Biochemical and Biophysical Research Communications 425 (2012) 413–418

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The role of MAP4K3 in lifespan regulation of *Caenorhabditis elegans*

Maruf H. Khan [a], Matthew J. Hart [b,*], Shane L. Rea [a,*]

[a] Barshop Institute for Longevity and Aging Studies, Department of Physiology, University of Texas Health Science Center, San Antonio, TX 78240, USA
[b] Barshop Institute for Longevity and Aging Studies, Department of Molecular Medicine, University of Texas Health Science Center, San Antonio, TX 78240, USA

ARTICLE INFO

Article history:
Received 6 July 2012
Available online 27 July 2012

Keywords:
MAP4K3
Target of rapamycin
mTOR

ABSTRACT

The TOR pathway is a kinase signaling pathway that regulates cellular growth and proliferation in response to nutrients and growth factors. TOR signaling is also important in lifespan regulation – when this pathway is inhibited, either naturally, by genetic mutation, or by pharmacological means, lifespan is extended. MAP4K3 is a Ser/Thr kinase that has recently been found to be involved in TOR activation. Unexpectedly, the effect of this protein is not mediated via Rheb, the more widely known TOR activation pathway. Given the role of TOR in growth and lifespan control, we looked at how inhibiting MAP4K3 in *Caenorhabditis elegans* affects lifespan. We used both feeding RNAi and genetic mutants to look at the effect of MAP4K3 deficiency. Our results show a small but significant increase in mean lifespan in MAP4K3 deficient worms. MAP4K3 thus represents a new target in the TOR pathway that can be targeted for pharmacological intervention to control lifespan.

1. Introduction

Target of rapamycin (TOR) is a central regulator of cellular growth and development. It is sensitive to a variety of modulatory signals including growth factors, nutrients, and stress. In response to these varied inputs, TOR regulates an array of functions including mRNA translation, ribosomal biogenesis, cellular metabolism, senescence and autophagy [1,2]. Interestingly, TOR also plays a central role in aging. The lifespan increasing effect of dietary restriction that has been observed across a diverse range of species spanning the evolutionary spectrum, from yeast to mice, is mediated through downregulation of TOR [3–5]. Downregulation of insulin signaling or IGF-1 signaling also increases lifespan, in part through downregulation of TOR [6]. Similarly, inhibiting TOR by administration of the drug rapamycin has also been shown to increase lifespan [7].

Upregulation of TOR in response to insulin and other growth factors is mediated through PI3K (phosophoinositide 3-kinase) and MAPK (mitogen activated protein kinase) signaling pathways [1]. These pathways activate downstream kinases – PKB (protein kinase B) and ERK (extracellular signaling regulated kinase), respectively, which phosphorylate and inactivate TSC1/2 (tuberous sclerosis complex 1/2), an inhibitor of the small GTPase Rheb [8–11]. Inactivation of TSC1/2 thus leads to activation of Rheb which itself leads to an upregulation of TOR. Another important upstream signal that TOR responds to is availability of amino acids, since amino acids are fundamental nutrients for all cells. In conditions of amino acid depletion, TOR activity is rapidly inhibited [12]. Conversely, amino acid exposure enhances TOR activity, even in the presence of growth factors. Recent studies suggest that this modulation of TOR in response to amino acid sensing is mediated by another kinase, MAP4K3, using an alternate pathway that is independent of Rheb. Specifically, Findlay et al. showed that in HeLa cells activation of TOR in response to amino acid availability requires MAP4K3 [13]. Overexpression of MAP4K3 in serum starved cells was sufficient to activate TOR and this activation was not mediated by PI3K or ERK. Subsequent studies in *Drosophila* showed that MAP4K3 mutants display phenotypes characteristic of low TOR activity, including smaller wing size and slower larval development [14,15]. Interestingly, MAP4K3 mutants showed the same growth delay as wild-type *Drosophila* when cultured on amino acid-restricted food, further supporting the role of MAP4K3 as a probable nutrient sensor.

Given that MAP4K3 appears to be a positive regulator of TOR that promotes cell growth and development, and that inhibition of TOR leads to life extension, an obvious question, that surprisingly has remained unanswered, is does inhibition of MAP4K3 extend lifespan? In this study we have addressed this question using the nematode *Caenorhabditis elegans*, an organism which has contributed many landmark findings to the field of longevity research [16]. With respect to TOR biology, TOR inhibition in worms by siRNA leads to increased lifespan [17]. Mutation in Raptor, an important component of TOR signaling, has also been shown to extend lifespan in worms [18]. More recently Blackwell and colleagues [19] have shown that inhibition of TOR by the drug rapamycin increases lifespan in *C. elegans* and that this life extension is mediated through activation of SKN-1, a transcription factor that is

* Corresponding authors.
E-mail addresses: HartMJ@uthscsa.edu (M.J. Hart), reas3@uthscsa.edu (S.L. Rea).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.113

Table 1
Survival statistics, logrank analysis and COX PH model estimates – *gck-2* RNAi.

N2 + RNAi	N_{TOTAL}	$N_{CENSORED}$	Replicates	Mean life span (days)	Std. error (days)	Logrank vs N2 (*p*-Value)
Vector	113	10	2	22.2	0.5	–
1 in 10	119	18	2	21.7	0.5	0.875
1 in 2	112	11	2	21.6	0.4	0.289
gck-2	112	9	2	24.1	0.3	*<0.001*
COX proportional hazard model estimates						
Covariate (*gck-2 RNAi*)	Coefficient	Std. Error	*p*-Value	Hazard Ratio	95% Conf-L	95% Conf-U
1 in 10	−0.25	0.143	0.081	0.779	0.588	1.032
1 in 2	−0.16	0.144	0.267	0.853	0.643	1.13
gck-2	−0.613	0.144	*<0.001*	0.542	0.409	0.718

* $p < 0.05$ values in italics are significant.

Table 2
Survival statistics, logrank analysis and COX PH model estimates – *gck-2* mutants.

Strain	N_{TOTAL}	$N_{CENSORED}$	Replicates	Mean life span (days)	Std. error (days)	Logrank vs N2 (*p*-Value)
N2	120	15	2	17.8	0.4	–
gck-2(ok2867)	120	18	2	18.3	0.4	0.542
gck-2(tm2867)	120	21	2	19.2	0.5	*0.0106*
COX proportional hazard model estimates						
Covariate (Strain)	Coefficient	Std. error	*p*-Value	Hazard ratio	95% Conf-L	95% Conf-U
gck-2(ok2867)	−0.064	0.14	0.648	0.938	0.712	1.235
gck-2(tm2867)	−0.329	0.141	*0.02*	0.72	0.546	0.949

* $p < 0.05$ values in italics are significant.

the functional ortholog of Nrf-2 and which both act to upregulate protective genes in response to environmental stress and which is the functional ortholog of Nrf-2. Dietary restriction has also been shown to increase lifespan in worms, and TOR is suggested to be involved in mediating this effect [20,21]. In the present study we exploit the fact that *C. elegans* contains only a single MAP4K3 gene (*gck-2)* to investigate the effects of this novel TOR regulator on lifespan.

2. Materials & methods

2.1. C. elegans maintenance

The following worm strains were used in this study: N2 Bristol, VC2167 [*gck-2(ok2867)V*] and TM2537 [*gck-2(tm2537)V*]. TM2537 was obtained from the National Biosource Project for *C. elegans* (Japan). All strains were maintained at 20 °C, on lawns of OP50 *Escherichia coli* spread on NGM agar plates, using standard worm culture techniques [26].

2.2. Feeding RNAi

A *gck-2* feeding RNAi construct was retrieved from the Ahringer RNAi library [27], and corresponds to clone JA:ZC404.9 (target location is shown in Fig. 2A). The identity of this construct was confirmed by sequencing. Feeding RNAi and RNAi dilution testing was performed as described previously [28].

2.3. Lifespan analysis

Lifespan studies were undertaken as described [28]. Briefly, large populations of N2 or mutant animals were synchronized at the L1 stage without the use of bleach, cultured until adulthood on NGM agar plates, then transferred to fresh NGM plates or RNAi plates (~70 worms/condition) for lifespan analysis. Worms were scored every other day. Significance testing was undertaken using the Logrank and Cox Proportional Hazard Model functions of SigmaPlot 11.0 (Systat Software, Inc. San Jose, CA). Survival statistics are summarized in Tables 1 and 2.

2.4. PCR and RT-PCR analysis

Deletion alleles of the *gck-2(ok2867)* and *gck-2(tm2537)* mutant strains were confirmed by PCR using the following primer pair: 5′-CCGTATTGGATGGCTCCGGAAGTTG-3′ and 5′-CCCTGGATAGCTGTTAGTCGATCC-3′. Primer locations and fragment sizes are provided in Fig. 2 and Supplementary Fig. 1. To determine if mutant *gck-2* transcripts were stable, total mRNA (500 ng) was extracted from 100 worms using Trizol reagent (Life Technologies, Carlsbad, CA) and then converted to cDNA using Superscript VILO cDNA synthesis kit (Life Technologies) and the reverse primer of. Semi-quantitative PCR was then undertaken using an ABI GeneAmp 2700 thermal cycler (Life Technologies) and the following primer pair: 5′-ACTTCTGCAACGTGTCGGCTCC-3′ and 5′-TCTCGGCACACGAAGGCGAT-3′.

3. Results

3.1. RNAi knockdown of gck-2 results in a modest enhancement of survival

To determine if inhibition of MAP4K3 lengthened *C. elegans* lifespan, we used a bacterial feeding RNAi to titer *gck-2* mRNA levels in wild type (N2) worms. Three RNAi dilutions were tested; in all instances empty vector-containing HT115 bacteria served as diluent (target to vector ratio – 0:1, 1:10, 1:2, and 1:0). Only the undiluted *gck-2* RNAi resulted in a significant increase in mean lifespan (Fig. 1). This effect was marginal (5%), albeit significant (*p*-value 0.01) and reproducible (Table 1). Interestingly, the increase in mean lifespan was not accompanied by an increase in maximum lifespan, suggesting *gck-2* RNAi improved healthspan only.

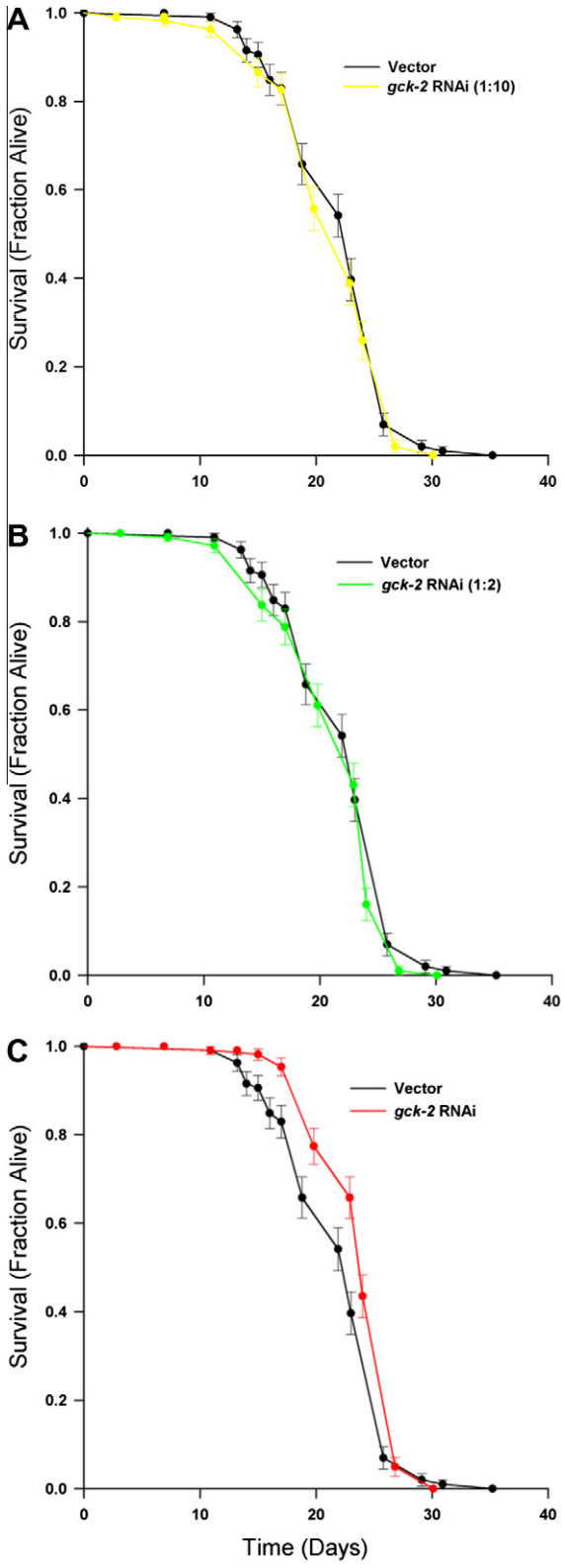

Fig. 1. *gck-2* RNAi increases the lifespan of wild type animals. Three different doses of a feeding RNAi construct targeting *gck-2* was fed to N2 worms from the time of hatching: (A and B) 1/10th strength and one-half strength RNAi, respectively, diluted with vector-only containing bacteria, and (C) full strength RNAi. Shown are survival plots (±SEM at each time point) representing pooled data from replicate experiments for each *gck-2* RNAi condition ($n \sim 60$ worms/replicate). Only N2 worms exposed to full strength *gck-2* RNAi exhibit a significant extension of healthspan relative to vector-only fed worms (*logrank test*, $p = 0.0001$). Full lifespan summary statistics are provided in Table 1.

3.2. gck-2(ok2867) and gck-2(tm2537) encode in-frame GCK-2 deletions

To determine if worms that are genetically-deficient for *gck-2* also have increased longevity, we examined two homozygous mutant strains – one containing the *ok2867* allele and the other the *tm2537* allele (Fig. 2). Both contain partial deletion mutations that disrupt the *gck-2* coding sequence. Specifically, *ok2867* contains a 549 bp deletion that results in an in-frame disruption of the conserved Ser/Thr kinase domain of GCK-2 (Fig. 2B and C). *tm2537* contains a 565 bp in-frame deletion that results in a prematurely truncated GCK-2 protein completely lacking the conserved citron homology (CNH) domain (Fig. 2B and C). We confirmed the presence of both mutations using PCR (Fig. 3A, primer locations are shown schematically in Fig. 2B). In the absence of an antibody to test if the *ok2867* and *tm2537* alleles each express their mutant protein, we instead tested if mutant mRNA was stably transcribed from each locus. Both alleles encode mRNA species that appear not to be degraded by nonsense-mediated decay (NMD) (Fig. 3B, primer locations described in Fig. 2B). This PCR-based assay was semi-quantitative and the results in Fig. 3B suggest both mutants respond to *gck-2* locus disruption by elevating transcript levels.

3.3. Genetic mutation of gck-2 results in an allele-specific increase in C. elegans survival

We next tested whether *gck-2* mutants differed in their post-larval survival relative to wild type animals. When all three lines were cultured on NGM agar plates coated with *E. coli* (OP50), only the *tm2537* mutant showed an increase in mean lifespan; *ok2867* mutants showed no significant increase or decrease in survival relative to wild type animals (Fig. 4, Table 2). Similar to the situation using the *gck-2* feeding RNAi, the effect of the mutant *tm2537* allele on survival was small (~7%), albeit significant ($p < 0.02$), and reproducible. Also similar to our observation with *gck-2* RNAi fed worms, the improvement was only in mean survival, there was no increase in maximum lifespan in the *tm2537* mutant strain.

4. Discussion

It is well established that inhibition of TOR extends lifespan when disrupted in a panoply of species. Rapamycin, a TOR inhibitor, is the only drug presently known that reproducibly extends lifespan in wild type mice, even when administered late in life [7]. In the present study we sought to determine if a recently-identified activator of TOR, MAP4K3, also plays a central role in lifespan control. We focused our efforts on the nematode *C. elegans*. Using a feeding RNAi targeting *gck-2*, we observed that loss of MAP4K3 resulted in a small but significant effect on worm survival. Specifically, but somewhat unexpectedly, we observed that only the mean lifespan of *gck-2* RNAi-treated animals increased; their maximal lifespan was unchanged. A similar result was obtained when we tested mutant *gck-2(tm2537)* animals. Over the course of these lifespan studies we observed that both groups of animals were motile longer than control animals (this effect was not quantified) – suggesting that disruption of normal MAP4K3 function in worms extends healthspan, but not lifespan. Intriguingly, when we tested a second genetic mutant, *gck-2(ok2867)*, these animals showed no difference in lifespan relative to N2 control worms. Why these mutants exhibit a different phenotype from the other tested worms likely lay in the nature of their unique mutation (refer to Fig. 2).

Both *gck-2(tm2537)* and *gck-2(ok2867)* encode shortened versions of the MAP4K3 protein (Supplementary Fig. 1). *gck-2(tm2537)* mutants lack the citron homology (CNH) domain but encode an intact Ser/Thr kinase domain. Fortuitously,

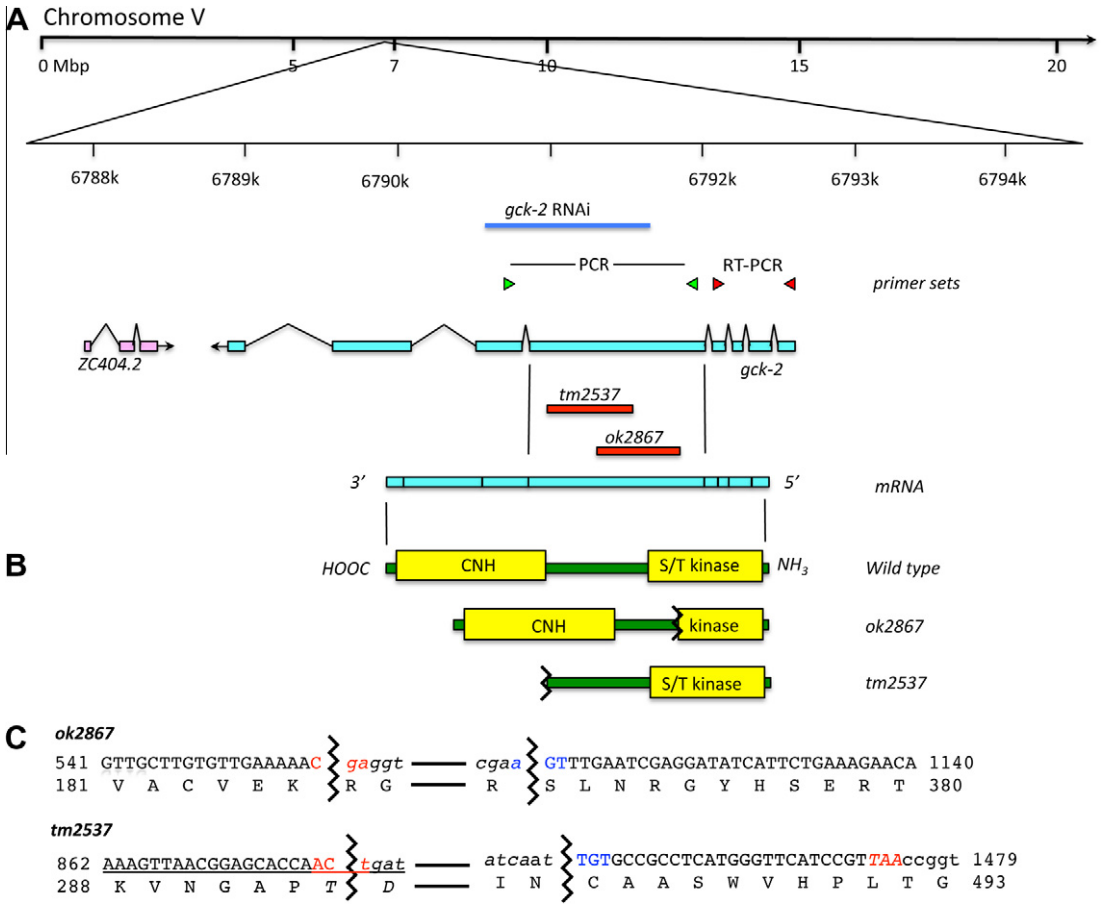

Fig. 2. Schematic representation of *gck-2* reagents. (A) Architecture of *gck-2* genomic locus in *C. elegans*. RNAi target sequence (dark blue), PCR and RT-PCR primers (triangles), location of deletion mutations (red), and spliced mRNA are shown. (Mbp: Mega basepairs; k: kilo). (B) Wild type and predicted mutant GCK-2 protein products relative to spliced *gck-2* mRNA. (CNH: citron homology domain; S/T kinase: Serine/threonine kinase domain). (C) DNA (and corresponding protein) fusion sequences resulting from *ok2867* and *tm2537* deletion mutations. Deleted residues are abbreviated between sawtooth lines. *tm2537* results in an out-of-frame fusion that stops at +8 amino acids (TAA). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

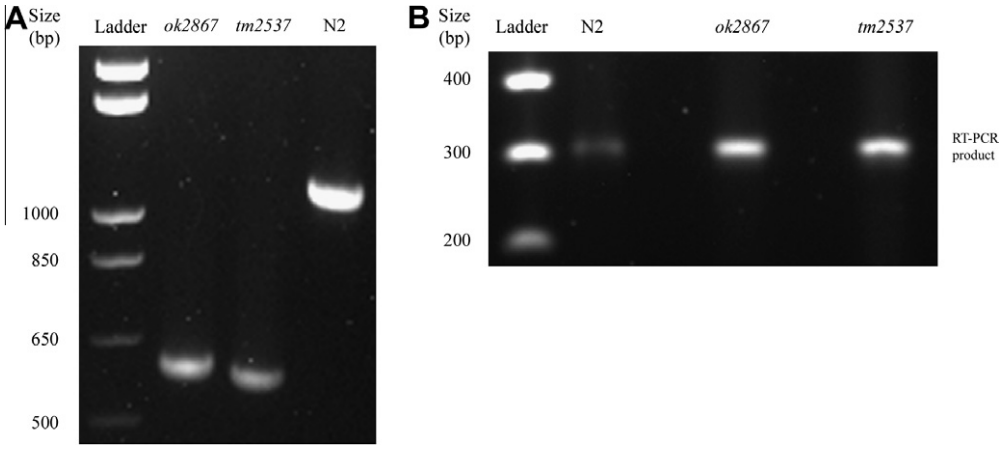

Fig. 3. *gck-2* Deletion Analysis and Transcript Abundance, in *gck-2(ok2867)* and *gck-2(tm2537)* mutant worms. (A) PCR confirmation of mutant alleles in *gck-2(ok2867)* and *gck-2(tm2537)* worms. Primer locations are shown schematically in Fig. 2B. PCR product sizes are 608, 592 and 1157 bp for *ok2867*, *tm2537* and N2 (control worms), respectively. (B) The presence of a *gck-2* transcript in both mutant strains was confirmed using semi-quantitative RT-PCR. As expected, a PCR product of 306 bp was detected in all three strains.

gck-2(ok2867) mutants generate an in-frame protein deficient in half of the residues required for the kinase domain but containing all of the residues needed for the CNH domain. The CNH domain is a poorly characterized protein–protein interaction motif that is known to bind small GTPases [22]. Rag proteins are small GTPases that positively regulate TOR activity in response to amino acid availability [23]. Recently, Bryk et al. showed that MAP4K3 binds to two Rag GTPases – Rag A and Rag C, and that amino acid deprivation caused a reduction in this binding [15]. Both Rag A and Rag C are conserved in *C. elegans*. These findings present a simple

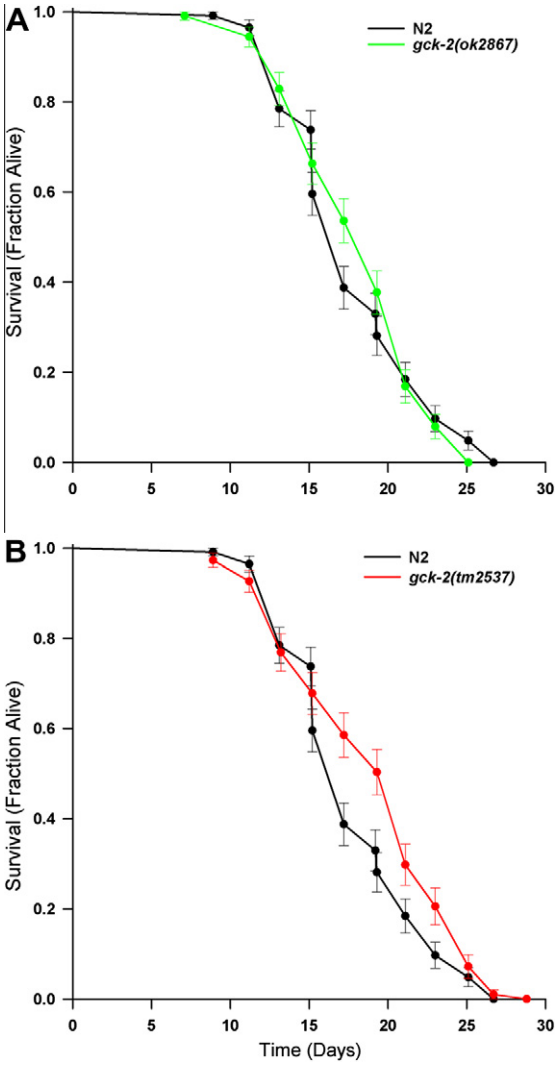

Fig. 4. Lifespan analysis of genetically-defined *gck-2* mutant worms. (A) *gck-2(ok2867)*, (B) *gck-2(tm2537)* and N2 (control) worms were cultured on NGM agar plates coated with OP50 *E. coli* and lifespan measured (see Section 2). Shown are survival plots (±SEM at each time point) representing pooled data from replicate experiments ($n \sim 60$ worms/replicate). Only *gck-2(tm2537)* mutant worms exhibited a significant extension of healthspan relative to control worms (*logrank test*, $p = 0.0106$). Full lifespan summary statistics are provided in Table 1.

hypothesis for why *gck-2(tm2537)* mutants, but not *gck-2(ok2867)* mutants, have increased average survival – absence of a functional CNH domain in MAP4K3 interferes with the ability of *tm2537* mutants to fully activate Rag A and Rag C, leading to reduced TOR activity.

The role of MAP4K3 in TOR regulation is not fully understood, and there may be other confounding functions of MAP4K3 that are responsible for the differences that we have observed in *gck-2(tm2537)* and *gck-2(ok2867)* average survival. For example, Lam et al., studying *Drosophila*, both in a cell culture model and *in vivo*, showed that MAP4K3 activation can induce apoptosis [24]. This functionality obviously contrasts with that of TOR, which inhibits apoptosis when activated [25]. Induction of apoptosis in the fly model was, however, shown to be independent of TOR. Paradoxically, both loss of function of MAP4K3 in *Drosophila*, as well as its overexpression, results in reduced wing size. In the case of overexpression, the reduction in wing size is due to apoptosis induction. Such conflicting roles of MAP4K3 may help explain why we do not observe a more prominent increase in lifespan in worms following loss of MAP4K3. A third point worth noting is that MAP4K3 represents one of two major pathways that funnel divergent signals into TOR - the second is represented by Rheb. It is well appreciated that *C. elegans* lack TSC1 and TSC2, negative regulators of Rheb, and this may be a another reason why loss of MAP4K3 in worms does not have a more prominent life extending effect.

In summary, we have shown that loss of MAP4K3 in *C. elegans* leads to a small but significant increase in median survival without increasing maximal lifespan. The CNH domain of MAP4K3 may be crucial in mediating this effect. Further studies will be aimed at determining if MAP4K3 similarly regulates lifespan in other species, and the extent to which MAP4K3 might serve as drugable target for modulating healthspan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.113.

References

[1] J. Avruch, K. Hara, Y. Lin, M. Liu, X. Long, S. Ortiz-Vega, K. Yonezawa, Insulin and amino-acid regulation of mTOR signaling and kinase activity through the Rheb GTPase, Oncogene 25 (2006) 6361–6372.
[2] T. Weichhart, Mammalian target of rapamycin: a signaling kinase for every aspect of cellular life, Methods Mol. Biol. 821 (2012) 1–14.
[3] M. Wei, P. Fabrizio, J. Hu, H. Ge, C. Cheng, L. Li, V.D. Longo, Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9, PLoS Genet. 4 (2008) e13.
[4] M. Kaeberlein, R.W. Powers 3rd, K.K. Steffen, E.A. Westman, D. Hu, N. Dang, E.O. Kerr, K.T. Kirkland, S. Fields, B.K. Kennedy, Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients, Science 310 (2005) 1193–1196.
[5] S. Honjoh, T. Yamamoto, M. Uno, E. Nishida, Signalling through RHEB-1 mediates intermittent fasting-induced longevity in *C. elegans*, Nature 457 (2009) 726–730.
[6] L. Partridge, N. Alic, I. Bjedov, M.D. Piper, Ageing in *Drosophila*: the role of the insulin/Igf and TOR signalling network, Exp. Gerontol. 46 (2011) 376–381.
[7] D.E. Harrison, R. Strong, Z.D. Sharp, J.F. Nelson, C.M. Astle, K. Flurkey, N.L. Nadon, J.E. Wilkinson, K. Frenkel, C.S. Carter, M. Pahor, M.A. Javors, E. Fernandez, R.A. Miller, Rapamycin fed late in life extends lifespan in genetically heterogeneous mice, Nature 460 (2009) 392–395.
[8] A. Garami, F.J. Zwartkruis, T. Nobukuni, M. Joaquin, M. Roccio, H. Stocker, S.C. Kozma, E. Hafen, J.L. Bos, G. Thomas, Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2, Mol. Cell 11 (2003) 1457–1466.
[9] B.D. Manning, A.R. Tee, M.N. Logsdon, J. Blenis, L.C. Cantley, Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway, Mol. Cell 10 (2002) 151–162.
[10] C.J. Potter, L.G. Pedraza, T. Xu, Akt regulates growth by directly phosphorylating Tsc2, Nat. Cell Biol. 4 (2002) 658–665.
[11] L. Ma, Z. Chen, H. Erdjument-Bromage, P. Tempst, P.P. Pandolfi, Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis, Cell 121 (2005) 179–193.
[12] K. Hara, K. Yonezawa, Q.P. Weng, M.T. Kozlowski, C. Belham, J. Avruch, Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism, J. Biol. Chem. 273 (1998) 14484–14494.
[13] G.M. Findlay, L. Yan, J. Procter, V. Mieulet, R.F. Lamb, A MAP4 kinase related to Ste20 is a nutrient-sensitive regulator of mTOR signalling, Biochem. J. 403 (2007) 13–20.
[14] M. Resnik-Docampo, J.F. de Celis, MAP4K3 is a component of the TORC1 signalling complex that modulates cell growth and viability in *Drosophila melanogaster*, PLoS ONE 6 (2011) e14528.
[15] B. Bryk, K. Hahn, S.M. Cohen, A.A. Teleman, MAP4K3 regulates body size and metabolism in *Drosophila*, Dev. Biol. 344 (2010) 150–157.
[16] H.A. Tissenbaum, Genetics, life span, health span, and the aging process in *Caenorhabditis elegans*, J. Gerontol. A Biol. Sci. Med. Sci. 67 (2012) 503–510.
[17] T. Vellai, K. Takacs-Vellai, Y. Zhang, A.L. Kovacs, L. Orosz, F. Muller, Genetics: influence of TOR kinase on lifespan in *C. elegans*, Nature 426 (2003) 620.
[18] K. Jia, D. Chen, D.L. Riddle, The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span, Development 131 (2004) 3897–3906.
[19] S. Robida-Stubbs, K. Glover-Cutter, D.W. Lamming, M. Mizunuma, S.D. Narasimhan, E. Neumann-Haefelin, D.M. Sabatini, T.K. Blackwell, TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO, Cell Metab. 15 (2012) 713–724.
[20] T.L. Kaeberlein, E.D. Smith, M. Tsuchiya, K.L. Welton, J.H. Thomas, S. Fields, B.K. Kennedy, M. Kaeberlein, Lifespan extension in *Caenorhabditis elegans* by complete removal of food, Aging Cell 5 (2006) 487–494.

[21] M. Hansen, A. Chandra, L.L. Mitic, B. Onken, M. Driscoll, C. Kenyon, A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*, PLoS Genet. 4 (2008) e24.
[22] M. Punta, P.C. Coggill, R.Y. Eberhardt, J. Mistry, J. Tate, C. Boursnell, N. Pang, K. Forslund, G. Ceric, J. Clements, A. Heger, L. Holm, E.L. Sonnhammer, S.R. Eddy, A. Bateman, R.D. Finn, The Pfam protein families database, Nucleic Acids Res. 40 (2012) D290–301.
[23] Y. Sancak, T.R. Peterson, Y.D. Shaul, R.A. Lindquist, C.C. Thoreen, L. Bar-Peled, D.M. Sabatini, The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1, Science 320 (2008) 1496–1501.
[24] D. Lam, S. Shah, I.P. de Castro, S.H. Loh, L.M. Martins, Drosophila happyhour modulates JNK-dependent apoptosis, Cell Death Dis. 1 (2010) e66.
[25] S. Huang, L. Shu, J. Easton, F.C. Harwood, G.S. Germain, H. Ichijo, P.J. Houghton, Inhibition of mammalian target of rapamycin activates apoptosis signal-regulating kinase 1 signaling by suppressing protein phosphatase 5 activity, J. Biol. Chem. 279 (2004) 36490–36496.
[26] W.B. Wood (Ed.), The Nematode *Caenorhabditis elegans*, Cold Spring Harbor Laboratory, New York, 1988.
[27] R.S. Kamath, J. Ahringer, Genome-wide RNAi screening in *Caenorhabditis elegans*, Methods 30 (2003) 313–321.
[28] S.L. Rea, N. Ventura, T.E. Johnson, Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in *Caenorhabditis elegans*, PLoS Biol. 5 (2007) e259.