Review

Rho Family GTPases and Rho GEFs in Glucose Homeostasis

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on PAK2, but not PAK1 [54]. Glucose tolerance in vivo and the insulin-stimulated uptake of 2-deoxy-D-glucose into extensor digitorum longus muscle ex vivo were mildly impaired in mice lacking PAK2, but not PAK1, using muscle-specific deletion [54]. In addition to

reflect protein levels of Rho GEFs in these tissues. It is known, for example, that P-Rex2 protein levels are much higher in the liver than in skeletal muscle of mice [75], and Tiam1 protein is expressed in skeletal muscle, [76] despite the mRNA data suggesting otherwise. The mRNA data do, however, emphasize an important gap in current understanding: the liver is a major metabolic organ that expresses multiple Rho GEFs; however, there is, to our knowledge, no research data available yet on glucoregulatory roles of Rho GEFs in this organ. More details on the expression, general functions and mechanisms of regulation of these Rho GEFs are given in Section 4, where we describe their roles in glucose homeostasis.

Figure 2. Domain structure of Rho GEFs involved in glucose homeostasis. The Rho GEFs are classified into two families, 70 Dbl-type and 11 DOCK-type proteins in mammals. This figure depicts the domain structures of the nine mammalian Dbl-type GEFs and of yeast Dck1, a homologue of mammalian DOCK1, which have been implicated to date in the regulation of glucose homeostasis. Dbl-type Rho GEFs are characterized by a catalytic Dbl homology (DH) domain and a tandem membrane-targeting pleckstrin homology (PH) domain. DOCK-type Rho GEFs have a membrane-targeting DHR-1 domain and a catalytic DHR-2 domain. The structures of the DH and DHR-2 catalytic domains differ, but the guanine nucleotide exchange reaction they catalyze to activate Rho GTPases is the same. Most Rho GEFs harbor additional domains that aid in their regulation. Rho GEFs adopt an auto-inhibitory conformation that is relieved by the binding of signals to their regulatory domains. DH, Dbl homology. PH, pleckstrin homology. DEP, disheveled, EGL-10 and pleckstrin. PDZ, PSD 95, DLG, ZO-1 protein–protein interactions. PEST, motif rich in proline (P), glutamic acid (E), serine (S), and threonine (T).

Figure 3. Metabolic tissue distribution of mammalian Rho GEFs that are known to be involved in the regulation of glucose homeostasis. Nine mammalian Rho GEFs are currently known to regulate glucose homeostasis in vitro or in vivo. Their distribution in human metabolic tissues is shown here, as extracted from public database BioGPS (http://biogps.org, accessed on 10 April 2021) [74]. Data are mean mRNA expression values determined by Affymetrix microarray. Units are z-scores of mean fluorescence intensity, determined using multiple probes for each transcript and processed using gcrma algorithms. Genes with z-scores above 5, indicated by the stippled black line, are considered to be expressed in that tissue. The graph was drawn using GraphPad Prism 8.

2. Glucose Homeostasis

The human body is highly dependent on the tight regulation of glucose homeostasis. Glucose is an essential metabolic source of energy, and the majority of cells require glucose for metabolic function such as respiration, protein synthesis or energy storage as glycogen. The human brain accounts for 60% of all glucose uptake from the blood, and a further 25% is taken up by the liver and gastrointestinal tissues in the unstimulated and rested state [77]. Both of these absorption processes are insulin independent. Only 25% of glucose uptake is insulin dependent, with the majority of this occurring in adipose and skeletal muscle tissues. It is vital to maintain blood glucose within its physiological range, and in the fully-grown adult human, this is between 4 and 7.8 mM or 72.0 and 140.4 mg/dL, with fasting levels at approximately 5.5 mM/99 mg/dL [78]. Blood glucose levels that differ significantly above or below this range can lead to hyper- and hypoglycemia, respectively. The clinical symptoms of these conditions can range from mild, headaches and tiredness, to severe, coma and death, so it is important to maintain the levels within a narrow range. Following a meal, food is digested and the nutrients are absorbed into the blood circulation from the intestines. Blood glucose levels transiently rise postprandial (after a meal), but return to the resting baseline level due to homeostatic mechanisms. In the fasting state, blood glucose levels are maintained through the liver by glycogenolysis, breakdown of glycogen to glucose, and gluconeogenesis, glucose synthesis from non-carbohydrate sources such as amino acids.

The major organs involved in glucose homeostasis are the brain, digestive tract, pan-

3. Rho GTPases in Glucose Homeostasis

The control of glucose homeostasis by Rho family GTPases is an emerging field. Best understood are the roles of Rac1, Cdc42 and RhoA in insulin-dependent glucose uptake into adipose and skeletal tissues, and the roles of Rac1 and Cdc42 in glucose-stimulated insulin secretion by pancreatic cells. There is already an extensive, recent review by Møller et al. on the involvement of Rho GTPases in these processes [98]. This excellent review enabled us to keep the subsequent section brief, we will summarize key findings and update on new literature. The main body of our review will focus instead on Rho GEFs, which were not covered by Møller et al.

Both Rac1 and Cdc42 have been implicated in glucose-stimulated insulin secretion by pancreatic cells via their roles in actin rearrangement [99–102]. During the fasting state when blood glucose levels are low, the actin cytoskeleton of pancreatic cells prevents insulin storage vesicles from fusing with the plasma membrane and releasing insulin. An increase in blood glucose levels leads to actin cytoskeleton rearrangements, to allow granule fusion and thus insulin release. This granule translocation process has been shown to be dependent on both Cdc42 [102] and Rac1 [99] activation, and is likely mediated through the Rac effector PAK1 [50,103], although the precise mechanism is still unclear.

Skeletal muscle is essential for maintaining whole-body glucose homeostasis, as it accounts for the majority of insulin-stimulated glucose uptake [104,105]. Insulin activates Rac1 and RhoA in muscle cells [92,94,106]. It is accepted that actin remodeling by Rac1 is required for GLUT4 translocation to the sarcolemma [53,92,107]. Insulin-stimulated GLUT4 translocation allows glucose entry into skeletal muscle cells. Accordingly, many studies have identified Rac1 as an essential regulator of insulin-dependent glucose uptake into skeletal muscle [92,94,107,108]. These include genetic studies using tissue-specific inducible knockout of Rac1 in the skeletal muscle of mice [53].

Rac1 has also been implicated in insulin-independent mechanisms of glucose uptake in muscle cells. Sylow et al. found that Rac1 is activated in both mouse and human skeletal cells following muscle contraction induced by physical exercise. Muscle-specific inducible Rac1 deficiency in mice, or pharmacological inhibition of Rac1 with NSC23766 or a derivative of this compound decreased the contraction-stimulated glucose uptake [109]. AMP-activated protein kinase (AMPK) and Rac1 pathways were found to be important for the regulation of contraction-induced muscle glucose uptake ex vivo, in the muscle-specific AMPK 1 2 KO mouse [110]. The Rac1 inhibitor II and deletion of AMPK 1 2, independently, and additively, led to decreased 2-deoxy-D-glucose transport in soleus muscle ex vivo. Yet, when the authors investigated this in vivo using a muscle-specific kinase-dead AMPK mouse and inducible muscle-specific Rac1 KO mouse, they found that exercise-induced glucose uptake depends on Rac1 and not AMPK in mice [110]. These

elicit its effects on the actin cytoskeleton by the ROCK pathway and ezrin/radixin/moesin proteins [115].

Adipose tissue is essential for maintaining glucose homeostasis, and for the postprandial reduction in blood glucose levels. Rho GTPases play similar roles in adipose tissue, as in skeletal muscle, regulating glucose uptake through translocation of GLUT4. The literature is less extensive and more controversial, but overall glucose homeostasis in adipose tissue is thought to be regulated by different combinations of Rho GTPase family members compared to muscle cells [92

chromosome 20q13.1 that has been linked to type 2 diabetes in many studies [127], and SNPs in the perigenic region of *PREX1* have been proposed to associate with the likelihood of obesity developing into type 2 diabetes [128]. P-Rex1 is highly expressed in leukocytes and neurons, but is present at lower levels in many other cell types, whereas P-Rex2 is more widely expressed except in leukocytes. P-Rex-deficient mouse strains have revealed roles for P-Rex1 in the pro-inflammatory functions of leukocytes and developmental migration of melanocytes, and roles for P-Rex2 in neuronal morphology and plasticity, and in motor coordination [124]. Both P-Rex proteins are activated in response to the stimulation of G protein-coupled receptors (GPCRs), by the G subunits of heterotrimeric G proteins, and in response to the stimulation of PI3K-coupled receptors, by PIP_3, with additional regulation through phosphorylation and protein/protein interactions [124]. P-Rex family Rac GEFs are known to facilitate insulin signaling [124]. Upon insulin stimulation, P-Rex1 responds to the activation of PI3K and the production of PIP_3, and transmits the insulin signal through its catalytic Rac GEF activity. P-Rex2 may have this same role, but has additionally been identified to inhibit the tumor suppressor PTEN. PTEN metabolizes PIP_3

Figure 4. P-Rex family Rac GEFs in insulin signaling. Insulin activates the insulin receptor (IR), leading to receptor conformational changes which promote transphosphorylation and subsequent activation of the receptor tyrosine kinase activity. Insulin receptor substrate-1 (IRS1) is phosphorylated and recruits PI3K to the plasma membrane. Class 1 PI3Ks phosphorylate PtdIns(4,5)P$_2$ to generate PIP$_3$ [84]. PIP$_3$ promotes the translocation of PDK1 and Akt to the membrane through interaction with the PH domains, and phosphorylation of Akt by PDK1 and mTORC2 (PDK2) leads to the full activation of Akt. P-Rex1 and P-Rex2 are direct binding partners of mTORC1 and mTORC2 [132], both of which are

Figure 5. The roles of Rho family GEFs in pancreatic   cell glucose homeostasis. (**A**,**B**

4.1.3. P-Rex2 In Vivo

4.2.2. Vav2 in Pancreatic Cells

A study by Veluthakal et al. highlighted Vav2 as a GEF for Rac1 in glucose-stimulated insulin secretion, through the use of Vav2 siRNA knockdown in INS-1 832/13 pancreatic cells [101]. Using the Vav2:Rac1 interaction inhibitor EHop 016, and live cell imaging with Lifeact-GFP biosensor, the authors were able to show a marked reduction in F-actin depolymerization during the second phase of insulin secretion. This phase requires F-actin cytoskeleton remodeling to allow the movement of granules from intracellular to plasma membrane localization (Figure 5). It should be noted, however, that EHop 016 is unlikely to be a specific inhibitor of Vav2, as it was designed to block the binding of Rho GEFs to Rac [150], and as this review shows, other Rho GEFs are also implicated in the actin

granules in INS 832/13 cells and in rat islets of Langerhans [159]. Syed et al. proposed that hyperactivation of Tiam1/Rac1 may bring about apoptotic death of pancreatic cells, as NSC23766 treatment reduced the superoxide production and mitochondrial dysfunction brought about by stress-related levels of glucose and saturated fatty acids [160]. The potential protective role that inhibition of Tiam1/Rac1 might play, was further shown through use of an in vivo mouse model of type 1 diabetes (NOD mouse), where intraperitoneal daily injection of NSC23766 significantly prevented the development of spontaneous diabetes [161]. Furthermore, NSC23766 was used to implicate Tiam1/Rac1 in the development of diabetic retinopathy, through the activation of Nox2 and p38 MAP kinase leading to mitochondrial

-PIX in Pancreatic   cells

Kepner et al. identified   -PIX as a Cdc42 GEF in MIN6 pancreatic   cells [173]. siRNA-mediated knockdown of   -PIX reduced glucose-stimulated insulin secretion in these cells. Cdc42 is a key regulator of the secondary sustained phase of insulin release from granules, and leads to PAK, then Rac1 activation. Kepner et al. showed that caveolin-1, the main component of caveolae in the plasma membrane, binds Cdc42 in MIN6   cells. They showed that   -PIX competes with caveolin-1 for Cdc42 binding at the boundary between

70) and Vav2, enhanced insulin-stimulated Rac1 activation and insulin-dependent GLUT4 translocation to the plasma membrane. Expression of constitutively active Plekhg4 and siRNA knockdown led to increased and decreased GLUT4 translocation, respectively.

4.6.2. Plekhg4 in Adipocytes

The Satoh group have shown a role for Plekhg4 in GLUT4 translocation in adipocytes [186]. Knockdown of Plekhg4 by siRNA treatment in differentiated 3T3-L1 adipocytes reduced insulin-stimulated Rac1 activity, even in the presence of constitutively active Akt2, which would usually increase Rac1 activity during the process of GLUT4 translocation. This study suggested that Plekhg4 may lie downstream of Akt2 [186]; however, this is likely to be through an intermediator protein as the GEF harbors no consensus sequence for phosphorylation by Akt. Research is required to help decipher the mechanisms that regulate Plekhg4, to enable GLUT4 translocation in skeletal muscle cells and adipocytes.

4.7. PDZ-RhoGEF

Rho guanine nucleotide exchange factor 11 (PDZ-RhoGEF) is a ubiquitously expressed Dbl-type GEF that activates RhoA and is best known for its role in neurotrophin-induced neurite outgrowth [187,188]. Alongside the DH/PH domain tandem, PDZ-RhoGEF harbors

DOCK1 in the Yeast Stress Response

There are data suggesting that Rho5 may be implicated in the stress response of yeast cells to low glucose conditions [192]. Glucose starvation induces Rho5 relocation from the plasma membrane to the mitochondria, and this translocation is conferred by Dck1/Lmo1, implicating the GEF in the change from cytoplasmic glycolytic fermentation to oxidative phosphorylation where energy production is more efficient. It is unknown whether this translates to mammalian cells, as there are key differences between the main roles of mammalian Rac1 and yeast Rho5. For example, Rac1 in mammals primarily controls actin dynamics. However, this role is only weakly conserved in the yeast homologue [192].

4.9. Summary of Rho GEF Functions in Glucose Homeostasis

In summary, 10 different Rho GEFs have been implicated to date in the regulation of glucose homeostasis, nine of which are in mammals, and one, the DOCK1 homologue Dck1, is in yeast. Among the mammalian Rho GEFs, P-Rex1, Vav2, Vav3, Tiam1, Kalirin and Plekhg4 have been identified in GLUT4 translocation and/or insulin-stimulated glucose uptake in skeletal muscle or adipose tissue. The Rho GEFs P-Rex1, Vav2, Tiam1 and β-PIX have been implicated in the glucose-stimulated release of insulin by pancreatic β cells. In vivo studies in mice have shown the Rho GEFs P-Rex2, Vav2, Vav3 and PDZ-RhoGEF to be involved in glucose tolerance and/or insulin sensitivity, with deletion of these GEFs either contributing to the development of metabolic syndrome or protecting mice from this disease.

5. Conclusions and Future Challenges

We have summarized evidence for the emerging roles of Rho GTPases and Rho GEFs in metabolism and metabolic diseases. The study of Rho GTPases in glucose homeostasis has been limited by the wide-ranging essential cellular functions of these proteins, which mean that long-term downregulation or overexpression are prone to inducing non-physiological effects. Dominant-negative or constitutively active mutants are no longer widely used for similar reasons. Rho GTPases function by protein/protein interaction, and pharmacological inhibition is difficult to achieve with high specificity and efficacy. Some bacterial toxins are highly efficient inhibitors, but with considerable cytotoxic effects. Increased use of cell type-specific inducible deletion of the endogenous GTPase, as well as re-expression of mutants to physiological levels, may advance our understanding of the roles Rho GTPases play in metabolic processes, both in isolated cells and in animal models of metabolic disease. Although great steps have been taken to decipher the roles of Rho-GEFs in metabolism, these roles have been restricted to the relatively few examples discussed. Research should focus on other processes such as hormone secretion from the intestine, and the counter-regulatory responses to low glucose levels by glucagon. Global phosphoproteomic analysis of myoblasts differentiated from type 2 diabetic patient iPS cells, has recently revealed cell autonomous insulin resistance and dysregulation of a vast signaling network that goes beyond canonical insulin signaling [193]. There was reduced phosphorylation of proteins involved in Rho GTPase regulation, including the GEFs ARHGEF18 and ARHGEF10 and the Rac1 GAP ARHGAP17, whilst there was downregulation of DOCK7 [193]. These are exciting proteins for further research, as they may impact the actin cytoskeleton controlling glucose uptake, or indeed regulate glucose homeostasis through cytoskeleton-independent mechanisms.

The study of Rho GEFs promises to yield insights into specific signaling inputs into Rho GTPases in glucose homeostasis. As we have described, several Rho GEFs have been implicated in the regulation of glucose-stimulated insulin secretion in β cells (Figure 5), and in the regulation of GLUT4 translocation and glucose uptake in skeletal muscle and adipose tissue in response to various stimuli, amongst other processes. However, most work has been performed in vitro, and there is a distinct lack of in vivo experiments to show the true physiological relevance of both Rho GTPases and Rho GEFs in metabolism. Among the 10 Rho-GEFs that have been identified to date, the roles of P-Rex2, Vav2, Vav3

and PDZ-RhoGEF in glucose homeostasis mechanisms have so far been investigated using mouse models with whole-body deletions, and only Vav2 has been investigated using

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