Universiteit
Leiden
The Netherlands

The role of PRAS40 in insulin action : at the intersection of protein kinase B (PKB/Akt) and mamalian target of rapamyein (mTOR)

Nascimento, E.B.M.

Citation
Nascimento, E. B. M. (2010, September 9). *The role of PRAS40 in insulin action : at the intersection of protein kinase B (PKB/Akt) and mamalian target of rapamyein (mTOR)*. Retrieved from https://hdl.handle.net/1887/15934

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/15934

Note: To cite this publication please use the final published version (if applicable).

Chapter 1

General Introduction

1

Preface

Diabetes is taking on epidemic proportions not only in western society. According to the World Health Organization more than 180 million cases of diabetes have been reported and as an estimate for the coming years, the number of people with diabetes will be over 375 million by the year 2030. A deeper understanding of diabetes is thus required in order to device better treatment strategies or to even prevent the onset of diabetes. In this general introduction, an overview is provided explaining how normal glucose homeostasis is accomplished through the metabolic action of insulin and how deregulation of insulin action can result in insulin resistance and diabetes. Special attention will be focused on signal transduction of the insulin signalling pathway and protein kinase B.

Glucose homeostasis

All living cells use glucose as main or only source of energy. Maintenance of physiological glucose levels is thus essential for proper energy homeostasis. Breakdown of glucose via glycolysis to acetyl groups yields ATP. Subsequently, the remaining acetyl group is degraded by the Krebs cycle to CO_2 and NADH and $FADH_2$. Oxidation of the latter components by the mitochondrial respiratory chain yields additional ATP. Normal plasma glucose levels are maintained at 5 mM in the fasting state in an average adult human being. Regulation of glucose levels is tightly regulated by glucose itself and through stimulation of insulin secretion. It was in 1921 that Canadian scientists and Nobel price winners Frederick G. Banting and Charles H. Best successfully purified insulin from the pancreas and were able to treat a diabetic dog whose pancreas was removed.

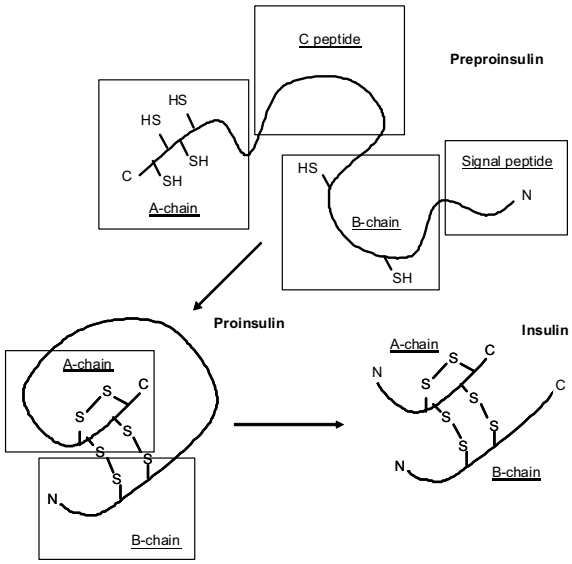

***Figure 1**. Overview of the human proinsulin precursor molecule. The A- and B-chain are linked together by disulfide bonds after removal of the C-peptide and signal peptide.*

The peptide hormone insulin (5.8 kDa) is produced in the islet of Langerhans by the β-cells. The name for insulin is derived from the latin word "insula" which means island. Insulin is synthesized from the proinsulin precursor molecule (Fig. 1). After removal of signal peptide and the C-peptide, the A- and B-chain are linked together by disulfide bonds. In general insulin induces an anabolic effect in cells and tissues. More specifically the effects of insulin include glucose uptake by muscle and adipose tissue, decrease of hepatic glucose production (gluconeogenesis), stimulation of cell growth and differentiation, storage of substrates in adipose tissue, liver and muscle (lipogenesis, glycogen and protein synthesis,

inhibition of lipolysis, glycogenolysis and protein breakdown), increased fatty acid synthesis and esterfication of fatty acids, increased amino acid uptake and vasodilatation (relaxation of vessel wall of blood arteries, allowing for more blood flow). Glucagon which is produced in the α-cells of the pancreas counteracts some of the metabolic effects of insulin. Glucagon as catabolic hormone and insulin as anabolic hormone are together able to regulate normal plasma glucose levels.

Insulin release is accomplished when blood glucose levels are increased. As a result, more glucose is taken up by the β-cell via glucose transporter GLUT2 and action of glucokinase. This results in an enhanced flux through the subsequent glycolytic pathway and citric acid cycle resulting in an increased ATP/ADP ratio. As a result the ATP-sensitive potassium (K^+) channels close, causing the cell membrane to depolarize. Depolarization of the cell membrane results in the opening of the voltage controlled calcium (Ca^{2+}) channels, allowing an influx of calcium. The increase of intercellular Ca^{2+} releases insulin from the stored vesicles (Fig. 2). By this mechanism, glucose levels are tightly regulated.

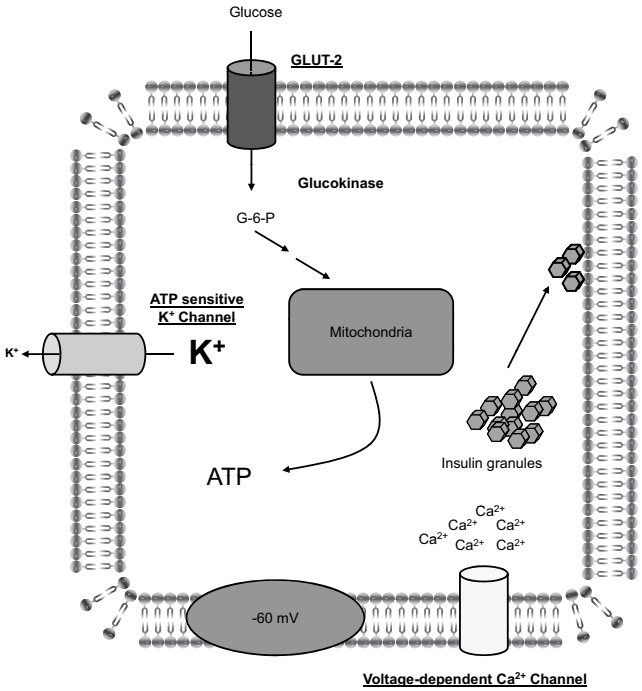

***Figure 2**. The process of glucose dependent insulin secretion from the pancreatic β-cell. The key players in insulin secretion from the β-cell are glucose transporter GLUT2, glucokinase, ATP-sensitive potassium (K^+) channel and voltage-dependent Ca^{2+} channel. See text for detailed description.*

Disturbance in glucose homeostasis: Diabetes mellitus

Diabetes is a disease state in which glucose concentrations in blood are chronically elevated (hyperglycaemia). Diabetes develops when the amount of secreted insulin by β-cells is insufficient for insulin action. The latter is dependent on the degree of insulin resistance in various insulin sensitive tissues. Different forms of diabetes have been identified. In type 1 diabetes mellitus (T1DM), the β-cells are destroyed causing an absolute insulin deficiency, resulting in hyperglycaemia. T1DM manifests itself around puberty. In case of type 2 diabetes mellitus (T2DM), a patient is still able to produce insulin, however the secreted amounts are not sufficient to lower blood glucose levels to the appropriate physiological level because the major target organs for insulin action (liver, skeletal muscle and adipose tissue) are unable to fully respond to insulin. The latter condition is thus called insulin resistance. Onset of T2DM is at a much later stage in life, however this phenomenon is currently also affecting (obese) youngsters. The acute complications arising from a total absence of insulin, as seen in T1DM, are hyperglycaemia, ketoacidosis and nonketotic hyperosmolar coma. Untreated long term hyperglycaemia induces microvascular damage, leading to blindness and kidney failure. Furthermore, it induces damage to the long sensory nerves resulting in foot problems. In addition, the insulin resistant state as seen in T2DM is a major risk factor for macrovascular complications, increasing death risk due to cardiovascular problems. Treatment of T1DM involves supplementation with insulin by injection, while treatment of T2DM can be controlled by dietary change, medication or injections with insulin depending on the severity. Other types of diabetes have been described. During pregnancy, women can develop diabetes which resembles very much to T2DM and is therefore called gestational diabetes. Many of these women develop T2DM later in life. A mutation in a single gene is can also be sufficient to give rise to diabetes eg. Maturity Onset Diabetes of the Young (MODY) (1-4) and Maternally Inherited Diabetes and Deafness (MIDD) (5). Latent Autoimmune Diabetes in Adults (LADA) is a form of T1DM which progresses very slowly, thus causing confusion with T2DM. The distinction between LADA and T2DM can be made, since in LADA antibodies against glutamatic acid carboxylase are present (6).

Downstream signal transduction from the insulin receptor

In order to better understand how insulin action is accomplished and how insulin action is altered under the insulin resistant state, a deeper understanding of insulin signalling is required. Insulin induces multiple responses (both mitogenic and metabolic) and often the nature of these response is tissue specific. Downstream signalling described below is generally linked to the metabolic effects of insulin. However how different signalling intermediates are able to contribute to the various insulin-stimulated responses is far from complete. The very first step in insulin action is accomplished by binding of insulin to its

corresponding receptor. Binding results in the activation of a downstream signalling cascade which starts at the insulin receptor.

Insulin receptor The insulin receptor (IR) is part of a subfamily of receptor tyrosine kinases which include the insulin receptor-related receptor (IRR) and the insulin-like growth factor (IGF)-I receptor. The receptor is made up out of two extracellular α-subunits and two transmembrane β-subunits resulting in a $\alpha_2\beta_2$ heterotetrameric complex which are joined by disulfide bonds. The α-subunit is able to inhibit the tyrosine kinase activity of the β-subunit. Thus binding of insulin to the α-subunit actives the tyrosine kinase of the β-subunit. The receptor undergoes a series of intramolecular transphosphorylation reactions in which one β-subunit phosphorylates its adjacent partner on specific tyrosine residues.

The activated IR is now able to phosphorylate tyrosine residues on intracellular substrates that include member of the insulin receptor substrate family (IRS1-6 (7-11)), Grb2-associated binder-1 (Gab-1 (12)), Cas-Br-M (murine) ecotropic retroviral transformin sequence homologue (Cbl (13)), adaptor containing PH and SH2 domains (APS (14)), Src homology 2 contaning protein (Shc (15)) and signal regulatory protein (SIRP) family members. IRS and Shc are recruited to the juxtamembrane region via a common NPXY motif, while APS is able to directly bind to the activation loop. The phosphotyrosine residues are recognized by molecules which contain Src homology 2 (SH2) domains like small adaptor proteins Gbr2 and Nck, the SHP2 protein tysoine phosphatise and the p85 regulatory subunit of the class I_A phosphatidylinositol 3'-kinase (PI3K).

Phosphatidyl inositol 3'-kinase (PI3K) The PI3K enzyme is composed out of a regulatory and a catalytic unit. The PI3K family is composed of class I_A, class I_B, class II and class III and the various classes display different substrate specificity. The class I_A is the most important with respect to metabolic action after binding of insulin to the IR and is composed of p110α catalytic subunit and its associated regulatory subunit p85. Binding of p85 to p110α stabilizes p110α and thus inactivates its kinase activity. Upon stimulation, p110α is not inhibited by p85 leaving kinase activity of p110α uninhibited (16). At the plasma membrane, PI3K phosphorylates phosphoinositides on the 3'-OH (D3) position of the inositol ring to generate second messengers phosphatidylinositol-3,4-bisphosphate (PI-3,4-P_2) and phosphatidylinositol-3,4,5-triphosphate (PIP_3) from substrates phosphatidylinositol-4-phosphate (PI-4-P) and phosphatidylinositol-4,5-bisphosphate (PIP_2). Phosphatase and tensin homolog (PTEN) dephosphorylates the 3'-OH position of the inositol ring, acting as a suppressor to PI3K. Proteins with a Pleckstrin homology (PH) domain can bind and localize to the lipid second messenger PIP_3 (review in (17)). The AGC kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) and downstream

phosphorylation substrate protein kinase B (PKB/Akt) are both recruited to the plasma membrane via their PH domain (Fig. 3).

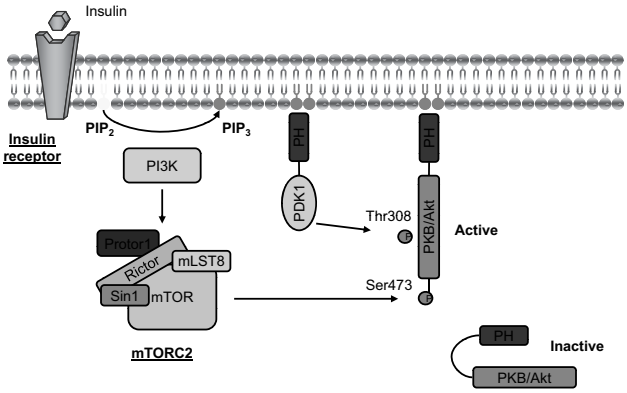

Figure 3. *Activation of protein kinase B (PKB/Akt) after insulin binding to the insulin receptor. For detailed description see text.*

Protein kinase B (PKB/Akt) The Ser/Thr kinase protein kinase B is composed of a N-terminal PH domain, a C-terminal catalytic domain and a hydrophobic motif and is highly conserved among different species (18). Generated PIP_3 is able to recruit PKB/Akt via its PH domain to the plasma membrane. For full potential of PKB/Akt, phosphorylation occurs at two different residues; phosphorylation of Thr308 in the catalytic domain occurs via PDK1 and phosphorylation of Ser473 in the hydrophobic motif site occurs via PDK2 (mTORC2 (19;20)). Once phosphorylated, PKB/AKT dissociates from the membrane and is able to phosphorylate targets in the cytoplasm and the cell nucleus (Fig. 4). In mammals three genes encode for the PKB/Akt family: *Akt1* (PKBα/Akt1), *Akt2* (PKBβ/Akt2) and *Akt3* (PKBγ/Akt3). The isoforms are products from distinct genes and overall they share a high degree of amino acid identity (>80%). PKBα/Akt1 and PKBβ/Akt2 are expressed ubiquitously, while expression of PKBγ/Akt3 is highest in brain and testes. Studies from knockout animals demonstrate that PKB/Akt is important in development and (glucose) metabolism. $Akt1^{-/-}$ mice are smaller compared to their wild type littermates and male $Akt1^{-/-}$ mice have increased apoptosis in the testes. Mouse embryonic fibroblasts from $Akt1^{-/-}$ mice are more sensitive to apoptotic stimuli (21). $Akt2^{-/-}$ mice are both hyperglycaemic and hyperinsulinaemic (22). $Akt3^{-/-}$ mice have smaller brains compared to wild type animals (23;24). All single knockout animals are viable indicating that PKB/Akt isoforms can probably compensate for the lack of another. The double knockout for Akt1 and Akt2 has a severe phenotype which includes atrophy of skin and skeletal muscle, dwarfism and early neonatal lethality (25). Double knockout for Akt2 and Akt3 results in animals with glucose and insulin intolerance and reductions in size and weight of brain and

testes (26). Considering the different phenotypes of the knockout mice, PKB/Akt signalling has been linked to malignancies, insulin resistance/diabetes, muscle atrophy, Alzheimer disease and chronic obstruct pulmonary disease (27-33).

PKB/Akt and PKB/Akt substrates in cellular processes and disease

PKB/Akt is protein kinase able to regulate a wide range of cellular processes: (glucose) metabolism, apoptosis, proliferation and angiogenesis. In order to achieve these effects different PKB/Akt substrates have been identified and linked to these processes. PKB/Akt specifically phosphorylates substrates that contain the following amino acid motif: Arg-Xaa-Arg-Xaa-Xaa-pSer/pThr (34;35). The number of substrates is ever increasing and a combined search of “protein kinase B”, "phosphorylation” and “substrate” in Pubmed results in over 1500 hits.

The tuberous sclerosis complex (TSC) is a downstream phosphorylation target of PKB/Akt. Phosphorylation of TSC2 by PKB/Akt results in an inhibition of TSC activity (36). As a result, Ras homolog-enriched in the brain (Rheb)-GDP is converted into Rheb-GTP, thus allowing Rheb-GTP to activate mammalian target of rapamycin (mTOR) (37;38). mTOR is a component of two distinct complex called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The mTORC1 complex is sensitive for mTOR inhibitor rapamycin, while mTORC2 is not. mTORC1 is composed out of mTOR, regulatory associated protein of mTOR (Raptor), mammalian LST8/G-protein β-subunit like protein (mLST8/GβL) and proline-rich PKB/Akt substrate of 40 kDa (PRAS40) (39;40). Direct substrates for mTORC1 include p70 S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein-1 (4EBP1). Via this way mTORC1 is able to regulate protein synthesis.

With respect to metabolism PKB/Akt is able to phosphorylate glycogen synthase kinase (GSK) which results in the downstream phosphorylation and inactivation of glycogen synthase (41). Via phosphorylation of PKB/Akt substrate of 160 kDa (AS160), PKB/Akt is able to regulate GLUT4 mediated glucose uptake (42). The forkhead box O (FOXO) transcription factors play an important role as PKB/Akt substrates controlling gene expression. Nuclear exclusion of FOXO inhibits gluconeogenesis while promoting β-cell function (43;44). FOXO proteins exert many different functions and are also able to regulate expression of Bcl2 family members thereby regulating apoptosis (review in (45)).

Involvement of PKB/Akt in the process of programmed cell death or apoptosis can be mediated by phosphorylation and inhibition of pro apoptotic mediators such as Bad, MDM2 (46), FOXO and IκB kinase (IKK (47)). When dephosphorylated, Bcl2 family member Bad is able to complex with Bcl_{XL} resulting in apoptosis. However phosphorylation of Bad on Ser136 results in 14-3-3 binding and thus inhibits apoptosis (48).

In cell cycle control, PKB/Akt can phosphorylate cyclin-dependent kinase (CDK) inhibitors $p21^{CIP1/WAF1}$ (49) and $p27^{KIP1}$ (50) which results in the cytoplasmic retention of these proteins, since they are able to bind 14-3-3. PKB/Akt is thus able through $p21^{CIP/WAF1}$ able to inhibit cell cycle arrest.
The list of identified PKB/Akt phosphorylation substrates is always increasing of which a small sample is included in the following list: ATP-citrate lyase (51), WNK1 (52), NDRG2 (53), PRAS40 (54), Synip (55), filamin C (56), JCF1 (57), VCP (58-60), PIKfyve (61), YB1 (62;63), Par-4 (64). However from the more recent PKB/Akt substrates it not always clear in which cellular processes they play a role.

Rationale and outline of thesis

Impairment of glucose metabolism is associated with decreased activity of PI3K and downstream signalling partner PKB/Akt. The PI3K and PKB/Akt signalling pathway is able to exert numerous different effects mediated through specific PKB/Akt substrates. A lot of distinct functions can be described to PKB/Akt substrates, however from a lot PKB/Akt substrates it remains unclear what their specific function is. Therefore in this thesis, the focus is on PKB/Akt substrate PRAS40 and examine how phosphorylation of this substrate is altered under conditions of insulin resistance. Next we examine the function of PRAS40 in respect to insulin action. In **chapter 2**, an overview is provided on different literature concerning PRAS40. Special attention will be paid to PRAS40 and the role of this protein in the regulation of mTORC1. In **chapter 3**, phosphorylation of PRAS40 on Thr246 both *in vitro* and *in vivo* is examined. Using an animal model for insulin resistance we have assessed phosphorylation status of PRAS40 under insulin resistance. **Chapter 4** describes phosphorylation of PRAS40 on Ser183 and the importance of Thr246 in this process. **Chapter 5** describes the subcellular localisation of the PRAS40 protein. In **chapter 6**, we investigate the involvement of PRAS40 in palmitate-induced insulin resistance. All the obtained results are summarised and discussed in **chapter 7**.

References

1. Vaxillaire,M, Boccio,V, Philippi,A, Vigouroux,C, Terwilliger,J, Passa,P, Beckmann,JS, Velho,G, Lathrop,GM, Froguel,P: A gene for maturity onset diabetes of the young (MODY) maps to chromosome 12q. *Nat Genet.* 9:418-423, 1995
2. Yamagata,K, Oda,N, Kaisaki,PJ, Menzel,S, Furuta,H, Vaxillaire,M, Southam,L, Cox,RD, Lathrop,GM, Boriraj,VV, Chen,X, Cox,NJ, Oda,Y, Yano,H, Le Beau,MM, Yamada,S, Nishigori,H, Takeda,J, Fajans,SS, Hattersley,AT, Iwasaki,N, Hansen,T, Pedersen,O, Polonsky,KS, Bell,GI, .: Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). *Nature* 384:455-458, 1996
3. Vionnet,N, Stoffel,M, Takeda,J, Yasuda,K, Bell,GI, Zouali,H, Lesage,S, Velho,G, Iris,F, Passa,P, .: Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature* 356:721-722, 1992
4. Yamagata,K, Furuta,H, Oda,N, Kaisaki,PJ, Menzel,S, Cox,NJ, Fajans,SS, Signorini,S, Stoffel,M, Bell,GI: Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). *Nature* 384:458-460, 1996
5. van den Ouweland,JM, Lemkes,HH, Ruitenbeek,W, Sandkuijl,LA, de Vijlder,MF, Struyvenberg,PA, van de Kamp,JJ, Maassen,JA: Mutation in mitochondrial tRNA(Leu)(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. *Nat Genet.* 1:368-371, 1992
6. Zimmet,PZ, Tuomi,T, Mackay,IR, Rowley,MJ, Knowles,W, Cohen,M, Lang,DA: Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. *Diabet.Med.* 11:299-303, 1994
7. Sun,XJ, Rothenberg,P, Kahn,CR, Backer,JM, Araki,E, Wilden,PA, Cahill,DA, Goldstein,BJ, White,MF: Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* 352:73-77, 1991
8. Sun,XJ, Wang,LM, Zhang,Y, Yenush,L, Myers,MG, Jr., Glasheen,E, Lane,WS, Pierce,JH, White,MF: Role of IRS-2 in insulin and cytokine signalling. *Nature* 377:173-177, 1995
9. Lavan,BE, Lane,WS, Lienhard,GE: The 60-kDa phosphotyrosine protein in insulin-treated adipocytes is a new member of the insulin receptor substrate family. *J.Biol Chem.* 272:11439-11443, 1997
10. Fantin,VR, Sparling,JD, Slot,JW, Keller,SR, Lienhard,GE, Lavan,BE: Characterization of insulin receptor substrate 4 in human embryonic kidney 293 cells. *J.Biol Chem.* 273:10726-10732, 1998
11. Cai,D, Dhe-Paganon,S, Melendez,PA, Lee,J, Shoelson,SE: Two new substrates in insulin signalling, IRS5/DOK4 and IRS6/DOK5. *J.Biol Chem.* 278:25323-25330, 2003
12. Lehr,S, Kotzka,J, Herkner,A, Sikmann,A, Meyer,HE, Krone,W, Muller-Wieland,D: Identification of major tyrosine phosphorylation sites in the human insulin receptor substrate Gab-1 by insulin receptor kinase in vitro. *Biochemistry* 39:10898-10907, 2000
13. Baumann,CA, Ribon,V, Kanzaki,M, Thurmond,DC, Mora,S, Shigematsu,S, Bickel,PE, Pessin,JE, Saltiel,AR: CAP defines a second signalling pathway required for insulin-stimulated glucose transport. *Nature* 407:202-207, 2000
14. Ahmed,Z, Smith,BJ, Kotani,K, Wilden,P, Pillay,TS: APS, an adapter protein with a PH and SH2 domain, is a substrate for the insulin receptor kinase. *Biochem.J.* 341 (Pt 3):665-668, 1999
15. Gustafson,TA, He,W, Craparo,A, Schaub,CD, O'Neill,TJ: Phosphotyrosine-dependent interaction of SHC and insulin receptor substrate 1 with the NPEY motif of the insulin receptor via a novel non-SH2 domain. *Mol Cell Biol* 15:2500-2508, 1995
16. Okkenhaug,K, Vanhaesebroeck,B: New responsibilities for the PI3K regulatory subunit p85 alpha. *Sci.STKE* 2001:E1, 2001
17. Corvera,S, Czech,MP: Direct targets of phosphoinositide 3-kinase products in membrane traffic and signal transduction. *Trends Cell Biol* 8:442-446, 1998

18. Fayard,E, Tintignac,LA, Baudry,A, Hemmings,BA: Protein kinase B/Akt at a glance. *J.Cell Sci.* 118:5675-5678, 2005
19. Sarbassov,DD, Guertin,DA, Ali,SM, Sabatini,DM: Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307:1098-1101, 2005
20. Jacinto,E, Facchinetti,V, Liu,D, Soto,N, Wei,S, Jung,SY, Huang,Q, Qin,J, Su,B: SIN1/MIP1 Maintains rictor-mTOR Complex Integrity and Regulates Akt Phosphorylation and Substrate Specificity. *Cell* 2006
21. Chen,WS, Xu,PZ, Gottlob,K, Chen,ML, Sokol,K, Shiyanova,T, Roninson,I, Weng,W, Suzuki,R, Tobe,K, Kadowaki,T, Hay,N: Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. *Genes Dev.* 15:2203-2208, 2001
22. Cho,H, Mu,J, Kim,JK, Thorvaldsen,JL, Chu,Q, Crenshaw,EB, III, Kaestner,KH, Bartolomei,MS, Shulman,GI, Birnbaum,MJ: Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* 292:1728-1731, 2001
23. Tschopp,O, Yang,ZZ, Brodbeck,D, Dummler,BA, Hemmings-Mieszczak,M, Watanabe,T, Michaelis,T, Frahm,J, Hemmings,BA: Essential role of protein kinase B gamma (PKB gamma/Akt3) in postnatal brain development but not in glucose homeostasis. *Development* 132:2943-2954, 2005
24. Easton,RM, Cho,H, Roovers,K, Shineman,DW, Mizrahi,M, Forman,MS, Lee,VMY, Szabolcs,M, De Jong,R, Oltersdorf,T, Ludwig,T, Efstratiadis,A, Birnbaum,MJ: Role for Akt3/Protein kinase B gamma in attainment of normal brain size. *Molecular and Cellular Biology* 25:1869-1878, 2005
25. Peng,XD, Xu,PZ, Chen,ML, Hahn-Windgassen,A, Skeen,J, Jacobs,J, Sundararajan,D, Chen,WS, Crawford,SE, Coleman,KG, Hay,N: Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone developement, and impeded adipogenesis in mice lacking Akt1 and Akt2. *Genes & Development* 17:1352-1365, 2003
26. Dummler,B, Tschopp,O, Hynx,D, Yang,ZZ, Dirnhofer,S, Hemmings,BA: Life with a single isoform of Akt: Mice lacking Akt2 and Akt3 are viable but display impaired glucose homeostasis and growth deficiencies. *Molecular and Cellular Biology* 26:8042-8051, 2006
27. Hennessy,BT, Smith,DL, Ram,PT, Lu,Y, Mills,GB: Exploiting the PI3K/AKT Pathway for Cancer Drug Discovery. *Nat.Rev.Drug Discov.* 4:988-1004, 2005
28. Liu,H, Hong,T, Wen,GB, Han,J, Zhuo,D, Liu,Z, Cao,W: Increased Basal Level of Akt-Dependent Insulin Signalling May Be Responsible for the Development of Insulin Resistance. *Am.J.Physiol Endocrinol.Metab* 2009
29. Medina,EA, Afsari,RR, Ravid,T, Castillo,SS, Erickson,KL, Goldkorn,T: Tumor necrosis factor-{alpha} decreases Akt protein levels in 3T3-L1 adipocytes via the caspase-dependent ubiquitination of Akt. *Endocrinology* 146:2726-2735, 2005
30. Sandri,M, Sandri,C, Gilbert,A, Skurk,C, Calabria,E, Picard,A, Walsh,K, Schiaffino,S, Lecker,SH, Goldberg,AL: Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 117:399-412, 2004
31. Lee,HK, Kumar,P, Fu,Q, Rosen,KM, Querfurth,HW: The insulin/Akt signalling pathway is targeted by intracellular beta-amyloid. *Mol Biol Cell* 20:1533-1544, 2009
32. Griffin,RJ, Moloney,A, Kelliher,M, Johnston,JA, Ravid,R, Dockery,P, O'Connor,R, O'Neill,C: Activation of Akt/PKB, increased phosphorylation of Akt substrates and loss and altered distribution of Akt and PTEN are features of Alzheimer's disease pathology. *J.Neurochem.* 93:105-117, 2005
33. Bozinovski,S, Vlahos,R, Hansen,M, Liu,K, Anderson,GP: Akt in the pathogenesis of COPD. *Int.J.Chron.Obstruct.Pulmon.Dis.* 1:31-38, 2006
34. Alessi,DR, Caudwell,FB, Andjelkovic,M, Hemmings,BA, Cohen,P: Molecular basis for the substrate specificity of protein kinase B; comparison with MAPKAP kinase-1 and p70 S6 kinase. *FEBS Lett.* 399:333-338, 1996

35. Obata,T, Yaffe,MB, Leparc,GG, Piro,ET, Maegawa,H, Kashiwagi,A, Kikkawa,R, Cantley,LC: Peptide and protein library screening defines optimal substrate motifs for AKT/PKB. *J.Biol.Chem.* 275:36108-36115, 2000
36. Manning,BD, Logsdon,MN, Lipovsky,AI, Abbott,D, Kwiatkowski,DJ, Cantley,LC: Feedback inhibition of Akt signalling limits the growth of tumors lacking Tsc2. *Genes Dev.* 19:1773-1778, 2005
37. Bai,X, Ma,D, Liu,A, Shen,X, Wang,QJ, Liu,Y, Jiang,Y: Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. *Science* 318:977-980, 2007
38. Proud,CG: mTOR, unleashed. *Science* 318:926-927, 2007
39. Wullschleger,S, Loewith,R, Hall,MN: TOR signalling in growth and metabolism. *Cell* 124:471-484, 2006
40. Sancak,Y, Thoreen,CC, Peterson,TR, Lindquist,RA, Kang,SA, Spooner,E, Carr,SA, Sabatini,DM: PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell* 25:903-915, 2007
41. Welsh,GI, Wilson,C, Proud,CG: GSK3: a SHAGGY frog story. *Trends Cell Biol* 6:274-279, 1996
42. Kane,S, Sano,H, Liu,SC, Asara,JM, Lane,WS, Garner,CC, Lienhard,GE: A method to identify serine kinase substrates. Akt phosphorylates a novel adipocyte protein with a Rab GTPase-activating protein (GAP) domain. *J.Biol.Chem.* 277:22115-22118, 2002
43. Puigserver,P, Rhee,J, Donovan,J, Walkey,CJ, Yoon,JC, Oriente,F, Kitamura,Y, Altomonte,J, Dong,H, Accili,D, Spiegelman,BM: Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. *Nature* 423:550-555, 2003
44. Kitamura,T, Nakae,J, Kitamura,Y, Kido,Y, Biggs,WH, III, Wright,CV, White,MF, Arden,KC, Accili,D: The forkhead transcription factor Foxo1 links insulin signalling to Pdx1 regulation of pancreatic beta cell growth. *J.Clin.Invest* 110:1839-1847, 2002
45. Burgering,BM, Medema,RH: Decisions on life and death: FOXO Forkhead transcription factors are in command when PKB/Akt is off duty. *J.Leukoc.Biol* 73:689-701, 2003
46. Mayo,LD, Donner,DB: A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc.Natl Acad.Sci.U.S.A* 98:11598-11603, 2001
47. Ozes,ON, Mayo,LD, Gustin,JA, Pfeffer,SR, Pfeffer,LM, Donner,DB: NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature* 401:82-85, 1999
48. Zha,J, Harada,H, Yang,E, Jockel,J, Korsmeyer,SJ: Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). *Cell* 87:619-628, 1996
49. Xia,W, Chen,JS, Zhou,X, Sun,PR, Lee,DF, Liao,Y, Zhou,BP, Hung,MC: Phosphorylation/cytoplasmic localization of p21Cip1/WAF1 is associated with HER2/neu overexpression and provides a novel combination predictor for poor prognosis in breast cancer patients. *Clin.Cancer Res.* 10:3815-3824, 2004
50. Shin,I, Yakes,FM, Rojo,F, Shin,NY, Bakin,AV, Baselga,J, Arteaga,CL: PKB/Akt mediates cell-cycle progression by phosphorylation of p27(Kip1) at threonine 157 and modulation of its cellular localization. *Nat Med.* 8:1145-1152, 2002
51. Berwick,DC, Hers,I, Heesom,KJ, Moule,SK, Tavare,JM: The identification of ATP-citrate lyase as a protein kinase B (Akt) substrate in primary adipocytes. *J.Biol.Chem.* 277:33895-33900, 2002
52. Jiang,ZY, Zhou,QL, Holik,J, Patel,S, Leszyk,J, Coleman,K, Chouinard,M, Czech,MP: Identification of WNK1 as a Substrate of Akt/Protein Kinase B and a Negative Regulator of Insulin-stimulated Mitogenesis in 3T3-L1 Cells. *J.Biol.Chem.* 280:21622-21628, 2005
53. Burchfield,JG, Lennard,AJ, Narasimhan,S, Hughes,WE, Wasinger,VC, Corthals,GL, Okuda,T, Kondoh,H, Biden,TJ, Schmitz-Peiffer,C: Akt mediates insulin-stimulated phosphorylation of Ndrg2: evidence for cross-talk with protein kinase C theta. *J.Biol.Chem.* 279:18623-18632, 2004
54. Kovacina,KS, Park,GY, Bae,SS, Guzzetta,AW, Schaefer,E, Birnbaum,MJ, Roth,RA: Identification of a proline-rich Akt substrate as a 14-3-3 binding partner. *J.Biol.Chem.* 278:10189-10194, 2003
55. Yamada,E, Okada,S, Saito,T, Ohshima,K, Sato,M, Tsuchiya,T, Uehara,Y, Shimizu,H, Mori,M: Akt2 phosphorylates Synip to regulate docking and fusion of GLUT4-containing vesicles. *J.Cell Biol.* 168:921-928, 2005

56. Murray,JT, Campbell,DG, Peggie,M, Alfonso,M, Cohen,P: Identification of filamin C as a new physiological substrate of PKBalpha using KESTREL. *Biochem.J.* 384:489-494, 2004
57. Johnson,JL, Pacquelet,S, Lane,WS, Eam,B, Catz,SD: Akt Regulates the Subcellular Localization of the Rab27a-Binding Protein JFC1 by Phosphorylation. *Traffic.* 6:667-681, 2005
58. Klein,JB, Barati,MT, Wu,R, Gozal,D, Sachleben,LR, Jr., Kausar,H, Trent,JO, Gozal,E, Rane,MJ: Akt mediated VCP phosphorylation regulates its association with ubiquitinated proteins. *J.Biol.Chem.* 2005
59. Klein,JB, Barati,MT, Wu,R, Gozal,D, Sachleben,LR, Jr., Kausar,H, Trent,JO, Gozal,E, Rane,MJ: Akt-mediated valosin-containing protein 97 phosphorylation regulates its association with ubiquitinated proteins. *J.Biol Chem.* 280:31870-31881, 2005
60. Vandermoere,F, Yazidi-Belkoura,I, Slomianny,C, Demont,Y, Bidaux,G, Adriaenssens,E, Lemoine,J, Hondermarck,H: The valosin-containing protein (VCP) is a target of Akt signalling required for cell survival. *J.Biol Chem.* 281:14307-14313, 2006
61. Berwick,DC, Dell,GC, Welsh,GI, Heesom,KJ, Hers,I, Fletcher,LM, Cooke,FT, Tavare,JM: Protein kinase B phosphorylation of PIKfyve regulates the trafficking of GLUT4 vesicles. *J.Cell Sci.* 117:5985-5993, 2004
62. Sutherland,BW, Kucab,J, Wu,J, Lee,C, Cheang,MC, Yorida,E, Turbin,D, Dedhar,S, Nelson,C, Pollak,M, Leighton,GH, Miller,K, Badve,S, Huntsman,D, Blake-Gilks,C, Chen,M, Pallen,CJ, Dunn,SE: Akt phosphorylates the Y-box binding protein 1 at Ser102 located in the cold shock domain and affects the anchorage-independent growth of breast cancer cells. *Oncogene* 24:4281-4292, 2005
63. Bader,AG, Vogt,PK: Phosphorylation by Akt disables the anti-oncogenic activity of YB-1. *Oncogene* 2007
64. Goswami,A, Burikhanov,R, de Thonel,A, Fujita,N, Goswami,M, Zhao,Y, Eriksson,JE, Tsuruo,T, Rangnekar,VM: Binding and phosphorylation of par-4 by akt is essential for cancer cell survival. *Mol.Cell* 20:33-44, 2005