

# Separating cause from effect: how does insulin/IGF signalling control lifespan in worms, flies and mice?

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Ageing research has been revolutionized by the use of model organisms to discover genetic alterations that can extend lifespan. In the last 5 years alone, it has become apparent that single gene mutations in the insulin and insulin-like growth-factor signalling pathways can lengthen lifespan in worms, flies and mice, implying evolutionary conservation of mechanisms.

Importantly, this research has also shown that these mutations can keep the animals healthy and disease-free for longer and can alleviate specific ageing-related pathologies. These findings are striking in view of the negative effects that disruption of these signalling pathways can also produce. Here, we summarize the body of work that has led to these discoveries and point out areas of interest for future work in characterizing the genetic, molecular and biochemical details of the mechanisms to achieving a longer and healthier life.

**Keywords:** ageing, *Caenorhabditis elegans*, *Drosophila*, insulin/IGF signalling, lifespan, mice.

## Introduction

Experiments with model organisms are providing ever-increasing evidence that signal reduction through the insulin and insulin-like growth factor (IGF) signalling (IIS) pathways acts to prolong lifespan by delaying the effects of ageing. This discovery is particularly striking in light of the dramatic, detrimental effects that can also occur when IIS function is altered, including death during embryogenesis, diabetes and cancer [1–3]. In addition, alterations of pathway activity can affect reproduction [4], stress resistance [5, 6] and metabolic phenotypes [2]. Modulation of IIS activity can thus have many different phenotypic effects. Recent work is beginning to unveil the causal connections between them, and thus to throw light on the important issues of whether modulation of IIS in the right tissue, at the right time,

to the right extent can offset the negative effects of advancing age without the detrimental side-effects of altering IIS.

A key aspect to this research has been the use of the relatively simple invertebrate models: the nematode worm *Caenorhabditis elegans* and the fruit-fly *Drosophila melanogaster*. Indeed, the short lifespan, ease of handling and genetic accessibility of the worm enabled the discovery of the first long-lived IIS mutant (*age-1*), which was found using a longevity screen that would not have been possible in rodents [7]. This mutant was later isolated and mapped to the worm phosphoinositide-3-kinase (PI3K; Fig. 1) [8, 9], which supported parallel findings in the worm demonstrating that mutation of the insulin receptor (DAF-2) could increase lifespan and that this effect depended on the forkhead transcription factor

DAF-16 [10–13]. In 2001, it was shown that mutation of the insulin receptor (InR) or its substrate CHICO could extend the lifespan of flies [14, 15] (Fig. 1), which suggested that the ability of lowered IIS activity to extend lifespan was conserved over large evolutionary distances. Finally, rodents were also shown to live longer if either the insulin or IGF signalling pathways were disrupted [16–19], indicating that the evolutionary conservation extends to mammals, raising the possibility that the human ageing process could be accessible to intervention through this pathway. These findings also opened the way for simpler, invertebrate model organisms to be used to open the black box of molecular mechanisms of ageing, and to determine patterns of causality between the many phenotypic effects of IIS. The first important insights have come from studies using conditional and/or tissue-specific knockdown of signalling pathway components.

An important feature of long-lived IIS mutants is that, where studied, they enjoy a longer ‘healthspan’ as well as longer lifespan. For example, work with *C. elegans* has shown that long-lived IIS mutants remain active at later ages [10, 20], and studies of mice null for the insulin receptor substrate 1 (IRS1) have shown that they possess greater indices of health and lower levels of pathology at later ages [18]. In addition, some studies have demonstrated protective effects of IIS mutants that extend lifespan in invertebrate models against specific ageing-related diseases such as cancer [21, 22], Alzheimer’s Disease [23] and cardiac failure [24]. Research on the interaction between IIS and the aetiology of such age-related diseases should therefore reveal information about possible ways to improve health during ageing. This area of research, in particular, is poised to be accelerated by the work with invertebrates, where multiple models of ageing-related disease are now available [25–29].

In this review, we summarize the IIS interventions that have so far been shown to increase lifespan in worms, flies and mice (see Fig. 1 and Table 1 for a summary of the lifespan-extending interventions that have been described for the three species). In addition, we describe how further analysis of these models

has led to the discovery of candidate, common molecular processes that could underlie the lifespan-extension. We also suggest some future research strategies that could help to separate the pleiotropic, detrimental effects of IIS mutations from those that result in increased healthy lifespan.

### **Multiply encoded pathway components display functional specialization**

An interesting, and in some cases striking, feature of the IIS-encoding genes is their multiplicity. An extreme example of this expansion is the 39 *C. elegans* insulin-like genes so far identified [5]. Given the difficulties of working on so many similar genes, with possible functional redundancy between them, it is understandable that there has been relatively little work on dissecting their ability to play specialized roles. It is nonetheless clear that some ligands act as receptor agonists, whilst others act as antagonists [30–32]. *Drosophila* has seven genes encoding insulin-like peptides (DILPs) and they show different spatiotemporal patterns of expression during development, indicating that they may have different functional outputs [33, 34]. Ablation of the cells expressing only three of the seven DILPs is sufficient to increase longevity [35]. Future work on the individual roles of each of the DILPs on lifespan and other traits will be important for unravelling patterns of causality.

In contrast to the two invertebrates, mice have only one insulin ligand and two IGF ligands but, unlike the invertebrates, they have multiple isoforms of the cellular signalling components. Due to their clinical importance in growth, diabetes and tumour progression, there has been a great deal of work on these genes, and several of their functional specializations have already been described. For instance, there are four insulin receptor substrate (IRS) genes, and knockout of each one separately produces phenotypes that range from mild growth defects only, for IRS-4 males, to severe insulin resistance and associated diabetes mellitus, which results in dramatically shortened lifespan in IRS-2 knockout males [18, 36]. Recently, IRS-1 global null, female mice have been shown to have extended lifespan [18], which contrasts

with the very short lifespan of IRS-2 null mice. However, when the IRS-2 knockout is brain-specific, both heterozygous and homozygous mice are long-lived [19]. Thus modulation of the activity of specific IIS components in specific tissues is required to extend lifespan.

### The timing requirements of IIS modulation for lifespan-extension

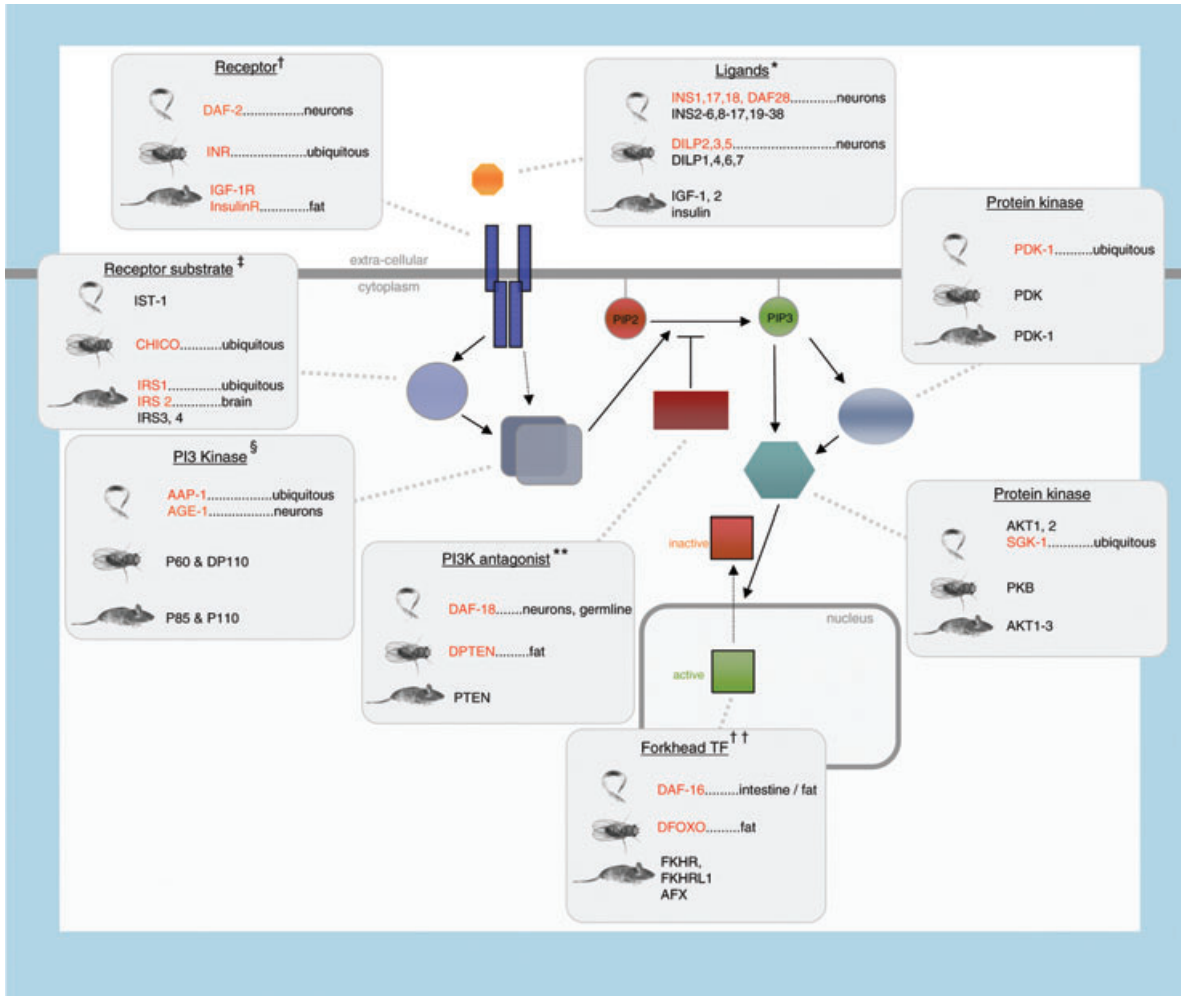
Many of the IIS mutants that have been examined alter the pathway's activity throughout the life history. Recent work has started to refine the analysis by determining the timing requirements for altered IIS to extend lifespan. An initial study took advantage of the fact that gene activity can be turned down in *C. elegans* by double-stranded RNA interference, induced by feeding the worms on mutant bacteria. Decreasing IIS activity during adulthood, particularly during the reproductive period, was required to extend lifespan, with IIS in the pre-adult, developmental period playing no role [37] (Table 1). More recently, adult-specific overexpression of dFOXO in flies was also demonstrated as sufficient to extend lifespan [38, 39] as was adult-specific overexpression of dPTEN [38] as well as ablation of the insulin producing neurons (median neurosecretory cells or mNSCs) in the last stage of preadult development [35] (Table 1). Interestingly, dFOXO most effectively extended lifespan when overexpressed during the reproductive period [40]. Thus, it appears that lowering IIS signalling during early adulthood only is sufficient for lifespan extension in invertebrates. It is now of great interest to discover both the reasons for this timing effect and if it also occurs in rodents. An important additional outcome of the worm and fly studies was to show that the adult-specific IIS interventions did not affect reproductive capacity [37–39]. Thus, adult-specific IIS modulation offers real promise for pharmacological interventions to improve late-life health without affecting important aspects of early life physiology.

### Tissue-specific IIS modulations that extend lifespan

For the insulin pathway mutants that have so far been tested in the three model organisms, lowered IIS

activity in neuronal and fat tissues appears to be particularly important to the longevity phenotypes of the three species (Fig. 1 and Table 1).

Two studies have specifically examined the importance of different tissues for the longevity effect of reduced IIS in worms. In the first, tissue-specific replacement of the wild-type *age-1* or *daf-2* gene in neurons of a global *age-1* or *daf-2* mutant animal could shorten the lengthened lifespan of the mutants back to the wild-type level, which was not the case for expression of these genes in intestine or muscle alone [41]. Thus, when neurons were normal for IIS in an otherwise IIS mutant background, the animals' lifespan was reduced to normal. This led to the proposal that lowering IIS in these cells had a neuroprotective role and that this was sufficient for the full effect of the pathway to extend lifespan. In contrast, it has been reported, using several different methods, that lowered IIS in all tissues *except* neurons produces a large lifespan extension phenotype [42]. This same report also went on to show that the majority of the IIS effect on longevity was localized to intestinal cells and, unlike the former article, that neuronal IIS had only a small effect on lifespan. Discrepancies between the results of these two studies could reflect nonspecific, detrimental effects of gene overexpression, branching of the IIS pathway below *daf-2* or *age-1* or a variable contribution from cell nonautonomous effects of the tissue-specific genetic manipulations depending upon the genotype of the rest of the worm. A role of neuronal tissue in longevity has also been highlighted for flies [35] and mice [19]. In flies, mNSC ablation extends lifespan, presumably by reducing circulating insulin ligands that in turn reduce peripheral insulin signalling [35]. In mice, brain-specific disruption of the IRS2 is proposed to protect the brain from age-related hyperinsulinaemia [19]. The exact functional overlap between these observations in the different species is not clear, especially since neuronal tissue is not uniform and so each of these different interventions will be likely to affect the physiology of each organism in different ways. However, there appear to be two themes to the interventions listed above: (i) appropriate levels of insulin signalling in the brain can have a neuroprotective



**Fig. 1** Comparison of the insulin-like growth-factor signalling (IIS) signaling pathway components in worms, flies and mice indicating the components of which that extend lifespan when mutant. Signaling through the IIS pathway is initiated by binding of the insulin and insulin-like peptide ligands to their appropriate receptors. This induces receptor dimerization and auto-phosphorylation of the cytoplasmic domain. Phosphotyrosines become bound by the insulin receptor substrate (IRS) family of proteins which in turn interacts with the phosphatidylinositol-3-OH-kinase (PI3K) via its regulatory domain (p85). PI3K activation directly by the insulin receptor is possible in worms and flies. The catalytic subunit (p110) of PI3K is recruited to the plasma membrane where it phosphorylates phosphatidylinositol-(4,5)-bisphosphate (PIP2) to phosphatidylinositol-(3,4,5)-trisphosphate (PIP3). This activity is antagonized by the PTEN phosphatase. Elevated PIP3 recruits AKT (PKB) to the plasma membrane where it enables its further activation by the phosphoinositide-dependent kinase (PDK). Activated AKT (and SGK in worms) functions to phosphorylate and inactivate the FOXO transcription factor resulting in its exclusion from the nucleus. Thus, elevated IIS activity is associated with reduced involvement of FOXO in transcriptional regulation, whereas reduced IIS activity results in increased involvement of FOXO in transcriptional regulation. Species-specific names for each of the pathway components are indicated. Red text indicates long-lived mutants, whilst text following leaders shows the most detailed tissue-specific intervention for that mutation that can also extend lifespan. In some cases, the tissues are sufficient and necessary, but not always. The references for these more specific interventions are described in detail in Table 1. Ubiquitous means intervention affects expression of the gene throughout the whole organism.

effect that is positive for lifespan; and (ii) reducing systemic insulin signalling, which is controlled by the invertebrate brain, can have life-preserving effects.

Although mice are physiologically different, in that ligand release is largely from the pancreas and liver and perhaps a minor contribution from the brain [43]

**Table 1** Tissue-specific long-lived IIS mutants referred to in Fig. 1

Pathway component	Organism(s)	Tissue	Intervention	Key phenotypes	References
*IIS ligands	Worm	Neurons	RNAi against <i>ins-7</i>	Increased lifespan	[31]
		Neurons, vulval muscle, intestine	Overexpression of <i>ins-1</i>	Increased lifespan	[32]
		Neurons, intestine	RNAi against <i>ins-18</i>	Increased lifespan	[30]
	Fly	Neurons	Genetic ablation of the insulin producing cells	Male and female lifespan extension; reduced female fecundity; altered fat, trehalose and glycogen levels; resistant to paraquat, starvation and xenobiotics; resistant to age-related heart function decline; sensitive to heat and cold stress	[24, 35, M. Piper, L. Partridge, unpublished data]
†Insulin receptor	Worm	Neurons	Early adult only, RNAi	Lifespan extension No effect on fecundity Resistant to oxidative stress	[37]
			Replacement of functional <i>daf-2</i> in neurons of mutant	Shortened lifespan (implying functional neuronal <i>daf-2</i> is required for normal signaling)	[41]
	Mouse	Fat (white adipose)	Homozygous deletion	Lifespan extension calculated using data from both sexes pooled Normal body size at birth, to smaller in late life Reduced fat mass	[16]
‡Insulin receptor substrate	Mouse	Brain	Brain-specific <i>Irs2</i> heterozygotes or homozygous deletion	Male and female lifespan extension; Homozygous mice infertile Increased weight and adiposity with age Insulin resistance and glucose intolerant Resisted age decline in activity	[19]
§PI 3 Kinase	Worm	Neurons	Replacement of functional <i>age-1</i> in neurons of mutant	Shortened lifespan (implying functional neuronal Age-1 is required for normal signaling)	[41]
**PI 3 Kinase antagonist (PTEN)	Worm	Neurons & germline	<i>daf-18</i> mutation	Suppressed <i>daf-2</i> mutant longevity phenotype	[117, 118]
	Fly	Fat	Fat-body specific induction	Male and female lifespan extension	[38]
††Forkhead transcription factor	Worm	Intestine/fat	Replacement of functional <i>daf-16</i> in intestine	Enabled IIS-mutant longevity and germline ablation longevity	[42]
	Fly	Fat	Fat-body over-expression; early adulthood only	Female lifespan increased; no effect on fecundity; Increased oxidative stress resistance	[38–40]

it seems that moderately lowered circulating IIS ligands is a general correlate for long life as it is associated with other interventions that extend mouse lifespan (e.g. dietary restriction [44] and reduced activity of the somatotropic axis [45–47]).

The second tissue highlighted by tissue-specific interventions that extend lifespan is fat (Fig. 1). Fat-specific knockout of the insulin receptor in mice [16] (Fig. 2) can extend lifespan, as can fat-specific overexpression of the pathway antagonists dPTEN or dFOXO in flies [38–40]. In worms, it has also been shown that there is a requirement for DAF-16 in the intestine (also adipose tissue) for enhanced longevity [42] (Fig. 1 and Table 1). This worm study also showed that overexpression of DAF-16 in intestinal cells of wild-type worms could activate DAF-16 activity in other tissues, indicating a cell nonautonomous effect of intestinal IIS activity [42]. Thus, there is evidence that a secondary signal, which originates in the fat tissue and involves the IIS at least in part, activates downstream processes in other tissues to coordinate organismal longevity. The fat tissue should therefore be the site of investigation into the exact nature of this secondary signal.

In addition to the role of these two evolutionarily conserved tissues in lifespan control, there is a third tissue that plays a role in IIS-altered worm longevity: the gonad (Fig. 1) [48, 49]. Ablation of the germline in worms extends lifespan in a manner that depends on the intestinal IIS as well as a nuclear hormone receptor (NHR) called DAF-12 [49]. A similar study in flies could not reproduce the life-prolonging effect of germline ablation, indicating that the two models differ in the specific roles and possible patterns of molecular signalling from the germline [50]. Interestingly, another study has reported that transplanting ovaries from young mice into aged females could extend the remaining lifespan of the recipient [51]. Thus, a signal from the gonad may well play a role in integrating lifespan and reproduction between species, but in different ways. In spite of this, it is important to note that reduced reproduction is not necessary for IIS to extend lifespan (see below).

A great deal of the work that has been presented so far is based on genetic analyses. Whilst these have brought a wealth of understanding, they are only one part of the physiological picture and therefore cannot provide us with a complete understanding of



**Fig. 2** Photographs depicting long-lived insulin-like growth-factor signalling (IIS)-mutant worms, flies and mice. The left panel shows a *Daf-2* mutant worm adult and a *Daf-2* mutant dauer (right of panel). A dauer is a developmentally arrested stage that is stress resistant and long-lived and typical of IIS-mutant worms. The middle panel shows a long-lived *chico*<sup>1</sup> homozygous female dwarf (right of panel) next to a wild-type female. The right panel shows a control and a long-lived fat-specific insulin receptor knockout (right of panel) mouse [16]. In this case, the long-lived mouse is smaller due to a lower fat content than control and not due to a developmental defect. Thus, it is possible to lower IIS activity in a manner that extends lifespan with no discernible detrimental effect on other aspects of physiology. Mouse photograph kindly provided by S.J. Russell and C.R. Kahn.

longevity. In order to gain a detailed mechanistic understanding of how lifespan is extended, biochemical and molecular genetic studies are also required. As our understanding deepens, these should also help uncover why some of the genetic data currently appears inconsistent or contradictory as well as providing methods for how longevity-assurance processes could be manipulated pharmacologically.

### Searching for the mechanisms downstream of IIS that effect long life

In addition to increasing lifespan, reducing IIS has many other effects on physiology and a great deal of work has focussed on whether these other phenotypes that accompany increased longevity are causal or simply correlated. As IIS coordinates nutritional availability and growth, it is unsurprising that the initial reports of long-lived invertebrates contained effects on metabolic reserves (increased fat content) as well as altered development, decreased adult body size and reduced fecundity [8, 14, 15] (see Fig. 2 for illustration). However, with time, various reports have shown that these phenotypes are separable from enhanced longevity [38–41, 52], which is also true of the long-lived IIS-mutant mice [16, 17, 19].

Several traits associated with increased longevity, such as increased resistance to stress, are candidates for the mechanism by which IIS increases lifespan. From an evolutionary perspective, oppositely regulating environmental stress resistance and developmental growth has the advantage of maximizing survival in both harsh and beneficial environments. Delayed ageing might hence be brought about as a by-product of ectopically inducing the organism's natural ability to cope with insults that cause molecular damage but in the protected environment of the laboratory [53]. Thus, identifying the system(s) that enhances stress resistance could also identify the cause(s) of longevity. Due to the wide range of possible stressors, this has become a large area of research that covers an increasing number of treatments for each of the model organisms. It cannot, therefore, be treated in detail in this review. However, of relevance here are treatments

that could point to an evolutionarily conserved mechanism of longevity.

Taking a candidate mechanism approach, a host of studies have identified enhanced oxidative stress resistance in long-lived IIS-mutant worms, flies and mice (e.g. [14, 15, 17, 54]). This mechanism was predicted by the 'free radical' and 'oxidative stress' theories of ageing in which reactive by-products of oxidative metabolism cause irreparable damage to macromolecules that accumulates with age [55, 56]. However, experiments examining whether enhancing such defences can increase the lifespan of wild-type animals have met with a mixture of both positive and negative results in each of the models (e.g. see [57–59] for worms, [60–64] for flies and [65, 66] for mice). This may simply be because we do not know enough about the relationship between stress resistance and longevity to establish causality. Alternatively, it may mean that lifespan is enhanced by other mechanisms.

Recently, a hypothesis-free approach has been taken to search for candidate longevity-assurance mechanisms, using whole-transcriptome microarrays. Several studies have been reported using long-lived worm IIS mutants [31, 67, 68]. These have generated lengthy lists of gene changes spanning a variety of functions. Interestingly, these lists do include several genes (such as superoxide dismutase) that function in oxidative stress resistance, supporting the hypothesis that they are important for lifespan extension. Nonetheless, when the effect of altering expression of these genes has been investigated directly, their effect on longevity has, at best, been minor [31]. This has been taken to indicate that many genes are required to act additively for the full lifespan effect caused by IIS mutations. This is perhaps understandable in light of how complex a phenotype ageing is. In addition to this complexity, lifespan is likely to be sensitive to the spatiotemporal effects of altering expression of these genes. In summary, it seems unlikely that a signalling pathway as far reaching as IIS will signal to one or a few gene products to slow ageing.

One way of capturing the complexity that altered ageing may demand is to perform higher-level functional

analyses of the array data to extract changes that occur in groups of genes whose products are involved in a coordinated function. This approach has revealed a set of genes (including those encoding cytochrome P450s, short-chain oxidoreductases, UDP-glucuronosyl transferases, glutathione S-transferases and solute transporters), which act to detoxify lipophilic xenobiotic compounds, as a candidate mechanism for longevity assurance [68, 69]. What is more remarkable about these genes is that their coordinated regulation recurs in long-lived IIS mutant flies and long-lived growth-hormone defective mice with lowered levels of IGF-1 [70, M. Piper, L. Partridge, unpublished data]. They are thus components of an evolutionarily conserved defence process that potentially acts against damaging compounds that contribute to ageing. In support of this array-based prediction, it has recently been shown that long-lived growth-hormone-releasing hormone mutant 'Little' mice are resistant to treatment with several toxic xenobiotic compounds [71] as are long-lived IIS-mutant flies [M. Piper, L. Partridge, unpublished data]. It now remains to be tested if an enhanced capacity to detoxify xenobiotics is sufficient to extend lifespan. In addition to xenobiotic detoxification, reduced protein synthesis has been identified as a putative evolutionarily conserved mechanism for lifespan extension [70]. Whilst there is direct experimental evidence for this in worms [72–74], this process remains untested in flies and mice. It is unknown how reducing protein synthesis might act to extend lifespan, but it is interesting that this intervention confers increased heat-stress resistance on the worms, indicating enhanced somatic maintenance [73]. This is perhaps connected to the observation that overexpressing protein chaperones can extend both worm [75, 76] and fly [77, 78] lifespan. Alternatively, reduced protein synthesis may trigger an increase in protein turnover rates via autophagy and the proteasome. Autophagy, in particular, has attracted interest as a longevity-assurance mechanism, because it has been shown to be required for the long life of IIS-mutant worms [79]. It is thought it may act to cleanse cells of damaged proteins and organelles as a by-product of its action in recycling nutrients [80]. Finally, microarray data has also identified enhanced immunity as a candidate longevity assurance process in flies [M. Piper, L. Partridge, unpubl. data]. A recent

report has indeed confirmed that long-lived IIS-mutant flies are more resistant to infection than controls [81], which concurs with data from IIS-mutant worms [82] and mice [18] as well as dwarf mice with lowered circulating insulin and IGF-1 [45]. To date, evidence in flies indicates that enhanced immunity may not extend normal lifespan [83, 84] but, similar to oxidative stress resistance, the exact method for manipulating immunity may be critical for observing longer life.

In most of these cases, higher-level functional analyses reveal complex processes involving many genes that may not be functionally important when altered individually. This makes genetic manipulation a difficult proposition because groups of genes would need to be manipulated together in the correct tissues to have the same effect on ageing as IIS. To address this, two analytical approaches can be taken.

First, analyses of transcriptome data to uncover over-represented transcription factor binding sites amongst co-regulated genes may provide the means to harness coordinated gene changes of transcripts encoding diverse functions. Amongst the transcriptional elements over-represented in the worm and fly IIS-mutant arrays, a conserved 'GATA' element has emerged [31, 68, M. Piper, L. Partridge, unpubl. data]. A family of transcription factors can bind this element to play a role in the development of worms, flies and mice [85, 86]. In addition, GATA factors are involved in controlling genes required for the invertebrate innate immune response [87, 88] as well as the control of reproductive protein synthesis in flies [89]. This element and its cognate binding proteins are therefore good candidates for the control of several aspects of physiology in a manner that interacts with IIS and perhaps lifespan as well. It is also interesting that this family of transcription factors are the downstream targets of TOR signalling in yeast [90] and mosquitoes [91] to signal dietary nitrogen availability. TOR is an evolutionarily conserved signalling protein that can extend lifespan in yeast [92, 93] worms [94] and flies [95] via an unknown mechanism. Perhaps one or more of these GATA transcription factors contribute to the lifespan enhancement seen in both the TOR and IIS mutant animals.

Secondly, tissue-specific information about expression profiles will be critical to capture the spatial requirements of IIS-induced changes. Tissue-specific transcriptome profiles are routinely generated in rodent studies, but have only recently been used in flies [96–98]. Although extremely labour-intensive, it is important that future work characterizes the transcriptional effects of fat-specific dFOXO overexpression in flies, since this appears to extend lifespan via a secondary signal (see above) [38–40]. Furthermore, it would be interesting for the same array profile to be generated from the fat tissues of long-lived, fat-specific insulin-receptor mutant mice [16] to look for evolutionarily conserved changes between the fly and mouse. These data, together with other tissue-specific changes, as well as knowledge of the transcription factors active in these tissues, should provide real tools with the potential to manipulate the downstream effects of IIS critical for modulating lifespan.

#### Altered IIS and its protective effects on physiology

In IIS mutant animals with extended lifespans the usual decline in physiological systems with age is slowed. In order to understand and potentially treat ageing-related disorders, it is important to study the mechanisms of IIS action (such as those mentioned above) in conjunction with their specific effects on different areas of physiology. For certain types of decline, such as bone loss, it is necessary to use mammalian models, but for others the invertebrate organisms can be used to make more rapid progress and to provide directions for future work on mammals. Recently this has become realistic for some age-related diseases, with the development of molecular models for complex dysfunctions such as neurodegeneration [99, 100]. Indeed, it is already known that long-lived IIS-mutant worms are less prone to aggregation of the Alzheimer's  $A\beta_{1-42}$  peptide [23] and it remains to be tested if this is also true for fly models [101, 102] in order to direct research with mouse models [103, 104]. Other models of neurodegeneration (such as Parkinson Disease and Huntington Disease) have also become available in the invertebrates [102, 105–114] and will be similarly useful to aid research in mammals. In other studies, IIS mutant

worms, flies and mice have all been shown to resist declining mobility with age [10, 18, 115, 116] and recent work has shown aged IIS-mutant flies are at lower risk of induced heart failure [24, 26]. Thus it appears that future work on how the quality of aged life is affected by IIS and how this could lead to potential drug treatments will also have the three model species of ageing at its focus, with the heady prospect of a broad-spectrum, preventative medicine for the diseases of ageing.

#### Conclusions

Ageing is a complex process that was once thought to be beyond the reach of genetic alteration. However, with the combined use of worms, flies and mice, it has now become established that single gene alterations that lower IIS activity can extend both the quantity and quality of life. Thus, ageing is genetically accessible.

Since these initial discoveries, important aspects of the timing and tissue-specific requirements of these interventions have come to light. These have shown that genetic manipulations of the signalling pathway during early adulthood in neuronal or fat tissues are sufficient to alter lifespan in each of the three species. These appear to act in communication with one another via IIS as well as at least one other signal to achieve the effect of enhanced longevity. At present it is unknown what the nature of this second signal is. Remarkably, IIS manipulations have also shown that it is possible to alter lifespan independently of many of the detrimental pleiotropic effects that alteration of IIS can have on physiology. Thus there is a real possibility that ageing and its detrimental effects could be manipulated by precise pharmacological interventions. Exactly where and to what functions these interventions should be targeted has been the subject of intense research. Molecular evidence points to three possibly evolutionarily conserved mechanisms: enhanced xenobiotic detoxification, decreased protein synthesis and enhanced immunity. It now remains for future work to characterize how these mechanisms may be involved and how they might be appropriately manipulated to alter ageing.

There is no mistaking the enormity of the task ahead for ageing research, but with the aid of detailed comparative interspecies research, the evolutionarily conserved pathway to a longer, healthier life certainly seems a realistic possibility.

### Conflict of interest statement

No conflict of interest was declared.

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