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S6K1: reducing the RiSKs of aging

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Caloric restriction (CR) can protect against aging and disease in a number of model systems, including primates (Colman et al., 2009). However, the underlying mechanisms by which CR affects mammalian life span remain unclear. In lower organisms, a number of potential mechanisms have been postulated to regulate life span. Included in these are the insulin and insulin-like growth factor (IGF-1) signaling (IIS) pathways and the mammalian target of rapamycin (mTOR) pathway. Although the roles of these pathways in mammalian aging are not well understood, dissection of the insulin signaling pathways using knockout mouse models implicates IIS as a crucial regulator of life span in mice. For example, haploinsufficiency of the IGF-1 receptor (*Igf1r*) and global deletion of insulin receptor substrate 1 (*Irs1*) are both reported to increase longevity in mice (Holzenberger et al., 2003; Selman et al., 2008).

The ribosomal S6 protein kinases (RSKs) phosphorylate ribosomal protein S6 (Rps6) and consist of two subfamilies, $p90^{rsk}$ and $p70^{rsk}$. The ribosomal protein S6 kinase 1 (S6K1) is one of two mammalian $p70^{rsk}$ proteins, acting to converge growth factor, hormonal, nutrient and energy signals in order to maintain cellular homeostasis. S6K1 is a downstream effector of both the IIS and mTOR pathways. Reducing the activity of S6K1 homologs in yeast, nematodes and fruit flies promotes longevity (Stanfel et al., 2009). Reduced mTOR-S6K1 signaling has also been proposed as a crucial determinant of the longevity promoting effects of CR. The recent report that rapamycin (an inhibitor of mTOR complex 1) can increase the life span of mice highlights a role for the mTOR-S6K1 pathway in mammalian aging (Harrison et al., 2009). Several other proteins [e.g. sirtuin 1 (SIRT1), hypoxia-inducible factor-1α (HIF-1α) and AMP-activated protein kinase (AMPK)] have also been suggested to mediate the effects of CR on aging (Guarente and Picard, 2005; Greer et al., 2009). Similar to S6K1, these proteins also respond to alterations in energy balance and stress to maintain cellular homeostasis. However, how S6K1 interacts with these proteins and its relative importance to mammalian aging remains unclear (Stanfel et al., 2009).

A report in a recent issue of *Science* shows that loss of S6K1 increases the life span of female mice. By following *S6K1*$^{-/-}$ mice and their wild-type (WT) littermate controls to term, Selman et al. show that deletion of S6K1 results in an increase in both median and maximum life span in female mice (by 19% and 10%, respectively), but not in male mice (Selman et al., 2009). Interestingly, similar sexual dimorphism has been reported for other long-lived IIS mouse mutants (Holzenberger et al., 2003; Selman et al., 2008). Consistent with their longevity, female *S6K1*$^{-/-}$ mice showed improvements for several age-sensitive biomarkers, including motor and neurological function; general activity and exploratory drive; immunological profile; and bone density. Furthermore, aged female *S6K1*$^{-/-}$ mice also exhibit reduced adiposity, and improved glucose tolerance and insulin sensitization. Similarly, young male *S6K1*$^{-/-}$ mice fed a high-fat diet also displayed reduced adiposity and increased insulin sensitivity relative to WT mice (Um et al., 2004). This finding supports the notion that loss of S6K1 affects similar pathways in both sexes but is only optimal to promote longevity in female mice.

The improvements in age-related pathology that are observed in *S6K1*$^{-/-}$ mice are similar to those seen in WT mice under CR. Selman et al. also compared the hepatic gene expression profiles in *S6K1*$^{-/-}$ mice with those observed in WT mice under long-term CR. Of the genes with altered expression in *S6K1*$^{-/-}$ mice, the gene categories that were most over-represented were found to overlap significantly with those that were over-represented among the CR-regulated genes. The hepatic transcript profiles of *S6K1*$^{-/-}$ mice also show similarity to those reported for long-lived *Irs1*$^{-/-}$ mice (Selman et al., 2008). Considering both comparisons, significant correlations were observed in terms of gene activation and repression. This suggests that S6K1, CR and IIS act through a common mechanism to mediate effects on aging.

The peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC1α), AMPK and SIRT1 (an NAD^+-dependent deacetylase) signaling pathways communicate to regulate downstream targets that are crucial for cellular energy metabolism, which is thought to influence aging (Canto et al., 2009). The expression patterns of target genes in the peripheral metabolic tissues of female *S6K1*$^{-/-}$ mice were consistent with their improved vigor and longevity. For example, PGC1α target gene expression is increased in the liver and muscle of *S6K1*$^{-/-}$ mice. White adipose tissue (WAT) also exhibited increased expression of PGC1α targets, but to a lesser extent. However, *S6K1* null WAT did display a notable increase in the transcripts for the α2 catalytic and β1 regulatory subunits of AMPK. In fact, AMPK activity is increased in the WAT, muscle and liver of *S6K1*$^{-/-}$ mice (Aguilar et al., 2007).

Selman et al. hypothesized that the increase in peripheral AMPK activity may explain the longevity of female *S6K1*$^{-/-}$ mice. The group studied long-lived *C. elegans rsks-1(ok1255)* mutants, which lack the single worm S6K1 homolog (Pan et al., 2007). The *rsks-1* mutants increase phosphorylation of the worm AMPK catalytic subunit AAK-2, a marker of increased AMPK activity. Selman et al. generated *C. elegans* mutants lacking both *aak-2* and *rsks-1* to determine the relative contribution of AAK-2 in mediating the effects of *rsks-1* on longevity. Loss of *aak-2* expression completely reversed the longevity of *rsks-1* mutants. It also reversed the body size and fecundity defects that are usually characteristic of *rsks-1* mutants. These findings imply that loss of S6K1 increases AMPK signaling, which promotes longevity in both worms and mice.

The mTOR or IIS signaling pathways are postulated to regulate life span (Holzenberger et al., 2003; Selman et al., 2008; Stanfel et al., 2009). S6K1 is a common downstream effector of both of these pathways, but its role in aging is undefined. Selman et

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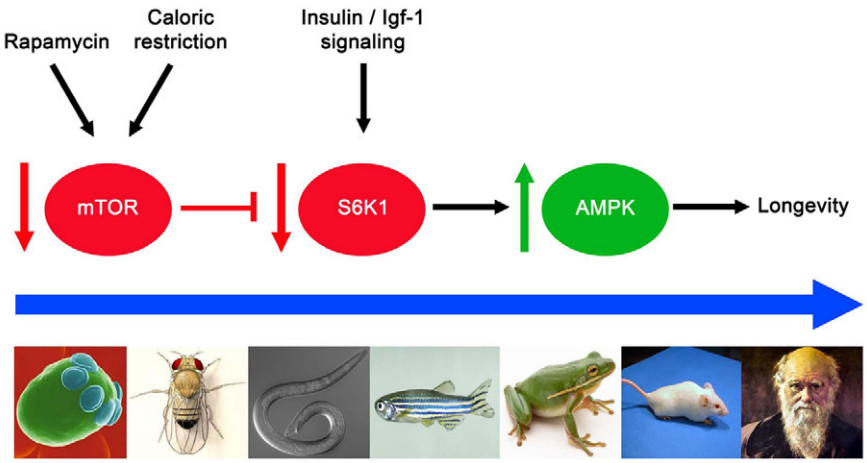

Fig. 1. The activation of AMPK following the loss of S6K1 may be an evolutionarily conserved pathway that protects organisms from aging and aging-associated phenotypes.

al. show that loss of S6K1 or *rsks-1* (in mice or worms, respectively) results in AMPK activation and longevity (Fig. 1). Consistent with the ability of rapamycin to promote longevity more readily in female mice (Harrison et al., 2009), only female *S6K1*$^{-/-}$ mice are long lived. However, activation of AMPK in response to S6K1 loss does not appear to be gender specific (Aguilar et al., 2007). Hence, it would be desirable to determine why AMPK activation is not sufficient to promote longevity in male *S6K1*$^{-/-}$ mice. Regardless, similar gender bias has also been reported for long-lived mouse mutants of the IIS pathways (Holzenberger et al., 2003; Selman et al., 2008). Hence, downregulation of S6K1 may mediate the abilities of both CR and rapamycin to increase life span. However, this raises the question of 'how does loss of S6K1 lead to AMPK activation?' Determining the downstream effects of S6K1 deficiency in mammalian cells will be essential in understanding how AMPK is activated. Both mRNA translation and protein synthesis are modulated by S6K1 in response to mTOR signaling. Reduced mTOR-S6K1 signaling in yeast is associated with a global decrease in mRNA translation, although some mRNAs are still preferentially translated (Steffen et al., 2008). It may be that loss of S6K1 causes preferential translation of certain mRNAs, including that encoding AMPK. Interestingly, AMPK activation in cancer cells following metformin treatment results in downregulation of S6K1 and general translation (Zakikhani et al., 2006). Such findings suggest that a feedback mechanism exists between S6K1 and AMPK to regulate AMPK levels. Understanding this interplay in different cell types will be essential for successful pharmacological intervention aimed at modulating S6K1 and AMPK activity.

SIRT1 activation may also mediate the longevity promoted by CR (Guarente and Picard, 2005). As with AMPK, SIRT1 activation mediates the metabolic response to stress, starvation or CR at the level of transcription through activation of the PGC1α coactivator (Canto et al., 2009). Gene expression in the liver and muscle of *S6K1*$^{-/-}$ mice shows elevation of a number of PGC1α target genes, suggesting increased SIRT1 activity. Resolving the interplay between AMPK and SIRT1 (and possibly other proteins) may be crucial in understanding how mTOR-S6K1-AMPK signaling regulates longevity. One hypothesis is that loss of S6K1 promotes cellular autophagy. In contrast to IIS and mTOR signaling, autophagy is a catabolic process engaged by cells in response to nutrient starvation. Interestingly, autophagy has been reported to mediate the ability of CR to extend life span in *C. elegans* (Jia and Levine, 2007).

This research conducted by Selman et al. highlights an evolutionary conserved role for S6K1 in aging. Perhaps pharmacological modulation of S6K1 and AMPK activity could eventually protect mammals from aging and prove effective in the treatment of aging-related diseases.

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