TOR Signaling in *Caenorhabditis elegans* Development, Metabolism, and Aging

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ABSTRACT The **T**arget **o**f **R**apamycin (TOR or mTOR) is a serine/threonine kinase that regulates growth, development, and behaviors by modulating protein synthesis, autophagy, and multiple other cellular processes in response to changes in nutrients and other cues. Over recent years, TOR has been studied intensively in mammalian cell culture and genetic systems because of its importance in growth, metabolism, cancer, and aging. Through its advantages for unbiased, and high-throughput, genetic and *in vivo* studies, *Caenorhabditis elegans* has made major contributions to our understanding of TOR biology. Genetic analyses in the worm have revealed unexpected aspects of TOR functions and regulation, and have the potential to further expand our understanding of how growth and metabolic regulation influence development. In the aging field, *C. elegans* has played a leading role in revealing the promise of TOR inhibition as a strategy for extending life span, and identifying mechanisms that function upstream and downstream of TOR to influence aging. Here, we review the state of the TOR field in *C. elegans*, and focus on what we have learned about its functions in development, metabolism, and aging. We discuss knowledge gaps, including the potential pitfalls in translating findings back and forth across organisms, but also describe how TOR is important for *C. elegans* biology, and how *C. elegans* work has developed paradigms of great importance for the broader TOR field.

KEYWORDS WormBook; *Caenorhabditis elegans* development; metabolism; aging; TOR; TORC1; TORC2; nutrient signaling; growth regulation; Raptor; Rictor; DAF-15; Rheb; Rheb-1; RagA; RAGA-1; RagC; RSKS-1; S6 kinase; Nprl2; NPRL-2; Nprl3; NPRL-3; sphingolipid

TABLE OF CONTENTS

doi: https://doi.org/10.1534/genetics.119.302504
Manuscript received September 27, 2018; accepted for publication July 18, 2019
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CONTENTS, *continued*

TOR is a serine/threonine kinase that was first discovered as a **T**arget **O**f **R**apamycin in yeast, and the mammalian TOR homolog was identified soon after in studies using cultured cells (Kunz *et al.* 1993; Blenis 2017; Sabatini 2017) . TOR is also commonly referred to as mTOR (mammalian, or mechanistic, target of rapamycin). Extensive work in yeast and mammalian cell culture led to the identification of two mutually exclusive TOR-binding proteins, Raptor (**R**egulatory **A**ssociated **P**rotein of m**TOR**) and Rictor (**R**apamycin-**I**nsensitive **C**ompanion of m**TOR**) (Hara *et al.* 2002; Kim *et al.* 2002; Loewith *et al.* 2002; Sarbassov *et al.* 2004). The association of TOR with each of these binding proteins defined the two TOR complexes: TOR Complex 1 (TORC1, containing TOR and Raptor) and TOR Complex 2 (TORC2, containing TOR and Rictor), each of which have distinct functions and signaling activities (Saxton and Sabatini 2017) (Figure 1).

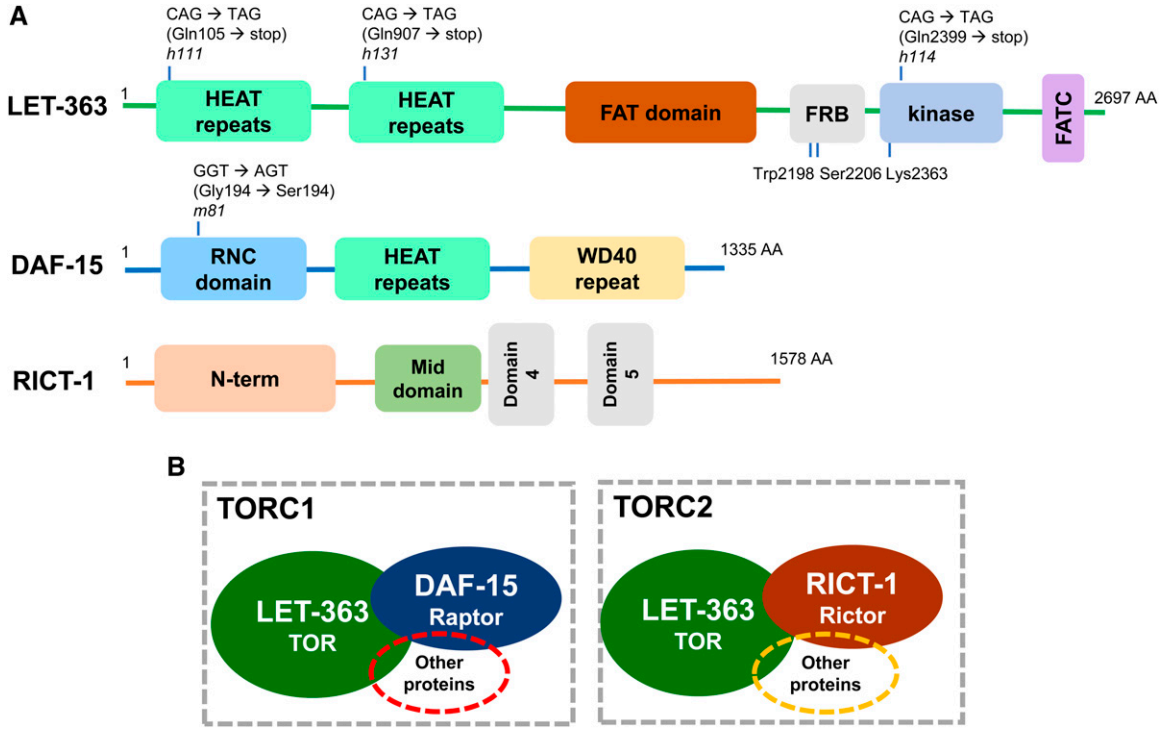

Figure 1 Core components of TOR signaling in *C. elegans*. (A) Cartoon diagram of the protein structures and domains in LET-363/TOR, DAF-15/Raptor, and RICT-1/Rictor [adapted from Long *et al.* (2002) and Jia *et al.* (2004)]. Mutant alleles (*h111*, *h131*, *h114*, and *m81*) and key conserved residues (Trp2198, Ser2206, and Lys2363) are indicated. HEAT repeats are named for four proteins (Huntingtin, EF3, PP2A, and TOR1) that contain this repeat structure. The RICT-1 domains are less characterized (domain identities taken from InterPro/European Molecular Biology Laboratory-European Bioinformatics Institute). (B) TORC1 is defined as the complex containing LET-363/TOR and DAF-15/Raptor. TORC2 is defined as the complex containing LET-363/TOR and RICT-1/Rictor. It is expected that other proteins are found in these complexes and required for TOR signaling. Please see the text for more discussion. AA, amino acid; FAT, focal adhesion-targeting domain; FATC, focal adhesion-targeting C-terminal domain; FRB, FKBP-Rapamycin-Binding domain (where FKBP stands for FK506-binding protein); RNC, Raptor N-terminal CASPase-like domain; TOR, Target of Rapamycin; TORC, TOR Complex; WD40 repeat, ~40AA motif that terminates in W-D.

TOR complexes are widely described as signaling systems that sense the levels of various nutrients, energy, and growth factors, and instruct changes in downstream activities involved in development, reproduction, metabolism, behavior, stress responses, and aging (Menon and Manning 2013; Saxton and Sabatini 2017) (Figure 2). In essence, the TOR complexes are critical because they drive growth, development, and anabolic metabolism, but reductions in TOR activity can also have profound consequences by leading to mobilization of mechanisms that protect cells and organisms from stress. Understanding how TOR complexes function is therefore of high significance in our pursuit of mechanisms that underlie aging and various human diseases. While mechanistic studies using yeast and mammalian tissue culture have made much progress in understanding the fundamental cellular functions and molecular mechanisms of TOR signaling, studies using model organisms have increasingly become important to study functions of TOR complexes *in vivo* under specific physiological conditions. The genetically amenable organism *Caenorhabditis elegans* has been an outstanding model system for new discoveries in animal development, metabolic regulation, aging, and neuronal functions. Since the connections between nutrient availability and these physiological functions present many unresolved disease-related biological problems that involve TOR functions, the worm system provides unique opportunities to learn about the physiological functions of TOR complexes. In particular, research in *C. elegans* pioneered the study of how metazoan life span can be increased by decreasing TOR activity, and *C. elegans* has continued to be a major contributor in understanding the mechanisms involved (Figure 2).

TOR Signaling Complexes are Conserved in *C. elegans*

C. elegans has orthologs of TOR, Raptor, Rictor, and many other conserved regulators of TORC1 and TORC2 activities (Table 1). Note that in many cases the null phenotype of these genes is not yet known.

Identification of key components

LET-363/TOR: The ortholog of TOR in *C. elegans*, LET-363 (Figure 1), was named based upon its lethal mutant phenotype (Howell *et al.* 1987), and later identified as a TOR protein in a sequence homology search for phosphatidylinositol

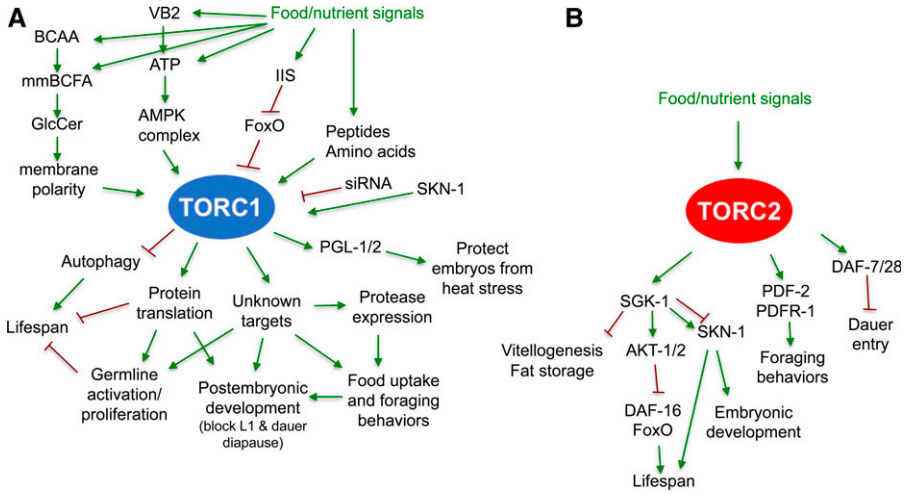

Figure 2 Abbreviated illustration of pathways, or cellular processes, identified to act upstream or downstream of TORC1 (A) and TORC2 (B) in *C. elegans*. These models are based mainly upon genetic analyses but also incorporate mechanistic findings from other systems. In many cases, *C. elegans* researchers analyzed the TORC1 or TORC2 function under starvation or dietary restriction conditions, when food/nutrient signals were absent or reduced. Arrows indicate the positive regulation or input, whereas T-bars indicate negative regulation, with neither necessarily indicating direct regulation. Please see the text for more discussion on these pathways and downstream physiological functions. TOR functions are notably complex and wide ranging, extending beyond the canonical functions that have been identified in other organisms. For example, the stress response transcription factor SKN-1 has three different roles regarding the two TOR complexes: it promotes transcription of several TORC1 components, its target genes are activated when TORC1 or translation is inhibited, and it is regulated downstream of TORC2 (see sections *Roles of TORC2 in regulating development and behaviors* and *Life span extension and increased stress resistance from TORC1 inhibition*). BCAA, branched-chain AAs; GlcCer, glycosylceramide; IIS, insulin/IGF signaling; mmBCFA, monomethyl branched-chain fatty acid; TOR, Target of Rapamycin; TORC, TOR Complex; VB2, vitamin B2.

kinase-related proteins (Long *et al.* 2002). Strong conservation was found in all key domains including the kinase domain, HEAT repeats [named for four proteins (Huntingtin, EF3, PP2A, and TOR1)], and the FKBP-rapamycin-binding (FRB), FAT (focal adhesion-targeting), phosphatidylinositol kinase homology, and FAT C-terminal domains [Figure 1; see Long *et al.* (2002) for more details]. The expression of a GFP reporter driven by a *let-363* promoter began in the comma-stage embryo, and was subsequently seen at all stages and in all major tissues (and perhaps all cells). The *let-363* gene is clearly essential for animal development as multiple different null mutants (obtained from heterozygous mothers) arrested at the L3 larval stage (Long *et al.* 2002). The mutants also displayed increased refractile and autofluorescent intestinal granules, decreased intestinal cytoplasmic volume with increased gut lumen size, increased fat storage, as well as compromised digestion. These phenotypes were also seen in worms with the *let-363* gene knocked down by bacteria feeding-mediated RNA interference (RNAi) (Long *et al.* 2002). A stronger embryonic lethal phenotype was reported after maternal injection of *let-363* double-stranded RNA [Sönnichsen *et al.* (2005) and WormBase], suggesting there could be additional maternally provided functions of *let-363/Tor* in embryos.

DAF-15/Raptor: In response to food deprivation, *C. elegans* enter an alternative developmental pathway in which they become specialized dauer larvae (rather than L3 larvae) after the second larval molt (Hu 2007) (see section *Roles of TORC1 in regulating development and behaviors* for more description). *daf-15* was named based upon its loss-of-function mutant phenotype, which is constitutive abnormal **da**uer **f**ormation (Albert and Riddle 1988). *daf-15(m81)* missense mutants segregated from heterozygotes arrested postembryonic development at the L2 molt with some dauer-like morphological features. However, unlike typical dauer-constitutive mutants, *daf-15(m81)* mutants displayed sporadic feeding and increased body size (the potential connection between TOR and dauer formation is discussed further in section *Roles of TORC1 in regulating development and behaviors*.). Structural similarity between DAF-15 and mammalian Raptor was later recognized in one of the studies that first identified mammalian Raptor (Hara *et al.* 2002) (Figure 1). *daf-15* RNAi resulted in L3 larval arrest with additional phenotypes including increased refractile and autofluorescent granules in the intestine, decreased intestinal cell cytoplasm with increased intestinal lumen, increased fat storage, and increased hypodermal granules (Hara *et al.* 2002; Jia *et al.* 2004). Since essentially all of these phenotypes are common between *daf-15(RNAi)* and *let-363(RNAi)*, these findings were consistent with the predicted roles of *let-363*/Tor and *daf-15/raptor* in *C. elegans* TORC1.

RICT-1/Rictor: The *C. elegans* putative rictor ortholog, RICT-1 (Figure 1), was identified in two forward genetic screens for genes that impact fat storage (Jones *et al.* 2009; Soukas *et al.* 2009). Loss-of-function alleles of *rict-1*, independently isolated by the two laboratories, displayed increased body fat, developmental delay (slow growth), and decreased body size. *rict-1(RNAi)* phenocopied the high-fat phenotype of the mutants and did not further enhance the phenotype of *rict-1(lf)* mutants. These *rict-1* alleles can have varied effects on life span, depending upon diet and other conditions (see *Role of TOR signaling in aging and stress response*). Notably, unlike loss of *let-363/Tor* or *daf-15/raptor*, these alleles of *rict-1* did not result in larval arrest, suggesting that TORC2 is not essential for larval development. *rict-1* promoter-driven GFP reporters were expressed in the intestine, hypodermis, and neurons (Jones *et al.* 2009; Soukas *et al.* 2009). *rict-1* expression driven by intestinal specific promoters in

Table 1 Major players in TOR signaling

Gene	Sequence name	Mammalian homolog	References
Complex components			
let-363	B0261.2	*TOR*	Long *et al.* (2002)
daf-15	C10C5.6	*RAPTOR*	Hara *et al.* (2002)
rict-1	F29C12.3	*RICTOR*	Jones *et al.* (2009), Soukas *et al.* (2009)
mlst-8	C10H11.8	*mLST8*	Jones *et al.* (2009)
sinh-1	Y57A10A.20	*mSIN1*	Soukas *et al.* (2009)
No homolog identified		*DEPTOR*	
No homolog identified		*PRAS40*	
Interactors			
raga-1	T24F1.1	*RAGA/RAGB*	Schreiber *et al.* (2010)
ragc-1	Y24F12A.2	*RAGC/RAGD*	Fukuyama *et al.* (2012), Robida-Stubbs *et al.* (2012)
rheb-1	F54C8.5	*RHEB*	Honjoh *et al.* (2009)
No homolog identified		*LAMTOR1*	
lmtr-2	Y97E10AR.7	*LAMTOR2*	Kim *et al.* (2018)
lmtr-3	C06H2.6	*LAMTOR3*	Shaye and Greenwald (2011), Kim and Guan (2019)
T08A11.1	T08A11.1	*DEPDC5*	Kim *et al.* (2018)
nprl-2	F49E8.1	*NPRL2*	Zhu *et al.* (2013)
nprl-3	F35H10.7	*NPRL3*	Zhu *et al.* (2013)
F39C12.1	F39C12.1	*MIOS*	
Y32H12A.8	Y32H12A.8	*WDR24*	Kim *et al.* (2018)
No homolog identified		*WDR59*	
npp-18	Y43F4B.4	*SEH1L*	Kim *et al.* (2018)
npp-20	Y77E11A.13	*SEC13*	Galy *et al.* (2003)
F13H10.3	F13H10.3	*SLC38A9*	Kim *et al.* (2018)
sesn-1	Y74C9A.5	*SESN2*	Yang *et al.* (2013)
No homolog identified		*CASTOR1*	
No homolog identified		*CASTOR2*	
F54B3.1	F54B3.1	*SZT2*	Kim *et al.* (2018)
No homolog identified		*KPTN*	
No homolog identified		*ITFG2*	
No homolog identified		*C12orf66*	
Substrates			
pgl-1	ZK381.4	none	Zhang *et al.* (2018)
pgl-3	C18G1.4	none	Zhang *et al.* (2018)
rsks-1	Y47D3A.16	*S6K*	Long *et al.* (2002)
ifet-1[a]	F56F3.1	*4E-BP*	Li *et al.* (2009), Nukazuka *et al.* (2011)
atg-13	D2007.5	*ATG13*	Tian *et al.* (2010)
sgk-1	W10G6.2	*SGK1*	Hertweck *et al.* (2004)
pkc-2	E01H11.1	*PKC(βδζ)*	Islas-Trejo *et al.* (1997)
akt-1	C12D8.10	*AKT*	Paradis and Ruvkun (1998)
akt-2	F28H6.1	*AKT*	Paradis and Ruvkun (1998)
Regulators			
aak-1	PAR2.3	*PRKAA(1,2),AMPK*	Fukuyama *et al.* (2012)
aak-2	T01C8.1	*PRKAA(1,2),AMPK*	Fukuyama *et al.* (2012)
daf-18	T07A9.6	*PTEN*	Fukuyama *et al.* (2012)
daf-16	R13H8.1	*FoxO*	Jia *et al.* (2004)
No homolog identified		*TSC1*	
No homolog identified		*TSC2*	

This table includes the *C. elegans* homologs of genes that encode proteins identified as components, interactors, substrates, or regulators of mTORC1 or mTORC2. They are organized by their putative roles in TOR signaling, although many need further characterization to determine function. "No homolog identified" means no apparent homolog based on ortholog prediction programs (Kim *et al.* 2018), but it is possible that there is a functional ortholog that is too diverged to identify in this manner. The references listed correspond to the related *C. elegans* studies. TOR, Target of Rapamycin; TORC, TOR Complex.

[a] *ifet-1* has also been known as *spn-2*.

the form of extrachromosomal arrays was sufficient to rescue the fat-storage phenotype in *rict-1(-)* mutants (Soukas *et al.* 2009), suggesting that *rict-1* may act in the gut to regulate fat storage. While mammals have three genes that encode Rictor proteins, *C. elegans* appears to have only one protein that shares this structural and functional similarity (Figure 1).

Biochemical analysis of the two TOR complexes: The worm field is still in an early stage of characterizing the TOR complexes by biochemical methods. Targeted co-immunoprecipitations using transgenes expressing tagged proteins have been encouraging, successfully pulling down LET-363::FLAG with DAF-15::Myc or RICT-1::HA (Nukazuka *et al.* 2011), which supported the prediction that *C. elegans* has both canonical

TOR complexes, as seen in mammals (Figure 1). RICT-1 and DAF-15 were also among proteins recently identified as LET-363 interactors in *C. elegans* embryos in an IP experiment using FLAG::LET-363, confirming protein binding with endogenous proteins of the TORC1 and TORC2 complexes (Zhang *et al.* 2018). This work also demonstrated for the first time the kinase activity of LET-363 with known targets in *C. elegans*. By immunoprecipitation of FLAG::LET-363, followed by a radiolabeled kinase assay, PGL-1 and PGL-3, two RGG-domain P granule assembly proteins with RNA endonuclease activities (Kawasaki *et al.* 1998; Aoki *et al.* 2016), were shown to be phosphorylation targets of LET-363.

Other conserved TOR components and key interactors: The proteins discussed below have been identified as members of the mTOR complexes, or key interacting proteins, in mammalian and/or yeast studies (Table 1). However, in most cases functional conservation has not been fully demonstrated in *C. elegans*.

MLST-8: The LST8 (aka GβL) protein is present in both TORC1 and TORC2 complexes in mammals (Kim *et al.* 2003), and genetic evidence suggests that *C. elegans* MLST-8 (MTor-associated protein, LST8 homolog) also functions in both complexes. Knockdown of *mlst-8* by RNAi produced many of the same phenotypes seen with *rict-1(-)* alleles (Jones *et al.* 2009), but not the larval arrest that resulted from reduced *let-363* or *daf-15* (although null alleles remain to be characterized). Biochemical assays using transgenic proteins have shown that knockdown of *mlst-8* eliminated binding of LET-363::FLAG to RICT-1::HA and increased binding of LET-363::FLAG to DAF-15::Myc (Nukazuka *et al.* 2011), and phenotypes suppressed by *rict-1* RNAi were also suppressed by *mlst-8* RNAi (Ruf *et al.* 2013). These findings suggested that MLST-8 is required for the proper formation of TORC2, which is consistent with a role of LST8 in mTORC2-related functions reported in mammals (Guertin *et al.* 2006). However, *mlst-8(RNAi)* phenocopied loss of other TORC1-related genes in suppressing accumulation of germline PGL (P GranuLe) protein-containing granules, suggesting that MLST-8 plays a role in TORC1 function in the embryo (Zhang *et al.* 2018). This is consistent with the association of LST8 with mammalian TORC1, making it interesting to further investigate the possible role MLST-8 plays in *C. elegans* TORC1 formation or activity.

SINH-1: SINH-1/mSin1, named for mammalian *s*tress-activated protein kinase (SAPK)-*in*teracting protein, is an mTORC2 component that is required for mTORC2 assembly and function (*e.g.*, Yang *et al.* 2006). *C. elegans sinh-1* (Sin-1 homolog) was identified in an RNAi screen, with reduced *sinh-1* extending mean life span, increasing thermotolerance and stress resistance, and enhancing dauer formation (Hansen *et al.* 2005). Phenotypes suppressed by *rict-1* RNAi were also partially suppressed by *sinh-1* RNAi (Ruf *et al.* 2013). *sinh-1(pe420)* null mutants are viable, unlike *let-653/Tor* or *daf-15/raptor* mutants, and share several other phenotypes with *rict-1* mutants, including viability, increased fat storage, and similar performance in associative learning assays (Sakai *et al.* 2017). Tissue-specific transgenes were used to show that expression of *sinh-1* in the intestine or neurons was sufficient to rescue the learning defect of the mutant (Sakai *et al.* 2017), suggesting that *sinh-1* and *rict-1* can function in some of the same tissues. All of these findings are consistent with a role for SINH-1 in TORC2.

RAGA-1 and RAGC-1: The Rag GTPases (RAS-related GTP-binding protein) are well characterized in mammalian cells as positive regulators of the TORC1 kinase, through which it is activated by amino acid (AA) availability signals (Jewell *et al.* 2013). In mammals, the Rag proteins function as obligate heterodimers that are made up of a member from each of two protein families, RagA/RagB and RagC/RagD, but in *C. elegans* only one homolog from each family is present (RAGA-1 and RAGC-1). RAGA-1/RagA was identified in an RNAi screen for improved locomotion in aged *C. elegans*, and studies using null mutations and transgenes containing putative dominant negative and gain-of-function (gf) mutations suggested that RAGA-1/RagA activity negatively impacts life span (Schreiber *et al.* 2010). The *raga-1(gf)* transgene was also used to putatively "hyperactivate" TORC1 to suppress the larval arrest caused by a loss-of-function mutation in a fatty acid elongase *elo-5* (Zhu *et al.* 2013). RNAi knockdown of *raga-1* and *ragc-1* revealed that a reduction in RagA/C function resulted in increased autophagy, decreased mRNA translation, and increased life span and stress tolerance, as observed with reduced TORC1 activity (Robida-Stubbs *et al.* 2012). Loss or reduction of the *ragc-1*, *raga-1*, and *rheb-1* gene activities all suppressed the ectopic germline proliferation seen in animals with deletion mutations in both of the AMPK genes (*aak-1* and *aak-2*), or in a *daf-18*/PTEN deletion mutant (Fukuyama *et al.* 2012), which is consistent with TORC1 having functions downstream of these factors. RAGA-1 and RAGC-1 were identified as weak binding partners with FLAG:LET-363, and loss of *raga-1* and *ragc-1* phenocopied loss of other TORC1-related genes in suppressing accumulation of PGL granules, consistent with these proteins being positive regulators of TORC1 in the embryo (Zhang *et al.* 2018).

The developmental defects produced by knocking down *raga-1* or *ragc-1* (slowed development, and modestly reduced body size and reproduction) are not as severe as those seen with knockdown of *let-363/TOR* or *daf-15/raptor*, suggesting that TORC1 may have residual activities that do not depend upon RAG-mediated signaling. Consistent with this idea, a deletion mutation in *raga-1* slows but does not block larval development, and extends adult life span (Schreiber *et al.* 2010). However, it is not clear whether RAGA-1/RagA and RAGC-1/RagC form an obligate heterodimer in *C. elegans* as in mammals, so potential redundancy between the two Rag proteins cannot be excluded.

RHEB-1: Rheb is another member of the Ras superfamily of small GTPases that is a critical positive upstream regulator of TORC1 in multiple organisms (Jewell *et al.* 2013; Saxton and

Sabatini 2017). *rheb-1* encodes the *C. elegans* ortholog of Rheb (Reiner and Lundquist 2018). A Prheb-1::RHEB-1::GFP reporter was expressed at all stages and in all cells (Honjoh *et al.* 2009). *rheb-1(RNAi)* resulted in mild developmental phenotypes (Honjoh *et al.* 2009), and a putative *rheb-1(gf)* mutant transgene appeared to promote TORC1 activation (Zhu *et al.* 2013). The effects of *rheb-1* on aging and life span extension are discussed in a later section (*Importance of TORC1 regulation in life span extension mechanisms*). *rheb-1 (RNAi)* has also been shown to phenocopy knockdown of other TORC1-related genes in suppressing accumulation of PGL granules, suggesting that RHEB-1 functions with TORC1 in the embryo (Zhang *et al.* 2018).

TSC proteins (or lack thereof): In mammals and *Drosophila*, the TSC1 and TSC2 (Tuberous sclerosis) proteins indirectly inhibit TORC1, and mediate its functional interaction with growth factor signaling pathways (Dibble and Cantley 2015). *C. elegans* lacks orthologs of both TSC1 and TSC2 (Table 1), which is highly intriguing given the conservation of signaling pathways connecting to these proteins. TSC1 and TSC2 form a complex and act as GTPase-activating proteins (GAPs) for the Rheb GTPase. Structural similarities between the TSC1-TSC2 complex and RalGAPα-RalGAPβ propelled one study that indicated a regulatory role of RalGAP proteins (and Ral GTPase) on TORC1 activity, in both mammalian cells and *C. elegans* (Martin *et al.* 2014). Furthermore, analysis of sequence alignments between *C. elegans*, *Drosophila*, and mammalian Rheb-family proteins has led to the argument that *C. elegans* RHEB-1 has converged toward RAL-1, while the *Drosophila* and mammalian Rheb proteins have not converged toward their Ral proteins (Reiner and Lundquist 2018). This observation was used to support the model that *C. elegans* RHEB-1 and RAL-1 are jointly repressed by the RalGAPs HGAP-1 and HGAP-2, which might be a reasonable explanation for the loss of the TSC proteins in nematodes. It would be interesting to test whether HGAP-1 and HGAP-2 have TSC-like activity on the RHEB-1 GTPase in addition to their roles on RAL-1 in *C. elegans*. Additionally, RNAi knockdown of *ral-1* extends life span (Kim and Sun 2007; Martin *et al.* 2014), suggesting that both RAL-1 and RHEB-1 might promote TORC1 activity in *C. elegans*.

NPRL-2/3: NPRL-2 and NPRL-3 are orthologs of the NPR2 and NPR3 proteins, respectively, which were first identified as negative regulators of TORC1 in yeast (Neklesa and Davis 2009). In *C. elegans*, *nprl-3* was identified in an unbiased genetic screen as a suppressor of the growth-arrest phenotype caused by depleting monomethyl branched-chain fatty acids (mmBCFAs) (see *Upstream inputs to TOR signaling*), with *nprl-2* having a similar suppressor role (Zhu *et al.* 2013) (Figure 3A). Additional assays using RNAi or hyperactivation of TORC1 supported the idea that NPRL-2/3 are negative regulators of TORC1 in the *C. elegans* intestine. Consistent with these findings in yeast and *C. elegans*, a parallel study initiated by a biochemical approach identified mammalian NPRL2 and NPRL3 (named as part of the GATOR1 complex), as negative regulators of TORC1 (Bar-Peled *et al.* 2013). More specifically, in the mammalian study GATOR1 was found to be the GAP for RagA/C GTPases. Therefore, disruption of GATOR1 results in activation of RagA/C, which in turn activates TORC1 (Saxton and Sabatini 2017). While such a GAP function has not yet been demonstrated for *C. elegans* NPRL-2/3, the negative regulatory nature of the NPRL-2/3 complex rendered it an excellent tool in analyzing TORC1-related functions in *C. elegans* (Zhu *et al.* 2013; B. Qi *et al.* 2017; Zhang *et al.* 2018).

Rapamycin

Rapamycin is an antifungal metabolite produced by *Streptomyces hygroscopicus* that is well known for its inhibitory effects on mTOR in mammalian, *Drosophila*, and yeast cells (Huang *et al.* 2003; Li *et al.* 2014; Kennedy and Lamming 2016). Rapamycin is widely used as an immunosuppressant in humans, and is of great interest as a paradigm for an antiaging drug (see *Life span extension and increased stress resistance from TORC1 inhibition*). In mammals, a complex between rapamycin and the cellular protein FKBP12 (FK506-binding protein 12) binds to the FRB domain of TOR, thereby inhibiting the TORC1 kinase in a manner that affects some substrates more severely than others (Huang *et al.* 2003). *C. elegans* LET-363 is highly related to TOR proteins from other species, with conservation that extends to the FRB domain (Figure 1A) (Long *et al.* 2002). However, binding of rapamycin to an FKB protein remains to be demonstrated in *C. elegans*, and an unambiguous FKBP12 ortholog has not yet been designated. Yet, a number of FKBP family members are present in *C. elegans*, of which FKB-2 is more similar to human FKBP12 than is the FKBP12 ortholog in *Saccharomyces cerevisiae* (*FPR1*) (Pemberton and Kay 2005), in which genetic analyses initially revealed TOR to be the biological target of FPR1/rapamycin action (Kunz *et al.* 1993; Blenis 2017). Therefore, it appears likely that the mechanisms through which rapamycin inhibits TORC1 in other species are conserved in *C. elegans*.

While the evolutionary conservation of TORC1 and its sensitivity to rapamycin suggested that rapamycin would be likely to reduce TORC1 activity in *C. elegans*, the impact of rapamycin on *C. elegans* growth is limited, and initial efforts to elicit a phenotype related to *let-363(-)* (*e.g.*, larval arrest) by several methods and concentrations failed (Long *et al.* 2002). A later study revealed a stage-dependent, dose-dependent rapamycin effect in adult worms, caused by treatment with a high dose (100 μM) of rapamycin, resulted in upregulation of genes that are activated by genetic TORC1 inhibition, increase in life span, reduction in translation, and resemblance to reducing TORC1/2 activities in genetic interaction tests (see *Life span extension and increased stress resistance from TORC1 inhibition*) (Robida-Stubbs *et al.* 2012). The high dosage used in this study (much higher than that used in mammalian cell culture) suggested that in *C. elegans* the bioavailability of rapamycin is poor, as is typical for many compounds. While *C. elegans* has been a useful

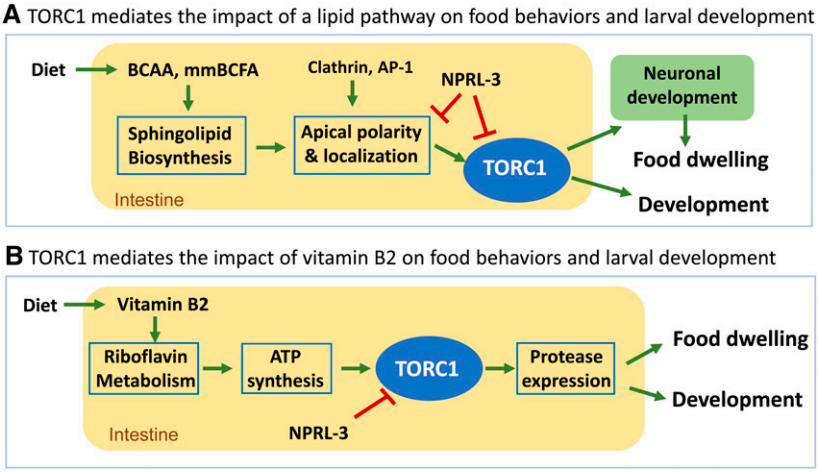

Figure 3 Proposed role of intestinal TORC1 in mediating the impact of lipids (A) and vitamin B2 (B) on animal development, and food behavior. Neuronal function related to food behaviors has been linked to the axis in (A) but . . . expression in (B). BCAA, branched-chain amino acids. mmBCFA, monomethyl branched-chain fatty acids. mmBCFA are derived from BCAA and both can be obtained from diet. BCAA, branched-chain AAs; mmBCFA, monomethyl branched-chain fatty acid; TORC, Target of Rapamycin Complex.

system for studying how rapamycin acts as an antiaging drug (see *Life span extension and increased stress resistance from TORC1 inhibition*), given the lack of a developmental phenotype and its high cost, the value of rapamycin for studying TOR functions in this organism is limited, particularly compared to the many genetic tools that are available. To date, no analyses of other chemical inhibitors of TOR have been reported in *C. elegans*.

Upstream inputs to TOR signaling

In mammals and other organisms, the TOR complexes have been characterized as nutrient-sensing centers that respond to changes in various nutrients, energy, and growth factors (Menon and Manning 2013; Saxton and Sabatini 2017). Here, we review studies in *C. elegans* that aim to connect upstream nutrient status to *in vivo* physiological functions (Figure 2).

AAs and peptides: A plethora of literature in the TOR field is devoted to understanding how TOR senses the availability of AAs (Jewell *et al.* 2013). The prevailing model is that AAs impact the localization of mTORC1, whereby high AA levels induce a relocation of mTORC1 from the cytosol to the lysosome, thereby activating the complex. This relocation is mediated by the Rag family of small GTPases (see *Other conserved TOR components and key interactors*), which can bind Raptor to recruit mTORC1 to the lysosome (Jewell *et al.* 2013; Saxton and Sabatini 2017).

As a powerful genetic model organism, *C. elegans* is potentially an excellent system to study the physiological role of AA sensing by TORC1. However, at this point, the specific connection between AA availability and TORC1 signaling in *C. elegans* is not yet well established. This may partly be due to the difficulty of establishing a good synthetic culturing medium where, unlike with live bacterial food, the AA level may be reduced. Instead, the upstream connections to AAs or proteins have been mostly indirect, and were first analyzed with the investigation of the oligopeptide transporter PEP-2/OPT-2/PEPT-1. One study showed that *pept-1* encodes a functional homolog of mammalian PEPT1/SLC15A1 by demonstrating that a *pept-1(-)* mutation causes loss of di- and tripeptide uptake, and developmental defects (Meissner *et al.* 2004). When loss of peptide uptake was combined with partial knockdown of *let-363*, an increase in the severity of the developmental retardation and intestinal phenotypes was observed. This result is consistent with *pept-1* acting upstream or downstream of, or in parallel with, TOR. The upstream model was supported by quantitative proteome analysis and transcriptome profiling that showed that *pept-1(-)* animals have reduced AA levels, which lead to reduced ribosome biogenesis and protein translation downstream of TOR (Geillinger *et al.* 2014). However, interestingly, the same group had suggested earlier that PEPT-1 acts downstream of the TOR complexes, based upon how RNAi knockdown of key TOR complex components reduced PEPT-1 protein levels and peptide uptake (Benner *et al.* 2011). These studies on PEPT-1 perhaps pointed out the complex interplay between diet, nutrient sensing, and protein expression and function, where feedback loops can complicate interpretation of epistasis experiments, especially when such experiments utilize RNAi or nonnull mutants.

While depleting AAs from the diet is difficult in *C. elegans*, raising the AA level by dietary supplementation, or genetic mutations, in the AA catabolic pathway can generate significant information regarding the functional relationships between AAs and TOR for specific physiological functions. In one study, AA supplementation was part of a series of tests leading to the model that TORC1 mediates the impact of AA availability to promote hypodermal P and M blast cell release from the quiescent state (Fukuyama *et al.* 2015) (Figure 4A) (also see *Roles of TORC1 in regulating development and behaviors*). Elevation of AA levels was also employed in two other studies to analyze the role of TOR signaling in aging, with very different conclusions about the relationship between AAs and TOR. In one study, the authors provided evidence that elevation of branched-chain AAs (BCAAs) in specific neurons caused by mutating a key BCAA catabolic gene (*bcat-1*) lead to increased life span in a *let-363/TOR*-dependent manner

(Mansfeld *et al.* 2015). Interestingly, whereas activation of TOR by AAs fits the with current models, this role of TOR signaling in promoting life span is in contrast to the numerous other studies indicating that TORC1 opposes longevity (see *Life span extension and increased stress resistance from TORC1 inhibition*). In another study, supplementation of heat-killed *Escherichia coli* with most L-AAs in liquid culture was also shown to extend life span (Edwards *et al.* 2015). However, circumstantial evidence led to the suggestion that AA supplementation may inhibit TOR signaling, which is in contrast to the consensus in the field. Such a result done with heat-killed bacteria could be subject to alternative explanations (B. Qi *et al.* 2017; Qi and Han 2018). It may be worth noting that excess AAs may induce complex metabolic responses in animals, including the activities in AA catabolic pathways and AA transport systems that are known to have profound impacts on various physiology (*e.g.*, Tărlungeanu *et al.* 2016).

The studies of TOR sensing of AAs in *C. elegans* have been wisely focused mainly on specific physiological functions such as development and aging. However, the potential to identify new insights regarding the mechanistic aspects of AA sensing has been somewhat limited by the fact that direct readouts of TOR activity have not been well established (discussed more below). In addition, the study of AA sensing by TORC1 in *C. elegans* has not yet addressed localization changes of the complex in specific tissues and under specific physiological conditions, even though the live animal system may permit new insights beyond what has been found in cultured cells.

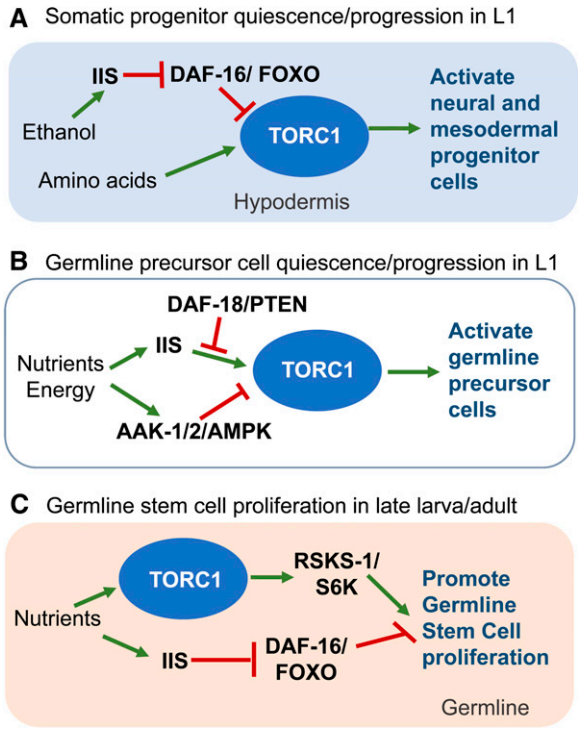

Figure 4 Proposed roles of TORC1 in three specific developmental events. Available experimental evidence supports a site of action for TORC1 in the hypodermis for (A) and in the germline for (B) and (C). IIS, insulin/IGF signaling; TORC, Target of Rapamycin Complex.

AMPK/ATP: In other species, the AMP-activated kinase (AMPK) has been shown to negatively regulate mTORC1 in response to the AMP:ATP energy ratio, primarily by phosphorylation and activation of TSC2, and TSC-independent phosphorylation and inhibition of Raptor [reviewed by Garcia and Shaw (2017)]. Since unambiguous TSC orthologs have not yet been identified in *C. elegans* [see *TSC proteins (or lack thereof)*], the mechanism by which TORC1 senses AMPK activity is unclear. In *C. elegans*, the homologs of the mammalian AMPK catalytic α subunit (*aak-1* and *aak-2*), the regulatory β subunit (*aakb-1* and *aakb-2*), and the γ subunit (*aakg-1*, *aakg-2*, *aakg-3*, *aakg-4*, and *aakg-5*) are conserved [reviewed by Ahmadi and Roy (2016)]. Work in *C. elegans* has explored the AMPK-TOR connection through the study of several specific developmental events. Specifically, one study observed a critical role for AMPK in germline quiescence, in L1 larvae under starvation-induced diapause, as mutating both *aak-1* and *aak-2* increased germline cell number in these developmentally arrested animals (Fukuyama *et al.* 2012) (Figure 4B). Knocking down *let-363* or other TORC1 components suppressed the germline defects of *aak-1/2* double mutants, consistent with the model that AMPK inhibits germline proliferation (promotes germline quiescence) by repressing TORC1 activity in L1 diapause induced by food deprivation. Similarly, two other studies provided genetic data that suggest that the AMPK-TORC1 axis regulates gonadogenesis and aging (Yuan *et al.* 2013; Ishii *et al.* 2016). However, other genetic data point to a TORC1-independent role of AMPK as the lethality of *aak-1/2* double mutants was not suppressed by reducing TORC1 activity (Fukuyama *et al.* 2012). Other studies have addressed the impact of ATP on TORC1 activity, with a focus on life span and nutrient deprivation-induced behavioral changes (Chin *et al.* 2014; Fu *et al.* 2015; B. Qi *et al.* 2017) (discussed in *TOR signaling plays pivotal roles in regulating development and behaviors* and *Role of TOR signaling in aging and stress responses*).

Insulin/IGF signaling pathway: Studies in mammalian cells have revealed a complex functional relationship between the insulin/IGF signaling (IIS) pathway and TOR complexes (Dibble and Cantley 2015; Saxton and Sabatini 2017). The IIS pathway has been shown to act upstream of mTORC1 activity through a succession of negative regulatory events in which the Akt kinase of the IIS pathway inhibits TSC, which in turn inhibits Rheb activation (binding to GTP) and hence inhibits TORC1. In addition, other studies indicate an inhibitory feedback mechanism, where the IIS pathway is downregulated by mTORC1 (through phosphorylation of Grb10 that blocks insulin signaling) in regulation of glucose homeostasis and insulin resistance (Hsu *et al.* 2011; Yu *et al.* 2011). In addition, Akt has also been shown to be a downstream target of mTORC2 under certain conditions (Sarbassov *et al.* 2005). Thus, it is important that the study of the functional

relationship between these two pathways is linked to specific physiological functions.

The major components of the IIS pathway are conserved in *C. elegans* [DAF-2/IGFR (IGF receptor), DAF-16/FoxO, *etc.*) (Murphy and Hu 2013). Although the IIS and TOR pathways have been investigated for common roles in nutrient sensing in *C. elegans*, regulation of one by the other has not been clearly demonstrated [discussed in a WormBook chapter by Murphy and Hu (2013)]. It has been shown that DAF-16/FoxO negatively regulates the transcriptional expression of *daf-15*, although the study could not conclude if this regulation was direct or indirect (Jia *et al.* 2004). DAF-16-dependent regulation of *daf-15* was also shown to play a role in germline tumorigenesis (W. Qi *et al.* 2017). The genetic data in the study by Fukuyama *et al.* (2015) also suggested that the IIS pathway regulates somatic progenitor cell quiescence, partly through modulating TORC1 activity (Figure 4A). However, as we discuss below (see *Importance of TORC1 regulation in life span extension mechanisms*), a number of aging-related studies in *C. elegans* have indicated that the TORC1 and IIS pathways also function independently in important ways.

mmBCFA/GlcCer-dependent apical polarity: Monomethyl BCFAs (mmBCFAs), derived from BCAAs, are conserved fatty acids present in *C. elegans* and humans, but their physiological functions were essentially unknown before genetic work done in *C. elegans* showed that they are required for post-embryonic development (Kniazeva *et al.* 2004, 2012; Watts and Ristow 2017). Mutants for the fatty acyl elongase gene *elo-5* lacked mmBCFAs and arrested as early L1 larvae, but could be rescued by exogenous supplementation with mmBCFAs. Further analysis indicated that this role of mmBCFAs on early larval development is mediated by mmBCFA-derived glycosylceramide (d17iso-GlcCer), which in turn promotes intestinal TORC1 activity (Zhu *et al.* 2013) (Figure 3A). One piece of critical evidence is that the L1 arrest phenotype of either or both *elo-5(-)* and reducing the ceramide glucosyltransferase activity [*cgt-1(-)* and *cgt-3(RNAi)*] is suppressed by activation of TORC1 (see *Other conserved TOR components and key interactors*). Such an impact of lipids on TORC1 activity in the intestine appeared to be mediated by apical membrane polarity that in turn affected the localization of TORC1 regulators (*e.g.*, V-ATPase) at the apical membrane (Zhu *et al.* 2015) (Figure 3A). The authors have proposed that the d17iso-GlcCer biosynthesis pathway, along with the downstream membrane polarity and subsequent TORC1 activity, may serve as a mechanism to connect the availability of certain nutrients or metabolites to development and behaviors (also see *Roles of TORC1 in regulating development and behaviors*).

Downstream targets

LET-363/TOR is a serine/threonine kinase, but it is important to note that for almost all of the TOR-regulated processes in *C. elegans*, no protein has yet been conclusively demonstrated to be a direct phosphorylation target of either TORC1 or TORC2. In the sections below, we will review the genetic and functional evidence that does, or does not, support particular mechanisms and proteins being regulated by TOR in the worm (Figure 2).

S6K/RSKS-1: S6K is the common name given to the ribosomal protein S6 kinase (also known as p70S6K). *In vitro* analyses in mammalian cell culture and other model organisms revealed S6K to be a direct phosphorylation target of mTORC1, and measurement of this phosphorylation has become the primary assay for mTORC1 activity (Ma and Blenis 2009; Magnuson *et al.* 2012). It was natural for *C. elegans* researchers to test this connection genetically. An early study identified Y47D3A.16 (later named *rsks-1*) as the worm homolog of p70S6K (Long *et al.* 2002). As would be predicted (Figure 2A), a null *rsks-1* mutation does not generate all of the phenotypes associated with loss of *let-363/TOR* (Long *et al.* 2002). However, the sharing of certain specific functions (such as life span extension, translation regulation, and germline proliferation) and genetic interactions with other factors (such *pha-4*), along with the established kinase–target relationship in other organisms, has led to the model that RSKS-1 acts downstream of TORC1 for these functions (*e.g.*, Hansen *et al.* 2007; Pan *et al.* 2007; Sheaffer *et al.* 2008; Nukazuka *et al.* 2011; Korta *et al.* 2012). Also consistent with this idea, a genetic interaction in developing larvae, in which a *rsks-1*/S6K null mutation suppressed loss-of-function in the transcriptional regulator heat-shock factor (*hsf-1*), was phenocopied by RNAi against TORC1 components (*daf-15/raptor* and *ragc-1*/RagC), but not inhibition of translation (Chisnell *et al.* 2018). This suggests that RSKS-1/S6K and TORC1 act on a common downstream mechanism that is distinct from translation regulation. However, genetic epistasis analysis between TORC1 and RSKS-1 using a mutation that constitutively activates either kinase has not been performed.

Given the extensive literature in other species, it is tempting to assume that RSKS-1 must be a direct phosphorylation target of TORC1 in *C. elegans* (Lin *et al.* 2014; Nakamura *et al.* 2016; Chen *et al.* 2017). However, this idea is not yet supported by direct biochemical evidence, and is still largely based upon conserved sequence homology, genetic analyses, and related mutant phenotypes that are consistent with findings in other species (see also *Germline development* and *Life span extension and increased stress resistance from TORC1 inhibition*). Some studies have used anti-S6K antibodies to detect changes in RSKS-1 phosphorylation as evidence of TORC1 activity. However, sequence alignment and phosphoproteomic data mining indicate that the relevant RSKS-1 residues are either not phosphorylated in *C. elegans* (*Homo sapiens* T389/*C. elegans* T404) (Bodenmiller *et al.* 2008; Zielinska *et al.* 2009), or not mTORC1-dependent phosphorylation targets (*H. sapiens* S411/*C. elegans* S439) (Magnuson *et al.* 2012). These findings should be carefully considered if RSKS-1 phosphorylation is used as a readout for TORC1

activity in *C. elegans*. Direct biochemical tests of TORC1 activity in *C. elegans*, such as *in vitro* kinase assays or TORC1-dependent phosphoproteomic profiling, may be necessary for a definitive conclusion on this point.

4E-BP1/ eukaryotic initiation factor 4E: 4E-BP1, named for eukaryotic initiation factor 4E (eIF4E)-binding protein, is a translational repressor that has been shown to be a phosphorylation target of mTORC1 in mammalian cell culture (Ma and Blenis 2009; Magnuson *et al.* 2012). Although it was first thought that *C. elegans* lacked a homolog of 4E-BP1 (Long *et al.* 2002), a later study identified IFET-1 (aka SPN-2) as the worm 4E-BP1-like protein (Li *et al.* 2009). The sequence homology is very limited (which is likely why it was not identified earlier) and IFET-1/SPN-2 lacks the consensus eIF4E-binding motif, but the authors went further to demonstrate that IFET-1/SPN-2 can bind several *C. elegans* eIF4E homologs *in vitro*, demonstrating conservation of the function. Another group expressed a human 4E-BP1 protein, h4EBP1, in *C. elegans*, and observed significant reduction of its phosphorylation in *let-363(RNAi)* and *daf-15(RNAi)* worms, as well as expected phenotypes from *ifet-1(RNAi)* (Nukazuka *et al.* 2011). These data indicated conservation of the specificity of TOR kinase and supported a likely role of IFET-1/SPN-2 as 4E-BP1. However, these studies are not yet sufficient to make a firm conclusion, as phosphorylation of IFET-1/SPN-2 by TORC1 has not been demonstrated and the interpretation of the genetic phenotype was made with the assumption that IFET-1/SPN-2 is the worm 4E-BP1. An alternative possibility is that IFET-1/SPN-2 is not a direct TORC1 target, and that TORC1 directly phosphorylates and inhibits one of several eIF4E isoforms, such as IFE-2, that have been implicated in longevity in *C. elegans* (Jankowska-Anyszka *et al.* 1998; Syntichaki *et al.* 2007).

Protein translation: Promoting protein translation has been well established in multiple organisms as a central downstream function of TORC1, with multiple direct phosphorylation targets being involved in translation (Ma and Blenis 2009; Magnuson *et al.* 2012). In addition to those related to S6K and 4EBP, genetic data also support TORC1 regulation of translation in *C. elegans*. Loss-of-function analysis of several other homologs of TOR effectors identified in other organisms, such as initiation factors M110.4/*ifg-1*, Y37E3.10/*eif-2*α, and K04G2.1/*eif-2*β produced most of the *let-363* loss-of-function phenotypes (Long *et al.* 2002). Knocking down *ifg-1* and *eif-1* also resembles *raga-1* knockdown in increase of life span (Robida-Stubbs *et al.* 2012) (see *Life span extension and increased stress resistance from TORC1 inhibition*). Other evidence includes inhibition of translation by rapamycin treatment or *ragc-1* knockdown, and promotion of rRNA maturation in nucleoli (which is expected to affect protein translation) by *let-363* (Sheaffer *et al.* 2008; Robida-Stubbs *et al.* 2012). Sheaffer *et al.* (2008) also introduced the localization of FIB-1, a box C/D small nucleolar ribonucleoprotein, in nucleoli as a useful indirect readout of TORC1 activity. This was significant for the *C. elegans* field, in which a direct phosphorylation target of TORC1 has not yet been defined (Zhu *et al.* 2013).

Autophagy: Extensive studies in multiple organisms including *C. elegans* have indicated clearly that TORC1 inhibits autophagy (Toth *et al.* 2007; Hansen *et al.* 2008; Meléndez and Levine 2009). While the TORC1–autophagy axis has been extensively studied in aging regulation (see *Role of TOR signaling in aging and stress response*), it could also potentially influence other cellular events. In several cases, a GFP reporter of LGG-1 (a worm ATG8 protein) (a *plgg-1*::GFP::LGG-1 translational fusion transgene), which is commonly used as an autophagy marker (Meléndez *et al.* 2003; Tian *et al.* 2010), has been a helpful yet indirect way to evaluate TORC1 activity (*e.g.*, Meléndez *et al.* 2003; Hansen *et al.* 2008; Tian *et al.* 2010; Robida-Stubbs *et al.* 2012; Chin *et al.* 2014; B. Qi *et al.* 2017). Unlike in yeast and mammals, where TOR has been shown to directly phosphorylate several ATG proteins to regulate autophagy (ATG13, Ulk1/ATG1, and AGT14) (Jung *et al.* 2009, 2010; Russell *et al.* 2013, 2014), phosphorylation of corresponding proteins in *C. elegans* by TOR has not been biochemically demonstrated. In addition, consistent with studies in mammalian cells, regulation of the mRNA levels of *lgg-1* and other autophagy genes by TORC1 is at least partly mediated by the transcription factor HLH-30/TFEB in *C. elegans* (Lapierre *et al.* 2013; Settembre *et al.* 2013; Nakamura *et al.* 2016). Phosphorylation of TFEB by TORC1 regulates its subcellular localization and activity in mammals, but this has not yet been demonstrated in *C. elegans* (Napolitano and Ballabio 2016).

SGK-1: The *C. elegans* homolog of serum and glucocorticoid-induced kinase 1, *sgk-1*, was first characterized for a role in stress response and life span (Hertweck *et al.* 2004). Later independent studies in two laboratories showed that *sgk-1(-)* phenocopied *rict-1(-)* in fat accumulation and smaller body size (Jones *et al.* 2009; Soukas *et al.* 2009). These studies provided two lines of evidence to support that SGK-1 acts downstream of RICT-1 in the same pathway: loss-of-function alleles in *sgk-1* did not enhance the defects in *rict-1* mutants, and gain-of-function or overexpression of *sgk-1* suppressed the defects in *rict-1* mutants. This RICT-1-SGK-1 pathway appears to act in the intestine to regulate fat metabolism independently of DAF-16/FoxO. Several additional studies have presented data to support the idea that SGK-1 is a downstream factor of TORC2, even though no one has yet demonstrated direct regulation by phosphorylation in *C. elegans*. For example, one study linked TORC2 and SGK-1 in regulating mesendodermal embryonic development (Ruf *et al.* 2013), and another presented genetic data to support the idea that SGK-1 acts downstream of TORC2 in the intestine to regulate vitellogenesis and fat mobilization for oogenesis (Dowen *et al.* 2016). Finally, two studies that are discussed below (see *Roles of TORC2 in aging and stress responses)* indicate

that SGK-1 acts downstream of RICT-1 in determining life span (Mizunuma *et al.* 2014; Zhou *et al.* 2019).

P granule proteins: The *C. elegans* P granules are germline-specific granules containing a group of perinuclear RNAs and their binding proteins (Wang and Seydoux 2014). PGL-1 and PGL-3 are RGG-domain P granule components, which have recently been found to be direct targets of TORC1 in *C. elegans* embryos through immunoprecipitation and *in vitro* kinase assays (Zhang *et al.* 2018) (also see *Roles of TORC1 in regulating development and behaviors*).

TOR Signaling Plays Pivotal Roles in Regulating Development and Behaviors

Theoretically, there may be two types of developmental regulation by the TOR complexes. One type would be directly linked to their well-known roles as major "nutrient sensors," under which TORC1/2 may promote developmental events under conditions with sufficient nutrients, whereas TOR inhibition may arrest or alter developmental events when specific or overall nutrients are deprived. Studies in *C. elegans* have offered a few excellent examples [*e.g.*, TORC1 regulating postembryonic development and food behaviors in response to vitamin B2 (VB2) availability (B. Qi *et al.* 2017)]. The other type of developmental regulation would involve TOR complexes functioning as built-in machinery in a regulatory network that controls cell differentiation and developmental pattern formation without a direct link to nutrient availability. For example, the role of TORC2 in mesendodermal development in the embryo does not seem to have an obvious connection to nutrient availability (Ruf *et al.* 2013). However, these two types of functions may not be as distinct as they appear. First, "nutrient sensing" may often be indirectly mediated by metabolic pathways or cellular events that modulate the activity of TOR complexes, which is exemplified by the role of glucosylceramide and apical membrane polarity in mediating the effect of fatty acid availability on TORC1 and postembryonic development (Zhu *et al.* 2015). Second, the connections to nutrient components in some cases are yet to be identified (*e.g.*, regulation of vitellogenesis by TORC2; Dowen *et al.* 2016). Given that the connection between nutrient availability and development is a relatively new and exciting research frontier, TOR functions in development will continue to be an attractive research topic in the *C. elegans* field.

Through its well-documented anabolic functions in mammalian and *Drosophila* cells, TORC1 regulates cell/organ size independently of the cell cycle so that flies with reduced TOR activity are smaller because they have smaller rather than fewer cells (Tumaneng *et al.* 2012; Lloyd 2013). Many studies have observed that TORC1 is required for completion of *C. elegans* larval growth and development, but comparatively little has been done to investigate its possible effects on cell or body size. It has been noted that distal germ cell size is smaller in *rsks-1* mutants, though not in *daf-2*/IIS mutants, which also slow the mitotic germ cell cycle (Korta *et al.* 2012). Mutation of *raga-1* modestly reduces adult body size, an effect that appears to derive from the importance of RAGA-1 for larval development (Schreiber *et al.* 2010). One speculative possibility is that cellular growth regulation might be less plastic in *C. elegans* than in more complex organisms, possibly because of constraints imposed by the worm cuticle or specific aspects of *C. elegans* body-size regulation, a process that is not well understood.

Roles of TORC1 in regulating development and behaviors

Germline development: *C. elegans* L1 larvae hatch with two primordial germ cells, Z2 and Z3, which then begin to proliferate mitotically as long as conditions are favorable (Kimble and Crittenden 2005). When *C. elegans* hatch in the absence of food, they enter an L1 diapause or arrest state, and suspend growth until food is available (Baugh 2013). During this L1 diapause, primordial germ cells arrest in G2 phase, with arrest dependent upon DAF-18/PTEN (a negative regulator of the IIS pathway) and on *aak-1/2/*AMPK, but not dependent on DAF-16/FoxO (Fukuyama *et al.* 2006, 2012). Loss-of-function mutations in *aak-1/2* or *daf-18* resulted in ectopic germline proliferation in the absence of food, and this ectopic proliferation was partially suppressed by RNAi targeting components of TORC1. Indeed, AMPK and PTEN have been shown to negatively regulate mTORC1 in mammals (Feng *et al.* 2007). The *C. elegans* studies suggest that TORC1 activity may be suppressed by AMPK to maintain germline quiescence during L1 diapause (Figure 4B). Whether TORC1 acts cell autonomously in the germline for this function has not yet been firmly addressed by experiments (Fukuyama *et al.* 2012).

Beginning in the L3 stage, and continuing into adulthood, some germ cells exit mitosis and enter meiosis, and eventually give rise to sperm in the L4 stage or oocytes in the adult (Hubbard and Greenstein 2005). GLP-1/Notch pathway signaling is required to prevent differentiation of germline progenitors. TGF-β and MAPK pathways also act to prevent differentiation (*e.g.*, Lee *et al.* 2007; Dalfó *et al.* 2012). In addition, the IIS pathway and TORC1 act in the germline to positively regulate cell cycle progression in the larval germline, though independently (Michaelson *et al.* 2010; Korta *et al.* 2012) (Figure 4C).

Based on the marked reduction of the larval germline progenitor pool in *rsks-1(−)* and the viability of the null mutant, the specific role of TORC1 and of this putative TORC1 target was further investigated. Among many defects, L3/L4-stage *rsks-1(−)* worms displayed a reduced number of germline progenitors, and this defect was rescued by germline-specific expression of *rsks-1*. The authors found that *let-363/Tor (RNAi)* and *daf-15/raptor(RNAi)* also reduced progenitor number, but less severely than *rsks-1(−)*. However, the phenotype of a double-mutant combination of *rsks-1(−)* and *ife-1(-)*, an eIF4E ortholog that acts in germline progenitors, closely resembled the phenotype of *let-363/Tor* or *daf-15/raptor* RNAi,

suggesting the possibility that TORC1 acts primarily via 4E-BP and S6K in this context. Further tests demonstrated that nutrients impact the germline progenitors in a *rsks-1*-dependent manner. When the conserved, putative TORC1 phosphorylation site of RSKS-1 was mutated in a *rsks-1(+)* transgene (T404A) (Schalm and Blenis 2002) it no longer rescued the larval germline progenitor accumulation phenotype (Korta *et al.* 2012). Although this result seems to support the idea that RSKS-1 is a phosphorylation target of TORC1, as mentioned earlier (see *S6K/RSKS-1*), biochemical analyses have not indicated that this T404 residue is a TORC1 phosphorylation site in *C. elegans*. In addition to a role in promoting germline cell cycle progression, RSKS-1 also promotes germline stem cell (GSC) maintenance in conjunction with GLP-1/Notch, a role that does not appear to be shared by TORC1 (Korta *et al.* 2012). This specific role is also germline autonomous and is dependent on residue T404 (Roy *et al.* 2018).

C. elegans germline tumor formation has become a significant model for the study of tumorigenesis (Singh and Hansen 2017). The IIS signaling target DAF-16/FoxO plays both cell autonomous and nonautonomous roles in germline proliferation, and tumor formation. DAF-16/FoxO activity inhibits germ cell proliferation in *gld-1(lf)* mutants (Pinkston *et al.* 2006), whereas DAF-16/FOXO activity from a transgene expressed in the hypodermis can induce the germline tumor phenotype (Qi *et al.* 2012). W. Qi *et al.* (2017) screened for kinases involved in this latter DAF-16/FOXO-dependent tumor formation in L3 staged larvae, and found that reducing *rsks-1* and genes for TORC1 components moderately suppressed the tumorous phenotype, which is consistent with the known roles of mTORC1 in cell growth and cancer in humans (W. Qi *et al.* 2017). Based on the genetic data, W. Qi *et al.* (2017) suggested a model where DAF-16/FOXO activity (along with a TGF-β pathway) in the hypodermis may promote germline proliferation in part by upregulating the transcription of TORC1 pathway components *daf-15*, *rsks-1*, and *rheb-1* (W. Qi *et al.* 2017). This functional relationship between DAF-16 and TORC1 is consistent with earlier findings on several somatic developmental events (see *Upstream inputs to TOR signaling*).

Through a different approach, one study made an interesting finding regarding the role of germline small RNAs in regulating TORC1 (Barberán-Soler *et al.* 2014). PRG-1 and CSR-1 are two germline-specific Argonaute proteins that are required for proper germline development; *prg-1(-)* mutants displayed a partial sterility phenotype that progressively worsened over multiple generations. Via RNA-sequencing profiling of *prg-1* mutants, Barberán-Soler *et al.* (2014) discovered abnormal splicing products of *let-363/Tor* and an abnormal presence of a male germline-specific endo-siRNA produced from the antisense strand of a *let-363* intron. RNAi against the intron containing this siRNA in *prg-1(-)* animals restored normal *let-363* splicing and expression, and thereby reversed the sterility phenotype in *prg-1(-)* mutants. Additional genetic tests led to the model that PRG-1 and CSR-1 regulate germline development by antagonistically regulating the splicing, and expression, of *let-363* through modulating the activity of this siRNA. Whether such regulation is conserved in mammals would be an interesting question to address.

Embryonic development: *C. elegans* with a null allele in *let-363/TOR* arrest development at the third postembryonic stage (L3) (Long *et al.* 2002) but *let-363/TOR(RNAi)* by injection caused embryonic lethality (Sönnichsen *et al.* 2005; WormBase), suggesting that certain embryonic functions of TOR are masked by maternal rescue in the null mutants. Additional roles could also be masked by genetic redundancy, under which the effect of knocking down single genes may only be observed when another contributing gene is also compromised. As discussed below (see *Roles of TORC2 in regulating development and behaviors*), a critical role of TORC2 in embryogenesis was identified in a sensitive suppressor assay. Therefore, additional roles of TORC1 during embryogenesis may yet to be identified by employing genetic suppressor or enhancer screens.

TORC1 requirements could also depend on environmental conditions. For example, a recent study identified two P granule proteins as TOR phosphorylation targets and indicated a role of TORC1 in protecting embryogenesis from stress (Zhang *et al.* 2018). Specifically, under heat stress, TORC1-dependent phosphorylation of P granule components PGL-1 and PGL-3 led to the formation of P granules that are resistant to autophagy, and increased embryo viability.

Postembryonic development and behavior: The L3 arrest of *let-363(lf)* and *daf-15(lf)* mutants indicates roles of TORC1 in promoting larval growth and development (see *Identification of key components*). The observation that *let-363(RNAi)* suppresses L1 larval lethality associated with a *pha-4/FOXA* mutation also suggests a role of TOR in early larval development (Sheaffer *et al.* 2008). Whether and how TORC1 perceives the availability of specific nutrients to instruct postembryonic development are challenging questions, which are well suited for *C. elegans* researchers to address.

Dauer formation: In response to food deprivation, *C. elegans* arrest their development and form specialized dauer larvae after the second molt to extend their survival until they encounter new food (Hu 2007). Dauer larvae are morphologically and behaviorally distinct from typical L3 larvae, and are highly resistant to stress; they can survive for months until conditions improve, whereupon they reenter the reproductive life cycle. More than 40 *daf* genes, including many acting in the IIS and TGF-β pathways, were defined based on either constitutive dauer formation under abundant food (Daf-c phenotype) or failure to form dauer under food deprivation (Daf-d phenotype). The mutant phenotypes of *daf-15/raptor* and *daf-9* (encoding cytochrome P450 family protein) were distinct from other *daf-c* mutants in that the "dauer-like" arrested worms appeared to be in an intermediate state, with only a subset of dauer characteristics (Albert and Riddle 1988; Jia *et al.* 2004). *daf-15(RNAi)* generated larval arrest phenotypes resembling those caused by *let-363/TOR(RNAi)* (Hara *et al.* 2002; Long *et al.* 2002), which, along with other data presented below, support an essential role of

TORC1 in regulating postembryonic larval growth. However, an additional role of TORC1 in dauer formation is also suggested by genetic data beyond just the "dauer-like" morphology of *daf-15(lf)* mutants: mutation or RNAi knockdown of *let-363* appears to enhance the dauer-constitutive phenotype of a *daf-2/IGFR* mutant, and *daf-15* expression is regulated by *daf-16/FOXO* (Vellai *et al.* 2003; Jia *et al.* 2004). Further studies may be necessary to confirm these interactions, which have important implications. A potential role of TORC1 in inhibiting dauer entry would raise the questions of whether TORC1 directly responds to food cues in this capacity, and what target(s) TORC1 might act on to influence the L3 *vs.* dauer decision.

Food deprivation-induced L1 diapause: Besides dauer formation, food deprivation-induced L1 diapause is also an excellent system to study how the postembryonic developmental program is regulated by nutrient/food availability (Baugh 2013). While extensive early studies uncovered the critical role of the IIS pathway in regulating L1 diapause (Baugh and Sternberg 2006; Zhang *et al.* 2011; Baugh 2013), more recent work has indicated the role of TORC1 in somatic cells. Specifically, newly hatched L1 larvae halted development in the absence of food (M9 solution), but supplementing the M9 solution with AAs and ethanol was sufficient to reactivate the quiescent somatic progenitor cells (the P, M, and Z1/Z4 cell lineages) (Fukuyama *et al.* 2015). It was further shown that ectopic expression of a putative activated *raga-1* transgene in the hypodermis (and not the intestine or neurons) was sufficient to stimulate M and P cell progression, and these effects were suppressed by RNAi against *let-363/TOR* or *daf-15/raptor*. This study also provided evidence that TORC1 may act downstream of IIS and DAF-16/FOXO to mediate the ethanol effect on the activation of somatic progenitor cells. These and additional results led to an interesting model where AA levels are monitored by TORC1 in the hypodermis to regulate somatic progenitor cell progression (Fukuyama *et al.* 2015) (Figure 4A).

Lipid deficiency-induced developmental arrest and foraging behavior change: When *C. elegans* embryos are deficient for mmBCFAs, they uniformly arrest postembryonic development after hatching, a state resembling food deprivation-induced L1 diapause, with the exception that the lipid deficiency also dramatically altered *C. elegans* food-seeking behavior (Kniazeva *et al.* 2008, 2015). The lipid deficiency also impairs survival of the arrested animals (Cui *et al.* 2017). Extensive further studies, including the isolation of a suppressor mutation in *nprl-3*, suggested that this growth arrest is due to a lack of d17iso-GlcCer and insufficient TORC1 activity (Kniazeva *et al.* 2008; Zhu *et al.* 2013) (also see *Upstream inputs to TOR signaling*) (Figure 3A).

Behavioral changes in mmBCFA- or d17iso-GlcCer-deficient L1 larvae also could be attributed to insufficient TORC1 activity (Kniazeva *et al.* 2015). Larvae provided with a normal diet typically spend more time dwelling near the food and less time roaming away from the food, and neuronal circuits controlling such foraging behavior have been extensively analyzed (*e.g.*, Ben Arous *et al.* 2009; Milward *et al.* 2011; Flavell *et al.* 2013). In contrast, mmBCFA-deficient L1 larvae failed to respond to the bacterial food (reduced dwelling) unless mmBCFAs were added, or unless TORC1 activity was otherwise elevated via genetic manipulations (Kniazeva *et al.* 2015). Since behavioral defects are often the consequence of defects in neuronal development, regulation of behavior by TORC1 may be closely linked to its role in postembryonic development. Indeed, Kniazeva *et al.* (2015) found evidence that mmBCFA deficiency reduced the expression of a known regulator of neuronal differentiation (*ceh-36*), which partially contributes to the change in food behavior. Therefore, the behavior defect is at least in part due to a defect in neuronal development and TORC1 plays a critical role to mediate the impact of mmBCFAs on these behaviors (Figure 3A).

VB2 deficiency-induced developmental arrest and foraging behavior change: Dietary VB2 is essential for the normal developmental progression of *C. elegans* (B. Qi *et al.* 2017). It was shown that heat-killed bacteria lack sufficient VB2 to support worm growth, and that worms stop eating such food and change their foraging behavior to search rather than dwell (B. Qi *et al.* 2017). Furthermore, VB2-deficient worms showed reduced intestinal expression of several specific proteases, suggesting that they may not be able to properly digest food. Providing exogenous VB2 (or its derivative flavin adenine dinucleotide) could partially restore worm growth, behavior, and protease expression, but this effect was dependent on functional TORC1, as it was eliminated by *daf-15/raptor(RNAi)* or *ragc-1(RNAi)*. Genetic manipulations thought to increase TORC1 activity also restored worm growth, behavior, and protease expression. Based on these and other data, B. Qi *et al.* (2017) suggested that VB2-derived ATP stimulates TORC1 activity to upregulate intestinal protease expression (Figure 3B). Further work is needed to show just how the expression of these proteases is regulated by TORC1 and translated into a signal to change neuronal development or functions.

Vulva development: Signaling by LIN-3/EGF and the Ras-ERK pathway promotes development of the vulva, an epithelial tube used for egg laying (Sundaram 2013). In certain vulvaless mutants with reduced signaling, starvation has been shown to restore vulval fates (Euling and Ambros 1996). A recent study demonstrated that loss of *pept-1* was equivalent to starvation in suppressing the vulvaless phenotype caused by a partial loss-of-function allele in the *lin-3/EGF* gene (Grimbert *et al.* 2018) (see *Upstream inputs to TOR signaling* for discussion on *pept-1*). Moreover, *let-363(RNAi)* or *rsks-1(−)* also displayed significant suppression, albeit not as strong as that by *pept-1(-)*. This study suggested a potential role of TORC1 in repressing EGF signaling under nonstarved conditions, although the mechanism underlying such a role and its physiological significance remain to be investigated.

Roles of TORC2 in regulating development and behaviors

Fat mobilization to the germline during larval development: TORC2 has been shown to regulate reproductive development by acting in a somatic tissue (intestine). Lipid

transportation from the intestine to germ cells by vitellogenins is a critical step for reproductive development and this process, including vitellogenesis, is coordinated with postembryonic development (Lemieux and Ashrafi 2016; Watts and Ristow 2017). The IIS pathway was first identified to play an important role in the process (DePina *et al.* 2011). Through a tandem genetic screen, one study found that several factors that regulate developmental timing, including the microRNAs *let-7* and *lin-29*, act in the hypodermis to promote lipid mobilization in the intestine (Dowen *et al.* 2016). In the intestine, this signal is conveyed by TORC2, not TORC1, with SGK-1 and the transcription factor PQM-1 acting downstream of TORC2 to promote the expression of genes involved in vitellogenesis, and other activities needed for fat mobilization. Specific signals generated by the hypodermis that remain unidentified must therefore trigger activation of TORC2 nonautonomously in the gut, thereby tightly coordinating lipid mobilization for reproduction with development (Dowen *et al.* 2016; Weaver *et al.* 2016).

Embryonic development: A role for TORC2 in embryonic development was revealed through its genetic interactions with *skn-1* (Ruf *et al.* 2013). SKN-1 is a transcription factor that has been well characterized for its role in promoting mesodermal and endodermal cell fates, stress resistance, and life span (Blackwell *et al.* 2015). *skn-1(RNAi)* causes cell fate transformations and consequent embryonic lethality, but such lethality is partly suppressed by a *rict-1* loss-of-function mutation (Ruf *et al.* 2013). These genetic data suggested that TORC2 acts downstream of (or in parallel to) SKN-1 to repress a gene expression network necessary for both mesodermal and endodermal fates. SGK-1 appears to mediate many TORC2 functions, but in this case the connection with SGK-1, as well as the connection between SKN-1 and TORC2, remain to be explored.

Postembryonic development and foraging behavior: Loss of *rict-1* confers many phenotypes, including developmental delay and small body size. A targeted RNAi suppressor screen identified *dpy-21* as a suppressor of the *rict-1(-)* slow-growth phenotype (Webster *et al.* 2013). Further tests showed that additional members of the dosage compensation complex also suppressed the *rict-1* phenotypes, leading to the conclusion that the dosage compensation complex acts downstream of RICT-1/TORC2 to negatively regulate development. Since not all *rict-1(lf)* phenotypes were suppressed by this pathway, it was proposed that the roles of TORC2 in impacting life span and body size are carried out by independent mechanisms.

A recent study discovered a fascinating role of Rictor/TORC2 in the intestine in regulating dauer formation and foraging behavior (O'Donnell *et al.* 2018). Entry into dauer usually occurs in response to high temperatures and/or crowded conditions (Hu 2007). Loss-of-function mutations in *rict-1* or the likely TORC2 downstream target *sgk-1* drastically increased dauer formation at 27°, and this phenotype was rescued by intestinal expression of the corresponding gene. Further tests have indicated that TORC2 promotes the expression of DAF-7/TGF-β and an insulin-like peptide (DAF-28) to negatively regulate heat-induced dauer entry. Moreover, the study showed that RICT-1 is required for food-induced dwelling behavior, and provided genetic data to support a model in which Rictor inhibits foraging by promoting the signaling activity of neuropeptides PDF-1 and PDF-2 (O'Donnell *et al.* 2018). This study also raises interesting questions regarding how TORC2 perceives the levels of specific nutrients, and how intestinal TORC2 and SGK-1 regulate gene expression and activities in neurons. Interestingly, these data, along with the studies discussed above (see *Roles of TORC1 in regulating development and behaviors*), demonstrated roles of both intestinal TORC1 and TORC2 in regulating postembryonic developmental events (albeit distinct events) and foraging behaviors, raising fascinating questions regarding the differences between TORC1 and TORC2, and the physiological significance and mechanism for each system. In addition, another recent study showed that loss of several TOR-related proteins results in changes in "taste-associated learning," suggesting a potentially critical role of TORC2 in a sensory neuron's ability to respond to salt-level changes (Sakai *et al.* 2017).

In a study of the role of Rho GTPases in axon guidance, CDC-42-induced neuronal protrusions were found to be dependent on *rict-1*, but not *daf-15* nor *daf-16*, implicating TORC2 in these processes (Alan *et al.* 2013). Neuron-specific RNAi experiments lead to the conclusion that these are cell-autonomous activities in the PDE neuron.

Fat storage: Studies in mammals have indicated that both mTORC1 and mTORC2 promote lipogenesis and adipogenesis (Lamming and Sabatini 2013; Caron *et al.* 2015), but studies in *C. elegans* suggest that TORC2 instead inhibits fat storage. An apparent role of TOR in lipid metabolism in *C. elegans* was first revealed by observations that *let-363 (RNAi)* led to a significant increase in Nile Red staining (Vellai *et al.* 2003). Several years later, two independent forward genetic screens identified a fat storage-increase phenotype associated with loss of *rict-1* activity (by staining with Nile Red, Oil Red O, and boron-dipyrromethene (BODIPY)-labeled fatty acid dye) (Jones *et al.* 2009; Soukas *et al.* 2009). In addition, *rict-1* was also found to be required for roles of several genes in regulating Nile Red accumulation and autofluorescence of lysosome-related organelles (Soukas *et al.* 2013). Consistently, mutations in the TORC2 component *sinh-1* also caused an increase in body fat (Sakai *et al.* 2017). Both Jones *et al.* (2009) and Soukas *et al.* (2009) indicated that TORC2 acts mainly through SGK-1, although the two studies have different conclusions about the role of the AKT pathway, which is thought to be a major downstream target in mammals (Jones *et al.* 2009; Soukas *et al.* 2009; Saxton and Sabatini 2017). These studies may have revealed a unique aspect of TORC2 regulation of fat metabolism that is yet to be uncovered in mammals. Such a

function may be related to the role of TORC2 in stress responses, including coping with changes in nutrient availability, which may warrant future investigation.

Role of TOR Signaling in Aging and Stress Responses

For the last several years, TOR has been a prominent topic in the aging field in *C. elegans*, and beyond. One reason is that rapamycin represents the current "gold standard" for an antiaging drug. Rapamycin extends life span in mice, even when administered in later life (Harrison *et al.* 2009; Kennedy and Lamming 2016), and provides a model for elucidating how a drug that acts on a single defined target can extend life span. Mounting evidence indicates that rapamycin treatment or genetic TOR inhibition also improves multiple health-related parameters in mice, and even dogs (Wu *et al.* 2013; Kennedy and Lamming 2016; Urfer *et al.* 2017). TOR is also of great interest because it has been linked to aging in organisms ranging from single-cell eukaryotes to mice, and plays an important role in life span extension by other genetic or pharmacological interventions that promote longevity (Johnson *et al.* 2013; Antikainen *et al.* 2017). Of particular importance, there is general agreement in the aging field that reduced TOR activity is a major mediator of the beneficial effects of dietary restriction (DR) (Figure 5) (Kenyon 2010; Johnson *et al.* 2013). DR, defined as a reduction in nutrient intake that does not induce malnutrition, can extend life span robustly and confers metabolic benefits in essentially all eukaryotes. For many reasons, DR is not practical as an antiaging strategy to be adopted by humans, making it important to identify DR-related mechanisms that could be more realistic strategies for intervention. Given the importance of elucidating specific protective mechanisms acted upon by either rapamycin or DR, it is not surprising that TOR is very much in the spotlight.

The notion that life span can be altered by a single genetic mutation, and hence by intervention in a specific pathway, was shown to be true over two decades ago with the groundbreaking finding that *C. elegans* life span can be extended by reductions in IIS activity (Friedman and Johnson 1988; Kenyon *et al.* 1993; Dorman *et al.* 1995; Morris *et al.* 1996; Kimura *et al.* 1997; Lin *et al.* 1997; Ogg *et al.* 1997; Kenyon 2010; Shore and Ruvkun 2013). Arguably, *C. elegans* remains the premier model organism for genetic, metabolic, and pharmacological analyses of how aging can be slowed. It was in *C. elegans* that it was first demonstrated that life span can be extended when TOR is reduced (Vellai *et al.* 2003), and subsequent *C. elegans* studies have made many major contributions to our understanding of how TOR affects aging. Given its short life span and genetic tractability, the worm has been especially valuable for elucidating mechanisms through which reduced TOR signaling promotes longevity and stress resistance. In particular, novel insights into TOR functions that have been obtained in *C. elegans* include identification of mechanisms that function downstream of TOR to extend

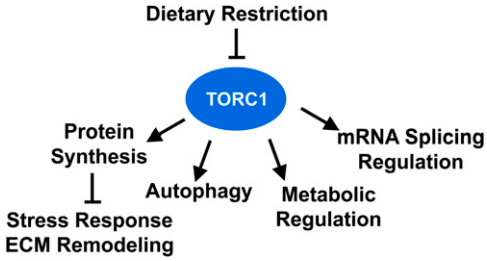

Figure 5 Processes through which TORC1 affects *C. elegans* life span. A partial list of major biological functions through which TORC1 modulates life span is shown. The critical role of TORC1 in dietary restriction is indicated, but TORC1 is also involved in other pathways that promote longevity. Please see the text for a more thorough discussion. ECM, extracellular matrix; TORC, Target of Rapamycin Complex.

life span, and teasing apart the effects of TORC1 and TORC2 on life span.

Life span extension and increased stress resistance from TORC1 inhibition

A good starting point for discussing how TORC1 influences *C. elegans* aging is to consider the effects of reducing or eliminating its activity, and treatment with rapamycin. The initial link between the TOR pathway and aging had been made in 2001 in *S. cerevisiae*, when it was shown that deletion of the S6 kinase ortholog Sch9 doubled the survival of cells in stationary phase, but at the time it was thought that Sch9 corresponded to AKT rather than S6K (Fabrizio *et al.* 2001). While TORC1 is required for *C. elegans* larval development and reproduction (see *Identification of key components*), it is possible to study its effects on *C. elegans* life span by examining animals in which its activity is disrupted partially, or by RNAi knockdown after development is complete. *C. elegans* thereby provided the very first evidence in any organism that TOR signaling affects aging, with the observation that RNAi against *let-363*/TOR or loss of one *daf-15*/*raptor* gene copy extended life span (Vellai *et al.* 2003; Jia *et al.* 2004). Subsequent work showed that in *C. elegans*, yeast, *Drosophila*, and mice, life span could be extended by reducing the activity of several TORC1 signaling components or by treatment with rapamycin (Johnson *et al.* 2013; Antikainen *et al.* 2017). Across the many *C. elegans* studies referenced in this article, genetic ablation of TORC1 pathway components typically extends mean life span by ~18–25%. While this degree of life span extension is considerably lower than is typically associated with DR or rIIS (> 50%) (Kenyon 2010; Moroz *et al.* 2014), a comparable increase in healthy human life span would generate considerable excitement! Ablation or inhibition of TORC1 signaling also improves *C. elegans* "healthspan," the length of time that the animal fails to show defined signs of aging or exhibits behaviors associated with youthfulness. For example, Rag GTPase knockdown or mutation dramatically delays the aging-related decline in various metrics of activity (Schreiber *et al.* 2010; Robida-Stubbs *et al.* 2012).

Less protein synthesis, reduced stress, and longer life: Across species, the biological process that is most solidly established as being regulated by TORC1 is mRNA translation

(Figure 5) (Johnson *et al.* 2013; Kennedy and Lamming 2016; Saxton and Sabatini 2017). This appears to be true in *C. elegans*, since functional studies of IFET-1/SPN-2 (Li *et al.* 2009) indicate that it is an eIF4E-binding protein. In the worm, the rate of protein synthesis, as indicated by ^{35}S incorporation, is reduced by adulthood RNAi knockdown of *let-363*/TOR, *ragc-1*/RagC, or *rsks-1*/S6K and by rapamycin treatment (Hansen *et al.* 2007; Robida-Stubbs *et al.* 2012). Although it has not been demonstrated conclusively that TORC1 directly phosphorylates RSKS-1/S6K in *C. elegans* (see *Downstream targets*), in this section we will consider it to be a putative TORC1 target because of the conservation of S6K being directly regulated by TORC1 in organisms as diverse as yeast and humans (Saxton and Sabatini 2017), the genetic evidence cited above (see *Downstream targets*), and the evolutionarily predicted role of RSKS-1/S6K in promoting ribosome function and protein synthesis (a function of TORC1 in other eukaryotes that is conserved in *C. elegans)*.

The intense level of interest in the TORC1 pathway, together with the identification of translation-related proteins in *C. elegans* genetic screens for life span extension or stress response activation, led a number of laboratories to investigate whether *C. elegans* life span can be increased simply by reducing the rates of protein synthesis (Henderson *et al.* 2006; Chen *et al.* 2007; Curran and Ruvkun 2007; Hansen *et al.* 2007; Pan *et al.* 2007; Syntichaki *et al.* 2007; Tohyama *et al.* 2008; Wang *et al.* 2010). These studies revealed that *C. elegans* life span and stress resistance are increased by mutations that decrease rates of translation initiation, or when translation is inhibited in adults by RNAi against ribosomal proteins or several different translation initiation factors. In agreement with these findings in the worm, genetic mutations that reduce translation also increase life span in yeast, *Drosophila*, and mice, suggesting that the relationship between protein synthesis and life span is evolutionarily conserved (Johnson *et al.* 2013).

The reduced rates of translation that result from TORC1 inhibition could influence aging in a number of ways. A reduction in protein synthesis lessens the burden of damaged or misfolded proteins, an important factor in aging (Kenyon 2010; López-Otin *et al.* 2013; Labbadia and Morimoto 2015; Solis *et al.* 2018). This might also result in a beneficial decrease in energy or resource demand. However, several studies indicate that the picture is more complex. Across species, a reduction in protein synthesis rates results in preferential translation of a subset of mRNAs that have particular structural features (Johnson *et al.* 2013; Steffen and Dillin 2016; Kapahi *et al.* 2017). These genes tend to be heavily weighted toward stress response proteins, providing a mechanism for defending the organism in times of resource limitation. This model is supported by work in *C. elegans*, in which inhibition of the translation initiation factor IFG-1/eIF4G resulted in preferential synthesis of many proteins that protect against stress (Rogers *et al.* 2011). This translational preference correlated with greater mRNA transcript length, suggesting a mechanism that may mediate part of this effect. In another potentially beneficial mechanism, in mammals TORC1 inhibition leads to derepression of translation elongation factor 2 kinase, a regulator that slows translational elongation, enhances translation accuracy, and in *C. elegans* contributes to life span (Xie *et al.* 2019).

Other studies have shown that transcription is altered when translation is reduced. Life span extensions arising from knockdown of some translation initiation factors are partially or fully dependent upon the transcription factor DAF-16/FOXO (Henderson *et al.* 2006; Hansen *et al.* 2007; Tohyama *et al.* 2008; Wang *et al.* 2010), and associated with increased DAF-16/FOXO transcriptional activity (Henderson *et al.* 2006). DAF-16/FOXO regulates many processes implicated in life span extension, including stress resistance, proteostasis, metabolism, and immunity (Kenyon 2010; Shore and Ruvkun 2013). DAF-16/FOXO also represents an important benchmark in the longevity field: it is inhibited by IIS in an evolutionarily conserved manner, and is fully required for reduced IIS and several other conditions to increase *C. elegans* life span (Figure 6), and in *Drosophila* and humans its FOXO orthologs are associated with longevity (Kenyon 2010; Shore and Ruvkun 2013; Fontana and Partridge 2015). Life span extension from reduced translation was also found to require the transcription factor SKN-1/Nrf, or overlapping functions of SKN-1/Nrf and DAF-16/FOXO (Wang *et al.* 2010). Alternatively spliced SKN-1/Nrf isoforms are orthologous to the mammalian transcription factors Nrf1 and Nrf2 (NF-E2-related factor), which mediate conserved responses to proteasomal and xenobiotic/oxidative stress, respectively (Blackwell *et al.* 2015; Lehrbach and Ruvkun 2016). SKN-1/Nrf is important in many contexts of *C. elegans* life span extension, including rIIS (Figure 6), and Nrf2 has been implicated in life span extension in *Drosophila* and mice (Blackwell *et al.* 2015). SKN-1/Nrf target genes are involved not only in stress resistance, but also in metabolism and immunity (Blackwell *et al.* 2015), and in *C. elegans* many are activated when translation is inhibited (Wang *et al.* 2010; Li *et al.* 2011). It is unknown how reduced translation causes these effects on transcription, although other transcription factors that defend against stress are known to be preferentially translated when translation rates are low (Harding *et al.* 2000; Johnson *et al.* 2013; Shpilka and Haynes 2018).

If TORC1 inhibition increases *C. elegans* life span in part by reducing translation (Figure 5), knockdown of TORC1-specific pathway components would be expected to phenocopy the effects described above. Accordingly, *daf-16*/FOXO mutation prevented life span extension from loss of one *daf-15/raptor* copy (Jia *et al.* 2004), and both DAF-16/FOXO and SKN-1/Nrf were required for longevity from knockdown of *raga-1*/RagA, *ragc-1*/RagC, *daf-15/raptor*, or *rheb-1* specifically during adulthood (Robida-Stubbs *et al.* 2012). RNAi against these TORC1 components activated SKN-1/Nrf and DAF-16/FOXO target gene transcription without increasing overall SKN-1/Nrf nuclear occupancy, as occurs when translation initiation is inhibited (Robida-Stubbs *et al.* 2012). These effects on gene expression were distinct from those

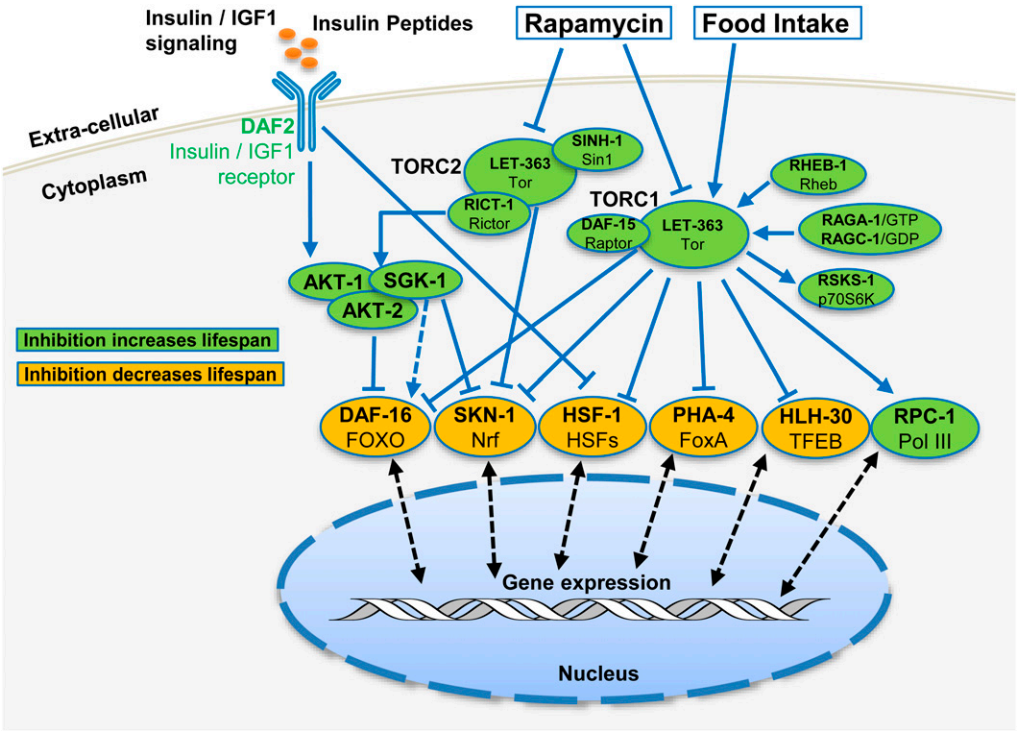

Figure 6 Regulatory mechanisms through which TOR signaling affects *C. elegans* life span. A partial list of transcription regulators through which TOR signaling affects life span is shown. Note the potential cross talk between TOR signaling and IIS that has been suggested by genetic analyses in *C. elegans*, and mechanistic studies in other species. Please see the text for a more thorough discussion. Pol, polymerase; TOR, Target of Rapamycin; TORC, TOR Complex.

that occur in response to reduced IIS. By several criteria, the effects of TORC1 inhibition on life span, stress resistance, and transcription were mimicked by inactivation of CGEF-1, a guanine nucleotide exchange factor that physically interacts with RHEB-1 in cell culture assays, and thus may be a new player in TORC1 signaling (Li *et al.* 2018). Together, the data suggest that SKN-1/Nrf- and DAF-16/FOXO-mediated transcriptional programs are critical for life span extension from reduced TORC1 activity, and are induced in response to reduced translation. These effects may be evolutionarily conserved, as Nrf2 targets are upregulated in livers from rapamycin-treated mice (Robida-Stubbs *et al.* 2012). Interestingly, when TORC1 activity is inhibited in *C. elegans* by *ragc-1* knockdown or rapamycin treatment, SKN-1 directly upregulates expression of multiple TORC1 pathway genes (*raga-1*/RagA, *daf-15/raptor*, and *rsks-1*/S6K), forming a feedback loop that might compensate by enhancing TORC1 activity (Robida-Stubbs *et al.* 2012).

Additional evidence supports the idea that reduced protein synthesis is a major mechanism through which TORC1 inhibition increases life span. In general, conditions that increase *C. elegans* life span decrease ribosome biogenesis and the size of nucleoli, where ribosomal components are synthesized and assembled, and small nucleoli are predictive of longevity across diverse eukaryotes (Tiku *et al.* 2017). The putative TORC1 target RSKS-1/S6K promotes ribosomal function and protein synthesis, and reducing its activity extends life span in yeast, *C. elegans*, *Drosophila*, and female mice (Selman *et al.* 2009; Fontana *et al.* 2010; Johnson *et al.* 2013). *C. elegans*, *Drosophila*, and yeast (chronological) life spans can be also extended by genetic inactivation of RNA Polymerase III (Pol III), which transcribes tRNA and 5S rRNA, and is required for wild-type (WT) levels of protein synthesis (Filer *et al.* 2017). *C. elegans* life span is extended when Pol III is inactivated in an apparently gut-specific manner (Filer *et al.* 2017), as is also true for *ragc-1*/RagC (Robida-Stubbs *et al.* 2012). Pol III transcription is increased by TORC1 acting directly at target gene loci, and in *Drosophila* life span extensions from Pol III inactivation and rapamycin are not additive, placing Pol III within the TORC1 pathway (Filer *et al.* 2017). As Pol III is a potentially druggable target, these results suggest a TORC1-based mechanism for extending life span that could be more specific than rapamycin (see below).

TORC1 and TORC2 affect longevity differently, with both inhibited by rapamycin in vivo: Many analyses of TOR and longevity in *C. elegans*, particularly earlier studies, developed models based upon knockdown of the TOR kinase (LET-363) itself. However, it is crucial to remember that *let-363*/TOR RNAi eliminates both TORC1 and TORC2, which have distinct functions (Figure 2 and Figure 6). The effects of TORC2 on life span, which are complex and will be discussed in detail below (see *Roles of TORC2 in Aging and stress responses*), can be seen in *let-363(RNAi)* animals. Adulthood RNAi against the TORC2 component *rict-1*/Rictor increases SKN-1/Nrf nuclear occupancy and extends life span independently of DAF-16/FOXO (Robida-Stubbs *et al.* 2012; Mizunuma *et al.* 2014). In striking contrast to the effects of inhibiting TORC1 alone, these events also occur with *let-363*/TOR RNAi (Vellai *et al.* 2003; Jia *et al.* 2004; Robida-Stubbs *et al.* 2012), as would be expected with both TORC1 and TORC2 being impaired

(Robida-Stubbs *et al.* 2012). Going forward, it will be important to remember that to draw conclusions about TORC1 functions from genetic studies, it is necessary to take advantage of the well-characterized genetic tools that are specific to the TORC1 pathway (*e.g.*, *daf-15*/*raptor*, *raga-1*/RagA, and *ragc-1*/RagC).

Epistasis analyses of TORC1 and TORC2 have been informative for understanding how the TOR inhibitor rapamycin acts *in vivo*. Rapamycin has been of great interest because of its effectiveness as a mammalian antiaging drug. It became possible to study rapamycin effects in *C. elegans* once it was found that very high concentrations were needed to achieve bioavailability, as indicated by the SKN-1 target gene activation that occurs when TORC1 is blocked (Robida-Stubbs *et al.* 2012). Importantly, *skn-1*/Nrf but not *daf-16*/FOXO was required for rapamycin to increase *C. elegans* life span, as was seen with *let-363*/TOR RNAi, consistent with the idea that both TORC1 and TORC2 are inhibited *in vivo* (Robida-Stubbs *et al.* 2012; Calvert *et al.* 2016). Moreover, like *rict-1*/Rictor RNAi, rapamycin induces SKN-1 nuclear accumulation when *C. elegans* is provided with particular food sources (see *Importance of TORC1 regulation in life span extension mechanisms*) (Robida-Stubbs *et al.* 2012). Why would rapamycin affect both TOR complexes in the worm? While rapamycin directly inhibits TORC1, in mammalian cells continued rapamycin treatment disrupts TORC2 complexes through its interactions with the TOR kinase, and in mice rapamycin thereby inactivates TORC2 along with TORC1 to an extent that varies among tissues (Lamming *et al.* 2012; Kennedy and Lamming 2016). Thus, impairment of both TOR complexes *in vivo* appears to be the rule rather than the exception for rapamycin and related compounds. Unraveling the biological effects of rapamycin and other TOR inhibitors on TORC1 and TORC2 is currently a topic of active investigation in the mammalian aging field (Kennedy and Lamming 2016). However, while the worm has proven valuable for examining effects of rapamycin *in vivo*, most *C. elegans* investigations of TORC1 *per se* use genetic approaches given the relative ease of genetics in the animal, the technical difficulty of administering rapamycin, and the understanding that rapamycin affects both TOR complexes (see *Rapamycin*).

Autophagy is critical: Genetic studies indicate that a functioning autophagy system is required for *C. elegans* life span to be extended by essentially any intervention, including rIIS and DR (Figure 5) (Hansen *et al.* 2018). Autophagy has similarly been implicated as broadly essential for life span extension in yeast and *Drosophila*, suggesting that its importance for longevity is evolutionarily conserved (Shpilka and Haynes 2018). In the setting of aging, autophagy may be critical for eliminating and recycling damaged proteins and organelles, and maintaining energy reserves. Autophagy also can have salutary effects on metabolism; for example, when reproduction is inhibited the resulting increase in autophagy upregulates expression of LIPL-4, a lysosomal acid lipase that promotes longevity through lipid-mediated signaling (Lapierre *et al.* 2011; Folick *et al.* 2015).

The role of autophagy in life span extension from reduced TORC1 activity has been of interest because TORC1 inhibits autophagy across eukaryotes, including *C. elegans* (see *Downstream targets*) (Hansen *et al.* 2008; Robida-Stubbs *et al.* 2012; Johnson *et al.* 2013). As would be predicted, genetic ablation of the autophagy-regulatory transcription factor HLH-30/TFEB (Figure 6) or of key autophagy proteins abrogates life span extension from knockdown or mutation of *let-363*/TOR, *daf-15*/*raptor*, or *rsks-1*/S6K (Hansen *et al.* 2008; Lapierre *et al.* 2013). Life span extension from *let-363* or *rsks-1*/S6K RNAi also requires the transcription factor PHA-4/FOXA (Figure 6), a regulator of autophagy that genetic analysis indicates is antagonized by TORC1 signaling (Sheaffer *et al.* 2008). Activation of PHA-4/FOXA by *let-363*/TOR knockdown has been linked to the kinase GCN-2, which regulates translation, thereby intertwining regulation of translation and autophagy by TOR (Rousakis *et al.* 2013). Additionally, life span extension from knockdown of *daf-15*/*raptor* or *let-363*/TOR depends upon the conserved kinase HPK-1, which is also required for autophagy to be increased when TORC1 signaling is reduced (Das *et al.* 2017). Finally, spermidine induces chromatin modifications that increase life span in yeast, *C. elegans*, *Drosophila*, and mice by upregulating autophagy (Eisenberg *et al.* 2009), and in *C. elegans* overexpression of HLH-30/TFEB on its own extends life span (Lapierre *et al.* 2013).

Together, the data cited above show that derepression and upregulation of autophagy is a major factor in life span extension from TORC1 inhibition. However, two recent *C. elegans* studies indicate that in some biological contexts, autophagy activation can be taken to an extreme. First, after reproduction ceases, yolk continues to be produced through autophagic breakdown of intestinal tissue, and prevention of this process appears to enhance both longevity and health (Ezcurra *et al.* 2018). In addition, as we describe below in "*Roles of TORC2 in aging and stress responses*," when TORC2 and SGK-1 activity are ablated genetically, mitochondrial permeability is increased, resulting in elevated autophagic activity that is deleterious (Zhou *et al.* 2019). This effect is a major contributor to the complex effects of TORC2 on *C. elegans* life span. Thus, while the bulk of evidence indicates that autophagy is an important driver of life span extension (Hansen *et al.* 2018), under some circumstances too much of a good thing can be harmful, suggesting that it could be advantageous to understand how to optimize activity of the various autophagic activities. It will be interesting to delve deeper into how the increase in autophagy that results from TORC1 inhibition affects specific tissues and parameters associated with pathology and health.

Other mechanisms through which TORC1 limits life span: TORC1 also limits *C. elegans* life span through mechanisms besides direct regulation of protein synthesis and autophagy (Figure 5). Adulthood RNAi knockdown of TORC1 pathway components (*daf-15*/*raptor* or *ragc-1*/RagC, and *rsks-1*/S6K) or rapamycin treatment induces HSF-1 (Figure 5) to activate

genes that are important for cytoplasmic proteostasis, and are required along with HSF-1 for the resulting life span extension (Seo *et al.* 2013). It remains to be determined how TORC1 inhibition activates HSF-1. The predicted TORC1 target RSKS-1/S6K limits longevity through two pathways that seem to be independent of protein synthesis regulation. First, like mammalian S6K, RSKS-1/S6K inhibits the energy sensor AMPK (see *Upstream inputs to TOR signaling*), which promotes *C. elegans* longevity, and is required for *rsks-1*/S6K mutation and some DR conditions to extend life span (Selman *et al.* 2009; D. Chen *et al.* 2013; Burkewitz *et al.* 2014; McQuary *et al.* 2016). When RSKS-1/S6K is inactivated, a conserved arginine kinase (ARGK-1) becomes expressed in a subset of glial cells, resulting in AMPK activation and delayed aging of the organism, presumably through tissue nonautonomous signaling (McQuary *et al.* 2016). Mutation of *rsks-1*/S6K impairs reproduction and growth independently of AMPK (Selman *et al.* 2009; McQuary *et al.* 2016), suggesting that RSKS-1/S6K may regulate translation independently of the latter kinase. In addition, genetic analysis suggests that TORC1 acts through RSKS-1/S6K to limit production of monounsaturated fatty acids that promote *C. elegans* longevity (Han *et al.* 2017). This intriguing study suggests that TORC1 might regulate production of signals that coordinate metabolism and aging across tissues. Finally, in mammals, TORC1 modulates several metabolic pathways and affects the phosphorylation status of hundreds of proteins (Hsu *et al.* 2011; Yu *et al.* 2011; Kennedy and Lamming 2016; Saxton and Sabatini 2017), suggesting that we have much to learn about how it acts in *C. elegans*. Therefore, it would be very surprising if TORC1 did not affect *C. elegans* metabolism in several additional ways that influence aging.

While most work in the aging field has focused on mechanisms that protect or repair cellular structures and functions, TORC1 inhibition also appears to enhance the functions of extracellular matrices (ECMs), which maintain tissue architecture, and are also critical for cell–cell communication and other functions (Figure 5). In *C. elegans*, diverse interventions that promote longevity, including DR and rapamycin treatment, delay a decline in adulthood expression of particular collagens and other ECM genes, and thus appear to promote ECM remodeling and cuticle strengthening that is important for life span extension (Ewald *et al.* 2015). It is not understood how these gene expression effects are mediated, but SKN-1/Nrf plays an important role. In mammals, various ECM parameters decline with age, most visibly represented by skin integrity, and in mice rapamycin treatment preserves tendon strength and elasticity (Wilkinson *et al.* 2012). This area of aging research is still in its infancy, but it appears that enhancement of extracellular structures is an important factor in the antiaging benefits of TORC1 inhibition and other interventions.

One intriguing theory posits that an important cause of aging is "run-on" activity of processes that are advantageous for development or reproduction, but later unneeded and possibly harmful (Williams 1957; Demidenko *et al.* 2009). While most of the field sees the relevant goal to be understanding how lower TORC1 activity leads to mobilization of processes that benefit the organism during aging, according to this idea it could be beneficial simply to reduce TORC1 activity below levels that might be maladaptive after growth and reproduction have been completed. These two views are not mutually exclusive, and the long list of mechanisms enumerated in this section illustrates how much we still have to learn, both about the causes of aging and how modulating TORC1 signaling can interfere with this process.

Importance of TORC1 regulation in life span extension mechanisms

DR: Under DR conditions, TORC1 is presumably inhibited because nutrient availability is reduced, making it a logical model that decreased TORC1 activity is an important mediator of DR life span extension (Figure 5) (Johnson *et al.* 2013; Fontana and Partridge 2015; Kapahi *et al.* 2017). Consistent with this idea, *let-363*/TOR knockdown failed to extend life span further in long-lived *eat-2* animals, in which an impairment of pharyngeal pumping may induce a DR-like state (Hansen *et al.* 2007). Genetic epistasis studies in yeast and *Drosophila* have yielded similar results (Kenyon 2010; Johnson *et al.* 2013). The transcription regulator hypoxia-inducible factor-1, which is regulated by TORC1 in mammals, modulates *C. elegans* life span downstream of *rsks-1*/S6K and is important in DR (Chen *et al.* 2009). DR longevity also partially depends upon DRR-2, a translation initiation factor that appears to function downstream of TORC1 (Ching *et al.* 2010). Other evidence implicating lower TORC1 activity in *C. elegans* DR came from a study that profiled mRNA levels over time when DR was imposed by food limitation (Hou *et al.* 2016). In a systems analysis of these gene expression data, many of the effects of DR could be mimicked by the expected combined effects of lower TORC1 and IIS activity, along with AMPK activation. Additionally, targeting these three regulatory "nodes" together resulted in an extremely long life span extension that was refractory to a further increase from DR (Hou *et al.* 2016). Cross talk among TORC1, IIS, and AMPK signaling has been demonstrated in mammals (Johnson *et al.* 2013; Kennedy and Lamming 2016; Sabatini 2017; Saxton and Sabatini 2017), and in *C. elegans* genetic evidence indicates that TORC1 signaling and IIS interact to process nutritional cues during larval development (Jia *et al.* 2004; Fukuyama *et al.* 2015) (Figure 4A and Figure 6). In addition, AMPK is important for synergistic life span extensions that result from double mutations in *rsks-1*/S6K and *daf-2*/IGFR (A. Chen *et al.* 2013b).

Given the evidence cited above, it appears likely that DR life span extension results from the effects of reduced TORC1 activity acting in parallel to rIIS, AMPK, and almost certainly additional mechanisms. However, inactivation of translation factors or *raga-1*/RagA can extend life span additively with some *C. elegans* DR conditions (Hansen *et al.* 2007; Schreiber

et al. 2010), seemingly arguing against the idea that TORC1 is a key player in DR. These last discrepancies may arise from differences among DR protocols and the essential impossibility of performing true epistasis experiments with a dietary intervention, and, in many cases, RNAi. Despite these last caveats, the idea that reduced TORC1 activity is critical in DR has been largely accepted (Johnson *et al.* 2013; Fontana and Partridge 2015; Kapahi *et al.* 2017).

Modulation of TORC1 also appears to be important for benefits of DR besides longevity. Inhibiting its activity improves healthspan parameters in *C. elegans* and mammals (see *Life span extension and increased stress resistance from TORC1 inhibition*) (Johnson *et al.* 2013; Kennedy and Lamming 2016), suggesting that TORC1 might be a major modulator of aging-associated disease in the setting of DR. In *C. elegans*, TORC1 inhibition and DR also dramatically improve function in an associative learning paradigm, through a neuronal signaling pathway that involves downregulation of a specific neuroinhibitory metabolite and is not required for life span extension (Vohra *et al.* 2017). TORC1 may thus be an important modulator of cognitive function independently of its effects on aging *per se*.

While it is clear that longevity and health can be enhanced by reducing TORC1 activity, some studies have revealed a positive role for TORC1 components during aging. In *C. elegans* and other species, DR can be imposed by intermittent fasting (IF), the repetitive deprivation of food for limited periods (Honjoh *et al.* 2009; Fontana and Partridge 2015; Kapahi *et al.* 2017). Life span extension from IF is suppressed by mutation of *rheb-1*, an effect that does not seem to be mediated through TORC1 regulation of translation (Honjoh *et al.* 2009). Perhaps analogously, TORC1 signaling in the intestine is required for a transcriptional response through which transient hypoxia extends *C. elegans* life span (Schieber and Chandel 2014).

It is unknown why inadequate TORC1 signaling limits life span extension in the above scenarios, but a recent *C. elegans* study identified a specific mechanistic parameter of "youthfulness" that is dependent upon TORC1. The relative representation of many alternatively spliced mRNAs changes during aging but DR delays this effect, resulting in the persistence of youthful splicing patterns (Heintz *et al.* 2017). This enhancement of mRNA splicing function appears to promote longevity, in keeping with the idea that longevity interventions in general may preserve mechanisms that maintain the fidelity of gene expression. Paradoxically, while it is generally accepted that DR reduces TORC1 activity, genetic inactivation of *raga-1* prevents DR from delaying the age-related decline in mRNA splicing. Thus, TORC1 signaling is essential for maintenance of this particular parameter of youth. Consistent with these findings, a recent study in mammalian cells revealed that S6 kinase promotes functional alternative splicing at key lipid metabolism genes by phosphorylating and regulating SRPK2, a kinase that modulates some mRNA splicing events (Lee *et al.* 2017). It will be interesting to elucidate how broadly TORC1 influences mRNA splicing, how this occurs, and how this contributes to the functions of TORC1 in growth, development, and aging. The idea that TORC1 is required for some mechanisms that promote longevity emphasizes the potential importance of determining the levels and tissue distribution of TORC1 activity most compatible with life span extension, and whether it might be possible to inhibit specific activities downstream of TORC1 in a way that promotes longevity and health without interfering with beneficial functions.

Reduced GSC number: TORC1 signaling has also been implicated in one of the most complicated mechanisms through which *C. elegans* life span can be extended, GSC ablation. When precursors to the germline are ablated with a laser or GSCs are genetically prevented from proliferating [conditions referred to here as GSC(-)], germ cells do not form, life span is increased by 25–40%, and resistance to multiple stresses is increased (Kenyon 2010; Lemieux and Ashrafi 2016; Hansen *et al.* 2018). This life span extension involves nuclear receptor and lipid signaling, and somatic activation of a network of numerous transcription factors that have been implicated in longevity or lipid metabolism: DAF-16/FOXO, DAF-12/FXR, PHA-4/FOXA, NHR-80/HNF4, NHR-49/PPARα, SKN-1/Nrf, HLH-30/TFEB, MML-1/Mondo, and MXL-2/Max (Hsin and Kenyon 1999; Lin *et al.* 2001; Goudeau *et al.* 2011; Lapierre *et al.* 2011; O'Rourke and Ruvkun 2013; Ratnappan *et al.* 2014; Steinbaugh *et al.* 2015). This complex response may have evolved to protect the animal under adverse conditions or to coordinate life span with reproduction, so that it can survive to reproduce when conditions are favorable (Kenyon 2010). Another possibility that is not mutually exclusive is that this response to GSC absence represents a metabolic defense against a glut of lipids and other resources that would normally have been allocated to reproduction (Steinbaugh *et al.* 2015; Lemieux and Ashrafi 2016). Evidence for an inverse relationship between GSC formation and life span has been also obtained in *Drosophila* and human males (Flatt *et al.* 2008; Min *et al.* 2012; Steinbaugh *et al.* 2015), suggesting that aspects of this relationship might be conserved.

Given that GSC(-) life span extension depends upon activation of PHA-4/FOXA, HLH-30/TFEB, and autophagy, it is not surprising that TORC1 signaling would be involved (Lapierre *et al.* 2011, 2013; Nakamura *et al.* 2016). Heterodimeric MML-1/Mondo:MXL-2/Max complexes regulate numerous genes involved in longevity and stress resistance, and are important in various contexts of life span extension (Johnson *et al.* 2014; Nakamura *et al.* 2016). In GSC(-) animals, MML-1/Mondo:MXL-2/Max increases HLH-30/TFEB nuclear accumulation and autophagy activity by inhibiting TORC1, as detected by phosphorylation of the predicted TORC1 target RSKS-1/S6K (Nakamura *et al.* 2016). This inhibition is mediated through MML-1/Mondo:MXL-2/Max transcriptionally repressing expression of leucyl tRNA synthase-1, a protein that activates TORC1 signaling (Nakamura *et al.* 2016).

While TORC1 is unequivocally central to the effects of GSC ablation on autophagy, it is unclear whether TORC1 signaling might be a more general mediator of GSC(-) life span extension. Consistent with this model, the life span of GSC(-) animals was not increased further by *let-363*/TOR knockdown (Lapierre *et al.* 2011). However, knockdown of *raga-1*/RagA or translation initiation factors extended life span additively with GSC loss, arguing against this idea (Robida-Stubbs *et al.* 2012). It is still unknown whether RSKS-1/S6K phosphorylation or other predicted markers of TORC1 signaling are actually reduced in GSC(-) animals. GSC loss decreases LET-363/TOR mRNA and protein levels compared to WT (Lapierre *et al.* 2011), but also eliminates around two-thirds of the cell bodies in the animal, making it difficult to draw clear conclusions from reduced expression of genes like *let-363*/TOR that are active in GCs (Steinbaugh *et al.* 2015). Germ cell number and total *let-363*/TOR mRNA levels are both decreased by DR in a genetically linked manner (Thondamal *et al.* 2014), consistent with the possibility that a major proportion of *let-363*/TOR mRNA expression derives from GCs. Finally, nuclear occupancy of SKN-1/Nrf and certain DAF-16/FOXO isoforms is induced by GSC loss, but not TORC1 inhibition (Lin *et al.* 2001; Robida-Stubbs *et al.* 2012; Steinbaugh *et al.* 2015; Wei and Kenyon 2016). Further work will be required to tease out the functions of TOR signaling in the complex network of overlapping gene expression responses that have been implicated in this very intriguing pathway.

TORC1 and IIS: In other organisms, the TORC1 and IIS pathways are directly integrated (see *Upstream inputs to TOR signaling* and Figure 6). While there is some evidence of integration between TORC1 and IIS pathways in *C. elegans* (see *Upstream inputs to TOR signaling* and Figure 6), these pathways also appear to act independently in some of their roles during *C. elegans* development (*e.g.*, Korta *et al.* 2012). Similarly, the TORC1 and IIS pathways affect various aging-related parameters independently. As noted above, genetic and expression profiling evidence suggests that these pathways function largely in parallel during DR (Hou *et al.* 2016), an idea consistent with the synergistic life span increase seen with the combination of *rsks-1*/S6K and *daf-2*/IGFR loss-of-function mutations (D. Chen *et al.* 2013). Earlier studies observed that *daf-2*/IGFR mutant life span was not increased by RNAi against *let-363*/TOR (Vellai *et al.* 2003) or *rsks-1*/S6K (Hansen *et al.* 2007), but this lack of synergy might have reflected incomplete RNAi penetrance or different conditions. IIS and TORC1 each oppose the activities of DAF-16/FOXO and SKN-1/Nrf, but appear to do so through different mechanisms (Robida-Stubbs *et al.* 2012). Additionally, rIIS modulates activity of a key innate immunity pathway by allowing DAF-16/FOXO to suppress food intake, but this does not occur when TORC1 activity is reduced by RNAi knockdown of *let-363*/TOR, Rag proteins, or *rsks-1*/S6K (Wu *et al.* 2019). A better understanding of differences and similarities between how life span is increased by rIIS and TORC1 inhibition may shed light on why the life span increases from rIIS are typically more robust (see *Life span extension and increased stress resistance from TORC1 inhibition*), and on fundamental mechanisms that are likely to be relevant to aging in more complex organisms.

Antiaging compounds: TORC1 has been implicated in *C. elegans* life span extension in response to a number of compounds besides rapamycin (Table 2). In many cases, the mechanisms involved are unknown, and the case that these compounds may inhibit TORC1 is limited to evidence that they do not extend life span further in genetic backgrounds in which TORC1 activity is reduced. A large-scale compound-testing project recently raised the bar for drawing mechanistic conclusions, by showing that effects on life span can vary widely among laboratories, strains, and conditions, and between *C. elegans* and the closely related nematode *C. briggsae* (Lucanic *et al.* 2017). Notwithstanding these caveats, the evidence for TORC1 involvement is particularly interesting and compelling for the diabetes drug metformin, and a set of natural metabolites that seem to mimic aspects of DR. We discuss those compounds below, and in Table 2 have listed them along with other compounds that seem to act through TORC1.

Metformin is a widely prescribed treatment for type 2 diabetes that increases sensitivity to insulin and may protect against some cancers [discussed in Castillo-Quan and Blackwell (2016), Wu *et al.* (2016), and Chen *et al.* (2017)]. It has been of great interest as a potential antiaging drug in part because it is well known to be safe. It is generally accepted that in humans, metformin alters mitochondrial function and increases AMPK activity, although its direct molecular target(s) remain unknown. In *C. elegans*, metformin seems to act as a DR mimetic, and extends life span and influences metabolism in part by altering metabolism of the bacterial food (Onken and Driscoll 2010; Cabreiro *et al.* 2013). Two recent *C. elegans* studies have looked more downstream, and elucidated mechanisms through which metformin regulates TORC1 along with AMPK. First, genetic screening identified the little-understood metabolic gene *CeACAD10/F37H8.3*, also known as *bigr-1*, as required for metformin action (Wu *et al.* 2016). Additional worm genetics, along with molecular analyses in mammalian cells, revealed the following pathway (Wu *et al.* 2016): by altering mitochondrial function, metformin impairs nuclear transport, thereby preventing RAGC-1/RagC from transiently passing through the nucleus. As a result, the Rag GTPase cannot become activated and TORC1 is inhibited, leading to *CeACAD10* expression being induced in part by SKN-1/Nrf, which is known to be regulated by TORC1 (Robida-Stubbs *et al.* 2012). This model was particularly surprising because it was previously unknown that appearance in the nucleus was needed for Rag GTPase activity (Castillo-Quan and Blackwell 2016; Wu *et al.* 2016). Another study that blended *C. elegans* and mammalian cell experiments concluded that metformin acts directly at the lysosome, and the vacuolar ATPase, to activate AMPK and inhibit TORC1, and increases life span through this pathway (Chen *et al.* 2017).

Table 2 Compounds proposed to increase *C. elegans* life span by reducing TORC1 signaling

Compound	Strain	Dose	Effect	Reference
Rapamycin (TOR inhibitor)	WT	100 μM	Up to 19% life span extension	Robida-Stubbs *et al.* (2012), Seo *et al.* (2013), Ewald *et al.* (2015), Xie *et al.* (2019)
Rapamycin (TOR inhibitor)	WT	10 μM	18.9% life span extension	Calvert *et al.* (2016)
Rapamycin (TOR inhibitor)	WT	100 μM	Taste-associative learning was disrupted	Sakai *et al.* (2017)
Sesamin (from sesame oil)	*daf-15*(+/−)	5.75 μg per plate	Life span extension blocked in *daf-15* heterozygotes	Nakatani *et al.* (2018)
10-Hydroxy-2-decenoic acid (royal jelly component)	*daf-15*(+/−)	25 μM	Life span extension blocked in *daf-15* heterozygotes	Honda *et al.* (2015)
α-Ketoglutarate (metabolite)	*let-363* RNAi	8 mM	Failed to increase the life span of *let-363* RNAi animals and inhibits TORC1 activity	Chin *et al.* (2014)
D-β-hydroxybutyrate (ketone body)	*rsks-1(ok1255)*	20 mM	Less life span extension in *rsks-1* mutants than in WT	Edwards *et al.* (2014)
LY-294002 (phosphoinositide 3-kinase inhibitor)	WT	100 μM	19% life span extension in WT, potential to target *let-363*	Calvert *et al.* (2016)
5-octanoyl salicylic acid (salicylic acid derivate)	WT	100 μM	Increases life span through autophagy and reduces S6K phosphorylation in mammalian cells	Shamalnasab *et al.* (2018)
EPEA (endocannabinoid)	*rsks-1(ok1255)*	50 μM	EPEA reduced life span in *rsks-1* mutants, which have low EPEA levels	Lucanic *et al.* (2011)
Metformin (type 2 diabetes drug for > 60 yr)	WT	50–100 mM	Life span extension; decreased Rag nuclear export and activity	Wu *et al.* (2016), Chen *et al.* (2017)

EPEA, eicosapentaenoyl ethanolamide; RNAi, RNA interference; TOR, Target of Rapamycin; TORC, TOR Complex; WT, wild-type.

The metabolic benefits of metformin in humans contrast with the effects of rapamycin, which can increase insulin resistance in mammals by reducing TORC2 activity (see *Importance of TORC1 regulation in life span extension mechanisms*). These elegant worm genetic studies of metformin have thus provided further incentive for the development of TORC1 inhibitors that do not affect TORC2.

Various natural metabolites seem to influence life span through TORC1 signaling (Table 2). The tricarboxylic acid cycle intermediate α-ketoglutarate (α-KG) extends *C. elegans* life span, with epistasis analyses suggesting that it acts as a DR mimetic (Chin *et al.* 2014). Unexpectedly, α-KG binds and inhibits ATP synthase (mitochondrial complex V). As a result, ATP levels are reduced and TORC1 is inhibited, as indicated by reduced levels of TORC1 target phosphorylation in mammalian cells. Consistent with the idea that α-KG extends life span by reducing TORC1 activity, in *C. elegans* α-KG increases autophagy levels, and does not extend the life span of *let-363/*TOR*(RNAi)* or *pha-4/*FOXA mutant animals. This life span extension is partially dependent upon *aak-2* (AMPK), a predicted TORC1 inhibitor (see *Upstream inputs to TOR signaling*). The oncometabolite 2-hydroxiyglutarate (2-HG) phenocopies these effects (Fu *et al.* 2015). D-β-hydroxybutyrate is synthesized in the liver during the fasting state (Chin *et al.* 2014). *N*-acylethanolamines (NAEs) are lipid signaling molecules that include endocannabinoids implicated in regulating energy balance (Lucanic *et al.* 2011). In *C. elegans*, DR and reduced TORC1 signaling each decrease NAE abundance, and administration of the NAE eicosapentaenoyl ethanolamide suppresses life span extension from *rsks-1* mutation, suggesting that these compounds act downstream of TORC1 to accelerate aging (Lucanic *et al.* 2011). These results support the model that conditions that deplete energy (*i.e.*, DR) or mimic this state extend life span at least partially through TORC1, and make it theoretically possible to promote longevity by developing strategies to alter their levels.

Roles of TORC2 in aging and stress responses

Compared to the progress made with TORC1, across eukaryotes less is understood about how TORC2 influences aging. This is an important question for the aging community beyond *C. elegans*, because all direct TORC1 inhibitors developed to date reduce TORC2 activity to some extent (Kennedy and Lamming 2016; Antikainen *et al.* 2017). By decreasing TORC2 activity, prolonged rapamycin treatment induces insulin resistance in mice, a side effect that could be particularly undesirable in humans (Lamming *et al.* 2012). Also in mice, rapamycin extends life span more effectively in females than males because male life span is reduced when TORC2 is blocked (Lamming *et al.* 2014).

Our understanding of how TORC2 influences aging and stress resistance has been developed most thoroughly in *C. elegans*, largely through genetic analyses of the TORC2-specific component RICT-1/Rictor and the TORC2 downstream phosphorylation target SGK-1 (see *Downstream targets*; Figure 2B and Figure 6). These studies indicate that the effects of TORC2 on life span are remarkably complex and involve at least three distinct mechanisms (Figure 7). First, RICT-1/Rictor and SGK-1 appear to function within a pathway in which a cold-sensitive TRP (transient receptor potential) channel (TRPA-1) acts in neurons and the intestine to promote life span by increasing the activity of DAF-16/FOXO, an apparent

direct target of SGK-1 (Figure 6 and Figure 7) (Hertweck *et al.* 2004; A. Chen *et al.* 2013; Xiao *et al.* 2013). This pathway is particularly important at lower temperatures, and at 15 or 20°, *rict-1* or *sgk-1* loss-of-function mutations dramatically decrease life span (A. Chen *et al.* 2013; Xiao *et al.* 2013; Mizunuma *et al.* 2014). Second, RICT-1/Rictor acts through SGK-1 to inhibit opening of the mitochondrial permeability transition pore (mPTP) and prevent excessive mitochondrial permeability (Zhou *et al.* 2019) (Figure 7). When *rict-1*/Rictor or *sgk-1* are ablated genetically, autophagy is elevated to levels that are harmful in this setting of increased mitochondrial permeability, apparently because mitophagy mechanisms that are supposed to clear damaged mitochondria are activated to excess. Third, and in contrast, TORC2/SGK-1 signaling limits life span and stress resistance by inhibiting nuclear localization of SKN-1/Nrf, also a direct phosphorylation target of SGK-1 (Figure 6 and Figure 7) (Tullet *et al.* 2008; Mizunuma *et al.* 2014). Interestingly, mutation of *rict-1*/Rictor or *sgk-1* is sufficient to release repression of SKN-1 only when the animals are propagated on certain food sources, including the standard RNAi feeding bacteria HT115 (Robida-Stubbs *et al.* 2012; Mizunuma *et al.* 2014).

The effects of TORC2/SGK-1 on life span therefore depend upon the balance among these three mechanisms (Figure 7), and are influenced not only by temperature, but also the bacterial food source (Soukas *et al.* 2009; Mizunuma *et al.* 2014; Xiao *et al.* 2015). Under most conditions, *rict-1* and *sgk-1* mutations decrease life span (Mizunuma *et al.* 2014; Zhou *et al.* 2019). However, at higher temperature (25°) the relative importance of the TRPA-1 and mPTP pathways seems to be decreased, and although *rict-1*/Rictor and *sgk-1* mutations still shorten life span with propagation on the standard strain OP50, these mutations actually increase life span through SKN-1/Nrf when the animals are fed HT115 (Soukas *et al.* 2009; Mizunuma *et al.* 2014). With HT115 feeding, *rict-1*/Rictor or *sgk-1* mutations also increase stress resistance in an SKN-1-dependent manner (Mizunuma *et al.* 2014). Similarly, although *rict-1*/Rictor or *sgk-1* RNAi extends life span when animals consume the HT115 RNAi strain (Robida-Stubbs *et al.* 2012; Mizunuma *et al.* 2014), life span is decreased when these knockdowns are performed using RNAi-competent OP50 (Xiao *et al.* 2015). This SKN-1-dependent pathway seems to be particularly important in the intestine, where SKN-1/Nrf is expressed, and does not require DAF-16/FOXO for life span extension (Robida-Stubbs *et al.* 2012; Mizunuma *et al.* 2014). Accordingly, as noted above (see *Life span extension and increased stress resistance from TORC1 inhibition*), by inhibiting TORC2, rapamycin treatment extends *C. elegans* life span independently of DAF-16/FOXO (Robida-Stubbs *et al.* 2012).

It is unclear whether other activities of TORC2 or SGK-1 might also influence longevity. For example, loss of *rict-1*/Rictor or *sgk-1* increases mitochondrial biogenesis, and affects the mitochondrial unfolded protein response (Gatsi *et al.* 2014), a stress defense mechanism implicated in aging (Shpilka and Haynes 2018). SKN-1/Nrf promotes both

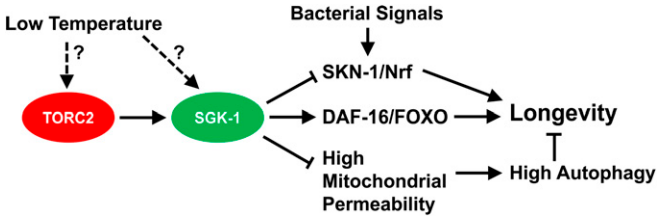

Figure 7 Processes through which TORC2 affects *C. elegans* life span. Three distinct pathways have been described through which TORC2 and SGK-1 influence life span. Whether life span is increased or decreased depends upon the balance among these mechanisms. Please see the text for a more thorough discussion. TORC, Target of Rapamycin Complex.

mitophagy and mitochondrial biogenesis (Palikaras *et al.* 2015), suggesting that SKN-1/Nrf might be involved in this effect. It also remains to be determined whether the influence of TORC2 on life span might also involve its regulation of fat metabolism, which is also mediated by SGK-1 (see *Downstream targets*) (Jones *et al.* 2009; Soukas *et al.* 2009; Dowen *et al.* 2016). SGK-1 appears to be activated directly not only by TORC2 but also by IIS (Figure 6) (Hertweck *et al.* 2004), although its role within the latter pathway has been controversial (A. Chen *et al.* 2013a). Interestingly, genetic analyses have revealed that TORC2/SGK-1 signaling interacts with the IIS pathway to regulate fat mobilization, mitochondrial function, and life span, suggesting that TORC2 signaling converges with IIS through its regulation of SGK-1 (Gatsi *et al.* 2014; Dowen *et al.* 2016). During development, RICT-1/Rictor inhibits dauer formation in part by signaling from the intestine to neurons to upregulate the insulin-like peptide DAF-28, providing an additional example of cross talk between TORC2 and IIS signaling (O'Donnell *et al.* 2018). However, although TORC2 signaling influences a number of aging-related signaling pathways, in contrast to TORC1 it does not appear to play a major role in DR longevity (Mizunuma *et al.* 2014). It seems unlikely that targeting TORC2 represents a promising strategy for promoting healthy human aging, but further analysis of this pathway in *C. elegans* should provide additional important models for how TORC2 might influence development, metabolism, and aging in higher organisms.

Perspectives

Over the past two decades or so, studies of the two TOR complexes have attracted numerous researchers working with yeast, cultured mammalian cells, and model organisms ranging from *C. elegans* to humans. In reviewing the literature, it is clear that a genetically amenable organism like *C. elegans* does not offer major advantages in uncovering the molecular or biochemical mechanisms underlying the formation, regulation, and activity of TOR complexes. However, *C. elegans* offers unique opportunities for scientists to analyze the regulation and functions of TOR complexes under physiological conditions, particularly in the contexts of development, aging, and responses to changes in nutrient availability and

environmental conditions. For example, the life span-related studies in C. *elegans* led the way in establishing the connection between TOR and aging, and have made breakthroughs and major contributions to understanding the role of TOR signaling in various nutritional, genetic, and pharmacological interventions that promote longevity, and in identifying downstream mechanisms that delay aging. *C. elegans* TOR studies of development, metabolism, and behavior have also made unique contributions in understanding the physiological roles of TOR signaling. These contributions from *C. elegans* are likely to grow as we begin to understand more about the complexities of TOR functions in growth, development, and metabolism.

In the next few decades, we expect that *C. elegans* researchers will continue to focus on uncovering and understanding the regulation, and activities, of TORC1 and TORC2 regarding specific physiological functions in live animals. Another important frontier will be to tease apart how TOR acts in different tissues to exert its biological effects. However, to enhance the contribution of these studies to the TOR field overall, worm researchers also should put forth greater effort to increase the depth of the studies regarding molecular mechanisms. Specifically, as we alluded to in this review, the connections between upstream nutrients and environmental cues to the TOR complexes are not well understood at the molecular level in *C. elegans*. Similarly, although we have revealed many interesting physiological outcomes of TOR signaling, the mechanisms by which they are executed through TORC1/2 activities, including direct downstream targets, remain to be further investigated in many cases. One notable advantage of *C. elegans* is that by utilizing CRISPR and leveraging its relatively rapid lifecycle, it will be possible to develop and test specific mechanistic models rapidly in the context of physiological settings *in vivo*. Overall, we expect TOR-related problems to continue to be popular topics in the *C. elegans* field that should continue to make important contributions to the nutrient-response, development, and aging research fields.

Acknowledgments

We thank Collin Ewald for helpful suggestions, and the editors and reviewers for expending great effort in critiquing and helping us improve the manuscript. T.K.B. and Z.W. are supported by funding from the National Institutes of Health (NIH) (AG-054215 and GM-122610). M.H. and A.K.S. are supported by funding from the NIH (GM-047869) and the Howard Hughes Medical Institute.

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Communicating editor: M. Sundaram