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

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Chapter 2

PRAS40: Target or Modulator of mTORC1 Signaling and Insulin Action?

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Abstract

Alterations in signalling via protein kinase B (PKB/Akt) and the mammalian target of rapamycin (mTOR) frequently occur in type 2 diabetes and various human malignancies. Proline-rich Akt substrate of 40 kDa (PRAS40) has a regulatory function at the intersection of these pathways. The interaction of PRAS40 with the mTOR complex 1 (mTORC1) inhibits the activity of mTORC1. Phosphorylation of PRAS40 by PKB/Akt and mTORC1 disrupts the binding between mTORC1 and PRAS40, and relieves the inhibitory constraint of PRAS40 on mTORC1 activity. This review summarizes the signalling pathways regulating PRAS40 phosphorylation, as well as the dual function of PRAS40 as substrate and inhibitor of mTORC1 in the physiological situation, and under pathological conditions, like insulin resistance and cancer.

Introduction

Proline-rich PKB/Akt substrate of 40 kDa (PRAS40) is a component of the mammalian target of rapamycin complex (mTORC) 1 (1-4). The catalytic subunit of mTORC1, mammalian target of rapamycin (mTOR), is shared with another multimeric protein complex, termed mTORC2. In addition to mTOR and PRAS40, mTORC1 consists of the regulatory associated protein of mTOR (raptor), and the mammalian ortholog of yeast Lethal with Sec13 protein 8 (mLST8, also known as Gβ1) (5-8). Signalling by mTORC1 is sensitive to rapamycin and regulates multiple cellular processes, such as mRNA translation, ribosome biogenesis, cell cycle progression, hypoxia, autophagy, mitochondrial function, lipid storage, and chronological lifespan through phosphorylation of multiple substrates (for review see (9-11)). The growing list of mTORC1-regulated proteins includes yin yang 1 (YY1), signal transducer and activator of transcription 3 (STAT3), serum- and glucocorticoid regulated kinase 1 (SGK1), PRAS40, phospholipase D2 (PLD2), hypoxia-inducible factor 1α (HIF1α), and Akt substrate of 160 kDa (AS160, also known as TBC1 domain family member D4 (TBC1D4)), in addition to the well characterized substrates, eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) and the p70 S6 kinases (S6K1 and S6K2) (12-22) (Table 1).

The mTORC2 complex contains mTOR, rapamycin-insensitive companion of mTOR (rictor), mSin1, mLST8, and proline-rich repeat protein-5 (PRR5, also known as protor) or PRR5-like (2;23-29). Active mTORC2 not only regulates actin polymerisation, but also promotes phosphorylation of the hydrophobic motifs of protein kinase B (PKB/Akt), and SGK1. Also phosphorylation of both the turn- and hydrophobic motifs within the protein kinase C (PKC) α isoform, and likely also within the PKC βI, βII, γ, and ε isoforms is mediated by active mTORC2 (30-36) (Table 1).

Table 1. *Overview of substrates for mTORC1 and mTORC2*

	Phosphorylation site	TOS motif	RAIP motif
mTORC1-regulated proteins:			
4EBP1, 2, 3	Thr37, Thr46, Ser65, Thr70	FEMDI	RAIP
HIF1α		FVMVL	
PLD2		FEVQV	
PRAS40	Ser183, Ser212, Ser221	FVMDE	KSLP
S6K1	Thr389	FDIDL	
S6K2	Thr388	FDLDL	
SGK1	Ser422		
STAT3	Ser727	FPMEL	RAIL
TBC1D4	Ser666	FEMDI	
YY1			
mTORC2-regulated proteins:			
PKB/Akt	Ser473	n.a.	n.a
PKCα	Thr638, Ser657	n.a	n.a
PKCβ1		n.a	n.a
PKCβ2		n.a	n.a
PKCγ		n.a	n.a
PKCε		n.a	n.a
SGK1	Ser422	n.a	n.a

mTOR and disease

Type 2 diabetes. Clinical insulin resistance of peripheral target tissues for insulin action, like the liver, skeletal muscle and adipose tissue, in combination with insufficient compensatory insulin secretion by the β-cells in the islets of Langerhans characterizes type 2 diabetes (T2D) (37). At the molecular level, both insulin resistance and T2D are often associated with an impaired activation of phosphatidylinositol 3'-kinase (PI3K) and its substrate PKB/Akt after insulin stimulation (38). In the liver, skeletal muscle, heart, and adipose tissue, the PI3K-PKB/Akt pathway regulates glucose metabolism (39;40). In the pancreas, the PI3K-PKB/Akt pathway promotes β-cell growth, proliferation, and survival (41). Activation of the PI3K-PKB/Akt pathway by insulin is mediated by recruitement of PI3K to the tyrosine phosphorylated insulin receptor substrates (IRS) 1 and 2 (42;43). Conversely, the induction of tyrosine phosphorylation of IRS1/2 is blunted upon serine phosphorylation of IRS1/2 (44-49). Serine phosphorylation of the IRS-proteins not only reduces the activation of the PI3K-PKB/Akt pathway by insulin, but also leads to proteasome-mediated protein degradation of IRS1/2 through interaction with 14-3-3 proteins (50-53).

Several studies on high-fat diet fat rodents show elevated activity of the mTORC1 signalling pathway (54-57). The sustained activity of S6K1 may abrogate insulin-mediated

activation of the PI3K-PKB/Akt pathway by inducing inhibitory serine phosphorylations on the insulin receptor and IRS1/2 (58-62). Accordingly, genetic ablation of S6K1 (63), or lowering mTORC1 activity with rapamycin (64) or chronic exercise (65) reduces IRS1 serine phosphorylation and reverses the inhibition of the PI3K-PKB/Akt pathway in the liver, skeletal muscle, and adipose tissue. In contrast, rapamycin treatment does not improve insulin sensitivity in ob/ob mice (66), indicating that rapamycin-insensitive protein kinases, such as *c-jun* N terminal kinase, inhibitor of kappa B kinase and PKC isoforms, might contribute to inhibition of insulin signalling (67). Alternatively, mTORC1 action may differ between tissues. For example, mTORC1 regulates mitochondrial function via the transcriptional regulators YY1 and PGC1α in skeletal muscle (68), and is crucial for β-cell survival and insulin biosynthesis in the pancreas (69-74). Recently, the tissue-specific regulation of metabolic control by mTORC1 has been reviewed extensively (75;76).

Cancer. Multiple human malignancies and inherited hamartoma syndromes show increased activity of mTOR (77-79). As will be described in more detail under "Regulation of mTORC1 activity", the tuberous sclerosis complex (TSC), a GTPase activating protein complex consisting of two subunits, TSC1 (also known as hamartin) and TSC2 (also known as tuberin) is a key upstream regulator of mTORC1 (reviewed by (80)). Various protein kinases, including PKB/Akt, AMP-activated kinase (AMPK), and extracellular-signal regulated kinase (ERK), affect the activity of TSC via phosphorylation of the TSC2 (81-84). In particular, hyperactivation of PKB/Akt is a common characteristic of human malignancies (85;86). Since PKB/Akt activates mTORC1 by phosphorylating TSC2 and mTORC2 acts a upstream activator of PKB/Akt, mTOR may function both upstream and downstream of PKB/Akt in the pathogenesis of human cancer as has been extensively reviewed by others (87-91).

The hamartoma syndrome tuberous sclerosis is characterized by inactivating mutations in TSC1 (92) or TSC2 (93). Other hamartoma syndromes have been linked to loss of function of tumour suppressors that regulate TSC activity (94;95). The Cowden syndrome can be ascribed to a loss of function of phosphatase and tensin homolog (PTEN), a phosphatase inactivating PI3K, the upstream regulator of PKB/Akt (for review see (96)). Inactivating mutations in LKB1, the upstream regulator of AMPK, underlie the Peutz-Jeghers syndrome (97;98). Finally, mutations in the neurofibromanin gene which encodes a GTPase activating protein for Ras, cause neurofibromatosis type 1 (99). The NF1 mutation results in high intracellular levels of active Ras that inactivate TSC2 through sustained activation of two Ras-effector pathways, the Raf-MEK-ERK- and the PI3K-PKB/Akt-pathway (100).

Regulation of mTORC1 activity

As summarized in Figure 1, activation of mTORC1 in response to anabolic stimuli, such as insulin and nutrients, like amino acids and glucose, involves the integration of multiple signalling pathways at the level of mTORC1 (101;102). Activation of the mTOR protein kinase occurs via binding of the GTP-bound form of the small GTP-binding protein Ras homolog-enriched in brain (Rheb) (103). To bring mTORC1 in the proximity of Rheb, amino acids are required (104). Amino acids increase the intracellular RagA- and RagB-GTP levels, thereby stimulating the binding of these small GTPases to raptor (105;106). The binding of the Rags serves to relocate mTORC1 to Rheb-containing peri-nuclear vesicular structures, thus allowing mTOR to interact with Rheb (107). The levels of GTP-bound Rheb are regulated by TSC, which acts as a GTPase activating protein on Rheb (108). Insulin inhibits TSC activity through PKB/Akt-mediated phosphorylation of TSC2 (109;110). As a result, Rheb is relieved from the inhibitory GTPase activity, thus allowing Rheb-GTP to bind and activate mTORC1. Glucose activates mTORC1 by inhibiting AMPK (111-113). AMPK, when activated such as in response to energy deprivation, activates TSC2 (114), thus promoting the hydrolysis of Rheb-GTP and inhibition of mTORC1.

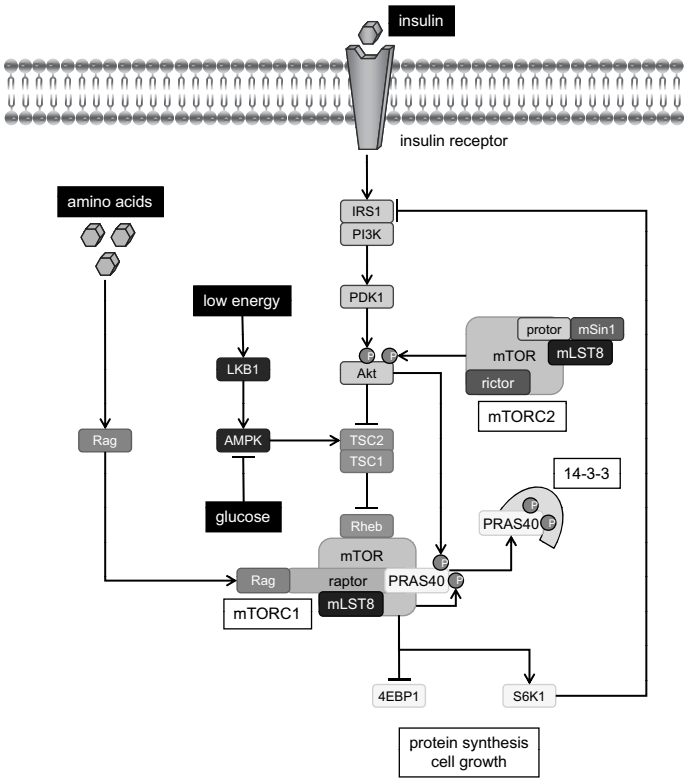

***Figure 1**. Regulation of mTORC1 activity by insulin, amino acids, glucose and energy deprivation. The activation of mTORC1 requires the binding of the small GTP-binding protein Rag and Rheb to mTORC1. The levels of GTP-bound Rag are enhanced by amino acids. Inhibition of the activity of TSC1/TSC2 complex increases cellular Rheb-GTP-levels. TSC2 is regulated by phosphorylation. AMPK-mediated phosphorylation of TSC2 leads to activation of TSC2 and inactivation of mTORC1. Phosphorylation of PKB/Akt on its turn inactivates TSC2, thus increasing Rheb-GTP levels. Full activation of mTORC1 further requires the dissociation of PRAS40 from mTORC1, which requires phosphorylation of PRAS40 by both PKB/Akt and mTORC1. When activated, mTORC1 regulates protein synthesis and cellular growth through phosphorylation of S6K1 and 4EBP1. Furthermore, the activated S6K1 exerts a negative feedback loop on IRS1 thereby blunting insulin-mediated phosphorylation of PKB/Akt.*

PRAS40

Identification of PRAS40. PRAS40 was originally described as a 40 kDa protein that binds to 14-3-3 proteins in lysates from insulin-treated hepatoma cells (115). PRAS40 is probably identical to the p39 protein that is phosphorylated in PC12 cells in response to nerve growth factor or epidermal growth factor (116). Finally, PRAS40 has been described as Akt1 substrate 1 (Akt1S1), a phosphoprotein identified from nuclear extracts from Hela cells (117).

PRAS40 was recognized as component of the mTORC1 complex following mass spectrometry analysis of mTOR immunoprecipitates (2;118-120). Subsequent western blot studies showed that PRAS40 preferentially interacts with raptor, but that it also binds to the kinase domain of mTOR (2;121-123). Compared to intact mTOR, PRAS40-binding to a kinase-dead mutant of mTOR is reduced (2). PRAS40 has not been found in rictor immunoprecipitates, indicating that PRAS40 is a component of mTORC1, and not of mTORC2 (2;124-126).

Structure and post-translational modification of PRAS40. The gene for PRAS40 is located on human chromosome 19q13.33 and encodes 3 transcript variants that differ in their 5'-UTR but result in the same 256 amino acid protein. Analysis of human, rat and mouse tissues demonstrates a ubiquitous expression of both PRAS40 mRNA and protein, with highest transcript levels found in human liver and heart (127;128). As shown in Figure 2A, the PRAS40 protein consists of two proline-enriched stretches at the aminoterminus with an as yet unknown function (129), but containing sequences that have the potential to bind proteins containing SH3- and/or WW-domains (130). The proline-rich region is followed by two short sequences that have been implicated mTORC1-binding and phosphorylation of mTORC1 substrates, i.e. an mTOR signalling- (TOS) and a potential RAIP-motif (131-133). The TOS motif is located between amino acids 129 and 133 (134-136), and is a common feature shared with multiple other mTORC1 substrates (Table 1). The Lys-Ser-Leu-Pro sequence located between amino acids 182 and 185 is similar to the RAIP-motif, which has been named after a short amino acid sequence identified in 4EBP1, Arg-Ala-Ile-Pro (137;138) (Table 1). The carboxyterminus of PRAS40 contains a sequence that matches the consensus for a leucine-enriched nuclear export sequence (NES), Leu-*xx(x)*-[Leu,Ile,Val,Phe,Met]-*xx(x)*-Leu-*x*-[Leu,Ile] (139). Finally, multiple residues within PRAS40 can become phosphorylated, including Ser183, Ser202, Ser203, Ser212, Ser221, and Thr246 (140-142) (Figure 2A).

Highly conserved homologues of PRAS40 have been identified down through *Danio rerio*. PRAS40 homologues almost identical to the human protein have been found in *Bos taurus*, *Mus musculus*, and *Rattus norvegicus*. The homologues from *Xenopus laevis* and *Danio rerio* completely lack the proline-enriched stretches at the aminoterminus, but are 60% and

44% identical to the carboxyterminal part of the human protein (143), and show conservation of the TOS, RAIP, and NES-motifs as well as the phosphorylation sites on Ser183, Ser221, and Thr246 (Figure 2B). The carboxyterminal part of PRAS40 also shows 58% and 46% similarity with the carboxyterminal part of the Lobe proteins from *Apis mellifera* and *Drosophila melanogaster* (144). The Lobe proteins lack the TOS motif and show less conservation of the NES. However, the RAIP motif as well as the equivalents of Ser183, Ser221, and Thr246 are preserved (Figure 2B). Human PRAS40 also has been reported to share some similarity with dauer or aging overexpression family member 5 (dao-5) from *Caenorhabditis elegans* and *Caenorhabditis briggsae* (145). However, dao-5 seems to lack preservation of the important regulatory motifs found in PRAS40 from higher organisms. Therefore, it remains unclear whether PRAS40 is also found in these lower eukaryotes.

Interaction with raptor. PRAS40 binds to the mTORC1 complex predominantly through interaction with raptor, and dissociates in response to the addition of insulin or amino acids (146-150). The interaction of PRAS40 with raptor requires an intact TOS-motif, as mutation of Phe129 to Ala greatly reduces the binding of PRAS40 to raptor (151-153). In addition to the TOS-motif, the binding of PRAS40 to raptor was found to require a sequence located between amino acids 150 and 234 of PRAS40 (154). Some studies have proposed a regulatory role for the RAIP motif of PRAS40. Mutation of Ser183 or Pro185 to Ala reduced the PRAS40-raptor complex formation (155), and substitution of Ser183 by Asp completely abrogated the interaction between PRAS40 and raptor (156). In 4EBP1, the RAIP-motif not only directs interaction with raptor, but also is critical for mTORC1-dependent phosphorylation of the protein (157-159). However, insulin and amino acids failed to promote phosphorylation of a mutant 4EBP1 in which the RAIP motif was replaced by the Lys-Ser-Leu-Pro sequence of PRAS40 (160). Thus, whereas phosphorylation of Ser183 seems to contribute disruption of the PRAS40-raptor complex (161;162), the function of the RAIP motif in the binding of PRAS40 to raptor still requires further analysis .

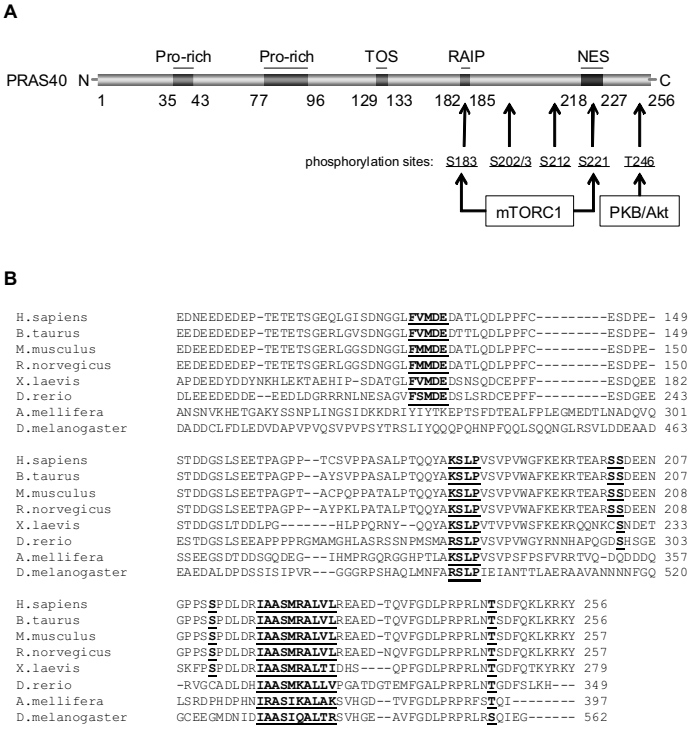

***Figure 2**. A. Primary structure of the human PRAS40 protein. PRAS40 consists two proline-rich stretches followed by a TOS-motif, a RAIP-motif and a nuclear export sequence. The arrows indicate the sites in PRAS40 that can be modified through phosphorylation by PKB/Akt, mTORC1, and as yet unidentified protein kinases. B. ClustalW alignment of the carboxyterminal part of the human PRAS40 protein (amino acids 101-256; NP_115751) with the PRAS40 homologues of Bos taurus (NP_001076903), Mus musculus (NP_080546), Rattus norvegicus (NP_001099729), Xenopus laevis (NP_001084778), Danio rerio (XP_692511), Apis mellifera (XP_623909) and Drosophila melanogaster (NP_524787). The amino acids comprising the TOS-motif, the RAIP-motif, the NES and the phosphorylation sites are depicted in bold and underlined.*

Subcellular localization of PRAS40. Although PRAS40 is part of the mTORC1 complex, which exerts its action predominantly in the cytosol, multiple components and regulators of mTORC1 signalling, including PI3K, Akt, TSC2, mTOR, raptor, and S6K, are found both in the cytoplasm and the nucleus (163-168). The presence of a NES in PRAS40 (Figure 2) suggests that shuttling of the protein may occur between the cytosolic compartment and the nucleus. Indeed, multiple studies report a nuclear localization of the protein. Notably, PRAS40 has been purified as nuclear phosphoprotein from Hela cells (169). Furthermore, staining A14 fibroblasts and E2 H9c2 cardiomyocytes with antibodies recognizing PRAS40 phosphorylated on Thr246 demonstrate a nuclear immunoreactivity that is promoted by insulin (170). Finally, immunohistochemistry studies on rat liver and heart, and mouse brain also demonstrate a predominant nuclear localization of Thr246-phosphorylated PRAS40 (171-173).

Regulation of PRAS40 phosphorylation

PRAS40 is phosphorylated on multiple sites in response to treatment of cells with growth factors like platelet-derived growth factor (PDGF), nerve growth factor (NGF), and insulin, as well as nutrients, such as glucose and amino acids (174-178). *In vivo* studies confirm PRAS40 as a physiological target for insulin action in human skeletal muscle and various rodent tissues, including skeletal muscle, adipose tissue, the liver, the heart and the arcuate nucleus (179;180) (EBMN and DMO, unpublished observations). Phosphorylation of PRAS40 induces 14-3-3 binding (181;182), and disrupts the interaction between raptor and PRAS40 (183-185).

Regulation of PRAS40-Thr246 phosphorylation. Thr246 of PRAS40 is embedded in a perfect and highly conserved consensus sequence for phosphorylation by PKB/Akt, i.e. Arg-*x*-Arg-*xx*-[pSer,pThr] (186) (Figure 1B). Indeed, incubation of PRAS40 with recombinant PKB/Akt promotes phosphorylation of Thr246 (187). Furthermore, activation of PKB/Akt alone is sufficient to induce Thr246 phosphorylation in NIH3T3 fibroblasts (188), whereas treatment of BT474 tumour cells with the PKB/Akt-inhibitor GSK690693 lowers phosphorylation of Thr246 (189). The amino acid context of Thr246 also displays similarities with the optimal phosphorylation site for the oncogene-encoded protein kinase PIM1 (190), and very recently PIM1 has been shown to phosphorylate Thr246 in *in vitro* kinase assays and following enforced expression of PIM1 in murine myeloid FDCP1 cells (191).

Studies in cultured cell lines show that Thr246 phosphorylation is promoted by insulin, NGF, and PDGF, and abrogated by the PI3K-inhibitors wortmannin and LY294002 (192-194). Furthermore, PDGF-mediated Thr246 phosphorylation is almost completely abrogated in embryonic fibroblasts derived from mice lacking both Akt1 and Akt2 (195;196).

The regulation of PRAS40-Thr246 phosphorylation by PKB/Akt seems dependent on phosphorylation of Ser473 of PKB/Akt by the mTORC2-complex. Inhibition of mTORC2 activity, either pharmacologically or by silencing of rictor, reduces Thr246-phosphorylation of PRAS40 (2;197). Finally, some studies show a partial inhibition of PRAS40-Thr246 phosphorylation by rapamycin (198-200). Although mTORC2 has been reported to be insensitive to rapamycin, prolonged exposure to rapamycin has been shown to inhibit mTORC2 activity in certain eukaryotic cell types (201;202). Alternatively, efficient phosphorylation of Thr246 by the PI3K-PKB/Akt-mTORC2 pathway may require phosphorylation of PRAS40 on other residues by mTORC1 (203;204).

Phosphorylation of PRAS40 by mTORC1. The observation that the interaction of PRAS40 with 14-3-3 proteins is completely dependent on the presence of amino acids, whereas the PKB/Akt-dependent phosphorylation of PRAS40 on Thr246 is only partially abrogated by amino acid deprivation, suggests that additional mTORC1-mediated phosphorylations are required for 14-3-3 binding to PRAS40 (205;206). Indeed, *in vitro* kinase assays on mTORC1 immunoprecipitates identified additional phosphorylation sites on Ser183, Ser202/Ser203, Ser212, and Ser221 in PRAS40 (207;208). In cultured cells, Ser183 is promoted by amino acids and insulin, and blunted by rapamycin, glucose withdrawal and amino acid starvation, thus providing further support that Ser183 is phosphorylated by mTORC1 (209;210). Although insulin was found to promote phosphorylation of Ser202/Ser203, Ser212, and Ser221 in HEK293 cells *in vivo*, only phosphorylation of Ser221 was sensitive to rapamycin (211). Therefore, the phosphorylation of Ser202/203 and Ser212 is probably mediated by as yet unknown protein kinases other than mTORC1.

Binding of PRAS40 with 14-3-3 protein. The binding of 14-3-3 proteins to PRAS40 is prevented by inhibition of PI3K-activity and amino acid deprivation (212-214). Accordingly, substitution of Ser221 or Thr246 by Ala in PRAS40 almost completely abolished the insulin-induced binding of 14-3-3 proteins to PRAS40 (215;216). Interestingly, mutations of Phe129 in the TOS-motif, or Ser183 or Pro185 in the potential RAIP-motif all prevented 14-3-3 binding and reduced Thr246 phosphorylation (217;218). Thus, although the binding of 14-3-3 proteins is clearly dependent on both PKB/Akt- and mTORC1-mediated phosphorylation of PRAS40, the precise contribution of the mTORC1-regulated sites requires further studies, such as analysis of Ser183 phosphorylation in PRAS40 mutants with a substitution of Ser221 or Thr246.
It has been proposed that 14-3-3 binding is important for mTORC1 activation by sequestering PRAS40 away from mTORC1, and thereby relieving any inhibitory action that PRAS40 has on mTORC1. Indeed co-expression of 14-3-3 enhances the phosphorylation of S6K1 induced by a constitutively active mutant of PKB/Akt (219). However, the inhibition of mTORC1 *in vitro* kinase activity, or mTORC1 signalling by PRAS40, was independent of the presence of or the ability to interact with 14-3-3 proteins (220;221). It is currently unknown whether 14-3-3 binding may serve to alter the subcellular localization of PRAS40. Therefore, the physiological significance of 14-3-3 binding to PRAS40 remains as yet unclear.

Cellular functions of PRAS40

Regulation of mTORC1 activity and cell growth. In addition to being a substrate for mTORC1, multiple studies also identify PRAS40 as a negative regulator of mTORC1 activity and cell growth. The presence of recombinant PRAS40 in *in vitro* kinase assays

inhibits both mTORC1 autophorylation and the induction of 4EBP1 and S6K1 phosphorylation (2;222;223). Accordingly, overexpression of PRAS40 lowered basal phosphorylation S6K1 and 4EBP1 in multiple cell lines (224-227). These initial reports suggest that PRAS40 functions as substrate inhibitor of mTORC1, and that phosphorylation of PRAS40, which results in dissociation of PRAS40 from mTORC1, relieves the inhibitory constraint on mTORC1. Indeed, silencing of PRAS40 increased basal S6K1 phosphorylation (228). A similar effect on S6K from *Drosophila melanogaster* was observed upon silencing of Lobe (229). Consistent with a role for PRAS40 as negative regulator of mTORC1, overexpression of PRAS40 led to a significant reduction in cell size (230;231). Reciprocally, silencing of Lobe in insect cells increased cell size (232). However, phorbol esters activate mTORC1 without affecting PRAS40 phosphorylation (233), leaving the possibility that the regulation of mTORC1 activity is stimulus-specific.
The inhibitory effect of PRAS40 on mTORC1 activity seems to require the interaction with raptor, as a PRAS40 mutant with Phe129 replaced by Ala, which cannot bind raptor, does not affect 4EBP1 phosphorylation *in vitro* and S6K1 phosphorylation *in vivo* (234). Also overexpression of another raptor-binding mutant, Ser183Asp PRAS40, had no inhibitory effect on S6K1 phosphorylation (235). Based on these findings, it has been proposed that PRAS40 functions as an inhibitor of substrate binding on raptor. Silencing of PRAS40 was found to enhance 4EBP1 binding to raptor, whereas recombinant wild type, but not Phe129Ala-PRAS40, competed with 4EBP1 binding to raptor (236). In line with this, overexpression of either 4EBP1 or S6K1 lowered the binding of PRAS40 to raptor and reduced mTORC1-mediated phosphorylation of PRAS40 on Ser183 (237). Another report, however, does not support a requirement for raptor-binding as the Phe129Ala PRAS40 mutant inhibited insulin-mediated 4EBP1 phosphorylation to a similar extent as wild type PRAS40 (238). The reason for this discrepancy is as yet unclear.
Strikingly, whereas silencing of PRAS40 has been found to enhance amino acid induced phosphorylation of 4EBP1 and S6K1 in one study (239), other reports show that both amino acid- and insulin-induced phosphorylation of 4EBP1 and S6K1 are reduced in the absence of PRAS40 (240-243). The requirement of PRAS40 for the phosphorylation of mTORC1 substrates, therefore, also suggests a role for PRAS40 in the assembly or integrity of the mTORC1 complex. Clearly, more studies are required to explain the discrepancies in literature and further detail the function of PRAS40 in the regulation of mTORC1.

Apoptosis. PRAS40 has been linked to the regulation of cell survival and apoptosis. Overexpression of PRAS40 protects neurons from apoptotic cell death transient focal cerebral ischemia or spinal cord injury (244;245). It has been proposed that ischemia and reperfusion-mediated PRAS40-Thr246 phosphorylation and subsequent 14-3-3 binding

play a critical role in the protection of neuronal cells from apoptosis induced by ischemia *in vivo* (246;247). Also Lobe has been implicated in the regulation of cell survival during eye development in *Drosophila melanogaster* (248). Conversely, lowering PRAS40 expression has been found to protect against the induction of apoptosis by tumour necrosis factor α or cyclohexamide (2). As rapamycin fails to mimic the pro-apoptotic effect of PRAS40 silencing, it was suggested that PRAS40 may regulate apoptosis independent of mTORC1. The role of mTORC1 signaling has not been determined in the other studies on the role of PRAS40 in apoptosis. Given the observed differences of the impact of PRAS40 silencing on mTORC1 signaling (249-253), the role of mTORC1 in the regulation of apoptosis by PRAS40 therefore requires further studies.

Deregulation of PRAS40 in disease

Insulin resistance. The induction of PRAS40-Thr246 phosphorylation by insulin *in vivo* is reduced in skeletal muscle, heart, liver, and adipose tissue from insulin-resistant high-fat diet fed rats, and skeletal muscle from ob/ob mice (254-256). Also incubation of rat soleus muscle with palmitate lowers the induction of PRAS40-Thr246 phosphorylation by insulin (257). Improving insulin sensitivity by weight loss improved the induction of PRAS40-Thr246 in human skeletal muscle response to hyperinsulinemia (258).

It is unclear whether the reduction in insulin-mediated PRAS40-Thr246 phosphorylation affects the activity of mTORC1, or results from hyperactivation of the mTORC1 pathway. It has been proposed that the increase in basal S6K1 phosphorylation caused by silencing of PRAS40 induces degradation of IRS1, which on its turn reduces insulin-induced phosphorylation of PKB/Akt (259). However, other studies could not demonstrate an affect of either silencing or overexpression of PRAS40 on PKB/Akt phosphorylation (260;261). Also acute treatment of ob/ob mice with rapamycin did not improve insulin sensitivity in ob/ob mice despite elevated activity of mTORC1 in skeletal muscle (262). A limitation of this study, however, is that the phosphorylation of IRS1, PKB/Akt and PRAS40 was not assessed after rapamycin treatment. Therefore, further studies, including the effects of alterations in insulin sensitivity on the other PRAS40 phosphorylation sites, are required to determine the physiological consequences of a reduced PRAS40-Thr246 phosphorylation in insulin resistance.

Cancer. Both the expression and Thr246-phosphorylation of PRAS40 are elevated in pre-malignant and malignant breast and lung cancer cell lines (263). Furthermore, levels of Thr246-phosphorylated PRAS40 were increased in meningiomas, and malignant melanomas (264;265). The increase in PRAS40 phosphorylation in during melanoma tumour progression was paralleled by increased Akt3 activity (266). However, also other oncogenic protein kinases, like PIM1 (267), may contribute to enhanced PRAS40-Thr246

phosphorylation as PRAS40-Thr246 phosphorylation in tumour was only lowered by incubation with wortmannin (268). Interestingly, lowering PRAS40-Thr246 phosphorylation associates with an increased sensitivity of tumour cells to pro-apoptotic stimuli (269), suggesting the PRAS40 plays a critical role in tumour cell survival.

Conclusions and perspectives

The identification of PRAS40 as regulator and substrate of both mTORC1 and PKB/Akt has added to the complexity of mTORC1 signalling. Future challenges lie in further detailing the impact of the alterations in PRAS40 phosphorylation, as observed in insulin resistance and cancer, on mTORC1-activity and insulin signalling through the IRS1-PKB/Akt pathway. Furthermore, it would be interesting whether PRAS40 also affects the activity of the growing list of substrates of mTORC1, and other mTORC1-regulated signalling pathways.

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