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The genetics of ageing

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The nematode *Caenorhabditis elegans* ages and dies in a few weeks, but humans can live for 100 years or more. Assuming that the ancestor we share with nematodes aged rapidly, this means that over evolutionary time mutations have increased lifespan more than 2,000-fold. Which genes can extend lifespan? Can we augment their activities and live even longer? After centuries of wistful poetry and wild imagination, we are now getting answers, often unexpected ones, to these fundamental questions.

For many years, molecular biologists interested in regulatory mechanisms did not study ageing, as the tissue decline associated with ageing suggested a passive, entropic process of deterioration that occurred in a haphazard way. We know now, however, that the ageing process, like so many other biological processes, is subject to regulation by classical signalling pathways and transcription factors. Many of these pathways were first discovered in small, short-lived organisms such as yeast, worms and flies, but a remarkable fraction turn out to extend lifespan in mammals as well. In this article, I describe these pathways and their regulation by environmental and physiological signals. I also discuss unsolved mysteries in the field and, finally, the outlook for drugs that could slow ageing in humans.

Many people assume that extending lifespan by slowing ageing would mean certain death from, say, Alzheimer's disease. Instead, we are finding that mutations that slow ageing also postpone age-related disease. This link raises the possibility of combating many diseases all at once by targeting ageing, their greatest risk factor. Fascinating as this topic is, age-related disease is not the focus of this article. Rather, its focus is ageing itself.

Pathways that regulate ageing

Many mutations that extend lifespan affect stress-response genes or nutrient sensors. When food is plentiful and stress levels are low, these genes support growth and reproduction. Under harsh conditions, their activities change: some are turned up and others are turned down, and as a consequence the animal undergoes a global physiological shift towards cell protection and maintenance. This shift protects the animal from environmental stresses and it also extends lifespan.

Nutrient and stress sensors mediate lifespan extensions that occur in response to many different environmental and physiological signals. The best known of these signals is dietary restriction, which extends lifespan in many species, from yeast to primates[1]. This effect was discovered in studies with rats during the Great Depression, amid concern that chronic hunger might shorten lifespan. Dietary restriction was initially assumed to extend lifespan simply by reducing the rate at which cellular damage accumulates over time as a result of nutrient metabolism. Recently, however, an elegant experiment with *Drosophila* showed that dietary restriction produces a rapid decrease in the mortality rate (the daily chance of death), suggesting that dietary restriction counteracts the causes of ageing in an acute manner[2]. We now know that the longevity response to dietary restriction is actively regulated by nutrient-sensing pathways involving the kinase target of rapamycin (TOR)[3–5], AMP kinase[6], sirtuins[7,8] and insulin/insulin-like growth factor (IGF-1) signalling[9,10]. Unexpectedly, which nutrient sensor is most important in extending lifespan in response to dietary restriction depends on the way that dietary restriction is imposed. In *C. elegans*, for example, one nutrient sensor extends lifespan in response to life-long food limitation, another in response to every-other-day feeding and a third if dietary restriction begins in middle age[11].

Many other conditions can increase lifespan, including heat[12–14] and oxidative stress[15], low ambient temperature, chemosensory signals[16], thermosensory signals[17], signals from the reproductive system[18,19] and reductions in the rates of respiration[19–21] or translation[3,22–24]. In most, if not all, of these cases, the longevity response is under active control by specific regulatory proteins.

Slowing ageing might seem like an overwhelming challenge, as the decline is so pervasive. So it is noteworthy that when we extend the lifespans of laboratory animals, we do not have to combat individually all the problems of age, such as the declining muscles, the wrinkled skin and the mutant mitochondria. Instead, we just tweak a regulatory gene, and the animal does the rest. In other words, animals have the latent potential to live much longer than they normally do.

Insulin/IGF-1 signalling

The first pathway shown to influence ageing in animals was the insulin/IGF-1 pathway[19]. In *C. elegans,* mutations that decrease the activity of *daf-2,* which encodes a hormone receptor similar to the insulin and IGF-1 receptors, more than double the lifespan of the animal, and mutations affecting the downstream phosphatidylinositol 3-kinase (PI(3)K)/AKT/PDK kinase cascade extend lifespan as well. The most remarkable thing about these (and many other) long-lived mutants is that they remain young long after normal worms look old[25,26]. (Imagine yourself, in your thirties, learning that your attractive young dinner date is actually 70.) Inhibiting insulin/IGF-1 signalling changes lifespan through changes in gene expression: through DAF-16, a FOXO transcription factor; the heat-shock transcription factor HSF-1; and SKN-1 (ref. 27), a Nrf-like xenobiotic-response factor. These transcription factors, in turn, upregulate or downregulate diverse genes that act cumulatively to produce large effects on lifespan. Downstream genes shown to be functionally significant include stress-response genes such as catalases, glutathione *S*-transferases and metallothioneins, as well as genes encoding antimicrobial peptides, chaperones, apolipoproteins, lipases[28] and channels. Surprisingly, genes normally expressed in the germ line are misexpressed in the somatic tissues of *daf-2* mutants, where they contribute to longevity[29]. This is intriguing because the germline lineage (traced from fertilized egg to fertilized egg) is immortal. However, unfertilized germ cells that express these germline genes do age, along with the rest of the animal, in a *daf-2*-dependent manner[25]. Thus, germline genes account for some, but not all, of the longevity mechanism. More generally, each downstream longevity gene has its own story, often

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completely unknown. Together, these genes constitute a treasure trove of discovery for the future. For example, some genes double their activity in a long-lived mutant; what would happen if their activity were increased tenfold?

Autophagy, a process that recycles cellular organelles, is also required for the inhibition of *daf-2* to extend lifespan[30]. Because it rejuvenates the cell, autophagy could be a powerful anti-ageing mechanism. But although DAF-16 is required for lifespan extension, DAF-16 itself is not required for autophagy in *C. elegans daf-2* mutants[31]. This suggests that autophagy might be acting to provide raw materials to be recycled into new protective proteins by the actions of DAF-16 and other transcription factors.

Perturbations in the insulin /IGF-1 pathway in one tissue reverberate through other tissues to establish a new homeostasis[19]. For example, in otherwise wild-type *C. elegans*, increasing the level of DAF-16 activity in just one tissue can increase DAF-16 activity elsewhere, through feedback regulation of insulin gene expression[32]. In addition, in *daf-16(−); daf-2(−)* double mutants, which do not live long, expressing *daf-16* exclusively in one tissue (such as intestinal/adipose tissue or neurons) can extend the lifespan of the whole animal. Therefore, in addition to regulating insulin gene expression, DAF-16 must also regulate downstream intercellular longevity signals that can act independently of *daf-16* in other tissues. DAF-16 is not the only transcription factor that can affect lifespan by influencing cells other than those in which it acts (that is, by acting cell non-autonomously): the same is true of HSF-1 (ref. 19) and SKN-1 (ref. 33). This complex network of tissue–tissue interactions may help to coordinate the rates of ageing of the different tissues.

A lifespan pathway that acts in a tiny roundworm composed largely of post-mitotic cells might not be expected to affect more complex animals. In fact, the insulin/IGF-1 pathway's effect on lifespan has been evolutionarily conserved[19,34] (Fig. 1). In *Drosophila*, inhibiting insulin/IGF-1 signalling systemically or increasing the activity of FOXO (the *Drosophila* orthologue of DAF-16) specifically in adipose tissue increases lifespan. And, as in worms, *Drosophila* FOXO can act cell non-autonomously by regulating insulin gene expression. In mice, a striking inverse correlation between IGF-1 levels and lifespan exists among inbred strains, strongly implicating IGF-1 in lifespan regulation[35]. In addition, mutations that inhibit the insulin receptor (specifically in adipose tissue[34]), the IGF-1 receptor[34,36], upstream regulators[34] and downstream effectors[34,37] can all extend lifespan (although not, for some genes, in all studies). Likewise, small dogs, which have a mutation that decreases IGF-1 levels, live longer than large dogs. Note, however, that in flies and mice, the effects of the insulin/IGF-1 pathway on body size and longevity have been uncoupled. And, at least in worms and flies, the pathway acts in adults, after growth has occurred, to affect lifespan[19].

In some ways, it is surprising that any insulin/IGF-1 pathway mutants live long, as these pathways are essential in all animals. In fact, knocking out the gene encoding the insulin receptor in the liver causes diabetes in mice. Thus, the precise type of perturbation in the pathway can have important consequences. Long-lived animals carrying mutations in some insulin/IGF-1 pathway genes are insulin sensitive, whereas, unexpectedly, mutations in other genes cause insulin resistance (a characteristic of individuals with type 2 diabetes)[34,37]. Perhaps as long as a mutation reduces the insulin/IGF-1 signalling flux and DAF-16/FOXO-like proteins are activated, the animal will undergo its physiological shift towards cell maintenance and live long. Consistent with this interpretation, inhibiting either insulin production or insulin receptor signalling in worms and flies increases lifespan[19], although the first perturbation should bring about insulin sensitivity and the second, insulin resistance.

Can a perturbation of insulin/IGF-1 activity increase lifespan in humans? The answer seems to be yes (Fig. 1). Mutations known to impair IGF-1 receptor function are overrepresented in a cohort of Ashkenazi Jewish centenarians[38] and DNA variants in the insulin receptor gene are linked to longevity in a Japanese cohort[39]. Variants of AKT and FOXO3A have been linked to longevity in three[40] and seven cohorts, respectively. The FOXO3A cohorts are located throughout the world: they include Hawaiians of Japanese descent[41], Italians[42], Ashkenazi Jews[40], Californians[40], New Englanders[40], Germans[43] and Chinese[44]. In the German cohort, the FOXO3A variants were even more frequent in centenarians than in 90 year olds, strengthening the case that these variants extend lifespan. FOXO1 gene variants have also been linked to longevity in American and Chinese cohorts[44,45]. It is striking that FOXO variants are so consistently associated with longevity. Perhaps this is because FOXO proteins act in many pathways to affect lifespan[19] (Fig. 2).

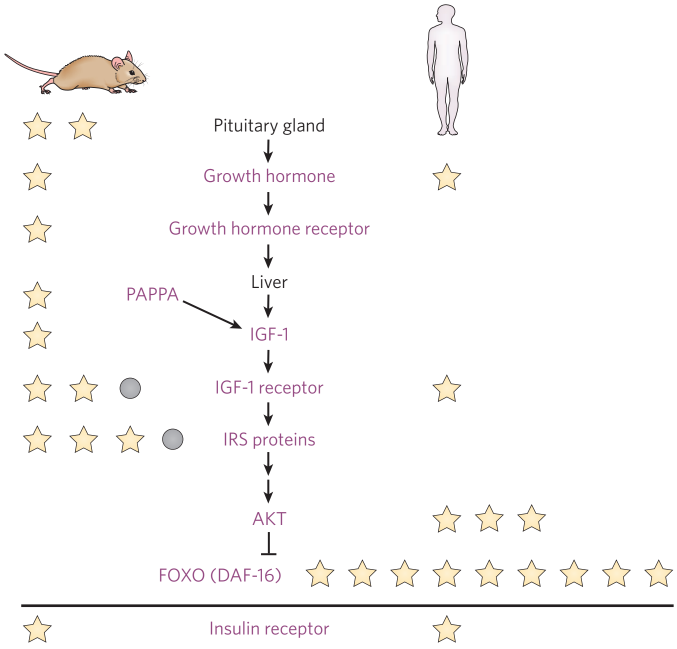

Figure 1 | Insulin/IGF-1 and FOXO signalling affects mouse and human lifespan. In mice and humans, plasma IGF-1 is produced by the liver in response to growth hormone secreted from the pituitary gland. In addition, PAPPA (pregnancy-associated plasma protein), a metalloproteinase that inactivates IGF-1 binding proteins, raises available IGF-1 levels. (Inactivation of PAPPA reduces IGF-1 signalling.) In response to IGF-1, the IGF-1 receptor activates a downstream signalling pathway containing several proteins that have been shown to affect lifespan. Shown in the figure are IRS (insulin-receptor substrate proteins 1 and 2) and AKT (a kinase that phosphorylates and inactivates FOXO transcription factors; originally called DAF-16 in *C. elegans*). These downstream signalling proteins are also components of insulin-response pathways (not shown). Unless noted otherwise, each star represents a long-lived mutant mouse strain (left) or a human cohort in which DNA variants are associated with exceptional longevity (right). Studies of the insulin receptor are indicated below the line. The star for mouse IGF-1 represents a study in which low IGF-1 levels were found to correlate with longevity among 31 inbred mouse lines, providing strong evidence that this hormone influences mouse longevity. One of the mouse IGF-1 receptor mutant strains lacked the receptor only in the nervous system, as did one of the IRS2 mutant strains. The long-lived insulin receptor mutant mice lacked the receptor only in fat tissue. Circles represent studies in which similar mutants were examined (sometimes in different genetic backgrounds) but lifespan extension was not observed. The human cohort in which IGF-1 receptor mutations were associated with exceptional longevity was noteworthy because one of the mutant receptors was analysed in cultured cells and was shown to have decreased function.

Because the insulin/IGF-1 pathway senses nutrients, and because this pathway also affects the development of *C. elegans* in response to nutrient limitation (discussed below), it is a good candidate for mediating the longevity response to dietary restriction[46] (Figs 3 and 4). In worms, flies and mice, it does indeed seem to fulfil this role, at least under certain conditions. To ask whether a life-extending or a progeric (rapid-ageing) mutation renders a dietary-restriction pathway constitutively active or inactive, one begins by determining whether the lifespan of the ageing mutant can be increased by dietary restriction. If not, then the gene is a candidate for a dietary-restriction gene. This interpretation is greatly strengthened if the gene's activity or expression is influenced by nutrients, and if other conditions can be shown to extend the mutant's lifespan,

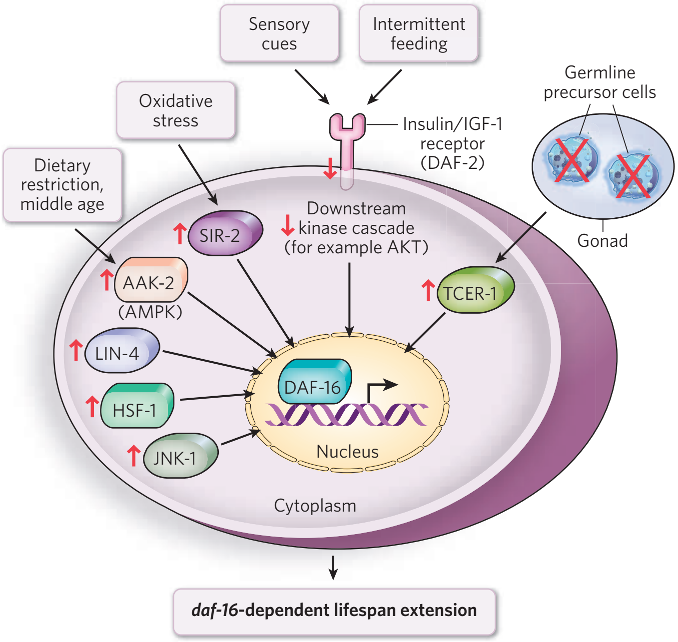

Figure 2 | The *Caenorhabditis elegans* transcription factor DAF-16/FOXO promotes longevity in response to many inputs. Arrows to the left of protein names depict increased or decreased gene expression. Overexpressing the sirtuin SIR-2, the heat-shock transcription factor HSF-1, the developmental-timing microRNA LIN-4, AAK-2 (a subunit of AMP kinase), Jun kinase 1 (JNK-1) or the predicted transcription elongation factor TCER-1 extends lifespan. Inhibiting the DAF-2 insulin/IGF-1 receptor, or components of its downstream kinase cascade also extends lifespan. In each case, the lifespan extension is DAF-16 dependent. Some of these proteins modulate DAF-16 activity directly, including AMP kinase (by phosphorylation), JNK-1 (by phosphorylation), SIR-2 (by deacetylation), AKT, a kinase in the insulin/IGF-1 pathway (by phosphorylation), and possibly TCER-1, which is required for specific DAF-16-dependent gene expression. Signals that activate these life-extending pathways include dietary restriction (starting in middle age, or via every-other-day feeding), oxidative stress, sensory cues and laser ablation of the germ-cell precursors within the developing gonad (indicated by the red crosses). Some of the proteins listed separately in the figure may act together in the same pathway. For example, HSF-1 is part of the DAF-2 pathway as it is required for *daf-2* mutants to live long. AAK-2/AMPK is partially required for *daf-2* mutants to live long, but TCER-1 and SIR-2 are not. Thus, these last two proteins do not act downstream of DAF-2. In many cases, the relevant genetic epistasis tests have not yet been performed.

indicating that the mutant is not just sick or intrinsically incapable of living longer. In worms, this type of analysis suggests that every-other-day feeding extends lifespan by inhibiting insulin/IGF-1 signalling[9]. In flies, long-lived insulin/IGF-1 pathway mutants respond to dietary restriction as though they were already somewhat diet restricted by their genotype[47,48], arguing that the two pathways overlap. Dietary restriction may also extend mouse lifespan by inhibiting insulin/IGF-1 signalling, as it does not extend the already long lifespans of mice with mutations in the gene for the growth hormone receptor[10,34]. (Growth hormone exerts its downstream effects by activating IGF-1 signalling.) Finally, dietary restriction may inhibit cancer by downregulating insulin/IGF-1 signalling, because it fails to inhibit cancers caused by mutations that constitutively activate the insulin/IGF-1 pathway[49].

In addition to eating less, smelling or tasting less (or differently) can also increase the lifespan of *C. elegans*[16], and this effect seems also to be due to decreased insulin/IGF-1 signalling. Sensory perception also influences lifespan in flies; in fact, dietary restriction does not extend lifespan as much if the flies are able to smell their food[50]. We do not know what happens in humans, except to say that if you smell the food you eat, your insulin levels rise even further. It is possible that a mechanism allowing changes in sensory cues to trigger a life-extending physiological shift towards cell protection evolved because it allowed the animal to sense and react quickly to deteriorating environmental conditions.

In mammals, insulin levels rise in response to glucose, and this rise might be predicted to shorten lifespan. In *C. elegans*, in fact, adding 2% glucose to the bacterial diet shortens lifespan by downregulating DAF-16/FOXO and HSF-1 activity[51]. Curiously, this effect involves the inhibition of aquaporin glycerol channels, which are also inhibited by glucose in mammals. In general, this finding might argue for a diet with a low glycaemic index. Unexpectedly, 2% glucose given to *C. elegans* insulin/IGF-1 receptor mutants almost completely suppressed their lifespan extension[51]. It is not clear why this is so, but if the same is true for mammals, then perhaps insulin/IGF-1 pathway mutant mice, whose lifespan extensions range from ~15% to 40%, would live longer on a diet with a lower glycaemic index. This leads to the counter-intuitive speculation that conditions that inhibit insulin receptor signalling in humans could actually promote longevity if the dietary glycaemic index were reduced.

How could longevity evolve? Insulin/IGF-1 biology provides a particularly interesting context in which to consider this question[19]. Because the force of natural selection tends to weaken with age, as fecundity declines and the chance of death from environmental hazard increases, evolutionary biologists have sought explanations that do not require selection for longevity itself. The finding that lifespan can be increased by pathways that shift physiology towards cell protection and maintenance provides an explanation that does not invoke selection for longevity per se, as these pathways could have evolved simply to allow animals to survive harsh, life-threatening environments. Once these protective pathways were in place, however, they would naturally have the potential to extend lifespan by counteracting internal metabolic wear and tear that accelerates ageing. Moreover, mutations that augmented the basal activity levels of these cell-protective pathways, through changes in regulators or downstream targets, might have increased lifespan during evolution.

Interestingly, *C. elegans* juveniles with sharply reduced insulin/IGF-1 signalling arrest growth during development and enter a long-lasting state of diapause called dauer. In nature, juvenile worms become dauers when insulin/IGF-1 signalling is reduced in response to harsh environmental conditions, resuming growth when conditions improve. Thus, in the worm, the expression of cell-protective lifespan-extending pathways induced by reduced insulin/IGF-1 signalling has been coupled to the expression of diapause traits that arrest growth, alter morphology and delay reproduction. In the context of dauer formation, further evolutionary refinement of this protective life-extending system could take place. Dauers with more effective survival mechanisms would pass on their protective alleles, as dauer formation takes place before sexual maturity. Subsequently, if this enhanced life-extension system were to be expressed independently of other dauer traits during adulthood, one might see large lifespan increases in normal-looking, fully fertile animals, which is indeed what we see in weak insulin/IGF-1 mutants[19]. A similar selective advantage could be conferred by any life-extending perturbation that enhances cell protection and delays reproduction, such as the longevity response to dietary restriction.

TOR signalling

The TOR kinase is a major amino-acid and nutrient sensor that stimulates growth and blocks salvage pathways such as autophagy when food is plentiful. Inhibiting the TOR pathway increases lifespan in many species, from yeast to mice[4,5,52–54] (Figs 3 and 4). TOR inhibition increases resistance to environmental stress[3], consistent with a physiological shift towards tissue maintenance. However, at least in *C. elegans*, TOR inhibition seems to activate a pathway that is distinct from the insulin/IGF-1 pathway, as it extends lifespan independently of DAF-16/FOXO[3,52,53].

Like inhibition of the insulin/IGF-1 pathway, TOR inhibition requires transcriptional changes in order to extend lifespan, at least in worms (where the PHA-4/FOXA transcription factor is required[55]) and in yeast[56]. TOR inhibition also has fascinating effects on translation that implicate respiration in the longevity response to dietary restriction[57]. In response to nutrients, TOR upregulates translation, in part by activating the ribosomal subunit S6 kinase and inhibiting 4E BP, a translation inhibitor. When nutrient levels and TOR activity fall, translation levels fall as well. This has an impact on lifespan, as inhibition of S6 kinase extends lifespan in yeast, worms, flies and mice[3–5,22,58], and inhibiting translation in many other ways has been shown to extend lifespan in yeast, worms and flies[3,5,22–24,59]. Surprisingly, in flies, messenger RNAs encoding components of the electron transport chain are relatively immune to this translational shutdown, and respiration increases in response to TOR inhibition. Preventing this increase in respiration prevents lifespan extension, and activating this translational shift artificially, by overexpressing 4E BP, increases lifespan[57]. Long-lived S6 kinase knockout mice have increased whole-body oxygen-consumption rates[60], suggesting that this mechanism for lifespan extension might be conserved.

TOR inhibition also stimulates autophagy, which, as in insulin/IGF-1 mutants, is required for lifespan extension (at least in worms and flies)[31,61,62]. The stimulation of autophagy by TOR inhibition may be indirect, effected by changes in gene regulation, as in worms it has been shown to require the PHA-4/FOXA transcription factor[31].

Of all the nutrient-sensing pathways, the TOR pathway has been most consistently linked to dietary restriction (Fig. 3). TOR inhibition mimics the physiological effects of dietary restriction, and in yeast, worms and flies, the lifespan extension produced by TOR inhibition is not further increased by dietary restriction[3–5,62]. In worms, as in flies, chronic dietary restriction extends lifespan, at least in part, by stimulating respiration[33,57]. The underlying mechanism does not rely on translational control alone, as it requires the transcription factor SKN-1. Long-lived S6 kinase mouse mutants exhibit gene expression patterns similar to those triggered by dietary restriction[58], suggesting that the TOR/S6 kinase pathway influences the response to dietary restriction in mammals as well.

Reducing methionine intake in mammals, or protein intake in flies, extends lifespan. Perhaps different kinds of diet trigger different cell-protective and longevity responses. The response to low insulin/IGF-1 signalling could be triggered by a low-glycaemic-index diet, and the response to low TOR could be triggered by amino-acid limitation. Hopefully, one day we will know what kind of diet will best keep us youthful and healthy.

AMP kinase

AMP kinase is a nutrient and energy sensor that activates catabolic pathways and represses anabolic pathways when the cell's AMP/ATP ratio rises. Overexpressing AMP kinase extends lifespan in *C. elegans*[14], and the anti-diabetic drug metformin, which activates AMP kinase, can extend lifespan in mice[63]. AMP kinase is also required for insulin/IGF-1 mutations to be able to extend worm lifespan[14], but exactly how it fits into this pathway is not known.

AMP kinase can also extend lifespan in response to dietary restriction (Figs 2 and 4). In *C. elegans,* the lifespan extension triggered when food limitation is initiated in middle age requires AMP kinase, which seems to act directly on DAF-16/FOXO to phosphorylate and activate it[6]. This AMP kinase pathway is not necessary for extension of lifespan by a continuous low availability of food[14,64] and, conversely, genes required for continuous food limitation to extend lifespan (*pha-4*/*FOXA*[65] and *skn-1*/*NRF*[33]) are not required for dietary restriction initiated in middle age to extend lifespan[11]. Thus, not only how much you eat, but when you eat, may influence which lifespan-extending pathways are activated (Fig. 4).

Sirtuins

Sirtuins are NAD$^+$-dependent protein deacetylases whose overexpression has been reported to extend lifespan in yeast, worms and flies[19]. How sirtuins influence lifespan is not yet clear[66]. In yeast, the sirtuin Sir2 has been proposed to extend lifespan by inhibiting the formation of toxic extrachromosomal ribosomal DNA circles, but it may have other functions that contribute to longevity. Interestingly, Sir2 promotes the segregation of damaged proteins (such as those that have been carbonylated) to mother cells during cell division[67]. But, although Sir2 overexpression extends lifespan, it does not enhance this segregation. A recent study

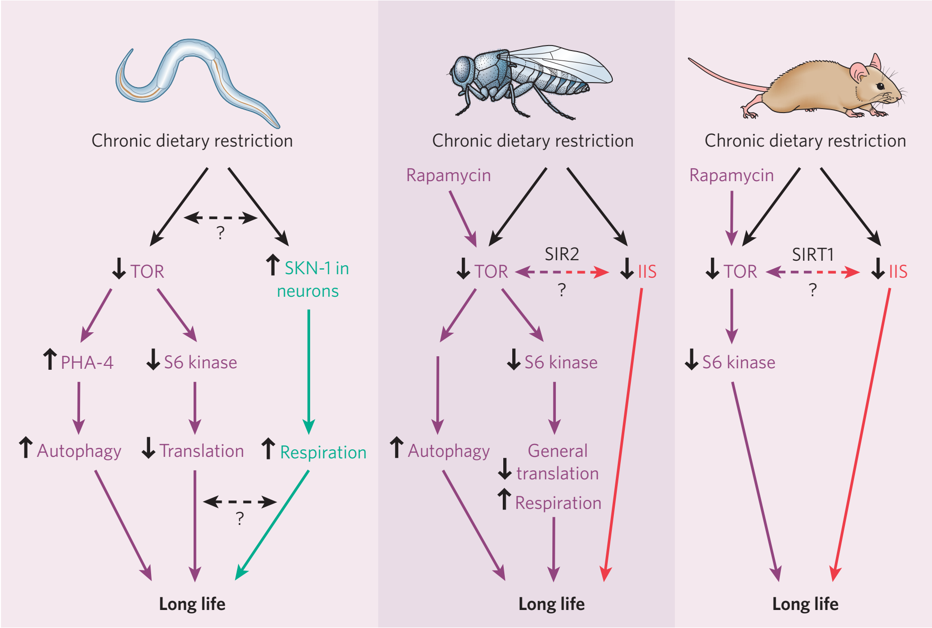

Figure 3 | Pathways that influence lifespan extension in response to chronic dietary restriction. In worms, flies and mice (as well as yeast; not shown), genetic and/or phenotypic analysis suggests that chronic dietary restriction increases lifespan by downregulating TOR activity. The TOR pathway (purple) is known to regulate autophagy and translation. The small arrows indicate upregulation or downregulation. In worms and flies, lifespan extension by means of TOR inhibition has been shown to require autophagy, and in all three species, inhibiting translation by inactivating the TOR target ribosomal S6 kinase increases lifespan. PHA-4 is required for autophagy in *Caenorhabditis elegans* TOR mutants. PHA-4 also affects expression of stress-response genes in response to dietary restriction; its effect on translation has not been examined. In flies, components of the respiratory electron transport chain escape translational inhibition when TOR activity is reduced, resulting in increased respiration. Chronic dietary restriction also increases respiration in worms, in response to activity of the SKN-1 transcription factor in neurons (green). In worms and flies, this increase in respiration has been shown to contribute to lifespan extension. Whether TOR affects respiration in worms, or SKN-1 affects respiration in flies, is not known. In flies and mice, chronic dietary restriction may increase lifespan, at least in part, by downregulating insulin/IGF-1 signalling (IIS, red). Sirtuins are required for chronic dietary restriction to extend lifespan in flies and mice, but whether they act in the insulin/IGF-1 and/or TOR pathways is not known.

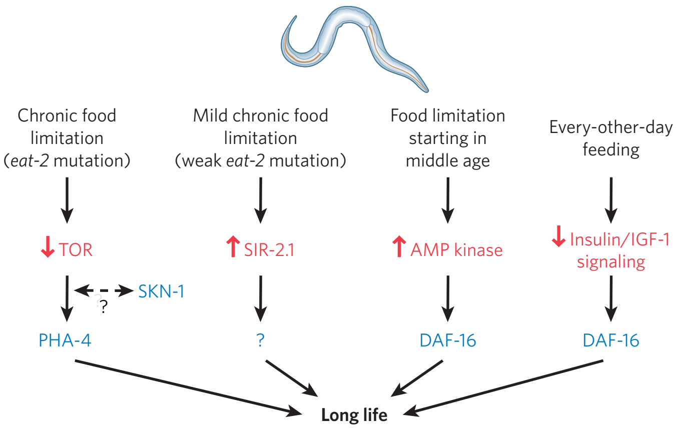

Figure 4 | Different *Caenorhabditis elegans* nutrient sensors (red) and transcription factors (blue) extend lifespan in response to different modes of dietary restriction. *eat-2* mutations, which inhibit feeding throughout life, are likely to increase lifespan by means of TOR inhibition, as TOR inhibition does not further increase the *eat-2* mutant lifespan. The PHA-4 transcription factor is required for TOR inhibition, and the *eat-2* mutation is required for extending lifespan. The SKN-1 transcription factor extends lifespan in response to chronic dietary restriction, but its relationship to TOR has not been examined. Curiously, a functional SIR-2.1 gene seems to be required for weak but not strong *eat-2* mutations to extend lifespan. When dietary restriction is initiated in middle age, functional genes for AMP kinase and DAF-16 are required for lifespan extension. Neither of these genes (nor *sir-2.1*) is required for strong *eat-2* mutations to extend lifespan. Every-other-day feeding is likely to extend lifespan by inhibiting insulin/IGF-1 signalling, as it does not further extend the long lifespan of *daf-2* mutants and it is largely dependent on *daf-16*. This lifespan extension also requires the small GTPase RHEB-1 (not shown), but it only partially requires TOR, a known RHEB target.

argues that Sir2 extends lifespan by maintaining gene silencing at telomeres during ageing[68]. This pathway could potentially affect metazoans, which do not have rDNA circles.

In *C. elegans,* overexpressing the sirtuin gene *sir-2.1* extends lifespan in what seems to be a straightforward way, by activating DAF-16/FOXO[19,69] (Fig. 2). SIR-2.1 is likely to activate DAF-16 directly, by deacetylation, as mammalian SIRT1 is known to deacetylate FOXO proteins in response to oxidative stress[19], which, in turn, shifts their target specificity towards genes involved in stress resistance. Oxidative stress may have similar effects in worms, as it stimulates the binding of SIR-2.1 to DAF-16 (ref. 69) and can extend lifespan in a *sir-2*-dependent and *daf-16*-dependent manner[15]. The finding that sirtuins can deacetylate FOXO proteins directly, as well as the fact that insulin/IGF-1 pathway mutants do not require *sir-2.1* for longevity, suggests that sirtuins may influence DAF-16/FOXO and lifespan independently of insulin/IGF-1 signalling in worms (Fig. 2). The situation with *C. elegans* SIR-2.1 is analogous to that of another stress regulator, Jun kinase 1 (JNK-1), which can extend lifespan in both worms and flies[19]. In both species, lifespan extension requires DAF-16/FOXO, which is phosphorylated directly by JNK-1. However, at least in flies, JNK-1 also influences insulin/IGF-1 signalling indirectly, though the action of lipocalin signals[70].

Long-lived flies that overexpress *Drosophila* SIR2 also overexpress a chaperone that prevents SIR2 from triggering cell death[71], so further study is required to confirm that SIR2 can extend fly lifespan. Whether FOXO is required for the longevity of these flies is not known, but another histone deacetylase, RPD3, and the tumour suppressor protein p53 seem to be involved[19].

Sirtuin overexpression has not been shown to extend lifespan in mammals. In one careful study, the plant polyphenolic compound resveratrol, which affects many processes in a sirtuin-dependent manner and has been reported to activate sirtuins, extended the lifespan of mice fed a high-fat diet but not that of mice fed a normal diet[72]. This finding does not necessarily mean that sirtuins cannot extend mammalian lifespan, as the relationship between resveratrol and sirtuins is unclear, and even in yeast, worms and flies, resveratrol does not always extend lifespan[66,73]. In particular, resveratrol may not actually activate sirtuins, at least not in a simple way (see page 480). Resveratrol stimulates mammalian SIRT1 to deacetylate a fluorescent substrate but not the native SIRT1 substrates that have been tested[66,73]. In addition, in *C. elegans*, SIR-2.1 extends lifespan through DAF-16/FOXO, but resveratrol does not[74]. Worse (for the model), resveratrol extends worm lifespan by upregulating a gene that is downregulated by SIR-2.1 overexpression[74]. Nevertheless, in some studies at least, resveratrol has been shown to require the activity of *sir-2.1* to extend worm lifespan. Likewise, many of the effects of resveratrol in mammalian disease settings require functional sirtuins, so there seems likely to be an interesting explanation.

As NAD$^+$ and NADH are important metabolic regulators, sirtuins are good candidates for proteins that respond to dietary restriction (Figs 3 and 4). In wild-type yeast, Sir2 is required for dietary restriction to extend lifespan[19,66]; in its absence, rDNA circles accumulate and kill the cells prematurely. However, whatever the effects of dietary restriction on NAD$^+$ and NADH levels may be, it is now clear that dietary restriction does not increase sirtuin activity in yeast[66,75,76]. Puzzles abound. For example, a recent study linked dietary restriction, TOR, nicotinamide (a sirtuin inhibitor) and sirtuins into a single pathway for lifespan extension[56]. It is not clear how to reconcile this study with those showing that Sir2 is not activated by dietary restriction and that dietary restriction can extend lifespan, apparently by means of TOR inhibition, in cells lacking sirtuins[4].

In worms, with the exception of mild chronic dietary restriction[77], all known modes of dietary restriction increase lifespan robustly in *sir2.1* deletion mutants[3,9,11] (Fig. 4). In flies, only one mode of dietary restriction has been tested, and here sirtuins were required for lifespan extension[7,19]. Likewise in mice, chronic dietary restriction cannot increase lifespan in the absence of SIRT1 (ref. 78). Consistent with sirtuins being nutrient sensors, they regulate a wide variety of metabolic and stress pathways, some in unexpected ways, in response to dietary restriction in mice.

Inhibition of respiration

A modest inhibition of respiration extends lifespan in a wide variety of species, including yeast, worms, flies and mice[19–21,79,80]. This seems contradictory to the finding that respiration promotes longevity in response to dietary restriction, but perhaps increasing respiration extends lifespan for one reason and inhibits it for another. Consistent with this idea, dietary restriction increases environmental stress resistance[3], but inhibition of respiration does not[19,81]. In any case, this finding may explain a classic phenomenon in nature: the general correlation in mammals between metabolic rate (which decreases with size) and lifespan (which tends to increase with size). Perhaps larger mammals live longer partly because their metabolic rates are lower. There are some exceptions to the rule, but they need not invalidate this hypothesis, as they can be explained by the actions of other longevity pathways. For example, small dogs live longer than large dogs because they have low IGF-1 levels.

In yeast, worms and mammalian cells, inhibiting respiration activates a conserved nuclear gene expression response called the 'retrograde response'. The retrograde response upregulates genes that activate alternative energy-generating pathways, as well as cell-protection pathways. In yeast and worms, mutations that inhibit either the entire retrograde response or individual retrograde-response genes can inhibit this lifespan extension[82,83]. Thus, inhibiting respiration, like the perturbations described above, also triggers a regulated longevity response. In worms (although curiously not in flies), respiration must be inhibited during development in order to increase lifespan[19,81], implying that a molecular memory of the event is set up. A similar situation might occur in mice[80]. In addition, at least in flies, inhibiting respiration in neurons alone extends lifespan[21], again suggesting a cell non-autonomous, regulated response.

Signals from the reproductive system

Physiologically speaking, animals must 'make choices' about how to deploy their resources. Does this mean that there must always be a trade-off between reproduction and longevity? Supporting this idea is the fact that in some species, individuals die soon after mating or have shorter lifespans as a consequence of mating. Likewise, strong insulin/IGF-1 pathway mutations delay and reduce reproduction[19]. However, recently, many examples of lifespan extension with little or no inhibition of reproduction have come to light[19]. Weak insulin/IGF-1 pathway mutations extend lifespan but have little effect on reproduction. (In fact, reproduction and lifespan are specified at different life stages by this pathway.) Moreover, some long-lived mutants have more progeny than normal. And in nature, guppies living with intensive predation have, when the predators are removed, both longer lifespans and more progeny than do guppies that naturally live without predators. Thus, longevity need not come at a cost to reproduction.

In *C. elegans,* removing the entire reproductive system does not extend lifespan, arguing against a simple reproductive trade-off. Nevertheless, signals from the reproductive system do influence ageing[18,19,84] (Fig. 5). When the animal's germ cells (but not its somatic reproductive tissues) are removed, its lifespan is extended by approximately 60%. Thus, an 'empty gonad' extends lifespan. Perhaps this phenomenon allows the animal to coordinate its rate of ageing with the timing of reproduction: if the germ line is not 'ready', the animal will 'wait' to age. This pathway, too, may have an ancient origin, as forcing germline stem cells to exit mitosis and enter meiosis during adulthood extends lifespan in both flies and worms[18,19,85]. Furthermore, FOXO activity, which is known to be required for the lifespan increase in worms, increases in both cases.

In *C. elegans*, germline loss activates DAF-16/FOXO by a novel mechanism[18]. An adaptor protein called KRI-1, as well as a transcription elongation/splicing factor homologue called TCER-1, is needed for loss of the germ line (but not inhibition of insulin/IGF-1 signalling) to trigger normal DAF-16 target gene expression and extend lifespan. The reproductive pathway may act additively with the *daf-2* pathway, as loss of the germ line greatly extends the already long lifespan of *daf-2* mutants. Thus, knowing more about this reproductive pathway may reveal powerful new ways of activating DAF-16/FOXO. Loss of the germ line influences the rest of the body through steroid signalling and through as-yet-unidentified signals that activate *tcer-1* gene expression. Although this longevity pathway is normally coupled to the germ line, it can be dissociated from it. For example, overexpression of TCER-1 can extend the lifespan of intact, fully fertile animals.

Loss of the germ line in *C. elegans* only extends lifespan in the presence of functional DAF-16-regulated lipase genes[18,28]. The role of fat metabolism in the germline pathway is not known, but one possibility seems attractive: in this pathway, DAF-16 acts primarily in the intestine (which is also the worm's adipose tissue) to extend the lifespan of the animal[18]. A life-extending lipase is upregulated by DAF-16 in the intestine in response to germline removal. Perhaps this lipase is part of a signalling pathway that allows DAF-16 activity in the intestinal/adipose tissue to influence the lifespan of other tissues (Fig. 5).

It is not clear whether a similar system exists in mammals, although the mammalian reproductive system can affect lifespan. If ovaries from young mice are transplanted into old recipients, the lifespans of the recipients are extended[18,19]. Human females lose their germ cells naturally as they age. Interestingly, if the somatic ovarian tissue is removed as well, mortality rates from many different causes rise[86]. It is too soon to know whether this phenomenon has more than a superficial similarity to the *C. elegans* situation, but it makes one wonder.

Telomeres

Because telomeres shorten with age, they have been seen as candidates for ageing determinants. They do act as such, but in a distinctive way. When mice are engineered to have longer telomeres, they live longer[87]. However, to live long, these mice must also be genetically modified to resist cancer. This requirement sets telomere-mediated lifespan extension apart from the other modes of lifespan extension discussed earlier. Dietary restriction, perturbations in nutrient sensors and reduced respiration all inhibit tumour formation[19,88]. (Mutations that increased lifespan during evolution probably also delayed cancer, as cancer incidence correlates tightly with physiological ageing in different species.) Together, these findings suggest that telomere lengthening does not increase lifespan by shifting the animal into a protective physiological state. Instead, it may do so for a different reason, for example by preventing stem-cell loss[87]. It would be interesting to learn whether very long lifespans (without cancer?) could be produced if telomere lengthening were combined with dietary restriction or mutations in nutrient sensors.

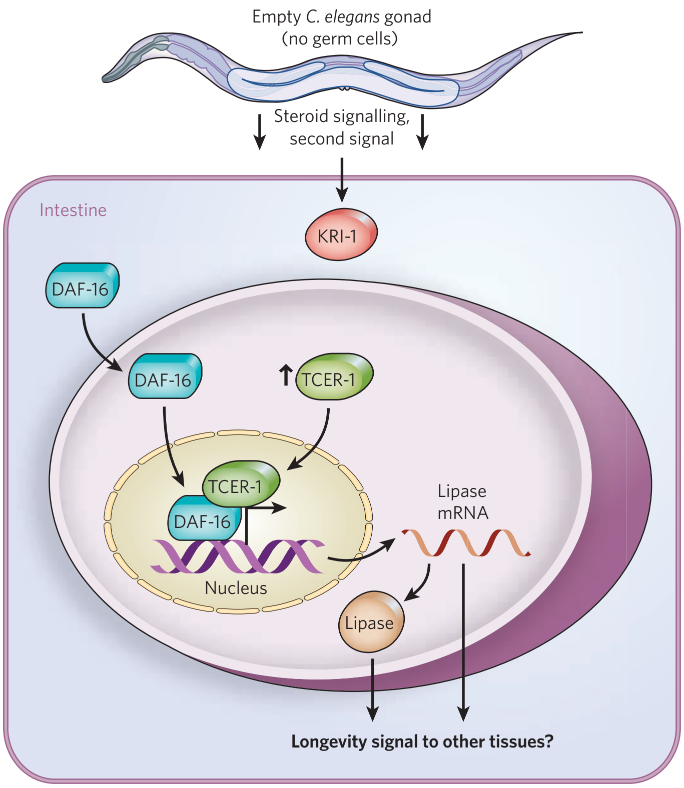

Figure 5 | Model for the regulation of lifespan by signals from the reproductive system. When the germ cells of *Caenorhabditis elegans* are absent but the somatic reproductive tissues are present, lifespan is extended. This lifespan extension requires the DAF-12 nuclear hormone receptor, genes that synthesize DAF-12 ligands, DAF-16/FOXO, KRI-1 (a conserved ankyrin-repeat protein) and TCER-1 (a putative transcription elongation factor). Loss of the germ line stimulates nuclear accumulation of DAF-16 and the upregulation of TCER-1 expression in intestinal cells (which is also the animal's adipose tissue). Both these events require the intestinal protein KRI-1. DAF-16 localization (but not TCER-1 upregulation) partially requires DAF-12-dependent steroid signalling, arguing that DAF-12 steroid signalling plus other, unidentified, factors convey information about the animal's reproductive status to the intestine. TCER-1 is required for specific DAF-16-dependent gene expression in these animals, suggesting that it may elongate mRNAs whose expression is initiated by DAF-16. Interestingly, neither KRI-1 nor TCER-1 (nor DAF-12) is required for the longevity of *daf-2*/insulin/IGF-1 pathway mutants. One of DAF-16's transcriptional targets encodes a lipase that is required for lifespan extension. As DAF-16 function in the intestine is sufficient for the entire animal to live long, it is possible that this lipase generates a downstream signal that triggers life-extending pathways in other tissues.

Mysteries

Some aspects of ageing remain particularly enigmatic. One of these is the all-important question of what actually causes ageing.

Causes of ageing

With age, protein homeostasis declines and damage accumulates. Thus, it seems self-evident that ageing is caused by macromolecular damage, and a show of hands at a recent ageing meeting confirmed that most people working in the field believe this. But we do not know for sure. And if this idea is correct, what is the damage that causes ageing? A prime candidate has been damage caused by reactive oxygen species (ROS), which are generated primarily during respiration. Many long-lived mutants are resistant to oxidative stress, and species of mammals that live longer tend to have cells that, when tested in culture, are more stress resistant[19]. However, given how ancient the respiratory chain is, one would imagine that mechanisms for controlling ROS very precisely would have evolved. As noted earlier, yeast cells segregate carbonylated proteins, which are produced by ROS, to the mother cell when they divide[67], and the level of protein carbonylation falls when embryonic stem cells differentiate[89]. Likewise, defective mitochondrial DNA is eliminated by a poorly understood quality-control mechanism in the germ line during reproduction[90,91].

Ideas about the role of ROS in ageing are in flux, and might be undergoing a paradigm shift[92]. First, a little ROS might be good for you (although high levels could still be harmful). For example, in worms, low levels of juglone, which generates ROS, extend lifespan[15]. 2-Deoxyglucose, which inhibits glycolysis, activates AMP kinase in worms and, as one might expect, extends lifespan. 2-Deoxyglucose also increases ROS and the expression of antioxidant genes, but if worms are given antioxidants along with 2-deoxyglucose, they do not live long[93]. Thus, ROS generated in response to 2-deoxyglucose have a key role in lifespan extension. In humans, exercise activates AMP kinase, which stimulates blood glucose uptake. Antioxidants prevent this stimulation of glucose uptake, suggesting that ROS may have a role here too. Overexpression of the antioxidant superoxide dismutase (SOD) extends lifespan in flies, but it also triggers expression of many FOXO target genes, suggesting that SOD targets a signalling pathway. Consistent with this idea, SOD expression exclusively in neurons is sufficient to extend life in *Drosophila*, suggesting cell non-autonomous signalling. Disabling of SOD genes can increase oxidative damage without shortening lifespan[19,92] and can even increase lifespan. Conversely, antioxidants can lower the level of oxidative damage without increasing lifespan. Although there is a correlation between stress resistance and longevity in many different mutants, these two phenomena can sometimes be uncoupled[19,92]. None of these findings rules out the idea that molecular damage causes ageing. In fact, that hypothesis cannot be disproved, as there could always be another form of damage that is the real culprit. At the very least, it would be nice to find a form of damage whose increase or decrease in mutants correlates consistently with lifespan, at least within a single species.

Time points

Time passes, and suddenly we need reading-glasses; then comes the menopause (for women) and fat redistribution. Why do these events occur when they do? One possibility is that over time the level of a rate-of-ageing regulator falls, crossing thresholds that trigger various aspects of ageing. In *C. elegans*, genes whose expression changes with age have been identified, and many of these genes contain a consensus GATA-factor binding site[94] that also binds DAF-16/FOXO[29]. DAF-16, in turn, is regulated during adulthood by a microRNA (lin-4), which also acts earlier to control developmental timing[95]. Could this microRNA (or something else) change DAF-16 or GATA factor levels over time, influencing the course of ageing? In yeast[68] and mice[96], sirtuin activity and sirtuin-dependent chromatin modifications decline with age, and in yeast at least, this change shortens lifespan. In mammals, circulating levels of Wnt signal proteins increase with age, and this increase triggers muscle stem-cell ageing[97].

What other age-related events are triggered by changes in the levels of regulatory molecules? And what, in turn, changes the regulators? Their levels could change simply because of a decline in protein homeostasis caused by molecular damage. Alternatively, their levels could potentially be subject to the action of a temporal regulatory cascade, somewhat analogous to the *per* system for circadian rhythms. The *per* system is in fact a candidate for such a regulatory mechanism, as mice mutant for transcription factors involved in circadian rhythms age prematurely[98].

Stopping the clock

There exists a seventeen-year-old girl who is still an infant[99]. What arrested her growth and development? Will she ever grow old? She could teach us something completely new.

Chance

In addition to genes and environment, another factor may affect ageing: chance[26]. Isogenic worms or mice do not all have the same lifespans, even in the same environment; this is why populations show lifespan curves rather than sharp corners. It is possible that a stochastic event — a metabolic insult or noise in the expression of a regulatory gene — flips an epigenetic switch or sets in motion a chain of events that promotes ageing. One way to investigate stochastic factors affecting lifespan would be to identify a marker that predicts, early in life, the subsequent rate of ageing and then sort animals according to this marker, learn how they differ, and make and test hypotheses. This might be possible: the rate of decline in movement during early ageing predicts a worm's lifespan[100], as does the AMP/ATP ratio, which rises with age[14]. Heat shock extends a worm's lifespan, and individuals with the strongest transcriptional response to heat shock live the longest[101]. Because chance could potentially have a large effect on lifespan, understanding how it may act is important.

Huge lifespan increases

Inhibiting respiration can further double the long lifespan of *C. elegans daf-2* mutants, and activating the reproductive longevity pathway in *daf-2* mutants extends lifespan by sixfold[19]. Completely knocking out the insulin/IGF-1 pathway's PI(3)K causes worms to enter, and then exit, the dauer stage and then go on to become adults that live ten times longer than normal[102]. In mice, lifespan extensions tend to be more modest, but combinations of mutations and dietary restriction can nearly double lifespan[34]. How are these very long lifespans produced? Do different, additive, lifespan pathways converge on the same longevity effectors? If so, the same mechanism that produces modest lifespan increases when a single pathway is altered will simply be augmented in these very long-lived animals. Alternatively, if different pathways extend lifespan by different mechanisms, then one expects their combination to produce very large increases due to cumulative effects. Perhaps very long lifespan increases can be produced in many different ways.

Evolution has generated great diversity in lifespan. For example, rats live 3 years; squirrels 25. In fact, longevity may be an 'easily evolvable' feature, as there are short-lived and long-lived insects, birds and mammals. Could lifespan have been rapidly extended by mutations in regulatory genes? We know that mutations in the IGF-1 gene extended the lifespans of small dogs, and we can plausibly hypothesize that mutations affecting respiration contributed to the long lifespans of large mammals. Further study may link more laboratory mutations to the evolutionary causes of longevity, and reveal whether evolution has also increased lifespan by means that have escaped our detection so far.

Prospects for interventions in humans

Until recently, the idea of lifespan extension was the stuff of fairy tales, not science. Why not? It may be that we had no role models to counter a natural conservatism: no 300-year-old creatures flying to the Moon, inventing Twitter and enjoying opera. Also, the idea that life extension must come with a trade-off is pervasive, though this objection begs the question of how we talented humans acquired our long lifespans in the first place.

Now we know that longevity can occur without debilitating trade-offs, and we have useful role models: healthy, long-lived worms, flies and mice. We have long-lived human FOXO variants. In fact, we may have drugs themselves. Rapamycin, an inhibitor of TOR, can extend the lifespan of mice even when administered late in life[54].

Because the US Federal Drug Administration does not recognize ageing as an indication (a condition to be treated), at least for now, drugs affecting ageing will be approved in the United States only if they affect disease. As many age-related diseases are delayed in long-lived mutants, drugs that slow ageing should gain approval this way. Rapamycin is already approved for human use, but for immune suppression, something that may preclude its effectiveness for lifespan extension. Another approved drug is predicted to disarm an aberrant nuclear lamin that causes Hutchinson–Gilford progeria[103]. Interestingly, the abundance of the aberrant lamin increases over the normal course of ageing, suggesting that this drug might keep us all younger. Drugs that target cholesterol-ester transport protein, whose inhibition is genetically linked to exceptional longevity[104], are in clinical trials for vascular risk reduction. Conversely, drugs now being developed to target age-related diseases, such as IGF-1 pathway inhibitors for cancer, may extend youthfulness and lifespan. In general, we do not know how potent these drugs might be, and because many stress and nutrient sensors are essential, they could have serious side effects. Still, given all we have learned, it seems reasonable to think that mutations in our future evolution could give us longer lifespans and that if they do, then drugs that mimic their effects should too. ■

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