Role of the clock gene *Rev-erb alpha* in metabolism and in the endocrine pancreas.

Short title: *Rev-erb alpha* in pancreatic alpha and beta cells

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ABSTRACT

Several hormones are regulated by circadian rhythms to adjust the metabolism to the light/dark cycles and feeding/activity patterns throughout the day. Circadian rhythms are mainly governed by the central clock located in the suprachiasmatic nucleus but also by clocks present in peripheral organs, like the endocrine pancreas. Plasma glucose levels and the main pancreatic hormones insulin and glucagon also exhibit daily variations. Alterations in circadian rhythms are associated with metabolic disturbances and pathologies like obesity and diabetes. The molecular components of central and peripheral clocks and their regulatory mechanisms are well established. Among the different clock genes, *Rev-erb alpha* is considered one of the key links between circadian rhythms and metabolism. *Rev-erb alpha* is a critical part of a negative feedback loop in the core circadian clock and modulates the clock oscillatory properties. Additionally, *Rev-erb alpha* plays an important role in the regulation of lipid and glucose metabolism, thermogenesis, adipocyte and muscle differentiation as well as mitochondrial function. In the endocrine pancreas, *Rev-erb alpha* regulates insulin and glucagon secretion and pancreatic β-cell proliferation. In the present review, we discuss all these subjects and, particularly, the role of the clock gene *Rev-erb alpha* in the endocrine pancreas.

Key Words: *Rev-erb alpha*, clock genes, glucose homeostasis, type 2 diabetes, insulin and glucagon secretion, metabolism.

INTRODUCTION

Glucose homeostasis is mainly regulated by the two main endocrine cell populations of the pancreas. Pancreatic α and β-cells are the most abundant cell types of the islet of Langerhans, and their opposite and complementary secretory activity is necessary for an adequate control of plasma glucose levels within a physiological range. Elevation of glucose concentrations stimulates insulin release from pancreatic β-cells, while hypoglycaemic conditions activate glucagon secretion from α-cells [1,2]. Among other metabolic functions, insulin promotes glucose uptake by the skeletal muscle and the adipose tissue, while inhibits hepatic glucose production. These processes lead to hypoglycaemic effects, decreasing plasma glucose concentrations. On the contrary, glucagon acts mainly on the liver, inducing glycogenolysis and gluconeogenesis and, subsequently, activating hepatic glucose output [1,2]. These processes lead to the rise of plasma glucose levels. In this manner, plasma glucose is regulated by the opposite role of insulin and glucagon and the complementary function of pancreatic α and β-cells at low and high glucose concentrations.

The abnormal function of the endocrine pancreas can result in impaired glucose tolerance and eventually, in type 2 diabetes. Metabolic conditions like obesity are associated with insulin resistance, which involves an attenuated action of this hormone on peripheral tissues. In response to this resistance, several morphological and functional adaptations allow the endocrine pancreas to secrete higher insulin levels to satisfy the increased demand imposed by insulin resistance in order to maintain normal plasma glucose levels [3,4]. When insulin resistance is not adequately compensated by an efficient pancreas adaptation, glucose tolerance becomes impaired. This situation can progress to overt hyperglycaemia and type 2 diabetes when β-cell function and/or viability become highly deteriorated. In addition to the pancreatic β-cell, alterations in

the pancreatic α-cell mass and glucagon secretion are also involved in the pathophysiology of diabetes [1,2]. It has been reported that increased plasma glucagon levels (in absolute terms or relative to those of insulin) can increase hepatic glucose output, exacerbating the hyperglycaemia in diabetes. Increased α-cell mass (relative to β-cells) and lack of suppression of glucagon secretion by hyperglycaemia may also contribute to excessive glucagon signalling [1,2]. Thus, both α and β-cells play a key role not only in glucose homeostasis but also in diabetes.

Circadian rhythms are important to adapt the metabolism to the changing conditions of the day/night and rest/activity daily cycles. In this regard, the pancreas function and the regulation of glucose homeostasis are also subjected to circadian control mechanisms [5]. Several features of the lifestyle of modern societies like shift working, jet lag, impaired sleeping or artificial light can negatively affect the optimal function of circadian rhythms. A growing body of evidence indicates that these alterations in the circadian regulation may be also associated to metabolic pathologies like obesity and type 2 diabetes [5]. In recent years, the molecular nature of the different circadian clocks located in the brain and in peripheral tissues has been revealed. At the molecular level, the main components of the core clock machinery are CLOCK and BMAL1, which regulate both the transcription of different genes involved in metabolism and the expression of other clock genes involved in two regulatory feedback loops of the molecular clock (Figure 1). In one of these loops, PER and CRY proteins repress CLOCK and BMAL1 expression, while in the other feedback mechanism, the nuclear receptors REV-ERB ALPHA and ROR ALPHA repress or activate *Bmal1*, respectively. Among the different genes involved in the control of the circadian system, we will discuss here the function of the clock gene *Rev-erb alpha* in metabolism and the pancreatic function.

Clock genes in glucose homeostasis and type 2 diabetes.

The plasma levels of glucose and several hormones involved in glucose homeostasis such as insulin and glucagon display circadian variations. Insulin peaks during the day to increase energy storage and utilization in humans [6-8]. In contrast, insulin secretion decreases and glucagon increases during night time to increase hepatic glucose production and maintain plasma glucose levels within a physiological range [8]. Thus, daily plasma oscillations in pancreatic hormones can be also regulated independent of fasting/feeding behaviour in animals and humans [7]. These daily cycles suggest an important role of the circadian regulation of glucose homeostasis, which involves the control by the suprachiasmatic nucleus (SCN) in addition to the sympathovagal balance of the autonomic system.

Alteration of circadian rhythms has been associated with obesity and diabetes [9]. Circadian disruption can occur by impaired function of the molecular circuitry in the different internal clocks and also by desynchronization between the SCN and the external environmental cues or the peripheral clocks. In modern societies, the natural circadian rhythms can be impaired by the current lifestyle: high-fat diet (HFD) and excessive calories, jetlag, shift work, sleep loss and exposure to light at night are among several factors that are considered to alter circadian rhythms, which may further contribute to the increasing epidemics in obesity and diabetes. For instance, shift workers have increased risk of developing cardiovascular disease, metabolic syndrome, cancer, obesity and type 2 diabetes [10-15] and alterations in the pancreatic β-cell responses, glucose and lipid metabolism [13,16,17]. Poor sleep or reducing sleeping time leads to increased body mass index, elevated ghrelin, reduced leptin levels and insulin resistance [18-20]. Moreover, first-degree relatives of patients with type 2

diabetes had shorter insulin secretion circadian cycles that lack real periodicity compared to healthy subjects [21].

Several animal models with genetic modifications in clock genes have revealed their key regulatory function in metabolism and glucose homeostasis. For instance, *Clock* mutant mice exhibited attenuated diurnal feeding rhythm, hyperphagia and obesity [22]. In addition, they developed a metabolic syndrome characterized by hyperleptinemia, hyperlipidemia, hepatic steatosis, hyperglycaemia, and hypoinsulinaemia. Mice deficient in *Bmal1* showed altered adipogenesis and hepatic carbohydrate metabolism [23,24]. Later studies showed that *Clock* mutant mice as well as global and pancreas-specific *Bmal1* knockout (KO) mice had clear diabetic features such as hyperglycaemia and hypoinsulinaemia [25]. Genetic manipulation of other clock genes has also demonstrated the importance of these genes in metabolism and energy homeostasis. *Per2* KO mice had altered lipid metabolism with reduced total triacylglycerol and nonesterified fatty acids [26]. Double *Cry1* and *Cry2* KO mice exhibited glucose intolerance and alterations in glucose and lipid metabolism [27]. *Cry1* overexpression in the liver down-regulated gluconeogenesis during fasting [28]. Additionally, this overexpression resulted in increased insulin sensitivity and lower glycaemic levels in diabetic animals [28]. Mice lacking the nuclear receptors REV-ERB ALPHA and ROR ALPHA decreased adiposity and HDL levels [29-31]. Altered lipid metabolism was also found in the double KO mice for *Rev-erb alpha* and *beta* [32]. Therefore, alterations in clock genes can lead to disturbances in energy and metabolism, which may promote metabolic diseases like obesity and type 2 diabetes.

Rev-erb alpha **and its function in metabolism**

The nuclear receptor REV-ERB ALPHA (also known as NR1D1) was first mapped to the reverse strand of the gene thyroid hormone receptor alpha and considered an orphan receptor. The endogenous ligand of REV-ERB ALPHA was later discovered to be the metabolite heme [33,34], which is an important molecule for cellular redox balance and mitochondrial function [35]. In addition to sensing the metabolic state of the cell, REV-ERB ALPHA has been proposed to link circadian rhythms to metabolism in several tissues [36]. In mice, *Rev-erb alpha* gene expression was found to exhibit circadian oscillations in different tissues including skeletal muscle, kidney and thymus [37,38], and the lack of this nuclear receptor shortened the period of behavioural rhythms, suggesting an important role in stabilizing circadian oscillations [31]. Mice lacking REV-ERB ALPHA showed impaired regulation of cholesterol, bile acid metabolism [30,39] and apolipoprotein CIII [40]. More recent studies using genomic techniques demonstrated that REV-ERB ALPHA bound genes were involved in lipid metabolism [41]. Besides regulating lipid metabolism, REV-ERB ALPHA was shown to regulate glucose *de novo* synthesis by heme-induced REV-ERB ALPHA activation and repression of the gluconeogenic *Pepck* gene in human hepatoma cells [34]. Studies in brown adipose tissue from mice lacking REV-ERB ALPHA demonstrated an important role of this nuclear receptor in body temperature by regulating *Ucp1* gene expression [42]. Additionally, mouse adipocytes cell lines showed a requirement of *Rev-erb alpha* in adipocyte differentiation and its interaction with PPARgamma [43]. In humans, different *Rev-erb alpha* polymorphisms were associated with obesity in heterogeneous populations [44-46]. Interestingly, *Rev-erb alpha* expression was upregulated in visceral adipose tissue from obese women as compared to lean individuals over the course of 24 h and this increased expression was associated with metabolic syndrome [47]. In subcutaneous adipose tissue from young obese subjects, a

positive correlation was found between *Rev-erb alpha* and the body mass index, highlighting the importance of this nuclear receptor in obesity and metabolic syndrome. Similar to liver and adipose tissue, *Rev-erb alpha* mRNA levels also oscillate in a circadian manner in mouse skeletal muscle [38]. Studies in C2C12 cells demonstrated that REV-ERB ALPHA is a repressor of genes involved in muscle differentiation [48]. Recently, it was shown that REV-ERB ALPHA can affect mitochondrial content and function by modulating the AMPK (AMP activated protein kinase) pathway in skeletal muscle [49]. REV-ERB ALPHA deficiency resulted in inhibition of the liver kinase b 1(LKB-1)-AMPK-NAD-dependent deacetylase sirtuin-1 (SIRT-1)–Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC-1 alpha) signalling pathways in skeletal muscle. Indeed, a previous study with mice treated *in vivo* with the AMPK activator AICAR suggested a link between the AMPK pathway and clock genes including *Rev-erb alpha* in mouse skeletal muscle [50]. The mechanism suggested was via AMPK gamma3 subunit, which was previously shown to control mitochondrial biogenesis [51].

Rev-erb alpha and its function in the molecular clock.

The central clock located in the SCN and the clocks located in peripheral organs have a common molecular structure consisting of transcriptional/post translational feed back loops that generate circadian rhythms. The core clock machinery composed of CLOCK and BMAL1 activates the transcription of other clock genes including *Per* and *Cry* (Figure 1). PER and CRY proteins repress CLOCK/BMAL1, inhibiting their own transcription, and allowing new cycles to start, which govern the circadian oscillatory pattern. The nuclear receptors REV-ERB ALPHA and ROR ALPHA are part of the additional feedback loop repressing or activating *Bmal1*, respectively. REV-ERB

ALPHA and REV-ERB BETA, a high similar transcription factor, were shown to play a crucial role in circadian rhythm generation [52]. Loss of REV-ERB ALPHA and REV-ERB BETA abrogated circadian gene expression in mouse embryonic fibroblasts [52]. Furthermore, genetic deletion of both *Rev-erb alpha* and *beta* in adult mice led to the loss of rhythmicity in the wheel running behaviour, demonstrating the importance of these two transcription factors as components of the clock machinery [32]. In both hypothalamic and peripheral clocks, *Rev-erb alpha* oscillates in a circadian manner. In the brain, *Rev-erb alpha* is expressed in the supraquiasmatic nucleus [31], hippocampus [53] and mid brain [54]. In the latter two areas, *Rev-erb alpha* repressed hydroxylase, the rate-limiting enzyme in dopamine biosynthesis [53,54]. In *Rev-erb alpha* null mice, the abnormalities of hippocampus functions caused impaired memory and novelty-induced hyperactivity [53], whereas abnormalities in mid brain led to aggression, anxiety and depression-like behaviours [54]. Pharmacological interventions using REV-ERB ALPHA and BETA synthetic ligands were shown to modulate circadian both behaviour and metabolism [55-59]. Administration of synthetic REV-ERB ligands in mice disrupted the circadian pattern of clock genes in the hypothalamus and altered the circadian behaviour, while improved dyslipidemia and hyperglycaemia in high fat diet treated mice [58]. In addition, synthetic REV-ERB ALPHA agonists increased mitochondrial content and exercise capacity in skeletal muscle [49].

Role of *Rev-erb alpha* in the pancreatic β-cell and insulin secretion.

The clock gene *Rev-erb alpha* is expressed in the rat pancreas [60], mouse pancreas [61] as well as mouse and human islets [25,61-63]. Its expression has been also reported in the insulin-releasing cell lines MIN6 [63] and INS-1 [64], which are derived from mouse and rat, respectively. Additionally, *Rev-erb alpha* expression was

found to follow a circadian pattern in the majority of these cell models. The role of *Rev-erb alpha* has been examined in MIN6 cells and in pancreatic islets using small interfering RNA (siRNA) [63]. When both isolated mouse islet cells and MIN6 cells were treated with a siRNA to down-regulate *Rev-erb alpha*, the expression of the lipogenic genes sterol regulatory element binding protein 1c (*Srebp-1c*) and fatty acid synthase (*Fas*) were decreased. *Rev-erb alpha* has been reported to have a key role in the regulation of lipid metabolism in the adipose tissue and the liver [29,32]. However, in contrast to the effects on mouse islets and MIN6 cells, REV-ERB ALPHA synthetic agonists inhibited the lipogenic genes *Srebp-1c* and *Fas* in liver and in insulinoma-derived INS-1 cells [58,64]. Thus, these effects might be species and tissue-specific. In MIN6 cells, down-regulation of *Rev-erb alpha* did not affect apoptosis but decreased proliferation, effect that was also observed in primary mouse pancreatic β-cells [63] (Figure 2). This is in line with findings obtained in isolated islets from *Clock* mutant mouse: the expression of different genes involved in islet growth and development like *CyclinD1*, *Pdx1* or *Hnf4α* was found decreased [25]. Given that *Rev-erb alpha* expression was also altered in these *Clock* mutant mice, it is plausible that the effect on proliferation found in MIN6 and primary β-cells was also related with changes in these genes regulating islet growth and development.

Furthermore, down-regulation of *Rev-erb alpha* in both MIN6 cells and isolated mouse islet cells led to impaired glucose-stimulated insulin secretion (GSIS) after 24 h of treatment with siRNA [63] (Figure 2). This effect was not associated to changes in insulin expression or insulin protein content. However, several genes like *Vamp3*, *Munc18*, *Snap25* and *Syntaxyn1A*, whose proteins are essential in exocytosis, exhibited a reduced expression. This down-regulated expression of exocytotic proteins has been also observed in pancreatic islets from *Clock* mutant mice [25], further indicating that

the clock machinery may have an important role in the later stages of GSIS. *Rev-erb alpha* expression was found to exhibit a circadian pattern in isolated islets from lean mice treated with a normal diet. However, HFD feeding to induce obesity was able to change the circadian expression found in isolated islets from lean mice [63]. These altered patterns after HFD feeding were also found in other clock genes like *Clock*, *Bmal1* and *Per1*. Similar HFD effects have been reported in other tissues like hypothalamus, adipose tissue and liver [65]. Interestingly, GSIS from islets of both lean and obese mice followed a similar circadian pattern as the one followed by *Rev-erb alpha* expression, further suggesting that the function of this clock gene may affect insulin secretion [63].

In addition to this long-term genomic effects, treatment of MIN6 cells with hemin, a natural ligand of REV-ERB ALPHA [33], as well as synthetic modulators of this clock gene, led to rapid GSIS changes [63]. In insulin-secreting INS-1 cells, the application of a REV-ERB ALPHA agonist also led to increased insulin release [64]. In agreement with the long-term effects mentioned earlier, these findings further supported the idea that *Rev-erb alpha* contributes to GSIS. Given that these effects with the REV-ERB ALPHA modulators were produced after 1 h of incubation with the different agents, it is very likely that altered GSIS in these conditions was the result of non-genomic rapid actions. In any case, whether these effects were directly mediated by REV-ERB ALPHA or by other mechanisms need to be elucidated. Indeed, several pleiotropic effects have been attributed to clock genes different to those regulating circadian systems, particularly in the case of *Rev-erb alpha* [5]. Additionally, hemin has been reported to block large conductance K^+ channels in neurons of the rat brain [66], which could affect electrical activity, calcium signals and insulin secretion in the pancreatic β-cell. Moreover, it has been suggested that hemin may rapidly affect the

activation of the MAPK (mitogen activated protein kinase) pathway in neurons of the SCN [67].

The regulation of *Rev-erb alpha* expression by extracellular messengers has been also studied in the pancreatic β-cell. *In vivo* treatment of mice with leptin for 5 days led to increased *Rev-erb alpha* mRNA levels in the pancreatic islets [63]. *In vitro* leptin application produced a similar effect in islets from wild-type and leptin-deficient *ob/ob* mice, but did not modify *Rev-erb alpha* expression in islets from *db/db* mice, which lack leptin receptors. This finding was also observed in insulin-producing MIN6 cells. While blockade of the JAK/STAT (janus kinase/signal transducer and activator of transcription) and the PI3K (phosphatidylinositide 3-kinase) pathways did not affect leptin actions, inhibition of the MAPK cascade completely abrogated leptin-induced *Rev-erb alpha* expression in isolated mouse islets [63]. It has been also reported in rat insulinoma-derived INS-1 cells that activation of melatonin receptors decreased the activity of the cAMP/protein kinase A/CREB (cAMP response element-binding protein) pathway, leading to augmented *Rev-erb alpha* expression and activity, while forskolin application induced the opposite effects [64].

Role of *Rev-erb alpha* in the pancreatic α-cell and glucagon secretion.

As mentioned earlier, pancreatic α-cells play a key role in glucose homeostasis and in the pathophysiology of diabetes [2]. Pancreatic α-cells augment their secretory activity at low glycaemic concentrations, increasing plasma glucagon levels, which activate hepatic glucose output. In diabetes, a relative or absolute hyperglucagonemia may aggravate the hyperglycaemia of these patients. Although glucagon secretion follows an oscillatory daily pattern [68], little is known about the presence of molecular oscillators in the pancreatic α-cell. The mouse-derived α-cell line αTC1-9 expresses

Clock, *Bmal1*, *Per1*, *Per2*, *Cry1*, *Cry2* and *Rev-erb alpha*. It was reported that the expression of this latter clock gene oscillated along the day at low glucose concentrations. However, elevation of glucose levels to 11 mM inhibited this oscillatory behaviour in αTC1-9 cells and decreased *Rev-erb alpha* mRNA levels in mouse pancreatic α-cell preparations enriched by fluorescence activated cell sorting [69]. Incubation of αTC1-9 cells with *Rev-erb alpha* siRNA for 24 h impaired glucagon secretion induced by low glucose levels (0.5 mM) (Figure 3). No effect was observed at 11 mM glucose, condition at which glucagon secretion is already highly inhibited [2]. After the siRNA treatment, several genes coding for exocytotic proteins like *Munc18* and *Syntaxin1A* were found decreased (Figure 3). This is in agreement with studies in pancreatic islets treated with *Rev-erb alpha* siRNA [63] and islets isolated from *Clock* mutant mice [25]. Although all these findings indicate that REV-ERB ALPHA may play an important function in exocytosis and glucagon secretion, further examination in primary pancreatic α-cells is still required. Another important aspect is the glucose regulation of *Rev-erb alpha* expression in pancreatic α-cells. A similar glucose modulation has been reported with *Per1*, *Per2*, *Dbp* and *Rev-erb alpha* in fibroblasts [70,71].

AMPK plays a key role in glucose-modulated glucagon secretion [72]. Its activation has been related with glucagon release from pancreatic α-cells at low glucose concentrations. Additionally, this protein has been implicated in the regulation of peripheral clocks located in liver, adipose tissue and skeletal muscle among others [50,71]. AMPK activation enables the clock gene *Cry* to transduce nutrient signals like glucose to circadian clocks, affecting *Rev-erb alpha* among other clock genes [71]. In αTC1-9 cells, AMPK activation by metformin prevented the inhibitory effect of high glucose levels on *Rev-erb alpha* expression [69], suggesting that AMPK also has an

299 important role in the pancreatic clock function. It has been shown that metabolic
300 sensing by the clock machinery may depend on changes in the cellular redox state,
301 involving nicotinamide phosphoribosyltransferase (NAMPT) and NAD-dependent
302 deacetylase sirtuin-1 (SIRT1) [73-76]. CLOCK:BMAL1 regulate NAMPT transcription
303 in a circadian manner, which is probably responsible for the oscillatory behaviour in
304 NAD^+ levels. NAD^+-sensitive SIRT1 also displays circadian changes and has been
305 found to affect CLOCK:BMAL1 activity by deacetylation of both BMAL1 and PER2
306 [73,74]. In αTC1-9 cells, *Nampt* and *Sirt1* expression decreased in the presence of high
307 glucose levels, effect that was prevented by metformin treatment [69]. Incubation of
308 αTC1-9 cells with a NAMPT inhibitor, led to a decrease in *Sirt1* and *Rev-erb alpha*
309 expression at low glucose concentrations, which was also accompanied by reduced
310 glucagon release. All these findings indicate that glucose can regulate glucagon
311 secretion via an AMPK/NAMPT/SIRT1 pathway that involves the participation of
312 REV-ERB ALPHA [69] (Figure 3). Similarly, this pathway and its modulation by
313 glucose restriction has been also reported in skeletal muscle [77,78]. Furthermore,
314 AMPK activation by metformin rescued the altered expression in several clock genes in
315 the white adipose tissue of obese mice [77]. These effects were mediated by the
316 AMPK/NAMPT/SIRT1 pathway, which is in line with the findings in αTC1-9 cells
317 [69]. Although the modulation of molecular clocks and circadian rhythms by AMPK
318 has been reported in several tissues [69,71,77], studies in heart, fat tissue and muscle
319 from AMPK knock out mice indicate that this AMPK modulation may be tissue-
320 specific [79].

321 Similarly to the situation discussed earlier for the β-cells [63], treatment of
322 αTC1-9 cells with synthetic modulators of REV-ERB ALPHA or its natural ligand
323 hemin showed rapid effects, which were unlikely to be genomic [69]. Hemin incubation

for 90 min increased glucagon release at both low and high glucose levels. In contrast, GSK4112, a REV-ERB ALPHA agonist [55], which was incubated for 60 min, only augmented glucagon release from αTC1-9 cells at low glucose concentrations. Glucagon secretion in pancreatic α-cells is largely controlled by Ca^{2+} signalling [2]. The agonist effect on glucagon secretion seemed to be mediated by Ca^{2+} signals, since its application rapidly increased the intracellular Ca^{2+} concentrations at low glucose levels [69]. The REV-ERB ALPHA antagonist SR8278 [80] produced the opposite effect on glucagon release and Ca^{2+} signalling in αTC1-9 cells. As we have commented earlier for the pancreatic β-cell, further studies are required to know whether these rapid effects were directly mediated by REV-ERB ALPHA or by other pathways. It would be interesting to analyze if these REV-ERB ALPHA modulators can regulate the activity of Ca^{2+} channels, as has been described for hemin in the case of neuron BK channels [66].

Conclusions and future perspectives.

REV-ERB ALPHA seems to form a robust link between circadian rhythms, internal clocks and metabolism. REV-ERB ALPHA plays a key function in the regulation of the clock machinery, since its transcription depends on CLOCK/BMAL1 activity and, at the same time, REV-ERB ALPHA proteins inhibit *Bmal1* expression, establishing a negative feedback loop. Moreover, *Rev-erb alpha* has been involved in lipid and glucose metabolism, thermogenesis, adipocyte and muscle differentiation as well as mitochondrial biogenesis. In humans, several *Rev-erb alpha* polymorphisms have been associated with obesity and metabolic syndrome. In the endocrine pancreas, the clock gene *Rev-erb alpha* exhibits a circadian pattern and regulates insulin and glucagon secretion, probably by modulating the expression of exocytotic proteins. A

metabolic challenge like HFD feeding in mice alters the normal expression of *Rev-erb alpha* in the pancreatic islets as well as their circadian insulin release. Additionally, it has been shown that this clock gene is involved in pancreatic β-cell proliferation. Thus, REV-ERB ALPHA plays a key role in several processes related with the physiology of pancreatic α and β-cells. Given that little information is still available about *Rev-erb alpha* regulation in the endocrine pancreas, much work is required to unravel the molecular mechanisms involved in the control and actions of this clock gene in α and β-cells.

Since REV-ERB ALPHA seems to be important in insulin secretion at high glucose concentrations and in glucagon release at low glucose levels, the specific modulation of this clock gene in both cell types may be of therapeutic interest in diabetes, obesity and metabolic syndrome. In this regard, it has been reported that treatment of diet-induced obese mice with synthetic REV-ERB agonists improved glycaemia and plasma lipids as well as decreased fat mass and body weight in these animals [58]. Additionally, activation of REV-ERB ALPHA by synthetic agonists increased muscle oxidative metabolism and exercise capacity by improving mitochondrial function [49]. Although these agonists seem to be useful to promote health benefits in metabolic disorders, a better understanding of the multiple REV-ERB effects on circadian physiology and metabolism in central and peripheral organs is required. This will be necessary to design specific strategies targeted to the different pathologies and tissues, like the endocrine pancreas, and to minimize non-desired actions and side effects.

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FIGURE LEGENDS

Figure 1. Molecular components of the core circadian clock. The positive arm, BMAL1 and CLOCK, drives the transcriptional expression of the negative arm, PERs and CRYs. PER and CRY proteins form a complex that inhibits their own CLOCK:BMAL1-induced transcription. The positive arm (CLOCK and BMAL1) also drives the transcriptional expression of CCGs, REV-ERBs and RORs. The later two inhibit and activate BMAL1 expression, respectively. BMAL1, brain and muscle ARNT-like 1; CLOCK, circadian locomotor output cycles protein kaput; PER, period homolog drosophila; CRY, cryptochrome; CCGs, clock controlled genes; REV-ERB, reverse-eritroblastosis virus; RORs, retinoic acid receptor-related orphan receptor; RORE, ROR response element.

Figure 2. Role of REV-ERB ALPHA in the regulation of pancreatic β-cell function. When plasma glucose concentrations rise, this sugar enters the cell through the glucose transporter 2 (GLUT-2) and is metabolized via glycolysis and the Krebs cycle. This results in the rapid increase of the ATP/ADP ratio, which induces the closure of ATP-sensitive K^+ channels (K_{ATP}). The blockade of these channels leads to plasma membrane depolarization, activating voltage-gated Ca^{2+} channels. The subsequent Ca^{2+} influx triggers insulin release through exocytosis of insulin secretory granules. REV-ERB ALPHA also regulates insulin secretion probably via modulation of the exocytotic process and the proteins involved. REV-ERB ALPHA also regulates β-cell proliferation and genes involved in lipid metabolism.

Figure 3. Role of REV-ERB ALPHA in the regulation of pancreatic α-cell function. Glucose is transported into the α-cells through the glucose transporter 1

405 (GLUT-1). According to the most cited model for the α-cell stimulus-secretion coupling
406 [81], at low glucose concentrations the ATP/ADP level is sufficient to partially close
407 the ATP-sensitive K^+ channels (K_{ATP}). This results in a small depolarization that
408 generates an intermediate membrane potential at which voltage-gated Na^+ and Ca^{2+}
409 channels are activated. The increase in the cytoplasmic Ca^{2+} concentration stimulates
410 glucagon secretion. Additionally, the low ATP concentrations would activate the
411 AMPK-NAMPT-SIRT1 pathway, which turns on the CLOCK/BMAL1 complex and,
412 consequently, increases REV-ERB ALPHA expression. REV-ERB ALPHA probably
413 regulates glucagon secretion via modulation of the exocytotic process. BMAL1, brain
414 and muscle ARNT-like 1; CLOCK, circadian locomotor output cycles protein kaput;
415 REV-ERB, reverse-eritroblastosis virus; AMPK, AMP activated protein kinase;
416 NAMPT, nicotinamide phosphoribosyltransferase; SIRT-1, NAD-dependent
417 deacetylase sirtuin-1.
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References

1. Marroqui L, Alonso-Magdalena P, Merino B et al. Nutrient regulation of glucagon secretion: involvement in metabolism and diabetes. Nutr Res Rev 2014; **27**: 48-62.
2. Quesada I, Tuduri E, Ripoll C et al. Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes. J Endocrinol 2008; **199**: 5-19.
3. Kahn SE, Zraika S, Utzschneider KM et al. The beta cell lesion in type 2 diabetes: there has to be a primary functional abnormality. Diabetologia 2009; **52**: 1003-1012.
4. Muoio DM, Newgard CB. Mechanisms of disease:Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol 2008; **9**: 193-205.
5. Vieira E, Burris TP, Quesada I. Clock genes, pancreatic function, and diabetes. Trends Mol Med 2014; **20**: 685-693.
6. Boden G, Ruiz J, Urbain JL et al. Evidence for a circadian rhythm of insulin secretion. Am J Physiol 1996; **271**: E246-252.
7. Kalsbeek A, Strubbe JH. Circadian control of insulin secretion is independent of the temporal distribution of feeding. Physiol Behav 1998; **63**: 553-558.
8. Marcheva B, Ramsey KM, Peek CB et al. Circadian clocks and metabolism. Handb Exp Pharmacol 2013; **217**: 127-155.
9. Gangwisch JE. Epidemiological evidence for the links between sleep, circadian rhythms and metabolism. Obes Rev 2009; 10 Suppl 2: 37-45.
10. Di Lorenzo L, De Pergola G et al. Effect of shift work on body mass index: results of a study performed in 319 glucose-tolerant men working in a Southern Italian industry. Int J Obes Relat Metab Disord 2003; **27**: 1353-1358.
11. Ellingsen T, Bener A, Gehani AA. Study of shift work and risk of coronary events. J R Soc Promot Health 2007; **127**: 265-267.
12. Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med. 2001; **58**: 747-752.
13. Knutsson A. Health disorders of shift workers. Occup Med (Lond) 2003; **53**: 103-108.
14. Leproult R, Holmback U, Van Cauter E. Circadian misalignment augments markers of insulin resistance and inflammation, independently of sleep loss. Diabetes 2014; **63**: 1860-1869.
15. Pan A, Schernhammer ES, Sun Q et al. Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. PLoS Med 2011; **8**: e1001141.
16. Caciari T, Tomei G, De Sio S et al. Evaluation of some cardiovascular risk parameters in health professionals exposed to night work. Ann Ig 2013; **25**: 23-30.
17. Scheer FA, Hilton MF, Mantzoros CS et al. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci U S A 2009; **106**: 4453-4458.
18. Donga E, van Dijk M, van Dijk JG et al. A single night of partial sleep deprivation induces insulin resistance in multiple metabolic pathways in healthy subjects. J Clin Endocrinol Metab 2010; **95**: 2963-2968.
19. Spiegel K, Knutson K, Leproult R et al. Sleep loss: a novel risk factor for insulin resistance and Type 2 diabetes. J Appl Physiol 2005; **99**: 2008-2019.

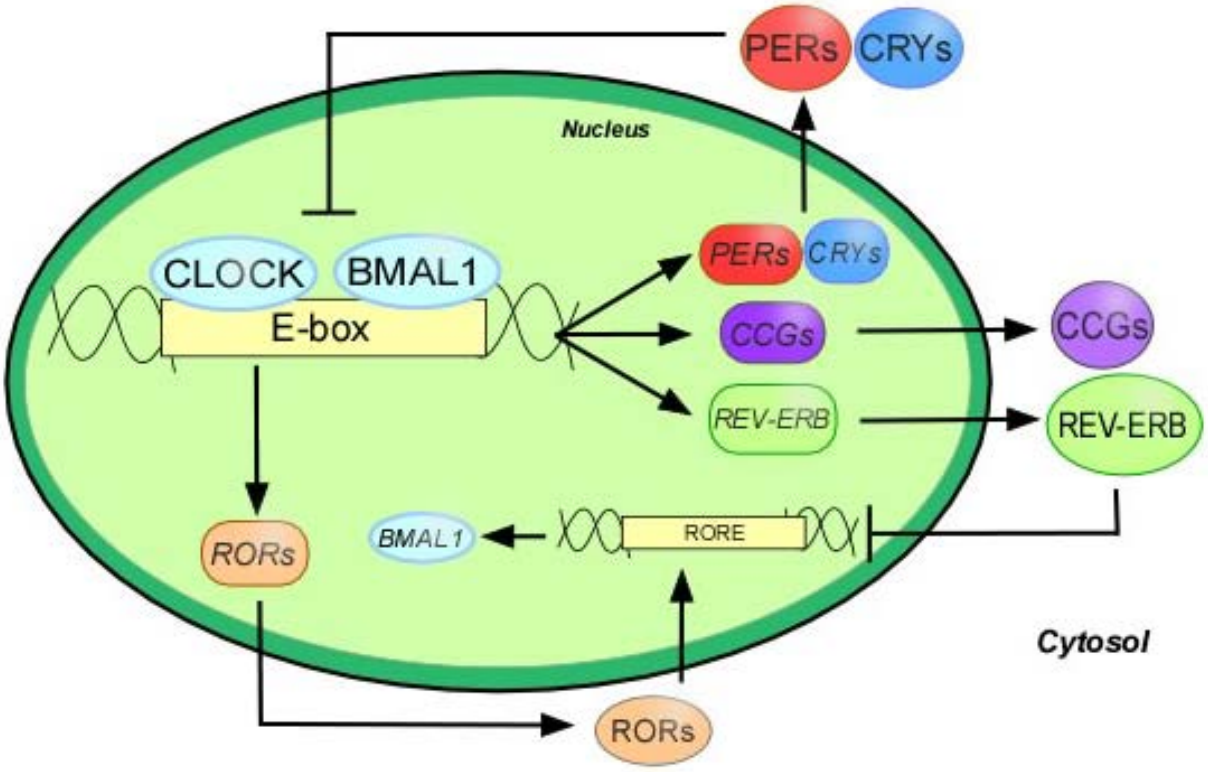
20. Taheri S, Lin L, Austin D et al. Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. PLoS Med 2004; **1**: e62.
21. Boden G, Chen X, Polansky M. Disruption of circadian insulin secretion is associated with reduced glucose uptake in first-degree relatives of patients with type 2 diabetes. Diabetes 1999; **48**: 2182-2188.
22. Turek FW, Joshu C, Kohsaka A et al. Obesity and metabolic syndrome in circadian Clock mutant mice. Science 2005; **308**: 1043-1045.
23. Rudic RD, McNamara P, Curtis AM et al. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. PLoS Biol 2004; **2**: e377.
24. Shimba S, Ishii N, Ohta Y et al. Brain and muscle Arnt-like protein-1 (BMAL1), a component of the molecular clock, regulates adipogenesis. Proc Natl Acad Sci U S A 2005; **102**: 12071-12076.
25. Marcheva B, Ramsey KM, Buhr ED et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature 2010; **466**: 627-631.
26. Grimaldi B, Bellet MM, Katada S et al. PER2 controls lipid metabolism by direct regulation of PPARgamma. Cell Metab 2010; **12**: 509-520.
27. Barclay JL, Shostak A, Leliavski A et al. High-fat diet-induced hyperinsulinemia and tissue-specific insulin resistance in Cry-deficient mice. Am J Physiol Endocrinol Metab 2013; **304**: E1053-1063.
28. Zhang EE, Liu Y, Dentin R et al. Cryptochrome mediates circadian regulation of cAMP signaling and hepatic gluconeogenesis. Nat Med 2010; **16**: 1152-1156.
29. Lau P, Fitzsimmons RL, Raichur S et al. The orphan nuclear receptor, RORalpha, regulates gene expression that controls lipid metabolism: staggerer (SG/SG) mice are resistant to diet-induced obesity. J Biol Chem 2008; **283**: 18411-18421.
30. Le Martelot G, Claudel T, Gatfield D et al. REV-ERBalpha participates in circadian SREBP signaling and bile acid homeostasis. PLoS Biol 2009; **7**: e1000181.
31. Preitner N, Damiola F, Lopez-Molina L et al. The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell 2002; **110**: 251-260.
32. Cho H, Zhao X, Hatori M et al. Regulation of circadian behaviour and metabolism by REV-ERB-alpha and REV-ERB-beta. Nature 2012; **485**: 123-127.
33. Raghuram S, Stayrook KR, Huang P et al. Identification of heme as the ligand for the orphan nuclear receptors REV-ERBalpha and REV-ERBbeta. Nat Struct Mol Biol 2007; **14**: 1207-1213.
34. Yin L, Wu N, Curtin JC et al. Rev-erbalpha, a heme sensor that coordinates metabolic and circadian pathways. Science 2007; **318**: 1786-1789.
35. Tsiftsoglou AS, Tsamadou AI, Papadopoulou LC. Heme as key regulator of major mammalian cellular functions: molecular, cellular, and pharmacological aspects. Pharmacol Ther 2006; **111**: 327-345.
36. Everett LJ, Lazar MA. Nuclear receptor Rev-erbalpha: up, down, and all around. Trends Endocrinol Metab 2014; **25**: 586-592.
37. Guillaumond F, Dardente H, Giguere V et al. Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. J Biol Rhythms 2005; **20**: 391-403.
38. Yang X, Downes M, Yu RT et al. Nuclear receptor expression links the circadian clock to metabolism. Cell 2006; **126**: 801-810.
39. Duez H, van der Veen JN, Duhem C et al. Regulation of bile acid synthesis by the nuclear receptor Rev-erbalpha. Gastroenterology 2008; **135**: 689-698.

40. Raspe E, Duez H, Mansen A et al. Identification of Rev-erbalpha as a physiological repressor of apoC-III gene transcription. J Lipid Res 2002; **43**: 2172-2179.
41. Feng D, Liu T, Sun Z et al. A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. Science 2011; **331**: 1315-1319.
42. Gerhart-Hines Z, Feng D, Emmett MJ et al. The nuclear receptor Rev-erbalpha controls circadian thermogenic plasticity. Nature 2013; **503**: 410-413.
43. Fontaine C, Dubois G, Duguay Y et al. The orphan nuclear receptor Rev-Erbalpha is a peroxisome proliferator-activated receptor (PPAR) gamma target gene and promotes PPARgamma-induced adipocyte differentiation. J Biol Chem 2003; **278**: 37672-37680.
44. Garaulet M, Smith CE, Gomez-Abellan P et al. REV-ERB-ALPHA circadian gene variant associates with obesity in two independent populations: Mediterranean and North American. Mol Nutr Food Res 2014; **58**: 821-829.
45. Goumidi L, Grechez A, Dumont J et al. Impact of REV-ERB alpha gene polymorphisms on obesity phenotypes in adult and adolescent samples. Int J Obes (Lond) 2013; **37**: 666-672.
46. Ruano EG, Canivell S, Vieira E. REV-ERB ALPHA polymorphism is associated with obesity in the Spanish obese male population. PLoS One 2014; **9**: e104065.
47. Vieira E, G Ruano E, Figueroa AL et al. Altered clock gene expression in obese visceral adipose tissue is associated with metabolic syndrome. PLoS One 2014; **9**: e111678.
48. Downes M, Carozzi AJ, Muscat GE. Constitutive expression of the orphan receptor, Rev-erbA alpha, inhibits muscle differentiation and abrogates the expression of the myoD gene family. Mol Endocrinol 1995; **9**: 1666-1678.
49. Woldt E, Sebti Y, Solt LA et al. Rev-erb-alpha modulates skeletal muscle oxidative capacity by regulating mitochondrial biogenesis and autophagy. Nat Med 2013; **19**: 1039-1046.
50. Vieira E, Nilsson EC, Nerstedt A et al. Relationship between AMPK and the transcriptional balance of clock-related genes in skeletal muscle. Am J Physiol Endocrinol Metab 2008; **295**: E1032-1037.
51. Garcia-Roves PM, Osler ME, Holmstrom MH et al. Gain-of-function R225Q mutation in AMP-activated protein kinase gamma3 subunit increases mitochondrial biogenesis in glycolytic skeletal muscle. J Biol Chem 2008; **283**: 35724-35734.
52. Bugge A, Feng D, Everett LJ et al. Rev-erbalpha and Rev-erbbeta coordinately protect the circadian clock and normal metabolic function. Genes Dev 2012; **26**: 657-667.
53. Jager J, O'Brien WT, Manlove J et al. Behavioral changes and dopaminergic dysregulation in mice lacking the nuclear receptor Rev-erbalpha. Mol Endocrinol 2014; **28**: 490-498.
54. Chung S, Lee EJ, Yun S et al. Impact of circadian nuclear receptor REV-ERBalpha on midbrain dopamine production and mood regulation. Cell 2014; **157**: 858-868.
55. Grant D, Yin L, Collins JL et al. GSK4112, a small molecule chemical probe for the cell biology of the nuclear heme receptor Rev-erbalpha. ACS Chem Biol 2010; **5**: 925-932.
56. Meng QJ, McMaster A, Beesley S et al. Ligand modulation of REV-ERBalpha function resets the peripheral circadian clock in a phasic manner. J Cell Sci 2008; **121**: 3629-3635.

57. Shin Y, Noel R, Banerjee S et al. Small molecule tertiary amines as agonists of the nuclear hormone receptor Rev-erbalpha. Bioorg Med Chem Lett 2012; **22**: 4413-4417.
58. Solt LA, Wang Y, Banerjee S et al. Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature 2012; **485**: 62-68.
59. Trump RP, Bresciani S, Cooper AW et al. Optimized chemical probes for REV-ERBalpha. J Med Chem 2013; **56**: 4729-4737.
60. Muhlbauer E, Wolgast S, Finckh U et al. Indication of circadian oscillations in the rat pancreas. FEBS Lett 2004; **564**: 91-96.
61. Muhlbauer E, Gross E, Labucay K et al. Loss of melatonin signalling and its impact on circadian rhythms in mouse organs regulating blood glucose. Eur J Pharmacol 2009; **606**: 61-71.
62. Pulimeno P, Mannic T, Sage D et al. Autonomous and self-sustained circadian oscillators displayed in human islet cells. Diabetologia 2013; **56**: 497-507.
63. Vieira E, Marroqui L, Batista TM et al. The clock gene Rev-erbalpha regulates pancreatic beta-cell function: modulation by leptin and high-fat diet. Endocrinology 2012; **153**: 592-601.
64. Nishiyama K, Hirai K. The melatonin agonist ramelteon induces duration-dependent clock gene expression through cAMP signaling in pancreatic INS-1 beta-cells. PLoS One 2014; **9**: e102073.
65. Kohsaka A, Laposky AD, Ramsey KM et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab 2007; **6**: 414-421.
66. Tang XD, Xu R, Reynolds MF et al. Haem can bind to and inhibit mammalian calcium-dependent Slo1 BK channels. Nature 2003; **425**: 531-535.
67. Guenthner CJ, Bickar D, Harrington ME. Heme reversibly damps PERIOD2 rhythms in mouse suprachiasmatic nucleus explants. Neuroscience 2009; **164**: 832-841.
68. Ruiter M, La Fleur SE, van Heijningen C et al. The daily rhythm in plasma glucagon concentrations in the rat is modulated by the biological clock and by feeding behavior. Diabetes 2003; **52**: 1709-1715.
69. Vieira E, Marroqui L, Figueroa AL et al. Involvement of the clock gene Rev-erb alpha in the regulation of glucagon secretion in pancreatic alpha-cells. PLoS One 2013; **8**: e69939.
70. Hirota T, Okano T, Kokame K et al. Glucose down-regulates Per1 and Per2 mRNA levels and induces circadian gene expression in cultured Rat-1 fibroblasts. J Biol Chem 2002; **277**: 44244-44251.
71. Lamia KA, Sachdeva UM, DiTacchio L et al. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science 2009; **326**: 437-440.
72. Leclerc I, Sun G, Morris C et al. AMP-activated protein kinase regulates glucagon secretion from mouse pancreatic alpha cells. Diabetologia 2011; **54**: 125-134.
73. Asher G, Gatfield D, Stratmann M et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell 2008; **134**: 317-328.
74. Nakahata Y, Kaluzova M, Grimaldi B et al. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 2008; **134**: 329-340.
75. Nakahata Y, Sahar S, Astarita G et al. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science 2009; **324**: 654-657
76. Ramsey KM, Yoshino J, Brace CS et al. Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science 2009; **324**: 651-654.
77. Canto C, Gerhart-Hines Z, Feige JN et al. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 2009; **458**: 1056-1060.

78. Fulco M, Cen Y, Zhao P et al. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. Dev Cell 2008; **14**: 661-673.
79. Um JH, Pendergast JS, Springer DA et al. AMPK regulates circadian rhythms in a tissue- and isoform-specific manner. PLoS One 2011; **6**: e18450.
80. Kojetin D, Wang Y, Kamenecka TM et al. Identification of SR8278, a synthetic antagonist of the nuclear heme receptor REV-ERB. ACS Chem Biol 2010; **6**: 131-134.
81. Ashcroft FM, Rorsman P. K(ATP) channels and islet hormone secretion: new insights and controversies. Nat Rev Endocrinol 2013; **9**: 660-669.

Figure 1.


PERs
CRYs
Nucleus
CLOCK
BMAL1
E-box
PERs
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CCGs
REV-ERB
REV-ERB
RORs
BMAL1
RORE
Cytosol
RORs

Figure 2.

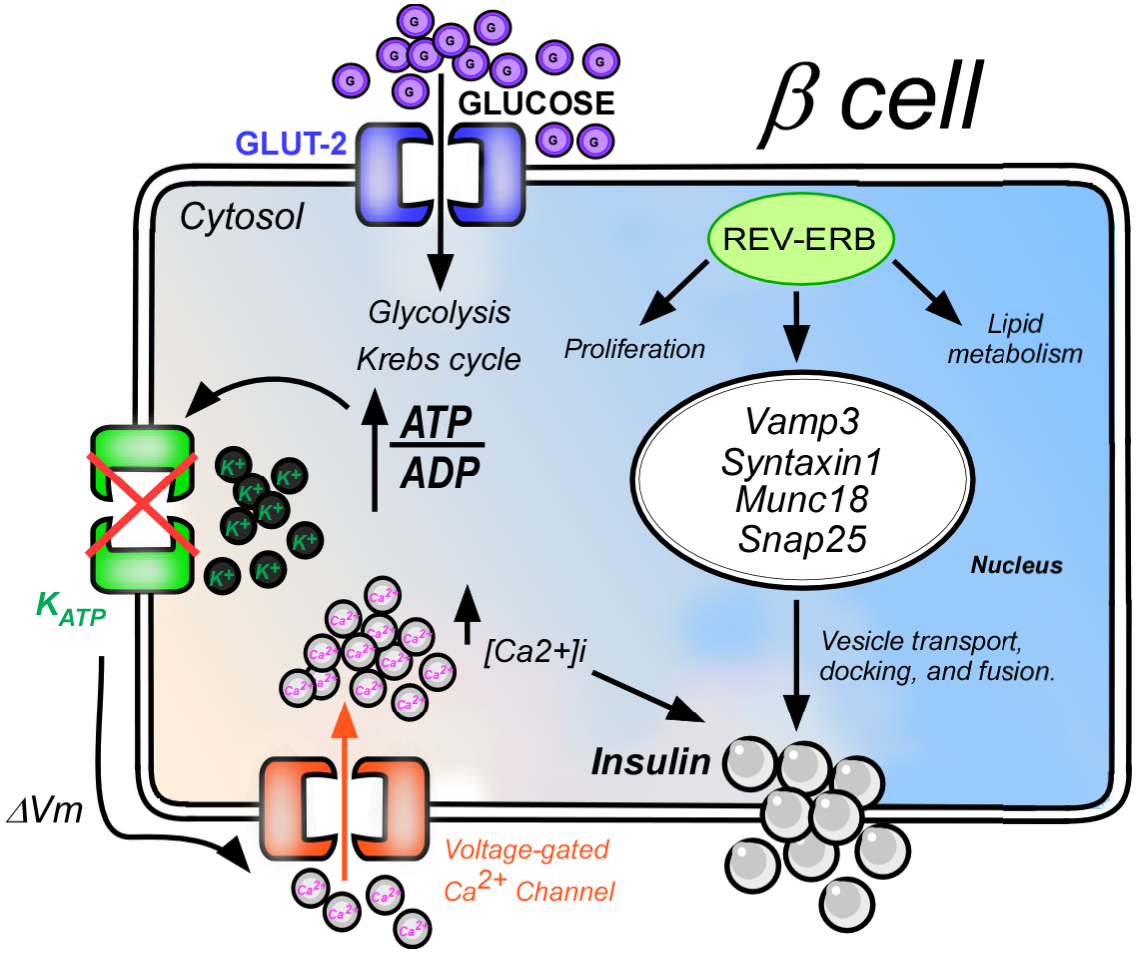
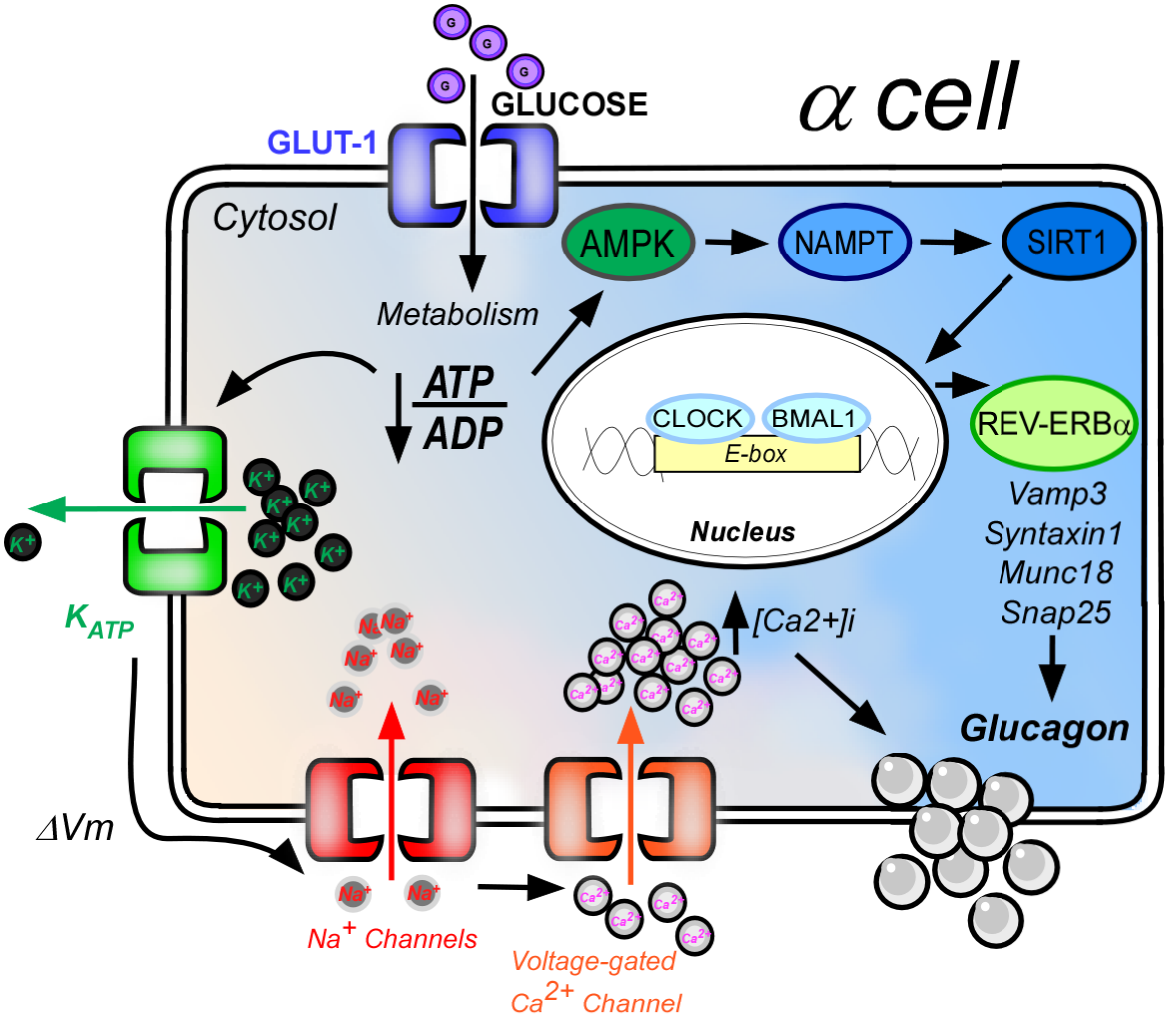

β cell
GLUCOSE
GLUT-2
Cytosol
Glycolysis
Krebs cycle
ATP
ADP
REV-ERB
Proliferation
Lipid metabolism
Vamp3
Syntaxin1
Munc18
Snap25
Nucleus
K_{ATP}
[Ca2+]i
Vesicle transport, docking, and fusion.
Insulin
ΔVm
Voltage-gated
Ca^{2+} Channel

Figure 3.


α cell
GLUCOSE
GLUT-1
Cytosol
AMPK
NAMPT
SIRT1
Metabolism
ATP
ADP
CLOCK
BMAL1
E-box
Nucleus
REV-ERBα
Vamp3
Syntaxin1
Munc18
Snap25
Glucagon
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ΔVm
Na+ Channels
Voltage-gated
Ca2+ Channel