Role of PRAS40 in Akt and mTOR signaling in health and disease

Claudia Wiza,1 Emmani B. M. Nascimento,2 and D. Margriet Ouwens1

1Institute of Clinical Biochemistry and Pathobiology, German Diabetes Center, Düsseldorf, Germany; and 2Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Submitted 21 December 2011; accepted in final form 20 February 2012

Wiza C, Nascimento EB, Ouwens DM. Role of PRAS40 in Akt and mTOR signaling in health and disease. Am J Physiol Endocrinol Metab 302: E1453–E1460, 2012. First published February 21, 2012; doi:10.1152/ajpendo.00660.2011.—The proline-rich Akt substrate of 40 kDa (PRAS40) acts at the intersection of the Akt- and mammalian target of rapamycin (mTOR)-mediated signaling pathways. The protein kinase mTOR is the catalytic subunit of two distinct signaling complexes, mTOR complex 1 (mTORC1) and mTORC2, that link energy and nutrients to the regulation of cellular growth and energy metabolism. Activation of mTOR in response to nutrients and growth factors results in the phosphorylation of numerous substrates, including the phosphorylations of S6 kinase by mTORC1 and Akt by mTORC2. Alterations in Akt and mTOR activity have been linked to the progression of multiple diseases such as cancer and type 2 diabetes. Although PRAS40 was first reported as substrate for Akt, investigations toward mTOR-binding partners subsequently identified PRAS40 as both component and substrate of mTORC1. Phosphorylation of PRAS40 by Akt and by mTORC1 itself results in dissociation of PRAS40 from mTORC1 and may relieve an inhibitory constraint on mTORC1 activity. Adding to the complexity is that gene silencing studies indicate that PRAS40 is also necessary for the activity of the mTORC1 complex. This review summarizes the regulation and potential function(s) of PRAS40 in the complex Akt- and mTOR-signaling network in health and disease.

PROLINE-RICH AKT SUBSTRATE OF 40 kDa (PRAS40) was first identified as a 14-3-3 binding protein in lysates from insulin-treated hepatoma cells (28) and is identical to the p39 protein that is phosphorylated in PC12 cells treated with nerve growth factor or epidermal growth factor (18) and the nuclear phosphoprotein Akt1 substrate 1 (AKT1S1) purified from Hela cells (3). Although originally described as substrate for Akt (28), analysis of mammalian target of rapamycin (mTOR) immunoprecipitates identified PRAS40 as a component and substrate of the mTOR complex 1 (mTORC1) (35, 42, 49, 51).

In addition to mTOR and PRAS40, the mTORC1 complex consists of regulatory-associated protein of mTOR (raptor), the mammalian ortholog of yeast lethal with Sec13 protein 8 (mLST8; also known as GβL), and DEP domain-containing mTOR-interacting protein (deptor) (61). Within mTORC1, raptor functions as a scaffold through regulation of the assembly of the mTORC1 complex, the recruitment of substrates, and direction of the subcellular localization (61). Both PRAS40 and deptor exert an inhibitory action on mTORC1 activity (61). Activation of mTORC1, which occurs in response to nutrients and growth factors, results in phosphorylation of both PRAS40 and deptor by mTORC1 (33, 61). This leads to dissociation of PRAS40 and deptor from the complex and relieves the inhibitory constraint on its activity (33, 61).

Through phosphorylation of a rapidly expanding list of protein substrates (9, 20, 59), mTORC1 participates in multiple cellular processes, including the regulation of cell size, mRNA translation, ribosome biogenesis, lipid biogenesis, vesicle-mediated transport, autophagy, and mitochondrial function (45, 61).

The catalytic subunit of mTORC1, the serine-threonine kinase mTOR, is shared with another large multimeric protein complex, mTORC2 (61). Although PRAS40 is absent in mTORC2, this complex shares mTOR, mLST8, and deptor with mTORC1 and further consists of the unique components rapamycin-insensitive companion of mTOR (rictor), mammalian stress-activated protein kinase-interacting protein, and proline-rich Akt substrate of 40 kDa; mammalian target of rapamycin...
abrogation of the insulin-signaling pathway regulating glucose metabolism (20, 33, 59). Furthermore, glucose-induced hyperphosphorylation of PRAS40 has been implicated in the progression of diabetic nephropathy (10). Deregulated activity of mTOR signaling (TOS) further fuels tumorigenesis through the mTORC1-dependent stimulation of cellular growth, cell proliferation, angiogenesis, and suppression of autophagy and the mTORC2-dependent regulation of proliferation, cell survival, and nutrient uptake (17, 61). These multiple aspects of mTORC1- and mTORC2-signaling pathways have been reviewed extensively by others (29, 45, 61). Here, we focus on one regulatory component of mTORC1 and downstream target of both mTORC1 and mTORC2, i.e., PRAS40. In this review, we summarize the regulation of PRAS40 activity and its potential function(s) in the complex Akt- and mTOR-signaling networks in health and disease.

Expression and Structure of PRAS40

The gene for PRAS40, located on human chromosome 19q13.33, encodes three transcript variants that differ in their 5′-untranslated region and result in 256 and 276 amino acid proteins. Although the PRAS40 mRNA and protein show a ubiquitous expression in human, rodent, and fly tissues (28, 32, 37), there is currently no information available related to the expression pattern of the various isoforms. The 276-amino acid protein differs from the 256-amino acid variant in a 20-amino acid extension at the amino terminus. As shown in Fig. 1, the amino terminal part of PRAS40 contains two proline-enriched stretches with an as-yet undefined function as well as sequences that have the potential to bind proteins containing Src homology 3 and/or WW domains (28). The proline-rich regions are followed by two short motifs implicated in mTORC1 binding, i.e., a TOS motif (amino acids 129–133 of the human PRAS40 protein) (14, 35, 54) and a Lys-Ser-Leu-Pro sequence (amino acids 182–185) showing resemblance to the RAIP motif, which has been named after a short amino acid sequence found in eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) (48). Furthermore, sequence analysis of the protein identifies a 10-amino acid stretch in the carboxy-terminal part of PRAS40 (amino acids 218–227 of the human 256-amino acid protein) that matches the consensus sequence for a leucine-enriched nuclear export sequence (NES), Leu-xx(x)−[Leu,Ile,Val,Phe,Met]−xx(x)Leu-x−[Leu,Ile] (33). Finally, phosphorylation of PRAS40 on multiple residues, including Ser88, Ser92, Ser116, Ser183, Thr198, Ser202, Ser203, Ser211, Ser212, Ser221, and Thr246, has been reported (9, 20, 28, 35, 53, 59).

Conservation of PRAS40

PRAS40 is highly conserved in higher species (33). Homologs almost identical to the human protein have been found amongst other species, including Pan troglodytes, Bos taurus, Mus musculus, and Rattus norvegicus (33). In these species, the longer forms with an extension at the aminoterminus have also been found. All phosphorylation sites are fully conserved in the PRAS40 variants in these higher species (Fig. 1) (33). In Xenopus laevis, Danio rerio and Drosophila melanogaster proteins, which are identical to the carboxy-terminal part of human PRAS40 but lack entirely the proline-enriched stretches found in the amino-terminal PRAS40 proteins from higher species, have been found (33, 37, 42). The proteins found in these species show conservation of the TOS and RAIP motifs as well as of the phosphorylation sites equivalent to the human residues Ser183, Ser221, and Thr246 (Fig. 1) (33). Notably, the Drosophila melanogaster protein dPRAS40 was identified originally as CG10109, a gene associated with the mutant Lobe phenotype (7, 37, 42). However, a loss of the genomic region containing the Lobe allele (Lrev6-3) is embryonically lethal and linked to a disturbed development of the ventral eye (7, 46, 55). In contrast, flies deficient in the dPRAS40 gene are viable and show no alterations in eye development compared with controls (37). Furthermore, CG10109/dPRAS40 expression did not rescue viability in the Lrev6-3 strain (37). This indicates that the phenotypes associated with a loss of the Lobe
PRAS40 interacts with 14-3-3 proteins and the mTORC1 complex (33, 52).

Interaction with 14-3-3 proteins. Using glutathione-S-transferase pulldown and far Western assays as well as coimmunoprecipitation experiments, PRAS40 has been found to interact with 14-3-3 proteins (13, 14, 18, 28, 53). Pretreatment of cells with phosphatidylinositol 3′-kinase (PI3K) inhibitors prevented insulin-induced phosphorylation of PRAS40 on Thr\textsuperscript{246} and 14-3-3 binding to PRAS40 (28). Accordingly, replacement of Thr\textsuperscript{246} by alanine resulted in a loss of the ability of PRAS40 to bind 14-3-3 proteins (14, 53). Importantly, the interaction between PRAS40 and 14-3-3 proteins is also dependent on amino acids (18). However, the induction of PRAS40-Thr\textsuperscript{246} phosphorylation by insulin is not prevented by amino acid deprivation. Therefore, additional mTORC1-mediated phosphorylations have been implicated in the formation of PRAS40/14-3-3 complexes (14, 18). Indeed, rapamycin partially blocks the interaction between PRAS40 and 14-3-3 proteins (14, 18). Also, the silencing of raptor substantially impaired the insulin-induced binding of PRAS40 to 14-3-3 proteins (14). Accordingly, insulin failed to promote 14-3-3 binding to PRAS40 mutants in which the mTORC1 phosphorylation sites Ser\textsuperscript{183} and Ser\textsuperscript{221} were substituted by alanines (14, 53). Except for Ser\textsuperscript{212}, which is not required for 14-3-3 binding (53), the involvement of the additional phosphorylation sites in PRAS40 remains to be investigated. Consistent with the idea that mTORC1-mediated phosphorylation of PRAS40 participates in the regulation of 14-3-3 binding, both mutation of Pro\textsuperscript{185} in the putative RAIP motif as well as of Phe\textsuperscript{129} in the TOS motif markedly impaired 14-3-3 binding to PRAS40 (14, 53).

Because overexpression of 14-3-3 proteins relieves the inhibitory action of PRAS40 on mTORC1 activity, it has been proposed that the binding of 14-3-3 proteins to phosphorylated PRAS40 serves to sequester PRAS40 away from mTORC1 (51). However, activation of mTORC1 by phorbol esters can occur independently of PRAS40 binding to 14-3-3 proteins (13). Therefore, additional studies seem to be required to assess whether 14-3-3 proteins are necessary for the activation of mTORC1 by other factors, like nutrients and growth factors. Furthermore, at least seven different 14-3-3 proteins have been characterized in humans. It remains to be addressed whether these variants impact on PRAS40 binding and possibly regulation of mTORC1 activity.

Interaction with mTORC1. The recent elucidation of the structure of the entire mTORC1 complex supports previous biochemical observations that PRAS40 interacts with mTORC1 through the binding to substrate binding site of raptor (14, 35, 42, 49, 51, 54, 57). A recent report also demonstrated an association between dPRAS40 and raptor in Drosophila melanogaster (37). The interaction between PRAS40 and raptor is weakened by insulin and to a lesser extent by amino acids, indicating that phosphorylation of PRAS40 results in dissociation of PRAS40 from mTORC1 (12, 14, 42, 51, 54). The dissociation of PRAS40 would then allow the binding and activation of mTORC1 substrates like ribosomal protein S6 kinase 1 (S6K1) and 4E-BP1 to the substrate binding site of raptor. Critical functions for the interaction between PRAS40 and raptor have been ascribed to the TOS and RAIP motif as well as the region located between amino acids 150 and 234 of PRAS40 (14, 35, 51, 54). It should be noted that some reports also implicate the kinase domain of mTOR in the binding of PRAS40 to mTORC1 (12, 49, 51). In cells overexpressing mutant forms of mTOR with a deletion or inactivation of the carboxy-terminal kinase domain, the amount of PRAS40 in mTOR immunoprecipitates decreased (49, 51). Furthermore, phosphorylation mimicking substitutions of two newly identified phosphorylation sites within the mTOR kinase domain, Ser\textsuperscript{183} and Thr\textsuperscript{2164}, reduced whereas substitution of these amino acids by alanine enhanced the presence of raptor and PRAS40 in mTOR immunoprecipitates (12). It seems likely that these findings can be explained by the identification of the carboxy-terminal domain of mTOR as raptor-binding domain (57).

Regulation of PRAS40 Phosphorylation

Phosphorylation of PRAS40 on multiple residues critically determines the function of the protein because it promotes the binding to 14-3-3 proteins and weakens the interaction with mTORC1. Stimuli that enhance PRAS40 phosphorylation include growth factors, such as insulin, NGF, and PDGF, as well as nutrients, such as glucose and amino acids (33, 52). Although PRAS40 is phosphorylated on multiple sites, only the pathways regulating Ser\textsuperscript{183} and Thr\textsuperscript{246} phosphorylation are well characterized because of the availability of commercial antibodies recognizing these phosphorylated residues. Phosphorylation of PRAS40-Thr\textsuperscript{246} by Akt-dependent and -independent pathways. As shown in Fig. 2, the major kinase promoting PRAS40-Thr\textsuperscript{246} phosphorylation is Akt. The activation of Akt in response to insulin involves the binding of insulin to the insulin receptor. This leads to tyrosine phosphorylation and activation of the insulin receptor itself and of multiple substrates, such as the insulin receptor substrate (IRS) proteins (47). The tyrosine-phosphorylated IRS proteins recruit PI3K to the plasma membrane, where activated PI3K phosphorylates phosphatidylinositol-4,5-biphosphate to form phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P\textsubscript{3}] (47). PI(3,4,5)P\textsubscript{3} binds to the pleckstrin homology domains of phosphoinositide-dependent kinase 1 (PDK1) and Akt and mediates the phosphorylation of Akt on Thr\textsuperscript{308} by PDK1 (47). Studies in cultured cell lines show that the induction of PRAS40-phosphorylation on Thr\textsuperscript{246} is prevented by P3K inhibition (28, 32, 34). Furthermore, the PDGF-induced phosphorylation of PRAS40 on Thr\textsuperscript{246} is almost completely abrogated in embryonic fibroblasts lacking both Akt1 and Akt2, whereas inducible activation of Akt alone is sufficient to promote PRAS40-Thr\textsuperscript{246}.
phosphorylation in NIH3T3 fibroblasts (28). In line with the regulation of PRAS40-Thr\(^{246}\) phosphorylation by the PDK1/PI3K/Akt pathway, tumor cells harboring constitutively active mutants of PI3K or Akt or a loss of phosphatase and tensin homolog (PTEN), which dephosphorylates PI-3,4,5-P\(_3\), display elevated levels of Thr\(^{246}\)-phosphorylated PRAS40, which can be lowered by PDK1 or Akt inhibitors (2, 4, 6, 15, 39). For phosphorylation of most downstream substrates, Akt requires phosphorylation of Ser\(^{473}\) by mTORC2 (Fig. 2). This also applies to PRAS40 since inhibition of mTORC2, either pharmacologically or through silencing of rictor, reduces the phosphorylation of PRAS40 on Thr\(^{246}\) (20, 49, 50, 59). Other stimuli promoting PRAS40-Thr\(^{246}\) through the Akt pathway include glucose and ceramide-1-phosphate (11, 16).

Although Akt is the major pathway regulating PRAS40-Thr\(^{246}\) phosphorylation, PRAS40 can also be phosphorylated...
on Thr\textsuperscript{246} by Akt-independent mechanisms in tumor cells (21). Accordingly, the proto-oncogene PIM1 promotes phosphorylation of Thr\textsuperscript{246} in vitro kinase assays and following enforced expression of PIM1 in myeloid factor-dependent cell progenitor cells (60). In the heart, leucine was found to promote PRAS40-Thr\textsuperscript{246} phosphorylation via a pathway that requires PI3K and PDK1 but was independent of Akt (43). PDK1 is a key regulator of the so-called AGC kinases. Although a role for S6K1, protein kinase A, and protein kinase C in the regulation of leucine-mediated PRAS40-Thr\textsuperscript{246} was excluded in this report, these findings implicate a role for members of the AGC protein kinase family in the phosphorylation of PRAS40-Thr\textsuperscript{246}. In support of this, protein kinase A has been linked to the induction of PRAS40-Thr\textsuperscript{246} phosphorylation in thyroid cell response to thyroid hormone treatment and elevation of intracellular cAMP levels (5).

**Phosphorylation of PRAS40-Ser\textsuperscript{183} by mTORC1.** Phosphorylation of PRAS40-Ser\textsuperscript{183} in cultured cells is promoted by insulin and amino acids and blunted by wortmannin, rapamycin, glucose withdrawal, and amino acid starvation (34, 35). Furthermore, insulin infusion was found to enhance PRAS40-Ser\textsuperscript{183} phosphorylation in human skeletal muscle as well as in rat cardiac and skeletal muscle (34). So far, only mTORC1 has been identified as an upstream regulator of PRAS40-Ser\textsuperscript{183} phosphorylation (53). Insulin-induced activation of mTORC1 occurs via binding of the GTP-bound form of Rheb to mTORC1. The intracellular levels of Rheb-GTP are regulated by the Akt-mediated phosphorylation of tuberous sclerosis complex 2 (TSC2; Fig. 2). This leads to inactivation of the TSC complex, which acts as a GTPase-activating protein on Rheb, and thus an increase in Rheb-GTP levels. The amino acid-mediated activation of mTORC1 may not involve Rheb directly. However, amino acids are indispensable for the activation of mTORC1 by insulin and other growth factors and serve to bring mTORC1 in the vicinity of Rheb-GTP, which is localized in the lysosomal membrane (26, 61). The recently identified “Rag-Ragulator” complex plays a key role in recruiting mTORC1 to the lysosomal membrane (26). The “Ragulator” complex, consisting of MPI, p14, and p18, targets the Rag-GTPase complex, consisting of RagA/B and RagC/D, to the lysosomal membrane (26). Amino acids increase the intracellular levels of RagA/B-GTP, thereby facilitating the interaction between RagC/D and raptor and thus the activation of mTORC1 (Fig. 2) (26).

**Phosphorylation of PRAS40 on other residues by mTORC1.** Beside inducing PRAS40-Ser\textsuperscript{183} and -Thr\textsuperscript{246} phosphorylation, phosphopeptide mapping of human embryonic kidney (HEK)-293 cells metabolically labeled with \[^{32}P\]orthophosphate revealed that insulin also promotes the phosphorylation of PRAS40 on Ser\textsuperscript{183}, Ser\textsuperscript{202}, Ser\textsuperscript{203}, Ser\textsuperscript{212}, and Ser\textsuperscript{221} (53). Phosphorylation of these amino acids was also induced in in vitro kinase assays on raptor immunoprecipitates, suggesting that mTORC1 mediates these additional phosphorylations (53). In this report, only the insulin-mediated phosphorylations on Ser\textsuperscript{183} and Ser\textsuperscript{221} were found to be sensitive to rapamycin (53). Accordingly, phosphoproteomic screens aimed at characterizing mTORC1- and mTORC2-regulated phosphoproteins on insulin-treated HEK cells showed that rapamycin downregulated the phosphopeptide corresponding to PRAS40-Ser\textsuperscript{183} and that the dual mTORC1/mTORC2-inhibitor Torin-1 prevented the insulin-mediated phosphorylation of Ser\textsuperscript{183} and Thr\textsuperscript{246} (20). In contrast, phosphoproteomic analysis of rat livers following fasting and refeeding showed that rapamycin downregulated the phosphorylation of the sites corresponding to Ser\textsuperscript{183} as well as Ser\textsuperscript{202}, Ser\textsuperscript{203}, Ser\textsuperscript{211}, and Ser\textsuperscript{212} of human PRAS40 (9). Furthermore, in mouse embryonic fibroblasts lacking an upstream activator of mTORC1, TSC2, the phosphopeptides corresponding to Ser\textsuperscript{188}, Ser\textsuperscript{192}, Ser\textsuperscript{202}, Ser\textsuperscript{203}, Ser\textsuperscript{212}, Ser\textsuperscript{215}, and Thr\textsuperscript{198} of human PRAS40 were downregulated by rapamycin (59). In another report, Torin-1 downregulated the phosphorylation of Ser\textsuperscript{183}, Thr\textsuperscript{246}, Ser\textsuperscript{202}, and Ser\textsuperscript{212} in wild-type embryonic fibroblasts, but upregulation of these phosphorylation sites in cells lacking TSC2 could not be confirmed (20). Finally, in wild-type embryonic fibroblasts, insulin-induced phosphorylation of Ser\textsuperscript{188}, Ser\textsuperscript{192}, Ser\textsuperscript{202}, Ser\textsuperscript{203}, Ser\textsuperscript{212}, and Thr\textsuperscript{246} was downregulated by a dual mTORC1/mTORC2 inhibitor, Ku0063794 (59). Thus, although these studies identify mTORC1 as potential regulator of the additional phosphorylation sites on PRAS40, further studies toward their regulation, for example, by using antibodies recognizing these phosphorylated residues, seem to be required at least to clarify some of the discrepancies that emerged in the phosphoproteomic studies.

**Cellular Functions of PRAS40**

**Effects on mTORC1 activity.** Knockdown of PRAS40 has been reported to increase the basal phosphorylation of the mTORC1 substrates S6K and 4E-BP1 in various cell types, including mouse embryonic fibroblasts, HT-29, HeLa, HepG2, HEK-293, mesangial cells, and 3T3-L1 adipocytes (11, 35, 42, 51). Similarly, silencing dPRAS40 increased S6K phosphorylation and cell diameter in cultured Drosophila cells (42). In line with these observations, overexpression of PRAS40 blunted the insulin-mediated phosphorylation of the mTORC1 substrates S6K and 4E-BP1 in cultured 3T3-L1 adipocytes and HEK-293 cells (14, 35, 42, 54) and reduced the cell size of HEK-293T cells and rat embryonic fibroblasts (51). Furthermore, tissue-specific overexpression of dPRAS40 led to tissue undergrowth, which could be ascribed to reduced cell size rather than apoptosis, whereas ubiquitous overexpression of dPRAS40 reduced the size of the entire animal and caused pupal lethality (37). Collectively, these findings support the idea that PRAS40 acts as a negative regulator of mTORC1 activity and that the phosphorylation-dependent dissociation of PRAS40 from raptor relieves an inhibitory constraint on mTORC1 activity.

Yet other reports argue against this proposed function of PRAS40 in mTORC1 activation. For example, the dissociation of PRAS40 from mTORC1 has been found to promote 4E-BP1 binding to raptor but not to affect basal or insulin-mediated S6K and 4E-BP1 phosphorylation in 293E cells (38). Also, in C\textsubscript{2}C\textsubscript{12} myoblasts but not in differentiated C\textsubscript{2}C\textsubscript{12} myotubes, knockdown of PRAS40 did not affect basal or insulin-like growth factor I-induced phosphorylation of the mTORC1 substrates S6K and 4EBP1 despite protein synthesis being reduced and cell diameter being increased (25). Finally, there are reports indicating that PRAS40 is essential for mTORC1 activity. In HEK-293 cells, the silencing of PRAS40 was found to impair the amino acid- and insulin-mediated phosphorylation of 4E-BP1 and the S6K substrate ribosomal protein S6 (14). In line with these findings, one study demonstrated that
silencing PRAS40 led to increased AMP kinase-mediated phosphorylation of TSC2, which results in inhibition of mTORC1 (19).

A recent report on Drosophila shed light on these seemingly contrasting findings regarding the function of PRAS40 in mTORC1 signaling (37). Deficiency of dPRAS40 did not impact on TORC1 during the growth of the animal. However, in contrast to the situation in larvae, the absence of dPRAS40 led to a dramatic elevation in basal S6K phosphorylation in the ovaries of adult flies but not in the rest of the animal (37). The authors ascribed these tissue-specific effects of dPRAS40 to alterations in the posttranslational modification of dPRAS40 in the ovary compared with the rest of the animal (37). Furthermore, the levels of phosphorylated S6K in the ovaries of dPRAS40-deficient females could not be enhanced further upon activation of the PI3K pathway (37). These molecular alterations were accompanied by increased fertility since dPRAS40-deficient females had larger ovaries and laid more eggs compared with controls. Importantly, removing one copy of the S6K gene in dPRAS40-deficient females normalized fertility, thereby indicating that the main physiological function of dPRAS40 is to act as an inhibitor of the TORC1 pathway (37). In addition, these findings suggest that posttranslational modification, such as phosphorylation, may be critical for the activity of PRAS40 toward mTORC1. Therefore, to clarify the function of PRAS40 in more detail in mammalian cell types, experiments using mutant forms of PRAS40 in addition to the silencing of PRAS40 should be considered.

Effects on insulin signaling. As for the effects on mTORC1 activity, contrasting data have also been reported for a regulatory role of PRAS40 in insulin action. In 3T3L1 adipocytes and HepG2 cells, silencing PRAS40 resulted in a decreased insulin-mediated phosphorylation of Akt (51). These results were ascribed to increased phosphorylation of S6K1, which via the induction of serine phosphorylation of IRS-1 resulted in degradation of IRS-1 and insulin resistance. Decreased IRS-1 expression and reduced basal phosphorylation of Akt were also observed following PRAS40 knockdown in C2C12 myoblasts (19). However, in these studies, these effects were paralleled by a reduced mTORC1 activity. In contrast, in HEK-293 cells neither overexpressing nor silencing PRAS40 affected the phosphorylation of Akt in response to insulin. Thus, as for the effects of PRAS40 on mTORC1 signaling, the potential regulatory role in insulin action also requires further analysis.

Effects on apoptosis and cell cycle progression. Overexpression of PRAS40 reduced neuronal apoptotic cell death in mice after transient focal cerebral ischemia (40, 41) and enhanced the survival of motor neurons after spinal cord injury in rats (58). These neuroprotective effects were accompanied by an increased binding of phosphorylated PRAS40 to 14-3-3 proteins (58). Furthermore, inhibition of PI3K increased apoptosis of motor neurons following spinal cord injury (58). Silencing PRAS40 increased apoptosis and lowered cell viability in melanoma cells and reduced tumor development in mice by increasing apoptosis levels rather than altering the proliferation rates in melanoma tumors (30). In contrast to these studies, one report ascribes a proapoptotic function to PRAS40, because silencing of PRAS40 was found to prevent the induction of apoptosis in HeLa cells in response to tumor necrosis factor-α or cyclohexamide treatment (49). Finally, silencing PRAS40 in C2C12 myoblasts had no effect on apoptosis but reduced the number of cells and the rate of proliferation due to an increased number of cells remaining in the G1 phase (25).

Dysregulation of PRAS40 Phosphorylation in Disease

Cancer. Human cancers frequently show a sustained activation of PTEN/PI3K/Akt- and mTORC-mediated signaling pathways (4, 61). Consequently, elevated PRAS40-Thr246 phosphorylation has been reported in several cancer cell lines as well as in meningiomas and malignant melanomas (21, 23, 24, 30, 52). Furthermore, phosphorylation of PRAS40-Thr246 has been used as a biomarker for evaluating the effects of novel inhibitors targeting components of the PTEN/PI3K/Akt- and mTORC-mediated signaling pathways in human cancer. In this respect, multiple studies showed that the PRAS40-Thr246 phosphorylation state could predict hyperactivation of the PTEN/PI3K/Akt pathway in multiple cancer cell types as well as their sensitivity to inhibitors of components of these signaling pathways (2, 4, 17, 56). Furthermore, PIM1-mediated hyperphosphorylation has been reported in radiation-resistant, non-small-cell lung cancer cells (27).

Insulin resistance. Insulin resistance in rodent models and type 2 diabetes is characterized by a reduced insulin-mediated activation of the PI3K/Akt pathway regulating amongst other glucose metabolism. Accordingly, the induction of PRAS40-Thr246 phosphorylation by insulin was reduced in target tissues for insulin action from rodent models of insulin resistance, such as adipose tissue; skeletal muscle, the liver, and the heart (31, 32, 36). In vitro, exposure of rat soleus muscle or fibroblasts to palmitate was found to reduce insulin-mediated PRAS40-Thr246 phosphorylation (1, 34). Conversely, enhancing insulin sensitivity by weight loss through a very low-calorie diet improved the induction of PRAS40-Thr246 phosphorylation in skeletal muscle following hyperinsulinemia in obese patients with type 2 diabetes (22).

Diabetic nephropathy. Hyperglycemia contributes to the development of diabetic nephropathy among others via the induction of hypertrophy of the mesangial cells in the kidney. Increased phosphorylation of PRAS40 was found to associate with renal hypertrophy in streptozotocin-induced diabetes in rats (11). In vitro studies showed that high glucose promoted the phosphorylation of PRAS40 in mesangial cell via activation of Akt that could be ascribed to reductions in PTEN expression resulting from the induction of microRNA 21 as well as elevated expression of the proto-oncogene DJ-1 (8, 10). Silencing PRAS40 was found to mimic the effects of high glucose on hypertrophy in mesangial cells, suggesting that inactivation of PRAS40 by glucose-mediated phosphorylation could participate in the development of renal cell pathologies in patients with diabetes (11).

Concluding Remarks

PRAS40 is among the most prominent Akt and mTORC1 substrates being phosphorylated in response to nutrient and growth factor stimulation in eukaryotic cells. Consequently, phosphorylated PRAS40 has emerged as a robust biomarker for pathological conditions associated with alterations in Akt and mTORC1 activity as well as effectiveness for inhibitors of these pathways. Intriguingly, the cellular function of PRAS40 has not been completely elucidated in mammalian cells. Yet genetic evidence from Drosophila supports the concept that
PRAS40 functions as a regulator of TORC1 signaling. Importantly, the observed regulation of TORC1 activity by dPRAS40 was tissue specific and seemed to be determined by tissue-specific differences in the posttranslational modification of dPRAS40. Since most studies toward the function of PRAS40 in mammalian cells have been performed by silencing the PRAS40 gene, studies employing overexpression of mutant forms of the protein could be fruitful to clarify the function of PRAS40 in further detail. Furthermore, the results obtained in flies indicate that the generation of mouse models with a (tissue-specific) PRAS40 deficiency or overexpression could be a promising tool to gain further insight into the function of this protein acting at the intersection of the Akt- and mTORC1-signaling pathways.

GRANTS

This work was supported by the Federal Ministry of Health, the Ministry of Innovation, Science, Research, and Technology of the German State of North-Rhine Westphalia, and the German Center for Diabetes Research (Deutsches Zentrum für Diabetesforschung).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.W. and D.M.O. drafted the manuscript; C.W., E.B.N., and D.M.O. edited and revised the manuscript; C.W., E.B.N., and D.M.O. approved the final version of the manuscript.

REFERENCES

31. Miller AM, Brestoff JR, Phelps CB, Berk EZ, Reynolds TH, 4th. Rapamycin does not improve insulin sensitivity despite elevated mammal-


