APPL1: role in adiponectin signaling and beyond

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Deepa SS, Dong LQ. APPL1: role in adiponectin signaling and beyond. Am J Physiol Endocrinol Metab 296: E22–E36, 2009. First published October 14, 2008; doi:10.1152/ajpendo.90731.2008.—Adiponectin, an adipokine secreted by the white adipose tissue, plays an important role in regulating glucose and lipid metabolism and controlling energy homeostasis in insulin-sensitive tissues. A decrease in the circulating level of adiponectin has been linked to insulin resistance, type 2 diabetes, atherosclerosis, and metabolic syndrome. Adiponectin exerts its effects through two membrane receptors, AdipoR1 and AdipoR2. APPL1 is the first identified protein that interacts directly with adiponectin receptors. APPL1 is an adapter protein with multiple functional domains, the Bin1/amphiphysin/rvs167, pleckstrin homology, and phosphotyrosine binding domains. The PTB domain of APPL1 interacts directly with the intracellular region of adiponectin receptors. Through this interaction, APPL1 mediates adiponectin signaling and its effects on metabolism. APPL1 also functions in insulin-signaling pathway and is an important mediator of adiponectin-dependent insulin sensitization in skeletal muscle. Adiponectin signaling through APPL1 is necessary to exert its anti-inflammatory and cytoprotective effects on endothelial cells. APPL1 also acts as a mediator of other signaling pathways by interacting directly with membrane receptors or signaling proteins, thereby playing critical roles in cell proliferation, apoptosis, cell survival, endosomal trafficking, and chromatin remodeling. This review focuses mainly on our current understanding of adiponectin signaling in various tissues, the role of APPL1 in mediating adiponectin signaling, and also its role in the cross-talk between adiponectin/insulin-signaling pathways.

adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain, and leucine zipper motif

A DECREASE IN ADIPONECTIN LEVEL is observed in various clinical conditions like type 2 diabetes, obesity, insulin resistance (4, 67, 70, 148), cardiovascular disease (93, 134), and hypertension (1). Adiponectin is an adipokine synthesized and secreted mainly by adipose tissue (76, 142). However, recent reports indicate that adiponectin is also synthesized by cardiomyocytes, skeletal muscle, osteoblasts, placenta, and pituitary (11, 19, 40, 90, 133, 137, 146). Adiponectin exerts its beneficial effect on metabolism by improving insulin sensitivity, glucose tolerance, and lipid profile and by decreasing atherosclerosis and inflammation (77). Adiponectin is a 30-kDa protein having an NH2-terminal collagen domain and a COOH-terminal globular domain. In serum, adiponectin exists either as multimers of full-length adiponectin or as globular adiponectin generated by the cleavage of full-length adiponectin by neutrophil elastase and other proteases (52, 76, 163). Adiponectin exerts its effect mainly by activating AMP-activated protein kinase (AMPK), p38 mitogen-activated protein kinase (MAPK), and peroxisome proliferator-activated receptor-α (PPARα) in skeletal muscle and liver, thereby decreasing the level of glucose and lipid in vivo. This effect of adiponectin is mainly through enhancement of fatty acid oxidation and glucose uptake in muscle and inhibition of gluconeogenesis in liver (172, 173), which are mediated by two cell surface receptors, AdipoR1 and AdipoR2 (173), by a direct interaction with the extracellular COOH terminus of these receptors (110, 173). APPL1 is the “missing link” in the adiponectin-signaling cascade, transmitting signals from adiponectin receptors to downstream targets by directly interacting with the NH2-terminal intracellular region of AdipoR1 and AdipoR2 (110).

APPL was initially identified as an Akt2-binding protein in a yeast-two hybrid screening (119) and is named after its unique structure, an adaptor protein containing pleckstrin homology (PH) domain, phosphotyrosine binding (PTB) domain, and leucine zipper motif. Because of its ability to interact with the tumor suppressor protein, deleted in colorectal cancer (DCC), APPL is also known as DCC-interacting protein (DIP-13α) (101). DIP-13β is the isof orm of DIP-13α (101), and currently, APPL/DIP-13α is known as APPL1, whereas DIP-13β is called APPL2 (110, 116). Encoded in the chromosomal region 3p14.3–21.1 (119), human APPL1 containing 709 amino acids is highly hydrophilic and has no transmembrane domain (101). APPL1 does not possess a classical nuclear localization signal or nuclear export signal; however, nuclear localization in response to epidermal growth factor (EGF) or oxidative stress has been reported (116). APPL1 interacts with nearly 14 proteins, including membrane receptors and signaling molecules in various signaling pathways to mediate apoptosis (101), cell proliferation, chromatin remodeling (116), endosomal localization of proteins (161), and cell survival...
Recently, emerging data suggest that APPL1 also plays a key role in regulating metabolism and insulin sensitivity by mediating adiponectin signaling (28, 110). We begin this review by giving an overview of the current understanding of adiponectin signaling in various tissues. The main focus of this review is on the role of APPL1 in adiponectin and insulin-signaling pathways and the mechanism by which APPL1 exerts the insulin-sensitizing effects of adiponectin.

ADIPONECTIN SIGNALING IN VARIOUS TISSUES AND THE ROLE OF APPL1

Adiponectin exhibits antidiabetic, anti-inflammatory, and antithrombogenic effects and also functions as an insulin sensitizer; hence, it is a novel therapeutic target for diabetes and metabolic syndrome (76, 86, 128). Adiponectin also plays a central role in energy homeostasis through its action in hypothalamus, and a new role for adiponectin as a “starvation gene” has been proposed (78, 92). In the following section, we will review the signal transduction of adiponectin in different tissues and the role of APPL1 in mediating the effects of adiponectin.

Skeletal Muscle

In type 2 diabetes and obesity, impairment of glucose and lipid metabolism results in the accumulation of lipid in insulin target tissues leading to insulin resistance (79, 147). AdipoR1 is the most abundantly expressed adiponectin receptor in skeletal muscle, one of the insulin target tissues. Most of the adiponectin effects in muscle are mediated by globular adiponectin, since globular adiponectin has a higher affinity for AdipoR1 (173). In C2C12 myocytes, adiponectin activates AMPK, p38 MAPK, and PPARα ligand activities, which increase fatty acid oxidation and glucose uptake in these cells (173). AMPK is a key sensor of cellular energy status (61), and adiponectin-mediated activation of AMPK in skeletal muscle increases glucose uptake, fatty acid oxidation, and lactate production (172). p38 MAPK regulates various cellular processes, including inflammation, cell differentiation, cell growth, and cell death and is activated in response to a variety of extracellular stimuli, including metabolic stress (126). In C2C12 myocytes, globular adiponectin-mediated fatty acid oxidation and glucose uptake are not solely by activation of AMPK but also by p38 MAPK activation (173). A study by Yoon et al. (181) suggests that, in C2C12 myotubes, adiponectin-stimulated fatty acid oxidation is by the sequential activation of AMPK, p38 MAPK, and PPARα. PPARα is a key regulator of fatty acid oxidation and p38 MAPK phosphorylates and activates PPARα and its coactivator PPARγ coactivator-1α (8, 135). Unlike activated AMPK and p38 MAPK, PPARα is involved only in fatty acid metabolism, not glucose uptake, in response to adiponectin stimulation (173).

Skeletal muscle has a relatively high expression of APPL1 and AdipoR1, and in C2C12 myotubes interaction between APPL1 and AdipoR1 is stimulated by adiponectin (110). APPL1 acts as a positive regulator of adiponectin signaling in muscle cells, since overexpression of APPL1 in C2C12 myotubes significantly increases phosphorylation levels of AMPK and p38 MAPK. Overexpression of APPL1 (ΔPTB) (AdipoR1-binding deficient mutant of APPL1) or suppression of APPL1 by siRNA failed to mediate adiponectin-stimulated AMPK and p38 MAPK activation. Suppression of APPL1 in C2C12 myotubes significantly attenuated adiponectin-stimulated phosphorylation of AMPK, p38 MAPK, acetyl-CoA carboxylase (ACC), and fatty acid oxidation, suggesting the crucial role of APPL1 in mediating adiponectin-regulated lipid metabolism in skeletal muscle cells (110).

Adiponectin decreases the resting blood glucose level in rodents and also protects animals with diet-induced obesity from developing insulin resistance (52, 173, 174). This glucose-lowering effect of adiponectin is mainly through glucose uptake in skeletal muscle by AMPK activation (173) and also by glucose transporter 4 (GLUT4) membrane translocation (22). Both full-length and globular adiponectin stimulate GLUT4 membrane translocation in L6 cells (110). Whereas overexpression of APPL1 enhances GLUT4 membrane translocation, suppression of APPL1 significantly reduces GLUT4 membrane translocation in L6 cells and also adiponectin-stimulated glucose uptake in C2C12 myotubes (110). Adiponectin-stimulated GLUT4 membrane translocation is partially through activated AMPK and p38 MAPK, whereas the rest is through the small GTPase Rab5. Adiponectin stimulated the interaction between APPL1 and Rab5, and the disruption of APPL1-Rab5 interaction blocked adiponectin-mediated translocation of GLUT4 to the membrane (110). Adiponectin-stimulated p38 MAPK activation is inhibited by the overexpression of dominant negative Rab5, indicating that activation of p38 MAPK by adiponectin is partly through APPL1-Rab5 association (110).

Vascular Endothelium

A decreased level of circulating adiponectin is correlated with inflammation and increased incidence of cardiovascular disease (9, 112, 134, 144). In mice, adiponectin deficiency causes severe neointimal thickening due to vascular smooth muscle cell proliferation in injured arteries. Adiponectin supplementation attenuates this effect by suppressing vascular smooth muscle cell migration (111). This protective effect of adiponectin is mediated by increasing the production of nitric oxide (NO) through activation of AMPK and endothelial NO synthase (eNOS) (26, 94, 131, 155, 169). NO protects the endothelial cells by inhibiting platelet aggregation, monocyte adhesion, and smooth muscle cell proliferation and also by increasing vasodilation (59, 72, 131, 169). Cheng et al. (28) found that in human umbilical vein endothelial cells the effects of adiponectin are mediated through adiponectin receptors AdipoR1 and AdipoR2. Their study identified APPL1 as a signaling protein mediating the downstream signaling events from adiponectin receptors to eNOS for NO production. Similar to the finding by Mao et al. (110) in C2C12 myotubes, in human umbilical vein endothelial cells also the cytoplasmic tail of adiponectin receptors interacts with APPL1. The critical role of APPL1 in adiponectin-induced vasodilation is demonstrated by the inability of adiponectin to induce NO production in APPL1 knockdown cells. Suppression of APPL1 expression resulted in a significant decrease in the phosphorylation of AMPK and eNOS and a decrease in the complex formation between eNOS and heat shock protein 90, which in turn decreases NO production, in response to adiponectin (28). In db/db mice, adiponectin-induced vasodilation is significantly lower than in their lean littermates, and the expression level of APPL1...
mRNA is selectively decreased in small mesenteric arteries in these animals (28). This suggests a direct relationship between decreased APPL1 expression and impaired vasodilation, which in turn leads to endothelial dysfunction in diabetes.

In addition to its protective effect against atherosclerosis (4, 66, 128), adiponectin also exhibits anti-inflammatory effects, especially in endothelial cells and macrophages (128, 129, 180). Whereas the high-molecular-weight form of adiponectin has anti-inflammatory and antiapoptotic roles (87), the globular domain of adiponectin has cytotoxic effects (100, 125, 130). An inverse relationship exists between inflammation and adiponectin level, since chronic inflammatory diseases such as coronary heart disease are characterized by hypoadiponectinemia (143). Not only adiponectin but also its receptors AdipoR1 and AdipoR2 are downregulated in chronic inflammation associated with diabetes, obesity, and insulin resistance (77). Interleukin-18 (IL-18) is a proinflammatory and proapoptotic cytokine, the increased expression of which is correlated with acute coronary syndromes (89, 108). Treatment of endothelial cells with IL-18 causes cell death by inhibiting Akt activity and also by inducing phosphatase and tensin homolog activation through IκB kinase-nuclear factor-κB (NF-κB)-dependent pathway. Adiponectin treatment blocks IL-18-mediated cell death through APPL1-mediated activation of AMPK. This in turn activates Akt and inhibits IκB kinase-NF-κB phosphatase and tensin homolog signaling and caspase-3 activation (23). The reversal of IL-18-mediated cell death by adiponectin is APPL1 dependent, since APPL1 knockdown reverses adiponectin’s prosurvival effects by inhibiting AMPK phosphorylation (23).

Hypothalamus

Hypothalamus plays a major role in the regulation of food intake and body weight. An increase in the activity of AMPK in arcuate hypothalamus has been shown to stimulate food intake (20, 60, 117). Kubota et al. (92) demonstrated that administration of adiponectin stimulates food intake and decreases energy expenditure by increasing the phosphorylation levels of AMPK and its downstream target ACC in hypothalamus and thereby plays an important role in maintaining energy homeostasis. Both AdipoR1 and AdipoR2 are expressed in the paraventricular nuclei of hypothalamus (35, 92) and also in the neurons of arcuate and lateral hypothalamic nuclei (35). Stimulation of food intake by adiponectin is mediated specifically through AdipoR1 in hypothalamus (92). APPL1, the mediatior of adiponectin signaling, is also present in brain (110). Coope et al. (35) demonstrated that following an intracerebroventricular injection of adiponectin to rat hypothalamus, AdipoR1 and AdipoR2 interact with APPL1. The adiponectin injection to hypothalamus promoted anorexigenic condition in rats with a 40% reduction in food intake. In addition, adiponectin injection also increases signal transduction through the classical insulin- and leptin-signaling pathways, and these effects of adiponectin are mediated through AdipoR1, not AdipoR2 (35). Adiponectin injection to the hypothalamus increases phosphorylation levels of insulin receptor substrate (IRS)-1, IRS2, Akt, ERK, forkhead transcription factor 1 (FOXO1), JAK2, and signal transducer and activator of transcription 3, indicating the existence of cross-talk between adiponectin-insulin and adiponectin-leptin pathways in hypothalamus (35).

Liver

In liver, adiponectin-stimulated activation of AMPK decreases gluconeogenesis by attenuating the expression level of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (172), thereby reducing glucose level in vivo. db/db Mouse liver has decreased mRNA expression levels of AdipoR1 and AdipoR2, and overexpression of AdipoR1 or AdipoR2 in the liver of db/db mice markedly improves glucose intolerance and insulin resistance (175). Overexpression of AdipoR1 activates AMPK, reduces hepatic glucose production, and increases fatty acid oxidation. On the contrary, overexpression of AdipoR2 activates PPARα pathway, which increases fatty acid oxidation and inhibits inflammation and oxidative stress (175). Thus, in liver, AdipoR1 and AdipoR2 differ in their signaling pathways. In agreement with these findings, AdipoR1 knockout mice have increased expression of gluconeogenic enzymes in liver with impaired glucose tolerance and insulin resistance. AdipoR2 knockout mice have normal hepatic glucose production with increased insulin levels (175). AdipoR2 knockout mice are also resistant to high-fat-induced obesity and insulin resistance (14, 102). Whether APPL1 is involved in mediating these downstream effects of adiponectin through AdipoR1 and AdipoR2 in liver is unknown. However, overexpression of APPL1 in mouse hepatocyte cells can activate p38 MAPK, and adiponectin treatment could further enhance this effect, suggesting that APPL1 plays a role in adiponectin signaling in liver (110).

Adipocyte

Both AdipoR1 and AdipoR2 are expressed in adipose tissue (12). In rat adipocytes, global adiponectin treatment increases glucose uptake by activating AMPK (168). In 3T3-L1 adipocytes, overexpression of adiponectin promotes adipocyte differentiation, increases lipid content, and enhances insulin sensitivity (54). These studies suggest that adiponectin exerts autocrine effects on adipocytes. ob/ob Mice overexpressing adiponectin are obese with higher contents of adipose tissue than ob/ob littermates. Hence, adiponectin promotes storage of fat preferentially in adipose tissue and thereby increases insulin sensitivity (86). AdipoR1 male knockout mice have increased total body fat mass, reproductive white adipose tissue, perirenal white adipose tissue, and brown adipose tissue, and this fat accumulation is due to decreased energy expenditure (14). Although presence of APPL1 in adipocytes is demonstrated (110, 140), it is not known whether adiponectin signaling in adipocytes is mediated through APPL1. A role for APPL1 in insulin signaling in adipocytes is reported. In primary rat
adipocytes, APPL1 forms a complex with Akt2 that is dissociated with insulin treatment (140). In 3T3-L1 adipocytes, APPL1 is involved in insulin-mediated Akt phosphorylation, glucose uptake, and GLUT4 membrane translocation (140).

Brain

Adiponectin, AdipoR1, and AdipoR2 are expressed in osteoblasts (11, 105, 127, 146), suggesting that adiponectin has functions in bone. Osteoblast is a direct target of adiponectin, since adiponectin induces osteoblast proliferation through c-jun NH2-terminal kinase pathway and differentiation through p38 MAPK pathway, and these effects of adiponectin are mediated through AdipoR1 (105). Adiponectin plays a role in regulation of bone mass, since it increases bone mass by suppressing osteoclastogenesis and by activating osteoblastogenesis (125). Adiponectin-mediated activation of AMPK promotes differentiation and mineralization in MC3T3-E1 cells, and knockdown of AdipoR1 abrogates these effects (80). Adiponectin has a protective effect on bone metabolism in patients with type 2 diabetes, since adiponectin is positively associated with bone mineral density at the distal radius in these patients (154). In diseases associated with cytokine activation, adiponectin acts as a regulator of bone resorption by inhibiting tumor necrosis factor-α and receptor activator of NF-κB ligand-induced osteoclastogenesis through activation of AMPK (176). Expression of APPL1 mRNA in human bone marrow, which contains the precursors for osteoblasts and osteoclasts, has been reported (29). Since adiponectin can activate AMPK in bone cells through adiponectin receptors, the involvement of APPL1 in this activation can be expected.

Kidney

Expression of adiponectin receptors AdipoR1 and AdipoR2 in kidney is reported (145). A decrease in AMPK activity due to adiponectin deficiency causes albuminuria, which is an increased risk factor for cardiovascular diseases and is also associated with diabetes and obesity (145). Decreased AMPK activity causes podocyte dysfunction by increasing oxidative stress, and either administration of adiponectin or activation of AMPK reverses this effect. In podocytes, adiponectin exerts a direct effect through adiponectin receptors since both AdipoR1 and AdipoR2 are expressed in podocytes. Although the expression level of AdipoR1 in kidney and podocyte is comparable to that in liver, expression level of AdipoR2 is much lower (145). Although APPL1 is present in kidney (44), it is not known whether the effects of adiponectin are mediated through APPL1.

Other Tissues

Besides its major role in metabolism, adiponectin also regulates a wide range of biological functions, including tumor progression and pituitary hormone secretion (84, 88, 137). This finding is supported by a widespread expression of AdipoR1 and AdipoR2 not only in skeletal muscle, liver, brain, and heart but also in kidney, lung, spleen, testis (173), pituitary, pancreatic β-cells, and placenta (19, 85, 137). Interestingly, mRNA and protein expression of APPL1 is also reported in a wide range of tissues, including those expressing adiponectin receptors (29, 110). However, it is unknown whether APPL1 participates in the mediation of adiponectin signal through AdipoR1 and AdipoR2 in tissues that coexpress APPL1 and adiponectin receptors.

MECHANISM OF ADIPONECTIN-STIMULATED AMPK ACTIVATION MEDITATED THROUGH APPL1

Activation of AMPK by adiponectin is a key step in mediating most of the effects of this adipokine (Fig. 1). AMPK is a key sensor of cellular energy status, and activation of AMPK shuts down ATP-consuming processes like biosynthesis, cell growth, and proliferation while switching on catabolic pathways that generate ATP (61, 156). However, the molecular mechanism of adiponectin-mediated AMPK activation through APPL1 is unknown. The finding that selective phosphoinositide 3-kinase (PI3K) inhibitors can abolish adiponectin-stimulated AMPK phosphorylation in endothelial cells suggests a role for PI3K in AMPK activation (26, 169). The involvement of PI3K is further supported by the finding that activation of AMPK/eNOS pathway by metformin is dependent on PI3K (38). In addition, both p85 and p110 subunits of PI3K interact with APPL1 (119, 177). However, there is no evidence to show that PI3K is a direct upstream kinase of AMPK. In general, AMPK is activated either by an increase in cellular AMP/ATP ratio or by its upstream kinases LKB1 or calmodulin-dependent protein kinase kinase (13, 61). At the moment, it is unclear whether PI3K can activate AMPK via LKB1 or calmodulin-dependent protein kinase kinase and whether adiponectin has any effect on APPL1-PI3K interaction. Solving these questions will help the mechanism of adiponectin-mediated AMPK activation be fully understood.

APPL1 AS A MEDIATOR OF INSULIN SIGNALING

The location of adiponectin gene chromosome 3q27 is the susceptibility locus for type 2 diabetes and other metabolic syndromes (37, 139, 153). The mRNA expression and secretion of adiponectin is significantly decreased in type 2 diabetic patients and animal models of insulin resistance (4, 67, 166, 178). Interestingly, the insulin-sensitizing PPARγ agonist thiazolidinedione increases adiponectin level in animal models and human patients (107). Replenishment of adiponectin restores insulin sensitivity in high-fat-diet-fed and lipotropic mice (171). Thus, adiponectin level and insulin sensitivity are directly correlated, which suggests the insulin-sensitizing effect of adiponectin (76). APPL1 is expected to play a role in insulin-signaling pathway since it interacts with Akt and PI3K (119). Activation of Akt2 by insulin stimulation plays a critical role in glucose transport (63, 74, 91). Akt is activated by phosphorylation at Thr308 by phosphoinositide-dependent kinase (PDK)1 (3, 151) and at Ser473 by PDK2 (43). PDK1 activation occurs by its binding to phosphatidylinositol 3,4,5-phosphate [PtdIns(3,4,5)P3] generated by PI3K, which is activated by binding to tyrosine-phosphorylated IRS (50, 53). Glucose clearance in response to insulin is mediated through the glucose transporter, GLUT4, that moves from intracellular membrane compartment to the cell surface during insulin stimulation. Mao et al. (110) demonstrated that APPL1 plays an important role in insulin signal transduction in C2C12 myocytes since overexpression of APPL1 stimulates insulin-mediated Akt phosphorylation, whereas APPL1 knockdown or overexpression of APPL1 (∆PTB) mutant significantly reduces this effect in C2C12 myocytes. Similarly, in 3T3-L1 adipocytes, suppression of APPL1 attenuated Akt phosphorylation, GLUT4 translocation,
and glucose uptake (140). Insulin stimulation has been shown to dissociate APPL1-Akt complex in primary rat adipocytes and skeletal muscle (140), which is in agreement with the previous finding that only the inactivated form of Akt can interact with APPL1 (119). These findings suggest a direct involvement of APPL1 in insulin-stimulated GLUT4 translocation and glucose uptake.

**APPL1 AS A MEDIATOR OF INSULIN-SENSITIZING EFFECT OF ADIPONECTIN**

Adiponectin is a well-documented insulin sensitizer, and adiponectin exerts this effect by 1) decreasing the triglyceride content in insulin-sensitizing tissues and thereby upregulating insulin signaling, 2) increasing fatty acid combustion and energy utilization by activating AMPK, 3) increasing fatty acid oxidation and glucose uptake by activating AMPK (76). By itself, adiponectin is unable to phosphorylate Akt, although a cotreatment of C2C12 myotubes with adiponectin and insulin showed a synergistic increase in Akt phosphorylation, and this synergism disappeared in APPL1 knockdown cells (110). Hence, APPL1 plays a critical role in the cross-talk between adiponectin- and insulin-signaling pathways. APPL1-mediated cross-talk between insulin- and adiponectin-signaling pathways could be a novel mechanism for the insulin-sensitizing effect of adiponectin (110). The ability of adiponectin to decrease the phosphorylation of p70 S6 kinase (S6K) at Thr389 is a key step in this cross-talk (164), since S6K activation has a negative correlation with IRS-1 tyrosine phosphorylation and downstream signaling (62, 165). In addition to S6K, adiponectin treatment also decreased the phosphorylation of IRS-1 at Ser302 and Ser636/639 (164), which has inhibitory effects for insulin-stimulated Akt activation (15, 62, 157, 165). This decrease in S6K and IRS-1 Ser phosphorylation by adiponectin are mediated through AMPK. Binding of adiponectin to its receptor activates AMPK, which in turn activates AMPK phosphorylates endothelial nitric oxide (NO) synthase (eNOS), leading to the formation of eNOS-heat shock protein 90 (Hsp90) complex and generation of NO to mediate the vasodilating effect of adiponectin. 4) Activated AMPK inhibits IKK-NF-κB-phosphatase and tensin homolog (PTEN) pathway, mediating the cytoprotective effect of adiponectin. 5) Binding of adiponectin to AdipoR1 stimulates glucose transporter 4 (GLUT4) translocation and glucose uptake through phosphorylated AMPK, phosphorylated p38 MAPK, and Rab5.

**APPL1: AN ADAPTOR PROTEIN WITH MULTIPLE FUNCTIONAL DOMAINS**

Three major functional domains are present in APPL1, the NH2-terminal Bin1/amphiphysin/rvs167 (BAR) domain (ini-
APPL1 belongs to a subfamily of BAR domain proteins in which the BAR and PH domain appear in tandem (16, 58, 106). The other members of this subfamily are APPL2, the GTPase-activating proteins centaurin-β1, -β2, and -β5, and oligophrenins (58). In general, the BAR domain is implicated in diverse biological processes like sensing and inducing membrane curvature, small GTPase binding (55, 58, 95, 186), transcriptional repression, apoptosis, and secretory vesicle fusion (39, 136). The BAR domain of APPL1 located near the NH₂ terminus spans 251 amino acids and is unique in having four α-helices (97, 185) compared with three α-helices in the BAR domains of Bin1/amphiphysin II and endophilin (21, 55). APPL1-BAR domain is involved in the binding of the small GTPase Rab5 (116) and also in the formation of homo- or heterodimer with the BAR domain of APPL2 (29, 97, 124). APPL1-BAR dimer is crescent shaped, and only the first three α-helices contribute to the structure of the concave inner face of dimer (97, 185). The fourth α-helix in the BAR domain is not necessary for dimerization or membrane targeting of APPL1 (29).

Unlike the BAR domain, PH domain of APPL1 is homologous to those in other proteins (97). In general, the PH domain targets proteins to specific membrane compartments by increasing the lipid specificity of the BAR domain (132) and also in GTPase binding (96). In APPL1, the BAR and PH domains function as a single unit rather than two individual domains (97, 116, 185). In cantaurin-β2 and oligophrenin, the other two members in the APPL family, the BAR-PH domain is involved in the tubulation of membranes, and hence, it is possible that APPL1 BAR-PH domain may induce membrane curvature (132). In APPL1 dimer, the PH domain of each monomer is positioned at the opposite ends of the BAR dimer like stretched arms, giving a crescent shape for the BAR-PH dimer with the concave face mediating membrane interaction (97, 185). The BAR-PH domain of APPL1 is involved in Rab5 binding (97, 116, 185), and Rab5 specifically binds the PH domain with marginal extension to the neighboring BAR domain (185). In agreement with this, a triple mutation in APPL1-PH domain (K280E/Y283C/G319R) disrupts its binding with Rab5 (116). A role for PH domain in membrane targeting is suggested, since isolated PH domain of APPL1 binds PtdIns(3)P, PtdIns(4)P, PtdIns(5)P, PtdIns(3,4)P², and PtdIns(3,5)P², which are the phospholipids in plasma membrane, early endosomes, late endosomes/multivesicular bodies, endoplasmic reticulum, Golgi, and the nucleus (29). The BAR and BAR-PH domains of APPL1 also interact with PtdIns(3,4,5)P³, consistent with the predicted membrane-binding function of BAR-PH domain of APPL1 (97). Consistent with this finding, isolated APPL1-PH is localized in plasma membrane, cytosolic vesicles, and nuclear and perinuclear structures (29), suggesting that BAR-PH domain is involved mainly in the membrane targeting of APPL1.

The general function of PTB domain is to act as an adaptor or scaffold for the binding of proteins, particularly those in signaling pathways. The PTB domain of APPL1 is located near the COOH terminus, away from BAR-PH domain, making it an easily accessible structure for its binding partners. This domain interacts with a diverse set of receptors, including netrin-1 receptor DCC (101), nerve growth factor (NGF) receptor TrkA (98, 161), follicle-stimulating hormone (FSH) receptor (FSHR) (123, 124), and adiponectin receptors AdipoR1 and AdipoR2 (28, 110). APPL1-PTB domain also interacts with various signaling proteins, such as Akt (119, 124, 140), PI3K subunits (119, 177), oculocerebrorenal syndrome of Lowe (OCRL), and inositol polyphosphate-5-phosphatase (INPP5B) (44), indicating the ability of APPL1 to act as an adaptor or scaffold protein for distinct signaling pathways. The secondary structure of APPL1-PTB domain is similar to the PTB domain of adaptor protein Shc (119), which recognizes phosphorylated tyrosine residues within the consensus sequence NPXPhosphoY (where Φ is a hydrophobic amino acid, X refers to any amino acid, and N, P, and Y represent Asn, Pro, and Tyr, respectively) (82, 184). However, APPL1-PTB domain-interacting proteins do not contain this consensus sequence, indicating that APPL1-PTB domain-mediated interaction is through a novel, unidentified mechanism.

![Diagram of APPL1 Domain Structure](https://example.com/diagram.png)
AdipoR2 as bait. In C2C12 myocytes, APPL1 interacts directly with G protein-coupled receptors and contain seven transmembrane domains with an intracellular NH2 terminus and an extracellular COOH terminus end (173). They are structurally and functionally different from the ADIPONECTIN- AND INSULIN-SIGNALING PATHWAYS

We have previously shown that APPL1 interacts with adiponectin receptors in adiponectin-signaling pathway and with signaling molecules Akt and PI3K in the insulin-signaling pathway. APPL1 and AdipoR2

AdipoR1 and AdipoR2

AdipoR1 and AdipoR2 are the two transmembrane receptors mediating the effects of adiponectin (173). AdipoR1 and AdipoR2 are highly related structurally and share 67.5% identity of protein sequence and are conserved from yeast to human (173). They are structurally and functionally different from the G protein-coupled receptors and contain seven transmembrane domains with an intracellular NH2 terminus and an extracellular COOH terminus end (173). AdipoR1 is expressed ubiquitously with high expression in skeletal muscle, and AdipoR2 is expressed predominantly in liver (173). Using a yeast-two hybrid study, we found that adiponectin interacts with the COOH-terminal extracellular part of AdipoR1 (110). We also identified APPL1 as an AdipoR1- and AdipoR2-interacting protein using NH2-terminal intracellular portion of AdipoR1 or AdipoR2 as bait. In C2C12 myocytes, APPL1 interacts directly with AdipoR1 through its COOH-terminal PTB domain, and adiponectin stimulates this interaction (110). A similar interaction of APPL1 with AdipoR1 and AdipoR2 stimulated by adiponectin is observed in endothelial cells (28). Although adiponectin-stimulated phosphorylation of AdipoR1 was expected to mediate AdipoR1-APPL1 interaction, the phosphorylation of AdipoR1 remains unchanged after adiponectin stimulation, and mutations of three tyrosine residues in the NH2 terminus of AdipoR1 did not alter its binding to APPL1, indicating the existence of an unidentified mechanism for APPL1-AdipoR1 interaction (110). Similarly, APPL1 interacts with DCC (101) and FSHR (123) by a phosphorylation-independent mechanism. Homo- or heterodimer formation by AdipoR1 and AdipoR2 (173) could be a possible mechanism by which APPL1 recognizes its receptors, as reported for FSHR (123). It is likely that oligomerization of receptors by adiponectin leads to curvature in the membrane, which is sensed by the APPL1 dimer via its BAR-PH domain to mediate downstream signals. However, whether adiponectin stimulates the oligomerization of its receptors is currently unknown.

PI3K

PI3K is a critical component in the insulin-signaling pathway mediating a wide range of functions of insulin. PI3K catalyzes the phosphorylation of D-3 position of inositol ring in phosphoinositides (167). PI3K contains a 110-kDa catalytic subunit and an 85-kDa regulatory subunit that is constitutively associated with the p110 subunit. The SH2 domain in p85 subunit recognizes phosphorylated IRS-1, activated by insulin receptor or insulin-like growth factor I (IGF-1) receptor. This follows the recruitment of IRS proteins along with p110 subunit to the plasma membrane (7, 122), where it catalyzes the phosphorylation of PtdIns(4,5)P2 to PtdIns(3,4,5)P3. PtdIns(3,4,5)P3 can interact with PH domains of various proteins and thereby orchestrate a range of signaling pathways (160). APPL1 interacts with p110α (119) and p85 (177) subunits of PI3K; however, the interacting domain in APPL1 is not known. APPL1 tethers inactive Akt2 to p110α (119) and is an important adaptor protein in IGF-I-mediated Akt activation through PI3K (177). IGF-I-mediated Akt phosphorylation is enhanced by APPL1 in a PI3K-dependent manner, and knockdown of APPL1 abrogates this effect (177). It is not clear how APPL1 activates Akt; it is likely that the PH domain of APPL1 binds PI3K and serves as an adaptor to recruit PI3K to the cell membrane, where PI3K is activated by IGF-I receptor or insulin receptor, leading to Akt activation (177).
APPL1 also plays a critical role in cell survival during development of zebrafish, and knockdown of APPL1 induces apoptosis, an effect mediated through Akt (141). APPL1 is specifically required for Akt activity and cell survival, since APPL1 knockdown significantly reduces the level of Akt phosphorylation (141). In addition, APPL1 knockdown significantly attenuates GSK-3β activation, a downstream target of Akt implicated in cell survival (75, 109). Activation of TSC2, another downstream target of Akt implicated in growth control (109), is unaffected by suppression of APPL1. Hence, APPL1 regulates the substrate specificity and activity of Akt. Endosomal localization of APPL1 is required for its activity (116), and APPL1-mediated Akt phosphorylation occurs only through endosomal APPL1, not through the soluble or nuclear APPL1 (141). Consistent with this, only GSK-3β, not TSC2, is localized in endosomes. Besides the Akt-mediated survival pathway mediated through GSK-3β, APPL1 also mediates cell survival through FOXO1a, another direct substrate of Akt (124).

EXPRESSION, CELLULAR LOCALIZATION, AND REGULATION OF APPL1

Protein and mRNA expression of APPL1 has been reported in various human and mouse tissues (29, 110, 119). In HeLa cells, APPL1 is present in both cytoplasm and nucleus as punctuate structures, and in the cytosol it is located on membrane structures like endosomes with high concentrations in tubular-vesicular structures under the plasma membrane (116). Interestingly, localization of APPL1 undergoes dramatic changes with respect to environmental stimuli. In HeLa cells, serum starvation shifts APPL1 localization entirely to the cytosol, and treatment with H2O2, simulating oxidative stress, translocates APPL1 from cytosol to the nuclei (116). In HeLa cells, in response to EGF treatment, APPL1 translocates from punctate cytosolic structures to a subset of EGF-containing endosomes and then to the nuclei and, finally, back to membrane structures in cytoplasm (116). This whole cycle of translocation occurs in 30 min, indicating the presence of efficient machinery for the import and export of APPL1 to and from the nuclei, although APPL1 does not contain a nuclear localization signal or nuclear export signal. Although endosomes act as the shuttling cargo for APPL1 import to nuclei (116), the mechanism of APPL1 export from the nuclei is currently unknown. Truncation studies have shown that BAR-PH domain determines the membrane anchoring of APPL1 (116). In primary rat adipocytes, the majority of APPL1 is localized in the cytosol, with small proportions in nuclear/mitochondrial fraction and light microsomal fraction. In these cells, insulin treatment increased the proportion of APPL1 in light microsomes, plasma membrane, and nuclear/mitochondrial fraction (140). On the contrary, in cultured adipocyte, this increase was primarily observed only in light microsomes and plasma membrane (140). However, chronic insulin treatment for 6–24 h caused a translocation of APPL1 to nucleus (140). During early embryogenesis in zebrafish, a ubiquitous expression of APPL1 is observed, with elevated expression in telencephalon, pronephros, olfactory organ, and neural tube, and is also present in the characteristic endosomes beneath the plasma membrane (141).

Single nucleotide polymorphisms (SNPs) within AdipoR1 and AdipoR2 gene are risk factors for type 2 diabetes in white populations (36, 150, 162), and SNPs in AMPK gene are also associated with alterations in plasma lipids and increased risk for type 2 diabetes (65, 170), which suggests that adiponectin-signaling pathway contributes to genetic regulation of metabolism. APPL1 locus, a 78-kb genomic region, on chromosome 3p21.1-p14.3 is not a susceptibility region for type 2 diabetes (149). Four common SNPs (rs6774584, rs3087684, rs17791685, and rs528035) were identified in healthy white populations, and this genetic variation within the APPL1 locus does not contribute to insulin resistance, changes in lipid metabolism, or inflammatory parameters, indicating that APPL1 gene may not play a major role in the development of prediabetic conditions (149). Interestingly, studies by Fang et al. (45) in a Chinese population indicate that two SNPs (rs3806622 and rs4640525) in APPL1 gene are correlated with body fat distribution in type 2 diabetes.

APPL1 AS AN ADAPTOR IN MULTIPLE SIGNALING PATHWAYS

To date, nearly 14 proteins have been reported to associate with APPL1 in various types of cells, and they could be categorized into three different groups: 1) membrane receptors, 2) signaling proteins, and 3) others.

Membrane Receptors

**DCC.** DCC is a candidate tumor suppressor gene (47) that encodes a type 1 membrane protein (31). It acts as a receptor for netrin-1 and plays important roles in axon outgrowth and cell migration in the developing nervous system (46, 83). The tumor suppression effect of DCC is through induction of apoptosis (27, 51, 115), and Liu et al. (101) identified APPL1 as a binding partner of DCC. The interaction between APPL1 and DCC enhances DCC-mediated apoptosis in the colon adenocarcinoma cell line DLD1. Cytoplasmic domain of DCC interacts with the COOH-terminal region of APPL1 (454–646 amino acids), which contains the PTB domain. DCC-induced apoptosis is dependent on APPL1, since inhibition of endogenous APPL1 blocks apoptosis (101). The molecular mechanism by which APPL1 induces apoptosis through DCC is not clear. It could be through caspase-9 (101), since the same region in DCC (1,243–1,264 amino acids) interacts with both APPL1 and caspase-9 (51).

**Androgen receptor.** Androgen receptor (AR) is an androgen-dependent transcription factor belonging to the nuclear receptor superfamily (25), which plays an important role in male sexual differentiation and prostate cell proliferation. Binding of the ligand testosterone to AR attaches it to the androgen response element on the 5′-promoter of the target gene, resulting in the modulation of cell growth (24, 69). AR can be regulated by growth factor-signaling pathways, which contributes to the development and progression of prostate cancer (48). PI3K/Akt pathway suppresses AR transactivation (99), and APPL1 can enhance Akt-mediated suppression of AR transactivation in prostate cancer cell lines (177). AR does not directly interact with APPL1; rather, Akt acts as a bridge to mediate APPL1-AR interaction (177). Overexpression of APPL1 suppresses the expression of p27Kip1, a target gene of PI3K/Akt signaling (177). Hence, APPL1 controls AR transactivation by regulating PI3K/Akt pathway and downregulating the gene expression of p27Kip1.
FSHR. During follicular development of the ovary, selection of a dominant follicle capable of ovulation is through a competing phenomenon of apoptosis and cell survival (121) mainly by the action of FSH and its receptor FSHR. FSH is capable of activating MAPK and Akt pathways through FSHR (32, 57). Using yeast two hybrid screen, Nechamen et al. (123) identified APPL1 as a FHSR-interacting protein. The COOH-terminal part of APPL1 (461–709 amino acids) containing the PTB domain interacts with FSHR, and this interaction is stimulated by FSH. Surprisingly, FSH also stimulates the interaction between FSHR and the APPL1 NH2-terminal end containing BAR-Phoenix (1–460 amino acids). In 293 FSHR cells, FSH treatment phosphorylates and inactivates FOXO1a, a downstream effector of the antiapoptotic PI3K/Akt signaling pathway (123). FOXO1a is involved in the transcription of proteins involved in apoptosis, and hence, its activation by PI3K/Akt pathway leads to suppression of apoptotic gene expression (18) and ultimately cell survival. FSH regulated APPL1-FSHR association could be the survival mechanism for dominant follicle selection. However, it is not known how FSH stimulates APPL1-FSHR interaction. FOXO1a has been shown to interact directly with FSHR but not with APPL1 (124). FSH stimulation has been reported to enhance constitutive formation of FSHR oligomers, and this oligomerization may provide a mechanism for signal transduction (123).

TrkA. The TrkA receptor tyrosine kinase is a receptor for neurotrophin NGF transducing signaling cascades required for survival, differentiation, and growth of neurons during development (10, 159). Following activation by ligand binding, TrkA is internalized and the signal is transduced as endosomes (68). Within the endosomes, activated TrkA together with the components of the TrkA signaling pathway form a signaling unit that is propagated along the axon to cell body (41, 179). GIPC1 is a scaffolding protein that is constitutively associated with TrkA and is involved in the recruitment of other proteins involved in endocytosis and early trafficking events (5, 103, 104). Lin et al. (98) found that, in NGF-treated cells, APPL1 is present in endosomal fractions along with phosphorylated TrkA and GIPC1. APPL1 associates with TrkA either directly through the COOH-terminal PTB domain (472–709 amino acids) or indirectly through GIPC1. The COOH-terminal end of APPL1 associates with the postsynaptic density protein, Drosophila disc large tumor suppressor, and zonula occludens-1 protein (PDZ) domain of GIPC1 (98). In the early stages of endosomal trafficking, APPL1 interacts with TrkA through GIPC1 (98). During trafficking, GIPC1 dissociates from the endosomes (5), and APPL1 may interact directly with TrkA at this stage. Through these interactions, APPL1 is involved in the NGF-mediated Akt and ERK phosphorylation (98), necessary for neuronal survival and neuritogenesis (81). Suppression of APPL1 attenuates these effects (98), suggesting that APPL1-mediated signaling is essential for neuronal survival and neuritogenesis.

**Signaling Proteins**

Rab. Recently, endosomes have emerged as an important machinery for signal transduction from cell membrane to the nuclei (42, 113). The small GTPase Rab5 has been identified as a key regulator of transport from the plasma membrane to early endosomes, and continuous cycles of GDP/GTP exchange and hydrolysis regulate endocytosis (138). Rab5 is involved in the organization of early endosomes that are enriched in PtdIns(3)P and a set of PtdIns(3)P-binding effectors (34, 183). Miazynska et al. (116) identified APPL1 as a Rab5 effector localized in endocytic compartments receiving internalized EGF. EGF internalization releases APPL1 from membranes, which depends on the GTPase cycle of Rab5, and translocates APPL1 to the nuclei. In the nuclei, APPL1 interacts with components of the nucleosome remodeling and histone deacetylase complex (NuRD/MeCP1) necessary for efficient cell proliferation (116). The interaction with Rab5 is part of the control mechanism to coordinate the release of APPL1 from endosomes and its trafficking following growth factor stimulation. APPL1 interacts with Rab5 through the NH2-terminal BAR-PH domain (97, 116, 185). Three mutations in the PH domain of APPL1 (K280E, Y283C, and G319R) abolished the binding of APPL1 to Rab5, and these mutants accumulated in the cytosol and nucleus, which resulted in decreased rate of cell proliferation. Hence, Rab5-dependent localization of APPL1 is necessary for downstream cytoplasmic interactions and also for transmitting proliferative signals. Rab5 also plays a role in glucose metabolism in response to insulin and adiponectin stimulations. Rab5 has been shown to regulate GLUT4 internalization by an insulin-dependent mechanism in 3T3-L1 adipocytes (71). It is not known whether APPL1 has any role in this process. In L6 cells, adiponectin-stimulated interaction between APPL1 and Rab5 plays a role in adiponectin-regulated GLUT4 translocation (106).

GIPC1. Originally identified as a binding partner of the G protein-signaling protein GAIP, GIPC1 plays an important role in TrkA-mediated NGF signaling (98). The PDZ domain of GIPC1 interacts with the COOH-terminal PDZ binding domain of APPL1 (a 3-amino acid, Ser-Glu-Ala domain at the COOH terminus of APPL1), and a mutant lacking the four COOH-terminal amino acids did not bind GIPC1 (161). During activation of TrkA with NGF, GIPC1 and APPL1 relocate from cytoplasm to the endocytic vesicles carrying TrkA. Knocking down APPL1 inhibits NGF-induced GIPC recruitment to endosomes, which in turn inhibits MAPK activation and neurite outgrowth (161). Hence, recruitment of GIPC1 to TrkA-containing endosomes by APPL1 plays an important role in TrkA trafficking and signaling.

Rab21. Rab21 is a member of the Rab5 subfamily, and APPL1 is a Rab21 effector since it binds Rab21 in a GTP-dependent manner (185). Rab21 and Rab5 exhibit differential binding affinity toward various APPL1 mutants (185), but the physiological significance of APPL1-Rab21 interaction is not known.

OCRL and INPP5B. OCRL is an inositol 5-phosphatase, mutation of which causes OCRL, characterized by congenital cataracts, mental retardation, and renal Fanconi syndrome (6). OCRL mutation is also identified in Dent disease, a condition associated with the loss of low-molecular-weight proteins and electrolytes in urine (64). OCRL is implicated in membrane trafficking from endosomes to Golgi and is a Rab effector that binds clathrin and clathrin adaptors (33, 73, 158). INPP5B is another inositol 5-phosphatase similar to OCRL, and COOH-terminal region of both INPP5B and OCRL binds APPL1 (44). A short peptide comprising 11 amino acids in human APPL1 (403–413 amino acids), located between the PH and PTB

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domains, is the minimal binding site for OCRL and INPPB5. Interestingly, phosphorylation at Ser410 catalyzed by protein kinase A inhibited the binding of OCRL to the 11-mer peptide, suggesting that modification in APPL1 regulates its interaction with OCRL (44). Three single amino acid mutations in the COOH-terminal of OCRL are associated with Lowe syndrome (120), and these mutants do not bind APPL1, indicating that impairment in APPL1-OCRL interaction plays a major role in this disease. OCRL in the endocytic clathrin coated pits has shown to bind APPL1 on early endosomes, and APPL1-OCRL association is important for trafficking and signaling in kidney and brain (44). APPL1 participates in the formation of a protein network along with OCRL and GIPC, an oligomeric endocytic adaptor protein implicated in receptor endocytosis and recycling in brain and kidney, and disruption of this network is implicated in the renal defects and mental retardation in Lowe syndrome.

Others

**NuRD/MeCP1.** NuRD/MeCP1 is an important regulator of chromatin structure and gene expression (49). In response to EGF or oxidative stress, APPL1 is transported from early endosomes to nuclei, where it interacts with NuRD/MeCP1 (116). It is not known whether APPL1 directly associates with NuRD/MeCP1 and which domain of APPL1 mediates this interaction. Mass spectra analysis of the interacted NuRD/MeCP1 revealed six out of 10 components of the complex, viz., Ptd/MTA2, p66, HDAC1 and/or HDAC2, Rab46, Rab48, and MBD3. Histone deacetylase (HDAC) activity is an essential factor for the progression of cell cycle and development (2, 182). The nuclear localization of APPL1 together with its binding to NuRD/MeCP1 complex suggests a role for APPL1 in cell proliferation. In agreement with this, APPL1 knocking down cells are less proliferative with less number of cells entering the S-phase (116). Hence, APPL1 is a critical element of signaling pathways leading to cell proliferation.

**APPL2.** Initially identified because of its high homology with APPL1, APPL2/DIP-13β (101) shares 54% identity with APPL1 and is encoded in human chromosome 12, with the translated protein containing 664 amino acids (116). Both APPL1 and APPL2 share the same domain structure except that, in APPL2, 45 amino acids in the COOH terminus are absent compared with APPL1. Unlike APPL1, APPL2 posses a putative nuclear localization signal and is more concentrated in the nucleus with marginal distribution in the cytoplasm as punctate structures (116). APPL1 forms a complex with APPL2, and the NH2-terminal region of APPL1 (1–460 amino acids) containing the BAR domain is implicated in this dimerization (124). The BAR domain, devoid of the fourth α-helix, is the minimal requirement for the dimerization of APPL isoforms to form homo- and heterodimers (29). The coexpression of APPL1 and APPL2 mRNAs in various human and mouse tissues suggests that APPL homo- and hetero-oligomer may be present in many tissues, whereas some tissues (kidney and lung in humans and lung, uterus, prostate, and certain breast tissues in mouse) may be rich in APPL1 isoforms or APPL2 isoforms (bone marrow and fetal brain in humans) (29). However, the potential function of APPL homo- or hetero-oligomer is not clear. Like APPL1, APPL2 PH and PTB domains also mediate phosphoinositide binding (29) and interact with Rab5 (116), NuRD/MeCP1 (116), GIPC1 (161), and FSHR (124) but not with Akt2 (124). APPL2 may also play a role in cell proliferation, since knockdown of APPL2 reduced the number of cells entering the S-phase (116).

**CONCLUDING REMARKS**

Adiponectin is a promising therapeutic target for the treatment of insulin resistance, type 2 diabetes, and related metabolic diseases. Identification of APPL1 as a mediator of adiponectin signaling enhances our current understanding on adiponectin and insulin-signaling pathways. APPL1 is indispensable for the signaling and insulin-sensitizing effect of adiponectin. However, our current knowledge of the mechanism by which APPL1 regulates adiponectin signaling is limited, and many questions need to be answered for a better understanding of this adaptor protein. How does APPL1 activate its downstream targets in response to adiponectin stimulation? What posttranslational modifications exist in APPL1, and how do these modifications affect the interaction with its binding partners? What is the functional significance of homo-oligomer of APPL1 or hetero-oligomers of APPL isoforms, and how are they regulated? How does APPL1 recognize adiponectin receptors? Do adiponectin receptors undergo endocytosis following adiponectin stimulation, and is endosomal localization of APPL1 necessary for adiponectin signaling? Do APPL1-dependent and/or -independent pathways exist for adiponectin signaling in tissues like liver, kidney, and bone? What is the physiological significance of this adaptor protein in metabolism? APPL1 knockout or conditional knockout mice will be helpful models to study the physiological significance of APPL1 in vivo. Since most drugs used to treat type 2 diabetes act as insulin sensitizers, similarly to adiponectin, it will be interesting to test whether they also modulate the expression or function of APPL1. Overexpression of APPL1 in C3H12 myoblasts could mimic the effect of adiponectin, and decreased expression of APPL1 in the small mesenteric arteries of db/db mice is related to defects in vasodilation, suggesting that regulation of APPL1 expression or function is a promising approach to enhance insulin sensitivity. This opens an avenue for the search of factors (like diet and exercise) and development of small molecules that can modulate expression and function of APPL1 in insulin target tissues. More insight into the regulation and expression of APPL1 will help to develop new strategies for treating type 2 diabetes and metabolic syndrome.

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