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Inhibition of the mechanistic target of rapamycin (mTOR) - Rapamycin and beyond

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Abstract

Rapamycin is an FDA-approved immunosuppressant and anti-cancer agent discovered in the soil of Easter Island in the early 1970s. Rapamycin is a potent and selective inhibitor of the mTOR (mechanistic Target Of Rapamycin) protein kinase, which acts as a central integrator of nutrient signaling pathways. During the last decade, genetic and pharmaceutical inhibition of mTOR pathway signaling has been found to promote longevity in yeast, worms, flies and mice. In this chapter, we will discuss the molecular biology underlying the effects of rapamycin and its physiological effects; evidence for rapamycin as an anti-aging compound; mechanisms by which rapamycin may extend lifespan; and the potential limitations of rapamycin as an anti-aging molecule. Finally, we will discuss possible strategies that may allow us to inhibit mTOR signaling safely while minimizing side effects, and reap the health, social and economic benefits from slowing the aging process.

Introduction

The mechanistic target of rapamycin (mTOR) is a phosphatidylinositol 3-kinase (PI3K)-like serine/threonine protein kinase that is conserved in eukaryotes including yeast, worms, flies and mammals. mTOR was discovered as a result of the search for the target of rapamycin, a polyketide produced by *Streptomyces hygroscopicus* which originally attracted attention due to its ability to inhibit the growth of *C. albicans* and other fungi (Vezina et al. 1975). It was soon determined that rapamycin also activity against mammalian cells, with effects on both cell size and proliferation, leading to its development as an immunosuppressant (Seto 2012). Its immunosuppressive effects led to very cautious exploration of the potential of rapamycin as an anti-cancer agent, but several rapamycin derivatives, including Everolimus and Temsirolimus, as well as rapamycin itself (Sirolimus) are FDA-approved both as immunosuppressants and anti-cancer agents. Rapamycin has attracted significant interest with the finding in 2009 that rapamycin treatment can extend robustly extend the lifespan of mice (Harrison et al. 2009). In this chapter, we discuss the molecular biology of mTOR, research into the mechanism by which mTOR inhibition promotes lifespan, the side effects of rapamycin, and possible ways in which rapamycin or alternative strategies to inhibit mTOR signaling may enable us to extend human lifespan and healthspan.

Molecular biology of rapamycin

The mTOR protein kinase is found in two evolutionarily conserved protein complexes with distinct functions, substrates, and sensitivity to rapamycin (Figure 1). mTOR complex 1 (mTORC1) consists of the mTOR protein kinase, RAPTOR, and mLST8, along with the regulatory proteins PRAS40 and DEPTOR. mTORC1 plays a key role in the regulation of translation and cell growth through substrates that include S6 kinase 1 (S6K1) and the eukaryotic initiation factor eIF4E binding protein 1 (4E-BP1) (reviewed in (Caron et al. 2015)). Other mTORC1 substrates include unc-51 like autophagy activating kinase 1 (ULK1), a key regulator of autophagy, TFEB, a regulator of lysosome biogenesis, and Grb10, an insulin-receptor binding protein (Hsu et al. 2011; Kim et al. 2011; Settembre et al. 2012). The activity of mTORC1 towards many substrates is acutely sensitive to rapamycin, but mTORC1 also possess rapamycin-resistant activity towards certain substrates (Thoreen et al. 2012; Kang et al. 2013).

The activity of mTORC1 is dependent upon its localization to the lysosome by the Rag/Ragulator complex, where it can interact with its activator Rheb, but these proteins are not strictly speaking components of mTORC1 itself (Sancak et al. 2010; Bar-Peled and Sabatini 2014). The regulation of mTORC1 activity is extremely complex, but in brief, the Rag/Ragulator complex recruits mTORC1 to the lysosome when amino acids and glucose are plentiful, while the TSC1/2 complex, which negatively regulates mTORC1 signaling, departs from the lysosome in response to insulin (Bar-Peled and Sabatini 2014; Menon et al. 2014). The regulation of the Rag/Ragulator complex has been a subject of intensive investigation, resulting in the identification of the additional upstream regulators of mTORC1 signaling, including the GATOR1/2 complex and Sestrin1-Sestrin3 (Bar-Peled et al. 2013; Chantranupong et al. 2014).

mTOR complex 2 (mTORC2) consists of mTOR, RICTOR, mLST8, PROTOR1/2, and mSin1, as well as the regulatory protein DEPTOR. In contrast to mTORC1, mTORC2 is relatively resistant to the effects of rapamycin both *in vitro* and *in vivo*, but can be disrupted by prolonged treatment (Sarbassov et al. 2006; Lamming et al. 2012). mTOR complex 2 (mTORC2) regulates a diverse set of substrates downstream of the insulin/IGF-1 receptor, the best characterized of which include AKT on residues T450, S473, and S477/T479, serum/glucocorticoid regulated kinase (SGK) S422, and protein kinase C (PKC)-α (Guertin et al. 2006; Garcia-Martinez and Alessi 2008; Ikenoue et al. 2008; Liu et al. 2014a). More recently, mTORC2 has been demonstrated to regulate control of other PKC family members, including PKCδ and PKCζ, and also regulates the stability of insulin receptor substrate 1 (IRS1) via phosphorylation of the ubiquitin ligase subunit Fbw8 (Gan et al. 2012; Kim et al. 2012; Li and Gao 2014). It is apparent from this diverse set of substrates that mTORC2 is a key effector of the insulin signaling pathway.

While the pathway that mediates activation of mTORC2 by the insulin/IGF-1 receptor is not fully understood, at least some mTORC2 localizes to the mitochondrial-associated endoplasmic reticulum membrane, and the activity of mTORC2 may be dependent upon its association with ribosomal subunits (Zinzalla et al. 2011; Betz et al. 2013). A variety of other proteins, including TSC1/2, P-rex1, Rac1, Sestrin3, and XPLN have also been

implicated in the regulation of mTORC2 (Hernandez-Negrete et al. 2007; Huang et al. 2008; Saci et al. 2011; Khanna et al. 2013; Tao et al. 2014). However, a cohesive model integrating all of these additional proteins is still lacking.

Rapamycin treatment extends lifespan

The role of the mTOR signaling pathway in longevity was first discovered in 2003 in *C. elegans*; mutation of worm mTOR or RNAi against mTOR more than doubled lifespan (Vellai et al. 2003). A similar effect was found in *Drosophila*, where expression of dominant negative mTOR or S6K similarly increased lifespan (Kapahi et al. 2004). The interest surrounding the mTOR signaling pathway increased still further when inhibition of mTOR signaling in yeast was found to increase both chronological and replicative lifespan (Kaeberlein et al. 2005; Powers et al. 2006). Importantly, Kaeberlein and colleagues found that calorie restriction (CR), an intervention that extends lifespan in yeast as well as mammals, was unable to extend the lifespan of long-lived *tor1Δ* yeast.

A CR diet has been the gold standard for lifespan interventions since its discovery in the 1930s, and extends the lifespan of yeast, worms, flies, mice, dogs, and non-human primates (reviewed in (Lamming and Anderson 2014)). The mechanism behind the effects of a CR diet on lifespan have been elusive and highly debated, and the possibility that CR might function by inhibiting mTOR pathway signaling spurred significant effort into understanding how the mTOR signaling pathway regulates lifespan. It also suggested the possibility that rapamycin, as an inhibitor of mTOR, could function as a small molecule CR mimetic and extend lifespan. Indeed, rapamycin was soon shown to extend lifespan in yeast (Powers et al. 2006; Lamming et al. 2007).

While interest in testing rapamycin in flies and worms was intense, and has now been shown to extend lifespan (Bjedov et al. 2010; Robida-Stubbs et al. 2012), investigation of the effects of rapamycin on lifespan jumped directly to mice with the aid of the NIA Interventions Testing Program (ITP). The ITP was able to solve the technical challenges of delivering rapamycin to mice in chow by microencapsulating it in an enteric polymer to protect rapamycin from the acidic environment of the stomach. In 2009, the ITP published the first of several manuscripts on the effects of rapamycin on mice, demonstrating that rapamycin could extend the lifespan of genetically heterogeneous HET3 mice when treatment began at 20 months of age (Harrison et al. 2009). Subsequent studies by the ITP determined that rapamycin had a similar effect on lifespan when delivered starting at 9 months of age (Figure 2A, 2B), and that the response to rapamycin was dose-dependent fashion (Miller et al. 2011; Miller et al. 2014).

In addition to HET3 mice, rapamycin has now been shown to extend the lifespan of both male and female C57BL/6J mice (Fok et al. 2014b; Zhang et al. 2014), male C57BL/6J Rj mice (Neff et al. 2013), female 129/Sv mice (Anisimov et al. 2011), and female FVB/N HER-2/neu mice (Popovich et al. 2014). Fascinatingly, all studies that have compared the effect of rapamycin on both males and females have found that rapamycin promotes longevity in females more effectively than in males (Figure 2C). We will discuss a possible reason for this effect below, but it is interesting to note that many genetic interventions in the

insulin/IGF-1/mTOR signaling pathway also show greater benefits in females than males (Figure 2C). This includes mice null for either *Irs1* or *S6K1* (Selman et al. 2009; Selman et al. 2011) and mice heterozygous for both *mTOR* and *mLST8* (Lamming et al. 2012).

How does rapamycin extend lifespan, and what can it teach us?

Due to the involvement of mTOR in so many key physiological processes, rapamycin has many different biological effects in pathways that are important in health and longevity. Interestingly, while rapamycin initially attracted attention as a CR mimetic, a microarray and metabolome study found that rapamycin and CR have surprisingly divergent effects on gene expression and metabolites in the liver (Fok et al. 2014a). As we have previously detailed (Lamming et al. 2013), the proposed mechanisms by which rapamycin extends lifespan include suppression of cancer, inhibition of translation, maintenance of protein quality, and effects on stem cells. We provide a brief overview of these areas with the latest research on these areas below.

Cancer

Cancer is an important cause of mortality in both mice and humans. Overall, rapamycin and derivatives such as Everolimus and Temsirolimus have been only modestly effective in humans (Miller et al. 2011), although targeted use of rapamycin against cancers with hyperactivating mutations in the mTOR protein kinase shows significant promise (Grabiner et al. 2014; Wagle et al. 2014). Approximately 70% of HET3 mice die from lymphoma, hemangiosarcoma, and lung carcinoma, the frequency of which is not significantly shifted by rapamycin (Miller et al. 2011), suggesting that rapamycin does not prevent cancer. Rapamycin significantly reduces the proportion of 16 month old mice with cancer or precancerous lesions, suggesting that rapamycin does delay cancer in mice, and it has been argued that the effect of rapamycin may be limited to delaying cancer, not aging (Neff et al. 2013). However, it is clear that rapamycin delays many forms of age-dependent changes and preserves healthspan (Wilkinson et al. 2012). While the anti-cancer effect of rapamycin may be important, it likely does not account for the full effects of rapamycin on the aging process.

Protein translation

mTORC1 is an important regulator of protein translation through two distinct mechanisms: the regulation of ribosomal biogenesis via S6K1, and the regulation of mRNA translation mediated by the 4E-BPs. The importance of translation in regulating longevity in model organisms is undisputed, as experiments in *S. cerevisiae*, *C. elegans*, and *D. melanogaster* clearly demonstrate (Kapahi et al. 2004; Hansen et al. 2007; Syntichaki et al. 2007; Steffen et al. 2008; Zid et al. 2009). In these experiments, deletion or RNAi-mediated knockdown of specific ribosomal proteins or translation initiation factors extend lifespan. In yeast, the lifespan extension resulting from reduced expression of large ribosomal subunits is dependent upon the increased translation of the transcriptional activator GCN4, providing a mechanistic explanation for how the efficiency of translation initiation can regulate lifespan (Steffen et al. 2008). However, this has not been demonstrated in higher organisms. Indeed, recent findings in *C. elegans* demonstrate that mTOR pathway inhibition can further

promote longevity in long-lived *C. elegans* with RNAi-depressed translation initiation factors (Hansen et al. 2007; Syntichaki et al. 2007). Moreover, a 50% decrease in protein translation is not sufficient to extend *C. elegans* lifespan (Hansen et al. 2007).

In mice, it is unclear if decreased protein translation is sufficient to extend lifespan. While deletion of *S6K1* significantly extends lifespan (Selman et al. 2009), initial studies found that loss of *S6K1* does not impair protein translation in skeletal muscle (Mieulet et al. 2007). While yeast lacking *Rpl22a* have extended lifespan, loss of *Rpl22* in mice has essentially no effect on translation due to compensatory expression of a paralog, *Rpl22l1* (O'Leary et al. 2013). A more recent study found that rapamycin does decrease skeletal muscle protein synthesis, but the amount of the change is very small, and rapamycin does not decrease protein synthesis in heart (Drake et al. 2013). A study comparing the effect of rapamycin and *S6K1* deletion in multiple tissues of mice found that although a single dose of rapamycin did decrease translation in multiple tissues, chronic treatment with rapamycin for four weeks did not (Garelick et al. 2013). Moreover, mice lacking *S6K1* have normal translational activity and respond normally to rapamycin, suggesting that the benefits of chronic rapamycin on lifespan are not due to decreased translation (Garelick et al. 2013).

Protein quality

Maintaining protein quality is an important challenge during aging. One of the most interesting effects of rapamycin that was recently discovered is that rapamycin treatment of aged mice rejuvenates the aging heart proteome. Despite increased protein half-life, the hearts of rapamycin treated mice had a decreased abundance of damaged proteins (Dai et al. 2014). Such a change could result from increased clearance of damaged proteins.

One of the ways in which damaged proteins are cleared is autophagy, a process in which proteins, especially damaged ones, are broken down to their constituent amino acids. mTOR normally suppresses autophagy by phosphorylating S757 of Ulk1, a kinase required for initiation of autophagy (Kim et al. 2011). In *C. elegans*, autophagy is required for either CR or mTOR inhibition to extend lifespan (Hansen et al. 2008). Autophagy is upregulated during CR in mice, and may mediate the beneficial effects of CR on many organ systems, including the liver (Cuervo et al. 2005; Zhang and Cuervo 2008; Kume et al. 2010; Han et al. 2012). The regulation of autophagy is likely a critical part of how rapamycin promotes lifespan, and it may also impact cancer, as stimulation of autophagy may be an important mechanism of tumor suppression (White et al. 2010).

A second way in which damaged proteins are cleared is proteasome activity, which has been shown to be important in yeast lifespan (Kruegel et al. 2011). Enhanced proteasome activity has also been found in the exceptionally long-lived naked mole rat (Rodriguez et al. 2012). It was recently found that rapamycin boosts proteasome activity in the brains of female mice treated with rapamycin (Rodriguez et al. 2014), suggesting that regulation of proteasome activity may be important for lifespan.

Stem cells and cell senescence

Loss of stem cell proliferative capacity, either due to a decrease in stem cell number or decreased potency, may explain many of the phenotypes of aging. Some of the first work on

mTOR signaling in stem cells was performed with hematopoietic stem cells (HSCs), which exhibit age related declines in self-renewal and function. The function of HSCs declines during aging, and Chen et al. determined that mTOR signaling was elevated in HSCs from aged mice, and that treatment with rapamycin restored self-renewal of aged HSCs (Chen et al. 2009). Similarly, rapamycin treatment or CR increases the self-renewal of aged intestinal stem cells (Yilmaz et al. 2012). More recent experiments conducted *in vitro* have found that rapamycin can preserve mesenchymal stem cell self-renewal and prevent epithelial stem cell senescence (Iglesias-Bartolome et al. 2012; Gharibi et al. 2014). In both cases, this appears to be largely a result of decreased damage from reactive oxygen species rather than more general protection from aging. Stem cells remain an important research area for the biology of aging, and hopefully more *in vivo* data will determine if rapamycin can protect or even rejuvenate other populations of stem cells. *In vivo* data suggests that rapamycin may increase transcription of oxidative stress response genes in *C. elegans* and mouse liver (Robida-Stubbs et al. 2012).

Will the side effects of rapamycin limit its use as a human anti-aging therapeutic?

Although rapamycin shows many beneficial effects in mice, in humans rapamycin and rapamycin derivatives are used primarily as immunosuppressants following organ transplantation, and in the treatment of several specific types of cancer, including renal cell carcinoma, pancreatic neuroendocrine tumors, and HER2-negative breast cancer (Pusceddu et al. 2014). Serious side effects in humans include an increased incidence of viral and fungal infections including pneumonia, chronic edema, painful oral aphthous ulceration, and hair loss (Mahe et al. 2005; McCormack et al. 2011). Metabolic effects of long-term rapamycin treatment have also been observed, including decreased insulin sensitivity, glucose intolerance and an increased risk of new-onset diabetes (Johnston et al. 2008). Finally, rapamycin treatment of mice consistently benefits females more than males, suggesting the possibility that rapamycin treatment of humans may show a similar sexually dimorphic effect on healthspan and lifespan.

Short-term treatment with rapamycin is acceptable in the context of cancer treatment and organ transplantation, and might be acceptable for short-term treatment of specific age-related pathologies. For instance, 10 weeks of rapamycin treatment reverses age-related cardiac hypertrophy and diastolic dysfunction in aged mice while rejuvenating the heart at the level of the proteome (Dai et al. 2014). However, short-term rapamycin treatment is likely to be insufficient in the case of many age-related diseases, including Alzheimer's disease. While prophylactic dosing with rapamycin in mouse models of Alzheimer's disease significantly reduces amyloid-β, plaques, tangles and cognitive defects, dosing older mice has no beneficial effects (Spilman et al. 2010; Majumder et al. 2012). The risks of long-term prophylactic treatment with rapamycin are therefore likely to be unacceptable.

One area in which the side effects of rapamycin treatment may be less important is in the treatment of diseases of rapid aging such as Hutchinson-Gilford Progeria Syndrome (HGPS). HGPS is a rare, fatal genetic disorder resulting from a mutation in *LMNA*, with no

known treatment or cure. The cause of death in most cases of HGPS is progressive arterial occlusive disease, with death from heart attack or stroke occurring at an average age of 13 years (Varga et al. 2006). Treatment of human HGPS fibroblasts and mice lacking *Lmna*, with rapamycin reverses HGPS phenotypes at the cellular level and promotes lifespan and health at the organismal level (Cao et al. 2011; Ramos et al. 2012). While long-term treatment with rapamycin poses risks, the fatal nature of HGPS suggests that clinical trials of rapamycin in HGPS patients should be considered.

The majority of the data on the side effects of rapamycin have come from mice and from relatively sick humans, not from relatively healthy humans, and healthy humans might experience fewer serious side effects. The potential benefits of rapamycin are so large that trials in other mammals, which may be better models for humans, are getting underway. Rapamycin pharmacology studies in a non-human primate, the marmoset, show that rapamycin can be dosed to socially-housed marmosets for over a year without causing anemia, fibrotic lung changes, or mouth ulcers (Tardif et al. 2015). The effects on metabolism and immunity in marmosets, however, are as yet unknown. Also, these marmosets have been maintained in a relatively pathogen-free environment, not the relatively pathogen-rich environment in which humans live. To address some of these issues, a new study at the University of Washington will test the effect of rapamycin on aging phenotypes in pet dogs (Check Hayden 2014). While these experiments have the potentially to significantly advance our understanding of the real-world effects of rapamycin, they must be pursued cautiously, as negative outcomes such as the development of diabetes in pet dogs could taint the public perception of rapamycin as a pro-longevity intervention.

Intermittent rapamycin treatment: a way to sidestep side-effects

How can we utilize the exciting potential of rapamycin to reap the Longevity Dividend? Importantly, recent discoveries suggest that at least some of the negative side effects of rapamycin may be separable from its deleterious side effects. In particular, it was recently discovered that long-term treatment with rapamycin disrupts not only mTORC1, but also disrupts mTORC2 *in vivo* in multiple tissues, including the liver, white adipose tissue, and skeletal muscle (Lamming et al. 2012). Research from many labs has identified many positive roles for mTORC2 in health and longevity, and negative consequences from its disruption.

Specifically, hepatic mTORC2 is important for the regulation of gluconeogenesis, and disruption of hepatic mTORC2 by rapamycin causes hepatic insulin resistance and decreased glucose tolerance (Lamming et al. 2012; Lamming et al. 2014a). mTORC2 is also important in the proper functioning and proliferation of beta cells (Zahr et al. 2008; Yang et al. 2012). The critical role of mTORC2 in the immune system has only been recently uncovered, with mTORC2 playing a role in the function, proliferation and differentiation of T cells, B cells, and macrophages (Haxhinasto et al. 2008; Maier et al. 2012; Powell et al. 2012; Byles et al. 2013). A significant decrease in T cell number and the expansion of Tregs are the likely cause of many of the effects of rapamycin on the immune system (Powell et al. 2012), and mTORC2 activity normal suppresses the differentiation of Tregs (Haxhinasto et al. 2008). Finally, mTORC2 is extremely important in male longevity, and genetic depletion

of *Rictor*, a key component of mTORC2, severely shortens male, but not female, lifespan (Lamming et al. 2014b).

It is possible that this male-specific effect of mTORC2 inhibition on lifespan explains the sexually dimorphic impact of rapamycin and genetic inhibition of insulin/IGF-1/mTOR signaling pathway on lifespan, but the mechanistic basis for this sexually dimorphic effect remains unknown. Many of the physiological effects of hepatic *Rictor* deletion are mediated by reduced Akt activity; however, mice heterozygous for *Rictor*, while having decreased male longevity (Figure 3A), have normal Akt activity (Lamming et al. 2014b). A recent publication examining the lifespan of mice heterozygous for *Akt1* found that these mice had a significant increase in lifespan (Figure 3B) (Nojima et al. 2013). It is therefore likely that one or more additional mTORC2 substrates mediate the decreased male lifespan resulting from *Rictor* depletion. While the role of the mTORC2 substrate SGK in mammalian lifespan is not known, recent findings in *C. elegans* suggest that SGK may play an important role in determining lifespan (Mizunuma et al. 2014).

Since many of the negative side-effects of rapamycin are mediated by inhibition of mTORC2, drugs that specifically inhibit mTORC1 without inhibiting mTORC2 could allow us to realize the full power of rapamycin (Lamming et al. 2013). We have reported that the rapamycin analogs everolimus and temsirolimus have a decreased impact on glucose tolerance in male C57BL/6J mice, suggesting that these analogs may have a reduced impact on mTORC2, but this remains to be proven (Arriola Apelo et al. 2016). Unfortunately, while the scientific literature suggests several compounds specifically inhibit mTORC1, we have found that some of these results may be specific to particular cell lines or time points. Regrettably, the efforts of the pharmaceutical industry have been focused on the development of mTOR kinase inhibitors such as Torin 1, PP242, KU63794, and WYE354 for the treatment of cancer (Liu et al. 2012). These inhibitors are extremely effective at inhibiting both mTOR complexes, and are therefore likely to have increased side effects as compared to rapamycin.

A more promising strategy is the possibility that intermittent rapamycin treatment might be sufficient to extend lifespan, while minimizing the time period that an individual might be immunosuppressed or at risk of diabetes. Recent findings that the effects of rapamycin on (at least) glucose tolerance and mTORC1 signaling can be washed out within a few weeks suggest this may be a feasible approach (Yang et al. 2012; Liu et al. 2014b). A fairly intensive dosing schedule (two weeks on, two weeks off) extends the lifespan of inbred female 129/Sv mice (Anisimov et al. 2011), but this dosing schedule still leads to mice spending more than 50% of their life exposed to rapamycin and subject to glucose intolerance, in addition to any other metabolic and immunological impact. We recently identified an intermittent rapamycin treatment regimen with decreased metabolic and immunological effects (Arriola Apelo et al. 2016), but it remains to be determined if this regimen can extend lifespan and healthspan.

Sustainable dietary interventions to inhibit mTORC1

An alternative approach that has not been fully explored is the possibility of inhibiting mTORC1 by altering the diet. mTORC1, but not mTORC2, is specifically sensitive to glucose and amino acid levels among other stimuli (Bar-Peled and Sabatini 2014). Interventions that focus on either amino acid sensing or on the availability of glucose and amino acids may act to inhibit mTORC1 signaling. A CR diet has been suggested to function in part by lowering fasting blood glucose levels, which is one of the most well-documented, reproducible and widely conserved response to a CR diet in mammals (Lamming and Anderson 2014). Treatment with acarbose, a compound that acts to slow glucose uptake from food, has been shown to extend lifespan (Harrison et al. 2014), and it will be interesting to learn the effect of acarbose on mTORC1 activity.

mTORC1 activity in cultured cells is extremely sensitive to leucine (Long et al. 2005), and in rodents, the branched-chain amino acids - leucine, isoleucine, and valine - promote mTORC1 activity in the liver, skeletal muscle, adipose tissue and the pancreas (Blomstrand et al. 2006; Sans et al. 2006; Li et al. 2011; Xiao et al. 2011). Consumption of leucine also significantly affects mTORC1 activity in humans (Moberg et al. 2014). A short-term protein free diet leads to a significant decrease in mTORC1 signaling (Harputlugil et al. 2014), and we recently found that a low protein diet can inhibit mTORC1 signaling in both the tumors and somatic tissues of a mouse xenograft model (Lamming et al. 2015).

Recent studies have clearly demonstrated that a low protein diet significantly extends rodent lifespan and is associated with reduced cancer and mortality in humans (Levine et al. 2014; Solon-Biet et al. 2014), although it is not clear if this effect is mediated by mTOR signaling. Low protein diets may be an attractive and more sustainable alternative to CR in humans (Fontana and Partridge 2015). A CR diet is extremely difficult for humans in the developed world to maintain, surrounded by the sights and smells of abundant food. In contrast, vegan diets may be maintainable, and it has been suggested that such plant based diets may be particularly low in methionine (McCarty et al. 2009), which when restricted significantly extends the lifespan of rodents (Anthony et al. 2013). Diets restricted in specific amino acids are often used in the treatment of inborn errors of metabolism, suggesting diets with reduced dietary protein or specific amino acids may be sustainable intervention for a large population.

Conclusion

There has been significant excitement over the discovery that rapamycin can prolong rodent lifespan and may be a potent anti-aging drug. While rapamycin is very promising, its side effect profile may limit its clinical utility for the treatment of diseases of aging in humans. While it is still unclear how mTOR inhibition extends lifespan, it appears that many of the side effects are mediated by the “off-target” inhibition of mTORC2, which the beneficial effects are primarily mediated by inhibition of mTORC1. Alternative dosing strategies for rapamycin that limit its effects on mTORC2, or the development of compounds that specifically inhibit mTORC1, may allow us to fully realize the health, social and economic

benefits of slowed aging. While we await these developments, consumption of a low-protein diet may promote health, perhaps in part by inhibiting mTORC1 signaling.

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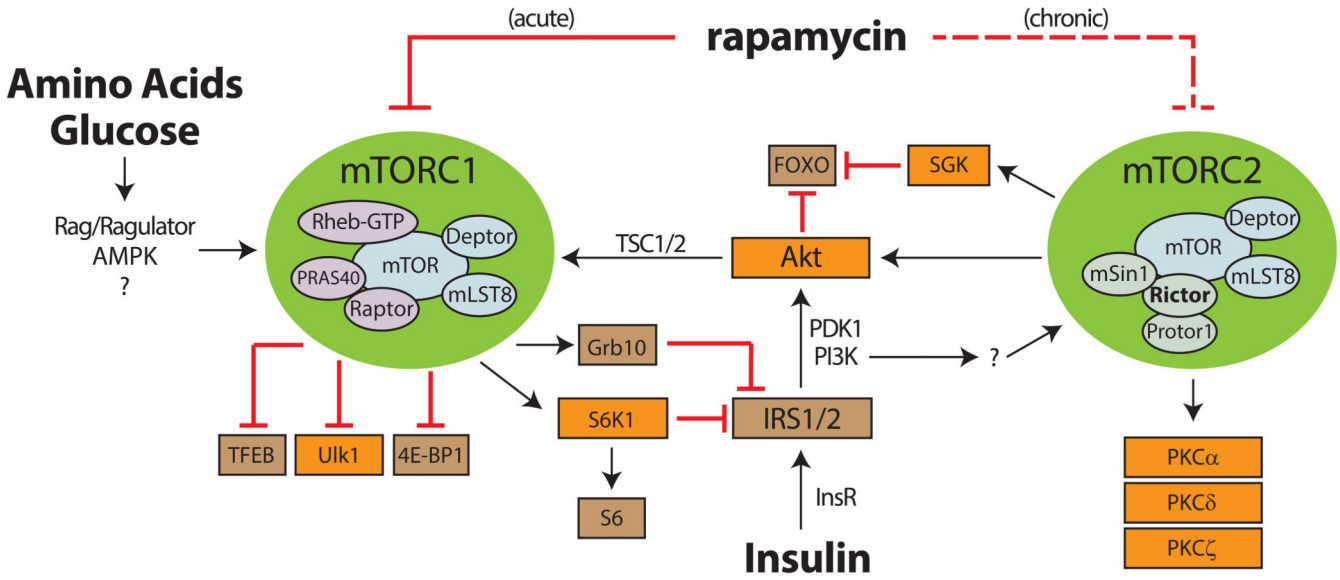

Figure 1.

The mTOR signaling pathway. Rapamycin is an acute inhibitor of mTOR complex 1 (mTORC1), which phosphorylates substrates including S6K1, 4E-BP1, TFEB, Ulk1, and GRB10. Rapamycin dosed chronically also inhibits mTOR complex 2 (mTORC2), which regulates the phosphorylation of Akt, SGK, and members of the PKC family. mTORC2 is primarily responsive to insulin/IGF-1 signaling, while mTORC1 is sensitive to insulin as well as amino acids and glucose.

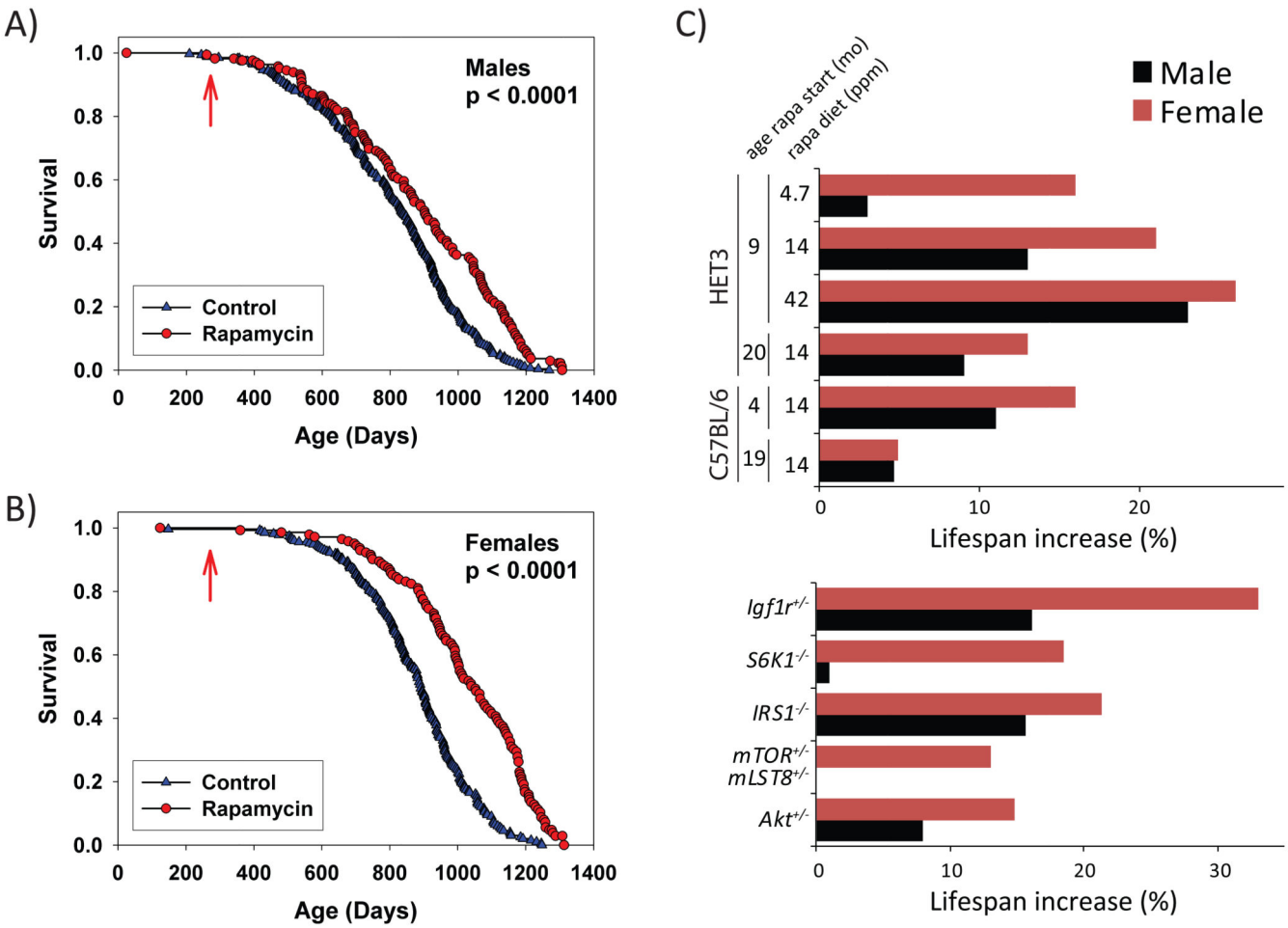

Figure 2.

Sexually dimorphic impact of rapamycin and genetic interventions in the insulin/IGF-1/mTOR signaling pathway. A,B) Treatment with 14ppm rapamycin begun at 9 months of age extends the lifespan of genetically heterogeneous HET3 A) Males and B) Females. C) Rapamycin and genetic interventions in the insulin/IGF-1/mTOR signaling pathway that promote lifespan have a stronger effect on median female lifespan than on male lifespan. Data taken from (Holzenberger et al. 2003; Harrison et al. 2009; Selman et al. 2009; Selman et al. 2011; Lamming et al. 2012; Nojima et al. 2013; Fok et al. 2014b; Miller et al. 2014; Zhang et al. 2014). Mean lifespan is shown for HET3 mice initiated on 14ppm rapamycin at 20 months of age as median is not available. Panels A and B from (Miller et al. 2011), used with permission.

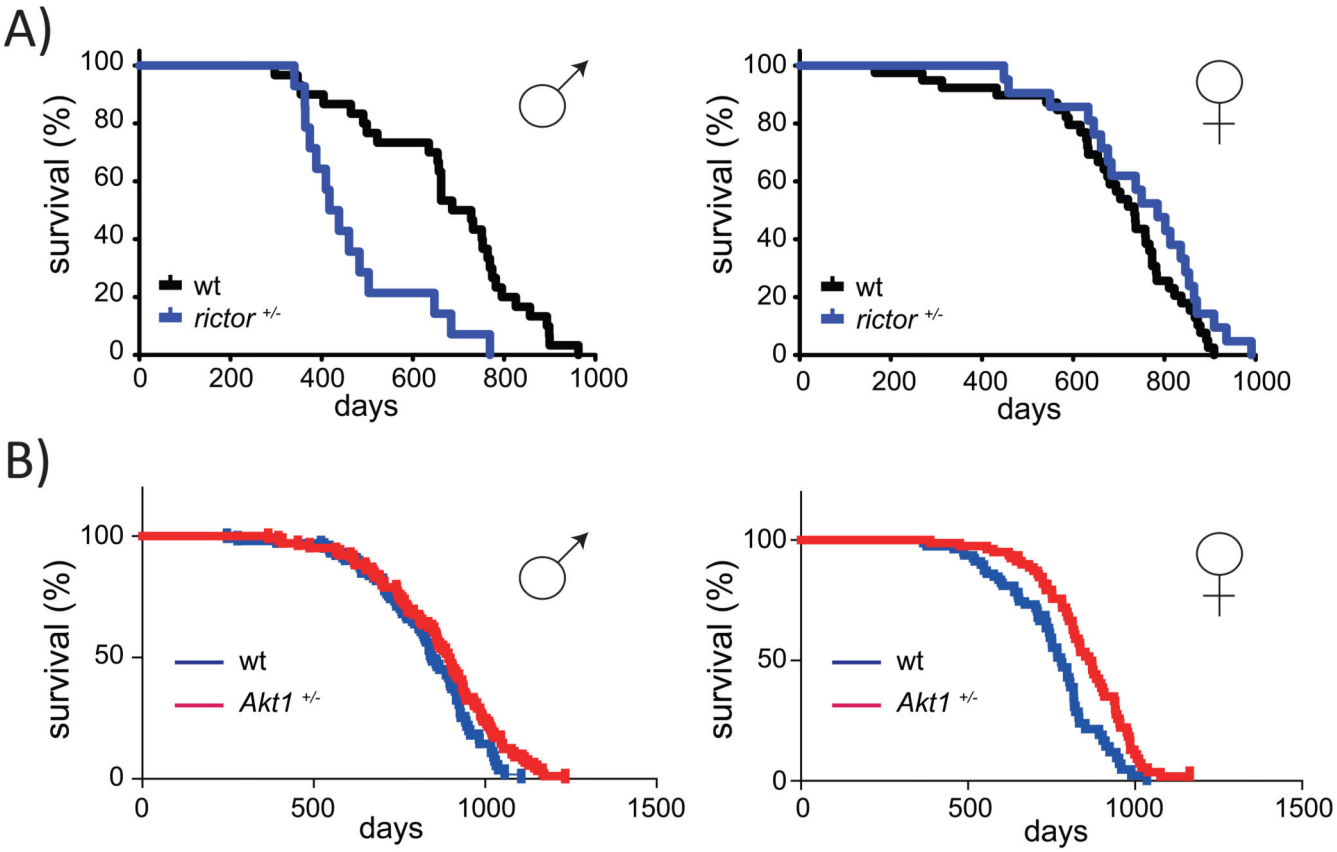

Figure 3.

Haploinsufficiency of *Rictor*, but not *Akt*, significantly decreases male lifespan. A) Kaplan-Meier plots showing lifespans of male and female mice heterozygous for *Rictor*. B) Kaplan-Meier survival plots showing lifespans of mice heterozygous for *Akt*. Panel A from (Lamming et al. 2014b), Panel B adapted from (Nojima et al. 2013), used with permission.