Drosophila as a model for ageing

Matthew D.W. Piper[1] & Linda Partridge[2, 3, *]

1. School of Biological Sciences, Monash University, Clayton, Victoria, 3800, Australia
2. Max Planck Institute for Biology of Ageing, Köln, 50931, Germany.
3. Institute of Healthy Ageing and Department GEE, UCL, Darwin Building, Gower Street, London, WC1E 6BT, UK

* correspondence: Partridge@age.mpg.de

Abbreviations: 4EBP – translation initiation factor 4E binding protein; AMPK – AMP activated protein kinase; AOP – anterior open; ATF4 – activating transcription factor 4; CHOP – C/EBP homologous protein; CR – calorie restriction; DR – dietary restriction; eIF – eukaryotic translation initiation factor; ERK – extracellular signal-regulated kinase; ETS – E-twenty six; FKH – forkhead; FOXO – forkhead BoxO; GCN – general control non-derepressible; IIS – insulin and IGF-1 like signaling; ilp – insulin-like peptide; PI3K – phosphoinositide 3-kinase; ROS – reactive oxygen species; mTOR – mechanistic target of rapamycin; ORF – open reading frame; REPTOR – repressed by TOR; S6K – S6 protein kinase; TSC – tuberous sclerosis complex

Abstract

Drosophila melanogaster has been a key model in developing our current understanding of the molecular mechanisms of ageing. Of particular note is its role in establishing the evolutionary conservation of reduced insulin and IGF-1-like signaling in promoting healthy ageing. Capitalizing on its many advantages

for experimentation, more recent work has revealed how precise nutritional and genetic interventions can improve fly lifespan without obvious detrimental side effects. We give a brief summary of these recent findings as well as examples of how they may modify ageing via actions in the gut and muscle. These discoveries highlight how expanding our understanding of metabolic and signaling interconnections will provide even greater insight into how these benefits may be harnessed for anti-ageing interventions.

Introduction

Drosophila melanogaster has been used as a model organism for ageing research for more than 100 years. Possibly the first quantitative account of *Drosophila* lifespan under lab conditions was reported by Roscoe Hyde in 1913, in which he observed, and correctly outlined, how hybrid vigour could account for apparent lifespan extension when crossing two inbred lines (Hyde, 1913). A more systematic programme of work on *Drosophila* ageing, which established many of the conditions we still employ today, was initiated in 1921 by Raymond Pearl & Silvia Parker. In their first article (Pearl and Parker, 1921), they report a catastrophic air conditioning failure that destroyed their colony of mice, which were intended for ageing studies and, after consultation with Prof Morgan and Dr Loeb, they took the decision to use *Drosophila* as their model for lifespan research. In this and 13 subsequent articles during the course of 14 years, Raymond Pearl and co-authors outline the basic dietary requirements of *Drosophila* for survival, as well as the effects of repeated anaesthesia (with ether), inbreeding depression, adult housing density and life-stage-specific temperature variations on adult lifespan.

At a similar time, the effect of temperature on the duration of life was reported (Loeb and Northrop, 1916), and established that a 10°C reduction in temperature resulted in an approximate doubling of lifespan, thus conforming to the same temperature coefficient as enzyme reactions in solution. Since flies are ectotherms, these findings promoted the concept that lifespan could be determined by an organism's rate of living (Pearl, 1928) as if it was dictated by the consumption of a substrate which was available in a predetermined quantity, or was related to the products/by-products of metabolic biochemistry.

The rate of living can dictate lifespan if the damaging by-products of aerobic metabolism as well as mechanical damage accumulate to the point of being overwhelming (Harman, 1956). Biological organisms fight back against such damage by enzyme systems and small molecules to mop up and/or repair chemical damage, as well as by the replacement of damaged cells with new cells derived from stem cells. Whether or not this damage is the fundamental cause of ageing, or if inappropriate

and uncontrolled continuation of growth is key (Blagosklonny, 2006), the incentive to maintain biological systems in working order and thus ensure longevity is provided by the imperative to reproduce. Thus, in an organism like *Drosophila* where there is no recognised advantage of parental or grand-parental care, evolutionary selection pressure drops after the age of first reproduction and physiological systems become freer to decline (Medawar, 1952; Rose et al., 2008; Williams, 1957), which defines ageing.

Flies show physiological signs of ageing

Ageing can be described as the decline in function over time that leads to reduced fertility and eventual death. The characteristics of population survival for *Drosophila* can reveal information about the progression of ageing as well as any additional, life-shortening effects from non-ageing related deaths due to things such as poor genetic stock and/or environmental factors (Pearl and Parker, 1921; Piper and Partridge, 2016). A typical, healthy and well-maintained outbred *Drosophila* population will have a median lifespan of approximately 70 days and maximum of approximately 90 days at 25°C (Ziehm and Thornton, 2013; Ziehm et al., 2013).

At a more detailed level of physiological decline, numerous markers of ageing-related loss of function can be observed. These include changes to metabolism (reduced resting metabolic rate, decreased protein and fat synthesis), behavior (reduced feeding, courtship and exploration, and increased sleep fragmentation), reduced stress resistance, reduced reproductive capacity (reduced egg laying and hatching success, decreased sperm and accessory fluid production and sperm competitive success), altered neuronal function (impaired learning and memory), modified physical activity (impaired negative geotaxis, reduced voluntary flying and walking), reduced immune capacity, progressive dysplasia and reduced barrier function in the gut, and compromised cardiac function (Figure **1** (Gargano et al., 2005; Grotewiel et al., 2005; Iliadi et al., 2012; Tamura et al., 2003). These features are important to study for two reasons: first, to understand how any lifespan-modifying intervention may affect the progression of these metrics of "health" over time, and; secondly, ascertaining if any of these changes play a causal role in the demographic ageing of

the fly and thus could be targeted by interventions to slow ageing.

Evolutionary conservation of the genetic basis of ageing

Given the discovery that ageing is under genetic control and that different organisms have evolved vastly different lifespans, it is evident that ageing would be modifiable by genetic manipulations. From 1983-1993 the first single gene mutations to extend lifespan were identified and characterized in worms as defective in components of the insulin and IGF-1 like signaling (IIS) pathway (Friedman and Johnson, 1988; Kenyon et al., 1993; Klass, 1983). In 2001, amongst the first fly mutants to extend lifespan two were found (Clancy et al., 2001; Tatar et al., 2001), both of which also acted to reduce IIS. Subsequently, mutations in mouse insulin or IGF-1 signaling were also shown to extend life (Blüher et al., 2003; Holzenberger et al., 2003), and subsequent genome wide association studies link polymorphisms in the insulin pathway transcription factor FOXO with length of life in humans (Anselmi et al., 2009; Flachsbart et al., 2009; Kuningas et al., 2007; Pawlikowska et al., 2009; Willcox et al., 2008). Thus, at least some aspects of the genetic basis of ageing are evolutionarily conserved, establishing small, short-lived invertebrates, such as *Drosophila*, as useful tools for examining the molecular mechanisms of ageing.

Why use flies in ageing research?

Drosophila have many advantages for use in ageing research and, as highlighted above, when used in conjunction with the nematode worm *Caenorhabditis elegans*, as well as other short-lived invertebrates such as the baker's yeast *Saccharomyces cerevisiae*, they are an extremely effective tool for studying evolutionarily conserved aspects of ageing (Kennedy et al., 2017). Where yeast can rapidly provide information about aspects of cellular ageing, the additional interactions at play within and between tissues of a multicellular, differentiated, organism (such as IIS) can be modeled in worms and flies. As a general rule, worms live for ~3 weeks and flies for ~3 months. When combined for their individual experimental strengths, the invertebrates can function as an effective pipeline of discovery of evolutionarily conserved

interventions to enhance lifespan, which can be targeted for experiments in the longer lived vertebrate systems, such as killifish (lifespan ~6-8m), mice (~3y) and rats (~3y).

The particular features of *Drosophila* that make it an effective model for ageing research are: its low cost of rearing and housing, the absence of regulatory oversight for their use in experiments, the ease of generating large populations, its well defined dietary requirements, its easily quantified reproductive output, its distinct tissues that can be dissected and genetically manipulated, and a large collection of readily available genetic tools, including CRISPR reagents for genome editing as well as constructs for over-expressing or knocking down any gene in a tissue and timing specific manner. Fly tissues are equivalent to many of those found in mammals, including the heart and kidney, both absent in *C. elegans*, and a high proportion (77%) of genes associated with ageing-related diseases in humans are expressed in the equivalent fly tissues (Kennedy et al., 2017). Most importantly, their relatively short lifespan means they can be used in repeated rounds of experimentation to refine conditions that maximise life.

However, some of these advantages double as disadvantages. In particular, the fact that *Drosophila melanogaster* is so small makes characterizing health with ageing a difficult prospect. What's more, no one knows what flies die of, although recent studies examining increased gut dysplasia and leakiness with ageing in females may give clues (Rera et al., 2013). Probably the simplest, and most popular, non-destructive assay for ageing-related health is to measure the ability of flies to climb (negative geotaxis) (Gargano et al., 2005). This yields a combined measure of neuronal and muscular function and, by this measure, IIS mutants that are long lived are also healthier for longer (Gargano et al., 2005).

Delivering healthy ageing without side effects

A principle goal of biogerontology is to shift the emphasis from understanding and treating the symptoms associated with ageing to comprehending the underlying molecular mechanisms with the hope of using this knowledge to design therapeutic approaches that prevent or slow the appearance of multiple ageing-related symptoms

and thus compress morbidity. A key aspect of this work is to understand the interrelationship between the benefits offered by interventions into ageing and their associated costs, in order to maximize health improvement with fewest side-effects. With increasingly fine-scaled adjustments to experimental conditions, for example dietary composition, and with interventions targeted to specific tissues and cell types at specific times during the life cycle, there is increasing evidence that broad-spectrum amelioration of the effects of ageing can be achieved (Giannakou et al., 2004; Grandison et al., 2009; Mair et al., 2003). In this endeavor, model organisms including *Drosophila* are key, since it is only through many rounds of experimentation with slightly adjusted conditions, that such discoveries have been made.

Outline and aims

Recently, nine hallmarks of ageing have been recognized as of widespread occurrence in different organisms (López-Otín et al., 2013), with experimental evidence available to indicate that their occurrence contributes to the progression of ageing related functional decline. These hallmarks are: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication. A central assumption in the field of biogerontology, is that ageing results from a combination of these mechanisms and that the various hallmarks are interconnected, with different types of loss of function interacting to cause the ageing process.

In this review, we highlight recent research with *Drosophila* that has contributed to our current understanding of the molecular mechanisms of ageing, and interventions that can ameliorate its effects. In particular, we focus on some of the ways by which the various hallmarks of ageing in *Drosophila* are shown to be interconnected, with the intention of identifying nodes of control that might be fruitful for future research. In doing so, we have not been exhaustive in our coverage of how *Drosophila* has contributed to our knowledge of the mechanisms of ageing, and nor do we cover the contribution of the fly to our understanding of how ageing has evolved. For additional

information and related topics, we point the reader to several other reviews(Barnes and Partridge, 2003; Gems and Partridge, 2013; Kapahi et al., 2016; Kirkwood, 2002; Kirkwood and Shanley, 2005; Partridge, 2001; Partridge and Gems, 2002; Rose, 1994)

Dietary manipulations and lifespan

One of the most important discoveries in the field of ageing research is that mild dietary restriction (DR; often called calorie restriction (CR)), without malnutrition, can extend lifespan. The first reported instance is most usually attributed to Clive McCay for his work on white rats (McCay et al., 1935), and since then DR has been implemented successfully to extend lifespan in yeast (Jiang et al., 2000), worms (Klass, 1977), flies (Chapman and Partridge, 1996) and monkeys (Colman et al., 2009; Mattison et al., 2012) although here the benefits appear to be lessened by increased age-related frailty (Hultström, 2015). Independent of the longevity effects, diet restriction in monkeys is thought to extend healthspan (Mattison et al., 2012, 2017). Interestingly, work from flies also indicates that DR reduces age-specific mortality rapidly, and apparently overturns the effects of dietary history (Mair et al., 2003) – a finding that promises DR benefits at any stage in life.   As a result of these collective findings, and because food manipulation is accessible to anyone who chooses, DR in some form has become a relatively mainstream activity for health-aware humans that hold out the long term hope of gaining extra years of healthy life (Rizza et al, 2014).

Diet Balance

Recent work with model organisms, in particular *Drosophila* and mice, has begun to reveal that reduced intake of specific nutrients, rather than of overall calories, mediates the health benefits of DR, with dietary protein playing a key role (Grandison et al., 2009; Mair et al., 2005; Miller et al., 2005; Zimmerman et al., 2003). Furthermore, altering the proportions of the macronutrients (protein, lipid, carbohydrate) in a diet that is consumed *ad libitum* can induce an extension of

lifespan equivalent to that seen when restricting access to all dietary nutrients (Lee et al., 2008; Mair et al., 2005; Skorupa et al., 2008; Solon-Biet et al., 2014). It is not clear if altered diet balance extends life by the same or different mechanisms as traditional DR/CR protocols, since the precise molecular and physiological mechanisms involved have not been identified in either case. A further complication is that, at least in mice, DR usually involves a feed/fast cycle in the experimental animals, since they consume all of their restricted diet as soon as it is supplied to them and fast for the remainder of the 24h period (Speakman et al., 2016). Whatever the mechanisms at work, from the point of view of humans, most of whom find it impossible to comply with a DR regime, changing diet balance can be considered a more manageable practice than simply eating less food. Much effort is thus focused on developing diets in which the nutritional components have been manipulated to mimic the effects of fasting for improving long-term health (eg (Cheng et al., 2017)).

For those experiments where the dietary macronutrient balance for flies and mice has been altered, the optimum for lifespan falls at a point where the protein content, as a proportion of total dietary energy, falls below the optimum for reproduction (Jensen et al., 2015; Lee et al., 2008; Mair et al., 2005; Skorupa et al., 2008; Solon-Biet et al., 2015, 2014). This pattern of responses to diet balance has also been observed for ants (Dussutour and Simpson, 2012), crickets (Harrison et al., 2014; Maklakov et al., 2008) and the Queensland fruit fly (Fanson et al., 2009). Given the strength of this trend and its apparent evolutionary conservation, it is interesting to probe the mechanisms by which protein is such a strong determinant of lifespan.

Several studies have manipulated individual amino acids in the diets of flies and mice, and shown that single essential amino acid dilutions can extend lifespan. By far the most consistent is the effect of reducing the essential amino acid methionine which extends life of both flies and mice, but is accompanied by lowered egg laying in flies, and reduced growth rate and enhanced early life mortality in mice (Grandison et al., 2009; Miller et al., 2005). The downstream mechanism mediating the effects of this intervention are not clear, but much attention has been paid to methionine's critical roles in translation initiation, its degradation to cysteine via the transulfurylation pathway and the subsequent role of its catabolites in protein

methylation and cellular detoxification. Each of these functions could act to modify a hallmark of ageing.

Given the dozens of nutrients involved and their interacting effects on each other in the context of whole organism physiology, there are doubtless additional subtle nutritional interactions that can modify lifespan. While this opens up a vast research opportunity, it also raises an important issue in the field of *Drosophila* research: there are dozens of "standard" *Drosophila* diets (Piper and Partridge, 2007) in use by different laboratories and there is little consistency in how these are reported. If extremely small differences in nutrients, such as the dilution of a single amino acid, can modify lifespan, then it is highly likely that each different study using a different diet comprised of natural ingredients will yield some degree of lifespan variation. While holidic diets can be used to get around this problem (Piper et al., 2014), the expense and complexity of their preparation can be off-putting. A more reasonable expectation is that all components of complex diets are made explicit upon publication and basic nutritional analyses of the constituents are made available.

Nutrient signaling pathways and lifespan

The long history and vast body of research showing that diet modifies lifespan leads inevitably to the conclusion that nutrient sensors and their downstream signalling pathways mediate these responses. Here we present knowledge about the major nutrient signaling pathway discoveries that have been shown to extend *Drosophila* lifespan.

Insulin / IGF-1 like signaling

Insulin is typically associated with maintenance of glucose homeostasis, a function that is at least partially conserved in *Drosophila* (Graham and Pick, 2017). It also has important roles in growth, reproduction, adult health and ageing (Hafen, 2004; Nässel and Broeck, 2016). The *Drosophila* genome encodes 8 insulin-like peptides (*ilps 1-8*) as well as a single insulin receptor and the intracellular components of the canonical PI3K branch (Nässel and Broeck, 2016). Upon activation, these transduce a signal to phosphorylate and inactivate the transcription factor FOXO by nuclear

exclusion (Puig et al., 2003). Together, this system is thought to fulfill the combined functions of mammalian insulin, IGF-1 and relaxins (Nässel and Broeck, 2016). Since the first report of mutations in IIS that extend fly lifespan (Clancy et al., 2001; Tatar et al., 2001), most other pathway components have also been reported to modulate lifespan (Proshkina et al., 2015) in a manner that requires the FOXO transcription factor (Slack et al., 2011).

Given the goal of biogerontology to find interventions that promote healthy ageing without imposing costs, the large family of *Drosophila ilp* genes is a tantalizing target for intervention. Although there are clear examples of at least some of the ilps having redundant roles (Grönke et al., 2010) there is evidence that each peptide may elicit different outputs for adaptation to specific conditions: each possesses its own characteristic pattern of expression that varies with developmental time (Brogiolo et al., 2001), tissue (Chintapalli et al., 2007) and in response to complex nutritional stimuli (Post and Tatar, 2016). Genetic manipulation of the *ilps* has revealed their various roles in growth (Boulan et al., 2015), sugar homeostasis (Graham and Pick, 2017), reproduction (LaFever and Drummond-Barbosa, 2005) and healthy ageing (Grönke et al., 2010; Wessells et al., 2004). Most relevant for this discussion, individually knocking down *ilp2* but no other *ilp*, is sufficient to extend fly lifespan and this effect is augmented by additional knockdown of *ilps 3* and *5* (Grönke et al., 2010). These mutants also exhibit enhanced resistance to stressors such as heat, lipophilic toxins and ROS, and also have lowered fecundity and elevated body fat. It is possible to dissociate each of these physiological changes from the longevity phenotype (Broughton et al., 2005; Giannakou et al., 2004; Slack et al., 2011), indicating that precise interventions to alter subsets of change caused by FOXO activation will benefit lifespan without dramatic side effects.

Specific over-expression of FOXO in the gut and fat tissues is sufficient to extend fly lifespan (Giannakou et al., 2004; Hwangbo et al., 2004). By examining the direct transcriptional outputs of activated FOXO in these tissues, Alic et al (2014) identified 5 transcription factors that may mediate the resultant lifespan extension (Alic et al., 2014). One of these, an ETS-family transcriptional repressor called AOP, has a similar set of transcriptional targets as FOXO. Activating AOP in the gut and fat

tissues, either directly by over-expression (Alic et al., 2014) or indirectly by inhibiting RAS/ERK signaling (Slack et al., 2015), revealed that AOP could also extend lifespan. Due to its key function in cancer, RAS/ERK signaling has been the target of a great deal of drug development, and Slack *et al* also showed that one of the FDA approved drugs to reduce its activity, trametinib, could extend fly lifespan when administered in the food (Slack et al., 2015). This is an important finding as its action may well be relevant to protect higher organisms from ageing and joins a short list of FDA approved pharmaceuticals with demonstrated anti-ageing properties (Roth and Ingram, 2016).

Given that AOP and FOXO work coordinately to extend life when over-expressed in gut and fat tissues, Alic *et al* characterized the overlap in their transcriptional outputs to seek out changes that could coordinate their roles for longer life (Alic et al., 2014). One of these genes, *Obp99b*, is a possible humoural factor that could be important for inter-tissue signaling. This gene has also recently been found to be strongly up-regulated in a *Drosophila* model of slowed ageing through reproductive diapause (Kučerová et al., 2016). This gene awaits further investigation.

Mechanistic Target of Rapamycin (mTOR) signaling

mTOR is an amino acid sensitive signaling kinase with an essential role in growth (Saxton and Sabatini, 2017). Genetically reducing mTOR function was first shown to extend life in worms (Vellai et al., 2003), an effect that is evolutionarily conserved to yeast (Kaeberlein et al., 2005), flies (Kapahi et al., 2004) and mice (Lamming et al., 2012).

mTOR is generally regarded as the predominant amino acid sensing and signaling molecule in cells and so it is unsurprising to find that it has been closely linked with longevity in response to changes in dietary macronutrient composition. In particular, for flies, both activation of the mTOR suppressor TSC2 or administration of the mTOR inhibiting drug rapamycin can overcome the lifespan shortening effects of high dietary yeast (the flies' only source of protein) or elevated dietary amino acids (Bjedov et al., 2010; Emran et al., 2014; Kapahi et al., 2004). A similar association

between dietary protein, mTOR function and lifespan has also been found for mice (Solon-Biet et al., 2014).

Rapamycin, which inhibits TOR activity, can extend lifespan in yeast, (Medvedik et al., 2007), worms (Robida-Stubbs et al., 2012), flies (Bjedov et al., 2010) and is one of most heavily studied drugs to increase lifespan of mice (Miller et al., 2014). In a recent meta-analysis of 29 mouse lifespan experiments using rapamycin, it was found to extend life robustly, but effect size varied with gender (greater effects in females than males) and genetic background (Swindell, 2016). Rapamaycin is an FDA approved drug with potential for treating ageing in humans. Indeed pre-treatment with rapamycin can rescue the lowered immune response of elderly subjects to immunization against influenza to youthful levels (Mannick et al., 2014).

Suppressing mTOR has the effect of generally reducing translation as well as activating autophagy, two heavily studied phenomena thought to lead to longer life by enhancing proteostasis (Taylor and Dillin, 2011). This increasingly popular concept captures the beneficial effects of many longevity interventions across different model organisms and thus qualifies as one of the nine hallmarks of ageing (López-Otín et al., 2013). The most commonly studied molecular targets of mTOR phosphorylation are the translational activator S6 kinase and the translational repressor 4EBP. Knocking down S6K has been reported to be sufficient to extend life in both flies (Kapahi et al., 2004) and mice (Selman et al., 2009) and its suppression is required for rapamycin to extend fly lifespan (Bjedov et al., 2010). 4EBP was also identified as involved in the lifespan response to rapamycin (Bjedov et al., 2010) and DR (Zid et al., 2009), but in a condition-specific manner (Partridge et al., 2011; Tatar, 2011). Finally, functional autophagy is required for lifespan extension by rapamycin treatment (Bjedov et al., 2010), and overexpression of autophagy components *ATG1* (Ulgherait et al., 2014) or *ATG8a* (Simonsen et al., 2007) is sufficient to prolong fly lifespan. Thus, dietary and/or drug treatments to enhance autophagy have become an attractive prospect for healthy ageing.

The polyamine spermidine can enhance lifespan in an autophagy dependent manner in yeast, worms and flies (Eisenberg et al., 2009), and two drugs identified in a screen

for molecules to enhance autophagy, AUTEN-67 and AUTEN-99, can also extend fly lifespan (Kovács et al., 2017; Papp et al., 2015). In all three studies, the lifespan of control flies was extremely short and displayed signs of non-ageing-related deaths, indicating that follow up work is warranted. In the case of spermidine, the evidence for its anti-ageing properties is strengthened by the evolutionary conservation of its effects, which have recently been expanded to include enhanced healthy ageing and lifespan of mice (Eisenberg et al., 2016). This same study also notes that high spermidine intake in humans is associated with lowered risk of cardiovascular disease (Eisenberg et al., 2016). Together, these data promote the case that reduced activity of mTOR lengthens life via enhanced proteostasis, and especially via enhanced autophagy.

In addition to its effects on translation and autophagy, mTOR signals to control other cellular functions, including transcription, through all three RNA polymerases (Ghosh et al., 2014; Iadevaia et al., 2014; Marshall et al., 2012). For the Pol II-related transcription factors, Bülow et al, (2010) identified the forkhead transcription factor FKH (Bülow et al., 2010) and Tiebe et al (2015) identified 2 transcription factors REPTOR and REPTOR-BP as important for larval growth and interacting with FOXO to regulate overlapping sets of genes (Tiebe et al., 2015). None of these interactions have yet been tested in the context of mTOR-mediated longevity. Of the transcription factors noted above that are targeted by mTOR for altered activity, FKH is a candidate for lifespan responses to nutrition since its worm orthologue pha4 is required for DR to prolong life (Panowski et al., 2007). Additionally, a recent study in flies has shown that knocking down the GATA transcription factors serpent or GATAe can suppress the lifespan shortening effects of high dietary amino acids (Dobson et al., 2016). The GATA family of transcriptional regulators modify amino-acid-sensitive transcription in a TOR-dependent manner in yeast and mosquitoes (Cooper, 2002; Park et al., 2006) and have also been associated with longevity in worms (Budovskaya et al., 2008; Zhang et al., 2013). Thus, the various transcriptional outputs of mTOR signaling are worthy of additional investigation for their impact on longevity.

GCN2/ATF4

Another important amino acid sensor for adaptation to nutritionally imbalanced diets is the evolutionarily conserved protein kinase GCN2. Its function was first characterized in yeast, where it was shown to be activated by uncharged tRNAs and to phosphorylate and inactivate the translation initiation factor eIF2 (Dever et al., 1992; Wek et al., 1989), thus reducing general translation. At the same time, small ORFs in the 5' UTR of some genes cause their expression to be selectively up-regulated (Abastado et al., 1991). The GCN4 transcription factor is the product of one such up-regulated gene and it functions to up-regulate dozens of genes involved in amino acid and purine biosynthesis (Hinnebusch, 1988). ATF4 is the fly and mammalian orthologue of GCN4 and its expression is also enhanced by translational control following phosphorylation of eIF2alpha by one of a family of stress responsive kinases that includes a GCN2 orthologue (Vattem and Wek, 2004).

Initial studies on GCN2 in mice indicated that it was required for rapid rejection of amino acid imbalanced diets (Hao et al., 2005; Maurin et al., 2005), but more recent work has shown these effects are likely to act via a non-GCN2-dependent route (Leib and Knight, 2015). A similar role for fly GCN2 has also been reported for larval feeding in *Drosophila* (Bjordal et al., 2014), but this remains to be investigated in adults.

In flies, GCN2 was recently found to be required for longevity in response to yeast restriction, and under these conditions, it phosphorylated eIF2alpha, which resulted in up-regulation of 4EBP (Kang et al., 2017). These conditions were associated with repression of global translation, with the exception of selected proteins whose expression was enhanced (Kang et al., 2017). It will be interesting in future to understand how these up-regulated proteins might function to enhance lifespan. An attractive target is *Sestrin2*, which in mammals is induced by ATF4 in response to amino acid stress and acts to repress mTOR function (Ye et al., 2015). In flies, sestrin has been implicated in ageing-related physiological decline via its feedback inhibition of mTOR (Lee et al., 2010). It will also be interesting to examine the tissue requirements of this pathway's protective effects as well as any humoural signals it

may trigger, especially in light of the recent finding that fat-specific amino acid reduction acts via GCN2 to control germline stem cell maintenance in the ovary (Armstrong et al., 2014).

Finally, ATF4 and its target gene CHOP have been found in mouse livers to have higher expression in five different treatments known to extend lifespan (caloric restriction, rapamycin treatment, methionine restriction, litter crowding and acarbose treatment (inhibitor of carbohydrate absorption)) (Li et al., 2014). Thus, GCN2 and other stress responsive kinases that activate ATF4 are candidate mechanisms for a convergence of protective effects across the different longevity models.

AMPK

The AMP activated protein kinase (AMPK) senses cellular energy status by monitoring intracellular AMP+ADP:ATP levels and is critical in balancing the use and storage of energy generating molecules. In flies, reduced AMPK function results in starvation sensitivity, hyperactivity, hyperphagia and abnormal lipid accumulation – all proposed to be signs that the flies experience symptoms of mild starvation due to inappropriate use of energy stores (Johnson et al., 2010).

Over-expression of AMPK is sufficient to extend lifespan in worms and flies (Apfeld et al., 2004; Greer et al., 2007; Mair et al., 2011; Stenesen et al., 2013; Ulgherait et al., 2014). In flies, up-regulating the AMPK alpha subunit in the fat body, muscle (Stenesen et al., 2013), neurons or intestine (Ulgherait et al., 2014) has been reported to extend lifespan. Interestingly, neuronal-specific over-expression of AMPK results in, and requires, enhanced neuronal autophagy for prolonged life, and also enhanced autophagy and delayed barrier dysfunction in the ageing gut (Ulgherait et al., 2014). This is linked to decreased *ilp2* levels, suggesting that reduced systemic insulin is responsible for coordinating inter-tissue activation of autophagy. In worms, neuronal

AMPK also appears to regulate a systemic signal to promote longevity in peripheral tissues by AMPK activation (Burkewitz et al., 2015). If activation of autophagy is the key lifespan preserving mechanism downstream of activated AMPK, this could occur via its inhibitory action on mTOR (Howell et al., 2017) or via the direct action of AMPK to phosphorylate and activate ATG proteins, which has been observed in mammals (Egan et al., 2011; Kim et al., 2011). Recently, in worms, both AMPK and DR have also been implicated in suppressing ageing-related loss of splicing fidelity, which is also sufficient to enhance lifespan (Heintz et al., 2016).

Metformin is a widely prescribed antidiabetic drug with a range of molecular activities including a reduction in signaling through insulin, IGF-1, mTOR, and AMPK, inhibition of the mitochondrial ETC, as well as a reduction in both ROS production and DNA damage (Barzilai et al., 2016). Given this hit list of longevity assurance mechanisms, it is unsurprising to find numerous reports from both worms (Cabreiro et al., 2013; Haes et al., 2014; Onken and Driscoll, 2010) and mice (Anisimov et al., 2008, 2011) that metformin administration can extend healthy lifespan and delay several markers of ageing related decline (Martin-Montalvo et al., 2013). Indeed, there is strong interest in advancing this drug as a treatment for ageing in humans, with planning and fundraising underway to establish a trial of metformin treatment on 3,000 65-79 year olds, following up for incidences of cardiovascular disease, cancer, dementia and mortality (Barzilai et al., 2016).

Surprisingly, the only report on metformin from *Drosophila* indicates no benefits to lifespan for a range of doses that were shown to be absorbed and stimulate AMPK (Slack et al., 2012). Possible explanations could include the absence of a metformin-sensitive microbiota, which was a requirement for lifespan extension in worms (Cabreiro et al., 2013), or because the experiments were done with outbred flies under dietary conditions already optimized for lifespan, and hence there was no additional effect of the drug to further extend their already long lifespan.

Tissues setting a limit on lifespan

In simple terms, ageing is characterized by varying failure rates across different tissues. If this is translatable to the invertebrate models, then targeting a single organ for protective genetic changes could extend life in one of two ways. Either it improves only the function of the organ to which it is targeted, and if that organ is lifespan-limiting, it will extend life until the next most limiting organ fails. Alternatively, if the target organ can coordinate general physiology to function better for longer, an extension of life and healthspan should be observed. While these two alternatives serve as a useful starting point, there is undoubtedly a more complex interplay between tissues in ageing. Obviously, organism-wide rather than a tissue-specific maintenance of function is more desirable, since it should protect against the intrinsic heterogeneity with which ageing manifests between individuals. Here, we present the findings from two tissue systems in flies, one capable of regeneration and the other post-mitotic, and how targeting them for anti-ageing interventions can extend lifespan. It is clear from both that we are only beginning to understand how each might coordinate whole-organism ageing.

The fly gut

The repeated observation that the gut is a key organ in which genetic modifications can regulate lifespan has highlighted this organ as an interesting target for further investigations. In particular, reducing gut IIS ((Giannakou et al., 2004; Hwangbo et al., 2004); gut and fat tissues), RAS/ERK signalling ((Slack et al., 2015); gut and fat), mTOR signalling ((Kapahi et al., 2004); muscle, gut and nervous system), or activating AMPK and the associated enhancement of autophagy (Ulgherait et al., 2014) is sufficient to extend *Drosophila* lifespan.

The gut is an important homeostatic organ that must balance apparently conflicting roles: to facilitate digestion and absorption as well as providing an accommodating environment for microbes that aid digestion, while at the same time acting as a barrier in the first line of defense against ingested toxins and

pathogens (Lemaitre and Miguel-Aliaga, 2013). The gut is also interesting because it is one of the few sites in *Drosophila* that houses a reservoir of active stem cells, making it a focal tissue for understanding the evolutionarily conserved role of the division of stem cells as a key player in ageing (López-Otín et al., 2013). Finally, the gut is also an important endocrine organ, being the production site for numerous signaling peptides involved in metabolic homeostasis (Lemaitre and Miguel-Aliaga, 2013). Thus, there are many reasons why maintaining healthy gut function can serve as a potent enhancer of fitness, and dysfunction can have profound consequences for organismal health. It is entirely possible that maintaining gut function for longer could enhance organismal lifespan either directly by enhancing its digestive and barrier function and / or indirectly via its role in regulating systemic metabolic homeostasis.

Soon after intestinal stem cells were first described in *Drosophila* (Micchelli and Perrimon, 2005; Ohlstein and Spradling, 2005), their over-proliferation and mis-differentiation was reported to increase in occurrence with ageing (Biteau et al., 2008; Choi et al., 2008). This has the consequence of compromising barrier function (Rera et al., 2012), with the implication that the gut may limit lifespan by allowing the infiltration of infection. Indeed, there is a strong correlation between the degree of gut proliferation and organismal lifespan (Biteau et al., 2010) as well as between the onset of gut microbial dysbiosis and lifespan (Biteau et al., 2010; Clark et al., 2015; Guo et al., 2014). Protecting flies against gut dysplasia or microbial dysbiosis, either by modifying local inflammatory signaling (Li et al., 2016) or by maintaining innate immunity in a youthful state for longer (Chen et al., 2014; Guo et al., 2014), has beneficial effects for gut microbial homeostasis and can enhance fly lifespan.

Given these important functions, it is not surprising to find that the gut is also critical in mediating the longevity effects of diet. The necessity to maintain dynamic gut growth differs markedly between male and female flies due to their differing nutritional requirements for gamete production. In contrast to

males, females resize their gut in response to nutrient quality and mating status, and thus can balance the benefits of a large gut for better nutrient extraction against the high metabolic costs of maintaining a large gut when nutritional resources do not warrant it. However, this resizing capacity comes at the cost of greater susceptibility to later life overgrowth (Hudry et al., 2016; Regan et al., 2016). Male and female flies also respond differently to DR: male lifespan is extended much less than that of females (Magwere et al., 2004). This may be because the protective effect of low nutrient diets against over-proliferation is more potent in females than in males, an argument that is supported by the finding that males with feminized guts have greater lifespan extension upon DR than non-modified males (Regan et al., 2016).

The degree to which gut dysfunction is generally limiting for lifespan is not clear, and it is possible to find situations where less age-related gut dysplasia and reduced barrier dysfunction do not correlate with longer life (eg male guts are visibly less overgrown and provide a better barrier with age than those of females, and yet males are the shorter-lived sex; (Regan et al., 2016)). But in the cases where gut function does appear to limit lifespan, it is yet to be clarified if prolonged maintenance fundamentally slows systemic ageing, or if it simply prolongs life until other organ failure(s) causes death. For this, a detailed understanding of gut derived signals (eg Obp99b in response to modified FOXO / AOP function) will be key for future work. Whatever the case, the gut is an important place to understand the details of how ageing alters stem cell function, as well as the more complex association with the ageing microbiota and immune system.

Muscle

Loss of muscle mass and function is a major contributor to the physical decline that occurs with ageing in humans. In flies, muscles contribute a large percentage of body mass and they have many structural similarities with those of mammalian muscles. One major difference, however, is the lack of regenerative capacity due to the absence of stem cells, thus rendering the fly

muscle a model of only some aspects of muscle ageing. However, this presents an opportunity to understand how manipulating ageing related dysfunction in non-replacing muscle cells can mediate systemic changes in healthy ageing (Demontis et al., 2013).

One of the first indications that *Drosophila* muscle can play an important role to slow ageing was uncovered with the discovery that adult, muscle-specific over-expression of dFOXO could extend fly lifespan (Demontis and Perrimon, 2010). This, at least in part, was proposed to be due to enhanced autophagy acting to improve proteostasis with ageing. Critically, this muscle-specific intervention triggered protective effects across the whole organism (eg enhanced autophagy) via an unknown signal that reduced feeding and thus promoted the benefits of DR. Another unknown, muscle-derived signal has been shown to extend lifespan in response to mild muscle-specific mitochondrial dysfunction (Owusu-Ansah et al., 2013) and, although the signals' identities are not known, in both cases they are thought to act indirectly to reduce systemic insulin signaling. Muscle tissue is also thought to coordinate organismal health by secreting the myokine myoglianin (Demontis et al., 2014). Muscle-specific over-expression of myoglianin or its transcriptional regulator Mnt was shown to be associated with reduced global rRNA, enhanced climbing ability with age and longer lifespan. These data indicate autocrine and endocrine effects of muscle to preserve body function. It will be interesting in future studies to understand exactly how these anti-ageing signaling signals work throughout the organism.

Understanding the metabolic constraints on improving ageing

The summaries above place the mechanisms that operate to enhance longevity into a hierarchy, with environmental factors and nutrient sensing higher up, and their effect on a broad network of metabolic and inter-tissue interactions lower

down. It is not surprising that the majority of discoveries have been made at the higher levels of this hierarchy, given the complexity involved at the lower levels, where disparate areas of physiology must coordinate to shape the long-lived phenotype.

The nine hallmarks of ageing cover a range of changes to the genome and its regulation, alterations to cellular function, and changes in tissues and the systemic environment (López-Otín et al., 2013). It is not known to what extent the molecular events that characterize each hallmark contribute to the overall physiological decline that leads to death, or how one hallmark influences the impact of another. Understanding their interconnection is, however, important for the future of designing therapeutic interventions that seek to slow ageing with minimal side effects. This work involves tracing the lineage of molecular events from the higher signaling levels to the downstream mechanisms. Most discoveries to date are the successes of reductionist approaches to ageing and yet the interconnections and constraints of metabolic and signaling pathways set a broader context to the way ageing hallmarks are expressed. This is illustrated by several recent discoveries showing that substrate availability can be key in dictating how maintenance of the epigenome can function to promote longevity (Berger and Sassone-Corsi, 2015; Brunet and Rando, 2017).

Recent work on *Drosophila* has shown that longevity is associated with lowered levels of free methionine (Kabil et al., 2011; Laye et al., 2015) to the extent that it is stoichiometrically limiting for translation and egg laying (Kabil et al., 2011; Piper et al., 2017). At the same time, and perhaps partially contributing to intracellular methionine limitation, methionine catabolism via the transulfurylation and associated pathways is enhanced, which results in increased substrates for methylation reactions (Figure **2**) (Larson et al., 2012; Parkhitko et al., 2016), as well as the production of glutathione and H_2S (Hine et al., 2015; Kabil et al., 2011), each of which can be protective against ageing. Since we know that diet-derived methionine is likely to be in limiting quantities when flies feed on natural diets (Grandison et al., 2009) it follows that the

establishment of methyl-derived epigenetic marks will be highly sensitive to variations in nutrition and metabolic status.

In other work, a metabolic limitation from central carbon metabolism in the form of cytosolic acetyl-CoA availability, has been shown to protect against ageing associated increases in histone acetylation (Peleg et al., 2016). Acetyl-CoA cannot cross the mitochondrial membrane and so must be exported from the mitochondrion as part of citrate shuttles, which also supply the cytosol with reducing power in the form of NADPH (Figure **2**). Limiting amounts of acetyl-CoA could have additional effects on ageing-related pathways beyond epigenetic marks, for example by reducing inhibitory acetylation of the pro-longevity transcription factor FOXO (van der Horst and Burgering, 2007) and relieving autophagy from acetylation-mediated repression (Mariño et al., 2014; Schroeder et al., 2014). Indeed, reducing neuronal cystoplasmic acetyl CoA in *Drosophila* is associated with enhanced autophagy (Eisenberg et al., 2014), and is sufficient to prolong life (Simonsen et al., 2007).

Together, these findings demonstrate how metabolic constraints, which are sensitive to an organism's nutritional status, can dictate the availability of activated C-1 and C-2 units that are required for chromatin maintenance and genomic stability, which in turn protects against ageing.

Metabolic pathways are networked by the shared use of many components, but principally by the relative abundance of ADP/ATP, NAD+/NADH and NADP+/NADPH (Nielsen, 2016). The inevitable consequence is that proper maintenance of these cofactor pairs is important for maintaining general metabolic homeostasis and thus will be important in longevity control (Bonkowski and Sinclair, 2016; Piper et al., 2005). Understanding and manipulating key points in metabolism, derived from genome scale metabolic modeling, may thus be a key future development towards identifying treatments for ageing, similar to the manner in which it has become commonplace in the search for therapeutic targets for cancer (Frezza et al., 2011; Nielsen, 2017; Yun et al., 2015).

Concluding remarks

Studies on the fruitfly *Drosophila* have been critical in developing our current understanding of the molecular basis of ageing. In particular, the combination of the fly's well defined nutritional requirements, its genetically accessible tissue systems and short lifespan make it ideal for developing precision interventions to extend healthy lifespan. Whilst reductionist approaches have been enormously successful in advancing knowledge to date, the work of developing a networked picture of how metabolism, signaling pathways and various tissues combine to produce longevity is only just beginning. For biogerontology to deliver on the promise of benefiting human aging, it will be important to understand how diverse aspects of metabolism are coordinated to achieve greater physiological health. This process will benefit from the development of large-scale metabolic models that pinpoint key nodes of control for intervention. For the same practical reasons that have underpinned its usefulness to date, these studies are particularly well suited to *Drosophila*.

References

Abastado, Miller, Jackson, and Hinnebusch (1991). Suppression of ribosomal reinitiation at upstream open reading frames in amino acid-starved cells forms the basis for GCN4 translational control. Mol Cell Biol *11*, 486–496.

Alic, N., Giannakou, M.E., Papatheodorou, I., Hoddinott, M.P., Andrews, T.D., Bolukbasi, E., and Partridge, L. (2014). Interplay of dFOXO and two ETS-

family transcription factors determines lifespan in Drosophila melanogaster. PLoS Genet. *10*, e1004619.

Anisimov, V., Berstein, L., Egormin, P., Piskunova, T., Popovich, I., Zabezhinski, M., Tyndyk, M., Yurova, M., Kovalenko, I., Poroshina, T., et al. (2008). Metformin slows down aging and extends life span of female SHR mice. Cell Cycle *7*, 2769–2773.

Anisimov, V., Berstein, L., Popovich, I., Zabezhinski, M., Egormin, P., Piskunova, T., Semenchenko, A., Tyndyk, M., Yurova, M., Kovalenko, I., et al. (2011). If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. Aging *3*, 148–157.

Anselmi, C., Malovini, A., Roncarati, R., Novelli, V., Villa, F., Condorelli, G., Bellazzi, R., and Puca, A. (2009). Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. Rejuv Res *12*, 95–104.

Apfeld, J., O'Connor, G., McDonagh, T., DiStefano, P., and Curtis, R. (2004). The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in C. elegans. Genes Dev *18*, 3004–3009.

Armstrong, A., Laws, K., and Drummond-Barbosa, D. (2014). Adipocyte amino acid sensing controls adult germline stem cell number via the amino acid response pathway and independently of Target of Rapamycin signaling in Drosophila. Development *141*, 4479–4488.

Barnes, A., and Partridge, L. (2003). Costing reproduction. Anim Behav *66*, 199–204.

Barzilai, N., Crandall, J.P., Kritchevsky, S.B., and Espeland, M.A. (2016). Metformin as a Tool to Target Aging. Cell Metab. *23*, 1060–1065.

Berger, S., and Sassone-Corsi, P. (2015). Metabolic Signaling to Chromatin. Csh Perspect Biol *8*, a019463.

Biteau, B., Hochmuth, C., and Jasper, H. (2008). JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging Drosophila gut. Cell Stem Cell *3*, 442–455.

Biteau, B., Karpac, J., Supoyo, S., DeGennaro, M., Lehmann, R., and Jasper, H. (2010). Lifespan Extension by Preserving Proliferative Homeostasis in Drosophila. Plos Genet *6*, e1001159.

Bjedov, I., Toivonen, J., Kerr, F., Slack, C., Jacobson, J., Foley, A., and Partridge, L. (2010). Mechanisms of Life Span Extension by Rapamycin in the Fruit Fly Drosophila melanogaster. Cell Metab *11*, 35–46.

Bjordal, M., Arquier, N., Kniazeff, J., Pin, J., and Léopold, P. (2014). Sensing of Amino Acids in a Dopaminergic Circuitry Promotes Rejection of an Incomplete Diet in Drosophila. Cell *156*, 510–521.

Blagosklonny, M. (2006). Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. Cell Cycle Georget Tex *5*, 2087–2102.

Blüher, M., Kahn, B., and Kahn, R. (2003). Extended Longevity in Mice Lacking the Insulin Receptor in Adipose Tissue. Science *299*, 572–574.

Bonkowski, M.S., and Sinclair, D.A. (2016). Slowing ageing by design: the rise of NAD(+) and sirtuin-activating compounds. Nat. Rev. Mol. Cell Biol. *17*, 679–690.

Boulan, L., Milán, M., and Léopold, P. (2015). The Systemic Control of Growth. Csh Perspect Biol *7*, a019117.

Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., and Hafen, E. (2001). An evolutionarily conserved function of the Drosophila insulin

receptor and insulin-like peptides in growth control. Curr Biol *11*, 213–221.

Broughton, S.J., Piper, M.D., Ikeya, T., Bass, T.M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D.J., Leevers, S.J., et al. (2005). Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands. Proc. Natl. Acad. Sci. U.S.A. *102*, 3105–3110.

Brunet, A., and Rando, T. (2017). Interaction between epigenetic and metabolism in aging stem cells. Curr Opin Cell Biol *45*, 1–7.

Budovskaya, Y.V., Wu, K., Southworth, L.K., Jiang, M., Tedesco, P., Johnson, T.E., and Kim, S.K. (2008). An elt-3/elt-5/elt-6 GATA Transcription Circuit Guides Aging in C. elegans. Cell *134*, 291–303.

Bülow, M.H., Aebersold, R., Pankratz, M.J., and Jünger, M.A. (2010). The Drosophila FoxA ortholog Fork head regulates growth and gene expression downstream of Target of rapamycin. PLoS ONE *5*, e15171.

Burkewitz, K., Morantte, I., Weir, H.J., Yeo, R., Zhang, Y., Huynh, F.K., Ilkayeva, O.R., Hirschey, M.D., Grant, A.R., and Mair, W.B. (2015). Neuronal CRTC-1 governs systemic mitochondrial metabolism and lifespan via a catecholamine signal. Cell *160*, 842–855.

Cabreiro, F., Au, C., Leung, K.-Y., Vergara-Irigaray, N., Cochemé, H., Noori, T., Weinkove, D., Schuster, E., Greene, N., and Gems, D. (2013). Metformin Retards Aging in C. elegans by Altering Microbial Folate and Methionine Metabolism. Cell *153*, 228–239.

Chapman, T., and Partridge, L. (1996). Female Fitness in Drosophila melanogaster: An Interaction between the Effect of Nutrition and of Encounter Rate with Males. Proc Royal Soc Lond B Biological Sci *263*, 755–759.

Chen, H., Zheng, X., and Zheng, Y. (2014). Age-Associated Loss of Lamin-B Leads to Systemic Inflammation and Gut Hyperplasia. Cell *159*, 829–843.

Cheng, C.-W.W., Villani, V., Buono, R., Wei, M., Kumar, S., Yilmaz, O.H., Cohen, P., Sneddon, J.B., Perin, L., and Longo, V.D. (2017). Fasting-Mimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes. Cell *168*, 775–788.e12.

Chintapalli, V., Wang, J., and Dow, J. (2007). Using FlyAtlas to identify better Drosophila melanogaster models of human disease. Nat Genet *39*, 715–720.

Choi, N., Kim, J., Yang, D., Kim, Y., and Yoo, M. (2008). Age-related changes in Drosophila midgut are associated with PVF2, a PDGF/VEGF-like growth factor. Aging Cell *7*, 318–334.

Clancy, D.J., Gems, D., Harshman, L.G., Oldham, S., Stocker, H., Hafen, E., Leevers, S.J., and Partridge, L. (2001). Extension of Life-Span by Loss of CHICO, a Drosophila Insulin Receptor Substrate Protein. Science *292*, 104–106.

Clark, R., Salazar, A., Yamada, R., Fitz-Gibbon, S., Morselli, M., Alcaraz, J., Rana, A., Rera, M., Pellegrini, M., Ja, W., et al. (2015). Distinct Shifts in Microbiota Composition during Drosophila Aging Impair Intestinal Function and Drive Mortality. Cell Reports *12*, 1656–1667.

Colman, R., Anderson, R., Johnson, S., Kastman, E., Kosmatka, K., Beasley, M., Allison, D., Cruzen, C., Simmons, H., Kemnitz, J., et al. (2009). Caloric Restriction Delays Disease Onset and Mortality in Rhesus Monkeys.

Science *325*, 201–204.

Cooper, T. (2002). Transmitting the signal of excess nitrogen in Saccharomyces cerevisiae from the Tor proteins to the GATA factors: connecting the dots. Fems Microbiol Rev *26*, 223–238.

Demontis, F., and Perrimon, N. (2010). FOXO/4E-BP Signaling in Drosophila Muscles Regulates Organism-wide Proteostasis during Aging. Cell *143*, 813–825.

Demontis, F., Piccirillo, R., Goldberg, A., and Perrimon, N. (2013). Mechanisms of skeletal muscle aging: insights from Drosophila and mammalian models. Dis Model Mech *6*, 1339–1352.

Demontis, F., Patel, V., Swindell, W., and Perrimon, N. (2014). Intertissue Control of the Nucleolus via a Myokine-Dependent Longevity Pathway. Cell Reports *7*, 1481–1494.

Dever, T., Feng, L., Wek, R., Cigan, A.M., Donahue, T., and Hinnebusch, A. (1992). Phosphorylation of initiation factor 2α by protein kinase GCN2 mediates gene-specific translational control of GCN4 in yeast. Cell *68*, 585–596.

Dobson, A., He, X., Blanc, E., Bolukbasi, E., and Feseha, Y. (2016). Ageing, TOR and amino acid restriction: a cross-tissue transcriptional network connects GATA factors to Drosophila longevity. BioRxiv.

Dussutour, A., and Simpson, S.J. (2012). Ant workers die young and colonies collapse when fed a high-protein diet. Proc. Biol. Sci. *279*, 2402–2408.

Egan, D., Kim, J., Shaw, R., and Guan, K.-L. (2011). The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. Autophagy *7*, 643–644.

Eisenberg, T., Knauer, H., Schauer, A., Büttner, S., Ruckenstuhl, C., Carmona-Gutierrez, D., Ring, J., Schroeder, S., Magnes, C., Antonacci, L., et al. (2009). Induction of autophagy by spermidine promotes longevity. Nat Cell Biol *11*, 1305–1314.

Eisenberg, T., Schroeder, S., Andryushkova, A., Pendl, T., Küttner, V., Bhukel, A., Mariño, G., Pietrocola, F., Harger, A., Zimmermann, A., et al. (2014). Nucleocytosolic Depletion of the Energy Metabolite Acetyl-Coenzyme A Stimulates Autophagy and Prolongs Lifespan. Cell Metab *19*, 431–444.

Eisenberg, T., Abdellatif, M., Schroeder, S., Primessnig, U., Stekovic, S., Pendl, T., Harger, A., Schipke, J., Zimmermann, A., Schmidt, A., et al. (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. Nat Med *22*, 1428–1438.

Emran, S., Yang, M., He, X., Zandveld, J., and Piper, M.D. (2014). Target of rapamycin signalling mediates the lifespan-extending effects of dietary restriction by essential amino acid alteration. Aging (Albany NY) *6*, 390–398.

Fanson, B., Weldon, C., Pérez-Staples, D., Simpson, S., and Taylor, P. (2009). Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (Bactrocera tryoni). Aging Cell *8*, 514–523.

Flachsbart, F., Caliebe, A., Kleindorp, R., Blanché, H., von Eller-Eberstein, H., Nikolaus, S., Schreiber, S., and Nebel, A. (2009). Association of FOXO3A variation with human longevity confirmed in German centenarians. Proceedings of the National Academy of Sciences *106*, 2700–2705.

Frezza, C., Zheng, L., Folger, O., Rajagopalan, K., MacKenzie, E., Jerby, L., Micaroni, M., Chaneton, B., Adam, J., Hedley, A., et al. (2011). Haem oxygenase is

synthetically lethal with the tumour suppressor fumarate hydratase. Nature *477*, 225–228.

Friedman, D., and Johnson, T. (1988). A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics *118*, 75–86.

Gargano, J., Martin, I., Bhandari, P., and Grotewiel, M. (2005). Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in Drosophila. Exp Gerontol *40*, 386–395.

Gems, D., and Partridge, L. (2013). Genetics of Longevity in Model Organisms: Debates and Paradigm Shifts. Physiology *75*, 621–644.

Ghosh, A., Rideout, E., and Grewal, S. (2014). TIF-IA-Dependent Regulation of Ribosome Synthesis in Drosophila Muscle Is Required to Maintain Systemic Insulin Signaling and Larval Growth. Plos Genet *10*, e1004750.

Giannakou, M.E., Goss, M., Jünger, M.A., Hafen, E., Leevers, S.J., and Partridge, L. (2004). Long-lived Drosophila with overexpressed dFOXO in adult fat body. Science *305*, 361.

Graham, P., and Pick, L. (2017). Drosophila as a Model for Diabetes and Diseases of Insulin Resistance. Curr. Top. Dev. Biol. *121*, 397–419.

Grandison, R.C., Piper, M.D., and Partridge, L. (2009). Amino-acid imbalance explains extension of lifespan by dietary restriction in Drosophila. Nature *462*, 1061–1064.

Greer, E., Dowlatshahi, D., Banko, M., Villen, J., Hoang, K., Blanchard, D., Gygi, S., and Brunet, A. (2007). An AMPK-FOXO Pathway Mediates Longevity Induced by a Novel Method of Dietary Restriction in C. elegans. Curr Biol *17*, 1646–1656.

Grönke, S., Clarke, D.-F., Broughton, S., Andrews, D., and Partridge, L. (2010). Molecular Evolution and Functional Characterization of Drosophila Insulin-Like Peptides. Plos Genet *6*, e1000857.

Grotewiel, M., Martin, I., Bhandari, P., and Cook-Wiens, E. (2005). Functional senescence in Drosophila melanogaster. Ageing Res Rev *4*, 372–397.

Guo, L., Karpac, J., Tran, S.L., and Jasper, H. (2014). PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. Cell *156*, 109–122.

Haes, W., Frooninckx, L., Assche, R., Smolders, A., Depuydt, G., Billen, J., Braeckman, B., Schoofs, L., and Temmerman, L. (2014). Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. Proc National Acad Sci *111*, E2501–E2509.

Hafen (2004). Cancer, type 2 diabetes, and ageing: news from flies and worms. Swiss Med Wkly *134*, 711–719.

Hao, S., Sharp, J., Ross-Inta, C., McDaniel, B., Anthony, T., Wek, R., Cavener, D., McGrath, B., Rudell, J., Koehnle, T., et al. (2005). Uncharged tRNA and Sensing of Amino Acid Deficiency in Mammalian Piriform Cortex. Science *307*, 1776–1778.

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology *11*, 298–300.

Harrison, S.J., Raubenheimer, D., Simpson, S.J., Godin, J.-G.J.G., and Bertram, S.M. (2014). Towards a synthesis of frameworks in nutritional ecology: interacting effects of protein, carbohydrate and phosphorus on field cricket fitness. Proceedings. Biological Sciences *281*, 20140539.

Heintz, C., Doktor, T.K., Lanjuin, A., Escoubas, C.C., Zhang, Y., Weir, H.J., Dutta, S., Silva-García, C.G., Bruun, G.H., Morantte, I., et al. (2016). Splicing factor 1 modulates dietary restriction and TORC1 pathway longevity in C. elegans. Nature.

Hine, C., Harputlugil, E., Zhang, Y., Ruckenstuhl, C., Lee, B.C., Brace, L., Longchamp, A., Treviño-Villarreal, J.H., Mejia, P., Ozaki, C.K., et al. (2015). Endogenous hydrogen sulfide production is essential for dietary restriction benefits. Cell *160*, 132–144.

Hinnebusch (1988). Novel mechanisms of translational control in Saccharomyces cerevisiae. Trends Genetics Tig *4*, 169–174.

Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Géloën, A., Even, P., Cervera, P., and Bouc, Y. (2003). IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature *421*, 182–187.

Van der Horst, A., and Burgering, B. (2007). Stressing the role of FoxO proteins in lifespan and disease. Nat Rev Mol Cell Bio *8*, 440–450.

Howell, J.J., Hellberg, K., Turner, M., Talbott, G., Kolar, M.J., Ross, D.S., Hoxhaj, G., Saghatelian, A., Shaw, R.J., and Manning, B.D. (2017). Metformin Inhibits Hepatic mTORC1 Signaling via Dose-Dependent Mechanisms Involving AMPK and the TSC Complex. Cell Metab. *25*, 463–471.

Hudry, B., Khadayate, S., and Miguel-Aliaga, I. (2016). The sexual identity of adult intestinal stem cells controls organ size and plasticity. Nature *530*, 344–348.

Hultström (2015). Caloric restriction reduces age-related but not all-cause mortality. Acta Physiol *214*, 3–5.

Hwangbo, D., Gershman, B., Gersham, B., Tu, M.-P., Palmer, M., and Tatar, M. (2004). Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature *429*, 562–566.

Hyde, R.R. (1913). Inheritance of the length of life in Drosophila ampelophila. Indiana Scientific Report *23*, 113–123.

Iadevaia, V., Liu, R., and Proud, C. (2014). mTORC1 signaling controls multiple steps in ribosome biogenesis. Seminars Cell Dev Biology *36*, 113–120.

Iliadi, K., Knight, D., and Boulianne, G. (2012). Healthy Aging – Insights from Drosophila. Frontiers Physiology *3*, 106.

Jensen, K., McClure, C., Priest, N.K., and Hunt, J. (2015). Sex-specific effects of protein and carbohydrate intake on reproduction but not lifespan in Drosophila melanogaster. Aging Cell *14*, 605–615.

Jiang, J., Jaruga, E., Repnevskaya, M., and Jazwinski, M. (2000). An intervention resembling caloric restriction prolongs life span and retards aging in yeast. Faseb J *14*, 2135–2137.

Johnson, E., Kazgan, N., Bretz, C., Forsberg, L., Hector, C., Worthen, R., Onyenwoke, R., and Brenman, J. (2010). Altered Metabolism and Persistent Starvation Behaviors Caused by Reduced AMPK Function in Drosophila. Plos One *5*, e12799.

Kabil, H., Kabil, O., Banerjee, R., Harshman, L.G., and Pletcher, S.D. (2011). Increased transsulfuration mediates longevity and dietary restriction in Drosophila. Proc. Natl. Acad. Sci. U.S.A. *108*, 16831–16836.

Kaeberlein, M., Powers, W., Steffen, K., Westman, E., Hu, D., Dang, N., Kerr, E., Kirkland, K., Fields, S., and Kennedy, B. (2005). Regulation of Yeast Replicative Life Span by TOR and Sch9 in Response to Nutrients. Science

310, 1193–1196.

Kang, M.-J.J., Vasudevan, D., Kang, K., Kim, K., Park, J.-E.E., Zhang, N., Zeng, X., Neubert, T.A., Marr, M.T., and Ryoo, H.D. (2017). 4E-BP is a target of the GCN2-ATF4 pathway during Drosophila development and aging. J. Cell Biol. *216*, 115–129.

Kapahi, P., Zid, B.M., Harper, T., Koslover, D., Sapin, V., and Benzer, S. (2004). Regulation of Lifespan in Drosophila by Modulation of Genes in the TOR Signaling Pathway. Current Biology *14*.

Kapahi, P., Kaeberlein, M., and Hansen, M. (2016). Dietary restriction and lifespan: Lessons from invertebrate models. Ageing Res. Rev.

Kennedy, B.K., Kaeberlein, M., and Partridge, L. (2017). Small metazoans. (The Gerontological Society of America), pp. 84–112.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature *366*, 461–464.

Kim, J., Kundu, M., Viollet, B., and Guan, K.-L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol *13*, 132–141.

Kirkwood, T. (2002). Evolution of ageing. Mech Ageing Dev *123*, 737–745.

Kirkwood, T., and Shanley, D. (2005). Food restriction, evolution and ageing. Mech Ageing Dev *126*, 1011–1016.

Klass, M. (1977). Aging in the nematode Caenorhabditis elegans: Major biological and environmental factors influencing life span. Mech Ageing Dev *6*, 413–429.

Klass, M. (1983). A method for the isolation of longevity mutants in the nematode Caenorhabditis elegans and initial results. Mechanisms of Aging and Development *22*, 279–286.

Kovács, T., Billes, V., Komlós, M., Hotzi, B., Manzéger, A., Tarnóci, A., Papp, D., Szikszai, F., Szinyákovics, J., Rácz, Á., et al. (2017). The small molecule AUTEN-99 (autophagy enhancer-99) prevents the progression of neurodegenerative symptoms. Sci Reports *7*, 42014.

Kučerová, L., Kubrak, O., Bengtsson, J., Strnad, H., Nylin, S., Theopold, U., and Nässel, D. (2016). Slowed aging during reproductive dormancy is reflected in genome-wide transcriptome changes in Drosophila melanogaster. Bmc Genomics *17*, 50.

Kuningas, M., Mägi, R., Westendorp, R., Slagboom, E., Remm, M., and van Heemst, D. (2007). Haplotypes in the human Foxo1a and Foxo3a genes; impact on disease and mortality at old age. Eur J Hum Genet *15*, 294–301.

LaFever, L., and Drummond-Barbosa, D. (2005). Direct Control of Germline Stem Cell Division and Cyst Growth by Neural Insulin in Drosophila. Science *309*, 1071–1073.

Lamming, D.W., Ye, L., Katajisto, P., Goncalves, M.D., Saitoh, M., Stevens, D.M., Davis, J.G., Salmon, A.B., Richardson, A., Ahima, R.S., et al. (2012). Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science *335*, 1638–1643.

Larson, K., Yan, S.-J.J., Tsurumi, A., Liu, J., Zhou, J., Gaur, K., Guo, D., Eickbush, T.H., and Li, W.X. (2012). Heterochromatin formation promotes longevity and represses ribosomal RNA synthesis. PLoS Genet. *8*, e1002473.

Laye, M.J., Tran, V., Jones, D.P., Kapahi, P., and Promislow, D.E. (2015). The effects of age and dietary restriction on the tissue-specific metabolome of

Drosophila. Aging Cell *14*, 797–808.

Lee, J., Budanov, A., Park, E., Birse, R., Kim, T., Perkins, G., Ocorr, K., Ellisman, M., Bodmer, R., Bier, E., et al. (2010). Sestrin as a Feedback Inhibitor of TOR That Prevents Age-Related Pathologies. Science *327*, 1223–1228.

Lee, K., Simpson, S., Clissold, F., Brooks, R., Ballard, W., Taylor, P., Soran, N., and Raubenheimer, D. (2008). Lifespan and reproduction in Drosophila: New insights from nutritional geometry. Proc National Acad Sci *105*, 2498–2503.

Leib, D.E., and Knight, Z.A. (2015). Re-examination of Dietary Amino Acid Sensing Reveals a GCN2-Independent Mechanism. Cell Rep *13*, 1081–1089.

Lemaitre, B., and Miguel-Aliaga, I. (2013). The Digestive Tract of Drosophila melanogaster. Annu Rev Genet *47*, 377–404.

Li, H., Qi, Y., and Jasper, H. (2016). Preventing Age-Related Decline of Gut Compartmentalization Limits Microbiota Dysbiosis and Extends Lifespan. Cell Host Microbe *19*, 240–253.

Li, W., Li, X., and Miller, R. (2014). ATF4 activity: a common feature shared by many kinds of slow-aging mice. Aging Cell *13*, 1012–1018.

Loeb, and Northrop (1916). Is There a Temperature Coefficient for the Duration of Life? P Natl Acad Sci Usa *2*, 456–457.

López-Otín, C., Blasco, M., Partridge, L., Serrano, M., and Kroemer, G. (2013). The Hallmarks of Aging. Cell *153*, 1194–1217.

Magwere, T., Chapman, T., and Partridge, L. (2004). Sex Differences in the Effect of Dietary Restriction on Life Span and Mortality Rates in Female and Male Drosophila Melanogaster. Journals Gerontology Ser *59*, B3–B9.

Mair, W., Goymer, P., Pletcher, S.D., and Partridge, L. (2003). Demography of dietary restriction and death in Drosophila. Science *301*, 1731–1733.

Mair, W., Piper, M.D., and Partridge, L. (2005). Calories do not explain extension of life span by dietary restriction in Drosophila. PLoS Biol. *3*, e223.

Mair, W., Morantte, I., Rodrigues, A.P., Manning, G., Montminy, M., Shaw, R.J., and Dillin, A. (2011). Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. Nature *470*, 404–408.

Maklakov, A., Simpson, S., Zajitschek, F., Hall, M., Dessmann, J., Clissold, F., Raubenheimer, D., Bonduriansky, R., and Brooks, R. (2008). Sex-Specific Fitness Effects of Nutrient Intake on Reproduction and Lifespan. Curr Biol *18*, 1062–1066.

Mannick, J., Giudice, G., Lattanzi, M., Valiante, N., Praestgaard, J., Huang, B., Lonetto, M., Maecker, H., Kovarik, J., Carson, S., et al. (2014). mTOR inhibition improves immune function in the elderly. Sci Transl Med *6*, 268ra179–268ra179.

Mariño, G., Pietrocola, F., Eisenberg, T., Kong, Y., Malik, S., Andryushkova, A., Schroeder, S., Pendl, T., Harger, A., Niso-Santano, M., et al. (2014). Regulation of Autophagy by Cytosolic Acetyl-Coenzyme A. Mol Cell *53*, 710–725.

Marshall, L., Rideout, E., and Grewal, S. (2012). Nutrient/TOR-dependent regulation of RNA polymerase III controls tissue and organismal growth in Drosophila. Embo J *31*, 1916–1930.

Martin-Montalvo, A., Mercken, E., Mitchell, S., Palacios, H., Mote, P., Scheibye-Knudsen, M., Gomes, A., Ward, T., Minor, R., Blouin, M.-J., et al. (2013). Metformin improves healthspan and lifespan in mice. Nat Commun *4*,

2192.

Mattison, J., Roth, G., Beasley, M., Tilmont, E., Handy, A., Herbert, R., Longo, D., Allison, D., Young, J., Bryant, M., et al. (2012). Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature *489*, 318–321.

Mattison, J.A., Colman, R.J., Beasley, T.M., Allison, D.B., Kemnitz, J.W., Roth, G.S., Ingram, D.K., Weindruch, R., de Cabo, R., and Anderson, R.M. (2017). Caloric restriction improves health and survival of rhesus monkeys. Nat Commun *8*, 14063.

Maurin, A.-C., Jousse, C., Averous, J., Parry, L., Bruhat, A., Cherasse, Y., Zeng, H., Zhang, Y., Harding, H., Ron, D., et al. (2005). The GCN2 kinase biases feeding behavior to maintain amino acid homeostasis in omnivores. Cell Metab *1*, 273–277.

McCay, C.M., Crowell, M.F., and Maynard, L.A. (1935). The effect of retarded growth upon the length of life span and upon the ultimate body size. Journal of Nutrition *10*, 63–79.

Medawar, P. (1952). An unsolved problem of biology: An Inaugural Lecture Delivered at University College, London, 6 December, 1951. (H.K. Lewis and Company),.

Medvedik, O., Lamming, D.W., Kim, K.D., and Sinclair, D.A. (2007). MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in Saccharomyces cerevisiae. PLoS Biol. *5*, e261.

Micchelli, C., and Perrimon, N. (2005). Evidence that stem cells reside in the adult Drosophila midgut epithelium. Nature *439*, 475–479.

Miller, R., Buehner, G., Chang, Y., Harper, J., Sigler, R., and Smith-Wheelock, M. (2005). Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. Aging Cell *4*, 119–125.

Miller, R.A., Harrison, D.E., Astle, C.M., Fernandez, E., Flurkey, K., Han, M., Javors, M.A., Li, X., Nadon, N.L., Nelson, J.F., et al. (2014). Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. Aging Cell *13*, 468–477.

Nässel, D., and Broeck, J. (2016). Insulin/IGF signaling in Drosophila and other insects: factors that regulate production, release and post-release action of the insulin-like peptides. Cell Mol Life Sci *73*, 271–290.

Nielsen, J. (2016). Systems Biology of Metabolism. Annu Rev Biochem *86*, 1–31.

Nielsen, J. (2017). Systems Biology of Metabolism: A Driver for Developing Personalized and Precision Medicine. Cell Metab *25*, 572–579.

Ohlstein, B., and Spradling, A. (2005). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature *439*, 470–474.

Onken, B., and Driscoll, M. (2010). Metformin Induces a Dietary Restriction–Like State and the Oxidative Stress Response to Extend C. elegans Healthspan via AMPK, LKB1, and SKN-1. Plos One *5*, e8758.

Owusu-Ansah, E., Song, W., and Perrimon, N. (2013). Muscle Mitohormesis Promotes Longevity via Systemic Repression of Insulin Signaling. Cell *155*, 699–712.

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

Papp, D., Kovács, T., Billes, V., Varga, M., Tarnóci, A., Jr, L., Puskás, L., Liliom, H., Tárnok, K., Schlett, K., et al. (2015). AUTEN-67, an autophagy-enhancing drug candidate with potent antiaging and neuroprotective effects. Autophagy *12*, 273–286.

Park, J.-H., Attardo, G., Hansen, I., and Raikhel, A. (2006). GATA Factor Translation Is the Final Downstream Step in the Amino Acid/Target-of-Rapamycin-mediated Vitellogenin Gene Expression in the Anautogenous Mosquito Aedes aegypti. J Biol Chem *281*, 11167–11176.

Parkhitko, A.A., Binari, R., Zhang, N., Asara, J.M., Demontis, F., and Perrimon, N. (2016). Tissue-specific down-regulation of S-adenosyl-homocysteine via suppression of dAhcyL1/dAhcyL2 extends health span and life span in Drosophila. Genes Dev. *30*, 1409–1422.

Partridge (2001). Evolutionary theories of ageing applied to long-lived organisms. Exp Gerontol *36*, 641–650.

Partridge, L., and Gems, D. (2002). Mechanisms of aging: public or private? Nat Rev Genet *3*, 165–175.

Partridge, L., Alic, N., Bjedov, I., and Piper, M.D. (2011). Ageing in Drosophila: the role of the insulin/Igf and TOR signalling network. Exp. Gerontol. *46*, 376–381.

Pawlikowska, L., Hu, D., Huntsman, S., Sung, A., Chu, C., Chen, J., Joyner, A., Schork, N., Hsueh, W.-C., Reiner, A., et al. (2009). Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. Aging Cell *8*, 460–472.

Pearl, R. (1928). The rate of living (London: University of London Press).

Pearl, R., and Parker, S.L. (1921). Experimental studies on the duration of life. I. Introductory discussion of the duration of life in Drosophila. The American Naturalist *55*, 481–509.

Peleg, S., Feller, C., Forne, I., Schiller, E., Sévin, D.C., Schauer, T., Regnard, C., Straub, T., Prestel, M., Klima, C., et al. (2016). Life span extension by targeting a link between metabolism and histone acetylation in Drosophila. EMBO Rep. *17*, 455–469.

Piper, M.D., and Partridge, L. (2007). Dietary restriction in Drosophila: delayed aging or experimental artefact? PLoS Genet. *3*, e57.

Piper, M.D., and Partridge, L. (2016). Protocols to Study Aging in Drosophila. Methods Mol. Biol. *1478*, 291–302.

Piper, M.D., Skorupa, D., and Partridge, L. (2005). Diet, metabolism and lifespan in Drosophila. Exp. Gerontol. *40*, 857–862.

Piper, M.D., Blanc, E., Leitão-Gonçalves, R., Yang, M., He, X., Linford, N.J., Hoddinott, M.P., Hopfen, C., Soultoukis, G.A., Niemeyer, C., et al. (2014). A holidic medium for Drosophila melanogaster. Nat. Methods *11*, 100–105.

Piper, M.D., Soultoukis, G.A., Blanc, E., Mesaros, A., Herbert, S.L., Juricic, P., He, X., Atanassov, I., Salmonowicz, H., Yang, M., et al. (2017). Matching Dietary Amino Acid Balance to the In Silico-Translated Exome Optimizes Growth and Reproduction without Cost to Lifespan. Cell Metabolism *25*, 610–621.

Post, S., and Tatar, M. (2016). Nutritional Geometric Profiles of Insulin/IGF Expression in Drosophila melanogaster. PLoS ONE *11*, e0155628.

Proshkina, E., Shaposhnikov, M., Sadritdinova, A., Kudryavtseva, A., and Moskalev, A. (2015). Basic mechanisms of longevity: A case study of Drosophila pro-longevity genes. Ageing Res Rev *24*, 218–231.

Puig, O., Marr, M., Ruhf, L., and Tjian, R. (2003). Control of cell number by Drosophila FOXO: downstream and feedback regulation of the insulin receptor pathway. Genes Dev *17*, 2006–2020.

Regan, J., Khericha, M., Dobson, A., Bolukbasi, E., Rattanavirotkul, N., and Partridge, L. (2016). Sex difference in pathology of the ageing gut mediates the greater response of female lifespan to dietary restriction. Elife *5*, e10956.

Rera, M., Clark, R., and Walker, D. (2012). Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. Proc National Acad Sci *109*, 21528–21533.

Rera, M., Azizi, M., and Walker, D. (2013). Organ-specific mediation of lifespan extension: More than a gut feeling? Ageing Res Rev *12*, 436–444.

Rizza, W., Veronese, N., and Fontana, L. (2014) What are the roles of calorie restriction and diet quality in promoting healthy longevity? Ageing Research Reviews *13*, 38-45.

Robida-Stubbs, S., Glover-Cutter, K., Lamming, D.W., Mizunuma, M., Narasimhan, S.D., Neumann-Haefelin, E., Sabatini, D.M., and Blackwell, T.K. (2012). TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. Cell Metab. *15*, 713–724.

Rose, M. (1994). Evolutionary biology of aging. Oxford University Press.

Rose, M., Burke, M., Shahrestani, P., and Mueller, L. (2008). Evolution of ageing since Darwin. J Genet *87*, 363–371.

Roth, G., and Ingram, D. (2016). Manipulation of health span and function by dietary caloric restriction mimetics. Ann Ny Acad Sci *1363*, 5–10.

Saxton, R.A., and Sabatini, D.M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. Cell *168*, 960–976.

Schroeder, S., Pendl, T., Zimmermann, A., Eisenberg, T., Carmona-Gutierrez, D., Ruckenstuhl, C., Mariño, G., Pietrocola, F., Harger, A., Magnes, C., et al. (2014). Acetyl-coenzyme A: a metabolic master regulator of autophagy and longevity. Autophagy *10*, 1335–1337.

Selman, C., Tullet, J., Wieser, D., Irvine, E., Lingard, S., Choudhury, A., Claret, M., Al-Qassab, H., Carmignac, D., Ramadani, F., et al. (2009). Ribosomal Protein S6 Kinase 1 Signaling Regulates Mammalian Life Span. Science *326*, 140–144.

Simonsen, A., Cumming, R., Brech, A., Isakson, P., Schubert, D., and Finley, K. (2007). Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult Drosophila. Autophagy *4*, 176–184.

Skorupa, D.A., Dervisefendic, A., Zwiener, J., and Pletcher, S.D. (2008). Dietary composition specifies consumption, obesity, and lifespan in Drosophila melanogaster. Aging Cell *7*, 478–490.

Slack, C., Giannakou, M., Foley, A., Goss, M., and Partridge, L. (2011). dFOXO-independent effects of reduced insulin-like signaling in Drosophila. Aging Cell *10*, 735–748.

Slack, C., Foley, A., and Partridge, L. (2012). Activation of AMPK by the Putative Dietary Restriction Mimetic Metformin Is Insufficient to Extend Lifespan in Drosophila. Plos One *7*, e47699.

Slack, C., Alic, N., Foley, A., Cabecinha, M., Hoddinott, M.P., and Partridge, L. (2015). The Ras-Erk-ETS-Signaling Pathway Is a Drug Target for

Longevity. Cell *162*, 72–83.

Solon-Biet, S., Walters, K., Simanainen, U., McMahon, A., Ruohonen, K., Ballard, J., Raubenheimer, D., Handelsman, D., Couteur, D., and Simpson, S. (2015). Macronutrient balance, reproductive function, and lifespan in aging mice. Proc National Acad Sci *112*, 3481–3486.

Solon-Biet, S.M., McMahon, A.C., Ballard, W.O.J., Ruohonen, K., Wu, L.E., Cogger, V.C., Warren, A., Huang, X., Pichaud, N., Melvin, R.G., et al. (2014). The Ratio of Macronutrients, Not Caloric Intake, Dictates Cardiometabolic Health, Aging, and Longevity in Ad Libitum-Fed Mice. Cell Metabolism *19*, 418–430.

Speakman, J.R., Mitchell, S.E., and Mazidi (2016). Calories or protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone. Exp Gerontol *86*, 28–38.

Stenesen, D., Suh, J., Seo, J., Yu, K., Lee, K.-S., Kim, J.-S., Min, K.-J., and Graff, J. (2013). Adenosine Nucleotide Biosynthesis and AMPK Regulate Adult Life Span and Mediate the Longevity Benefit of Caloric Restriction in Flies. Cell Metab *17*, 101–112.

Swindell, W. (2016). Meta-Analysis of 29 Experiments Evaluating the Effects of Rapamycin on Life Span in the Laboratory Mouse. Journals Gerontology Ser Biological Sci Medical Sci glw153.

Tamura, T., Chiang, A.-S., Ito, N., Liu, H.-P., Horiuchi, J., Tully, T., and Saitoe, M. (2003). Aging Specifically Impairs amnesiac-Dependent Memory in Drosophila. Neuron *40*, 1003–1011.

Tatar, M. (2011). The plate half-full: status of research on the mechanisms of dietary restriction in Drosophila melanogaster. Exp. Gerontol. *46*, 363–368.

Tatar, Kopelman, Epstein, Tu, M.-P., Yin, C.-M., and Garofalo (2001). A Mutant Drosophila Insulin Receptor Homolog That Extends Life-Span and Impairs Neuroendocrine Function. Science *292*, 107–110.

Taylor, R., and Dillin, A. (2011). Aging as an Event of Proteostasis Collapse. Csh Perspect Biol *3*, a004440.

Tiebe, M., Lutz, M., De La Garza, A., Buechling, T., Boutros, M., and Teleman, A. (2015). REPTOR and REPTOR-BP Regulate Organismal Metabolism and Transcription Downstream of TORC1. Dev Cell *33*, 272–284.

Ulgherait, M., Rana, A., Rera, M., Graniel, J., and Walker, D. (2014). AMPK Modulates Tissue and Organismal Aging in a Non-Cell-Autonomous Manner. Cell Reports *8*, 1767–1780.

Vattem, K., and Wek, R. (2004). Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. P Natl Acad Sci Usa *101*, 11269–11274.

Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A., Orosz, L., and Müller, F. (2003). Genetics: Influence of TOR kinase on lifespan in C. elegans. Nature *426*, 620–620.

Wek, Jackson, and Hinnebusch (1989). Juxtaposition of domains homologous to protein kinases and histidyl-tRNA synthetases in GCN2 protein suggests a mechanism for coupling GCN4 expression to amino acid availability. P Natl Acad Sci Usa *86*, 4579–4583.

Wessells, R., Fitzgerald, E., Cypser, J., Tatar, M., and Bodmer, R. (2004). Insulin regulation of heart function in aging fruit flies. Nat Genet *36*, 1275–1281.

Willcox, B., Donlon, T., He, Q., Chen, R., Grove, J., Yano, K., Masaki, K., Willcox, C., Rodriguez, B., and Curb, D. (2008). FOXO3A genotype is strongly associated with human longevity. Proc National Acad Sci *105*, 13987–13992.

Williams, G.C. (1957). Pleiotropy, Natural Selection, and the Evolution of Senescence. Evolution *11*, 398–411.

Ye, J., Palm, W., Peng, M., King, B., Lindsten, T., Li, M., Koumenis, C., and Thompson, C. (2015). GCN2 sustains mTORC1 suppression upon amino acid deprivation by inducing Sestrin2. Genes Dev *29*, 2331–2336.

Yun, J., Mullarky, E., Lu, C., Bosch, K., Kavalier, A., Rivera, K., Roper, J., Chio, I., Giannopoulou, E., Rago, C., et al. (2015). Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. Science *350*, 1391–1396.

Zhang, P., Judy, M., Lee, S.-J., and Kenyon, C. (2013). Direct and Indirect Gene Regulation by a Life-Extending FOXO Protein in C. elegans: Roles for GATA Factors and Lipid Gene Regulators. Cell Metab *17*, 85–100.

Zid, B.M., Rogers, A.N., Katewa, S.D., Vargas, M.A., Kolipinski, M.C., Lu, T.A., Benzer, S., and Kapahi, P. (2009). 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in Drosophila. Cell *139*, 149–160.

Ziehm, M., and Thornton, J. (2013). Unlocking the potential of survival data for model organisms through a new database and online analysis platform: SurvCurv. Aging Cell *12*, 910–916.

Ziehm, M., Piper, M.D., and Thornton, J.M. (2013). Analysing variation in Drosophila aging across independent experimental studies: a meta-analysis of survival data. Aging Cell *12*, 917–922.

Zimmerman, J., Malloy, V., Krajcik, R., and Orentreich, N. (2003). Nutritional control of aging. Exp Gerontol *38*, 47–52.

Figure Legends

Figure 1 Overview of the physiological and genetic factors that dictate *Drosophila* ageing.

(Bottom panel) During their 3-month lifespan, flies exhibit numerous signs of ageing, including changes to metabolism, tissue function, reproductive capacity, physical activity and behaviour. Numerous molecular interactions govern changes in ageing that have been grouped into nine hallmarks (identified in (López-Otín et al., 2013) and illustrated by the nine icons. Note that two are not relevant / have not been studied in *Drosophila* and have thus been moved to the bottom of the figure.) The names of *Drosophila* genes that can be manipulated to extend life are listed, having been categorized as belonging to one of the nine hallmarks. In cases where more categorization could have involved more than one hallmark, the most likely was selected and for those changes not belonging to a hallmark, the extra categories "altered metabolic homeostasis" and "other longevity mutants" were created. These changes can be found at various levels of physiological organization and signaling, reflecting a complex interconnection. There is a growing clarity in the way metabolic networks underpin these interconnections.

Figure 2 Ageing-related maintenance of the epigenome is linked to central metabolic pathways.

Manipulating the availability of activated C1 and C2 units, in the form of *S*-adenosyl methionine and acetyl-CoA respectively, have been uncovered as important regulators of lifespan in *Drosophila.* Importantly, these beneficial metabolic changes also result in improved maintenance of chromatin structure through modifications to the epigenome. This is thought to protect against transcriptional noise that grows with age. Expanding metabolic network models, and their associated effects on signaling, should reveal other metabolic characteristics of ageing and how critical intervention points may be used to slow their decline.