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REVIEW

The role of mammalian target of rapamycin (mTOR) in the regulation of pancreatic β-cell mass: implications in the development of type-2 diabetes

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Abstract Type-2 diabetes mellitus (T2DM) is a disorder that is characterized by high blood glucose concentration in the context of insulin resistance and/or relative insulin deficiency. It causes metabolic changes that lead to the damage and functional impairment of organs and tissues resulting in increased morbidity and mortality. It is this form of diabetes whose prevalence is increasing at an alarming rate due to the 'obesity epidemic', as obesity is a key risk factor in the development of insulin resistance. However, the majority of individuals who have insulin resistance do not develop diabetes due to a compensatory increase in insulin secretion in response to an increase in insulin demand. This adaptive response is sustained by an increase in both β-cell function and mass. Importantly, there is increasing evidence that the Serine/Threonine kinase mammalian target of rapamycin (mTOR) plays a key role in the regulation of β-cell mass and therefore likely plays a critical role in β-cell adaptation. Therefore, the primary focus of this review is to summarize our current understanding of the role of mTOR in stimulating pancreatic β-cell mass and thus, in the prevention of type-2 diabetes.

Keywords Type 2 diabetes · Obesity · β-cell mass · mTOR · TSC · Rheb · S6K · PKB · AMPK · RICTOR

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An introduction to mTOR

Mammalian target of rapamycin (mTOR, also known as FRAP, RAFT or RAPT) is a highly conserved Ser/Thr protein kinase, which integrates nutrient availability with hormonal/growth factor signalling to regulate cell growth, proliferation, viability and function (for reviews, see [69, 142, 187]). It assembles into two biochemically and functionally distinct multi-component complexes termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Fig. 1), which phosphorylate different substrates, are differentially regulated and vary in their degree of sensitivity to the immunosuppressive drug rapamycin. mTORC1 is rapidly inhibited by rapamycin, which acts by binding in a complex with FKBP12 to the FKBP12-rapamycin binding domain of mTOR [23]. In contrast, mTORC2 is resistant to acute rapamycin treatment, although prolonged rapamycin treatment can inhibit mTORC2 in some cell types, probably due to the binding of rapamycin-FKBP12 to unbound mTOR and the inability of this complex to be incorporated into nascent mTORC2 [137].

Mammalian target of rapamycin complex 1 (mTORC1)

mTORC1 is acutely activated by nutrients, growth factors and hormones (Fig. 2) and importantly, in the context of this review, has been shown to control both cell growth and proliferation of mammalian cells. This is mediated, at least in part, through the phosphorylation of its downstream targets eIF4E binding proteins (4EBPs) and the ribosomal protein S6 kinases-1 and -2 (S6K1/2), both of which impinge on the regulation of protein synthesis (Fig. 1).

S6K and 4EBPs are recruited to mTOR via the mTORC1 specific component RAPTOR [64, 86]. mTORC1

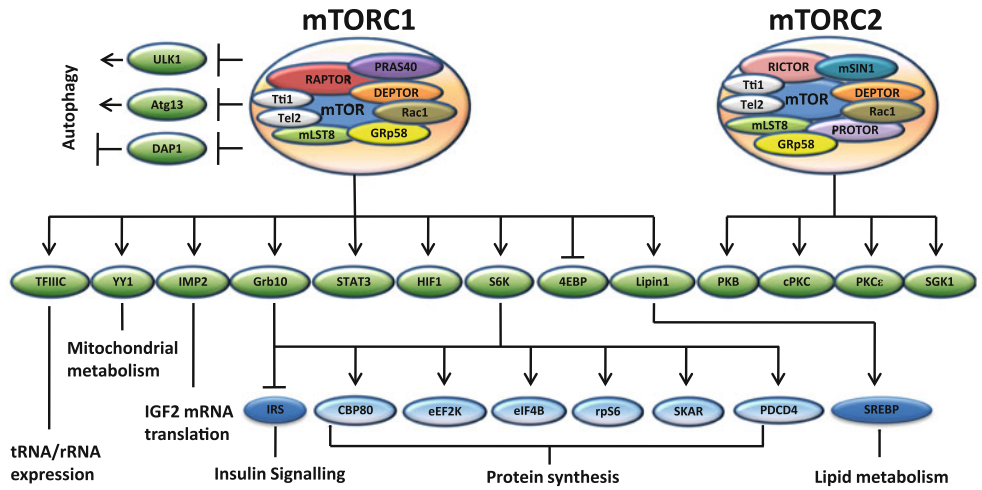

Fig. 1 Downstream substrates regulated by the mTOR complexes. Both mTOR complexes are comprised of mTOR, G_{β}-like protein mLST8 (for mammalian ortholog of lethal with sec 13, also known as GβL) [87], DEPTOR (for DEP domain-containing mTOR interacting protein) [118], GRp58 (for 58 KDa glucose-regulated protein, also referred to as ERp57) [124], Tti1 (Tel 2 interacting protein 1), Tel2 (telomere maintenance 2) [83] and Rac1 (Ras-related C3 botulinum toxin substrate 1) [132]. mTORC1 specific components include RAPTOR (for regulatory-associated protein of mTOR) [86] and PRAS40 (for Pro-rich Akt substrate of 40 kDa) [44, 112, 135, 166, 167]. mTORC2 specific components consist of RICTOR (for rapamycin-insensitive companion of TOR) [136], PROTOR (for protein observed with RICTOR) [114] and mSIN1 (mammalian stress activated protein kinase interacting protein 1) [45, 79, 179]. mTORC1 substrates include 4EBP (eukaryotic initiation factor 4E binding proteins) [9, 19] and S6K [ribosomal protein S6 (rpS6) kinase] [23, 28]. S6K phosphorylates rpS6 [6, 94], SKAR (S6K1 Aly/REF-like target) [126], eIF4B [125], PDCD4 (programmed cell death protein 4) [34], eEF2K [eukaryotic elongation factor 2 (eEF2) kinase] [17, 169], CBP80 (for 80 KDa nuclear cap-binding protein, also known as nuclear cap binding protein subunit 1 or NCBP1) [172] and insulin receptor substrates (IRS) [165]. The effect of mTORC1 on lipogenesis is mediated through the transcription factor SREBP (sterol regulatory element-binding protein) [38, 120], which has recently been shown to be controlled by mTORC1-mediated lipin1 nuclear import [119]. mTORC1 also upregulates the expression of genes implicated in mitochondrial metabolism through the YY1 (Ying Yang 1)-PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1-α) transcription factor complex [30]. mTORC1 also increases the expression of Grb10 (growth factor receptor-bound protein 10), which inhibits insulin signalling via the inhibition of IRS [70, 182]. Other mTORC1 targets include TFIIIC (transcription factor 3C) [85], IMP2 (insulin-like growth factor 2 (IGF2) mRNA binding protein) [31], STAT3 (signal transducers and activator 3) [181] and HIF1 (hypoxia-induced factor 1) [74]. Another major function of mTORC1 is the regulation of autophagy. mTOR directly phosphorylates ULK1 and Atg13 and inhibits autophagosome formation [48, 68, 82], although it also inhibits DAP1, a negative regulator of autophagy which is activated under nutrient deprivation, via direct phosphorylation on Ser3 and Ser51 [92]. mTORC2 is responsible for the phosphorylation and activation of several AGC (for protein kinase A, G and C) kinases, including PKB [79, 138], SGK1 (serum/glucocorticoid-induced kinase 1) [50], conventional PKCs (cPKC) and PKCε (one of the novel PKCs or nPKCs) [42, 75, 136]

phosphorylates S6K1 and S6K2 within their hydrophobic motif (HM), on Thr389 and Thr388, respectively, which is critical for their activation [115, 144]. Once activated, S6K1/2 can phosphorylate a number of proteins including: ribosomal protein S6 (rpS6) [6, 94], S6K1 Aly/REF-like target (SKAR) [126], eukaryotic initiation factor-4B (eIF4B) [125], programmed cell death protein 4 (PDCD4) [34], eukaryotic elongation factor 2 (eEF2) kinase (eEF2K) [17, 169], and 80KDa nuclear cap-binding protein (CBP80) [172] (Fig. 1). S6K1 can also phosphorylate IRS-1 on Ser307 [66] and Ser1101 [160], resulting in decreased insulin signalling. This acts as an important negative feedback mechanism in the regulation of mTORC1. Importantly, knock-out studies in *Drosophila* and mice have revealed that S6K1 plays an essential role in the regulation of cell size but not cell proliferation [105, 116, 144].

4EBPs, of which there are three isoforms (4EBP1, 4EBP2 and 4EBP3), act as repressors of cap-dependent translation by binding to and sequestering the mRNA cap binding protein eIF4E (for review, see [150, 171]). Upon phosphorylation of 4EBP by mTORC1, 4EBP is released from eIF4E [53], promoting the expression of highly cap-dependent proteins including the cell cycle proteins $p27^{kip1}$, $p21^{cip1/waf1}$, cyclin D1, D2, D3, E and A, leading to an increase in G_1 to S cell cycle progression (for review, see [170]). Knock-out studies in *Drosophila* indicate that 4EBP regulates cell growth and proliferation [104]. However, results from mouse embryonic fibroblasts (MEFs) lacking 4EBP indicate that 4E-BPs only influences proliferation in mammalian cells [35].

mTORC1 is activated upon association with the small G-protein Rheb (Ras homolog enriched in brain), when the latter is in its GTP bound state [49, 139, 185] (Fig. 2). Rheb is in turn regulated through the activity of its GTPase-activating protein (GAP), the tuberous sclerosis complex 1 and 2 (TSC1/2) [49, 139, 185]. Importantly,

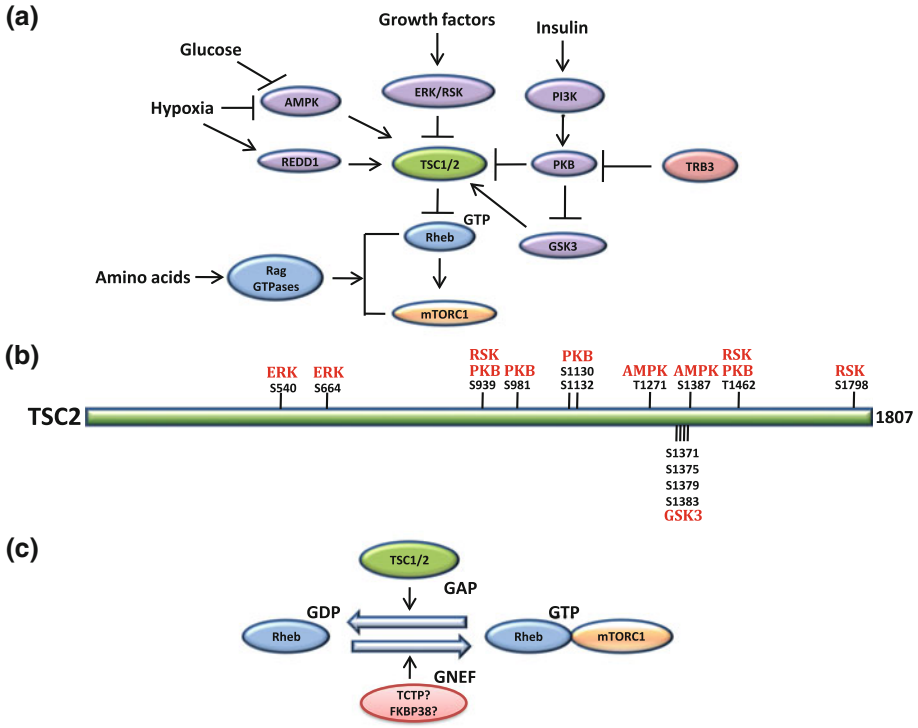

Fig. 2 Schematic representation of signalling pathways that regulates mTORC1. **a** mTOR senses a wide range of upstream signals such as: amino acids availability, which modulates the activity of mTORC1 through Rag GTPases [88, 134]; glucose or oxygen levels through AMPK [AMP (5′-adenosine monophosphate) activated protein kinase] [78] and REDD1 (regulated in development and DNA damage responses 1) [18, 81, 149, 173]; growth factors, which activate MAPK (mitogen-activated protein kinase) and stimulate mTORC1 via ERK (extracellular signal-regulated kinases) and RSK (p90 ribosomal protein S6 kinase) [21, 22, 43, 101, 127, 128]; insulin via activation of PI3K (phosphoinositide 3-kinase), PKB (protein kinase B, also referred to as Akt) [52, 141], and inactivation of GSK3 (glycogen synthase kinase 3) [77] [n.b. the activity of PKB can be suppressed by TRB3 (mammalian homolog of *Drosophila tribbles* 3) [36]]. These pathways impinge on TSC1/2 (tuberous sclerosis complex 1/2), a GTPase activating protein (GAP) of the small G protein Rheb (Ras homolog enriched in brain), and GTP bound Rheb in turn activates mTORC1. **b** Sites of phosphorylation on TSC2 and their respective kinases, adapted from [73]. **c** The regulation of Rheb. TSC1/2 acts as a GAP for Rheb [139, 185], whereas TCTP (translationally controlled tumor protein) [71] and FKBP38 (FK506 binding protein 38) [4], have been proposed to act as a GNEF for Rheb

growth factors, hormones and nutrients activate signalling pathways that lead to changes in the phosphorylation status of TSC2 (Fig. 2) and the inactivation of TSC1/2. This promotes the formation of Rheb-GTP and hence the activation of mTORC1. Although FKBP38 (FK506 binding protein 38) and TCTP (translationally controlled tumour protein) have been proposed to act as guanine nucleotide exchange factors (GNEF) for Rheb [4, 71], other studies have questioned this [164, 168].

Insulin, for example, can activate mTORC1 through the protein kinase B (PKB) (also known as Akt)-dependent phosphorylation of TSC2 on Ser939, Ser981, Ser1130, Ser1132 and Thr1462, which leads to the inactivation of TSC1/2 [32, 102, 121] (Fig. 2). PKB can also promote mTORC1 activation through the phosphorylation of mTORC1 specific component PRAS40 (for Pro-rich Akt substrate of 40 kDa) at Thr246 [93, 112, 135, 157, 166, 167], which results in its dissociation from mTORC1, thus preventing its inhibitory activity towards mTORC1 [112, 135, 157, 166, 167].

Growth factors can also activate mTORC1 via the inactivation of TSC1/2, but in this context, it is often via the ERK1/2 (extracellular signal-regulated kinase 1 and 2) and/or RSK (p90 ribosomal protein S6 kinase)-dependent phosphorylation of TSC2 at Ser540 and Ser664 [101], or Ser939, Thr1462 and Ser1798 [128], respectively (Fig. 2). In addition, both ERK and RSK are able to phosphorylate RAPTOR on sites that have been reported to promote the activation of mTORC1 [21, 22].

However, growth factor or hormonal activation of mTORC1 is dependent on the nutrient status of the cell. The presence of amino acids is an obligate requirement for the activation of mTORC1, regardless of stimuli. This amino acid "sensing" by mTORC1 occurs independently of TSC1/2 [148], but is dependent on a group of Ras-related small GTPases comprised of four proteins (Rag A, B, C and D) that form heterodimers [88, 134], which bind to a newly identified trimeric protein complex termed as "ragulator" [133]. These Rag GTPases are essential for the recruitment of mTORC1 to endosomal and lysosomal

compartments, where it encounters Rheb-GTP and is activated by the latter. Importantly, these Rags are activated by amino acids [88, 134]. Other proteins, including hVps34 [homologue of vacuolar protein sorting 34, or class III PI3K (phosphoinositide 3-kinase)] [110], Ca^{2+}/Calmodulin [61], MAP4K3 (mitogen-activated protein kinase kinase kinase kinase 3) [178] and IPMK (inositol polyphosphate multikinase) [89] have also been reported to impact on amino acids signalling to mTORC1. However, the exact mechanism by which amino acids are sensed and regulate mTORC1 is poorly understood and is the subject of intensive research.

mTORC1 also responds to changes in the energy status of the cell [33]. For example, glucose can stimulate mTORC1 through the inactivation of AMP (5′-adenosine monophosphate) activated protein kinase (AMPK) [78, 90], which responds to changes in the cellular AMP:ATP ratio (for a historical review, see [65]). AMPK suppresses mTORC1 activity via the phosphorylation of TSC2 on Thr1271 and Ser1387, which stimulates TSC1/2 GAP activity [78]. AMPK can also phosphorylate RAPTOR on sites that promote the inhibition of mTORC1 [62]. In addition, a decrease in the energy status of the cell can augment the expression of REDD1 (regulated in development and DNA damage responses 1) independently of AMPK and this has also been reported to activate TSC1/2 and inhibit mTORC1 [18, 149].

Mammalian target of rapamycin complex 2 (mTORC2)

mTORC2 has originally been identified as a positive regulator of actin cytoskeletal organization, polarization and cell migration [60, 80, 100, 136]. The mechanism by which mTORC2 mediates these effects is not fully understood, but it is known that mTORC2 is able to phosphorylate the turn motif (TM) and the hydrophobic motif (HM) of several AGC (for protein kinase A, G and C) kinases, including PKB [79, 138], SGK1 (serum/glucocorticoid-induced kinase 1) [50], conventional PKCs (cPKC) and PKCε [42, 75, 136], resulting in their stabilisation and activation (Fig. 1). For example, in the case of PKB, mTORC2 associates with polysomes and phosphorylates nascent PKB on its TM at Thr450, which promotes the correct folding of PKB and hence its stability [111]. Then, upon an appropriate stimuli, the mature PKB is translocated to the plasma membrane where it is activated via the phosphorylation on Thr308 (Activation-loop) by PDK1 [2] and Ser473 (HM) by mTORC2 [138].

Initially it was thought that mTORC2 was not activated by hormones and growth factors. However, it has recently been demonstrated that mTORC2 activity towards PKB on S473 is acutely stimulated by insulin or serum via a PI3 kinase-dependent mechanism [47], yet the mechanism by which PI3 kinase activates mTORC2 is far from understood, and may just be through the PIP3-dependent translocation of PKB to mTORC2 at the plasma membrane. However, as TSC1/2 deletion inactivates mTORC2 [72], the effects of PI3 kinase could potentially be through TSC1/2. Interestingly, siRNA-mediated knock-down of proteins involved in ribosome maturation and formation, such as mNIP7 (mammalian nuclear import 7 homolog), Rpl7 (ribosomal protein L7) or Rps16 (ribosomal protein S16), also abrogates mTORC2 activity, indicating that ribosomes may bind to and promote mTORC2 activity [186].

mTOR and its role in the regulation of pancreatic β-cell mass

During the development of type-2 diabetes mellitus (T2DM), age-related body weight gain and the loss of insulin sensitivity (insulin resistance) leads to an increase in insulin demand, which is met by an increase in secretory output maintained though increased β-cell mass and function through a process referred to as β-cell compensation (for reviews see [99, 122]). It is the inability of pancreatic β-cell to adequately compensate for this increase in insulin demand which results in the development of impaired glucose tolerance and ultimately T2DM.

Augmentations in β-cell mass can be mediated through: (1) increased rate of neogenesis (i.e., the generation of new β-cells from ductal stem cells); (2) increased rate of differentiation of pancreatic precursor cells; (3) transdifferentiation of exocrine cells; (4) increased rate of replication (hyperplasia); (5) expansion of cell size (hypertrophy); and (6) decreased rates of cell death (apoptosis) (for reviews, see [1, 15, 16, 29]).

Many growth factors, hormones and nutrients have also been shown to play an important role in stimulating increases in β-cell mass and many, if not all, activate mTORC1. For example, glucose, a potent in vivo stimulator of β-cell mass in rodents [12, 14, 113, 158], acutely up-regulates mTORC1 activity in isolated rat islets and rodent β-cell lines [7, 56, 95]. Moreover, it has been demonstrated that, in vitro, glucose can stimulate β-cell proliferation [7, 95] and protein synthesis [55, 176], an important hypertrophic stimuli [151], via a rapamycin sensitive pathway. The mechanism by which glucose activates mTORC1 in islets and β-cell lines has been reported to be mediated via the autocrine action of insulin [176]. This is likely via the activation of PKB (El Sayed NM, Moore CE and Herbert TP, unpublished data) and the inactivation of AMPK [54]. However, it has recently been reported, in a β-cell line derived from insulin receptor

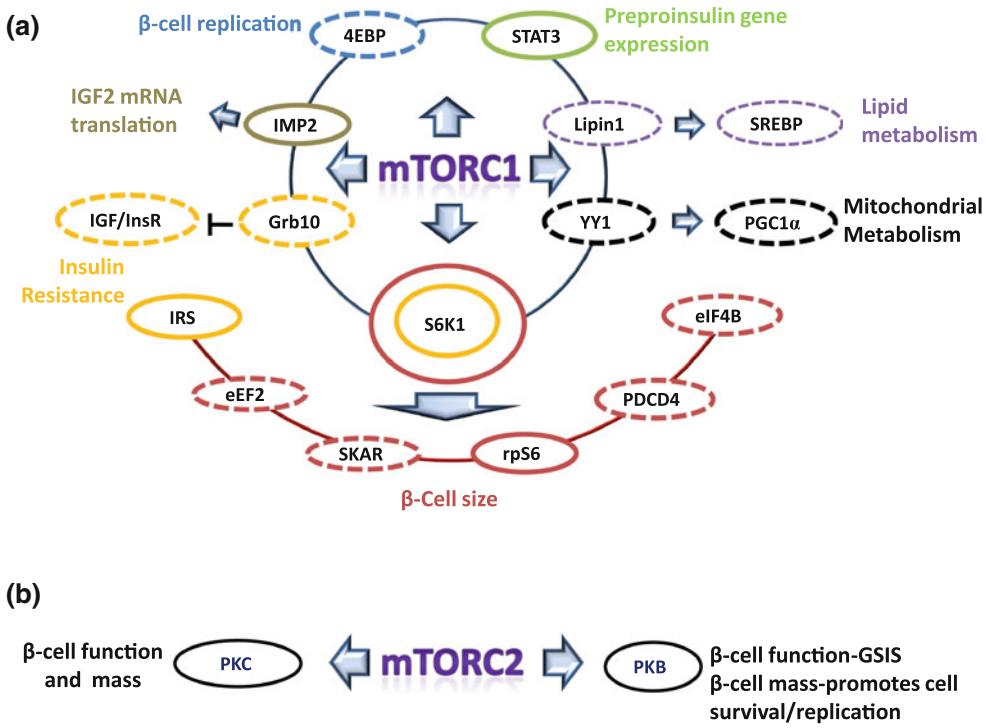

Fig. 3 Schematic representation of the involvement of protein targets downstream of mTORC1 and 2 in the control of pancreatic *β*-cell mass and function. Proteins circled in continuous line: function proved in *β*-cells; Proteins circled in dashed line: function reported in non *β*-cells, and yet to be studied in *β*-cells. **a** Effects of downstream targets of mTORC1 on *β*-cell function and mass. Using transgenic mouse models it has been shown that S6K1 [ribosomal protein S6 (rpS6) kinase 1] [116] and rpS6 [131] are crucial for the maintenance of *β*-cell size, although it remains a possibility that other S6K downstream targets, such as SKAR (S6K1 Aly/REF-like target) [126], eEF2 (eukaryotic elongation factor 2) [17, 169], PDCD4 (programmed cell death protein 4) [34] and eIF4B (eukaryotic initiation factor 4B) [125], also contribute to *β*-cell growth, presumably through their ability of regulating protein synthesis. Insulin resistance can be induced by the inhibition of IRS (insulin receptor substrate) resulted from the over-activation of S6K1 in *β*-cells [39]. Recently, it has also been reported that newly discovered mTORC1 substrate Grb10 (growth factor receptor-bound protein 10) negatively regulates insulin and IGF (insulin-like growth factor) signalling through the binding and inhibition of InsR (insulin receptor) and IGFR (IGF receptor) [70, 182], which may also contribute to insulin resistance. mTORC1-stimulated fat accumulation is driven by the activation of SREBP (sterol regulatory element-binding protein) [98, 145, 146] through the nuclear import of lipin1 [119]. It has been demonstrated in *β*-cells that mTORC1 directly phosphorylates IMP2 [insulin-like growth factor 2 (IGF2) mRNA binding protein] to promote IGF2 mRNA translation [31], and leptin-induced activation of STAT3 (signal transducers and activator 3) suppresses preproinsulin gene expression [96]. Furthermore, it can also be speculated that 4EBP [eukaryotic initiation factor 4E (eIF4E) binding proteins] controls *β*-cell proliferation, while YY1 (Ying Yang 1) and PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1-α) [30] may play roles in *β*-cell mitochondrial metabolism. Circle colours represent: *dark red* *β*-cell size; *yellow* insulin resistance; *black* mitochondrial metabolism; *tan* IGF2 mRNA translation; *blue* *β*-cell replication; *light green* preproinsulin gene expression; *purple* lipid metabolism. **b** Downstream of mTORC2. Gu et al. [59] have demonstrated that mTORC2 is important in *β*-cell replication. In addition, mTORC2 is essential for the maintenance of *β*-cell viability and GSIS (Barlow AD, Xie J and Herbert TP, unpulished data). mTORC2 also controls the folding and protein stability of PKCα (protein kinase C α), PKC*β* and PKCε [42, 75], which are known to play important roles in *β*-cell function and survival [13, 140]

knock-out mice, that glucose-stimulated mTORC1 activation is mediated via the MAPK pathway independently of PKB [7]. In the presence of glucose, the incretin hormone glucagon-like peptide-1 (GLP-1) is also a potent stimulator of *β*-cell mass in vivo [20, 153, 159, 177] and has been shown to potentiate glucose-stimulated mTORC1 activation in isolated islets and clonal cell lines via a PI3K dependent mechanism [95, 106]. Moreover, in vitro, GLP-1R agonists in the presence of glucose are able to enhance *β*-cell replication via an mTORC1-dependent pathway (Xie J and Herbert TP, unpublished data). Therefore, GLP1-stimulated *β*-cell replication in vivo is also likely to be mediated, at least in part, by the activation of mTORC1 [95].

Conversely, the inhibition of mTOR by rapamycin causes loss of *β* cell function and viability in pancreatic *β*-cell lines and murine and human islets [8, 41, 184], indicating that the maintenance of mTOR activity is critical for the integrity of the *β*-cell. It was assumed that these toxic effects of rapamycin on the *β*-cell were mediated by

Table 1 Phenotypes of selected transgenic mouse models

Transgenic mice		β-cell mass				Metabolic parameters				Pancreas/Islet insulin content	Glucose in ITT[d]	Susceptibility to exp. diabetes	References
Type	Specificity	Size	Proliferation	Apoptosis	Total mass	Blood glucose		Blood Insulin	In vivo GSIS				
						Fasted/Fed	OGTT/IGTT	Fasted/Fed	OGTT/IGTT				
S6K1$^{-/-}$	Global	↓	N/D	↔	↓ (endocrine mass)	Fasted: ↔	IGTT: ↑	Fasted: ↓	IGTT: ↓	↓	↓	↓ (HFD)	[116], [165]
S6KCA^{RIP}	β-cells	↑	↑	↑	↔	Fasted: ↓	IGTT: ↓	Fasted: ↑	IGTT: ↑	N/D	↔	N/D	[39]
rpS6$^{P-/-}$	Global	↓	↑	N/D	↔	Fasted/Fed: ↔	IGTT: ↑	Fasted: ↓	N/D	↓	↓	N/D	[131]
4EBP1$^{-/-}$	Global	N/D	N/D	N/D	N/D	Fasted/Fed: ↓	N/D	Fed: ↔	N/D	N/D	N/D	N/D	[162]
4EBP1$^{-/-}$ 4EBP2$^{-/-}$	Global	N/D	N/D	N/D	N/D	Fasted: ↔	IGTT: ↔	Fasted: ↑	N/D	N/D	↑	↑ (HFD)	[97]
Rheb (R3/R20[a])	β-cells	↑ (R3)	↔	↔	↑ (R3)	Fed (R20): ↓	OGTT and IGTT (R20): ↓	Fed (R20): ↑	OGTT and IGTT (R20): ↑	N/D	N/D	↓(R3) (STZ)	[63]
βTSC2$^{-/-b}$	β-cells; 4–52 weeks	↑	↑	↔	↑ (up to 52 weeks)	Fasted (8-52 weeks)/ Fed (4-52 weeks): ↓	IGTT (4,40 and 52 weeks): ↓	Fasted/ Fed (4 to 20 weeks): ↑	IGTT (12 weeks): ↑	N/D	N/D	N/D	[123]
	β-cells; ≤35 weeks	↑ (6 weeks)	N/D	↔ (5 weeks)	↑ (6 weeks)	Fed (4-32 weeks): ↓	OGTT (8 weeks): ↓	Fed (12 weeks): ↑	OGTT/IGTT (8 weeks): ↑	N/D	N/D	N/D	[143]
	β-cells; ≥35 weeks	↑ (40 weeks)	N/D	↑ (35 weeks)	↓ (40 weeks)	Fed (36-48 weeks): ↑	N/D	Fed (40-48 weeks): ↓	N/D				
RIP-TSC1cKO	β-cells	↑	↔	↔	↑	Fed: 4 weeks:↓; 8 and 20 weeks: ↔; 12–16 weeks: ↑	IGTT (4 weeks): ↓	Fed (4-24 weeks): ↑	IGTT (4 weeks): ↑	↑	↑ (≥8 weeks)	N/D	[107]
βLKB1KO	β-cells	↑	↑	N/D	↑	Fasted/fed: ↓	IGTT: ↓	Fasted/fed: ↑	IGTT: ↑	↑	↔	↓ (HFD)	[46],[58], [155]
βAMPKDKO	β-cells	↓	↑	↔	↔	Fasted/fed: ↑	IGTT: ↑	Fasted/fed: ↓	IGTT: ↓	N/D	↓	↓ (HFD)	[154]
βAMPK.CA (male)[j]	β-cells	↓	N/D	N/D	N/D	Fasted: ↔	IGTT: ↑ (3 months) or ↔ (6 months)	Fasted: ↓ (3 months)	IGTT: ↓ (3 months)	N/D	↔	↔ (HFD)	[154]
βPi3kr1 Pi3kr2DKO	β-cells	N/D	↑	↑	↓	↔	IGTT: ↑	↔	IGTT: ↓	N/D	↔	N/D	[84]
PTEN$^{+/-}$	Global	N/D	N/D	N/D	↔	Fasted/fed: ↓	IGTT: ↓	Fasted: ↓	IGTT: ↔	↔	↓	N/D	[174]
RIP*cre*$^{+}$*Pten*$^{fl/fl}$	β-cells and hypothalamus	↔	↑	↓	↑	Fasted: ↓	IGTT: ↔	Fasted: ↔	N/D	N/D	↓	↓ (STZ)	[152]
		↑	↑ (not significant)	↔ but ↓ if STZ treated	↑	Fasted: ↓	IGTT: ↓	Fasted: ↓	IGTT: ↔	↑	↓	↓ (STZ)	[108]
βRicKO[b]	β-cells	↔	↓	↔	↓	Fed:↑ (12 and 16 weeks)	IGTT: ↑	N/D	IGTT: ↓	↓	↔	N/D	[59]
Akt1$^{-/-}$	Global	N/D	N/D	↑ (testes and thymus)	N/D	Fasted/fed: ↔	OGTT/IGTT: ↔	Fasted: ↔	N/D	N/D	↔	N/D	[25], [27]
RIP-KdAkt	β-cells	↔	N/D	↔	↔	Fasted (4 month): ↔	IGTT (6–8 weeks): ↔	Fasted (4 months): ↔	IGTT (6-8 weeks): ↔	↔	N/D	↑ (HFD)	[10]
						Fed (4 month): ↑	IGTT (6 months): ↑	Fed (4 months): ↓	IGTT (6 months): ↓				
Myr-Akt1[c]	β-cells	↑	↔	↑ but ↓ if STZ treated	↑	Fasted/fed: ↓	IGTT: ↓	Fasted: ↑	IGTT: ↑	↑	↔	↓ (STZ) in reference [163]	[3], [163]
		↑	↑ (5 weeks)	N/D	↑	Fasted/fed: ↔	IGTT: ↓	Fasted: ↑	IGTT: ↑	N/D	N/D	↓ (STZ)	[11]
Myr-Akt1; S6K1$^{-/-f}$	β-cells[f]	↔[e]	↔[e]	N/D	N/D	Fasted/fed: ↔[e]	IGTT: ↑	Fasted: ↓	N/D	N/D	↓[e]	N/D	[3]
Myr-Akt1; S6K1$^{-/-}$; S6K2$^{-/-f}$	β-cells[f]	↓[e]	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	[3]

Table 1 continued

Transgenic mice		β-cell mass				Metabolic parameters				Pancreas/Islet insulin content	Glucose in ITT[i]	Susceptibility to exp. diabetes	References
Type	Specificity	Size	Proliferation	Apoptosis	Total mass	Blood glucose		Blood Insulin	In vivo GSIS				
						Fasted/Fed	OGTT/IGTT	Fasted/Fed	OGTT/IGTT				
$Akt2^{-/-}$[d]	Global	N/D	N/D	N/D	↑	Fasted/fed: ↑	OGTT: ↑	↑	N/D	N/D	↑	N/D	[26]
		N/D	N/D	↑[h] (24 weeks)	↓[h] (24 weeks)	Fasted/fed: ↑	OGTT: ↑ (7 weeks)	↑[g]; or ↓ after 8 weeks[h]	N/D	↓[h] (24 weeks)	↑	N/D	[51]
		N/D	N/D	N/D	N/D	N/D	OGTT: ↑	N/D	N/D	N/D	↑	N/D	[37]
		N/D	N/D	N/D	N/D	Fed: ↔ (6 months)	IGTT: ↑ (6 months)	Fed: ↑ (6 months)	IGTT: ↑ (6 months)	↑	↔	N/D	[24]

↑, increased compared to wild-type; ↓, decreased compared to wild-type; ↔, similar to wild-type; exp., experimental; HFD, high fat diet; GSIS, glucose-stimulated insulin secretion; IGTT, intraperitoneal glucose tolerance test; N/D, no data; OGTT, oral glucose tolerance test; RIP, rat insulin promoter; STZ, streptozotocin

[a] Rheb over-expression in mouse: R3 and R20 are two independent founder lines used in the study of [63]

[b] Global deletion of TSC2 or RICTOR causes embryonic lethality [147]

[c] Mice expressing myristoylated (constitutively active) Akt1

[d] Phenotypes of other PKB isoform-combined knock-out mice ($Akt1^{+/-}Akt2^{-/-}$, $Akt1^{-/-}Akt2^{+/-}$, $Akt2^{-/-}Akt3^{-/-}$, $Akt1^{+/-}Akt2^{-/-}Akt3^{-/-}$, $Akt2^{-/-}Akt3^{-/-}$ and others) have also been described [24, 37, 180]. Description of other mouse models related to PKB pathway (e.g., RIP-IGF1, $PTEN^{-/-}$, βGSK-3$\beta^{-/-}$, and others) can be found in recent reviews [1, 40]

[e] All parameters (↑, ↓ or ↔) are in comparison to Myr-Akt mice

[f] Myr-Akt (beta-cell specific) and $S6K1^{-/-}$ (and $S6K2^{-/-}$) (global deletion) mice were crossed to yield the Myr-Akt;$S6K1^{-/-}$ (and $S6K2^{-/-}$) mice

[g] These $Akt2^{-/-}$ mice was consistently hyperinsulinemic

[h] Plasma insulin levels from these $Akt2^{-/-}$ mice started to drop after 8 weeks, and they became hypoinsulinemic compared to their wild-type littermates after 24 weeks

[i] Blood glucose levels during insulin tolerance test (ITT) compared to wild-type animals

[j] Female βAMPK.CA (expressing constitutively active AMPK in β-cells) and βAMPK.DN (expressing dominant negative AMPK in β-cells) mice displayed no abnormalities compared to wild-type in IGTT or ITT

the inhibition of mTORC1. However, we have recently discovered that rapamycin also inhibits mTORC2 and that the toxic effects of rapamycin on β-cells are primarily mediated by the inhibition of mTORC2 (Barlow AD, Xie J and Herbert TP, unpublished data).

The roles of mTORC1 and 2 downstream targets in the regulation of pancreatic β-cell mass and function are summarized in Fig. 3.

Transgenic mouse models

Although in vitro studies have revealed important insights into the role and regulation of mTORC1 in β-cells, transgenic mouse models of upstream regulators and downstream effectors of mTOR have provided unequivocal evidence demonstrating that mTORC1/2 plays a critical role in the regulation of β-cell mass in vivo. What we consider to be the most pertinent examples are discussed below. However, we have also provided a comprehensive list of these transgenic mouse models and their phenotypes (see Table 1).

Tuberous sclerosis complex 1 and 2 (TSC1/2)

TSC1/2 is an upstream negative regulator of mTORC1 [76, 156], and loss of TSC2 leads to the constitutive activation of mTORC1 [57, 156, 183]. Importantly, the generation of β-cell specific TSC2 knock-out mice ($\beta TSC2^{-/-}$), from two independent groups, has revealed that TSC2 plays in important role in the regulation of β-cell size and mass [123, 143]. Rachdi et al. [123] reported that in 8 weeks old $\beta TSC2^{-/-}$ mice, β-cell mass is increased by over two-fold due to a doubling in β-cell size and proliferation and that this increase in β-cell mass is maintained for up to 52 weeks of age [123]. Shigeyama et al. [143] reported similar increases in β-cell size and mass in 6 weeks old $\beta TSC2^{-/-}$ mice but, in contrast to Rachdi et al. [123], β-cell mass had decreased dramatically (80% reduction compared to control) by 40 weeks of age likely due to an increase in apoptosis. This was accompanied by hypoinsulinemia and, as a consequence, hyperglycemia. The positive and negative effects of TSC2 knock-out on β-cell mass are likely mediated by mTORC1 as rapamycin treatment of young $\beta TSC2^{-/-}$ mice causes a reduction in β-cell mass, whereas rapamycin treatment (18–40 weeks) of Shigeyama et al.'s [143] $\beta TSC2^{-/-}$ mice resulted in the maintenance of β-cell mass and improved glyceamic control. The age-related decrease in β-cell mass observed in $\beta TSC2^{-/-}$ mice is likely due to the prolonged hyper-activation of mTORC1 resulting in feedback inhibition, possibly through S6K-dependent phosphorylation of IRS2.

β-cell specific TSC1 knock-out mice (RIP-TSC1cKO) also show an enhancement of β-cell size rather than number and this correlated with an augmentation in glucose stimulated insulin secretion (GSIS) in vivo and increased glucose clearance as determined by intravenous glucose tolerance test (IGTT) compared to WT mice [107]. As the observed enhancement of GSIS was eliminated by rapamycin treatment, the positive effects of TSC1 deletion are likely mediated by the activation of mTORC1.

Rheb

The over-expression of Rheb, the downstream target of the TSC1/2 complex, also results in the constitutive activation of mTORC1 [109, 148]. β-cell specific over-expression of Rheb in mice enhances β-cell mass by approximately 50% [63]. β-cell size was increased by up to 30%, whereas cell proliferation and cell viability were unaffected. This augmentation in β-cell mass correlates with improved glucose tolerance in oral glucose tolerance test (OGTT) and increased late phase GSIS in vivo compared to their wild-type littermates. These improvements in glucose tolerance and enhancement of GSIS were reversed upon rapamycin treatment, indicating that that these functional effects are likely mediated via the activation of mTORC1 [63].

Ribosomal protein S6 Kinase (S6K1/2) and ribosomal protein S6 (rpS6)

S6K1/2 are downstream targets of mTORC1 and S6K1 knock-out ($S6K1^{-/-}$) mice have reduced β-cell mass due to a reduction in β-cell size (a 24% decrease in comparison with the WT) [116]. In addition, the islets from these mice had decreased islet insulin content and the amount of insulin secreted per cell in response to glucose was significantly reduced. Conversely, the over-expression of a constitutively active form of S6K1 in mouse β-cells ($S6KCA^{RIP}$) results in an increase in β-cell size by approximately 50% [39]. These reports indicate that S6K1 is a positive effector of β-cell size and function. The effects of S6K1 on β-cell size may be mediated through the phosphorylation of rpS6, as non-phosphorylatable rpS6 knock-in mice ($rpS6^{P-/-}$) have smaller β-cells (a 35% decrease compared to WT) [131]. Yet, β-cell mass is unaffected due to a compensatory increase in β-cell number. Interestingly, β-cells from $rpS6^{P-/-}$ mice are smaller than those from $S6K1^{-/-}$ mice, indicating that other rpS6 kinases may be involved. However, there is no reduction in β-cell size or mass in S6K2 knock-out ($S6K2^{-/-}$) mice [117], although S6K2 is considered to be the major in vivo rpS6 kinase [117]. It is therefore possible that the difference in cell size between $rpS6^{P-/-}$ and $S6K1^{-/-}$ mice is mediated by an alternative S6K such as p90 ribosomal S6 kinase (RSK) or protein kinase A (PKA) [106, 129]. Then again, the effects of rpS6 and S6K on β-cell size may occur via distinct mechanisms [130]. For example, the effects of S6K on β-cell size could be mediated by alternative S6K substrates. Intriguingly, the depletion of SKAR [126] has been shown to reduce cell size in other cell types. Therefore, it would be of interest to determine whether these S6K substrates also play a role in the regulation of β-cell size.

Protein kinase B (PKB)

There are three isoforms of PKB: PKBα, PKBβ and PKBγ (also known as Akt1, Akt2 and Akt3, respectively). Although PKBα is the most abundant isoform in many mammalian tissues [25, 27], PKBβ is highly expressed in insulin responsive tissue [26, 51], whereas expression of PKBγ is restricted to the central nervous system and testis [91]. PKB, through its phosphorylation of TSC2 [76, 121] and PRAS40 [93, 112, 135, 157, 166, 167], is a well characterised positive regulator of mTORC1, and transgenic mice expressing constitutively active PKBα (Myr-Akt1) specifically in β-cells have increased β-cell mass, through an increase in β-cell size and proliferation, and an increase in function as manifested through increased high circulating insulin levels and improved glucose tolerance [3, 11, 163]. Interestingly, rapamycin treatment of Myr-Akt1 mice results in a decrease in β-cell proliferation and mass [5]. Yet, PKBα-dependent increase in β-cell size is unaffected by the deletion of S6K1 but is reduced by the deletion of both S6K1 and 2 [3], although β-cell size still remains bigger than that of their WT littermates [3]. This implies that other targets of PKBα are responsible for stimulating an increase in β-cell size. Indeed, the effects of rapamycin on β-cell mass in Myr-Akt1 mice is likely through the suppression of mTORC1-dependent activation of cdk4, and a reduction in cyclin D_2 and D_3 levels [5], possibly mediated by a decrease in cap-dependent translation via the hypophosphorylation of 4EBPs [9, 52]. Surprisingly, glucose homeostasis is maintained in PKBα knock-out ($Akt1^{-/-}$) mice [27], and no significant changes in neither β-cell size nor number was observed in mice expressing kinase dead PKBα (RIP-KdAkt), which has a dominant-negative phenotype (although defects in insulin secretion were observed) [10]. However, kinase-dead PKBα reduces endogenous PKB activity by no more than 80%, and therefore it is conceivable that the remaining 20% of PKBα activity is sufficient to maintain β-cell mass [10]. Deletion of PKBβ ($Akt2^{-/-}$) in mice resulted in a

reduction in β-cell mass concomitant with an increase in the rate of apoptosis [51], although in a previous study Akt2$^{-/-}$ mice had enhanced β-cell mass (increase in both size and number), likely due to the development of insulin resistance and subsequent β-cell compensation [26]. In PKBγ KO mice, glucose homeostasis is unaffected yet effects on β-cell mass were not reported [161].

AMPK/LKB1

In clonal β-cell lines, an increase in energy status through, for example, an increase in glucose concentration can lead to the inactivation of AMPK and the activation of mTORC1 [54, 103], and increased glucose concentration in vivo is a potent stimulator of β-cell mass [16]. Yet, β-cell specific AMPK catalytic subunits (α1 and α2) knock-out mice (βAMPKdKO) exhibit no change in β-cell mass, although a 36% reduction in β-cell size and a two-fold increase in the rate of β-cell proliferation was observed [154]. Surprisingly, no increase in rpS6 phosphorylation (a marker of mTORC1 activation) was observed and, therefore, changes in β-cell size and proliferation in the βAMPKdKO mice may not be caused by altered mTORC1 signalling. In addition, these mice had abnormal glucose tolerance and enhanced in vivo GSIS. In contrast, and seemingly at odds with the results reported in βAMPKdKO mice, β-cell area was reduced in male transgenic mice over-expressing constitutively-active AMPK in β-cells (βAMPK.CA), as were circulating insulin levels, resulting in glucose intolerance [154]. However, no significant changes in β-cell number or size were observed in female βAMPK.CA mice or in β-cells expressing dominant-negative AMPK (βAMPKDKO) [154].

Liver kinase B1 (LKB1) is a kinase which can phosphorylate and activates AMPK [67, 175] and β-cell specific LKB1 knock-out (βLKB1KO) mice have increased β-cell mass due to increased β-cell size [46, 58, 155] and proliferation [46, 155]. Moreover, these mice have improved glucose tolerance because of enhanced in vivo GSIS [46, 58, 155]. However, Sun et al. [155] observed a diminished in vitro GSIS in their βLKB1KO model, which parallels a reduction in the levels of glucose transporter 2 (GLUT2) and ATP-sensitive potassium channel (K_{ATP} channel) subunit Kir6.2. The effects of LKB1 depletion, at least on β-cell mass, are likely through mTORC1, as mTORC1 was shown to be activated in the β-cells of these mice [46, 58, 155], and the effects of LKB1 knock-out on β-cell mass were reversed by rapamycin [46, 58]. The differences observed between βLKB1KO and βAMPKdKO suggest that LKB1 activates signalling pathways independent of its established role in the activation of AMPK.

RICTOR

β-cell specific knock-out of RICTOR (βRicKO), an essential component of the mTORC2 complex, in mice, results in a reduction in β-cell mass due to an impairment in proliferation [59]. However, no changes in β-cell size or the rate of cell death were detected. These mice also had decreased pancreatic insulin content, moderate hyperglycemia and glucose intolerance. In islets isolated from βRicKO mice, the phosphorylation of PKB at Ser473, a target for mTORC2 and an important site for PKB activation, was not surprisingly compromised. Moreover, this correlated with an increase in FoxO1 nuclear localisation, which is known to be inhibited by PKB-dependent phosphorylation on Ser473. However, the phosphorylation of PKB at Thr308, another important site for PKB activation and which is mediated by PDK1, was enhanced. Therefore, it is possible that the increase in the phosphorylation of PKB at Thr308 may compensate for the loss of Ser473 phosphorylation and that PKB activity towards specific subset of substrates is maintained. Indeed, no change in mTORC1 activity was detected in islets isolated from βRicKO mice. Interestingly, in β-cell specific Pten (phosphatase and tensin homolog, which inhibits the activation of PKB through promoting the dephosphorylation of PIP3 and thereby prevents plasma membrane translocation of PDK-1 and PKB) and RICTOR double knock-out mice (βPtenRicKO), Thr308 phosphorylation on PKB was dramatically enhanced and this correlated with an increase in β-cell size [59]. Therefore, the authors concluded that phosphorylation of PKB at Thr308 (by PDK-1) drives cell size, whereas the phosphorylation of PKB on Ser473 (by mTORC2) drives cell proliferation [59].

Concluding remarks

Transgenic mouse models have clearly demonstrated that mTORC1 is a positive regulator of β-cell mass through stimulating an increase in both β-cell proliferation and size. It has also been shown in vitro that physiological stimulators of β-cell mass, such as GLP-1 and glucose, activate mTORC1 and stimulate β-cell replication via a rapamycin sensitive mechanism [95]. It is also likely that, in vivo, all growth factors, hormones and nutrients that stimulate increases in β-cell mass also require the activation of mTORC1. However, the chronic activation of mTORC1 may in due course lead to a decrease in β-cell mass mediated by a potent negative feedback mechanism. Therefore, it is possible that chronic excess of nutrient availability, as seen in obesity, may ultimately result in decreased mTORC1 function and β-cell mass. The molecular mechanisms by which mTORC1 stimulates β-cell mass is not

fully understood. Yet, it is likely that the effect of mTORC1 on β-cell size is primarily mediated by the activation of S6K1. Although the phosphorylation of rpS6, a substrate for S6K, has been implicated in increased β-cell size [131], it appears that S6K1 may stimulate β-cell size independently of rpS6. Therefore, S6K1-dependent increase in β-cell size may be mediated by the only known S6K1 specific substrate SKAR, which have been previously shown to be important in other cell types to regulate cell size [126]. How mTORC1 stimulates β-cell proliferation is unknown and clearly warrants further investigation. However, it may well be mediated by the phosphorylation of 4EBPs, which is known to stimulate cap-dependent translation and to increase the synthesis of proteins important in cell cycle progression [35]. Other substrates of mTORC1 may also play an important role in β-cell function such as the transcription factors STAT3 (signal transducers and activator 3) [181], which enhances proinsulin gene expression in clonal pancreatic β-cell lines and islets of Langerhans [96], or YY1 (Ying Yang 1), which stimulates mitochondrial gene expression and reduces oxygen consumption in respiration [30].

mTORC2 has also been shown to play a positive role in the regulation of β-cell mass through an increase in β-cell proliferation [59]. Surprisingly, although mTORC2 regulates the activity of PKB, a protein known to be critical in cell survival, β-cell viability is unaffected in RICTOR knock-out mice [59]. However, we have evidence that acute inactivation of mTORC2 causes loss of β-cell viability (Barlow AD, Xie J and Herbert TP, unpublished results).

In conclusion, both mTORC1 and 2 are required to maintain β-cell mass and function. Moreover, the activation of mTORC1 can stimulate increases in β-cell mass through an increase in both β-cell replication and an increase in cell size. Thus, the activation of mTORC1 may play an important role in β-cell compensation in response to an increase demand for insulin under conditions of insulin resistance and/or increased body mass. It is therefore possible that the activation of mTORC1 pharmacologically or using nutritional supplements may stimulate β-cell function and mass and therefore may be useful in the prevention and/or treatment of type 2 diabetes. On a cautionary note, it is worth mentioning that chronic nutritional activation of mTORC1 has been implicated in the development of insulin resistance [39, 160, 165], and that chronic activation of S6K1 can lead to its inactivation [39, 66, 160, 165] and a decline in both β-cell function and mass [39, 165]. However, there is no substantive evidence, as yet, that mTOR plays a role in β-cell dysfunction and death in the development of type 2 diabetes, although it is clearly worthy of investigation.

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