Genetic links between diet and lifespan: shared mechanisms from yeast to humans

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Abstract | Caloric restriction is the only known non-genetic intervention that robustly extends lifespan in mammals. This regimen also attenuates the incidence and progression of many age-dependent pathologies. Understanding the genetic mechanisms that underlie dietary-restriction-induced longevity would therefore have profound implications for future medical treatments aimed at tackling conditions that are associated with the ageing process. Until recently, however, almost nothing was known about these mechanisms in metazoans. Recent advances in our understanding of the genetic bases of energy sensing and lifespan control in yeast, invertebrates and mammals have begun to solve this puzzle. Evidence is mounting that the brain has a crucial role in sensing dietary restriction and promoting longevity in metazoans.

Feeding *ad libidum*
A feeding protocol in which an organism is allowed to eat as much as desired, given an unlimited food supply.

Hypothalamus
A region of the brain that regulates organismal homeostasis, in particular energy balance, by regulating behaviour and metabolism by endocrine and autonomic signalling.

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Dietary restriction (DR), the limitation of food intake below the *ad libidum* level without malnutrition, can extend the mean and maximum lifespan in every organism in which it has been tested, including yeast[1], worms[2], flies[3] and rodents[4]. Studies are currently underway to test the effect of DR on lifespan in primates, with promising preliminary results[5]. Perhaps even more significantly, DR has also been shown in animal models to slow the progression of, or even prevent entirely, a range of age-dependent pathologies, including cardiovascular disease[6], multiple types of cancer[7], several neurodegenerative disorders[8,9] and diabetes[10]. Short-term DR also reduces the risk of coronary disease and stroke in humans[6]. Clearly, identification of the genetic mechanisms that underlie the protective effects of DR would have profound implications for the development of new medical interventions for diseases of ageing. DR induces alterations in the physiology of many organ systems in rodents, which have been characterized extensively over the past several decades[11]. However, an understanding of which of these myriad changes are causally relevant to the increased longevity and improved health of DR animals has remained elusive.

By contrast, studies of model organisms during the past 15 years, principally the yeast *Saccharomyces cerevisiae*, the worm *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster*, have revealed several genetic mechanisms of lifespan control, many of which are conserved across taxa, including mammals (FIG. 1). One strategy to determine whether a given candidate gene functions in the same pathway as DR to regulate lifespan is to subject a mutant in the gene to DR and assess the normality of the response[12]. This approach has identified several genes that mediate DR longevity in yeast (see below). Until recently, however, little was known about the genetic mechanisms of DR longevity in metazoans. Remarkably, as described in this Review, recent findings suggest a neural basis of DR longevity in metazoans.

Here we provide an overview of genetic insights into the mechanisms of DR longevity in yeast, invertebrates and mammals. We begin by outlining energy-sensitive genetic pathways that mediate DR longevity in the organism in which it is best understood, yeast. We then review the roles of similar nutrient-sensitive pathways in invertebrate lifespan control, and describe recent direct evidence for central neuronal control of DR longevity in worms and flies. We next highlight similarities between DR longevity control pathways in lower organisms and genetic mechanisms of energy sensing in the mammalian hypothalamus. Finally, we speculate that many of the same mechanisms that control DR longevity in lower organisms also mediate this phenomenon in mammals by action in the hypothalamus.

Dietary restriction in yeast

The first genes that were shown to be involved in a DR-induced increase in lifespan were identified in the yeast *S. cerevisiae*[13]. In this organism, two different measures of lifespan exist: replicative and chronological. Replicative lifespan is defined as the number of daughter cells a mother cell can produce before senescing, whereas chronological lifespan refers to the duration of viability of stationary-phase cells. Both replicative and chronological lifespan can be modulated by many of the same genes that control metazoan lifespan[14,15]. It has been suggested that replicative lifespan is a better model of ageing, mitotically active animal cells, and that chronological lifespan is a better model of ageing postmitotic animal cells. However, most studies of yeast DR have been done in the context of replicative lifespan, so we refer chiefly to results that relate to this measure of lifespan.

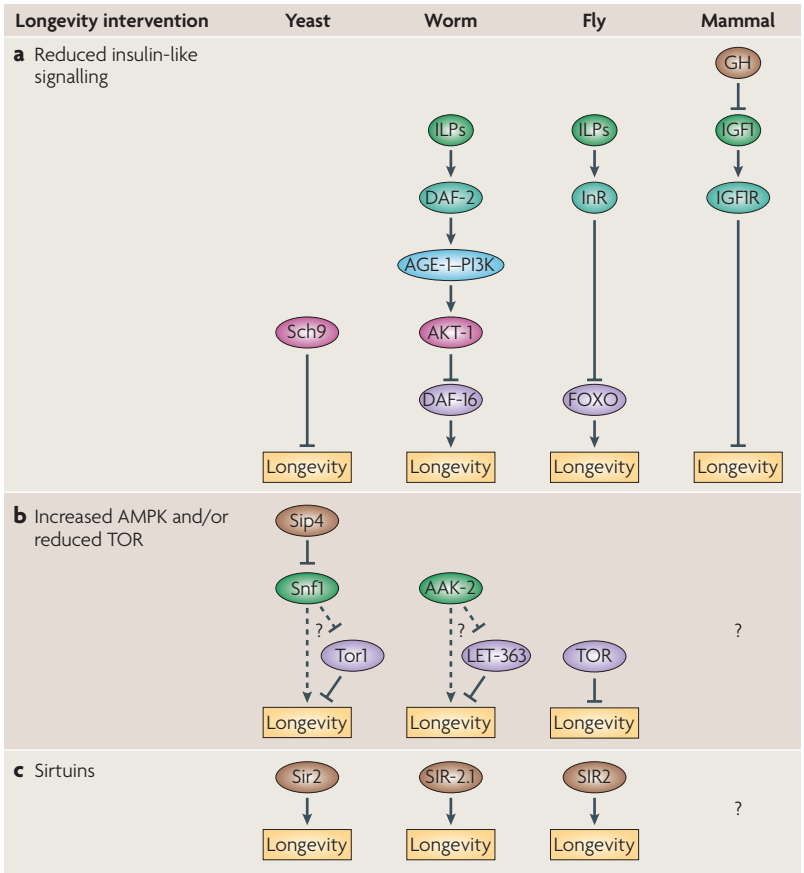

Figure 1 | **Conserved lifespan control pathways. a** | Insulin-like signalling accelerates ageing. *Saccharomyces cerevisiae* lacks the insulin receptor, but does possess kinases that function downstream of this receptor in other organisms, such as the AKT homologue Sch9. **b** | Target of rapamycin (TOR) signalling accelerates ageing. AMP-dependent protein kinase (AMPK) activation slows ageing, and may do so in part by inhibiting TOR signalling. AAK-2 in the worm is a homologue of the mammalian AMPK catalytic α-subunit. **c** | Sirtuins can extend lifespan in yeast, worms and flies. AGE-1, ageing alteration 1; DAF, abnormal dauer formation; FOXO, forkhead box, sub-group O; GH, growth hormone; IGF1, insulin-like growth factor 1; IGF1R, insulin-like growth factor 1 receptor; ILPs, insulin-like peptides; InR, insulin receptor; PI3K, phosphatidylinositol-3 kinase; Sip4, a C6 zinc-cluster transcriptional activator; Snf1, an AMP-activated serine–threonine protein kinase.

A report published in 2000 showed that reducing glucose concentration in the medium from 2% to 0.5% (moderate DR) could extend replicative lifespan, establishing a model of DR in yeast[13], and several genes that mediate DR longevity were identified using this model. More recently, however, it was found that more extreme glucose limitation, to 0.05% (severe DR), can induce a separate set of genes that also mediate lifespan extension[16–18].

Genetics of increased longevity under moderate DR. Moderate DR has been shown to cause an increase in replicative lifespan through a genetic pathway that is dependent on a shift of carbon metabolic flux away from anaerobic fermentation and towards aerobic respiration[1] (FIG. 2). Increased mitochondrial carbon metabolism was inferred from an increase in oxygen consumption during DR. This increase in respiration is necessary for DR to extend lifespan, because deletion of the cytochrome C1 gene *CYT1* suppresses respiration and prevents the longevity increase. Furthermore, blocking oxidative metabolism of carbon by deleting a subunit of the pyruvate dehydrogenase complex, encoded by *LAT1*, prevents moderate DR longevity, and *LAT1* overexpression increases the lifespan of cells grown in 2% glucose, but not cells subjected to moderate DR[18].

Reduced glycolysis and increased respiration during moderate DR raises the cellular NAD^+/NADH ratio. This elevated ratio is necessary and sufficient to increase longevity[19], and activates the lifespan-regulating NAD^+-dependent deacetylase silent information regulator 2 (Sir2) and its homologues, which drive increased lifespan[13]. Moderate DR also promotes Sir2 activity by inducing Pnc1, which catabolizes cellular nicotinamide, a Sir2 inhibitor[20]. Four genes with similarity to *SIR2*, named homologues of Sir2 (*HST1–4*), exist in yeast. Triple deletion of *sir2, hst1* and *hst2* suppressed moderate DR longevity in the hands of most investigators[18,21], although one group has reported an exception[22,23]. The reasons for this disparity in results are the subject of active debate[24], and a clear consensus has yet to be reached. One hypothesis is that differences in experimental protocol engage distinct longevity pathways (see below).

Yeast replicative lifespan is limited by the occurrence of recombination between the rDNA repeats, which leads to the excision of toxic, self-replicating extrachromosomal rDNA circles (ERCs) that accumulate specifically in the ageing mother cell[25]. One important mechanism by which DR-activated Sir2 extends lifespan is by the suppression of rDNA recombination and the consequent limitation of ERC formation[13]. However, because ERC accumulation has not been observed in other organisms, it is likely that the functions of Sir2 in DR longevity of other organisms proceed by different mechanisms[26]. One suggestion is that Sir2 genes have evolved to be connected to processes that limit lifespan in each organism, even if they are disparate[27].

Genetics of increased longevity under severe DR. More recently, it has been reported that a severe limitation of glucose, to 0.05%, produces an extension in yeast replicative lifespan that seems to require neither the electron

transport chain nor any sirtuin homologues[17,23,28]. Although the genetic underpinnings of this lifespan extension are not well understood, roles for the AKT homologue *SCH9* and the target of rapamycin (TOR) homologue *TOR1* have been suggested, because deletion of either of these genes produces a lifespan increase that cannot be further extended by growth in 0.05% glucose[17].

The interpretation of the *sir2*- and respiration-independent lifespan increase that occurs under severe DR has been a matter of recent debate, with two possibilities being proposed[28,29]. First, subtle *sir2*-independent lifespan increases might have been originally masked by suboptimal DR conditions at 0.5% glucose; alternatively, moderate and severe DR conditions might induce separate longevity pathways. We favour the second interpretation for several reasons, although it should be stressed that the existence of separate longevity pathways acting at different glucose concentrations has yet to be proven experimentally. First, numerous experiments from different laboratories have repeatedly confirmed that deletion of *sir2* and some of its homologues prevent lifespan extension by moderate but not severe DR[18,21]. Second, the NAD^+/NADH ratio was strongly increased in cells under moderate DR, but much less so in cells under severe DR, suggesting a different metabolic response to the two conditions[18]. Third, mutations that block moderate DR longevity, such as *cyt1Δ* and *sir2Δ* (REF. 1), had the opposite effect in severe DR, actually enhancing the longevity response[16,18]. This observation suggests that the moderate DR response genes inhibit the severe DR response genes, allowing the cell to fine-tune its responses to various levels of nutrient availability.

Notably, mutations that activate severe DR longevity pathways also generally increased the chronological lifespan of stationary-phase cells that lack nutrients entirely, whereas genes that extend replicative lifespan in moderate DR generally did not[30,31]. This observation supports the view that genes such as *SCH9* and *TOR1* might become increasingly important in lifespan control as food restriction becomes severe, whereas genes such as *SIR2* become less important (FIG. 2). Curiously, the expression of *SIR2* actually seems to shorten the lifespan of stationary-phase cells that have been incubated in water and therefore totally lack nutrients[31]. However, the relevance of this observation to beneficial DR in animals (as opposed to detrimental starvation) remains to be elucidated.

Interestingly, the increased lifespan of both moderate and severe DR depends on the pyruvate dehydrogenase subunit *LAT1* (REF. 18). It remains unclear how *LAT1* functions during severe DR to extend lifespan, because an intact ETC was not required[18]. Nevertheless, *LAT1* represents an apparent point of intersection between moderate and severe DR longevity pathways, and other examples of cross-regulation between the two DR longevity pathways might be found in future studies.

Genetic mimics of DR. In addition to the actual physical limitation of available glucose, mutation or overexpression of several genes that affect the transport or sensing of glucose has been shown to extend lifespan in yeast. Examples include: a hypomorphic mutation of the cell division cycle 25 gene, *cdc25-10*, which disrupts a glucose-sensing GTP–GDP-exchange factor; haem activator protein 4 (*HAP4*) overexpression, which increases mitochondrial biogenesis and respiration; and deletion of the hexokinase gene *HXK2*, which disrupts glucose entry into glycolysis[13]. As mentioned above, deletion of the AKT homologue *SCH9* also seems to mimic DR[17]. Interestingly, these mutations can be divided into different classes on the basis of genetic epistasis, and this also supports the view that there is more than one genetic pathway controlling nutrient-dependent lifespan. The first class of genetic manipulations, including the *cdc25-10* mutation and *HAP4* overexpression, share characteristics with the response to moderate DR: like moderate DR, *cdc25-10* and *HAP4* overexpression require *SIR2* and *LAT1* to increase lifespan[18]. Mutations that mimic the lifespan extension caused by severe DR, that is, that are *SIR2* independent but *LAT1* dependent, have not been described. *hxk2Δ* and *sch9Δ* fall into yet another class of nutrient-responsive longevity genes, as their effects on lifespan are independent of both *SIR2* and *LAT1* (REFS. 16–18).

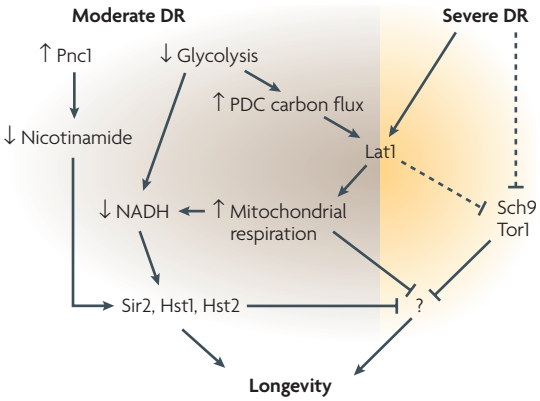

Figure 2 | **Responses to moderate and severe dietary restriction (DR) in yeast.** Different longevity pathways can predominate at moderate versus severe DR in yeast; pathways that potentially act in parallel are shown. In moderate DR, the longevity pathway on the left side of the figure is proposed to be the most important. Reduced glycolysis leads to a lowered cytoplasmic NADH concentration. Carbon flux through the pyruvate dehydrogenase complex (PDC), especially its subunit Lat1, is increased under moderate DR, which elevates mitochondrial respiration and further lowers NADH. The elevated NAD^+/NADH ratio increases the activity of the sirtuin Sir2 and/or the Sir2 homologues Hst1 and Hst2, increasing lifespan. Sir2 activity is also increased by Pnc1-mediated catabolism of nicotinamide, which is a Sir2 inhibitor. In severe DR, an apparently separate longevity pathway predominates, which extends lifespan without requiring any of the genes in the moderate DR longevity pathway, except for Lat1. Sch9 and Tor1 inhibit longevity, acting by a similar mechanism to that of severe DR and downstream of or in parallel with Lat1. The moderate DR longevity pathway seems to antagonize the severe DR longevity pathway, as severe DR extends lifespan much more effectively in strains with deletions in three sirtuin genes or genes required for function of the electron transport chain.

Table 1 | **Effects of dietary restriction on mammalian tissues**

Tissue	Effects of dietary restriction	References
Liver	Increase in gluconeogenesis and glycogenolysis	97
	Decrease in glycolysis	
Muscle	Increase in mitochondrial biogenesis and respiration	11,33,97
	Increase in β-oxidation of fatty acids	
	Increase in protein turnover	
Fat	Decrease in storage of triglycerides	97,98
	Decrease in secreted leptin	
	Increase in secreted adiponectin	
Pancreatic β-cells	Decrease in secreted insulin	97
Brain	Decrease in pituitary secretion of growth hormone, thyroid hormone, gonadotropins	97,99
	Increase in adrenal secretion of corticoids	
Whole organism	Increase in insulin sensitivity and decrease in blood glucose	97,33
	Increase in metabolism	

It will be important to resolve the extant controversies in the regulation of yeast lifespan at different levels of DR. It is generally agreed that moderate DR requires a functional electron transport chain for full effect, whereas severe DR does not. It is also likely that moderate DR requires Sir2 and possibly its paralogues, whereas severe DR does not. The PDC subunit *LAT1* may mediate both moderate and severe DR longevity. It is intriguing that severe DR may be mediated by genes of certain nutrient-sensitive pathways, such as *SCH9* and *TOR1*, because homologues of these genes have already been shown to regulate lifespan in higher organisms (see below). If two separate pathways do indeed regulate yeast DR, it will be important to establish which pathway is relevant to studies in higher organisms. Worms and mice that are subjected to longevity-promoting levels of DR show increased respiration[32–35], which is required in yeast for moderate DR longevity but not severe DR longevity (see above). It is therefore possible that moderate DR in yeast models the typical 30% food reduction that leads to rodent lifespan extension, whereas severe DR and stationary-phase ageing may engage survival mechanisms that are triggered by famine.

Metazoan mediators of DR longevity

Until recently, almost nothing was known of the genetic mediators of DR longevity in metazoans. However, much has been learnt about the genetic control of ageing in general, especially in *C. elegans* and *D. melanogaster*. Many of the genes that act as key regulators of lifespan also have known roles in nutrient sensing, including homologues of yeast genes that are known to be involved in DR longevity, such as *SCH9*, *TOR1* and *SIR2*. This overlap between genes that control metazoan lifespan and nutrient sensing suggests several candidate regulators of metazoan DR longevity, although few have been conclusively linked to DR longevity.

Homologues of the first DR effector gene to be identified, yeast *SIR2*, have been shown to have roles in DR in other organisms. In flies, SIR2 is required for DR longevity[36], although the tissue(s) in which this protein acts during DR is not known. Interestingly, a sirtuin-mediated neural activity has been suggested to act during mammalian DR after a study showed that *Sirt1*, the nearest mammalian homologue of yeast *SIR2*, was specifically required for the increase in spontaneous movement that is typically observed in diet-restricted animals[37]. In worms, *sir-2.1*, the closest homologue of yeast *SIR2*, can both positively and negatively affect lifespan in different contexts[24,38,39]. Worm SIR2 genes have not been convincingly connected with DR, although *sir-2.1* is the only one of the four worm *SIR2* homologues that has been tested. The balance of evidence in the literature argues against a role for *sir-2.1* in DR, with four groups finding that there is no requirement for *sir-2.1* in DR longevity[21,40–42]; however, one contrasting report found that DR longevity is partially dependent on *sir-2.1* (REF. 43).

Reduced TOR signalling extends lifespan in yeast[17], worms[44] and flies[45]. Although a role for TOR in mammalian lifespan control has not been investigated, this conservation across taxa suggests that TOR signalling is a candidate pathway for regulating mammalian lifespan. The mechanisms by which reduced TOR signalling increases lifespan are poorly understood, but one effect of reduced TOR signalling is reduced ribosome biogenesis, and reducing the expression of certain ribosomal genes extended the lifespan of both yeast[17] and worms[40]. DR did not further extend the lifespan of yeast, worms or flies with reduced TOR signalling[17,40,45], suggesting a common mechanism of action between these two interventions. Furthermore, whereas many interventions that extend worm lifespan depend on the forkhead transcription factor encoded by *daf-16* (REF. 46), both DR and reduced TOR signalling are notable exceptions[40,44,47,48], a fact that provides further support for the argument that TOR has a role in the DR-longevity pathway. The tissue(s) in which TOR acts to control worm and fly lifespan has not been identified.

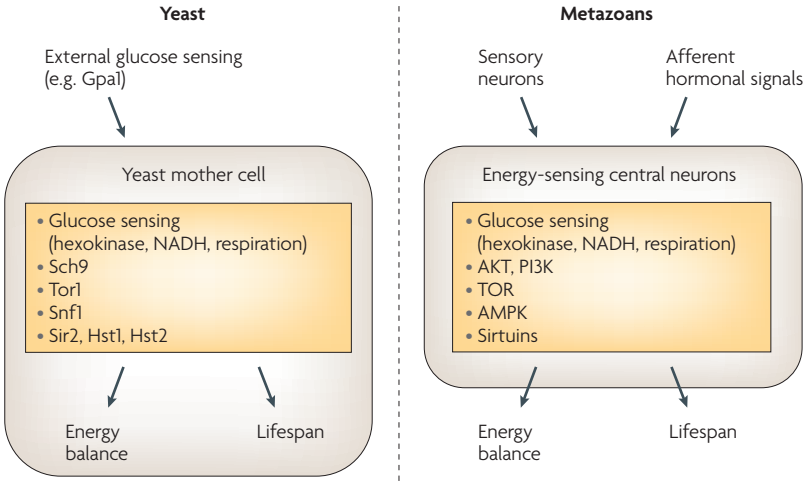

Figure 3 | **Potential parallel mechanisms of longevity control induced by dietary restriction (DR) in yeast and metazoans.** External nutritional cues are sensed in both cases, via the glucose sensor G-protein α subunit 1 (Gpa1) in yeast, and via sensory neurons in metazoans. Similarly, in both cases this environmental information is integrated with the cellular energy status, which is sensed by similar pathways. Glucose is sensed by a mechanism that depends on hexokinases to drive glucose metabolism, as well as on elevated NADH and mitochondrial activity. Nutrients are sensed and lifespan is controlled by homologues of AKT (such as yeast Sch9), target of rapamycin (TOR) and AMP-dependent protein kinase (AMPK). Sirtuins (such as Sir2 in yeast) are involved in some responses to DR in both systems. The primary difference between yeast and metazoan DR longevity control is that, in yeast, cellular energy-sensing pathways are coupled directly to cellular energy homeostasis and lifespan effectors whereas, in metazoans, cellular energy-sensing pathways are coupled to non-cell-autonomous signalling that achieves organismal energy homeostasis and lifespan control. A necessary consequence of this difference is that metazoan energy-sensing neurons receive additional afferent inputs from the periphery that are indicative of organismal energy status. PI3K, phosphatidylinositol-3 kinase; Snf1, an AMP-activated serine–threonine protein kinase; Hst, homologue of Sir2.

Another energy-sensitive kinase, AMP-dependent protein kinase (AMPK), has been shown to extend lifespan when hyperactivated in worms[49], although again the relevant tissue(s) of action is not known. Surprisingly, deletion of a worm AMPK α-subunit gene, *aak-2*, did not affect DR-induced longevity[50], although this might be due to redundancy with another α-subunit in the worm genome. An AMPK homologue has also been implicated in yeast longevity[51], although no role of AMPK in yeast DR has been reported. A more complete investigation of AMPK function during DR in metazoans and yeast is warranted.

The organism-wide coordination of metabolic responses to changes in nutritional status is mediated by hormonal signalling, and endocrine signalling is also crucial in the coordinate regulation of ageing across tissues. The *daf-2* insulin receptor homologue in *C. elegans* was one of the first genes to be shown to control metazoan lifespan[52]. Mutation of *daf-2* extends lifespan by reducing the activation of homologues of phosphatidylinositol-3 kinase (PI3K) and AKT, causing dephosphorylation and nuclear entry of *daf-16* (FIG. 1a). *daf-2* functions at least partly in neurons to regulate lifespan[53,54]. Because mutation of *daf-16* completely suppresses the longevity of well fed *daf-2* mutants[52], and because DR produces a normal lifespan extension in *daf-16* mutants[47,48], it has been argued that DR longevity is independent of insulin signalling in worms. However, several groups have observed significantly greater lifespan extension by DR in *daf-2* animals compared with the wild type[32,47], suggesting that insulin signalling might in fact antagonize DR longevity by a *daf-16*-independent mechanism. The identification of the yeast AKT homologue SCH9 as a possible inhibitor of DR longevity in that organism is also suggestive of a role for the insulin pathway in metazoan DR longevity[17]. Recently, it was shown that *pha-4*, which, like *daf-16*, encodes a Forkhead family transcription factor, is required for DR longevity in worms, although *daf-16* itself is not[55]. *pha-4* is homologous to mammalian forkhead box A (FOXA) genes, which regulate glucagon production and gluconeogenesis during periods of fasting.

The control of lifespan by insulin signalling is conserved in flies and mammals[46]. It has been argued that reduced insulin signalling and DR increase lifespan by a common mechanism in flies[56], although the interpretation of the data has been disputed[57]. Diet-restricted organisms show a gradual increase in lifespan up to a maximum at the optimal level of DR; beyond that level, lifespan is shortened owing to starvation. *Chico*^{1} mutant flies (the *chico* gene encodes a homologue of the insulin receptor substrate proteins) have reduced insulin signalling and are long-lived[58]. When these mutants were diet-restricted over a range of food levels, their lifespan dose-response curve was shifted relative to the wild type, such that the optimum DR lifespan was similar to the wild type in magnitude but occurred at a higher food level[56]. This was interpreted as indicating that reduced insulin signalling and DR promote longevity by the same mechanism. However, whether reduced insulin signalling normally mediates DR longevity in the wild type cannot be determined from this result alone, as the presence of a robust (albeit shifted) DR-response curve in *chico*^{1} flies is also consistent with an intact normal DR pathway that is distinct from insulin signalling. In addition, Tatar has argued that candidate DR genes should be tested only in the range of food concentrations that are greater than or equal to the optimum, because the mechanisms that promote survival in the starvation range might differ from those that promote longevity in the optimum-to-*ad libidum* range[57,59]. Using this criterion, the *chico*^{1} mutation and DR seem to act by different mechanisms, producing an additive effect on lifespan. Readers who are interested in further exploration of the complexities that are associated with lifespan mutant interaction studies are referred to REF 12, which is an excellent treatise. Ultimately, we believe that resolution of the best way to evaluate insulin signalling for a role in DR awaits elucidation of the precise pathways that are engaged during mild food limitation versus starvation.

Similarly to worms and flies, reducing the activity of the growth hormone (GH)–insulin-like growth factor 1 (IGF1) hormonal axis extends lifespan in mice[60]. The lifespan of prophet of pit 1 (*Prop1*^{df}) mutant mice, which have severe defects in pituitary production of GH (as well as prolactin and thyroid-stimulating hormone) and are long-lived[61], was further extended by DR[62], suggesting

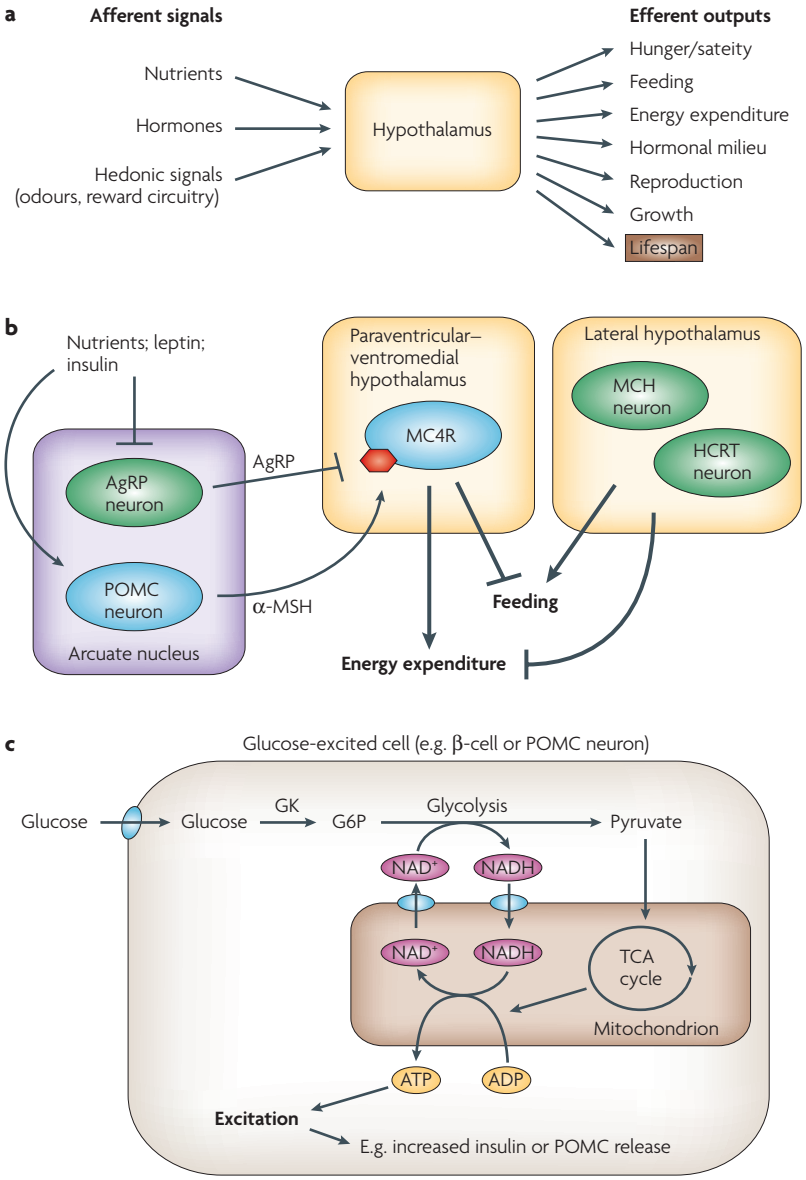

Figure 4 | **Homeostatic energy balance is maintained by the hypothalamus.** **a** | Afferent signals, such as nutrient availability, energy-status-dependent hormones from the periphery and signals from sensory and reward centres of the brain are integrated by the hypothalamus and translated into nervous and hormonal outputs that modulate multiple physiological and behavioural processes. **b** | Principal hypothalamic nuclei modulating feeding and peripheral energy expenditure. Orexigenic nuclei are coloured green and anorexigenic nuclei are coloured blue. Nutrients and the nutrient-dependent hormones leptin and insulin activate anorexigenic proopiomelanocortin (POMC) neurons and inhibit orexigenic agouti-related peptide (AgRP) neurons in the arcuate nucleus. When activated, POMC neurons secrete α-MSH (α-melanocyte stimulating hormone, a proteolytic cleavage product of POMC), which is an agonist of the melanocortin 4 receptor (MC4R) on anorexic neurons in the paraventricular and ventromedial hypothalamus. Activated AgRP neurons secrete AgRP, which antagonizes the MC4R. Orexigenic melanin-concentrating hormone (MCH) and hypocretin (HCRT) neurons in the lateral hypothalamus regulate energy balance in parallel with the MC4R neurons. **c** | Certain hypothalamic neurons are glucose-sensitive. Glucose-excited hypothalamic neurons sense glucose by a similar mechanism to that used by pancreatic β-cells. The pancreatic form of glucokinase (GK) drives glucose entry into the glycolytic pathway. Glycolysis elevates cytosolic NADH and pyruvate. Shuttling of cytoplasmic NADH into the mitochondria is essential for subsequent electron transport chain (ETC)-dependent production of ATP. Increased ATP concentration closes ATP-sensitive potassium channels, leading to neuronal activation.

that reduced GH–IGF1 signalling might increase lifespan by a different mechanism than DR. Paradoxically, mice that are long-lived owing to targeted disruption of the GH receptor[63] did not have an increased mean lifespan in response to DR[64], which would seem to indicate that reduced GH and DR in fact increase lifespan by overlapping mechanisms. The question of why *Prop1*^{df} mice responded to DR whereas GH receptor knockout mice did not has not been resolved, so it is still unclear whether there is any functional role of reduced GH level in mammalian DR longevity.

In summary, insulin signalling, TOR, AMPK and sirtuins all remain intriguing candidates for conserved regulators of DR longevity in metazoans. None has been conclusively ruled out, and there is positive evidence for roles of AKT, TOR and sirtuins in multiple species. Future exploration of the connections between nutrient-sensitive pathways and DR longevity in invertebrate models should improve our understanding of the mechanisms of mammalian DR.

Neural regulation of DR longevity in invertebrates

DR induces physiological changes in various mammalian tissues, with the net effect of producing an animal that is insulin sensitive and stress resistant (TABLE 1). Until recently, however, little was known about which organs were causally relevant to increased lifespan during DR. Several recent reports have established that the nervous system is an important regulator of invertebrate lifespan. In *C. elegans*, loss of chemosensory ability in all neurons dramatically extended lifespan[65], as did laser ablation of any of several individual neurons[66]. Similarly, in *D. melanogaster*, ablation of specific insulin-producing neurons in the head was sufficient to extend lifespan[67]. Strong evidence for a crucial role of neurons in mediating the DR longevity response of metazoans has recently been obtained from two invertebrate studies, one in flies and one in worms.

The fly study[68] reported two important findings. First, the authors showed that the odour of food alone was sufficient to reduce the longevity response of diet-restricted flies, suggesting that odour-sensitive neuronal pathways modulate DR longevity. Second, this study showed that mutation of a pan-neuronally expressed chemoreceptor called OR83B disrupted the function of many sensory neurons and extended lifespan significantly. These long-lived mutant flies responded less robustly to DR than the wild type, suggesting that the lifespan extension that is mediated by these neurons might overlap mechanistically with DR longevity. Interestingly, it was shown in a separate study that neuron-specific overexpression of the human uncoupling protein UCP2 in flies also extended lifespan[69]. The interaction of this lifespan extension with DR was not examined but, because UCP2 has an important role in hypothalamic nutrient sensing (see below), it is tempting to speculate that this neuronal UCP2 overexpression might have driven the fly nervous system to perceive hunger and activate related DR-like lifespan-extension pathways. Consistent with this interpretation, the endogenous *D. melanogaster* uncoupling protein UCP5 was required specifically in neurons to

Table 2 | **Conserved control of energy sensing, lifespan and dietary restriction (DR) longevity response**

Gene or metabolic process	Energy sensing				Lifespan control				DR longevity			
Organism	Y	W	F	M	Y	W	F	M	Y	W	F	M
Glucokinases and fructokinases	✓			✓	✓	✓			✓			
NADH level	✓			✓	✓				✓			
Respiration rate				✓	✓	✓	✓	✓	✓	✓		
Neuronal signalling	na	✓	✓	✓	na	✓	✓	✓	na	✓	✓	
InsR	na	✓	✓	✓	na	✓	✓	✓	na	✗?	✓?	✗?
PI3K		✓	✓	✓		✓						
Akt	✓	✓	✓	✓	✓	✓			✓			
FoxO1	na	✓	✓	✓	na	✓	✓		na	✗		
Sirtuins	✓	✓		✓	✓	✓	✓		✓	✗?	✓	
AMPK	✓	✓	✓	✓	✓	✓			✓	✗?		
TOR	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	

Y, yeast; W, worm; F, fly; M, mammal. ✓ indicates that a given gene or process is connected with energy sensing, lifespan control or DR longevity in the corresponding model organism. ✗ indicates that there is evidence that the given gene or process is not involved in DR longevity in the corresponding model organism. na, not applicable. Blank cells indicate that a potential interaction has not been tested. AMPK, AMP-dependent protein kinase; FOXO1, forkhead box transcription factor O1; InsR, insulin receptor; PI3K, phosphatidylinositol-3 kinase; TOR, target of rapamycin.

mediate normal adaptation to low-energy conditions[70], suggesting that uncoupling proteins are crucial for neuronal energy sensing in flies.

The second recent study that implicated neurons in the control of DR longevity was carried out in *C. elegans*. A pair of sensory neuroendocrine cells in the worm head, called the ASI neurons, is in many ways functionally analogous to the hypothalamus. The ASI neurons sense food in the environment and integrate this information with the intrinsic energy availability to modulate the hormonal signalling pathway that controls dauer entry in larvae and fat metabolism in adults[71–73]. A transcription factor gene called *skn-1* was shown to be required to act specifically in the ASI neurons to increase worm lifespan in response to DR[32]. Furthermore, ablation of the ASI neurons prevented the DR-longevity response. This and other studies have shown that, as in yeast, DR causes increased respiration in worms[32,34,35], and that this increase is required for longevity and mediated by *skn-1* activity in the ASI neurons. These findings suggest that the ASI neurons sense DR and, by a non-cell-autonomous mechanism, signal metabolic changes in peripheral tissues to cause longevity.

A role for the hypothalamus in DR longevity?

The recent reports described above that link energy-sensitive neurons to lifespan control suggest a model in which DR produces longevity in metazoans by a similar mechanism to that of yeast, except that the effectors of metazoan DR longevity are non-cell-autonomous signals from central neurons (FIG. 3). The discovery that central neurons mediate DR longevity in invertebrates raises the interesting possibility that the same is true in mammals.

The hypothalamus is the principal vertebrate brain region that is responsible for maintaining homeostatic organismal energy balance. This region detects changes in energy availability and mediates appropriate modifications of energy intake and expenditure through hormonal and autonomic signalling (FIG. 4a). Notably, the hypothalamus controls the release of GH, which has already been implicated in the mammalian DR response (described above), by the pituitary. For the remainder of this Review, we will present evidence that supports the hypothesis that the mammalian hypothalamus is a crucial regulator of mammalian DR longevity.

Energy-sensing hypothalamic nuclei and neuropeptides. Much has been learnt about the genetic mechanisms of hypothalamic energy sensing in recent years, a brief overview of which is presented here. The reader is directed to REFS 74–76 for further detail. The behaviour- and metabolism-modifying outputs of the hypothalamus result from the integrated activity of several populations of energy-sensitive neurons (FIG. 4b). The best characterized of these are two neuronal populations in the arcuate nucleus: the anorexigenic (appetite-suppressing) pro-opiomelanocortin (POMC) neurons and the orexigenic (apetite-stimulating) agouti-related peptide (AgRP) neurons[74]. Two additional populations of neurons in the lateral hypothalamus, expressing either hypocretin (HCRT) or melanin-concentrating hormone (MCH), also have a role in promoting feeding, as ablation of either the MCH gene or the HCRT neurons caused hypophagia and altered energy expenditure[77,78].

Afferent hormones modulate hypothalamic energy sensing. Hypothalamic energy-sensing pathways are modulated by an ever-expanding group of afferent hormones that carry information about the long- and short-term energy status of the body[74]. Two of the most important of these are leptin and insulin.

Leptin is produced by adipocytes in proportion to fat mass, and promotes anorexic hypothalamic signalling[74].

Uncoupling proteins
A family of mammalian proteins that dissipate the proton gradient across the mitochondrial membrane, necessitating more rapid respiration to maintain the rate of ATP production.

Dauer
An alternative larval stage of *Caenorhabditis elegans* that is adapted to survive adverse conditions.

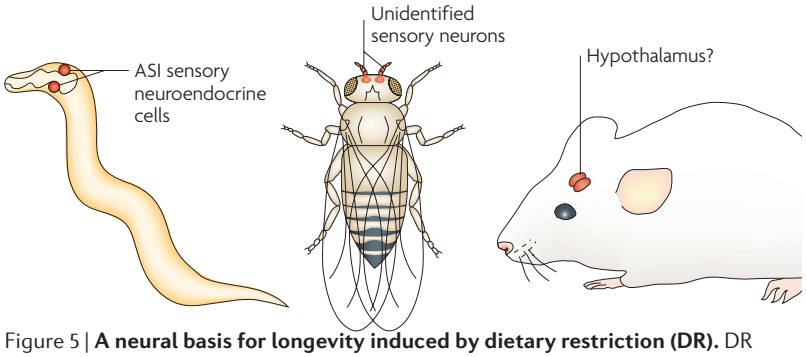

Figure 5 | **A neural basis for longevity induced by dietary restriction (DR).** DR longevity is mediated by energy-sensitive central neurons in worms, flies and possibly mammals. The ASI neurons mediate DR longevity in worms, and they signal non-cell-autonomously during DR to cause increased respiration in peripheral tissues and long life. Unidentified olfactory neurons mediate DR longevity in flies. The hypothalamus may mediate DR longevity in mammals (see main text for details).

Mutation of the leptin gene (*Lep*, also known as *ob*) caused hyperphagia and massive obesity[79], in large part through repression of anorexic AgRP neurons and activation of orexigenic POMC neurons[74,80]. Insulin also promotes hypothalamic anorexigenic signalling, but has a more minor role than leptin. Neuron-specific insulin receptor knockout caused mild obesity[81], and central administration of insulin caused feeding to be suppressed[82]. Insulin cooperates with leptin to promote anorexic and repress orexigenic neuropeptide expression in arcuate neurons by activating the PI3K–AKT–FOXO1 signalling cascade[83–86].

Leptin and insulin function in several other ways to promote hypothalamic anorexigenic signals. For instance, leptin and insulin both inhibit hypothalamic AMPK signalling[87]. AMPK controls hypothalamic energy sensing, acting both to promote expression of orexigenic peptides in the arcuate nucleus and to promote the appropriate response in the paraventricular receiving cells[87]. Leptin also upregulates TOR in the mouse hypothalamus[88], which promotes anorexic responses.

Cell-intrinsic sensing of energy availability. Hypothalamic neurons also integrate information about energy status by cell-autonomous mechanisms. One such mechanism is the sampling of the levels of certain key metabolites in the cell, including glucose, fatty acids, ATP and possibly amino acids[74–76]. The hypothalamus contains many glucose-sensitive neurons, which are either glucose-excited or glucose-inhibited. For example, anorexigenic POMC neurons are glucose-excited[89] and orexigenic AgRP neurons are glucose-inhibited[90]. Glucose-excited hypothalamic neurons sense glucose concomitantly with its metabolism, in a manner that is analogous to that of pancreatic β-cells[91–93] (FIG. 4c).

Recently, fasting was shown to induce expression of UCP2 in AgRP neurons[94]. In UCP2 knockout animals, fasting-induced mitochondrial uncoupling, mitochondrial biogenesis and orexigenic peptide expression were abolished, and the amount of rebound feeding following fasting was diminished. Thus, fasting-induced uncoupling of mitochondrial respiration might be crucial in the activation of orexigenic AgRP neurons. It is not known how increased uncoupling in these neurons leads to perception of a low-energy state, but it is worth noting that increased uncoupling should result in lower NADH levels and/or ATP levels, possibly reducing glucose inhibition and/or increasing AMPK activity.

DR longevity pathways in the hypothalamus? It will be apparent from the preceding discussion that there are considerable parallels between the genetic pathways in yeast that translate low glucose perception into long lifespan and the genetic pathways in the hypothalamus that translate perception of low energy availability into the appropriate efferent signals (FIG. 3; TABLE 2). Glucose sensing in both yeast and hypothalamic neurons is coupled to glucose metabolism in similar ways. Both cell types depend on a hexokinase enzyme to detect glucose, and use an elevated NADH level as a signal of high glucose. Increased respiration is a crucial step in sensing low nutrient levels in both yeast and AgRP neurons. In addition, *TOR1*/TOR, *SNF1*/AMPK and *SCH9*/AKT homologues have roles in both nutrient-sensitive longevity in yeast and nutrient sensing in the hypothalamus. Furthermore, several of the suspected signalling pathways in the DR longevity of invertebrates (for example, TOR and insulin) modulate hypothalamic energy sensing, and central neurons have been established as key sites of longevity control in diet-restricted invertebrates. However, we should point out that some of these mammalian energy-sensing mechanisms are not unique to the hypothalamus, so similar parallels with yeast energy sensing could also be drawn to other mammalian tissues. Nevertheless, the connection of central neuronal signalling to DR longevity in invertebrates, coupled with the similarities between the mechanisms that sense nutrients and affect DR longevity in lower organisms and the pathways that sense nutrients in the hypothalamus beg the question: is DR longevity in mammals mediated by hypothalamic signalling?

This question remains to be answered by future investigations. Recently, however, an encouraging direct connection between nutrient-sensitive cells of the hypothalamus and mammalian longevity was demonstrated[95]. UCP2 was overexpressed specifically in the orexigenic hypocretin neurons in the lateral hypothalamus of mice to bring about thermogenesis in the hypocretin neurons, thus raising the temperature of nearby temperature-sensitive centres of the hypothalamus and causing the core body temperature to be lowered. We note that overexpression of UCP2 would also be predicted to increase hunger perception in the hypocretin neurons. Consistent with this prediction, the transgenic mice showed mild hyperphagia: despite having a reduced daily calorie requirement due to a lowered core body temperature, transgenic mice ate as much food as the wild-type controls, and males gradually became obese as they aged. Remarkably, these mice also had a longer mean and maximum lifespan than controls, by 12% in males and 20% in females. So, altered gene expression in a single population of hypothalamic neurons is sufficient to modify energy balance and increase lifespan; whether the hypocretin neurons have a role in DR longevity remains to be tested.

In summary, it is worth considering how much studies in lower organisms might be telling us about the situation in mammals. There is evidence that many of the key genes that control yeast energy sensing and lifespan might also control one or both processes in invertebrates and mammals. The recent evidence for the crucial role of small populations of neurons in controlling DR longevity in invertebrates and perhaps mammals suggests that the problem of DR longevity can essentially be reduced to a cell-biological one. If we unlock the mechanisms of DR in lower organisms, we might be much closer to solving that problem in mammals than previously suspected.

Conclusions and future directions

We are beginning to arrive at a mechanistic understanding of how DR extends lifespan. On the basis of current evidence, it is tempting to speculate that the hypothalamus is a crucial site for the regulation of mammalian DR longevity. In invertebrates, energy-sensing central neurons have a crucial role in DR-induced longevity (FIG. 5), and an extensive catalogue of genes regulates ageing. For mammals, we have a detailed understanding of how energy-sensing pathways function in the brain, and there is considerable overlap between the genes that regulate lifespan in lower organisms and those that mediate hypothalamic energy sensing in mammals, which should provide fertile ground for future studies of the mechanism of DR. As a first step in evaluating the importance of the hypothalamus in DR longevity in mammals, DR responses should be tested in mutant animals with defective hypothalamic energy sensing.

If mammalian hypothalamic energy sensing can be convincingly linked to DR longevity, one exciting question that should be explored is whether the DR-longevity response is separable from the other hypothalamic outputs that are caused by perception of a low-energy balance, such as reduced peripheral metabolic rate and impaired reproduction. This will provide important information for potential future medical activation of putative hypothalamic DR-longevity pathways. Preliminary evidence suggests that the longevity effects of DR can be uncoupled from metabolic effects, because DR of genetically obese *ob/ob* mice resulted in a long lifespan, even though the diet-restricted *ob/ob* mice retained greater adiposity than the *ad libidum* wild-type[96]. Thus, DR might have effects on lifespan that are separate from low adiposity.

Invertebrate model organisms provide an attractive system in which to investigate the genetic pathways that act as effectors of DR longevity in peripheral tissues. Initial evidence suggests that genes and processes that are known to be crucial in yeast DR longevity, including increased respiration, sirtuins, TOR and AKT, might have conserved roles in invertebrate DR longevity. The high degree of conservation between yeast and metazoan DR that is suggested by these findings should encourage further characterization of DR regulators in yeast. Other regulators of invertebrate DR longevity include *pha-4*, *skn-1* and perhaps AMPK. For most of these genes, a definite role in invertebrate DR longevity and/or the tissue(s) of action have not been identified. It will be important for future investigations to identify the genes and tissues that are crucial in invertebrate DR to inform our understanding of mammalian DR longevity.

The recent discoveries of common pathways that regulate physiological ageing suggest that the regulatory proteins involved constitute attractive new targets for therapeutic interventions in age-related disease. Such DR mimetics may eventually offer an entirely new paradigm to extend the duration of optimal human health.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
aak-2 | *chico* | *CYT1* | *HXK2* | *LAT1* | *Lep* | *SCH9* | *daf-2* | *daf-16* | *pha-4* | *sir-2.1* | *Sirt1* | *skn-1* | *TOR1*
UniProtKB: http://ca.expasy.org/sprot
HCRT | leptin | MCH | OR83B | Sir2 | UCP2

ALL LINKS ARE ACTIVE IN THE ONLINE PDF