MicroRNAs: 'ribo-regulators' of glucose homeostasis

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metabolism. At the same time, they reveal differences in the action of Sirt1. It remains to be clarified whether the observed differences result from disparities in cell type–specific actions of Sirt1, the differentiation status of each type of cell in which it has been studied, the 'artificial' overexpression of Sirt1 in these model systems or a combination of these variables.

While these possibilities are explored, the data of Rodgers *et al.*[2] argue that Sirt1 decodes the nutritional status of the hepatocyte with the activation of gene programs that regulate hepatic glucose metabolism. These findings, together with the data of Picard *et al.*[7], may suggest that Sirt1 serves as a systemic metabolic master switch. This is especially exciting from the perspective that Sirt1 also modulates aging and may consequently serve as the key decoder that translates caloric restriction to increase in lifespan. Future research will establish to what extent interference with Sirt1-regulatory pathways in the liver and in other organs that regulate glucose homeostasis (**Fig. 1**) may lead to new therapeutic approaches to metabolic and age-related diseases and syndromes.

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Author's Comments

For a long time, we have devoted our research efforts to finding molecular mechanisms that underlie metabolic diseases—in particular, how nutrients are sensed by transcriptional regulators and control biological processes that are dysregulated in these diseases. Our work on PGC-1α and SIRT1 led us along a new route: exploring the metabolic alterations that occur in aging and aging-associated diseases. It is known that as we age, metabolic pathways are considerably altered by nutrients and hormones, but how this is controlled is not completely understood and has always been an attractive area of research. We knew that discovering new modulators for PGC-1α function in relation to metabolic diseases would be of importance. In this regard, we suspected that establishing the role of SIRT1—a protein that influences longevity in organisms such as yeast and worms—as an activator of PGC-1α through deacetylation could be a significant contribution to our understanding of how metabolic processes are altered in aging. That said, I wouldn't consider this a straightforward project; what really inspired us were the genetic data indicating that in lower organisms SIRT1 was connected to nutrient metabolism. These results compelled us to devote our efforts to finding metabolic regulators that would be a direct target of SIRT1 in mammals. The fact that PGC-1α is a SIRT1 substrate turned out to be a truly satisfying and exciting result.

Pere Puigserver, Johns Hopkins University School of Medicine

MicroRNAs: 'ribo-regulators' of glucose homeostasis

Benoit R Gauthier & Claes B Wollheim

Insulin secretion regulates glucose homeostasis and its dysregulation causes type 2 diabetes. Short noncoding microRNAs have now been shown to control exocytosis, the final event in insulin secretion. This discovery opens potential perspectives for diabetes therapy.

In recent years, our understanding of complex gene-regulatory networks governing cell physiology has rapidly evolved. Now, seemingly redundant, small noncoding RNAs have come into the limelight as major players in this process. In particular, short 21–22-nucleotide RNA molecules—the microRNAs (miRNAs)—have emerged as important regulators of genes involved in developmental timing, neuronal cell fate, apoptosis, proliferation, adipocyte differentiation and hematopoiesis[1,2]. miRNAs have also been associated with cancer[3,4] and neurological disorders[5], indicating a fundamental role of these small molecules in diverse biological processes. A study by Poy *et al.*[6] suggests that miRNAs are involved in yet another specialized cell function—exocytosis, the final step in the secretory pathway. The pancreatic islet–specific miRNA miR-375 regulates insulin exocytosis, a key determinant of blood glucose homeostasis, pointing to a possible involvement of microRNAs in type 2 diabetes.

Maintaining appropriate blood glucose levels depends on the fine regulation of insulin release; when glucose-stimulated insulin secretion fails, hyperglycemia ensues[7]. Like neurotransmitter release, the exocytosis of insulin-containing vesicles is regulated by the second messengers Ca^{2+}, cAMP and phospholipid derivatives, which control vesicle docking, priming and fusion with the membrane[8,9]. Stimulation with glucose leads to synthesis of ATP and, ultimately, depolarization of the plasma membrane and opening of Ca^{2+} channels that are located in close proximity to the vesicles, promoting their fusion. Defects in this stimulation-secretion coupling have been thought to be involved in certain forms of type 2 diabetes[10].

Insulin release also requires the movement of insulin granules toward the membrane, ensuring replenishment of the releasable pool of vesicles. This process involves microtubules and actin filaments (F-actin). Endocrine cells have a dense F-actin network beneath the membrane, the mesh size of which is smaller than insulin granules. If such a network were not constantly remodeled by depolymerization and repolymerization of F-actin, it would be impenetrable to granules and impede fusion[11].

Every step in the secretory pathway is tightly controlled at multiple levels, including the biosynthesis of insulin and secretory granule proteins, granule movement and exocytosis. Transcriptional regulation and the control of transcript stability and translational efficacy dictate the levels of insulin and other secretory granule proteins[12,13].

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Although miRNAs were initially described in *Caenorhabditis elegans* more than a decade ago, their presence in vertebrates was only confirmed in 2001 (ref. 14). In mammals, primary miRNAs are transcribed by RNA polymerase II and sequentially processed by two RNase III enzymes—Drosha and Dicer—to generate tiny 21–22-nucleotide double-stranded RNAs. Subsequently, the active single-stranded miRNA enters the RNA-induced silencing complex, which interacts with the 3′-untranslated region (UTR) of target mRNAs. The miRNA-mRNA sequence mismatch impairs translation initiation, owing to inhibition of eukaryotic initiation factor 4E/cap and poly(A) tail function[15]. In contrast to miRNA, short interfering RNAs (siRNAs) are not encoded by the genome, but arise from exogenous or endogenous, long, double-stranded RNAs. siRNAs achieve gene silencing by promoting cleavage of the perfectly matched mRNA-siRNA duplex. This different mode of action explains why siRNAs repress single genes, whereas miRNAs are promiscuous and can target up to 200 transcripts[16].

To date, 3,424 miRNAs have been deposited in the miRBase registry (http://microrna.sanger.ac.uk). From these, 326, 249 and 187 have been identified in human, mouse and rat, respectively[17]. *In silico* analysis has shown that about 20% of human genes are potentially regulated by miRNAs[18]. The challenge is to validate these targets and to define the functional impact of miRNAs.

Poy *et al.* have begun to unravel this puzzle by focusing on miR-375, one of the most abundant pancreatic islet–specific miRNAs in pancreatic cell lines. Overexpression of miR-375 in these cells resulted in decreased glucose-induced insulin secretion with no alterations in glucose-mediated production of ATP or rise in intracellular Ca^{2+}. In addition, repression of miR-375 increased glucose-stimulated secretion of insulin. These results suggested that miR-375 regulates a late step of insulin exocytosis, an idea confirmed by a marked decrease in Ca^{2+}-stimulated fusion of granules.

What are the targets of miR-375? Poy *et al.* focused on myotrophin, also called V-1, a protein previously implicated in exocytosis[19] that, on the basis of its sequence, could potentially be regulated by this miRNA (**Fig. 1**). Suppression of myotrophin by siRNA impaired glucose-stimulated secretion of insulin, mimicking the effects of miR-375 overexpression. The authors showed that interaction of miR-375 with the 3′-UTR of myotrophin represses its translation and inhibits insulin secretion.

The discovery of myotrophin as a regulator of secretion adds to the growing list of cytoskeletal components important for vesicular release. What mechanisms could account for this effect? Myotrophin interacts with CapZ, an actin-capping protein that inhibits F-actin assembly[20]. Although Poy *et al.* rule out potential alterations in the actin network, the large increase in docked secretory granules after overexpression of miR-375 might imply a failure of the actin mesh to rearrange and allow vesicle access to fusion sites. Furthermore, myotrophin could impinge on the function of the molecular motor myosin II in attracting vesicles to the membrane, as suggested for chromaffin cells by detailed analysis of release kinetics[21]. This remains to be confirmed in the beta cell.

Another interesting aspect of myotrophin not addressed by Poy *et al.* is its function as a transcription factor, as documented in cardiomyocytes. Indeed, cardiac hypertrophy is associated with activation of nuclear factor-κB (NF-κB) by myotrophin[22]. In beta cells, NF-κB activity has been associated with improved cytoskeleton, resulting in increased glucose-stimulated insulin secretion[23]. Conversely, inactivation of NF-κB in insulinoma cells and in mutant mice led to decreased glucose-stimulated insulin secretion[24]. It will be important to establish if the miR-375–mediated changes in myotrophin levels are relevant to these effects of NF-κB.

Regulation of myotrophin by miR-375 may only be the tip of a large iceberg, as Jak2, ubiquitin-specific protease 1 and adiponectin receptor 2 are additional *bona fide* targets of miR-375 (ref. 16). Of note, miR-124 and let-7b, a miRNA that is also abundantly expressed in islet cells, coordinately repress myotrophin, pointing to converging translational control of a single protein[16]. Multiple targeting of a transcript may ensure sequential miRNA actions and fine-tuning of gene expression[1].

The function and regulation of mammalian miRNAs remain to be studied in more detail. Moreover, their potential therapeutic value holds promise, as the *in vivo* injection of novel oligonucleotide-based 'antagomirs' can suppress the liver-specific miRNA miR-122, resulting in altered cholesterol biosynthesis[25]. Further insight into the regulation of beta cell–specific miRNAs must be investigated before the potential therapeutic use of antagomirs in type 2 diabetes.

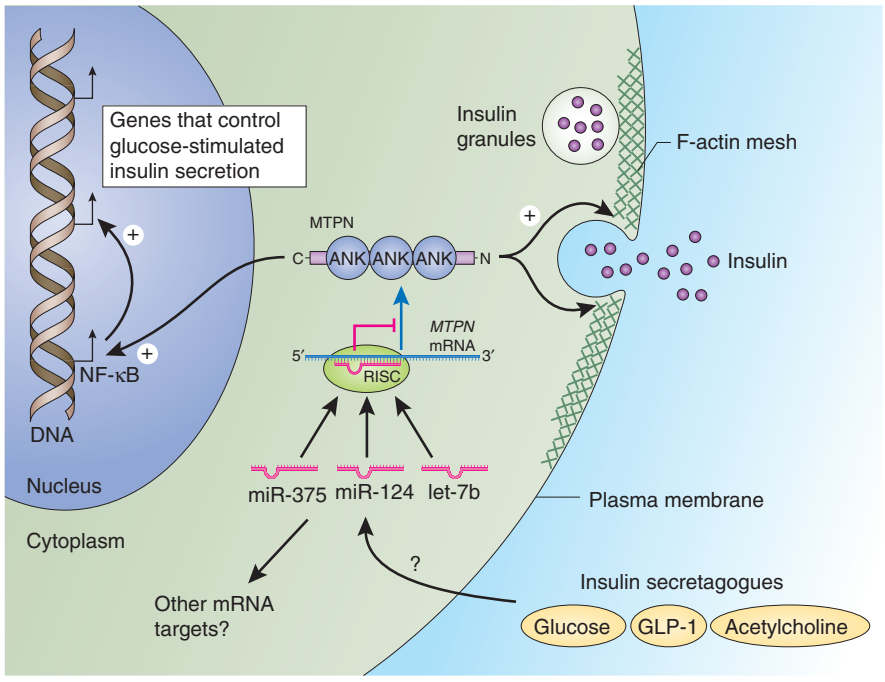

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Figure 1 MicroRNA-mediated regulation of insulin exocytosis in the pancreatic beta cell. The microRNAs miR-375, miR-124 and let-7b suppress the translation of mRNA encoding myotrophin (MTPN). Normally, MTPN interacts with cytoskeletal elements and is thought to open the F-actin mesh, allowing access of the secretory granules to exocytotic sites. Furthermore, MTPN may activate the transcription factor NF-κB in the nucleus. NF-κB is permissive to insulin secretion, probably by activating genes that control vesicle trafficking and exocytosis. RISC, RNA-induced silencing complex; ANK, ankyrin motifs; GLP-1, glucagon-like peptide-1.

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Foxa2, a novel transcriptional regulator of insulin sensitivity

Pere Puigserver & Joseph T Rodgers

The transcriptional network downstream of the insulin receptor is incompletely understood, but recent data identify a new player. Foxa2—a forkhead transcription factor—controls hepatic lipid metabolism in fasting and type 2 diabetes, improving insulin resistance in peripheral tissues.

Insulin resistance—a state of reduced responsiveness to insulin—is a major contributor to the pathogenesis of type 2 diabetes. Although we have made major advances in unraveling the underlying defects that cause insulin resistance, many of the pathways and regulators that connect insulin to its downstream metabolic effects are poorly understood. Deciphering these signaling targets is of crucial importance to understanding the molecular mechanisms of insulin resistance and the pathogenesis of diabetes. A recent report by Stoffel and colleagues fills a major gap in our understanding of insulin resistance[1]. They showed that the forkhead transcription factor Foxa2 is regulated by fasting, and, in type 2 diabetes, it controls the expression of key genes involved in fatty acid oxidation, ketogenesis and glycolysis. By modulating these metabolic pathways, Foxa2 improves insulin resistance in a variety of mouse models of diabetes. Their study provides a new connection between insulin signaling and Foxa2 transcriptional targets that are dysregulated in insulin resistance and type 2 diabetes.

Abnormal lipid accumulation in skeletal muscle and liver is associated with insulin resistance and is a strong predisposing factor for type 2 diabetes[2]. A crucial issue in understanding this relationship is the degree to which glucose and lipid metabolism are resistant to the action of insulin. For instance, in type 2 diabetes with moderate insulin resistance, hepatic glucose output is mainly insensitive to insulin, whereas rates of fatty acid synthesis and oxidation are still sensitive to it. By contrast, in cases of severe insulin resistance and in type 1 diabetes—in which insulin is not produced because of the destruction of pancreatic beta cells—glucose production and fatty acid oxidation are increased[3]. Uncontrolled rates of fatty acid

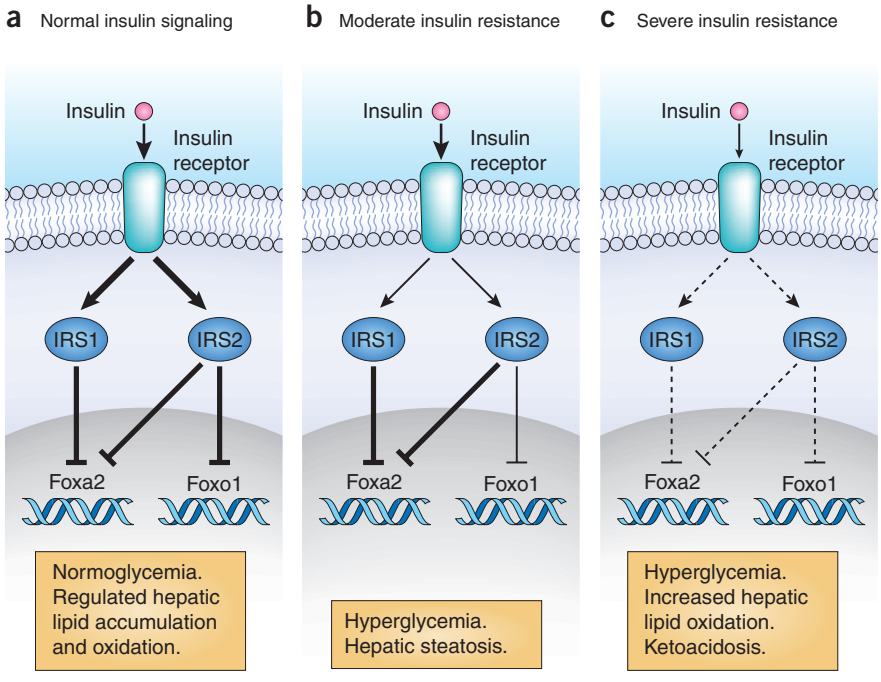

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Figure 1 Insulin regulates glucose and lipid metabolism through forkhead transcription factors. **(a)** Under normal conditions, the insulin pathway regulates Foxo1 and Foxa2, resulting in regulated glycemia and a balance of hepatic lipid accumulation and oxidation. **(b)** In moderate insulin resistance, reduced activity of the insulin pathway results in activation of Foxo1, leading to elevated gluconeogenesis and hyperglycemia. However, Foxa2 is more sensitive to insulin and therefore is still repressed, resulting in reduced lipid oxidation and hepatic steatosis. **(c)** In severe insulin resistance, the insulin pathway is barely active, and the constitutive activation of Foxo1 and Foxa2 result in elevated gluconeogenesis, hyperglycemia and high levels of lipid oxidation, leading to ketoacidosis.

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