Rapamycin, an inhibitor of mechanistic target of rapamycin (mTOR), has the strongest experimental support to date as a potential anti-aging therapeutic in mammals. Unlike many other compounds that have been claimed to influence longevity, rapamycin has been repeatedly tested in long-lived, genetically heterogeneous mice, in which it extends both mean and maximum life spans. However, the mechanism that accounts for these effects is far from clear, and a growing list of side effects make it doubtful that rapamycin would ultimately be beneficial in humans. This Review discusses the prospects for developing newer, safer anti-aging therapies based on analogs of rapamycin (termed rapalogs) or other approaches targeting mTOR signaling.

A brief history of rapamycin and mechanistic target of rapamycin

Rapamycin was discovered in the soil of Easter Island as a compound produced by Streptomyces hygroscopicus that was capable of inhibiting the proliferation of the yeast Candida albicans but did not affect the growth of bacteria (1). In mammals, rapamycin was found to inhibit the immune response and was subsequently adopted as a standard therapy to prevent graft rejection in transplant recipients and to treat autoimmune disorders (2, 3). Rapamycin also broadly inhibits the growth and proliferation of mammalian cells, spurring more recent interest in its use as a cancer therapy (4).

Mechanistically, rapamycin binds FKBP12, an immunophilin with prolyl isomerase activity. Two additional proteins required for its effects in yeast were identified in a genetic screen in 1991 and termed target of rapamycin 1 (TOR1) and TOR2 (5). During 1994 and 1995, three separate groups isolated a 289-kDa kinase that is bound and inhibited by the rapamycin-FKBP12 complex in mammalian cells (6–8). This kinase is now known as the mechanistic target of rapamycin (mTOR) and is approximately 40% homologous to Saccharomyces cerevisiae TOR proteins and highly conserved among eukaryotes.

mTOR is found in two complexes that have distinct functions and different sensitivities to the action of rapamycin. mTOR complex 1 (mTORC1; consisting of mTOR, raptor, mLST8/GβL, PRAS40, DEPTOR) plays a key role in the regulation of translation and cell growth via phosphorylation of substrates that include S6 kinase (S6K) and eukaryotic initiation factor eIF4E binding protein (4E-BP), and is potently inhibited by rapamycin. In contrast, mTORC2 (consisting of mTOR, rictor, mLST8/GβL, mSin1, protor, DEPTOR) regulates a diverse set of substrates, including AKT S473, serum/glucocorticoid regulated kinase, and PKC-β, and is acutely resistant to rapamycin, although it can become physically disrupted during chronic exposure. mTORCs receive inputs through a wide variety of signaling mechanisms and have roles in many aspects of physiology, which have been reviewed in depth (9). Briefly, mTORC1 responds to signals that include amino acids, glucose, WNT ligands, oxygen, cAMP, and insulin/IGF-1. The regulation of mTORC2 activity is less clear but may involve interaction with ribosomes (10). Insulin/IGF-1 signaling to mTORC1 is mediated in part by mTORC2 via AKT phosphorylation. In turn, mTORC1 activation feeds back to attenuate insulin/IGF-1 signaling via S6K1 and GRB10 (Figure 1 and ref. 11).

Connecting mTOR signaling to aging

A role for TOR signaling in aging was first revealed in 2003, when Vellai and colleagues showed that RNAi against let-363/CeTor significantly extended the life span of Caenorhabditis elegans and functioned independently fromdaf-16, a FOXO homolog that had previously been shown to influence life span (12). This was rapidly followed by the demonstration that genetic inhibition of TOR signaling extends life span in Drosophila melanogaster and the budding yeast S. cerevisiae (13, 14). Genetic inhibition of mTOR signaling in mammals is a delicate matter, as the mTOR protein kinase, raptor, rictor, and mLST8 are all essential for development (15). Recently, we demonstrated that female Mtor+/−/Mist8+/− mice have reduced mTORC1 activity and increased longevity, similar to the phenotype reported by Selman and colleagues for mice that lack S6K1, one of the principal substrates of mTORC1 (16, 17). Therefore, the link between mTOR signaling and longevity appears to be conserved from yeast to mammals (Table 1).

Effects of rapamycin on longevity

Rapamycin extends life span in yeast, worms, and flies (Table 2 and refs. 18–21). In 2009, rapamycin was shown to extend both mean and maximum life spans of male and female genetically heterogeneous mice (offspring of a four-way cross between long-lived, inbred strains) (22). Remarkably, the treatment was not initiated until the mice had reached an advanced age (20 months), roughly equivalent to a human age of 60 years. In a follow-up study beginning at 9 months of age, rapamycin extended median life span in males and females by 10% and 18%, respectively, and maximum life span by 16% and 13% (23). Rapamycin was microencapsulated in an enteric coating that enabled delivery in the food during these studies, and the blood level achieved was approximately three-fold higher than the typical therapeutic range for immunosuppression in humans (24).
Other studies have also found a positive effect of rapamycin on life span. Chen et al. found that rapamycin decreased mortality rate in aged male C57BL/6 mice (25). Anisimov et al. showed that rapamycin extends the maximum life span (mean life span of the last 10% surviving) in a short-lived, tumor-prone strain of mice (FVB/N HER-2/neu transgenic) (26). While this study provides strong evidence that rapamycin can be beneficial in the setting of cancer, the choice of strain makes it hard to separate anticancer effects from aging per se. However, rapamycin also extends life span in 129/Sv mice, an inbred strain with a more typical life span and tumor incidence (27). Impressively, 22.9% of the treated mice remained alive at the death of the last control animal.

Taken together, these observations make rapamycin the best-supported candidate for a mammalian longevity drug. Understanding its mechanism of action has the potential to offer insight into the nature of the underlying aging process and may lead to new therapeutic approaches to alleviate the burden of age-related diseases. However, the mechanism accounting for the anti-aging effects of rapamycin is not yet clear (Table 2).

**Potential mechanisms of life span extension by rapamycin**

**Anticancer effects.** Cancer is the most common cause of death for laboratory mice, and rapamycin is an anticancer drug. Therefore, it remains possible that life span extension by rapamycin is secondary to tumor suppression and unrelated to the underlying aging process. There are several reasons why we do not favor this model. First, the initial experiments linking rapamycin and mTOR inhibition to longevity were performed in organisms that are mainly postmitotic (worms and flies) or single celled (yeast) and therefore do not experience cancer. Second, rapamycin increases maximum longevity, providing support for the idea that it slows multiple age-related pathologies. Targeting a single disease should not substantially increase the life spans of the longest-lived individuals in a group, as the oldest individuals will be at very high risk for most or all causes of death unless the underlying aging process has been postponed. Third, rapamycin has been shown to delay multiple age-related changes in mice, including loss of stem cell function (25), cognitive decline (28), retinopathy (29), accumulation of subcellular alterations in the myocardium, liver degeneration, endometrial hyperplasia, tendon stiffening, and decline in physical activity (30). Moreover, rapamycin is therapeutic in rodent models of cardiac hypertrophy (31, 32) and neurodegenerative diseases (33–35), conditions that affect aging humans. While cancer prevention clearly plays a major role in the survival benefit conferred by rapamycin, it is important to understand that cancer is an age-related disease, and its prevention is an expected consequence of any therapy that slows aging.

**Translation.** mTORC1, via S6K and 4E-BP, plays a central role in the regulation of translation, and it is worth considering whether reduced protein synthesis per se might mediate the effects of rapamycin on longevity. For example, decreasing the overall rate of translation might allow better fidelity during synthesis and/or relieve stress on the mechanisms that degrade erroneous, misfolded, etc.
Recent findings challenge the view that translation per se is the key to the benefits of TOR/mTOR inhibition. While female mice lacking S6K1 have extended life spans, there is no discernible effect on overall translation, at least in skeletal muscle (41). In addition, the long life spans of worms that lack a key translation initiation factor are dependent on \textit{daf-16}, whereas life span extension due to deletion of translation initiation factors is dependent on \textit{S6K} (38). Moreover, life span extension in animals lacking S6K, ostensibly without affecting general translation, rapamycin has a more subtle effect, most likely because a subset of the functions of 4E-BP is rapamycin resistant (42, 43). Both rapamycin and complete mTOR inhibition preferentially suppress translation of mRNAs with 5′ terminal oligo-}

or damaged proteins (36). Indeed, experiments in \textit{S. cerevisiae}, \textit{C. elegans}, and \textit{D. melanogaster} have demonstrated that deletion or siRNA-mediated knockdown of ribosomal subunits, S6K, or translation initiation factors results in increased life span and S6K1 deletion extends life span in female mice, whereas 4E-BP deletion blocks the life-extending effects of caloric restriction (CR) in flies (13, 37–40).

Recent findings challenge the view that translation per se is the key to the benefits of TOR/mTOR inhibition. While female mice lacking S6K1 have extended life spans, there is no discernible effect on overall translation, at least in skeletal muscle (41). In addition, the long life spans of worms that lack a key translation initiation factor can still be further increased by TOR deletion, implying that distinct mechanisms are at play (38). Moreover, life span extension due to deletion of translation initiation factors is dependent on \textit{daf-16}, whereas life span extension by deletion of TOR, S6K, or ribosomal subunits is not, again pointing to the involvement of multiple distinct mechanisms (12, 37). Interestingly, reducing TOR using RNAi fails to further extend the life spans of \textit{eat-2} mutant worms, a model for CR, despite suppressing the already low rate of protein synthesis by an additional 49% (37). Furthermore, inactivation of the worm homolog of AMPK is sufficient to suppress life span extension in animals lacking S6K, ostensibly without affecting translation (16). Clearly the relationship between translation and longevity is more complex than initially supposed.

Translation of specific mRNAs may influence life span. While complete loss of mTOR function has a major effect on general translation, rapamycin has a more subtle effect, most likely because a subset of the functions of 4E-BP is rapamycin resistant (42, 43). Both rapamycin and complete mTOR inhibition preferentially suppress translation of mRNAs with 5′ terminal oligo-

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Genetic manipulation</th>
<th>Resulting change in life span</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yeast</strong></td>
<td>\textit{SCH9} homolog insertional mutant</td>
<td>Increase in mean chronological life span (30%)</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>\textit{SCH9} homolog deletion</td>
<td>Increase in mean chronological life span (30%)</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>\textit{SCH9} homolog deletion</td>
<td>Increase in mean replicative life span (18%)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>\textit{SCH9} homolog deletion</td>
<td>Nonsignificant increase in mean replicative life span</td>
<td></td>
</tr>
<tr>
<td></td>
<td>\textit{TOR} deletion</td>
<td>Increase in mean and maximum replicative life spans (20%)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>\textit{TOR} deletion</td>
<td>Increase in median chronological life span (2.7-fold)</td>
<td>113</td>
</tr>
<tr>
<td><strong>C. elegans</strong></td>
<td>\textit{TOR} (let-363) RNAi</td>
<td>Increase in mean life span (2.5-fold)</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>\textit{Raptor} heterozygous</td>
<td>Increase in mean life span (4%–34%) and maximum life span (39%–60%)</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>\textit{S6K} (rsk1) RNAi</td>
<td>Increase in mean life span (20%)</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>\textit{S6K} (rsk1) deletion mutant</td>
<td>Increase in mean life span (10%)</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>\textit{TOR} (let-363) RNAi</td>
<td>Increase in mean life span (7%–39%; nutrient-rich diet, 25°C)</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>\textit{S6K} (rsk1) RNAi</td>
<td>Increase in mean life span (4%–34%) and maximum life span (7%–39%; nutrient-rich diet, 25°C)</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>\textit{Rictor} (rict-1) deletion mutant</td>
<td>Decrease in median life span (24%–43%) and maximum life span (21%–32%; normal diet, 25°C)</td>
<td>114</td>
</tr>
<tr>
<td><strong>D. melanogaster</strong></td>
<td>\textit{dTSC1} overexpression</td>
<td>Increase in mean life span (14%; males)</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>\textit{dTSC2} overexpression</td>
<td>Increase in mean life span (12% at 29°C, 20% at 25°C; males)</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>\textit{dTSC1} knockdown</td>
<td>Prevention of eclosion</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>\textit{dTSC2} knockdown</td>
<td>Increase in mean life span (24% at 29°C, 26% at 25°C; males)</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>\textit{dTOR} (hypomorph)</td>
<td>Increase in median life span (20%)</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>\textit{d4E-BP} null</td>
<td>Decrease in mean life span (34%; males)</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>\textit{d4E-BP} overexpression</td>
<td>Decrease in mean life span (20%, 18%, and 10%, respectively; females only)</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>\textit{d4E-BP} weak activated</td>
<td>Decrease in mean life span (39%; males)</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>\textit{d4E-BP} strong activated</td>
<td>Increase in mean life span (15%)</td>
<td>115</td>
</tr>
<tr>
<td><strong>M. musculus</strong></td>
<td>Loss of S6K1</td>
<td>Increases in mean, median, and maximum life spans (20%, 18%, and 10%, respectively; females only)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>\textit{Mtor}^{-/-}\textit{Mist8}^{-/-} genotype</td>
<td>Increases in mean, median, and maximum life spans (14%, 13%, and 18%, respectively; females only)</td>
<td>17</td>
</tr>
</tbody>
</table>
Autophagy. Another effect of mTOR inhibition that has been linked to longevity is the induction of autophagy, a process by which cells recycle their proteins and organelles. Autophagy allows cells to survive nutrient-limited conditions and is a central mechanism by which damaged components are removed. Under conditions of nutrient sufficiency, mTOR phosphorylates and inhibits the autophagy-initiating kinase ULK1 (46). Inactivation of genes involved in autophagy decreases life span in yeast (chronological), mice, and Drosophila, and promotion of autophagy in the fly nervous system extends life span (47–49). Furthermore, autophagy is required for the extension by rapamycin of yeast chronological life span (47) and for life span extension by CR or genetic inhibition of mTOR signaling in worms (50).

In mammals autophagy also appears to play a significant role in the aging process. Most dramatically, the induction of autophagy is sufficient to rejuvenate the liver histology and function of aged mice (51). Furthermore, autophagy seems to be upregulated in CR mice, and to mediate some of the beneficial effects of a CR diet on the heart, liver, and kidneys (52–54). Cells from long-lived Snell dwarf mice also show evidence of increased autophagy (55). Cardiomyocytes isolated from aged mice have lower autophagy and exhibit defects in calcium handling, both of which are corrected by exposure to rapamycin ex vivo (56). However, increased autophagy may not always be beneficial, and indeed may contribute to the pro-aging phenotype of progeroid mice (57).

Interestingly, rapamycin ameliorates nuclear blebbing and premature senescence in cells derived from patients with Hutchinson-Gilford progeria, a rare premature aging syndrome (58). The disease results from a misspliced variant of lamin A, termed progerin, that accumulates to a large degree in patients and is also detected in FVB/N HER-2/neu transgenic mice.

### Table 2
Effects of rapamycin on longevity

<table>
<thead>
<tr>
<th>Species</th>
<th>Dosage</th>
<th>Resulting effect on life span</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. cerevisiae</em></td>
<td>0.1–1 ng/ml (0.11–1.1 nM)</td>
<td>Increase in chronological life span (up to 54%; area under the curve for the viable fraction of cells over 7 weeks)</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>1 nM 10 ng/ml (11 nM)</td>
<td>Extension of replicative life span (15%)</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Extension of replicative life span (19%–24%)</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td><em>C. elegans</em></td>
<td>100 μM</td>
<td>Extension of life span (19%)</td>
<td>21</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>1–400 μM</td>
<td>Extension of life span at 50, 200, and 400 μM (females) and 200 μM (males); effects up to 17% and 23% (median and maximum, respectively) on rich media and 54% and 36% during starvation (females)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>1–1,000 μM</td>
<td>Decrease in median life span at 500 μM (8.6% decrease) and 1,000 μM (18.4% decrease) (females; nutrient-rich 10% sugar/10% yeast diet)</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>200 μM</td>
<td>Increase in life span (males, 6.0%; females, 25.6%; standard diet); decrease (males, 11.7%) and increase (females, 56.5%) in life span (low-calorie diet); nonsignificant decrease in life span (males and females; high sugar/low protein diet)</td>
<td>118</td>
</tr>
<tr>
<td><em>M. musculus</em></td>
<td>14 mg/kg diet (~2.24 mg/kg/day)</td>
<td>Increase in mean life span (males, 9%; females, 13%) and maximum life span (males, 9%; females, 14%); treatment initiated at 20 months of age</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>in HET3 mice</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4 mg/kg every other day (intraperitoneal injection) in CS7BL/6 mice</td>
<td>30 weeks after the initiation of treatment 22- to 24-month-old mice, 2 of 10 control and 8 of 10 treated mice were surviving (only males studied)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>14 mg/kg diet (~2.24 mg/kg/day)</td>
<td>Increase in mean life span (males, 10%; females, 18%) and maximum life span (males, 16%; females, 13%); treatment initiated at 9 months of age</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>in HET3 mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5 mg/kg three times weekly for two weeks (subcutaneous), alternating with two weeks uninjected, in FVB/N HER-2/neu transgenic mice</td>
<td>Nonsignificant increases in mean and median life spans; increase in maximum life span (11%; only females studied)</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>1.5 mg/kg three times weekly for two weeks (subcutaneous), alternating with two weeks uninjected, in 129/Sv mice</td>
<td>Nonsignificant increases in mean and median life spans; increase in maximum life span (7.8%; only females studied)</td>
<td>27</td>
</tr>
</tbody>
</table>

Species Dosage Resulting effect on life span Reference

- *S. cerevisiae*: 0.1–1 ng/ml (0.11–1.1 nM) Increase in chronological life span (up to 54%; area under the curve for the viable fraction of cells over 7 weeks)
- 1 nM Extension of replicative life span (15%)
- 10 ng/ml (11 nM) Extension of replicative life span (19%–24%)
- *C. elegans*: 100 μM Extension of life span (19%)
- *D. melanogaster*: 1–400 μM (females) and 200 μM (males); effects up to 17% and 23% (median and maximum, respectively) on rich media and 54% and 36% during starvation (females)
- 1–1,000 μM Decrease in median life span at 500 μM (8.6% decrease) and 1,000 μM (18.4% decrease) (females; nutrient-rich 10% sugar/10% yeast diet)
- 200 μM Increase in life span (males, 6.0%; females, 25.6%; standard diet); decrease (males, 11.7%) and increase (females, 56.5%) in life span (low-calorie diet); nonsignificant decrease in life span (males and females; high sugar/low protein diet)
- *M. musculus*: 14 mg/kg diet (~2.24 mg/kg/day) in HET3 mice Increase in mean life span (males, 9%; females, 13%) and maximum life span (males, 9%; females, 14%); treatment initiated at 20 months of age
- 4 mg/kg every other day (intraperitoneal injection) in CS7BL/6 mice 30 weeks after the initiation of treatment 22- to 24-month-old mice, 2 of 10 control and 8 of 10 treated mice were surviving (only males studied)
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review series

in smaller amounts during normal cellular aging (59, 60). Rapamycin appears to stimulate clearance of progerin from diseased cells via autophagy, and thus may limit the normal age-related accumulation of progerin as well. Overall, the appropriate regulation of autophagy is likely to be a critical determinant of healthy aging.

Stem cell maintenance. Rapamycin has a number of interesting effects on stem cell function. Hyperactive signaling upstream of mTORC1 due to deletion of Pten, deletion of tuberous sclerosis 1 (Tsc1), or constitutive activation of AKT reduces the number and functional capacity of HSCs (61–63). Rapamycin treatment can restore normal self-renewal capacity in a subpopulation of mouse HSCs that have spontaneously high oxidative stress and reduced functional capacity (64). More recently, Chen et al. noted that mTORC1 activity is elevated in HSCs derived from aged mice, which display functional deficits reminiscent of those caused by Tsc1 deletion (25). Rapamycin restored functional capacity in HSCs from aged mice and boosted the immune response to influenza virus. Rapamycin also increases intestinal stem cell self-renewal via inhibition of mTORC1 in the adjacent Paneth cells, similar to effects that have been observed in CR animals (65). In addition, rapamycin enhances the reprogramming of somatic cells to generate induced pluripotent stem cells, suggesting a general promotion of stem cell function (66). On the other hand, rapamycin impairs pluripotency, reduces proliferation, and promotes differentiation in human embryonic stem cells (67, 68). In mouse embryonic stem cells, expression of pluripotency markers is more resistant to rapamycin treatment, yet cell size and proliferation are still reduced and differentiation is enhanced (67, 69). Intriguingly, rapamycin depletes leukemia-initiating cells and inhibits both the self-renewal and differentiation capacities of stem cells derived from infantile hemangioma, suggesting a protective effect against cancer stem cells (61, 70). Taken together, these results suggest that rapamycin modulates the behavior of stem cells and generally favors the retention of “stemness” and a more youthful phenotype in the adult stem cells types that have been studied.

Antiinflammatory mechanisms. The original clinical use of rapamycin as an immunosuppressant should not be overlooked when it comes to longevity. Chronic, low-grade inflammation is a feature of aging, and almost every chronic disease has an inflammatory component (71). A complete discussion of the immunological effects of rapamycin is beyond the scope of this Review, and the topic has been covered elsewhere (72). Importantly, the drug has both positive and negative effects on innate and adaptive immunity, with a net outcome that is more complex than simple immunosuppression, as exemplified by its ability to enhance the immunization of aged mice against influenza virus (25).

mTORC2-dependent mechanisms. Despite the high specificity of rapamycin for mTORC1 during acute treatment, chronic exposure can also inhibit mTORC2. This effect was first observed in certain cultured cell lines (73), and we have recently shown that it also occurs in vivo in multiple tissues including liver, muscle, and adipose (see Figure 2). It is currently unclear whether inhibition of mTORC2 plays a role in the pro-longevity effects of rapamycin. Female mice lacking S6K1 and female Mtor+/–Mlst8+/– mice are ostensibly long lived due to impairments in mTORC1-dependent signaling, but data from C. elegans suggest that inhibition of mTORC2 can also promote longevity (21, 74). Interestingly, life span extension by disruption of mTORC1 in worms requires skn-1 (the homolog of mammalian NRF1/2) and daf-16 (the homolog of mammalian FOXO), both transcription factors that control genes involved in stress defenses. Life span extension by rapamycin or mTORC2 disruption, however, requires only SKN-1. Consistent with a role for general stress defenses in the benefits of rapamycin, both worms and flies with impaired TOR function are stress resistant, and induction of NRF1/2 and FOXO target genes has been detected in the livers of mice treated with rapamycin (2 mg/kg daily for two weeks) (20, 21).

mTOR-independent mechanisms

Some in vivo effects of rapamycin may be independent of mTOR. FKBP12 proteins influence sodium and calcium currents in multiple excitable cell types, in part through binding to ryanodine receptors (75, 76). Moreover, rapamycin also binds to FKBP52, and analogs that favor interaction with FKBP52 over FKBP12 exhibit neuroprotective properties (77). Endocannabinoid signaling. Although rapamycin itself has not yet been tested, an intriguing connection between TOR and endocannabinoid signaling was recently described (78). Small molecules analogous to a mammalian endocannabinoid were identified in C. elegans, and depletion of these molecules was associated with life span extension by CR. One specific molecule, eicosapentaenoil
ethanolamide (EPEA), was also found to be lower in worms lacking S6K, and treatment with EPEA suppressed life span extension in both models while conferring increased susceptibility to heat stress. Clearly, there are many twists and turns remaining in the path to understanding life span extension by rapamycin, and the answers may offer insight into the 80-year-old mystery of how CR is able to delay mammalian aging.

**Rapamycin side effects**

Rapamycin is FDA approved for use as an immunosuppressant following transplant surgery and for the treatment of renal cell carcinoma, and has been used as a coating for coronary stents and carcinoma, and has been used as a coating for coronary stents and has clinical utility in these settings, it is unlikely to be approved for use as a preventative measure in healthy individuals due to substantial side effects.

One of the greatest concerns with rapamycin is its ability to suppress the immune system. Rapamycin extends the life spans of mice, but these studies have been performed in pathogen-free facilities. Studies have found that rapamycin boosts the function of the immune system against certain pathogens (80), but human data are often complicated by the frequent use of rapamycin in conjunction with other immunosuppressants. A carefully controlled study of the use of rapamycin in renal transplant recipients found that 34% of patients experienced viral infection, while 16% suffered from fungal infection (24). Clearly, there are significant risks associated with long-term rapamycin treatment outside of a sanitized laboratory environment.

Rapamycin is also very frequently associated with dermatological adverse events. In renal transplant recipients, rapamycin was found to lead to edema in 60% of patients and aphthous ulcers in 55% of patients (24). Mucositis and rash have been observed in other patient populations (79). Rapamycin treatment has been associated with hair and nail disorders, with 90% of patients experiencing alopecia (24), and with loss of testicular function and reduced male fertility in both humans and mice (30, 81).

In addition, rapamycin treatment leads to metabolic changes, including hyperlipidemia, decreased insulin sensitivity, glucose intolerance, and an increased incidence of new-onset diabetes (79, 82). We recently found that rapamycin treatment promotes stem cell self-renewal in the intestinal crypt (65), but chronic rapamycin treatment of humans has also been associated with gastrointestinal events including diarrhea. Cancer and transplant patients are willing to tolerate these side effects as well as anemia, renal toxicity, impaired wound healing, and joint pain because the benefits outweigh the risks (83). However, the trade-offs are far less likely to be considered acceptable by healthy individuals considering preventative measures.
inhibitors are now being developed (88), but even if sufficient selectivity is achieved, these compounds will require many years of development before FDA approval.

Fortunately, a number of FDA-approved compounds reduce mTORC1 activity. The most widely used by far is aspirin, which has been shown to decrease S6K phosphorylation in response to TNF-α signaling (89). Aspirin may act in part by inhibiting the phosphorylation of TSC1 by IKKβ (90), but it was recently demonstrated that aspirin can also activate AMPK (91). AMPK inhibits mTORC1 activity through two independent mechanisms, the activating phosphorylation of TSC2 and the inhibitory phosphorylation of raptor, an essential component of mTORC1 (92, 93). We might therefore expect other compounds that activate AMPK to specifically inhibit mTORC1 activity. In fact, this is the case: activation of AMPK by 5-aminoimidazole-4-carboxamide-1β-d-ribonucleoside (AICAR) results in decreased mTORC1 activity (94). Interestingly, aspirin influences longevity in rodent models, extending the average but not maximum life span of male mice (95), and has been found to decrease cancer-related and all-cause mortality in humans (96).

A screen of FDA-approved compounds for regulators of autophagy identified four compounds that reduce mTORC1 activity without affecting mTORC2: perhexiline, niclosamide, rottlerin, and amiodarone (97). Rottlerin regulates mTORC1 in a TSC-dependent fashion, but the mechanisms of action for perhexiline, niclosamide, and amiodarone are TSC independent (97). At least one natural product, phenethyl isothiocyanate, has also been shown to inhibit mTORC1 activity in a TSC-dependent manner (98). Given the wide variety of factors that can influence signal-}

**Conclusion**

Rapamycin shows significant promise in animal models as a pharmaceutical agent for the treatment of age-related disease. However, the significant side effects limit its long-term utility in humans. Similar problems are likely to emerge for rapalogs and mTOR kinase inhibitors. Moving forward, mTORC1-specific inhibitors that avoid disruption of mTORC2 signaling or that only reduce, rather than abolish, the activity of the mTORC1 pathway, may offer a safer method for the treatment of age-related diseases. The exploration of different dosing regimens for rapamycin and further testing of metformin have significant promise in this regard, but further research will be required to determine whether any of the available strategies for targeting mTOR will ultimately prove beneficial to human longevity and protect against age-related diseases.

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