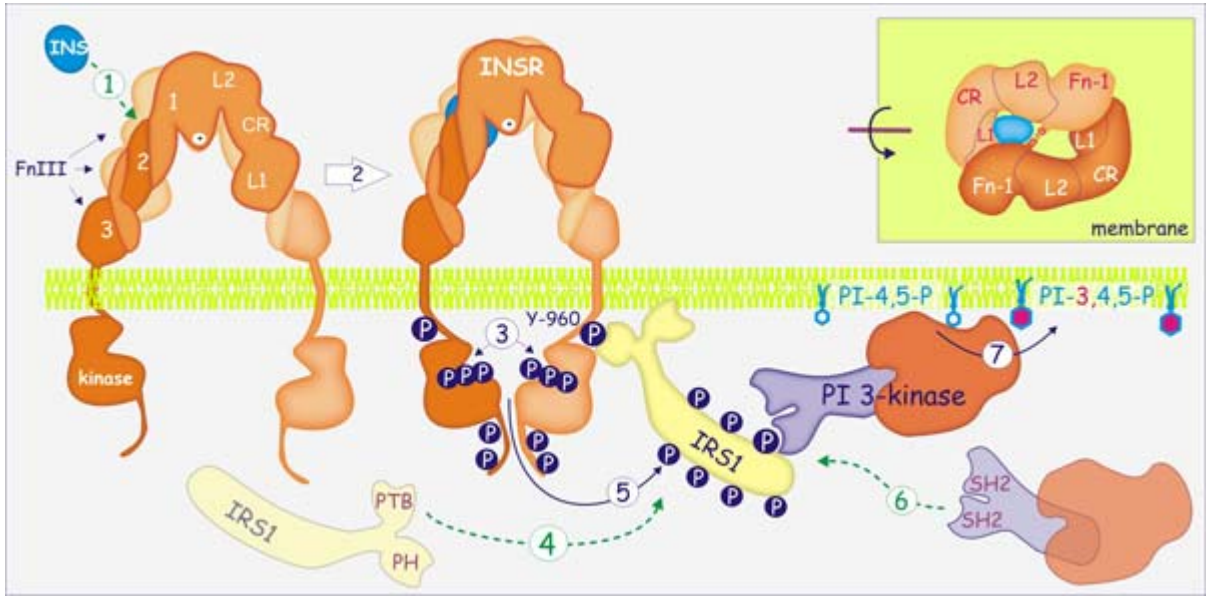


Figure 16a *Receptor signalling complex formation. Insulin binding to the receptor dimer (1) induces a conformational change (2) that causes transphosphorylation of the activation segment of the protein kinase domain at three tyrosine residues (3). Further phosphorylation follows on both sides of the catalytic domain. The IRS-1 binds pY960 with its PTB domain (4). Phosphorylation on further tyrosine residues follows (5)*

generating a docking site for the SH2 domain of the p85 regulatory subunit and activation of PI 3-kinase (6) leading to the phosphorylation, at the 3-position, of inositide lipids (7).

The signalling complex gives rise to a wide range of intracellular signals (see figure 16b) with a wide range of downstream effectors involved in a wide range of cellular responses. Here we simplify matters and present one linear pathway only leading to the activation of protein kinase B.

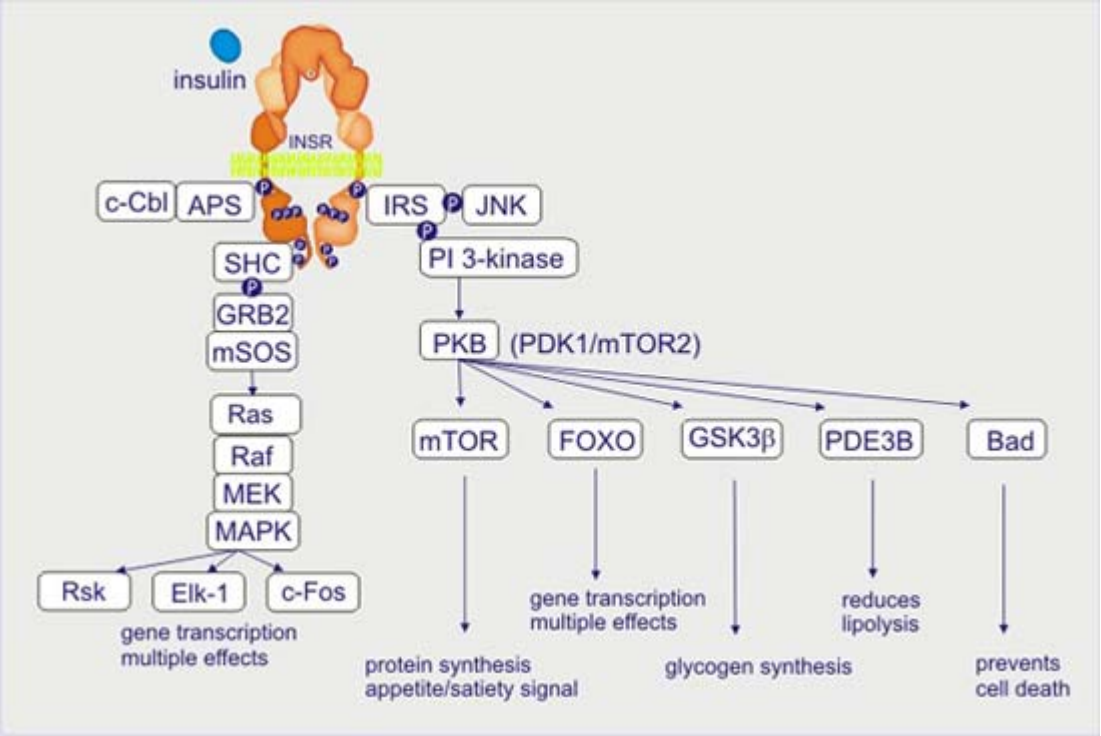


Figure 16b *multiple signals emanate from signalling complex attached to the activated insulin receptor*

PI 3-kinase mediated activation of protein kinase B (PKB)

Despite its affinity for inositol lipids, PI 3-kinase classifies as a classical protein kinase (based on its amino-acid sequence and tertiary structure). It forms a subfamily of protein kinases, divided into type 1, type 2 and type 3 (figure 17). The one activated by the insulin receptor (via IRS1) is a type I PI-3kinase.

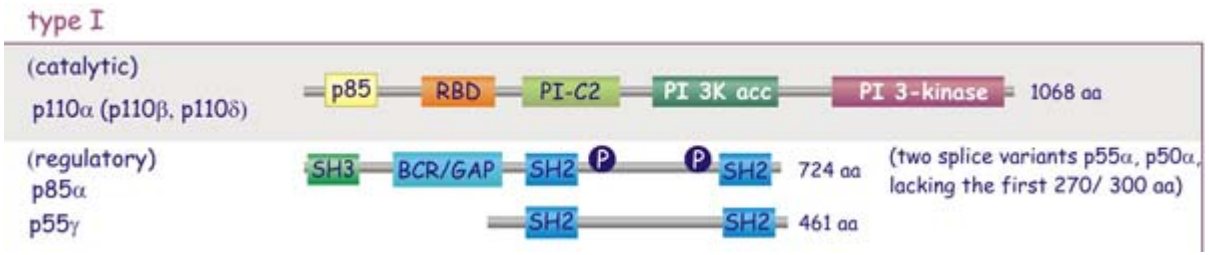
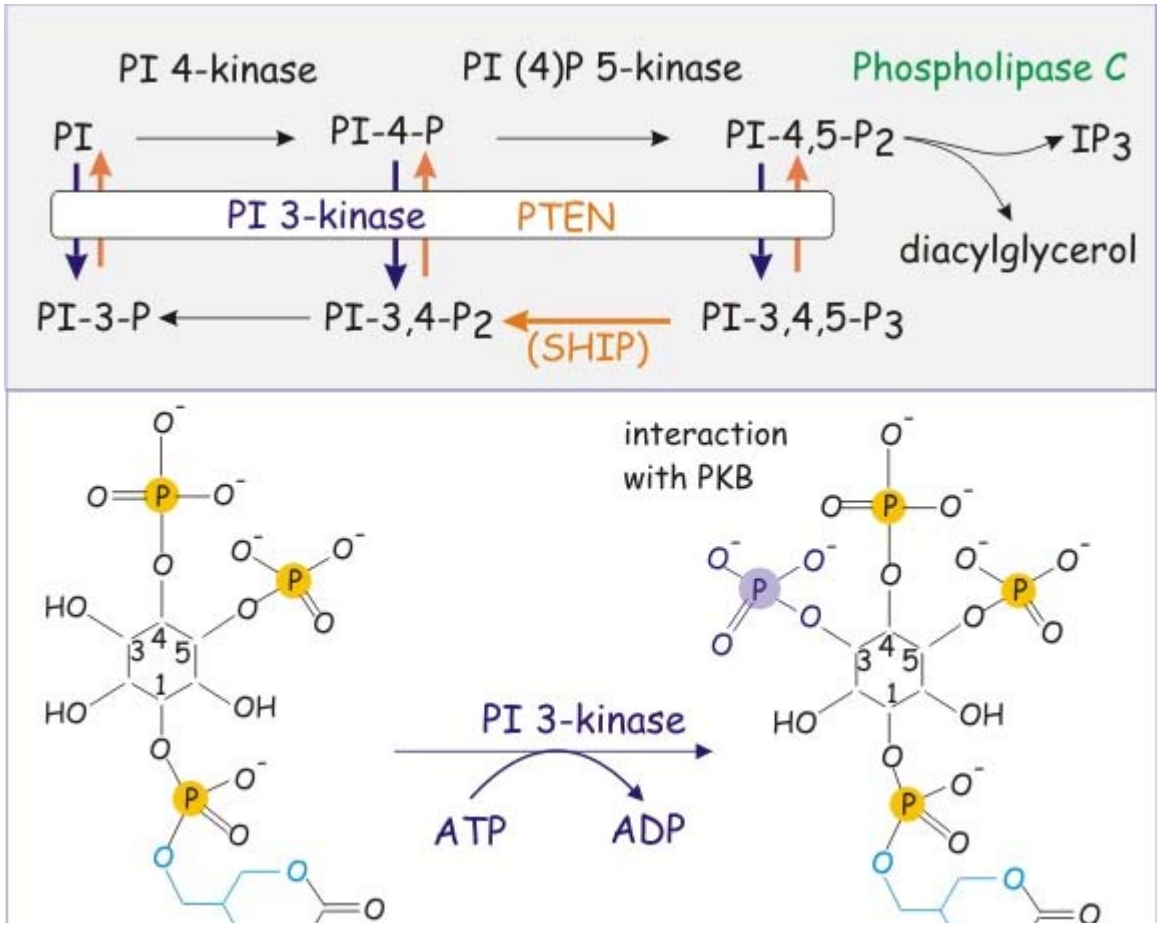


Figure 17 *domain architecture of type I PI 3-kinase (catalytic and regulatory domains). Click on the image (17b) to see the domain architecture of all members of the PI 3-kinase family.*

PI 3-kinase tranfers a phosphate (from ATP) to phosphatidyl-inositol at the 3'-position of the inositol ring (figure 18). The ensuing PI-3,4-bisphosphate or PI-3,4,5-trisphosphate act as binding sites for proteins carrying a Pleckstrin-Homology (PH) domain. Two such proteins are the protein kinases PKB and PDK1, both of which prefer PI-3,4-bisphosphate and both are recruited to the membrane where they meet each other. This meeting has important consequences for PKB.



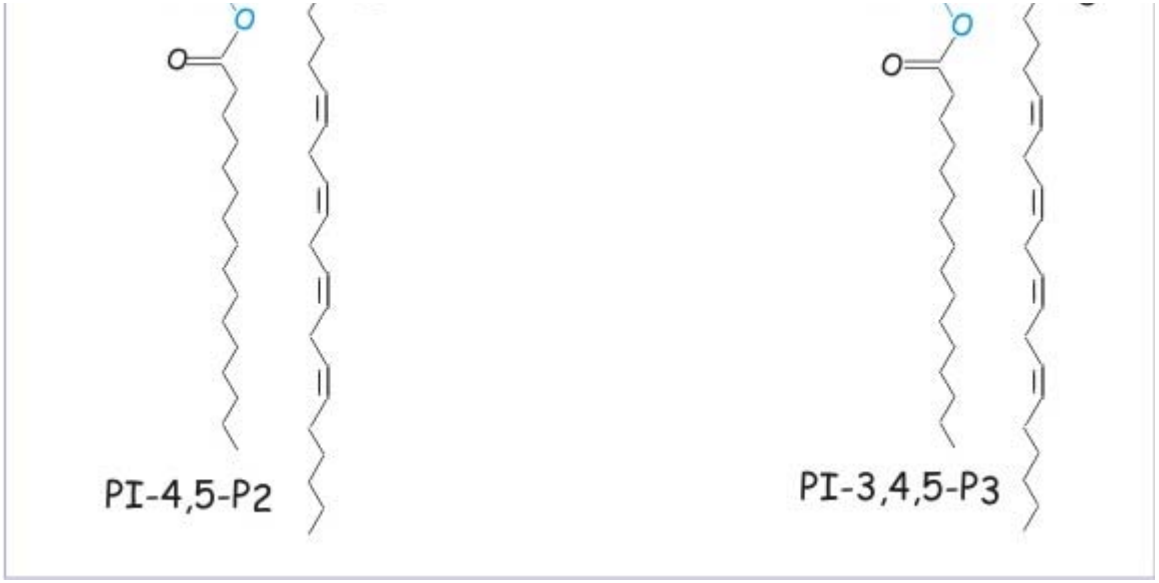


Figure 18 *Phosphoinositide metabolism and the structure of PI-3,4,5-trisphosphate. A reaction scheme outlining the substrates and endproducts of PI 3-kinase. PTEN and SHIP, in yellow, are phosphatases. PI 3-kinase phosphorylates the inositol ring at the 3′ position.*

PKB occurs in a non-active state. Both the coordination of ATP binding and the structural requirements for interaction with substrate are inappropriate for a transfer of phosphate (kinase reaction). PKB requires two modifications to become a competent protein kinase. Firstly it needs phosphorylation of a hydrophobic motif at the C-terminal. This occurs through the mTOR2 (complex 2, see above) but it is not yet clear how this is regulated. (NB: at the start of investigations of PKB activation, the putative protein kinase that phosphorylates the hydrophobic motif was named PDK2 (phosphoinositide dependent protein kinase-2)). The second phosphorylation occurs in the activation segment and this is made possible because of the mutually beneficial membrane-encounter between PDK1 and PKB (see figure 19). PKB facilitates full kinase expression of PDK1 by transiently laying its phosphorylated hydrophobic motif on the shoulder of PDK1. In return, PDK1 phosphorylates the activation segment of PKB. Once doubly-phosphorylated, PKB is less likely to remain membrane attached and is now fit to phosphorylate substrate in the cytoplasm (for as long as it does not encounter phosphatases that remove its activating phosphates).

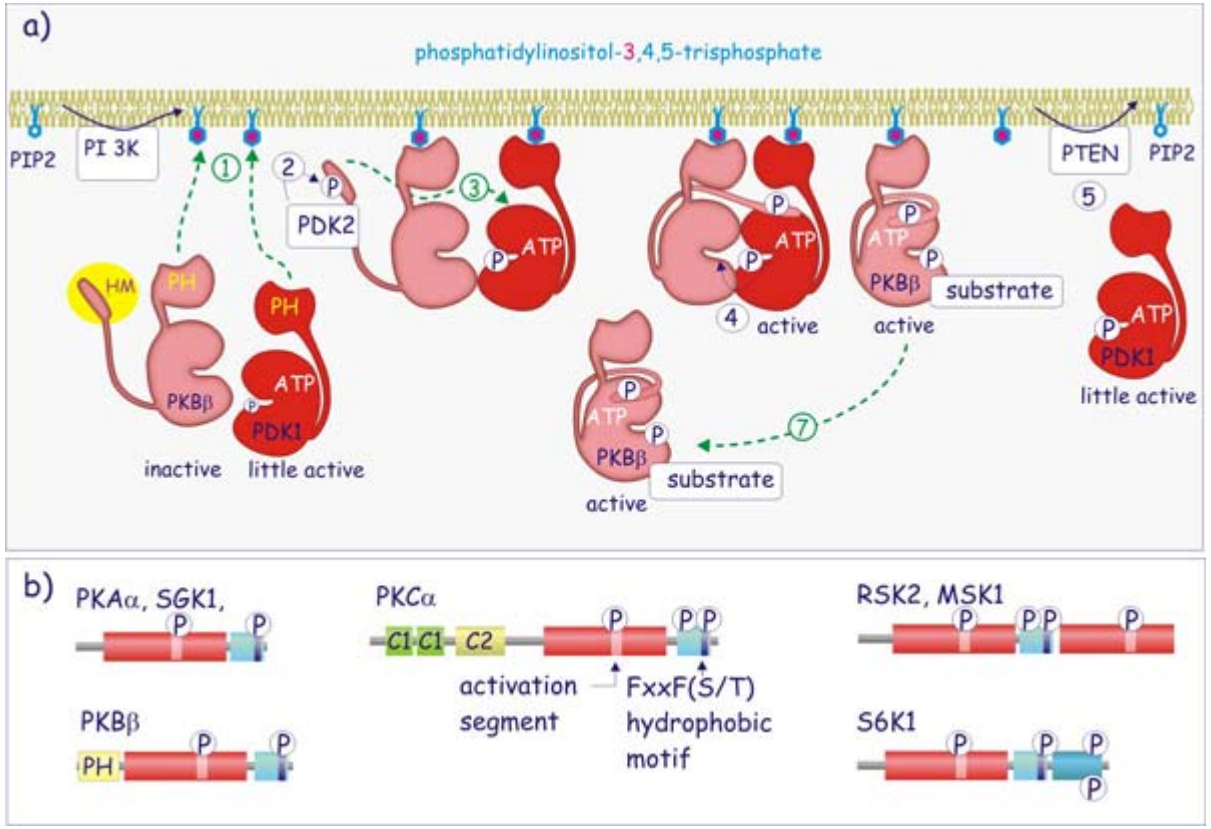


Figure 19 *(a) schematic representation of the activation mechanism of PKB. Membrane translocation of PKB and PDK1 occurs as a consequence of the production of phosphatidyl-3,4-bisphosphate (1). In this process, PKB is phosphorylated by mTOR2 (initially named PDK2). The hydrophobic motif now binds the neighbouring PDK1 and this renders the kinase fully active (3). In return PDK1 phosphorylates the activation segment of PKB (4). The doubly phosphorylated protein kinase can detach from the membrane and phosphorylate cytosolic substrates. (b) This dual kinase activation mechanism applies for a number of protein kinases, each having a hydrophobic motif with serine or threonine phosphorylation sites. These kinases are referred to as AGC kinases (PKA, PKG and PKC) and for these kinases PDK1 acts as the master switch. Note that PKB is unique in carrying a PH domain.*

PKB and Akt

Protein kinase B was discovered as a protein kinase with 73% sequence similarity to PKCε and 68% similarity to PKA and was therefore named PKB. It was also discovered as a viral oncogene of the acutely transforming retrovirus AKT8 and was therefore named v-akt. Three closely related variants of PKB exist. They are named PKBα, β and γ and are the same as Akt1, 2 and 3. In the SwissProt database they come under the name of the RAC subfamily of protein kinases (for Related to A and C protein kinase).

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PKB-mediated phosphorylation of FOXO1 and TSC1/2

In the context of regulation of food intake, two important substrates will be discussed below; the transcription factor FOXO1 and the GTPase activating protein TSC1/2.

1. FOX01, for forkhead box protein 1(also known as FKHR), is a transcription factor that normally shuttles between the nucleus and the cytoplasm. It plays an important role in the regulation of expression of pomc (gene coding for the precursor protein POMC), which is inhibited by FOXO1, and agrp (gene coding for AgRP), which is stimulated. When FOXO1 resides in the nucleus, it contributes to an orectic signal (apetite) (figure 20). Upon addition of insulin and subsequent activation of PKB, FOXO1 gets phosphorylated on two residues (T24 and T256) and this causes it to bind to the 14-3-3 protein which sequesters PKB into the cytoplasm. Loss of nuclear localization leads to a loss of agrp expression, and an increase in the expression of pomc, shifting the balance from orectic to anorectic signals (leading to fasting) (figure 21) (to refresh your memory see the section about the arcuate nucleus, target of ghrelin, lepmtin and insulin of team 2).

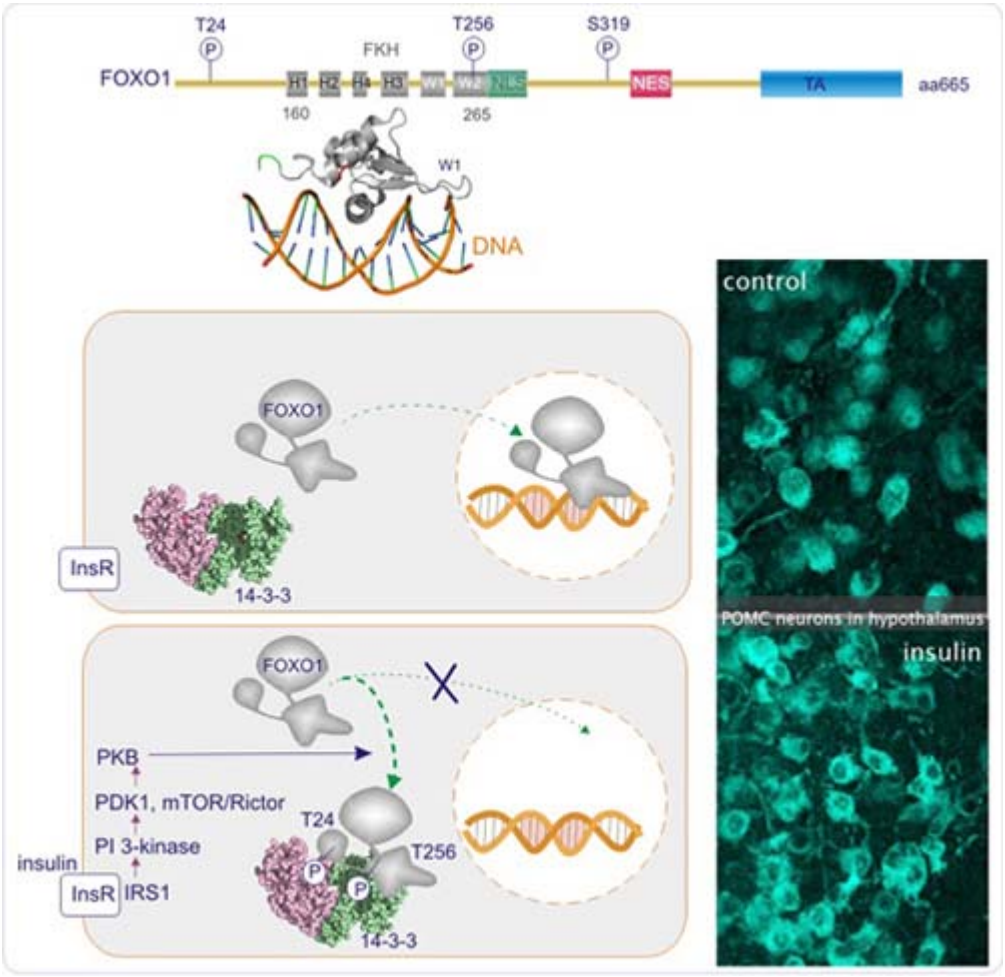

Figure 20 *Upper panel; domain architecture of FOXO1 and structure of the conserved forkhead segment (FKH, comprising H1-H4) bound to DNA. Lower panels; FOXO1 exists both in cytoplasm and in the nucleus (immunochemical staining of FOXO1 shows fluorescence in nucleus and fain staining in the cytoplasm). Addition of insulin leads to PKB-mediated phosphorylation which, in turn, leads to 14-3-3-mediated sequestration of FOXO1 in the cytoplasm (immunochemical staining shows nuclear exclusion, the fluoresence signal resides mainly in the cytoplasm).*

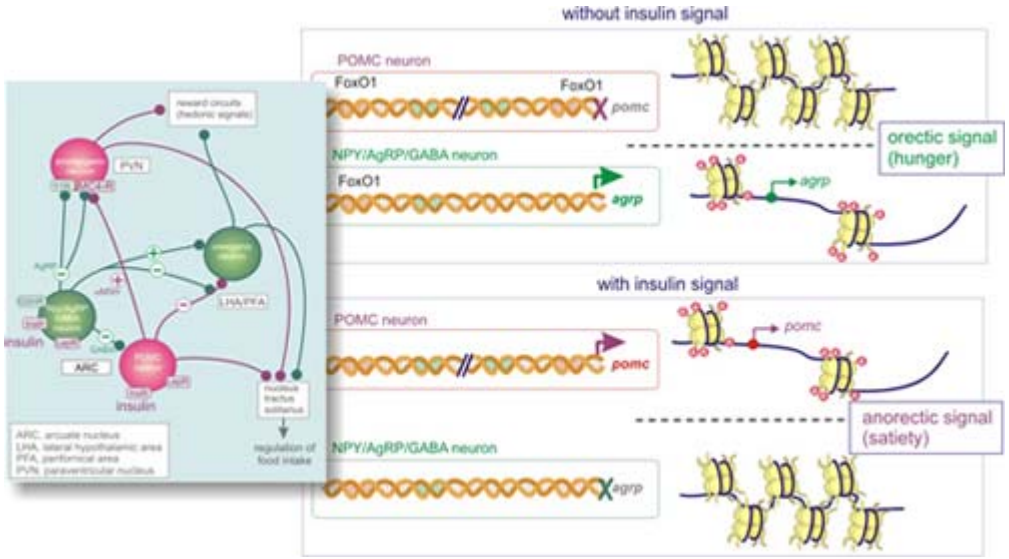

Figure 21 *Insulin shifts the balance of expression of neurotransmitters from a predominant orexigenic AgRP to a predominant anorexigenic POMC (leading to the production of a-MSH)*

2. TSC1/2 is a Rheb GTPase activating protein already discussed in the section about ghrelin. When phosphorylated on T1227 and S1345 by AMPK it becomes active whereas phosphorylation by PKB, on residues S939 and T1462, renders it inactive. Inactivation of TSC1/2 leads to accumulation of Rheb in its GTP bound state. This in turn brings about the activation of mTOR2 which, through an unknown mechanism, provides an anorectic signal (figure 22).

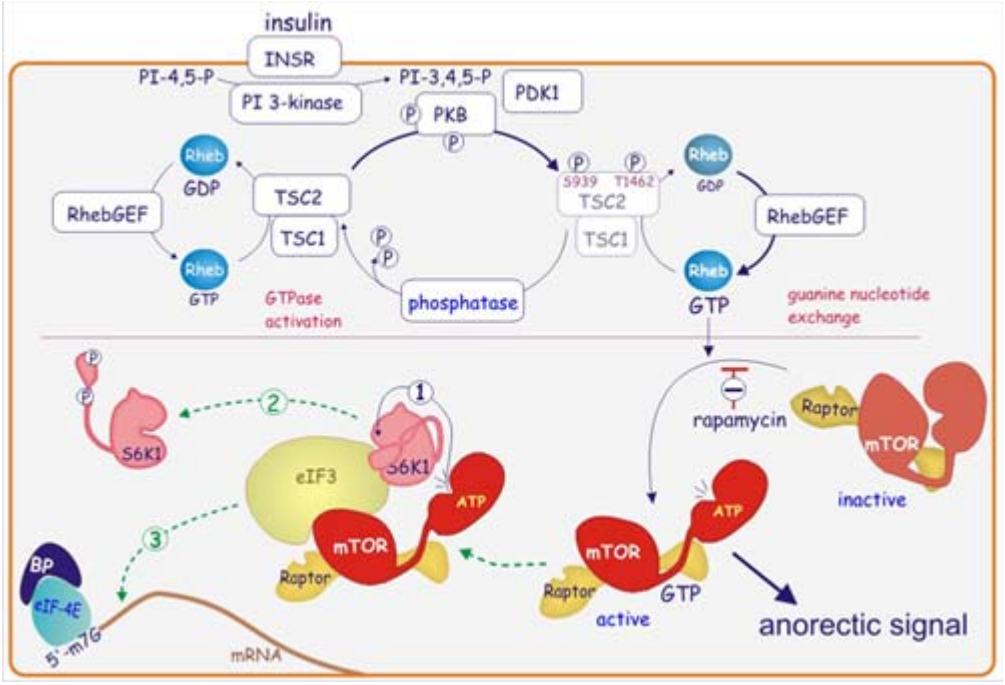

Figure 22 *Activation of PKB leads to phosphorylation and inactivation of TSC1/2 (GTPase activating protein). As a consequence Rheb accumulates in its GTP bound state and this causes the activation of mTOR1 (complex of mTOR, Raptor and Lst8). The orexigenic action of mTOR2 remains to be elucidated.*

Signalling by leptin

Leptin, from the Greek leptos, meaning thin, is the product of the obese gene (meaning, if mice lack expression of the gene product they get fat). Leptin is anorexigenic and congenital leptin deficiency in humans is associated with voracious appetite, morbid obesity, immunosuppression and hypothalamic (secondary) hypogonadism. Click here to see the effect of leptin on the Tasmanian devil (figure 23A).

Leptin is a 167 amino-acid peptide (small protein), which, with respect to its structure resembles, amongst others, interleukin-6 and human growth hormone. It is produced by adipose tissue (see leptin section of team 2). It binds to the Leptin receptor, but productive binding requires a co-receptor which has yet to be identified (figure 23). Five receptor isoforms have been identified, LRa-e. LRa lacks an intracellular domain and is abundantly expressed in brain capillary endothelium and peripheral organs. It is proposed to mediate leptin transport across endothelial cells. LRb is restricted to the hypothalamus, brainstem and key regions of the brain involved in the control of feeding, metabolism and neuroendocrine systems.

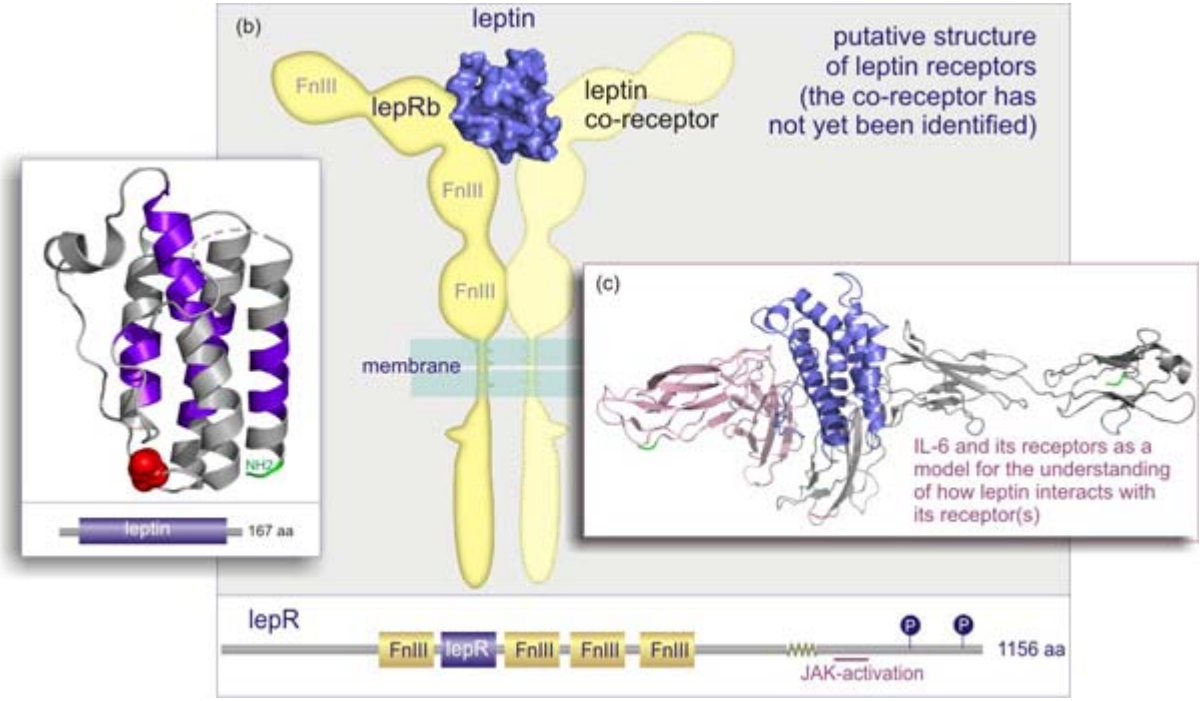

Figure 23 *(a) structure, a 4-helix bundle, and domain architecture of leptin (pdb: 1ax8) (b) leptin binds two transmembrane receptors; the leptin-receptor and an as yet to be identified second protein (co-receptor). The domain architecture reveals fibronectin domains and a unique leptin receptor domain. The leptin receptor reveals no catalytic activity in the cytosolic segment but has the capacity to bind the tyrosine protein kinase JAK. (c) IL-6 binding to its receptors act as a model for the understanding of how leptin works.*

Dimerization of the leptin receptor and its co-receptor lead to activation of the cytosolic tyrosine protein kinase JAK (janus kinase). This binds the receptor with its FERM domain and is normally kept in an auto-inhibited state (figure 24). Following dimerization, a change in the orientation of the intracellular segment of the receptor is anticipated which, in turn, leads to a liberation of the functional kinase domain. A transphosphorylation ensues (in the activation segment) and the active JAK kinase now phosphorylates the leptin receptor-b (LepRb) on three tyrosine residues (Y985, Y1138 and Y1077).

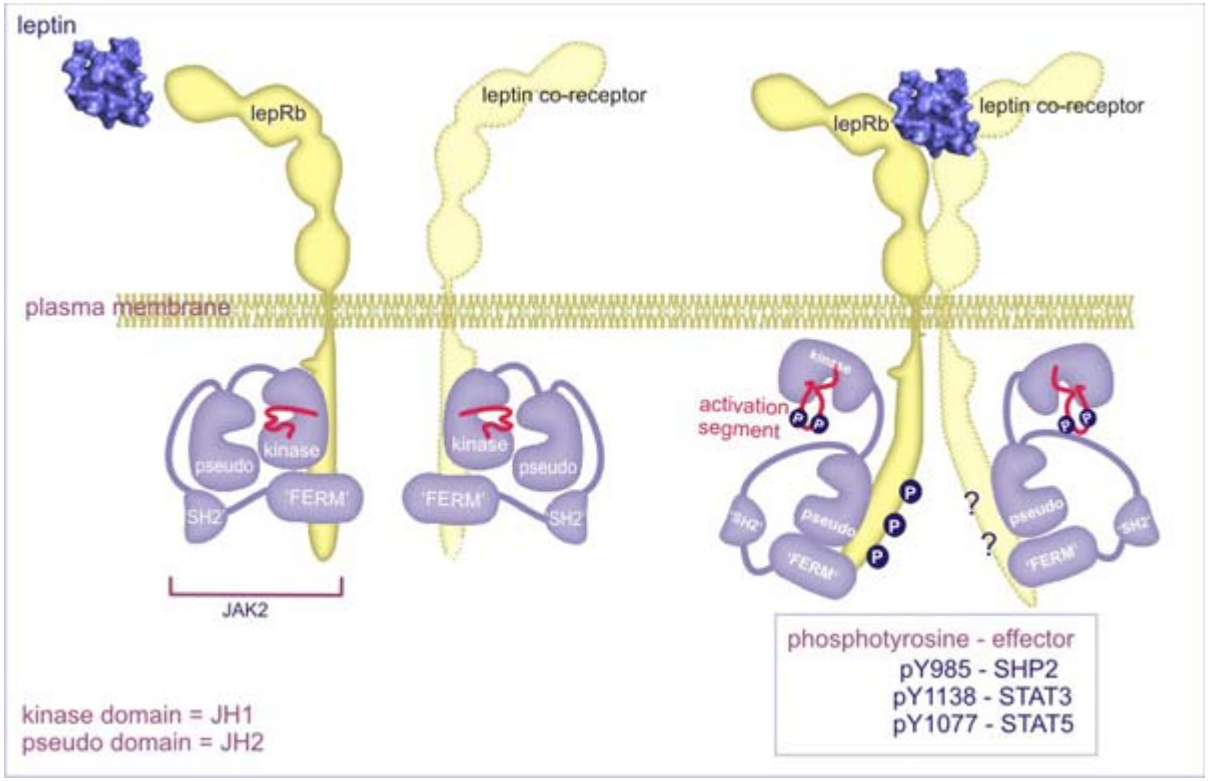

Figure 24 *leptin binding leads to a reorientation of the intracellular segments of the receptor (and co-receptor). This leads to liberation of the auto-inhibitory constraint imposed by the pseudo kinase domain of JAK2. Once liberated, transphosphorylation of the activation segment follows (one kinase phosphorylates the other), turning JAK2 fully competent to phosphorylate substrates such as the LepRb. The phosphotyrosines act as docking site for SH2- and PTB-domain containing proteins.*

JAK2 is a member of the Janus kinases (JAK1-3 and TYK2), which in turn constitute a subfamily of the big family of non-receptor tyrosine protein kinases (nrRTK) also known as cytosolic protein tyrosine kinases. JAK has two kinase domains of which only the N-terminal is functional (JAK Homology domain 1). The FERM domain binds the lepRb. For more information about this family of protein kinases click on figure 25 for an enlarged version. Chromosomal aberrations involving JAK2 are found in both chronic and acute forms of eosinophilic, lymphoblastic and myeloid leukemia.

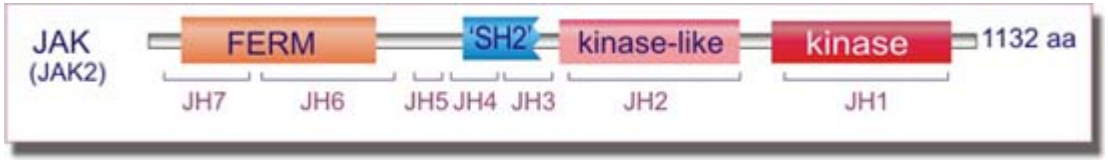

Figure 25 *domain architecture of JAK2. Note the presence of a non-functional kinase domain (pseudo kinase)*

Click on figure 25 to get an overview of the domain architecture of non-receptor tyrosine protein kinases

Leptin induces nuclear translocation of STAT3

- Phosphotyrosines and their immediate upstream or downstream amino-acids act as docking sites for proteins with an SH2- or a PTB-domain (see also the insulin receptor signalling complex above). One of these is the transcription factor STAT3 (Signal Transducer and activator of transcription), the other is the IRS2 protein (see two sections down). When recruited to the tyrosine phosphorylated receptor (by pY1138) STAT3 gets phosphorylated (on Y705 for human) by JAK2 and this leads to dimerization of the STAT3 proteins (whereby the SH2 of one binds the phosphotyrosine of the other and vice versa). The STAT3 dimers enter the nucleus and bind DNA, there to change gene transcription (figure 26).

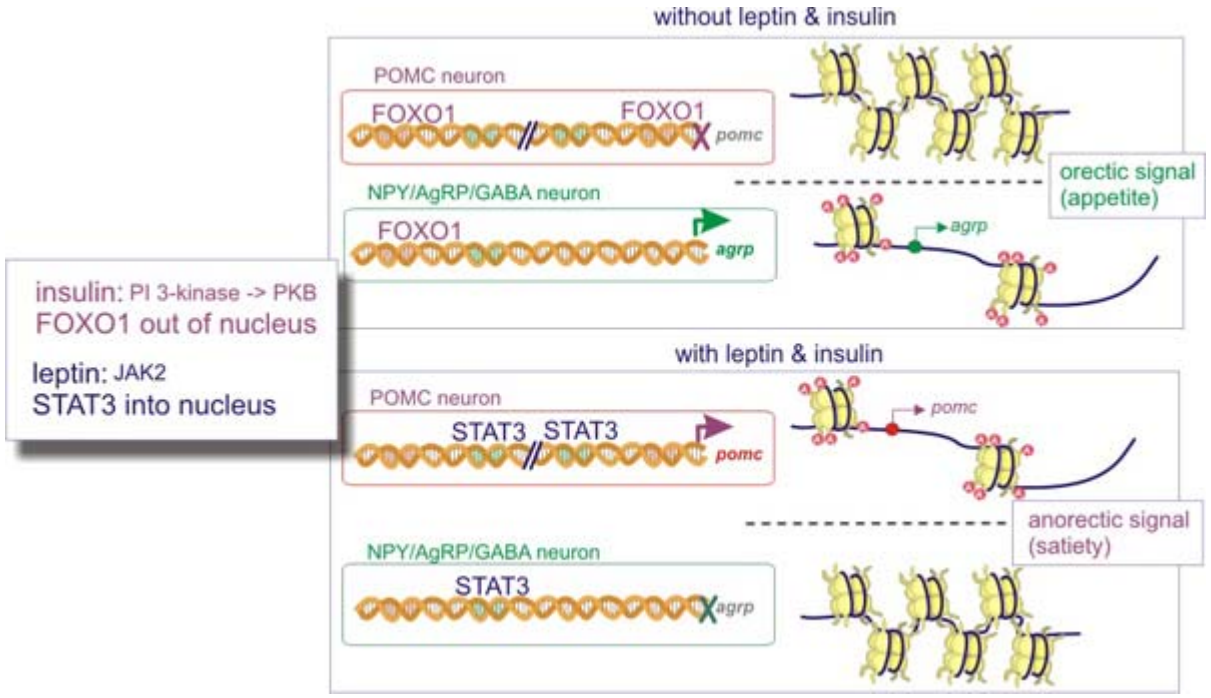

Figure 26 *STAT3, after being recruited to the leptin receptor (through binding to pY1138) gets phosphorylated by JAK2 on Y705 (human STAT3). This leads to dimerization and nuclear translocation of the transcription factor*

Leptin augments pomc expression and inhibits agrp

- The genes coding for pomc and agrp contain STAT binding sites which in the case of pomc leads to enhanced expression, DNA becomes accessible, whereas in the case of agrp the reverse occurs, DNA becomes inaccessible (figure 27). This entry of STAT3 induces a shift from a predominant

AgRP signal (orexigenic) to a predominant POMC signal (anorexigenic); leptin signalling (JAK2 to STAT3) synergizes with insulin signalling (INSR to PKB).

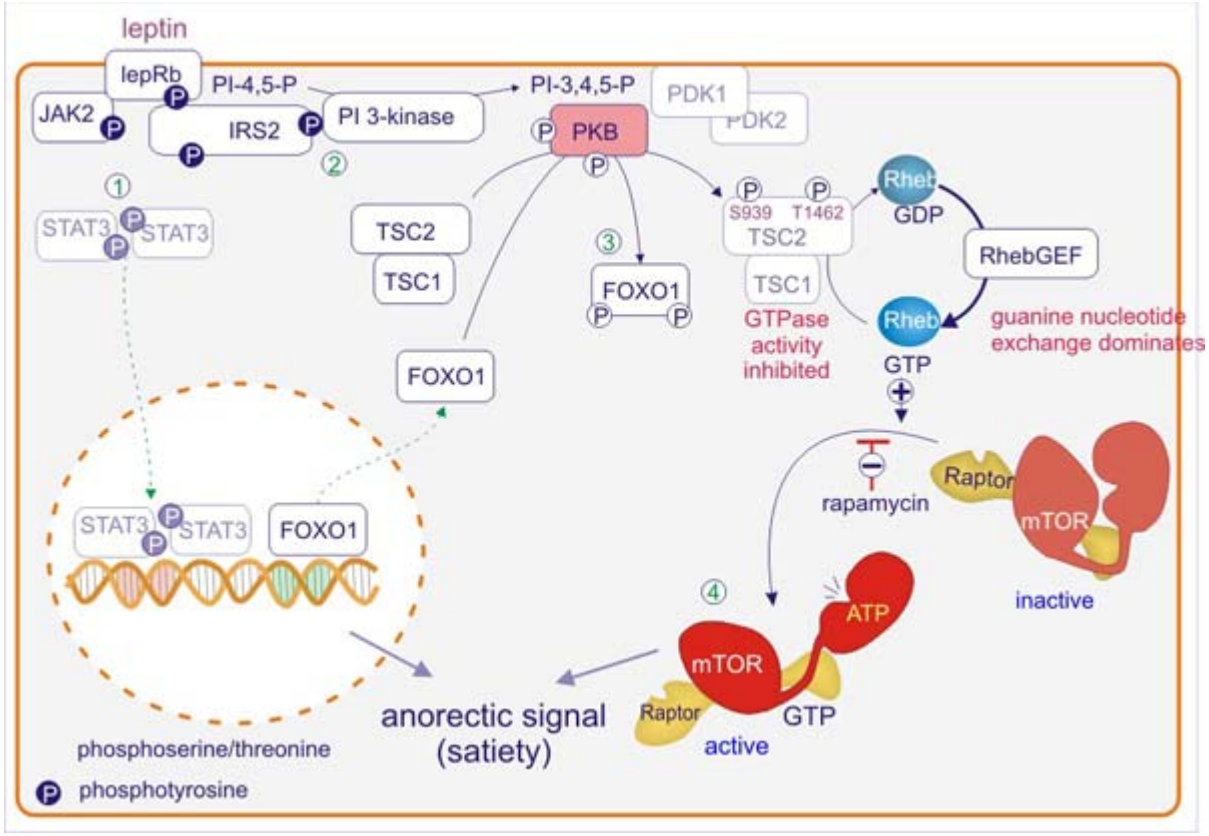

Figure 27 *Leptin signalling via JAK2 and STAT3 leads to activation of pomc expression and inhibition of agrp. Note that insulin-mediated exclusion of FOXO1 and leptin mediated inclusion of STAT3 into the nucleus act synergistically.*

Leptin, like insulin, activates the PI 3-kinase/PKB pathway in a JAK2-dependent manner

Leptin and insulin signalling pathways also synergize at the level of activation of PKB and mTOR. This occurs through phosphorylation of the docking protein IRS2 by JAK2. The ensuing tyrosine phosphoresidues bind the p85 regulatory subunit of PI 3-kinase and the same string of activities follow as described for insulin. It ends with phosphorylation and cytoplasmic retention of FOXO1, hence a block in expression of agrp, and activation of mTOR1 (mTOR, Raptor, Lst8 complex) (figure 28).

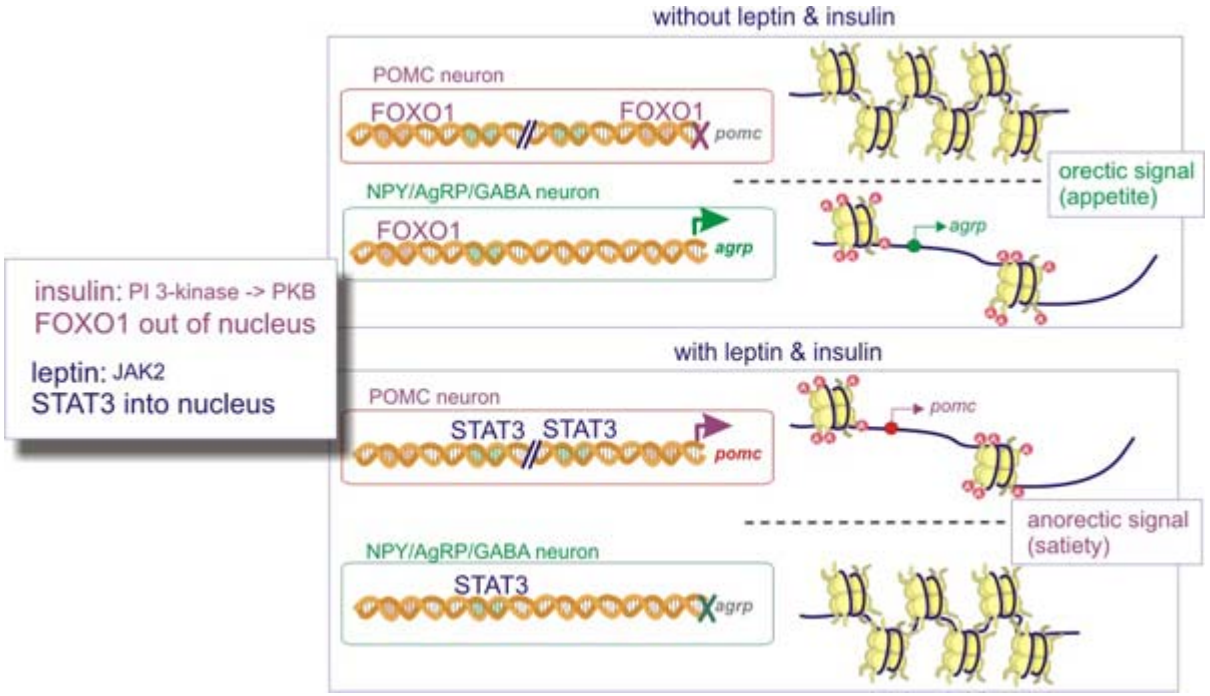

Figure 28 *leptin-mediated activation of PKB and mTOR also provide a anoretic signal leading to a sense of satiety.*

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