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JB Review

Two sides of lifespan regulating genes: pro-longevity or anti-longevity?

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Traditionally, ageing has been considered a passive and entropic process, in which damages accumulate on biological macromolecules over time and the accumulated damages lead to a decline in overall physiological functions. However, the discovery of a longevity mutant in the nematode *Caenorhabditis elegans* has challenged this view. A longevity mutant is a mutant organism, in which a reduction-of-function of a certain gene prolongs the lifespan. Thus, the discovery of longevity mutants has shown the existence of genes, which function to shorten lifespan in wild-type organisms, promoting extensive hunting for longevity-regulating genes in short-lived model organisms, such as yeast, worms and flies. These studies have revealed remarkable conservation of longevity-regulating genes and their networks among species. Decreased insulin/IGF-like signalling and decreased target of rapamycin (TOR) signalling are both shown to extend lifespan in evolutionarily divergent species, from unicellular organisms to mammals. Intriguingly, most of these longevity-regulating pathways reveal pro-longevity and anti-longevity effects on lifespan, depending on biological and environmental contexts. This review summarizes pleiotropic functions of the conserved longevity-regulating genes or pathways, focusing on studies in *C. elegans*.

Keywords: ageing/*C.elegans*/insulin/IGF-like signalling/pleiotropy/TOR.

Abbreviations: CR, calorie restriction; DR, dietary restriction; ETC, electron transport chain; IF, intermittent fasting; IIS, insulin/IGF-like signalling; NAD, nicotinamide adenine dinucleotide; TOR, target of rapamycin; Rheb, Ras homologue enriched in brain.

We accept ageing as inevitable, as it has been intuitively believed that cumulative damage to genome, proteins and lipids provokes an age-associated decline in cellular functions. However, each species has its own characteristic lifespan, suggesting that information coded by their genome defines lifespan as well as other physiological phenomena. A groundbreaking progress in ageing research is the discovery of *Caenorhabditis elegans* longevity mutants in the 1980s (*1*). The mutants live longer than the wild-type organism with a reduction-of-function mutation in certain genes. First, the extended lifespan was attributed to reduced fecundity or reduced food intake. Following studies revealed that one of the mutant strains lives longer independently of fecundity and food intake and the gene was named *age-1*. *age-1* encodes PI3K, a component in the insulin/IGF-like signalling (IIS) pathway. The study showed that IIS functions to limit lifespan in the wild-type organism in *C. elegans* (*2–4*). Surprisingly, it has also been revealed that lifespan is prolonged in yeast, flies and rodents when IIS is reduced although these organisms are totally different in their shape, size and life cycle (*5*). Based on these results, researchers have come to think that ageing is not a passive degenerative process, but a genetically regulated and evolutionarily conserved phenomenon. Extensive studies in invertebrate model organisms have then identified many pro-longevity pathways and anti-longevity pathways. Target of rapamaycin (TOR) and sirtuins are identified as negative (*6*, *7*) and positive regulators (*8*, *9*) of ageing, respectively, in several species, such as *Saccharomyces cervisiae*, *C. elegans* and *Drosophila melanogaster*. Recent studies in mice have also suggested that TOR signalling (*10–12*) and sirtuins (*13*, *14*) regulate mammalian ageing. However, detailed studies concerning the molecular mechanisms by which these genes regulate lifespan revealed that they have opposing effects on lifespan; while sometimes they prolong organism's lifespan, sometimes they limit it. Why most of longevity-regulating genes reveal the two sides of effects on lifespan? Addressing this question should help increase our understanding of the essence of ageing.

IIS

IIS is now widely accepted as a signalling pathway that plays a central role in the regulation of organism's growth and lifespan. There appeared breakthrough reports showing that worms with a mutation in *daf-2*, a *C. elegans* homologue of insulin/IGF receptor (*15*, *16*) and in *age-1*, *C. elegans* PI3 kinase (*3*, *4*) lead active and healthy lives twice as long as those of wild-type worms, demonstrating the involvement of IIS in ageing for the first time. In *C. elegans*, low IIS extends lifespan through several transcription factors: DAF-16, a

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FOXO transcription factor (*17*, *18*); HSF-1, the heat-shock transcription factor (*19*); and SKN-1, an Nrf-like xenobiotic-response factor (*20*). As knock-down or loss-of-function mutation of these transcription factors abolished longevity conferred by low IIS, the simplest idea was that the expression levels of pro-longevity genes and anti-longevity genes are high and low, respectively, in longevity mutants compared with wild-type animals (Fig. 1A). Thus, genome-wide analyses with microarray (*21–23*), SAGE (*24*), bio-informative analyses (*25*, *26*) and ChIP-chip (*27*) have been performed to identify essential genes for low IIS-induced longevity. These analyses have successfully identified genes whose expression levels are up- or down-regulated in longevity mutants; however, none of them is a sole, absolute pro- or anti-longevity gene. Rather, knock-down of each gene modifies *C. elegans* lifespan by piecemeal. Therefore, marked lifespan extension in low IIS mutants may results from accumulation of small effects on many different genes that act together (Fig. 1B).

Recently, quantitative mass spectrometry analysis has identified *daf-2*/*daf-16* target genes at protein level, *i.e.* at the final gene product level. However, this study does not necessarily identify a longevity promoting mechanism, but rather shows a compensatory mechanism in lifespan regulation. Most of the genes which are identified as more abundant in long-lived *daf-2* mutants than in wild-type worms are shown to function to reduce longevity (*28*). This result was opposite to what might be expected, and thus indicated the complexity of lifespan regulation. In other words, this might suggest that there exist several pathways which promote or limit longevity downstream of IIS.

It has also been shown recently that single genes can have opposing effects on lifespan, depending on genetic backgrounds. Prohibitins are ubiquitous, evolutionarily conserved proteins, which form a ring-like, high-molecular mass complex at the inner membrane of mitochondria. Knock-down of *phb-1* and *phb-2*, two subunits of prohibitin complex, shortens lifespan of wild-type worms through modulation of mitochondrial function and fat metabolism. However, the same procedure significantly extends lifespan of *daf-2* mutants, showing that the mitochondrial prohibitin complex limits longevity in *daf-2* mutants (*29*). Taken together, organism's lifespan is determined by the sum of small effects of many different genes, and each gene can have a different effect on lifespan, depending on biological contexts (Fig. 1C). This complexity makes it difficult to elucidate molecular bases underlying longevity even for the molecular functions of IIS, the most established and studied lifespan regulating pathway.

TOR signalling

As restriction of food intake delays ageing in yeast to mammals, energy metabolism is tightly associated with ageing. TOR is a serine/threonine kinase, which integrates and transmits signals from nutrients such as growth factors, amino acids and ATP levels to regulate cell survival and growth. TOR exerts these effects through changes in mRNA translation, ribosomal biogenesis, autophagy and metabolism. TOR activation under nutrient- and energy-replete conditions stimulates protein synthesis and cell growth through phosphorylation of ribosomal protein S6 kinase (p70S6K), eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and eukaryotic elongation factor 2 kinase (EEF2K) (*30*). Therefore, reducing activity of TOR is supposed to mimic nutrient-limited cellular conditions. Consistent with this, it was shown that inhibition of TOR or raptor, an essential component of TOR complex1, extends lifespan dramatically in *C. elegans*. (*6*, *7*). Similar to low IIS, low TOR signalling promotes longevity in other species, including yeast, flies and mammals. Under food-replete conditions animals age more rapidly with the high activity of TOR than do they under calorie-restricted conditions.

Recently, the dual role of TOR signalling in lifespan regulation was reported in *C. elegans*. The upstream activator of TOR, a low molecular weight GTPase Ras homologue enriched in brain (Rheb) (*31*), also accelerates ageing under over-nutrition in *C. elegans* (*32*). Intriguingly, Rheb is required for suppression of ageing in response to intermittent fasting, a kind of dietary restriction (*32*). These results are seemingly paradoxical; however, the authors showed that Rheb/TOR signalling suppresses two lifespan regulating pathways, IIS and FoxA signalling at the same time. Since IIS and FoxA have anti- and pro-longevity effects, respectively (*33*), it has been suggested that the balance of these antagonistic effects finally determines worm's lifespan. In addition to IIS and FoxA, various effectors of TOR signalling have also been shown to affect ageing. Among them, HIF-1 (hypoxia-inducible factor 1) (*34*) is of particular interest, as it has both pro- and anti-longevity effects (*35*).

HIF-1 is a heterodimeric protein complex composed of HIF-1α and HIF-1β subunits. The HIF-1α subunit is rapidly degraded under normoxic conditions, whereas it is stabilized and accumulates upon hypoxia. As a dimer, HIF-1α/HIF-1β translocates to the nucleus and induces expression of genes involved in adaptation to reduced oxygen availability. In mammalian cells, TOR is shown to up-regulate HIF-1α in its protein levels and activate its transcriptional activity (*36*). Recently, studies in *C. elegans* established a direct connection between HIF-1α and ageing. They reported that stabilization of HIF-1 protein (*C. elegans* HIF1-α) caused by loss-of-function mutations in the HIF-1 degradation system (*37*, *38*) or over-expression of degradation-resistant HIF-1 protein (*39*) extended lifespan. When over-expression of certain genes extends organism's lifespan, one would expect that their deletion should shorten lifespan. Surprisingly, however, independent studies reported that deletion of *hif-1* also extends *C. elegans* lifespan (*39–41*). [The dual role of HIF-1 in *C. elegans* lifespan is nicely reviewed by Leiser and Kaeberlein (*35*)].

These studies concerning TOR signalling and HIF-1 concurrently underscore the importance of 'basal' activities of these pathways. As TOR is activated by nutrients, people tend to regard TOR activity as almost zero under nutrient-depleted conditions.

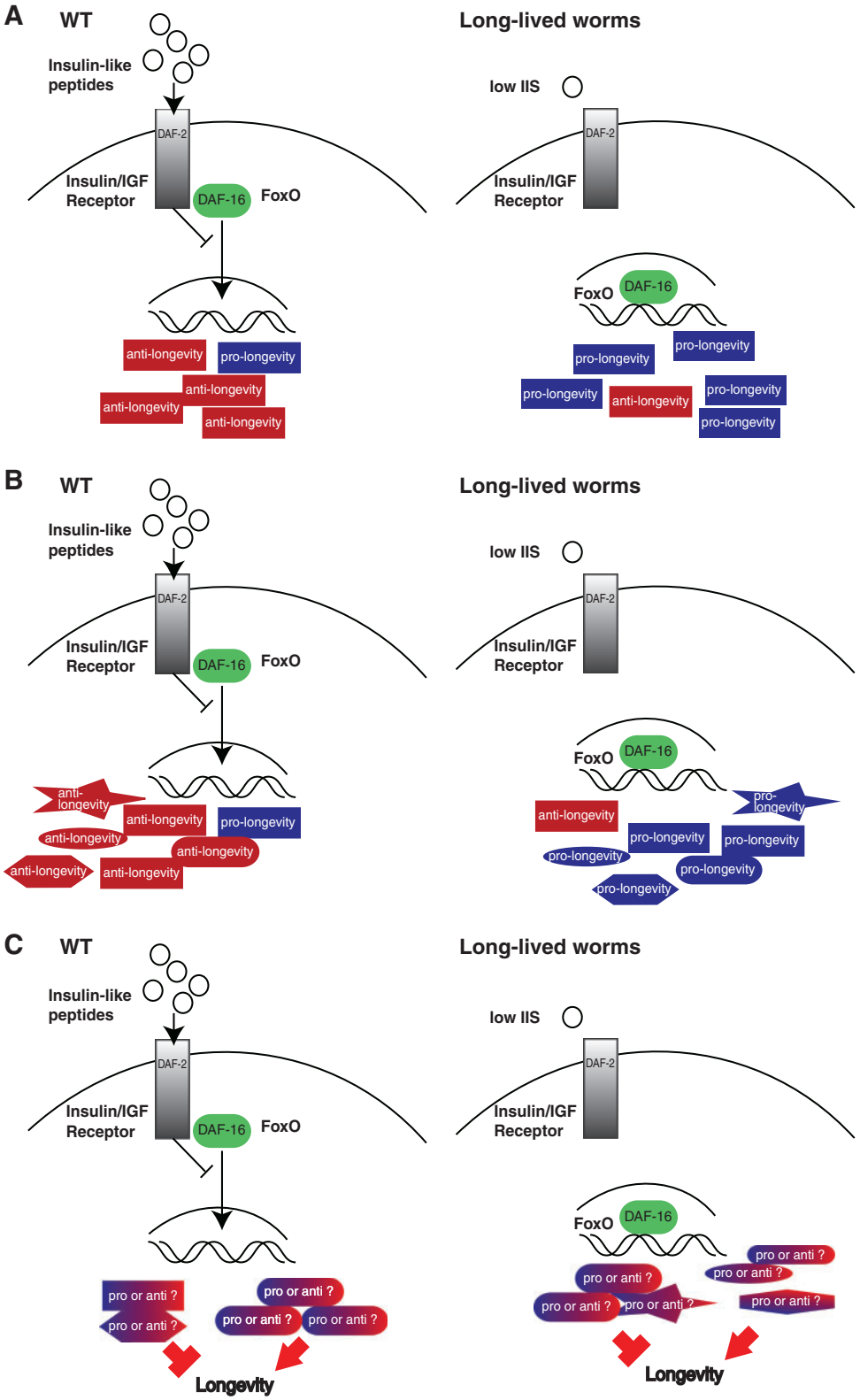

Fig. 1 Schematic representation of mechanisms underlying low IIS-induced longevity. It had been assumed that expression levels of a few genes that play critical roles in lifespan regulation are changed in longevity mutants (A). Genome-wide analyses suggest that low IIS-conferred longevity may result from accumulation of small effects on many different genes (B) and their effects on lifespan are variable rather than definitive, depending on biological contexts (C).

However, as it is shown that the Rheb/TOR axis is required for fasting-induced longevity, Rheb and TOR play an important role even when their activity remains low (*32*). Similarly, the observation that loss-of-function of HIF-1α extends lifespan under normoxia when HIF-1α is rapidly degraded should emphasize the importance of the basal activity of HIF-1α to shorten lifespan. Additional studies need

to be carried out to elucidate their functions at basal or high activity levels and a possible interaction between TOR and HIF-1 in ageing.

Sirtuins

Sirtuins are an nicotinamide adenine (NAD)-dependent protein deacetylase family. These enzymes constitute the class III histone deacetylases (HDACs) and are conserved from bacteria to humans (*9*). Unlike IIS and TOR signalling, they were identified as *positive* regulators of lifespan in the budding yeast *S. cerevisiae* for the first time. In yeast, ageing has been studied by two approaches. One is replicative lifespan. Mother cells of the yeast reproduce asymmetrically by originating buds (daughter cells). Daughter cells are smaller than mothers and can be easily recognized and removed by micromanipulation after budding occurs. Replicative lifespan is defined as the total number of daughter cells generated by a mother cell. The other is chronological lifespan, *i.e.* surviving periods of non-dividing populations. It was shown that sirtuins extend replicative lifespan by maintaining silent chromatin through the deacetylation of histones in yeast (*8*, *42*). Subsequent studies then showed that surtuins protect animals from ageing in worms (*43*), flies (*44*) and mammals (*13*, *14*).

Yeast cells uptake glucose through transporters. Limited glucose availability in media, calorie restriction (CR) in yeast, extends replicative lifespan. This CR-induced longevity in replicative lifespan was shown to be dependent on both NAD and Sir2, a member of sirtuins (*45*). Meanwhile, in the chronological lifespan, Sir2 null mutations caused a minor reduction in lifespan in one wild-type background or no significant changes in two wild-type strains (*46*). The same study also showed that the deletion of Sir2 extends further the chronological lifespan of long-lived mutant strains, Sch9 (a functional homologue of mammalian Akt) mutants and Cyr1 (adenylate cyclase) mutants, and starved wild-type strains (*46*), which means that under certain conditions in which yeast lives much longer than usual, Sir2 blocks extreme lifespan extension.

In *C. elegans*, *sir-2.1*, a homologue of yeast Sir2, was first reported to promote longevity. However, it can function to block longevity under specific conditions. In *C. elegans*, autophagy was reported to contribute to longevity conferred by low IIS (*47*). However, inactivation of autophagy-related genes after adult stages did not shorten lifespan of wild type animals (*48*). Rather, knock-down of several autophagy-related genes extended lifespan of *sir-2.1* null mutants by >40% (*48*). Although it is unclear whether the longevity effects were caused by suppression of autophagy or other side effects, this study suggests that *sir-2.1* can have anti-longevity effects under certain conditions in *C. elegans*, too. In summary, sirtuins were identified as positive regulators of lifespan, and they usually protect animals from ageing, but block abnormal longevity in specific cases in yeast and worms.

Dose-dependent effects

Why do these signalling pathways sometimes promote longevity, but sometimes limit it? As ageing is a complicated process, lifespan may be significantly influenced by minor differences among laboratories in culture conditions (*e.g.* liquid culture or solid culture, ways to remove offspring, food quality, food availability, temperature fluctuation, humidity, population density and so on). However, several studies described above demonstrated that one gene can prolong or restrict lifespan in a series of controlled experiments. Therefore, it is unlikely that these opposing effects merely result from experimental variabilities or errors.

Besides above signalling networks, extensive studies revealed that several physiological reactions or processes are important for ageing regulation. A systematic RNAi screen identifies critical roles for mitochondria and protein turnover in *C. elegans* longevity (*49*). In *C. elegans*, inhibition of mitochondrial components by RNAi increased lifespan with high probability (*50*). However, many mutations that disrupt the function of mitochondrial electron transport chain (ETC) in humans (*51*) and a few mutations in *C. elegans* (*52*) are known to be pathologically life shortening. It is not surprising that different mutations in ETC affect organism's lifespan differently, as ETC consists of five complexes with many subunits having diverse functions. However, knock-down of three genes, disruption of which in human leads to diseases (*53–55*) was reported to lengthen *C. elegans* lifespan (*50*, *56*, *57*). A recent study resolved the disparity between the increased lifespan in worms due to ETC knock-down and the diseases in humans due to ETC malfunction. The authors employed a 12-point dilution series of bacteria expressing dsRNA towards five ETC genes, and found that ETC activity affects lifespan of worms in a dose-dependent manner. They observed a three-phase lifespan response to increasingly greater inhibition by RNAi: at low levels of inhibition, there was no response, then as inhibition increased, lifespan monotonically lengthened. Finally, at the highest levels of RNAi inhibition, lifespan began to shorten (*58*). The results demonstrated that worms live shorter in both cases when ETC activity is higher or lower than optimal levels.

It is possible that another important process, protein turnover, works in a similar way. Autophagy, a bulk degradation system of macromolecules and organelles, has an important role in ageing (*47*). Autophagy is thought to promote both cell and organism survival by providing fundamental building blocks to maintain energy homeostasis during starvation. A previous study demonstrated that physiological levels of autophagy promote optimal survival of *C. elegans* during starvation, but either insufficient or excessive levels of autophagy render *C. elegans* starvation hypersensitive (*59*, *60*). Under different conditions, however, autophagy may instead act to promote cell death through an autophagic cell death pathway distinct from apoptosis. Therefore, similar to the case of mitochondrial ETC function, there may be an optimal level of autophagy for the normal ageing process.

Additionally, the other side of protein turnover, protein synthesis, might also affect lifespan in a dose-dependent manner. In yeast, deletion of *RPL31A* and *RPL6B*, which encode two components of the large ribosomal subunit, increased replicative lifespan substantially (*61*). In *C. elegans*, depletion of key components of the cognate translational machinery (eIF-4G and EIF-2B homologues) by RNAi increased lifespan (*62*). Subsequent studies have established the role of protein synthesis in accelerating ageing (*63–66*). These increases in lifespan may be caused by partial, not complete, inhibition of translation. Intriguingly, inhibition of two *C. elegans* translation initiators, eIF2β (*iftb-1*) or eIF4G (*ifg-1*), by RNAi extended lifespan of wild-type animals, but slightly shortened lifespan of long-lived, sterile *fer-15(b26); daf-2(mu150); fem-1(hc17) mutants* (*64*). As complete shutdown of translation is expected to be harmful to living organisms, protein synthesis may affect lifespan in a dose-dependent manner, such as ETC function and autophagy. It is noteworthy that these processes are mutually interacting. Obviously, autophagy and mitochondrial ETC play important roles in protein synthesis through amino acid metabolism and intracellular energy metabolism, respectively. Autophagy is proposed to decrease the potential oxidative damage due to defective mitochondria by promoting mitochondrial turnover. Conversely, ROS from mitochondria stimulates autophagy as a signal (*67*). Therefore, the optimal levels of these reactions or processes might be changeable depending on the levels of the others, environmental parameters and biological context, such as oxygen levels and fat contents. When we focus on the role of certain genes or pathways in lifespan regulation, we should consider their function in the whole system and evaluate their function through integrating numerous physiological processes.

Ageing has two aspects in higher eukaryotes, maintenance of individual cells and maintenance of homeostasis of tissues, organs and organisms. In short-lived model organisms, especially in *C. elegans* whose somatic cells are non-dividing, ageing is studied from the first aspect. The fact that mitochondrial ETC and protein turnover have been identified as longevity-affecting processes by systemic RNAi screens renews the importance of damages, which accumulate to macromolecules. Although it still remains unclear whether the damage accumulation is a primary cause, or results of, ageing, it is certain that many kinds of damages accumulate on cellular components over time, and once accumulated, they accelerate ageing, the ageing of individual cells.

Conclusion

As each species has its own characteristic lifespan, ageing is doubtlessly regulated by genomic information. Additionally, it is becoming increasingly clear that the longevity-regulating signalling networks are evolutionarily conserved. Global similarity in the transcriptional profiles of ageing between *C. elegans* and *D. melanogaster* was reported (*68*). Another network analysis revealed that human protein interaction networks show a high conservation in the ageing process with those of invertebrates (*69*), suggesting that similar changes occur at a molecular level during ageing in evolutionarily divergent species. Do they mean that an ageing process is actively programmed?

It is sometimes said that germ lines are immortal because they are transmitted to next generations indefinitely. In a similar context, it is also said that transformed cells do not age. These observations are sometimes interpreted as evidence that somatic cells are actively programmed to age, because ageing is not a necessary feature of eukaryotic cells. However, they might just show that eukaryotic cells can proliferate indefinitely depending on telomerase, which does not mean that post-mitotic cells can live forever. In the unicellular organism, budding yeast, mother cells rejuvenate their daughter cells by retaining damaged proteins in mother cells in a sirtuin-dependent manner (*70*), consistent with deleterious effects of damaged molecules. It is unknown that similar mechanisms work in other unicellular organisms; however, cell division itself can lower the concentrations of damaged cellular components by dilution because cells increase their contents by freshly synthesizing chromosomes, proteins and lipids before divisions. During development in multicellular organisms, embryos undergo cell divisions and increase their body mass. Thus, most of their body components are freshly synthesized. Therefore, in offspring, parental age effects can be reset by the developmental process itself, and germ cells themselves are also mortal, like somatic cells.

Previously, theoretical studies proposed the antagonistic pleiotropy theory of ageing, which says that ageing has evolved by natural selection (*71*, *72*). In this theory, organisms might have evolved for the sake of early reproduction and survival, sacrificing those late in life, as natural selection has favoured genes that exert beneficial effects early in life regardless of their impacts on later in life. Similarly, natural selection might have eliminated mutations which reduce fitness early in life more effectively than mutations which impair individual's survival after reproduction. In other words, organisms are optimized for early development and reproduction, but not for maintenance of individuals.

In this review, we summarized opposing effects of longevity-regulating genes or pathways on lifespan. It is not unusual that one signalling pathway is involved in divergent functions. For example, IIS and TOR in *C. elegans* play important roles in growth, reproduction, lipid metabolism and ageing. The important clue to understand ageing is to address the question of whether the opposing effects of IIS and TOR functions are specific to ageing among their functions. IIS regulates both organism's growth and ageing through the regulation of the activities of transcription factors. Are there also compensation mechanisms in the regulation of growth, as is the case of ageing regulation? Are there any genes that promote the growth of wild-type animals, but suppresses growth of low IIS mutants? If opposing and changeable effects are characteristic to longevity-regulating genes or pathways,

then the antagonistic pleiotropy theory of ageing can be strengthened. It is important to compare the functions of signalling pathways in ageing and those in other physiological processes.

A major remaining challenge is to understand the mechanism of how the species-specific lifespan is determined. Each species has its own body plan and lifespan. In general, larger animals tend to live longer (*73*). Thus, the species-specific body plan may be one of the determinants of species-specific lifespan, which also include the intra- and inter-cellular programmes concerning maintenance of individual cells, such as metabolic rates, genome maintenance, telomere length and stress resistance. Therefore, ageing can be understood as a result of sequential developmental processes, maintenance of homeostasis of organisms, such as cellular maintenance, tissue maintenance due to cell turnover, disease-preventing mechanisms including cellular senescence and apoptosis and their side effects.

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Conflict of interest
None declared.

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