Molecular mechanisms of dietary restriction in aging⸺insights from *Caenorhabditis elegans* research

LAN JianFeng, ZHANG Xuan & CHEN Di[*]

MOE Key Laboratory of Model Animal for Disease Study, Model Animal Research Center, Nanjing Biomedical Research Institute, Nanjing University, Nanjing 210061, China

Dietary restriction (DR) is one of the most robust environmental manipulations that not only extend life span but also delay the onset of age-related diseases in almost every species examined. *Caenorhabditis elegans* plays an important role in aging studies due to its simple life cycle, easy genetic manipulations and highly conserved genome. Recent studies have demonstrated that the beneficial effects of DR are mediated by the highly conserved transcription factors and signaling pathways in *C. elegans*. Here we review recent progress in the methodology and molecular mechanisms of DR using *C. elegans* as a model, as well as prospects for future research.

***C. elegans*, dietary restriction, aging, TOR pathway, insulin/IGF-1 signaling**

Aging is defined as functional decline accompanied with increased mortality rates over time. Non-communicable diseases, such as cardiovascular diseases, diabetes, cancer and neurodegenerative diseases, are the major causes of death in humans. The occurrence of non-communicable diseases shows exponential increase over age [1]. How does aging occur and develop? Are there genetic or environmental manipulations that can extend life span and delay age-related pathologies? Answers to these questions are important topics in basic biological and biomedical research because they are concerned with the molecular mechanisms of aging.

Aging problems are urgent to China. Demography analyses conducted by the United Nations indicated that the Chinese population is undergoing accelerated aging. Starting from 1980s, it only took 20 years for China to become one of the countries with an aging population, which is in-dicated by the fact that more than 10% of the whole population are people over 60 years old. It is expected that China will become the most aged country in 2030s [2]. Therefore, biological studies on how to slow aging and perhaps more importantly, how to delay or prevent age-related diseases should be one of the most important research fields in China.

Recent studies demonstrated that aging can be modulated by highly-conserved signaling pathways [3]. It has been shown that the target of rapamycin (TOR) pathway and insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS) play an important role in aging in nearly all the species studied [3,4]. Besides, environmental manipulations, such as changes in the diet and pharmaceutical treatments, can also significantly affect aging [5]. Dietary restriction (DR), reduced food intake without malnutrition, has been proven to be a robust environmental manipulation that delays aging. DR can be achieved by decreasing food intake known as caloric restriction (CR), reducing specific components in the

*Corresponding author (email: chendi@nju.edu.cn)

diet, or intermittent fasting (IF). DR not only extends life span, but also delays or prevents age-related diseases [4,6]. Using model organisms including yeast, *C. elegans*, *Drosophila* and mice, researchers have made significant progress in understanding DR at the molecular level in recent years. Although the beneficial effects of DR have been observed in many species, animals might show different response to DR depending on their genetic backgrounds [7,8]. Whether there are universal DR regimens that can be applied to all species requires better understanding of the molecular mechanisms of DR.

C. elegans is a free-living, soil nematode that has been widely used in biological research due to its short life cycle, ease of manipulation, complete cell lineage and highly conserved genome. The average life span of *C. elegans* is around 20 days under normal growth conditions. Due to powerful genetic, molecular and cellular tools, many signaling pathways that modulate aging were initially characterized in *C. elegans* [3,9]. In this article, we reviewed recent progress in elucidating the molecular mechanisms of DR using *C. elegans* as a model.

1 Different DR regimens in *C. elegans*

C. elegans is normally fed with *E. coli* on the solid agar based nematode growth media (NGM) in petri dishes in the lab [10]. Recently, researchers have developed various methods to create DR conditions in *C. elegans*. These DR regimens include using genetic mutants with reduced food intake, reducing food concentrations, depriving bacterial food completely, intermittent fasting, bacteria-free axenic culture media and so on. Here we compare these DR methods for their advantages and disadvantages in aging research.

The *eat-2* gene encodes a subunit of the nicotinic acetylcholine receptor. Mutations in *eat-2* lead to reduced pharyngeal pumping and therefore decreased food intake. *eat-2* mutants show extended life span and reduced fertility, which are typical features of animals under DR [11]. Thus, *eat-2* mutants have been used as a convenient genetic mimic of DR. However, researchers cannot choose the time to start or stop DR treatments when using the *eat-2* mutant to mimic DR. Moreover, there are many *eat* mutants with reduced pharyngeal pumping but not all of them show life span extension, suggesting they might modulate aging in a complicated manner. Since the *eat-2* null mutant is lethal, hypomorphic *eat-2* alleles are usually used in aging research. It is hard to draw valid conclusions in genetic epistasis studies when partial loss-of-function mutants were used.

Dilution of the bacterial food is one of the most popular methods to create DR conditions in *C. elegans*. The Dillin and Guarente labs have independently reported DR study results using worms grown in *E. coli* suspension at different concentrations. These methods were named bacterial DR (bDR) and liquid DR (lDR), respectively [12,13]. Compared with animals growing under *ad libitum* (AL) conditions in which they were free feeding, those treated with bDR or lDR showed more than 50%, robust life span extension [12,13]. However, *C. elegans* shows different morphology, behavior and life span in liquid culture compared with animals under standard lab culture condition on solid agar plates. Besides, it is much more challenging to handle worms in liquid culture. Therefore, the bDR and lDR methods have some limitations.

The Brunet lab developed a solid DR (sDR) method by feeding animals with *E. coli* at different concentrations on the regular NGM agar plates after animals finished reproduction [14]. The less than 30% life span extending effect of sDR is not as strong as bDR/lDR probably due to the late onset of DR treatment and/or the nutrients in the NGM plates that might promote *E. coli* growth.

The NGM plates contain peptone, which helps *E. coli* to grow. Thus, dilution of peptone (DP) is another way to create DR effects [15]. However, it is very difficult to connect the amount of peptone in the media with the *E. coli* concentrations in a linear relationship. Not surprisingly, this method caused big variations between experiments and thus its use is limited.

The Kaeberlein and Zou labs independently reported that complete dietary deprivation (DD) during adulthood can also create DR effects (50% life span extension) in *C. elegans* [16,17]. This method is easy to perform, and it creates a situation similar to null mutation that is helpful for genetic epistasis analysis. However, DD seems to be against the standard definition of DR, which is reduced food intake without malnutrition. Besides, starvation significantly reduces life span in most species. Therefore, whether discoveries made using this methodology can be applied to higher organisms remains to be determined.

Intermittent fasting (IF) is another method to create DR. Animals under IF were provided with *ad libitum* food but with periodical fasting. There is little or no overall decrease in the calorie intake compared with the controls. It has been reported that every other day or every two days fasting significantly extends life span by 50% in *C. elegans* [18]. This method is powerful regarding the life span extension, but it is a very laborious procedure. Besides, there are some differences between IF and chronic DR.

When animals grown in the bacteria-free axenic media, they also showed DR-related phenotypes, such as extended life span, reduced reproduction and so on [19]. This method is very useful to study the involvement of specific nutrients in aging since the media is chemically defined. However, it is a liquid based culture, which has limitations similar to those of the bDR/lDR methods.

Considering the advantages and disadvantages of these DR methods, we have developed a modified solid DR (msDR) regimen [20]. Based on the sDR method, we excluded peptone and added ampicillin in the media to prevent

E. coli from growing. We also changed the onset of DR treatment to Day 1 adulthood [20]. With these modifications, we observed more potent life span extension (~50%) compared to the sDR method, as well as typical DR phenotypes including increased stress resistance, decreased but prolonged reproduction [20].

Using these DR regimens, researchers have identified key regulators that mediate the beneficial effects of DR. Interestingly, some of these regulators showed methodology-dependent involvement in DR. Therefore, we propose that at least two or more DR regimens should be applied in aging research to determine whether the discoveries are relevant to the molecular mechanisms of DR in general.

2 Molecular mechanisms of DR in *C. elegans*

Recent studies indicated that the molecular mechanisms of DR involve highly conserved signaling pathways. Here we summarize the key regulators of DR response.

2.1 TOR pathway

Target of rapamycin (TOR) is a highly conserved serine/threonine kinase that qualitatively and quantitatively senses the changes in nutrients (amino acids, glucose, etc.) to promote cell growth. TOR is also subject to regulation via signaling pathways mediated by cellular factors, such as insulin, IGF-1, Wnt, and TGF-β. Moreover, environmental stress can also affect TOR activities [4]. It has been known that TOR functions through regulation of mRNA translation, autophagy, ER stress, sugar/lipid metabolism to affect cell growth and proliferation [4].

TOR binds to the regulatory associated protein of TOR (Raptor) and rapamycin-insensitive component of TOR (Rictor) to form the TOR Complex 1 (TORC1) and TOR Complex 2 (TORC2), respectively [21]. In *C. elegans*, *let-363* encodes TOR, whereas *daf-15* encodes Raptor. Mutations in *let-363* or *daf-15* lead to developmental arrest [22,23]. Inhibition of TORC1 either by RNAi knocking-down of *let-363* or by heterozygous mutants of *daf-15* significantly extends life span [22,24]. TOR plays important roles in regulating mRNA translation. In mammals, this effect is mediated by the ribosomal S6 kinase (S6K) and translational initiation factor 4E-binding protein (4EBP). The *C. elegans* genome does not have a 4EBP ortholog at least from the sequence similarity point of view. Therefore, S6K encoded by the *rsks-1* gene is the key mediator of TOR's function on mRNA translation in *C. elegans*. Deletion mutants of *rsks-1* show life span extension [25]. Interestingly, genes such as *pha-4*, *aak-2* and *egl-9* that are required for *rsks-1*-mediated life span extension are also involved in the DR response [12,14,20].

In *C. elegans* and *Drosophila*, DR or inhibition of TOR also activates autophagy to extend life span. It has been reported that inhibition of *bec-1*, an essential autophagy gene, suppresses the life span extension via TOR inhibition or the *eat-2* mutation in *C. elegans* [26]. Besides, several autophagy genes are transcriptionally activated by PHA-4, the key mediator of DR [26].

2.2 Insulin/IGF-1 signaling pathway

The highly conserved insulin/insulin-like growth facor-1 signaling (IIS) plays an important regulatory role in aging. Under high nutrient conditions, IGF-1 binds to the receptor tyrosine kinase encoded by *daf-2*, and activates a kinase cascade including AGE-1 (PI3K), PDK-1 (PDK) and AKT-1, 2 (PKB) to eventually phosphorylate the downstream DAF-16 (FOXO) transcription factor. This modification will inactivate DAF-16 by keeping in the cytoplasm. In the *daf-2* mutant, hypophosphorylated DAF-16 migrates into nuclei to regulate downstream gene expression and promote life span extension. In addition to DAF-16, there are other transcription factors such as HSF-1 and SKN-1 that function downstream of IIS [9].

DAF-16 is the key modulator of aging, and many genetic or environmental manipulations totally or partially depend on DAF-16 for their roles in life span determination. DAF-16 is only required for the life span extension under certain DR regimens such as sDR and IF [14,18], whereas it is not required for the life span extension induced by bDR, lDR, msDR and DD [12,16,20]. Recent studies showed that the sDR treatment activates DAF-16 via AMPK to extend life span [14], whereas IF treatment inhibits RHEB-1, an upstream activator of TOR, and inhibits transcription of the IGF-1 gene *ins-7* to activate DAF-16 and extend life span [18]. Besides, *daf-15*, which encodes the TOR interacting protein Raptor, is inhibited by DAF-16 at the transcription level [22]. These results have revealed that DAF-16 plays regulatory roles under DR via interacting with the TOR pathway.

hsf-1 encodes the ortholog of heat shock factor-1 in *C. elegans.* Mutations in *hsf-1* fully suppress the life span extension by *daf-2* mutants [27]. Recent studies reported that DD-mediated life span extension can be fully suppressed by the *hsf-1* mutant [28]. Protein aggregation as a consequence of protein misfolding is one of the major causes of neurodegenerative diseases. In *C. elegans*, DD functions through HSF-1 to delay age-related proteotoxicity [16,28].

2.3 Other key regulators of DR response

2.3.1 PHA-4/FOXA

pha-4 encodes a fork head box A (FOXA) transcription factor ortholog. It plays a critical role in the development of pharynx, the food intake organ of *C. elegans* [29]. Inhibition of *pha-4* by RNAi in the *rsks-1* mutant fully suppresses the life span extension [30]. Inhibition of *pha-4* can also suppress the life span extension by bDR or the *eat-2* mutant,

whereas it does not affect life span extension by reduced IIS, suggesting a specific regulatory role of PHA-4 in DR [12]. *pha-4* is up-regulated at the transcription level under DR, and it promotes the expression of specific superoxide dismutase genes under DR [12].

2.3.2   AAK-2/AMPKα

aak-2 encodes the α catalytic subunit of the key energy homeostasis regulator AMPK. When energy levels are low, as indicated by increased AMP:ATP ratio, AMP will bind to the γ regulatory subunit of AMPK, which leads to phosphorylation of the highly conserved Threonine 172 site on the α subunit. This modification results in the activation of AMPK, increased catabolism for more ATP production, temporarily decreased energy-consuming processes for less ATP consumption, and eventually restored energy homeostasis inside the organism [31]. Recent studies indicated that the *rsks-1* mutant showed significantly increased AAK-2 phosphorylation at the Threonine 172 site, and a deletion mutant of *aak-2* fully suppressed the life span extension by *rsks-1* [32]. Besides, it has also been reported that AAK-2 is required for sDR-mediated life span extension by phosphorylating and activating DAF-16 [14].

2.3.3   HIF-1/HIF-1α

The hypoxia inducible factor 1 (HIF-1) is a transcription factor complex that plays important roles in metabolism, cancer and many other biological processes. Under normal oxygen levels, the newly synthesized α subunit of HIF-1 is quickly hydroxylated at highly conserved proline residues, which leads to ubiquitination and proteasome-mediated protein degradation. In *C. elegans*, the *egl-9* gene encodes the proline hydroxylase that promotes HIF-1α degradation. Thus, HIF-1α is overexpressed in the *egl-9* deletion mutant [33]. Studies in *Drosophila* and mammalian cells showed that S6K promotes HIF-1α mRNA translation [34,35]. Interestingly, the mutation in *egl-9* suppressed life span extension by the *rsks-1* deletion in *C. elegans* [20]. A deletion mutant of *hif-1* that encodes HIF-1α showed life span extension under high nutrients, whereas the *hif-1* mutant did not show further life span extension under DR. Consistently, HIF-1 overexpression by the *egl-9* deletion suppressed the life span extension by various DR regimens, such as the msDR, DD and *eat-2* mutant. Therefore, inhibition of the TOR downstream effector HIF-1 can mimic DR [20]. Further studies demonstrated that inhibition of HIF-1 functions through reducing the endoplasmic reticulum unfolded protein response (ER UPR) to modulate aging. Moreover, HIF-1 functions in specific neurons and muscle to regulate DR-mediated life span extension [20].

2.3.4   SKN-1/Nrf

SKN-1 is another key transcription factor regulated by IIS as well as other signaling pathways. The mammalian ortholog of SKN-1 is the nuclear factor-erythroid-related factor (Nrf). SKN-1 is important for *C. elegans* embryonic development, oxidative stress response and aging. Previous studies demonstrated that SKN-1 is regulated by the DAF-2 downstream protein kinases, and mutations in *skn-1* can partially suppress life span extension by certain *daf-2* alleles [36]. Under lDR conditions, SKN-1 functions in the ASI neurons through cell-non-autonomous mechanisms to regulate mitochondrial electron transport chain (ETC) activities in the intestine, the major metabolic organ in *C. elegans*, to extend life span [13]. Life span extension by rapamycin treatments that inhibit TOR also requires SKN-1 activities [37].

2.3.5   NHR-62/HNF4α

Nuclear hormone receptors (NHRs) are transcriptional regulators that affect downstream genes' expression in response to developmental, environmental and nutrients signals. NHR-62, an HNF4α-like nuclear hormone receptor, has been identified as a mediator of DR-induced life span extension from an RNAi screen in the *eat-2* mutant. Further studies indicated that a deletion in *nhr-62* partially suppressed the prolonged longevity by bDR [38]. The *nhr-62* mutation also partially suppressed metabolic phenotypes of the *eat-2* mutant, such as decreased triglyceride and increased autophagy levels. Many genes induced by the *eat-2* mutant are dependent on NHR-62 [38]. These findings indicated a complicated network of hormonal and metabolic regulation of longevity in *C. elegans*.

Together, effects of TOR, IIS signaling pathways and other key aging regulators on longevity under normal and various DR conditions are summarized in Figure 1 and Figure 2, respectively.
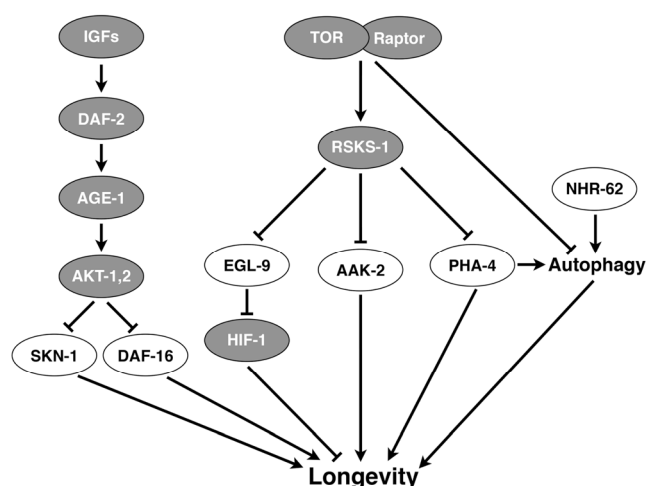

Figure 1 Effects of TOR and IIS pathways as well as other key regulators on aging under normal nutrient conditions. Inhibition of the IIS pathway results in de-repression of DAF-16 and SKN-1 and extended life span. Inhibition of the TOR pathway leads to activation of AAK-2 and PHA-4, inhibition of HIF-1, and activation of autophagy, which cause life span extension. NHR-62 may function through autophagy to promote longevity. Grey, pro-aging, life span-shortening factors. White, anti-aging, life span-extending factors.
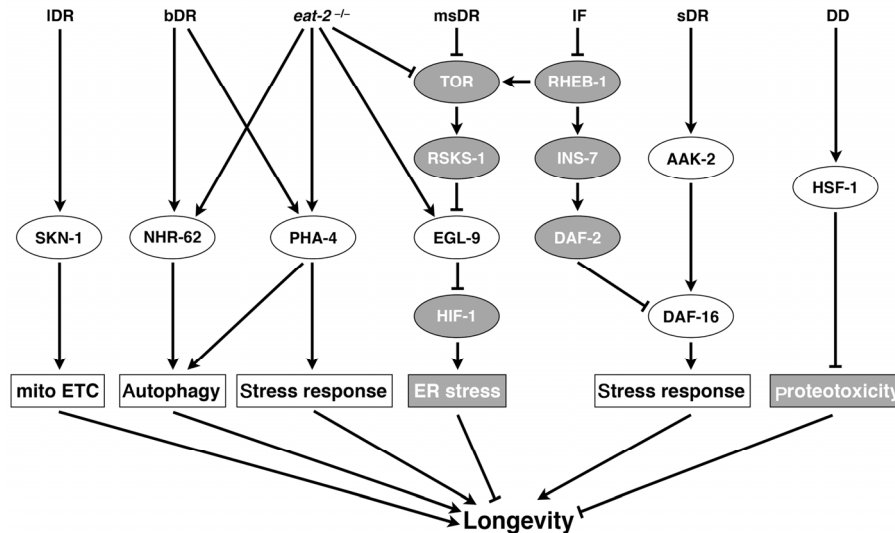
Figure 2 Effects of TOR and IIS pathways as well as other key regulators on aging in various DR regimens. lDR is mediated by SKN-1, bDR is mediated by PHA-4 and NHR-62; the *eat-2* mutant is mediated by TOR, PHA-4, EGL-9 and NHR-62; msDR is mediated by EGL-9; IF and sDR are mediated by DAF-16, and DD is mediated by HSF-1 for their effects on life span extension. SKN-1 functions through activating mitochondrial electron transport chain to extend life span. PHA-4 and DAF-16 function through regulating expression of distinct stress response genes to extend life span. EGL-9 functions through inhibiting HIF-1 and ER stress to extend life span. HSF-1 functions through reducing proteotoxicity to extend life span. NHR-62 functions through autophagy to extend life span. Grey, pro-aging, life span-shortening factors or biological processes. White, anti-aging, life span-extending factors or biological processes.

3   Perspective

Here we summarized recent progress on the molecular mechanisms of DR in *C. elegans*. In order to gain better understanding of the beneficial effects of DR, we propose to focus on the following aspects in future studies.

3.1   Different DR regimens

In order to study DR, researchers have independently developed multiple methods to reduce food intake in *C. elegans*. Most DR regimens require dilution of bacterial food to achieve reduced food intake. Currently, there is no easy and efficient way to quantitatively measure food consumption in *C. elegans*. The traditional method used the pharyngeal pumping rate as a rough indicator of the amount of food consumed by animals, but this method is not very accurate. Thus, there are large amount of variations among different experiments and different DR regimens, which makes experimental results less comparable and repeatable. Although the bacteria-free axenic media is hard to handle, it is a chemically defined culture that is very suitable to study the effects of specific nutrients on aging. Another resource is the *E. coli* genome-wide, single gene knockout mutant library created by microbiology researchers [39]. It may serve as very useful resource to study DR from the micronutrients point of view.

3.2   Key regulators of DR response and the downstream molecular mechanisms

Recent studies have identified several key mediators of the DR response. Knockout or knockdown of these genes can fully or partially suppress the DR-mediated life span extension. However, studies on DR enhancer genes, which further increase life span extension under DR when inhibited, have not been characterized. Identification of more suppressors and enhancers of DR by genetic studies will help us gain better understanding of the molecular mechanisms of DR.

The nutrients regulated TOR pathway mediates the beneficial effects of DR in many species. However, it has not yet been clear which downstream components are most relevant to DR. TOR-mediated regulation in mRNA translation should be the focus of future studies since inhibition of TOR, S6K or suppression of mRNA translation by other methods significantly extend life span across species [40]. How could inhibition of mRNA translation result in life span extension? There are two major hypotheses on this phenomenon. First, inhibition global mRNA translation will improve the quality of newly synthesized proteins and reduce the amount of misfolded, harmful proteins. Second, TOR and S6K might be able to regulate the translation of specific aging regulators. Therefore, functional genomic studies under DR, including transcriptome analysis (RNA Seq), translatome analysis (polysomal profiling coupled with deep sequencing) and proteome analysis (quantitative proteomics), will provide new insights into the molecular mechanisms of DR.

The IIS pathway also plays an important regulatory role in DR response. Interestingly, this regulation is achieved via interactions with the TOR pathway. We recently found that the *daf-2 rsks-1* double mutant, which simultaneously in-

hibits both IIS and TOR pathways, showed nearly 5-fold, synergistic life span extension [41]. Whether DR response is involved in this super long-lived phenotype needs to be further examined.

How could *C. elegans* sense changes in the amount of food in the environment through the nervous systems? Whether and how food signal could function cell non-autonomously to affect downstream tissues and modulate aging? Answers to these questions shall help us better understand DR.

3.3   DR, metabolism and reproduction

In addition to DR, TOR and IIS pathways also regulate metabolism, especially fat metabolism. Fat serves as the energy storage, cellular structure, and even signaling molecules to affect various biological processes. In *C. elegans*, inhibition of TOR or IIS results in increased fat accumulation [22,42]. Studies in *Drosophila* showed that DR by protein restriction significantly increases fat synthesis and breakdown, which are essential for the life span extension by DR [43]. Therefore, analyzing fat metabolism as a dynamic process via stable isotope labeling coupled with GC/LC-MS under DR will help us understand aging from the metabolic point of view.

Despite life span extension and delayed age-related pathologies, DR can cause harmful effects such as reduced reproduction. Whether there are genetic or pharmacological methods to dissect different outputs of DR should be characterized in future studies so that we can develop cures for aging without side effects.

In summary, studies on the molecular mechanisms of DR using *C. elegans* as a model have made significant progress. However, there are still fundamental questions remaining in the field. Further studies on these topics shall help us understand the nature of aging and provide novel targets to cure age-related diseases.

1   Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell, 2013, 153: 1194–1217

2   Daniels N. Global aging and the allocation of health care across the life span. Am J Bioethics, 2013, 13: 1–2

3   Kenyon C. The plasticity of aging: insights from long-lived mutants. Cell, 2005, 120: 449–460

4   Kapahi P, Chen D, Rogers AN, Katewa SD, Li PW, Thomas EL, Kockel L. With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. Cell Metab, 2010, 11: 453–465

5   Fontana L, Partridge L, Longo VD. Extending healthy life span—from yeast to humans. Science, 2010, 328: 321

6   Katewa SD, Kapahi P. Dietary restriction and aging, 2009. Aging Cell, 2010, 9: 105–112

7   Zhu CT, Ingelmo P, Rand DM. GxGxE for lifespan in *Drosophila*: mitochondrial, nuclear, and dietary interactions that modify longevity. PLoS Genet, 2014, 10: e1004354

8   Mulvey L, Sinclair A, Selman C. Lifespan modulation in mice and the confounding effects of genetic background. J Genet Genomics, 2014, 41: 497–503

9   Kenyon CJ. The genetics of ageing. Nature, 2010, 464: 504–512

10   Brenner S. The genetics of *Caenorhabditis elegans*. Genetics, 1974, 77: 71–94

11   Lakowski B, Hekimi S. The genetics of caloric restriction in *Caenorhabditis elegans*. Proc Natl Acad Sci USA, 1998, 95: 13091–13096

12   Panowski SH, Wolff S, Aguilaniu H, Durieux J, Dillin A. PHA-4/Foxa mediates diet-restriction-induced longevity of *C. elegans*. Nature, 2007, 447: 550–555

13   Bishop NA, Guarente L. Two neurons mediate diet-restriction-induced longevity in *C. elegans*. Nature, 2007, 447: 545–549

14   Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, Blanchard D, Gygi SP, Brunet A. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. Curr Biol, 2007, 17: 1646–1656

15   Hosono R, Nishimoto S, Kuno S. Alterations of life span in the nematode *Caenorhabditis elegans* under monoxenic culture conditions. Exp Gerontol, 1989, 24: 251–264

16   Kaeberlein TL, Smith ED, Tsuchiya M, Welton KL, Thomas JH, Fields S, Kennedy BK, Kaeberlein M. Lifespan extension in *Caenorhabditis elegans* by complete removal of food. Aging Cell, 2006, 5: 487–494

17   Lee GD, Wilson MA, Zhu M, Wolkow CA, de Cabo R, Ingram DK, Zou S. Dietary deprivation extends lifespan in *Caenorhabditis elegans*. Aging Cell, 2006, 5: 515–524

18   Honjoh S, Yamamoto T, Uno M, Nishida E. Signalling through RHEB-1 mediates intermittent fasting-induced longevity in *C. elegans*. Nature, 2008, 457: 726–730

19   Szewczyk NJ, Udranszky IA, Kozak E, Sunga J, Kim SK, Jacobson LA, Conley CA. Delayed development and lifespan extension as features of metabolic lifestyle alteration in *C. elegans* under dietary restriction. J Exp Biol, 2006, 209: 4129–4139

20   Chen D, Thomas EL, Kapahi P. HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in *Caenorhabditis elegans*. PLoS Genet, 2009, 5: e1000486

21   Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol, 2010, 12: 21–35

22   Jia K, Chen D, Riddle DL. The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. Development, 2004, 131: 3897–3906

23   Long X, Spycher C, Han ZS, Rose AM, Muller F, Avruch J. TOR deficiency in *C. elegans* causes developmental arrest and intestinal atrophy by inhibition of mRNA translation. Curr Biol, 2002, 12: 1448–1461

24   Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller F. Genetics: influence of TOR kinase on lifespan in *C. elegans*. Nature, 2003, 426: 620

25   Pan KZ, Palter JE, Rogers AN, Olsen A, Chen D, Lithgow GJ, Kapahi P. Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. Aging Cell, 2007, 6: 111–119

26   Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C. A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. PLoS Genet, 2008, 4: e24

27   Hsu AL, Murphy CT, Kenyon C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science, 2003, 300: 1142–1145

28   Steinkraus KA, Smith ED, Davis C, Carr D, Pendergrass WR, Sutphin GL, Kennedy BK, Kaeberlein M. Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in *Caenorhabditis elegans*. Aging Cell, 2008, 7: 394–404

29   Mango SE, Lambie EJ, Kimble J. The pha-4 gene is required to generate the pharyngeal primordium of *Caenorhabditis elegans*.

Development, 1994, 120: 3019–3031

30 Sheaffer KL, Updike DL, Mango SE. The target of rapamycin pathway antagonizes *pha-4/FoxA* to control development and aging. Curr Biol, 2008, 18: 1355–1364

31 Hardie DG. AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. Genes Dev, 2011, 25: 1895–1908

32 Selman C, Tullet JM, Wieser D, Irvine E, Lingard SJ, Choudhury AI, Claret M, Al-Qassab H, Carmignac D, Ramadani F, Woods A, Robinson IC, Schuster E, Batterham RL, Kozma SC, Thomas G, Carling D, Okkenhaug K, Thornton JM, Partridge L, Gems D, Withers DJ. Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. Science, 2009, 326: 140–144

33 Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, Ratcliffe PJ. *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell, 2001, 107: 43–54

34 Bernardi R, Guernah I, Jin D, Grisendi S, Alimonti A, Teruya-Feldstein J, Cordon-Cardo C, Simon MC, Rafii S, Pandolfi PP. PML inhibits HIF-1alpha translation and neoangiogenesis through repression of mTOR. Nature, 2006, 442: 779–785

35 Dekanty A, Lavista-Llanos S, Irisarri M, Oldham S, Wappner P. The insulin-PI3K/TOR pathway induces a HIF-dependent transcriptional response in *Drosophila* by promoting nuclear localization of HIF-alpha/Sima. J Cell Sci, 2005, 118: 5431–5441

36 Tullet JM, Hertweck M, An JH, Baker J, Hwang JY, Liu S, Oliveira RP, Baumeister R, Blackwell TK. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C.*

elegans. Cell, 2008, 132: 1025–1038

37 Robida-Stubbs S, Glover-Cutter K, Lamming DW, Mizunuma M, Narasimhan SD, Neumann-Haefelin E, Sabatini DM, Blackwell TK. TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. Cell Metab, 2012, 15: 713–724

38 Heestand BN, Shen Y, Liu W, Magner DB, Storm N, Meharg C, Habermann B, Antebi A. Dietary restriction induced longevity is mediated by nuclear receptor NHR-62 in *Caenorhabditis elegans.* PLoS Genet, 2013, 9: e1003651

39 Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol, 2006, 2: 2006.0008

40 Rogers AN, Chen D, McColl G, Czerwieniec G, Felkey K, Gibson BW, Hubbard A, Melov S, Lithgow GJ, Kapahi P. Life span extension via eIF4G inhibition is mediated by posttranscriptional remodeling of stress response gene expression in *C. elegans.* Cell Metab, 2011, 14: 55–66

41 Chen D, Li PW, Goldstein BA, Cai W, Thomas EL, Chen F, Hubbard AE, Melov S, Kapahi P. Germline signaling mediates the synergistically prolonged longevity produced by double mutations in *daf-2* and *rsks-1* in *C. elegans.* Cell reports, 2013, 5: 1600–1610

42 Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans.* Science, 1997, 277: 942–946

43 Katewa SD, Demontis F, Kolipinski M, Hubbard A, Gill MS, Perrimon N, Melov S, Kapahi P. Intramyocellular fatty-acid metabolism plays a critical role in mediating responses to dietary restriction in *Drosophila melanogaster.* Cell Metab, 2012, 16: 97–103