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mTOR signaling in growth control and disease

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Abstract

The mammalian target of rapamycin (mTOR) signaling pathway senses and integrates a variety of environmental cues to regulate organismal growth and homeostasis. The pathway regulates many major cellular processes and is implicated in an increasing number of pathological conditions, including cancer, obesity, type 2 diabetes, and neurodegeneration. Here, we review recent advances in our understanding of the mTOR pathway and its role in health and disease as well as aging. We further discuss pharmacological approaches to treat human pathologies linked to mTOR deregulation.

Introduction

Most organisms have evolved mechanisms for efficiently transitioning between anabolic and catabolic states, allowing them to survive and grow in environments in which nutrient availability is variable. In mammals, an example of such a mechanism is the signaling network anchored by the protein kinase mTOR (originally ‘mammalian TOR’, but now officially ‘mechanistic TOR’). This pathway, which responds to diverse environmental cues, controls many processes that generate or use large amounts of energy and nutrients. It is increasingly apparent that mTOR signaling impacts most major cellular functions, giving it an outsized role in regulating basic cell behaviors like growth (mass accumulation) and proliferation. Because mTOR deregulation occurs in human disease, including cancer, obesity, type 2 diabetes, and neurodegeneration, there are significant ongoing efforts to pharmacologically target the pathway. Here, we review our current understanding of the mTOR pathway and its role in health and disease, as well as discuss pharmacological approaches for modulating mTOR activity.

Overview of the mTOR pathway

mTOR is the target of a molecule named rapamycin or sirolimus, which is a macrolide produced by *Streptomyces Hygroscopius* bacteria and that first gained attention because of

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its broad anti-proliferative properties. In the early 1990s, genetic screens in budding yeast identified the TOR1 and TOR2 genes as mediators of the toxic effects of rapamycin on yeast (Cafferkey et al., 1993; Kunz et al., 1993). Shortly afterwards, biochemical approaches in mammals led to purification of mTOR and its discovery as the physical target of rapamycin (Brown et al., 1994; Sabatini et al., 1994; Sabers et al., 1995). mTOR is an atypical serine/threonine protein kinase that belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family and interacts with several proteins to form two distinct complexes named mTOR complex 1 (mTORC1) and 2 (mTORC2). The mTOR-containing complexes have different sensitivities to rapamycin as well as upstream inputs and downstream outputs (Figure 1, top panel).

Both mTOR complexes are large, with mTORC1 having six and mTORC2 seven known protein components. They share the catalytic mTOR subunit, and also mammalian lethal with sec-13 (mLST8, also known as GβL) (Jacinto et al., 2004; Kim et al., 2003), DEP domain containing mTOR-interacting protein (DEPTOR) (Peterson et al., 2009), and the Tti1/Tel2 complex (Kaizuka et al., 2010). In contrast, regulatory-associated protein of mammalian target of rapamycin (raptor) (Hara et al., 2002; Kim et al., 2002) and proline-rich Akt substrate 40kDa (PRAS40) (Sancak et al., 2007; Thedieck et al., 2007; Vander Haar et al., 2007; Wang et al., 2007) are specific to mTORC1, while rapamycin-insensitive companion of mTOR (rictor) (Jacinto et al., 2004; Sarbassov et al., 2004), mammalian stress-activated map kinase-interacting protein 1 (mSin1) (Frias et al., 2006; Jacinto et al., 2006), and protein observed with rictor 1 and 2 (protor1/2) (Pearce et al., 2007; Pearce et al., 2011; Thedieck et al., 2007) are only part of mTORC2. Figure 1 describes the known molecular functions of the mTOR complex components and the interaction sites between them (middle and bottom panels).

As discussed later, the effects of rapamycin on mTOR signaling are much more complex than originally anticipated and, surprisingly, almost 20 years after the discovery of mTOR, our understanding of its mechanism of action is still evolving. It is clear, however, that rapamycin forms a gain-of-function complex with the intracellular 12-kDa FK506-binding protein (FKBP12) protein (Brown et al., 1994; Sabatini et al., 1994). This complex directly interacts with and inhibits mTOR when it is part of mTORC1 but not mTORC2. Many mTORC1 functions are highly sensitive to rapamycin but exactly how the binding of FKBP12-rapamycin to mTORC1 inhibits its activity is unknown. Rapamycin may compromise the structural integrity of mTORC1 (Kim et al., 2002; Yip et al., 2010) as well as allosterically reduce the specific activity of its kinase domain (Brown et al., 1995; Brunn et al., 1997; Burnett et al., 1998).

Upstream regulators of mTORC1

mTORC1 is the better characterized of the two mTOR complexes and a remarkable feature of this branch of the pathway is the number and diversity of upstream signals it senses. The mTORC1 pathway integrates inputs from at least five major intracellular and extracellular cues--growth factors, stress, energy status, oxygen, and amino acids--to control many major processes, including protein and lipid synthesis and autophagy. The heterodimer consisting of tuberous sclerosis 1 (TSC1; also known as hamartin) and TSC2 (also known as tuberin) is a key upstream regulator of mTORC1 and functions as a GTPase-activating protein (GAP) for the Ras homolog enriched in brain (Rheb) GTPase. The GTP-bound form of Rheb directly interacts with mTORC1 and strongly stimulates its kinase activity. As a Rheb GAP, TSC1/2 negatively regulates mTORC1 by converting Rheb into its inactive GDP-bound state (Inoki et al., 2003a; Tee et al., 2003). To date, there is no credible evidence that a guanine nucleotide exchange factor (GEF) exists for Rheb.

TSC1/2 transmits many of the upstream signals that impinge on mTORC1 (Figure 2A), including growth factors, such as insulin and insulin-like growth factor 1 (IGF1), that stimulate the PI3K and Ras pathways. The effector kinases of these pathways—protein kinase B (Akt/PKB), extracellular-signal-regulated kinase 1/2 (ERK1/2), and ribosomal S6 kinase (RSK1)-- directly phosphorylate the TSC1/TSC2 complex to inactivate it and thus activate mTORC1 (Inoki et al., 2002; Ma et al., 2005; Manning et al., 2002; Potter et al., 2002; Roux et al., 2004). Akt also signals to mTORC1 in a TSC1/2-independent fashion by phosphorylating and causing the dissociation from raptor of PRAS40, an mTORC1 inhibitor (Sancak et al., 2007; Thedieck et al., 2007; Vander Haar et al., 2007; Wang et al., 2007). Pro-inflammatory cytokines, like tumor necrosis factor-α (TNFα), activate mTORC1 through a conceptually similar mechanism as growth factors: IκB kinase β (IKKβ) phosphorylates TSC1, causing TSC1/2 inhibition (Lee et al., 2007). Lastly, the canonical Wnt pathway, a major regulator of cell growth, proliferation, polarity, differentiation, and development, also activates mTORC1 through TSC1/2. In this case, Wnt signaling inhibits glycogen synthase kinase 3β (GSK3-β), which normally phosphorylates and promotes TSC2 activity (Inoki et al., 2006).

Like the growth factor inputs to mTORC1, many stresses also act, at least in part, through TSC1/2, with low energy and oxygen levels and DNA damage being the best characterized. Adenosine monophosphate-activated protein kinase (AMPK), in response to hypoxia or a low energy state, phosphorylates TSC2 and increases its GAP activity towards Rheb (Inoki et al., 2003b). Like Akt, AMPK also communicates directly with mTORC1; it phosphorylates raptor, leading to 14-3-3 binding and the allosteric inhibition of mTORC1 (Gwinn et al., 2008). Hypoxia also induces the expression of transcriptional regulation of DNA damage response 1 (REDD1), which activates TSC2 function in a still poorly understood manner (Brugarolas et al., 2004; DeYoung et al., 2008; Reiling and Hafen, 2004). DNA damage also signals to mTORC1 through multiple mechanisms, all of which require p53-dependent transcription. DNA damage induces the expression of *Tsc2* and *phosphatase and tensin homolog deleted on chromosome 10* (*Pten),* causing downregulation of the entire PI3K-mTORC1 axis (Feng et al., 2005; Stambolic et al., 2001) and activates AMPK through a mechanism that depends on the induction of *Sestrin1/2* (Budanov and Karin, 2008). Given that so many signals regulate mTORC1 through TSC1/2, it is surprising that we still do not know how TSC1/2 integrates, at the molecular level, the inputs to control its GAP activity towards Rheb. Furthermore, it is unclear if certain inputs are dominant over others and if cell type-dependent regulatory mechanisms exist.

Amino acids, particularly leucine and arginine, also activate mTORC1 and must be present for any upstream signal, including growth factors, to activate mTORC1 (Blommaart et al., 1995; Hara et al., 1998). Although it has been known for some time that amino acids act independently of TSC1/2 (Smith et al., 2005), the molecular mechanism through which mTORC1 senses intracellular amino acids remains a big mystery in the mTOR field. In 2008, two groups independently discovered that amino acid-dependent activation of mTORC1 requires the Rag GTPases (Kim et al., 2008; Sancak et al., 2008). Mammals have four Rag proteins, RagA to RagD, which form obligate heterodimers consisting of RagA or RagB with RagC or RagD (Figure 2A). The two members of the heterodimer appear to have opposite nucleotide loading states, so that when RagA/B is bound to GTP, RagC/D is bound to GDP and *vice versa*. Through an unknown mechanism, amino acids promote the loading of RagA/B with GTP, which enables the heterodimer to interact with the raptor component of mTORC1 (Sancak et al., 2008). This interaction results in the translocation of mTORC1 from a poorly characterized cytoplasmic location to the lysosomal surface, where the Rag GTPases dock on a multi-subunit complex called Ragulator (Sancak et al., 2010). Like the Rag GTPase, Ragulator is essential for the activation of mTORC1 by amino acids.

Why does translocation of mTORC1 to the lysosomal surface result in its activation? A current model posits that at the lysosomal surface mTORC1 can bind to and become activated by Rheb, which is found throughout the endomembrane system. Thus, the Rag and Rheb GTPases are part of a molecular AND gate: GTP-loaded Rheb only interacts with mTORC1 when the amino acid-sensitive Rag-Ragulator mechanism brings it onto the lysosomal surface, ensuring that mTORC1 activation occurs only if amino acids are available, irrespective of the presence of other positive signals.

The localization of the Ragulator and Rag GTPases to the lysosomal surface, but not on other endomembranes where Rheb also resides, suggests an important role for this organelle in amino acid sensing by mTORC1 pathway. Recent work proposes a *inside-out* model of amino acid sensing in which amino acids accumulate in the lysosomal lumen and initiate signaling through a mechanism requiring the vacuolar H^+-adenoside triphosphate ATPase (v-ATPase)(Zoncu et al., 2011). Depletion of v-ATPase subunits blocks amino acid-induced recruitment of mTORC1 to the lysosomal surface and downstream signaling. The v-ATPase directly interacts with the Ragulator, providing a physical link between the v-ATPase and the Rag GTPase on the surface of lysosomes (Figure 2A). The ATPase activity of the v-ATPase and the associated rotation of its V0 section appear to be essential to relay the amino acids signal from the lysosomal lumen to the Ragulator and Rag GTPases, but exactly how the v-ATPase functions to do so is unknown. Interestingly, the mTORC1 pathway regulates v-ATPase expression, suggesting that a feedback loop exists between mTORC1 and lysosome function (Duvel et al., 2010; Pena-Llopis et al., 2011).

Over the years a number of other proteins have been implicated in amino acid sensing by mTORC1, including mitogen-activated protein kinase kinase kinase kinase (MAP4k3) (Findlay et al., 2007), mammalian vacuolar protein sorting 34 homologue (hVPS34) (Nobukuni et al., 2005), and inositol polyphosphate monokinase (IPMK) (Kim et al., 2011) and if and how these molecules connect to the Rag-Ragulator system is not known. MAP4k3 is likely upstream of the Rag GTPases (Yan et al., 2010) but whether it interacts with them is not clear (Bryk et al., 2010; Yan et al., 2010).

Finally, phosphatidic acid (PA) has also been identified as an activator of mTORC1 (Fang et al., 2001). Although the role of PA in regulating mTOR is controversial, several reports show that exogenous PA or overexpression of PA-producing enzymes such as phospholipase D1 (PLD1) and PLD2 significantly increases mTORC1 activity (reviewed in (Foster, 2009)). PA activates mTOR signaling at least in part by stabilizing the mTOR complexes (Toschi et al., 2009).

Cellular processes downstream of mTORC1

Protein synthesis is by far the best characterized process controlled by mTORC1 (Figure 2B). mTORC1 directly phosphorylates the translational regulators eukaryotic translation initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) and S6 kinase 1 (S6K1), which, in turn, promote protein synthesis (reviewed in (Ma and Blenis, 2009)). The phosphorylation of 4E-BP1 prevents its binding to the cap-binding protein eIF4E, enabling it to participate in the formation of the eIF4F complex which is required for the initiation of cap-dependent translation. The activation of S6K1 leads, through a variety of effectors, to an increase in mRNA biogenesis, as well as translational initiation and elongation (Figure 2B). S6K1 was originally thought to control the translation of an abundant subclass of mRNAs characterized by an oligopyrimidine tract at the 5′ end (5′ TOP mRNAs) and which encode most of the protein components of the translational machinery. Although mTOR itself is key for the translational control of 5′TOP mRNAs, S6K1 and its substrate ribosomal protein S6 are not required for this process (Tang et al., 2001) and so how mTORC1 controls the translation of these mRNAs remains unknown. mTORC1 also upregulates the protein

synthesis machinery in other ways: (1) it activates the regulatory element tripartite motif-containing protein-24 (TIF-1A), which promotes its interaction with RNA Polymerase I (Pol I) and the expression of ribosomal RNA (rRNA) (Mayer et al., 2004); and (2) mTORC1 phosphorylates and inhibits Maf1, a Pol III repressor, and so induces 5S rRNA and transfer RNA (tRNA) transcription (Kantidakis et al., 2010; Shor et al., 2010). The overall role of mTORC1 in the regulation of mRNA translation is highly significant because specific, active-site inhibitors of mTOR that completely inhibit mTORC1 function, significantly reduce overall rates of protein synthesis in proliferating cells in culture (Thoreen et al., 2009; Yu et al., 2009).

In addition to regulating the production of proteins, mTORC1 also controls the synthesis of lipids required for proliferating cells to generate membranes (reviewed in (Laplante and Sabatini, 2009). To a large extent, mTORC1 acts through the sterol regulatory element binding protein 1/2 (SREBP1/2) transcription factors that control the expression of numerous genes involved in fatty acid and cholesterol synthesis (Figure 2). The inactive SREBPs reside on the endoplasmic reticulum (ER) and their proteolytic processing in response to insulin or sterol depletion releases an active form that travels to the nucleus to activate transcription. mTORC1 inhibition reduces SREBP1/2 levels as well as processing and markedly lowers the expression of lipogenic genes (Duvel et al., 2010; Li et al., 2010; Porstmann et al., 2008; Wang et al., 2011). mTORC1 appears to regulate SREBP function through several mechanisms, including, at least in some cell types, through S6K1 (Duvel et al., 2010; Li et al., 2011; Wang et al., 2011). In addition, mTORC1 phosphorylates Lipin-1, preventing it from entering the nucleus and suppressing SREBP1/2 function and levels (Peterson et al., 2011). mTORC1 also promotes the expression and activity of peroxisome proliferator-activated receptor γ (PPAR-γ), the master regulator of adipogenesis (Kim and Chen, 2004; Zhang et al., 2009).

Befitting a pathway that when active drives energy consumption, mTORC1 also positively regulates cellular metabolism and ATP production. mTORC1 increases glycolytic flux by activating the transcription and the translation of hypoxia inducible factor 1α (HIF1α) (Brugarolas et al., 2003; Duvel et al., 2010; Hudson et al., 2002; Laughner et al., 2001), a positive regulator of many glycolytic genes (Figure 2B). Another study reported that mTORC1 also increases mitochondrial DNA content and the expression of genes involved in oxidative metabolism, in part by mediating the nuclear association between PPAR-γ coactivator 1α (PGC1α) and the transcription factor Ying-Yang 1 (YY1), which positively regulate mitochondrial biogenesis and oxidative function (Cunningham et al., 2007). Additional evidence is needed to support this connection because the YY1 response element was not identified as a motif enriched in the promoters of mTORC1-regulated genes (Duvel et al., 2010) and little endogenous mTORC1 is found in the nucleus (Sancak et al., 2010; Sancak et al., 2008; Zoncu et al., 2011).

The discussion so far has focused on the positive effects of mTORC1 on anabolic processes, but mTORC1 also promotes growth by negatively regulating autophagy, the central degradative process in cells. Autophagy is required for the recycling of damaged organelles and for the organismal and cellular adaptation to nutrient starvation. Upon mTORC1 inhibition, autophagosomes form which then engulf cytoplasmic proteins and organelles and fuse with lysosomes, leading to the degradation of cell components and the recycling of cellular building blocks. In mammals, mTORC1 directly phosphorylates and suppresses ULK1/Atg13/FIP200 (unc-51-like kinase 1/mammalian autophagy-related gene 13/focal adhesion kinase family-interacting protein of 200kDa), a kinase complex required to initiate autophagy (Figure 2B) (Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009). As with the control of protein and lipid synthesis, mTORC1 is likely to impact autophagy through several mechanisms. For example, mTORC1 regulates death associated protein 1

(DAP1), a suppressor of autophagy (Koren et al., 2010) and, in a recent analysis of the mTOR-dependent phosphoproteome, WIPI2, a mammalian ortholog of Atg18—a regulator of early autophagosome formation in yeast—emerged as a potential mTOR effector (Hsu et al., 2011).

It is sometimes joked that 'mTOR regulates everything' and although this is, of course, not true it is remarkable how many major process the pathway does control. This perhaps is not so surprising considering that mTOR is one of the key sensors of nutritional status at the cellular and organismal levels and it is not hard to imagine why it is beneficial for many processes to be linked to the nutritional state.

Overview of the mTORC2 signaling network

Because acute treatment with rapamycin does not perturb mTORC2 signaling and FKBP12-rapamycin cannot bind to intact mTORC2, this complex was originally thought to be rapamycin-insensitive (Jacinto et al., 2004; Sarbassov et al., 2004). However, the situation turns out to be much more complex as long term treatment with rapamycin reduces mTORC2 signaling in some, but not all cell types, and does so by suppressing mTORC2 assembly (Phung et al., 2006; Sarbassov et al., 2006). Why there is cell type specificity to the rapamycin sensitivity of mTORC2 assembly is still unclear.

Compared to mTORC1, much less is known about the mTORC2 pathway. mTORC2 signaling is insensitive to nutrients, but does respond to growth factors like insulin through a poorly defined mechanism(s) that requires PI3K. One potential mechanism involves a novel role for ribosomes, as ribosomes are needed for mTORC2 activation and mTORC2 binds them in a PI3K-dependent fashion (Zinzalla et al., 2011).

mTORC2 controls several members of the AGC subfamily of kinases including Akt, serum- and glucocorticoid-induced protein kinase 1 (SGK1), and protein kinase C-α (PKC-α) (Figure 2). Akt regulates cellular processes such as metabolism, survival, apoptosis, growth, and proliferation through the phosphorylation of several effectors. mTORC2 directly activates Akt by phosphorylating its hydrophobic motif (Ser473), a site required for its maximal activation (Sarbassov et al., 2005). Defective Akt-Ser473 phosphorylation associated with mTORC2 depletion impairs the phosphorylation of some Akt targets, including forkhead box O1/3a (FoxO1/3a), while other Akt targets like TSC2 and GSK3-β remain unaffected (Guertin et al., 2006; Jacinto et al., 2006). The fact that Akt activity is not completely abolished in cells lacking mTORC2 likely explains these results. mTORC2 also directly activates SGK1, a kinase controlling ion transport and growth (Garcia-Martinez and Alessi, 2008). In contrast to Akt, SGK-1 activity is completely blocked by the loss of mTORC2. Because SGK1 controls FoxO1/3a phosphorylation on residues also phosphorylated by Akt, loss of SGK1 activity is probably responsible for the reduction in FoxO1/3a phosphorylation in mTORC2-depleted cells. PKC-α is the third AGC kinase activated by mTORC2. Along with other effectors such as paxilin and Rho GTPases, the activation of PKC-α by mTORC2 regulates cell shape in cell type-specific fashion by affecting the actin cytoskeleton (Jacinto et al., 2004; Sarbassov et al., 2004) (Figure 2B).

Role of mTOR signaling in cancer

Several observations support the importance of mTOR pathway in cancer pathogenesis. Many components of the PI3K signaling pathway, which is upstream of both mTORC1 and mTORC2 (Figure 2), are mutated in human cancers (Figure 2A). Additionally, the loss of *p53*, a very common event in cancer, promotes mTORC1 activation (Feng et al., 2005). In addition, several familial cancer syndromes arise from mutations in genes encoding proteins that lie upstream of the mTOR complexes, including *Tsc1/2*, *serine threonine kinase 11*

(*Lkb1)*, *Pten*, and *neurofibromatosis type 1* (*Nf1)* (Figure 2). Oncogenic activation of mTOR signaling induces several processes required for cancer cell growth, survival, and proliferation (Figure 3A).

A growing body of evidence points to the deregulation of protein synthesis downstream of mTORC1 at the level of 4E-BP1/eIF4E as playing a central role in tumor formation. Loss of 4EBP1/2 and the concomitant activation of cap-dependent translation promotes cell cycle progression and cell proliferation *in culture* (Dowling et al., 2010). 4E-BP1/eIF4E also mediates the effects of oncogenic Akt signaling on mRNA translation, cell growth, and tumor progression (Hsieh et al., 2010). Interestingly, the contribution of S6K1 and S6 to the oncogenic action of ERK and/or Akt appears limited, indicating that the signaling branches controlling protein synthesis downstream of mTORC1 are not equally required in oncogenesis (Hsieh et al., 2010; She et al., 2010). Exactly how the mTORC1/4E-BP1/eIf4E axis contributes to cancer is unclear. It is thought that eiF4E affects cell proliferation and tumorigenesis by promoting the translation of specific mRNAs coding for pro-oncogenic proteins regulating cell survival, cell cycle progression, angiogenesis, energy metabolism, and metastasis. Additionally, the increase in ribosome biogenesis linked to mTOR activation likely promotes cell proliferation by providing the machinery required to sustain high levels of cell growth.

An increase in *de novo* lipid synthesis is a hallmark of proliferating cancer cells (reviewed in (Menendez and Lupu, 2007)) and such cells must produce fatty acids to synthesize membranes. PI3K signaling promotes the activation of the pro-lipogenic factor SREBP1 and mTORC1 is required to relay oncogenic/growth factor signaling to SREBP1 (Duvel et al., 2010). SREBP1 also drives the expression of components of the oxidative branch of the pentose phosphate pathway, which controls the production of the reducing equivalents and ribose-5-phosphate needed for lipogenesis and nucleotide biosynthesis, respectively (Figure 3A) (Duvel et al., 2010). The inhibition of cell proliferation associated with SREBP1/2 depletion in *Tsc2* null cells indicates that mTORC1-driven cell proliferation requires the transcriptional program controlled by SREBP1/2.

The constitutive activation of PI3K-mTORC1 signaling in cancer cells strongly inhibits autophagy. How such impairment of autophagy affects cancer is unclear. Autophagy is a double-edged sword in tumorigenesis, acting both as a tumor suppressor and a protector of cancer cell survival. Mice deficient for essential components of the autophagy machinery have accelerated rates of spontaneous tumor development (reviewed in (Yang and Klionsky, 2010)). Autophagy-defective cells accumulate protein aggregates, damaged mitochondria, and reactive oxygen species, which are believed to promote DNA damage and tumorigenesis. Conversely, several evidences indicate that repressing autophagy may impair tumorigenesis by reducing the ability of cancer cell to survive in nutrient/energy poor conditions. For instance, *Tsc2* and *Lkb1* null cells are hypersensitive to energy deprivation-induced apoptosis (Inoki et al., 2003b; Shaw et al., 2004). The role of autophagy in mediating the effect of mTORC1 activation on cancer is probably context-specific, autophagy being important to prevent cancer initially but being required to protect cells when the tumor is established.

There is also emerging evidence for a role for mTORC2 in cancer. Many gliomas overexpress the mTORC2 subunit rictor and its forced overexpression promotes mTORC2 assembly and activity and endows cancer cells with increased proliferative and invasion potential (Hietakangas and Cohen, 2008; Masri et al., 2007). In mice, the development of prostate cancer induced by the loss of the tumor suppressor PTEN requires mTORC2 function (Guertin et al., 2009). These results support an important role of mTORC2 in promoting tumorigenesis and suggest that strategies aimed to reduce the activity of this

complex could have roles as anti-cancer therapies. Currently, however, there is no pharmacological way to inhibit mTORC2 without also affecting mTORC1 and the fact that both complexes share the same catalytic domain makes the prospect of developing an mTORC2-specific inhibitor daunting.

Targeting mTOR signaling for cancer therapy

The evidence linking activated mTOR signaling to cancer has generated significant interest in targeting the pathway for cancer therapy and many rapamycin analogs (rapalogs) are now tested in clinic. In 2007, the Food and Drug Administration (FDA) approved the rapalog Temsirolimus for the treatment of advanced stage renal cell carcinoma, becoming the first mTOR inhibitor approved for cancer therapy. Recently, the rapalog Everolimus was approved for the treatment of Tuberous Sclerosis Complex, a relatively rare genetic disease caused by mutations in *Tsc1/2*, in which patients develop non-malignant tumors in many organs, including the brain. There are an important number of clinical trials underway using rapalogs, which have shown promise in several malignancies that are often refractory to standard chemotherapies (reviewed in (Wander et al., 2011)).

While rapalogs have had some success in the clinic, they have shown only modest efficacy in tumors where they were expected to provide important benefits. Substantial work in pre-clinical models of cancer suggested that loss of PTEN or Von Hippel–Lindau (VHL) might represent biomarkers of rapalog sensitivity (Neshat et al., 2001; Thomas et al., 2006). Unfortunately, in the clinical setting the situation has clearly turned out to be more complex so that the identification of biomarkers that predict which tumors will respond to rapamycin-like molecules remains an unmet goal.

The presence of numerous negative feedback loops in the mTOR pathway may contribute to limit the therapeutic efficacy of rapalogs (Figure 3B). When activated by mTORC1, S6K1 directly phosphorylates the insulin receptor substrate-1 (IRS1), which promotes IRS1 degradation and reduces the ability of growth factors to signal downstream of receptor tyrosine kinase (RTK) (Harrington et al., 2004; Um et al., 2004) (Figure 3C). Additionally, mTORC1 negatively regulates growth factor signaling by directly phosphorylating IRS1 (Tzatsos and Kandror, 2006) and the RTK inhibitor growth factor receptor-bound protein 10 (Grb10) (Hsu et al., 2011; Yu et al., 2011) as well as by reducing the expression of the platelet-derived growth factor receptors (PDGFRs) through an unknown mechanism (Zhang et al., 2007). Although our understanding of the feedback loops from mTORC1 to RTK signaling has progressed, it is important to point out that formal evidence is missing showing that the activation of feedback signaling by rapamycin and its derivates limits the therapeutic potential of these molecules.

Another more likely reason why rapamycin may have limited efficacy in cancer treatment is the increasing realization that rapamycin only partially inhibits the phosphorylation of 4E-BP1 (Chresta et al., 2010; Feldman et al., 2009; Garcia-Martinez et al., 2009; Thoreen et al., 2009; Yu et al., 2009). As discussed above, the 4E-BP1/eIf4E axis plays an important role in cancer by controlling translation of various transcripts that promote cell proliferation and tumorigenesis.

With the rationale that the inhibition of both mTORC1 and mTORC2 would have a greater impact on cancer cells, several groups developed small molecules that directly inhibit mTOR kinase activity (Chresta et al., 2010; Feldman et al., 2009; Garcia-Martinez et al., 2009; Thoreen et al., 2009; Yu et al., 2009). These molecules, which function as ATP-competitive inhibitors of mTOR, block the phosphorylation of all known downstream targets of mTORC1 and mTORC2. As anticipated, these inhibitors do impair cell growth and proliferation *in vitro* and tumor growth *in vivo* to a much greater degree than rapamycin

(Falcon et al., 2011; Feldman et al., 2009; Garcia-Martinez et al., 2009; Janes et al., 2010; Thoreen et al., 2009; Yu et al., 2010; Yu et al., 2009). Because mTORC2 positively regulates Akt, it was originally thought that the suppression of mTORC2 by the mTOR kinase inhibitors would play a significant role in their greater effects compared to rapamycin (Figure 3D). Surprisingly, however, even in mTORC2-deficient cells these inhibitors cause a greater reduction in proliferation than rapamycin (Feldman et al., 2009; Thoreen et al., 2009). This led to the realization that mTOR kinase inhibitors exert their anti-proliferative effects primarily through suppression of rapamycin-resistant functions of mTORC1. Unlike rapamycin, these inhibitors completely block 4E-BP1 phosphorylation, which results in a stronger inhibition of cap-dependent translation. Moreover, mTOR kinase inhibitors induce a significantly broader transcriptional response compared with rapamycin; many genes with roles in tumor biology and metabolism are only affected by complete mTOR inhibition (Wang et al., 2011).

Although these results support the importance of mTORC1 inhibition in mediating the effects of mTOR kinase inhibitors on cancer, this does not mean that mTORC2 is not playing a role. In addition to its role on regulating cell survival downstream of Akt and SGK1, mTORC2 has also been shown to positively control vascular system formation (Guertin et al., 2006) and chemotaxism (Liu et al., 2010). This raises the possibility that mTORC2 inhibition could impair tumor formation/maintenance by blocking angiogenesis or by reducing the recruitment of immune cells to the tumors. *In vivo* work is needed to verify these hypotheses.

It is important to point out that the efficiency of mTOR kinase inhibitors, like that of rapamycin, may also be impaired by the activation of feedback loops. The elevation of RTK-PI3K-PDK1 (phosphoinositide-dependent kinase 1) activity in response to mTOR kinase inhibitors can promote Akt phosphorylation on Thr308, which may be sufficient to drive cell survival (Peterson et al., 2009). Ongoing clinical trials with mTOR kinase inhibitors will help to determine to what extent these feedback loops can impact the therapeutic potential of these molecules.

The similarity between the catalytic domains of mTOR and class I PI3K has also enabled the development of compounds that simultaneously inhibit both kinases. These molecules, which inhibit mTORC1, mTORC2 and PI3K, decrease the phosphorylation of Akt, S6K1, and 4E-BP1, and may be attractive molecules to target cancers driven by PI3K activation (Figure 3E)(Brachmann et al., 2009). Some studies indicate that such broad inhibition of cellular signaling may hurt normal cells, thus limiting the therapeutic window of these compounds (Janes et al., 2010). Nonetheless, phase I clinical trials with the dual PI3K/mTOR inhibitor NVP-BEZ235 (Novartis) or XL-765 (Exelixis) show promising efficiency in patients with various types of tumors (reviewed in (Vilar et al., 2010)).

mTOR signaling in tissues and its role in metabolic disease

In mammals, the transition between the fasting and fed states affects the circulating amounts of nutrients and growth factors. In turn, these changes determine if tissues orient their metabolism towards anabolic or catabolic processes. For example, high levels of nutrients and growth factors drive glycogen synthesis in muscle and liver, lipid uptake in adipose tissue, while reducing protein breakdown in muscle, gluconeogenesis in the liver, and lipolysis in adipose tissue. Because the mTOR pathway responds to nutrients and growth factors levels, its role in regulating metabolism has been of intense interest during the last few years.

An understanding of the role of mTOR in regulating metabolism *in vivo* has been limited by the fact that whole-body inactivation in mice of key components of the pathway causes

embryonic lethality (Gangloff et al., 2004; Guertin et al., 2006; Jacinto et al., 2006; Murakami et al., 2004; Shiota et al., 2006; Yang et al., 2006). The use of conditional null alleles of genes encoding mTOR pathway components has started to reveal new functions of this pathway in controlling metabolism in various tissues. The following section reviews current knowledge linking mTOR to tissue metabolism by focusing on the role of the pathway in the development of metabolic diseases like obesity, non-alcoholic fatty liver disease, insulin resistance, and type 2 diabetes.

mTOR in the brain – regulation of energy balance

The hypothalamus is an important region of the brain that integrates signals from circulating nutrients (glucose, amino acids, lipids) and hormones (leptin, insulin) to control energy balance. In particular, the arcuate nucleus (ARC) of the hypothalamus is a key node in the complex network that controls energy balance and affects the development of obesity. mTORC1 is active in the ARC and intracerebroventricular administration of leucine or leptin to rats promotes mTORC1 activity and reduces food intake in a rapamycin sensitive fashion (Cota et al., 2006) (Figure 4D). mTORC1 reduces food intake by promoting the expression of the orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the hypothalamus through an unclear mechanism that involves S6K1 (Blouet et al., 2008; Cota et al., 2008). Together, these results highlight the importance of hypothalamic mTORC1 signaling axis for the central regulation of energy balance by nutrients and hormones.

High fat feeding and obesity impair the central anorectic action of insulin and leptin, which likely promote obesity by deregulating the control of energy balance (reviewed in (Cota, 2009)). Interestingly, nutrient overload associated with high fat feeding blocks the ability of leptin to activate hypothalamic mTORC1 and to reduce food intake (Figure 4A)(Cota et al., 2008). This finding supports the possibility that deregulation in mTORC1 signaling could play a role in the development of obesity by favoring resistance to anorectic signals and by promoting hyperphagia following exposure to a high fat diet. Another interesting possibility is that genetic predispositions affecting the activity of mTORC1 in the hypothalamus could directly favor obesity/leanness by modulating the control of energy balance. Whether such predispositions exist is unknown.

mTOR in adipose tissue – regulation of adipogenesis and lipogenesis

mTOR signaling plays a fundamental role in adipogenesis (reviewed in (Laplante and Sabatini, 2009)), the process that leads to the formation of adipose tissue, the most important energy storage site in mammals. *In vitro*, the inhibition of mTORC1 blocks adipogenesis and impairs the maintenance of fat cells (Gagnon et al., 2001; Kim and Chen, 2004; Polak et al., 2008), while mTORC1 overactivation promotes adipogenesis (Zhang et al., 2009). Like with much of mTORC1 biology, there are likely many downstream effectors involved in the control of adipogenesis. S6K1 regulates the commitment of embryonic stem cell to adipogenic progenitors by regulating the expression of early adipogenic transcription factors (Figure 4B)(Carnevalli et al., 2010) and the 4E-BPs control the terminal differentiation of adipocytes through the translational control of the master regulator of adipogenesis, PPAR-γ (Carnevalli et al., 2010; Le Bacquer et al., 2007).

Mice with adipose-specific loss of the mTORC1 are lean and resistant to high fat diet-induced obesity (Polak et al., 2008) and have smaller and fewer adipocytes. On the other hand, mice with adipose-specific loss of mTORC2 have normal fat mass (Kumar et al., 2010), but a defect in adipose tissue Akt phosphorylation that translates into an increase in lipolysis and circulating free fatty acids (FFA).

The expansion of adipose tissue that characterizes the obese state represents the main risk factor for the development of insulin resistance and type 2 diabetes and mTORC1 is highly active in the tissues of obese and high fat-fed rodents (Khamzina et al., 2005; Tremblay et al., 2007; Um et al., 2004). Elevated circulating levels of insulin, pro-inflammatory cytokines, and nutrients (branch-chain amino acids and glucose), represent driving forces that likely promote mTORC1 activity in obese animals (Figure 2 and 4B). In addition to directly contributing to adipose tissue expansion through the activation of adipogenic/lipogenic factors, mTORC1 promotes insulin resistance in adipose tissue through the S6K1-mediated inhibition of insulin signaling (Um et al., 2004). The reduction in the action of insulin in adipose tissue likely exacerbates systemic insulin resistance by promoting FFA release by adipocytes, ectopic fat deposition, and lipotoxicity (reviewed in (Cusi, 2010)).

The high rate of protein synthesis associated with mTORC1 activation may also induce insulin resistance by promoting ER stress and the unfolded protein response (UPR) (Ozcan et al., 2008). ER stress is a condition that prevails in enlarged adipocytes where it impairs insulin signaling through the destabilization of IRS1 by c-Jun N-terminal kinase (JNK) (reviewed in (Hotamisligil, 2010)). To what extend mTORC1 activation in the adipose tissue of obese individuals promotes ER stress and insulin resistance remains to be determined.

mTOR in muscle – regulation of muscle mass, oxidative metabolism, and glucose homeostasis

In addition to responding to many of the same upstream signals described earlier, in muscle, mTORC1 also senses, through unknown mechanisms, mechanical contraction, which stimulates protein synthesis to drive muscle hypertrophy (reviewed in (Philp et al., 2011)) (Figure 4C). In mice, muscle-specific loss of mTORC1 reduces muscle mass and oxidative function and leads to early death (Bentzinger et al., 2008). In such mice, the expression of the mitochondrial transcriptional regulator PGC1-α decreases, which correlates with a reduction in oxidative metabolism. Previous work also points to a connection between mTORC1 and PGC1-α as rapamycin inhibits the complex of PGC1-α with YY1 (Cunningham et al., 2007). Loss of mTORC1 in muscle also reduces the intensity of the negative feedback loop to IRS1, which increases Akt activation and promotes glycogen accumulation in muscles. On the other hand, mTORC2 inhibition in muscle *in vivo* has no structural impact (Bentzinger et al., 2008; Kumar et al., 2008), but does cause a reduction in glucose uptake and thus mild systemic glucose intolerance.

Skeletal muscle is the major site of glucose disposal in response to food intake/insulin and an impairment of glucose uptake in this tissue contributes to type 2 diabetes. The high activation of mTORC1 in the muscle of obese and high fat-fed rodents drives S6K1-mediated feedback inhibition of insulin signaling, which reduces glucose uptake by the muscle and contributes to systemic insulin resistance (Figure 4C) (Khamzina et al., 2005; Um et al., 2004). Beyond its impact on glucose homeostasis, impaired insulin signaling in muscle may also contribute to the muscle loss observed in obesity/insulin resistance by promoting protein catabolism through the expression of ubiquitin ligases by FoxO1 (Wang et al., 2006). Such stimulation of protein catabolism could explain why high mTORC1 activity in the muscles of obese humans and mice does not translate into increased muscle mass. Strangely, despite high mTORC1 activity, high fat feeding, obesity, and type 2 diabetes impairs mitochondrial biogenesis/function in muscles (Mootha et al., 2003; Patti et al., 2003; Sparks et al., 2005). The reason for this paradox is unknown, but highlights the fact that mitochondrial biogenesis/function is not solely controlled by mTORC1 and that other signaling pathways certainly play important roles.

mTOR in the liver – regulation of ketogenesis and lipogenesis

The liver plays a central role in controlling glucose and lipid homeostasis in response to fasting and feeding. mTORC1 controls the hepatic production of the ketone bodies that peripheral tissues use as energy sources during fasting (Sengupta et al., 2010). mTORC1 activity is low during fasting and mice with constitutive activation of mTORC1 in the liver are unable to induce ketogenesis when fasted. mTORC1 impairs the activity of PPAR-α, the master transcriptional regulator of ketogenic genes, by promoting the nuclear accumulation of nuclear receptor co-repressor 1 (NcoR1) (Figure 4D). In addition to its role in controlling the hepatic response to fasting, mTORC1 also promotes anabolism in the fed state by controlling hepatic lipogenesis through the regulation of *Srebp1c* expression (Li et al., 2010; Yecies et al., 2011).

As in muscle and adipose tissue, mTORC1/S6K1 activity is high in the livers of obese rodents, which leads to the degradation of IRS1 and hepatic insulin resistance (Khamzina et al., 2005; Tremblay et al., 2007). The impairment of PI3K-Akt signaling in the liver promotes gluconeogenesis and contributes to the hyperglycemia and the hyperinsulinemia observed in insulin resistance/type 2 diabetes. Obesity is the major risk factor in the development of non-alcoholic fatty liver disease, a condition produced by fat accumulation in the liver and which can lead to serious complications including cirrhosis and hepatocellular carcinoma. The accumulation of triglycerides in the liver of obese humans is associated with the promotion of lipogenesis in hepatocytes (Donnelly et al., 2005). Although highly dependent on insulin for its activation, lipogenesis is paradoxically very active in the liver of insulin resistant rodents. Sustained activation of mTORC1 in response to high circulating levels of nutrients and pro-inflammatory cytokines is likely to exacerbate lipogenesis through the activation of SREBP1. Consistent with this idea, liver-specific deletion of mTORC1 significantly impairs SREBP1 function and makes mice resistant to the hepatic steatosis and hypercholesterolemia induced by a western diet (Peterson et al., 2011). Thus, elevated hepatic mTORC1 could explain why lipogenesis remains active while the suppression of glucose production becomes insulin resistant in the liver of obese/insulin resistant mice and humans (reviewed in (Brown and Goldstein, 2008)) (Figure 4D).

mTOR in the pancreas – regulation of β-cell mass, insulin secretion, and glucose homeostasis

The β-cells of the pancreas secrete insulin in response to nutrients and are essential in regulating glucose homeostasis. The fact that mTORC1 signaling controls growth in response to nutrients has generated interest in the potential role of this signaling node in the regulation of β-cell mass and function. In mice, constitutive activation of mTORC1 in β-cells causes a decrease in blood glucose, hyperinsulinemia, and improves glucose tolerance (Rachdi et al., 2008; Shigeyama et al., 2008). This phenotype is associated with an increase in β-cell size and number and can be reverted by rapamycin, indicating that mTORC1 is a key positive regulator of β-cell function and mass (Figure 4E). S6K1 appears to mediate some of the effects of mTORC1, as mice with loss of S6K1 have small β-cells and are glucose intolerant, hypoinsulinemic, and have impaired insulin secretion (Pende et al., 2000). Loss of mTORC2 in β-cells is linked to the reduction in Akt activity and to the activation of FoxO1, and causes mild hyperglycemia and glucose intolerance due to a reduction in β-cell mass, proliferation, and insulin production/secretion (Gu et al., 2011).

Peripheral insulin resistance and nutrient excess increase the pressure on pancreatic β-cells for the production of more insulin. The high demand for insulin induces β-cell hypertrophy and proliferation and increases the formation of new β-cells from progenitors, which culminates in the elevation of insulin production and secretion. This process is known as β-cell compensation. The chronic pressure on β-cells can ultimately lead to their exhaustion

and the development of type 2 diabetes. mTORC1 activity is elevated in the β-cells of genetically obese or high fat fed mice (Shigeyama et al., 2008). mTORC1 acts as a double edge sword in the regulation of β-cell mass and function in response to nutrient overload/insulin resistance (Figure 4E). Although mTORC1 positively regulates β-cell mass and insulin secretion, sustained activation of mTORC1/S6K1 signaling exacerbates insulin resistance in islets through the feedback inhibition of IRS1 and IRS2, which reduces cell survival and promotes apoptosis (Elghazi et al., 2010; Shigeyama et al., 2008). In support of this model, mice with constitutive activation of mTORC1 in β-cells have increased β-cells mass in the first phase of their life but upon aging become hyperglycemic and hypoinsulinemic due to the loss of β-cells (Shigeyama et al., 2008).

mTOR inhibitors in the treatment of metabolic diseases

Because the chronic activation of mTORC1 in the tissues of obese mice and humans appears to play a role in the development of insulin resistance and type 2 diabetes, the potential of rapamycin to improve metabolic parameters has been tested in a variety of animal models. Unexpectedly, treatment of rodents with rapamycin leads to a profound deterioration of the metabolic profile. Rapamycin reduces adipose tissue size and β-cell mass/function and causes hyperlipidemia, severe insulin resistance, glucose intolerance, and promotes hepatic gluconeogenesis. (Aggarwal et al., 2006; Cunningham et al., 2007; Fraenkel et al., 2008; Houde et al., 2010) (Figure 5A). An impaired metabolic profile was also observed in humans chronically treated with rapamycin (reviewed in (Stallone et al., 2009)). Many reasons could explain the inefficiency of rapamycin to improve insulin sensitivity and glucose/lipid homeostasis *in vivo*. First, the chronic inhibition of mTORC1 modulates many key processes (i.e., protein synthesis, autophagy, mitochondrial function/biogenesis) that are likely required for the maintenance of tissue functions. In this context, any positive effect associated with the reduction in the negative feedback loop from mTORC1/S6K1 to IRS may be Iost due to systemic metabolic dysregulation. A new study also indicates that chronic rapamycin treatment impairs whole-body insulin sensitivity at least by disrupting the integrity of mTORC2 and by blocking the ability of mTORC2-Akt to inhibit hepatic gluconeogenesis (Lamming et al., 2012 - in press). Although the use of rapamycin *in vivo* worsens systemic metabolism, it is reasonable to think that sub-optimal doses of rapamycin could improve metabolism in the context of obesity by normalizing, but not completely inhibiting, mTORC1 (Figure 5B). This strategy could also limit the inhibition of mTORC2-Akt caused by high doses of rapamycin. Following the same rational, it is possible that the antidiabetic drug metformin, which is known to negatively regulate the action of mTORC1, might improve metabolic profile by partially inhibiting mTORC1 signaling. Finally, inhibition of S6K1 downstream of mTORC1 could represent another interesting approach to improve insulin sensitivity without too many side effects. The recent availability of a new specific S6K1 inhibitor could be used to test this possibility (Pearce et al., 2010).

The chronic effect of mTOR kinase inhibitors on tissue metabolism and glucose/lipid homeostasis has not been extensively tested so far. One study reported an elevation in blood glucose in mice treated with the mTOR kinase inhibitor AZD8055 (Chresta et al., 2010), suggesting that these inhibitors may also impair metabolism. The negative impact mTOR kinase inhibitors on systemic metabolism is expected considering that these molecules are much better than rapamycin at blocking mTOR and Akt signaling.

Implications of mTOR signaling in neurodegeneration

Neurodegenerative diseases, such as Parkinson disease, Alzheimer disease, Huntington disease, amyotrophic lateral sclerosis, and frontotemporal dementia, are all associated with permanent loss of neuronal structure and functions. Genetic predispositions and aging represent the main risk factors for these diseases and a key pathological hallmark shared by

many of them is the accumulation of toxic protein aggregates, also known as inclusions. The inability of neurons to clear mutant and/or misfolded proteins leads to their aggregation and to the cellular damage that ultimately causes cell death.

Many lines of evidence now suggest that intracellular protein degradation pathways like autophagy and the ubiquitin-proteasome system are deregulated in neurodegenerative diseases and may play key roles in the etiology of these pathologies (reviewed in (Rubinsztein, 2006)). Because mTORC1 signalling is recognized as the most important regulator of autophagy, its implication in neurodegenerative diseases has been intensively investigated over the last decade. Autophagy serves as a major degradation pathway for the clearance of various aggregate-prone proteins and defects in the activation of autophagy are common to many neurodegenerative disorders. Additionally, deletion of the essential autophagy gene *Atg5* or *Atg7* in the central nervous system of mice promotes the accumulation of polyubiquitinated proteins and neurodegeneration, even in the absence of any disease-associated mutant proteins (Hara et al., 2006; Komatsu et al., 2006). These last observations support the notion that autophagy is essential for the survival of neural cells, and that an impairment of autophagy is implicated in the pathogenesis of neurodegenerative disorders. Interestingly, inhibition of mTORC1 with rapamycin promotes the autophagic degradation of aggregate-prone proteins *in vitro* and reduces the severity of neurodegeneration in several *in vivo* models (reviewed in (Sarkar and Rubinsztein, 2008)). Interestingly, rapamycin also reduces the aggregation of misfolded proteins by slowing protein synthesis, suggesting that other downstream effectors of mTORC1 signaling may play roles in the development of these pathologies (King et al., 2008).

In this context, the emergence of the new generation of mTOR kinase inhibitors is very exciting, as these new tools will help to clarify the role of mTOR signaling in neurodegeneration. Considering that mTOR kinase inhibitors are more efficient than the first generation of rapalogs in promoting autophagy (Thoreen et al., 2009) and blocking protein synthesis (Thoreen et al., 2009; Yu et al., 2009), it is reasonable to believe that these molecules could be even more efficient in treating diseases associated with the formation/accumulation of protein aggregates. From a systemic point of view, the use of mTOR kinase inhibitors over a long period of time could impair tissue and metabolism, as discussed in the previous section. The development of small molecules that selectively modulate the activity of proteins controlling autophagy downstream of mTORC1 represents a possible avenue to induce this process in a more specific fashion.

Implication of mTOR signaling in the aging process

Aging is the main risk factor for the development of various diseases including cancers, type 2 diabetes, cardiovascular and neurodegenerative diseases. Understanding the mechanisms regulating aging may help to delay the development of these pathologies and could ultimately extend human healthspan.

Dietary restriction (DR) is one of the most robust environmental manipulations to expend lifespan in various species (reviewed in (Kapahi et al., 2010)). Because TOR signaling controls cellular responses to nutrient availability, many groups have tested the possibility that this pathway could be an important player in the regulation of life span. Numerous reports indicate that inhibition of TOR activity induces life extension in yeast (Kaeberlein et al., 2005; Medvedik et al., 2007), worms (Vellai et al., 2003), and flies (Bjedov et al., 2010; Kapahi et al., 2004). In yeast, DR does not further extend the lifespan of in absence of the gene *TOR1*, one of the two *TOR* genes in yeast, suggesting that TOR inhibition and DR promote lifespan via a common mechanism (Kaeberlein et al., 2005). A similar effect has been seen in *C.elegans*, where dsRNA against TOR does not extend the lifespan of *eat-2*

mutant worms, which have impaired feeding behavior and represent a genetic model for DR (Hansen et al., 2008). However, rapamycin treatment does slightly extend the lifespan of flies subject to DR (Bjedov et al., 2010). Furthermore, inhibition of one of the principle targets of TOR signaling, S6K, extends the lifespan of an *eat-2 C.elegans* DR model (Hansen et al., 2007). These data suggest that, as with many other pathways (Greer and Brunet, 2009), DR treatment and TOR inhibition promote lifespan via overlapping, yet partially distinct pathways.

Recently, a multi-centric study from the National Institute on Aging, the Interventions Testing Program (ITP) reported that inhibition of mTOR with rapamycin expands maximal and median life span in mice (Harrison et al., 2009; Miller et al., 2010). Interestingly, this effect was observed even when the treatment was initiated late in life, i.e. 600 days, which corresponds roughly to an age of 60 years in humans. Although these results cannot be directly extrapolated to humans, they do suggest that mTORC1 inhibition may be efficient to treat age-related diseases even when the treatment is initiated in middle-aged humans.

As mentioned previously, rapamycin induces a diabetes-like syndrome by reducing β-cell mass and disrupting glucose and lipid homeostasis *in vivo*. Such profound deterioration of the metabolic profile is commonly associated with a reduction but not an increase in life span. The reason for this paradox is unclear but could be due the use of different formulations of rapamycin. In the longevity studies, rapamycin was microencapsulated and added to the food whereas the drug was administered daily by intraperitoneal injection in the other studies. The lower bioavailability and the different pharmacokinetic of the microencapsulated rapamycin have probably limited the exposure of the tissues to the drug, thus reducing its negative impact on metabolism. An exhaustive examination of plasma metabolites and insulin sensitivity in mice treated with rapamycin in the longevity studies is required to clarify this important issue. Overall, these observations support the idea that the dose of rapamycin has to be carefully adjusted in order to get benefic effects on both longevity and metabolism.

How mTORC1 inhibition increases longevity in mammals is an unresolved issue. In mice, rapamycin-mediated life extension is not associated with change in disease patterns or causes of death (Harrison et al., 2009; Miller et al., 2010). This indicates that rapamycin is likely to increase life span by slowing down age-related pathologies. It is possible that mTORC1 inhibition could prevent tissue degeneration by improving stem cell function. Chen *et al.* observed that old mice have elevated mTORC1 signaling in hematopoietic stem cells (HSC) and that induction of mTORC1 by *Tsc1* loss induces premature aging in HSC (Chen et al., 2009). Importantly, reducing mTORC1 signaling with rapamycin restores HSC self-renewal and hematopoietic function, improves immunity, and increases life span. This result confirmed previous observations showing that inhibition of mTORC1 prevents stem cell exhaustion and increases stem cell function *in vivo* (Castilho et al., 2009; Yilmaz et al., 2006). Interestingly, mTORC1 activity is also elevated in the liver of old mice. Such elevation in mTORC1 signaling impairs fasting-induced ketogenesis, which is a common phenotype associated with aging (Sengupta et al., 2010). Whether specific tissues or cell types play dominant roles in the effect of mTORC1 inhibition on longevity is unknown.

Which effectors downstream of mTORC1 modulate the aging process is still unknown (Figure 6). A reduction in S6K activity increases life span in various species and, in mice, S6K1 loss increases resistance to age-related pathologies (reviewed in (Kapahi et al., 2010)). The other classic mTORC1 substrate 4E-BP has also been shown to regulate the aging process. In flies, loss of 4E-BP reduces life extension induced by DR, whereas overexpression of a gain-of-function form of 4E-BP is sufficient to extend life span under rich nutrient conditions (Zid et al., 2009). The attenuation of mRNA translation, ribosome

biogenesis, and protein synthesis downstream of the mTORC1-S6K1 and -4E-BP1 axis, probably plays an important role in regulating life span as impairing these processes extends life in several species (reviewed in (Kapahi et al., 2010)). Importantly, mTORC1 may also control life span through complementary mechanisms that are not associated with modulation of protein synthesis. For instance, the promotion of autophagy linked to mTORC1 inhibition could mediate some effects of mTORC1 on longevity. Substantial evidence indicates that suppression of autophagy in worms blocks the life span extension mediated by TOR inhibition (Toth et al., 2008). Inducing autophagy could reduce aging by favoring the degradation of aberrant proteins and damaged organelles that are accumulating over time and impairing cellular fitness.

Perspectives

The last decade has seen a rapid rise in interest in and knowledge about the mTOR pathway. Much surely remains to be discovered, but we now know enough about the pathway and its function in normal and disease physiology that efforts to modulate it for therapeutic benefit can move forward in a more reasoned fashion. It is actually quite amazing how much has been learned using just rapamycin, considering how, in retrospect, its capacity to partially inhibit mTORC1 and mTORC2 and trigger numerous feedback signals greatly complicates the interpretation of its cellular effects. Undoubtedly, direct catalytic inhibitors of mTOR will continue to have a major impact on our understanding of the pathway and have already solved long-standing mysteries, like the mTOR-dependence but rapamycin-resistance of 4E-BP phosphorylation. It remains to be determined how broadly useful such molecules will be in the clinical setting. As discussed here, many common pathological conditions, including cancer and neurodegeneration, might benefit from mTOR inhibition. However, given the central role of mTOR in the basic physiology of all growing and dividing cells, it is also likely that very strong inhibition of mTOR will have considerable adverse effects in human beings. Although such effects may be tolerable in the treatment of acutely life-threatening diseases, like cancer, they may be more problematic for chronic conditions requiring long treatment times. In fact, it may be that partial inhibition of mTORC1 and mTORC2, as caused by rapamycin, is about as much mTOR inhibition that can be tolerated in a chronic setting. If that turns out to be the case, a better understanding of the functions of the mTOR-interacting proteins might allow for the development of allosteric modulators of the mTOR complexes that perturb their function towards only certain effectors.

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Abbreviation list

ACC	acetyl-coA carboxylase
ACLY	acyl-coA lyase
Bcl-2	B-cell CLL/lymphoma 2
Bim	BCL2-like 11
CBP80	cap binding protein 80
CDK2	cyclin-dependent kinase 2

cMyc	v-myc myelocytomatosis viral oncogene homolog
eEF2K	eukaryotic translation elongation factor 2 kinase
eEF2	eukaryotic translation elongation factor 2
Fas-L	Fas ligand
FASN	fatty acid synthase
FAT domain	FAT-carboxy terminal domain
FATC domain	FRAP-ATM-TTRAP domain
FRB domain	FKBP12-rapamycin binding domain
G6PD	glucose-6-phosphate dehydrogenase
GLUT4	glucose transporter 4
HEAT repeats	Huntingtin-Elongation factor 3-regulatory subunit A of PP2A-*TOR1* repeats
Mek	mitogen-activated kinase kinase
MMP9	matrix metallopeptidase 9
NF1	neurofibromatosis type 1
p27Kip	cyclin-dependent kinase inhibitor 1B
PDCD4	programmed cell death 4
PGD	phosphogluconate dehydrogenase
PIP2	phosphatidylinositol (4,5)-biphosphate
PIP3	phosphatidylinositol (3,4,5)-triphosphate
Ras	RAt Sarcoma
Rho	rhodopsin
SCD1	stearoyl-Coenzyme A desaturase 1
SKAR	S6K aly/REF-like target
Sos	son of sevenless

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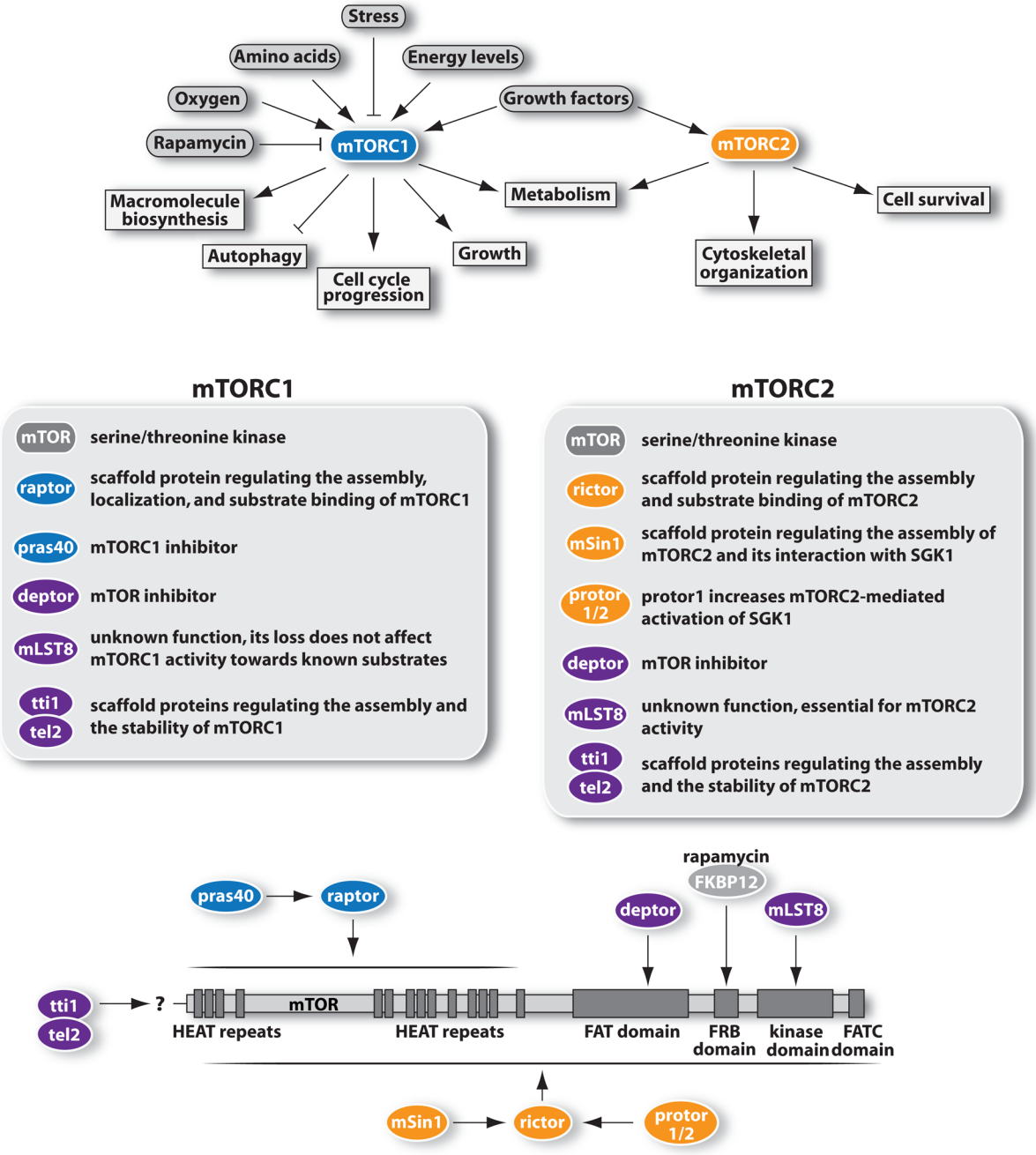

Figure 1. Overview of mTORC1 and mTORC2

The mTOR kinase nucleates two distinct protein complexes termed mTORC1 and mTORC2. mTORC1 responds to amino acids, stress, oxygen, energy, and growth factors and is acutely sensitive to rapamycin. It promotes cell growth by inducing and inhibiting anabolic and catabolic processes, respectively, and also drives cell cycle progression. mTORC2 responds to growth factors and regulates cell survival and metabolism, as well as the cytoskeleton. mTORC2 is insensitive to acute rapamycin treatment but chronic exposure to the drug can disrupt its structure. The middle panel describes the known functions of the protein components that make up the mTOR complexes and the bottom panel schematically

depicts their interaction sites. Refer to the text and abbreviation list for details about the complete name of proteins.

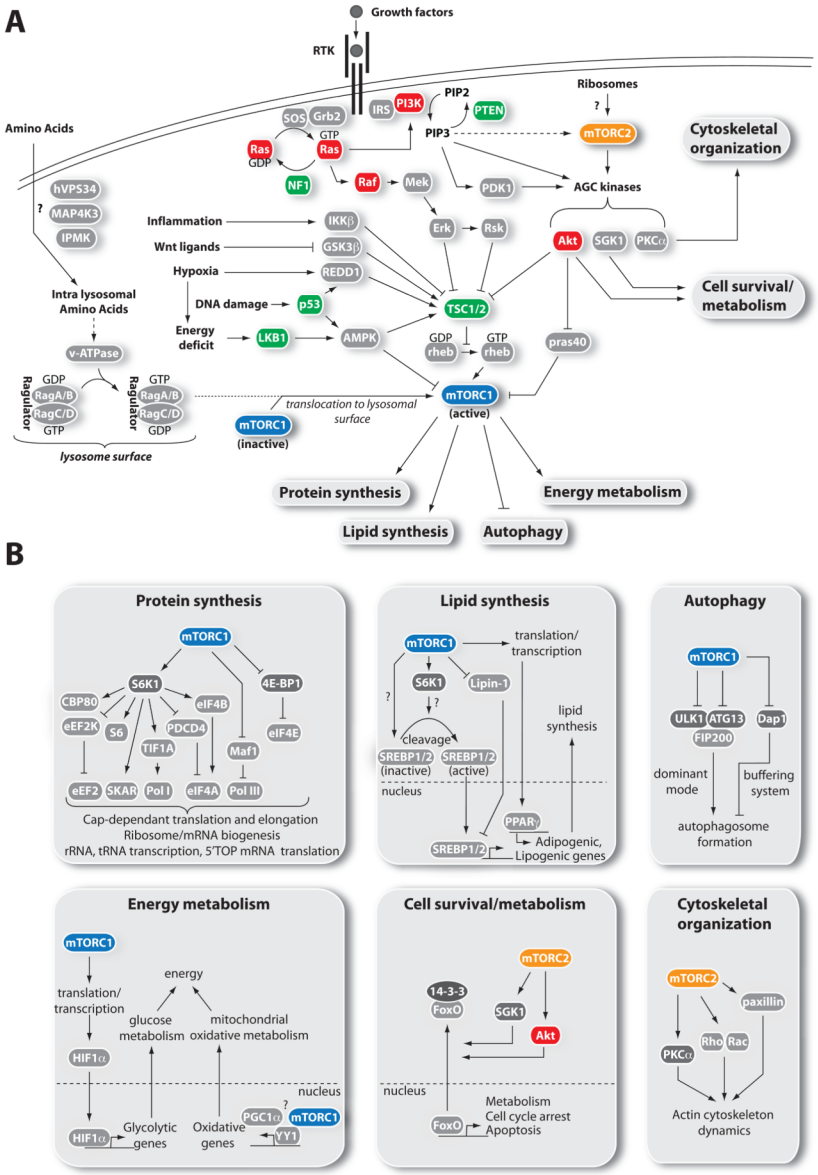

Figure 2. The mTOR signaling pathway

(A) The key signaling nodes that regulate mTORC1 and mTORC2.

(B) The key outputs of the mTORC1 and mTORC2 pathways.

See text for details. Refer to the text and abbreviation list for details about the complete name of proteins.

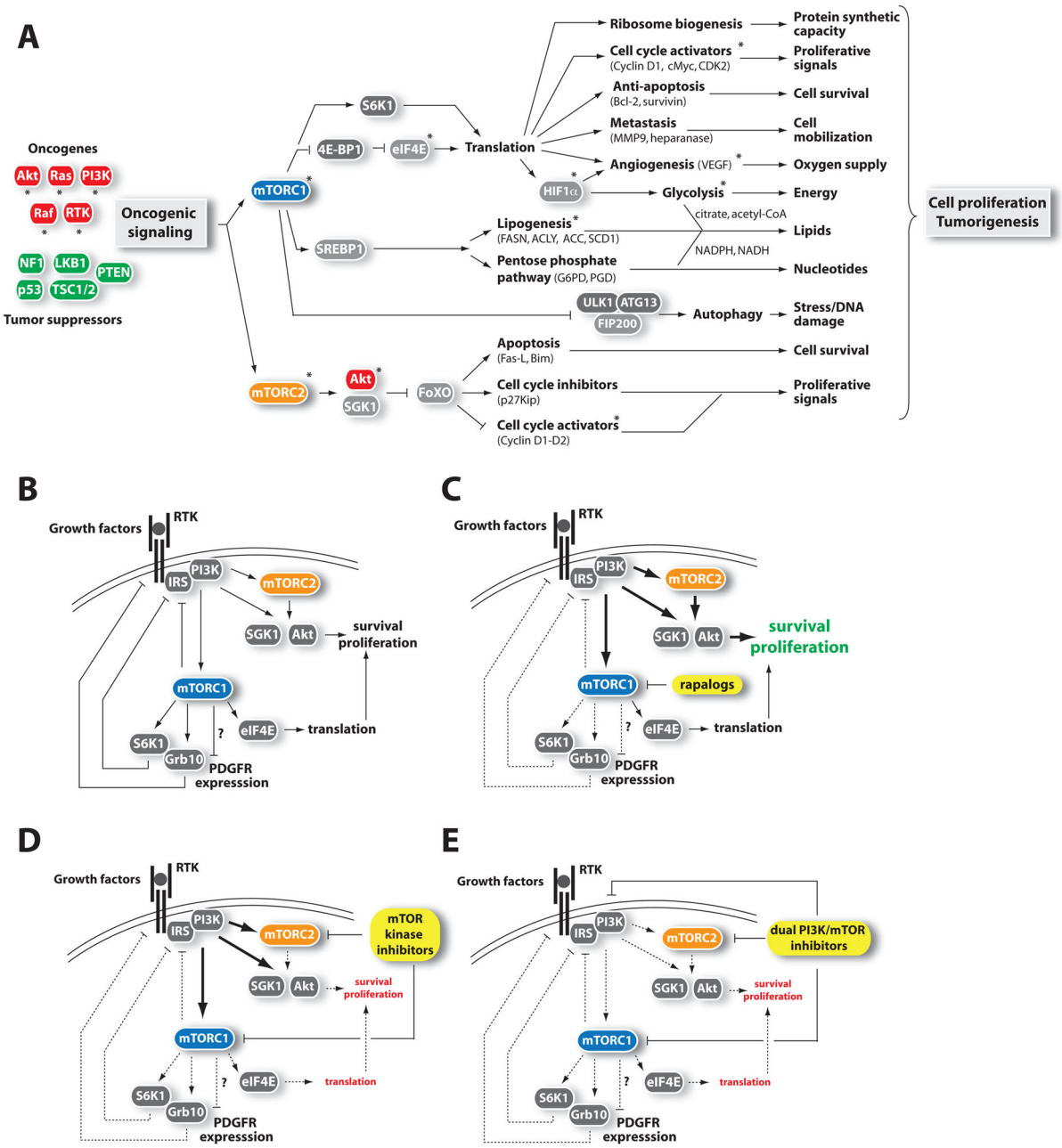

Figure 3. Connections of mTOR to cancer

(A) mTOR signaling promotes tumorigenesis. Oncogenes (red) or tumor suppressors (green) implicated in the control of mTOR signaling are indicated. Asterisk (*) denotes proteins currently targeted for cancer therapy.

(B) mTORC1 controls many negative feedback loops that regulate RTK-PI3K signaling.

(C) The inhibition of mTORC1 by rapalogs reduces the intensity of the negative feedback loops on RTK signaling, which promotes PI3K activation and cell survival. Because the rapalogs only partially inhibit 4E-BP1 phosphorylation, their impact on eiF4E-mediated protein translation is limited.

(D) By completely blocking mTORC1, mTOR kinase inhibitors strongly inhibit the 4E-BP1/eIF4E axis and protein synthesis. Additionally, mTOR kinase inhibitors can affect cell

survival and proliferation by blocking mTORC2-mediated Akt phosphorylation. The elevation in RTK-PI3K-PDK1 activity in response to mTOR kinase inhibitors can potentially reactivate Akt phosphorylation on Thr308, which may be sufficient to drive cell survival.

(E) Dual PI3K/mTOR inhibitors block all known outputs of the PI3K, mTORC1, and mTORC2 pathways.

Refer to the text and abbreviation list for details about the complete name of proteins.

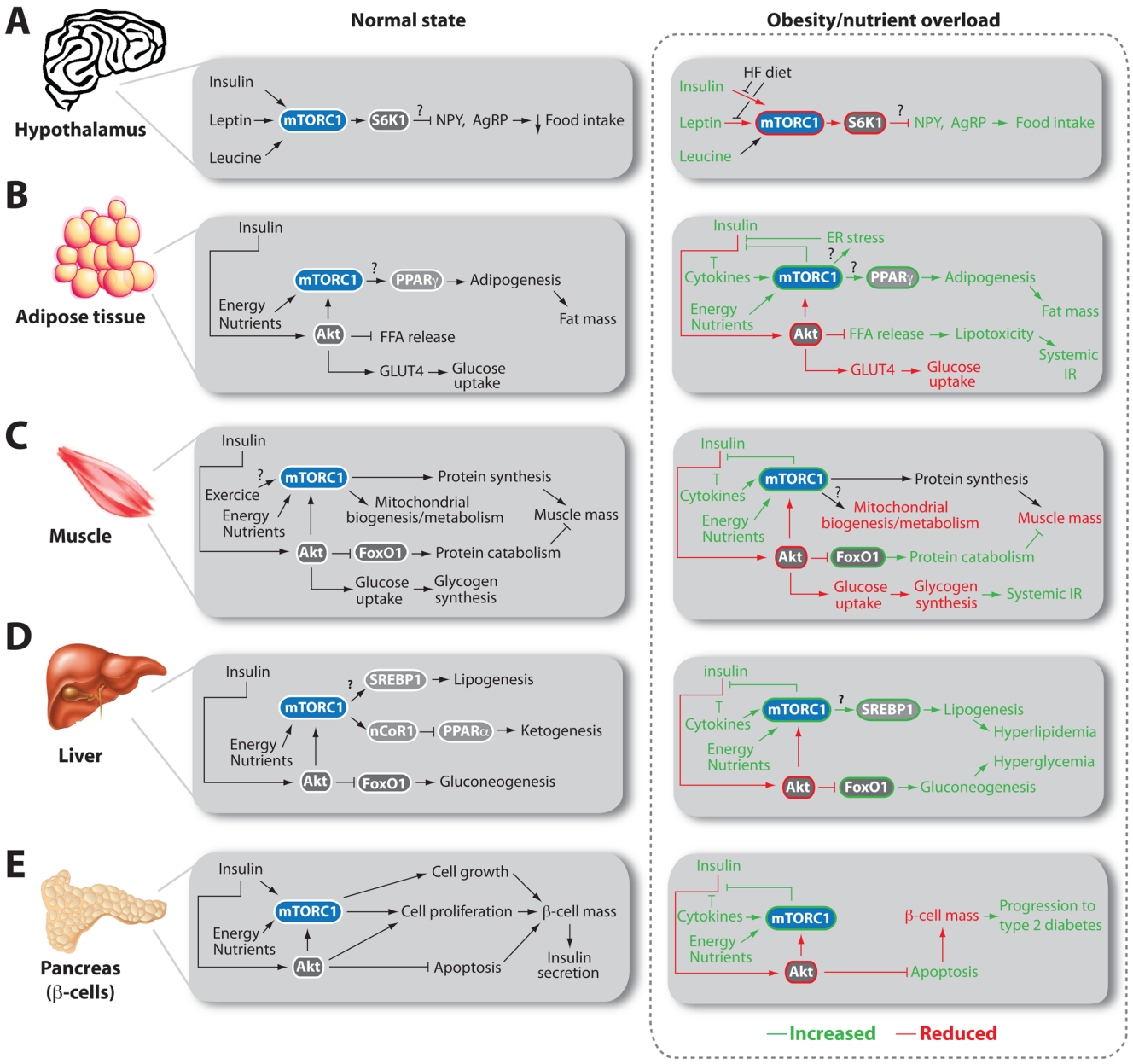

Figure 4. mTOR signaling and metabolism

The roles of mTOR signaling in the regulation of tissue metabolism in the normal (left side) or obese/nutrient overload state (right side).

(A) In the hypothalamus, mTORC1 activation reduces the expression of orexigenic peptides (NPY, AgRP) through an unclear mechanism that involves S6K1. High fat diets reduce the ability of leptin and insulin to promote mTORC1 activity and reduce food intake.

(B) In adipose tissue, mTORC1 activation promotes adipogenesis by activating PPAR-γ. mTORC2-Akt activation reduces lipolysis and promote glucose uptake. High circulating nutrients and cytokines promote mTORC1 activity in obesity, which inhibits insulin signaling and cause insuline resistance (IR) through various mechanisms.

(C) In muscles, mTORC1 play crucial role in regulating protein synthesis, mitochondrial biogenesis, and oxidative metabolism. Muscle contractions increase mTORC1 activity.

mTORC2-Akt activation induces glucose uptake and blocks protein catabolism. Similar to adipose tissue, the elevation of mTORC1 activity by obesity/nutrient overload blocks insulin signaling. The reduction in mTORC2-Akt action promotes protein catabolism and reduces glucose uptake, contributing to muscle mass loss and systemic IR.

(D) In the liver, mTORC1 activation reduces ketone body production by inhibiting PPAR-α activity. mTORC1 also promotes hepatic lipogenesis by activating SREBP1. Alternatively, mTORC2-Akt blocks FoxO1 activity and the activation of gluconeogenesis. Liver mTORC1 activity is elevated in obesity/overfeeding, which promotes hepatic IR, unrestrained gluconeogenesis, and hyperglycemia.

(E) In the pancreas, mTORC1 regulates β-cell mass by promoting β-cell growth and proliferation. mTORC1 is also important for insulin production/secretion. The mTORC2-Akt axis positively affects β-cell mass by promoting proliferation and survival. Obesity/nutrient overload drives mTORC1 activity in β-cells. Sustained activation of mTORC1 ultimately cause β-cell apoptosis by inhibiting Akt signaling. The loss of β-cells favors progression towards diabetes.

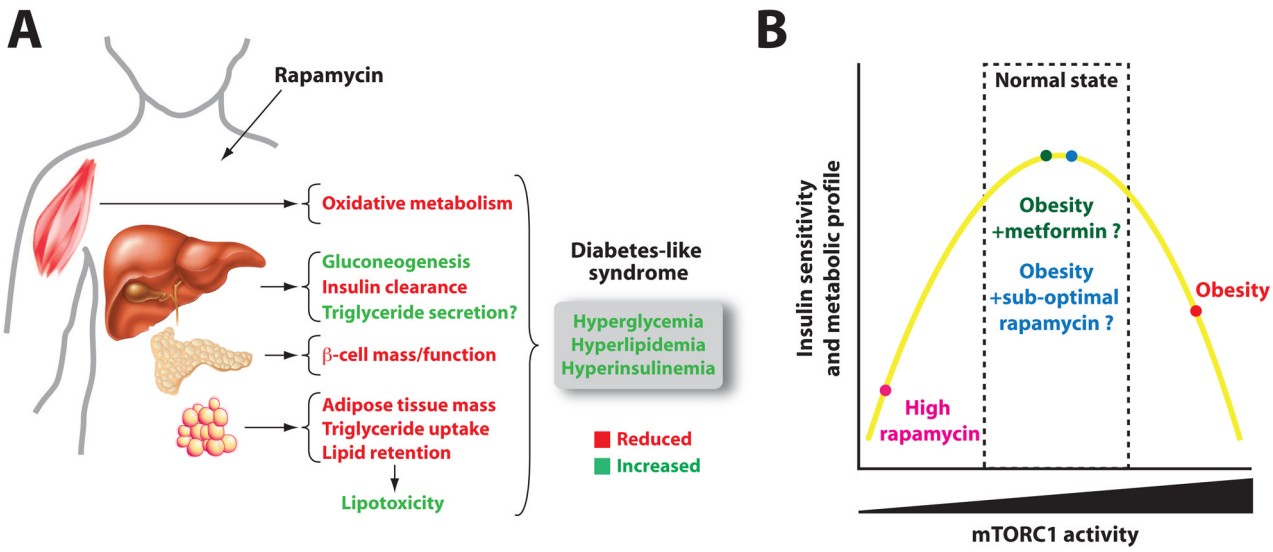

Figure 5. Rapamycin and the treatment of metabolic diseases

(A) Overview of the impact of rapamycin on organ and systemic metabolism. Rapamycin induces a diabetes-like syndrome by impairing the function of the muscles, liver, adipose tissue and pancreatic β-cells. The downregulated processes are in red and those upregulated in green.

(B) Illustration of the hypothesized relation between mTORC1 activation and insulin sensitivity/metabolic profile *in vivo*. The relation between mTORC1 activity and insulin sensitivity/metabolic profile probably follows a U-shaped curve, where too little or too much mTORC1 activity has a negative impact on systemic metabolism.

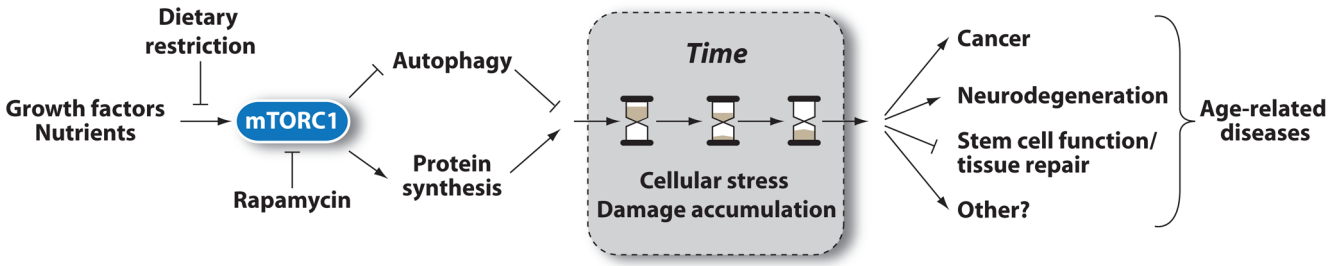

Figure 6. mTORC1 and aging

The activation of mTORC1 by growth factors and nutrients inhibits autophagy and promotes protein synthesis. Over time, this may promote cellular stress (protein aggregation, organelle dysfunction, oxidative stress), which might lead to damage accumulation, a reduction in cell function and thus promote the development of aging-related diseases. Also, mTORC1 activation induces stem cell exhaustion, which reduces tissue repair and promotes tissue dysfunction. Dietary restriction and rapamycin may delay aging and increase longevity by regulating these processes downstream of mTORC1.