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Pharmacological Lifespan Extension of Invertebrates

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Abstract

There is considerable interest in identifying small, drug-like compounds that slow aging in multiple species, particularly in mammals. Such compounds may prove to be useful in treating and retarding age-related disease in humans. Just as invertebrate models have been essential in helping us understand the genetic pathways that control aging, these model organisms are also proving valuable in discovering chemical compounds that influence longevity. The nematode *Caenorhabditis elegans* (*C. elegans*) has numerous advantages for such studies including its short lifespan and has been exploited by a number of investigators to find compounds that impact aging. Here, we summarize the progress being made in identifying compounds that extend the lifespan of invertebrates, and introduce the challenges we face in translating this research into human therapies.

Keywords

Aging; chemical screening; drug discovery; lifespan; *C. elegans*; *Drosophila*

1. Introduction

Aging is the single largest risk factor for chronic disease in developed countries and is consequently responsible for an enormous social and economic burden. The development of therapies or preventive measures aimed at reducing or delaying age-related disease must be a priority for the biomedical community. However, the traditional models of drug discovery are failing when it comes to the major chronic diseases of the elderly. The disappointing outcomes of dozens of phase III clinical trials in Alzheimer's disease and Parkinson's disease, among others, suggest a general failure in our understanding of the mechanisms at play (Sperling et al., 2011). This has led some commentators to ask whether targeting aging mechanisms might lead to better outcomes.

Novel compounds that slow aging are highly sought after due to their potential for treating age related diseases. Here, we argue that recent growth of a new subfield, the chemical biology of aging, will lead to the identification of candidate compounds and mechanistic insights that will ultimately propel forward treatments of age related diseases. While identifying compounds that slow the aging of mammals is undoubtedly more relevant for human drug development, the prohibitive cost of mouse aging studies, make it extremely

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unlikely that large scale chemical screens will be carried out in mice. Basic research in more cost effective model systems is therefore a critical starting point for identifying such compounds and elucidating their mechanism(s) of action. Cell culture and invertebrate model organisms provide opportunities to screen hundreds of thousands of chemical compounds in an efficient manner. Moreover, once candidate compounds are identified, the strengths of these model systems in molecular genetics, allows for rapid elucidation of the genetic pathways being targeted by these compounds.

In this review, we summarize the contribution of invertebrate models to our understanding of the pharmacology of aging, and speculate on the directions the field is headed in the imminent future. We will almost exclusively focus on *C. elegans* research, since most of the chemical biology of aging studies to date, have been conducted in the nematode. However, we will also discuss a limited number of pharmacological aging studies undertaken in the fruit fly *Drosophila melanogaster* (*D. melanogaster*).

2. General Consideration When Conducting Experiments to Identify Lifespan Extending Compounds

2.1 High-throughput chemical screens vs. candidate based approaches

In screening compounds for biological activity, investigators are often drawn to the idea of examining a wide range of chemical structures using high-throughput screens. Due to the prohibitive cost, labor and (for aging studies) the relatively long lifespan of the current vertebrate models, they are impractical for large scale chemical screens, particularly mice. Therefore, researchers have turned to *in vitro* and invertebrate models to conduct such large scale screens. It is routine for *in vitro* chemical screens to test tens of thousands, or even hundreds of thousands of synthetic and structurally diverse compounds.

Instead of high-throughput screens of synthetic chemical structures, some researchers have taken an alternative, more focused, candidate approach by screening individual classes of compounds or small libraries of compounds that are predicted to modulate biological processes such as oxidative stress, intracellular signaling and protein aggregation among others. This approach has worked very well in the *C. elegans* model. To our knowledge the vast majority of the chemicals that have been extensively characterized to modulate lifespan have stemmed from these targeted studies.

2.2 Choosing an appropriate model to identify lifespan extending compounds

2.2.1 In vitro based assays vs. whole organisms—*In vitro* biochemical or cell based assays have been the mainstay for decades of chemical screening. Such research has led to the identification candidate compounds that are of great interest to the aging community (Howitz et al., 2003) and will certainly continue to be an effective method of chemical screening. Chemical screens in simple eukaryotic models such as the brewers yeast (*Saccharomyces cerevisiae*) are particularly promising due to the ease of its culture, the wealth of information on the endogenous genetic pathways that contribute to lifespan, and the molecular tools available for the organism. Other cell based, and particularly *in vitro* biochemical assays can be designed to maximize specificity for particular targets. A major advantage of these assays is the opportunity to use human cells and recombinant proteins which provide direct relevance for the development of drugs for humans. Studies of the molecular genetics of aging have provided hundreds of potential gene product targets. Therefore, it is likely that *in vitro* biochemical assays which aim to target human homologs of these proteins, known to influence lifespan in model organisms, will lead to the discovery of compounds that hold great promise for treatment of age related diseases in humans.

Whole organism screens have some distinct advantages over cell-based assays, since they allow for the pharmacological investigation of complex phenotypes. Whole organism screens can be designed to inform on various discrete aspects of biology simultaneously, such as: growth rate, behavior, fertility, and specific pathological features. However, whole organism screens can present difficulty for the very reason that they are deemed attractive; while novel proteins or pathways can be identified that alter a complex phenotype of the whole organism, characterizing the mechanism of the action of the compound can be difficult due to the complexity of the very phenotype being assessed and the corresponding increase in off target opportunities.

2.2.2 Using *C. elegans* in chemical screens—Due to its ease of culture and short lifespan *C. elegans* is rapidly becoming the invertebrate model of choice for chemical tests on aging and age-related phenotypes. Indeed, *C. elegans* not only represents a model for assessing the biological effects of a large number of compounds, but the great number of genetic tools available in the nematode also make it a powerful system for determining the mechanism of action of known pharmacological treatments (Fitzgerald et al., 2006). The organism's relative simplicity and the wealth of knowledge of its biology, along with the large number of genetic tools available, make it an attractive organism for pharmacological research. The nematode has proved useful to assay both the compound's bioactivity and for determining its mechanism of action. Additionally, its rapid growth and high fecundity make *C. elegans* well suited for high-throughput chemical screens. Its utility as a pharmacological tool has been highlighted by its recent use to identify small molecules that; influence development, act as antifungals, inhibit neurotransmission, facilitate neuro-regeneration and act as toxic agents (Breger et al., 2007; Cao et al., 2010; Kokel et al., 2006; Kokel and Xue, 2006; Kwok et al., 2006; Moy et al., 2006; Samara et al., 2010). These diverse studies have demonstrated the versatility of the model for chemical testing and screening. This being said, worm idiosyncrasies should be minimized when testing the effects of chemicals on lifespan. This topic has previously been covered and we will refer to their arguments for consistency in the lifespan assays (Gruber et al., 2009). However, it is important to highlight here a particularly important feature of the worm that can confound chemical assays; the worm is unusual in animal models in its diet. *C. elegans* feeds on live bacterial cultures. This means there is an inherent possibility when observing a response to a chemical, that this is due in some way to the compounds interaction with the bacteria. This possibility should always be addressed. This can be done by growing and maintaining the worms on killed bacteria or by using bacteria free culture conditions during the chemical response assay.

While large-scale screens have been performed with *C. elegans* on other phenotypes, few researchers have reported large scale small molecule screens for compounds that can extend the lifespan of *C. elegans*. One of the first, and certainly the largest, was conducted by Petrascheck and colleagues who screened 88,000 chemical structures for the ability to extend *C. elegans* lifespan (Petrascheck et al., 2009). They identified numerous compounds with lifespan extending properties and presented a structure and mechanistic study for one of their hits from this screen. Interestingly, the authors focused on a hit that was structurally related to known human drugs and we will discuss their results in detail in the section below that relates to human drugs used to modulate invertebrate lifespan. To our knowledge there has yet to be a reported detailed characterization of an entirely novel structure to come out of a high throughput screen for longevity in *C. elegans*, likely because these high-throughput chemical lifespan screens have only recently been developed and implemented. Due to the vastness of possible chemical structures it seems certain that a large number that exhibit a positive influence on longevity remain to be identified. We expect that in the near future many novel chemical structures and descriptions of their mechanisms of action will emerge from these high-throughput screens that target aging.

2.2.3 Using *Drosophila* in chemical screens—Screening large numbers of compounds in *D. melanogaster*, is more difficult than in *C. elegans*, but the existence of complex behavioral phenotypes and several good models of human age-related diseases in *Drosophila*, make such challenging endeavors worthwhile. *Drosophila* has been successfully used to identify chemical inhibitors of functional protein domains involved in cell signaling (Chen et al., 2007) as well as compounds with therapeutic potential against fragile X syndrome (Chang et al., 2008) among others.

There is a long history of *Drosophila* being used to test for chemical effects on aging. *Drosophila* can be cultured as adults in large population cages which allows for the testing of bio-demographic effects. As early as 1948, the fruit fly was used to determine the biological effects of discrete chemical components of royal jelly, and to this end pantothenic acid was suggested to extend longevity of the fly (Gardner, 1948a, b). A recent review outlines the current strengths of the model and in particular sets out specific guidelines for evaluating the significance of chemical hits (Jafari, 2010). With the emergence of an increasing number of compounds shown to slow aging in *C. elegans*, *D. melanogaster* presents the opportunity to test whether the action of these compound on aging is species specific or is conserved.

3. Summary of Aging Pathways That Can Serve as Targets for Lifespan Extending Compounds

Many compounds have now been identified that extend the lifespan of model organisms. Some of these have been discovered by conducting chemical screens in *C. elegans*, and *Drosophila* as mentioned above. For some of these compounds we merely know that they are capable of extending lifespan, for others we understand the biological mechanisms and longevity pathways involved. Before introducing the myriad of compounds capable of influencing lifespan, we first summarize the biological pathways implicated in mediating the lifespan extension effects of these compounds.

3.1 Regulation of oxidative stress and its effect on lifespan

Macromolecular oxidative damage is a feature of aging and is clearly a major component of age-related disease (Beckman et al., 1997). It has long been suggested as a cause of aging and a major determinate of lifespan. However, genetic manipulation of antioxidant functions has not provided a clear case for causality particularly in mammals (Bokov et al., 2004; Perez et al., 2009). These negative studies have to be weighed carefully against the fact that oxidation chemistry is so integral to physiology and metabolism that definitive studies in this area are difficult to conceive and conduct. For example, we have an incomplete picture of endogenous oxidative defense and repair mechanisms making it difficult to predict outcomes of genetic manipulation. This picture is reflected in the mixed results obtained by genetic manipulations designed to modulate oxidative stress.

Since mitochondria are a major source of oxygen radicals such as diffusible hydrogen peroxide (H_2O_2), many studies have focused on manipulation of mitochondrial function. In mammals, the major enzyme that prevents oxidative damage in the mitochondria is Mn superoxide dismutase (MnSOD) which catalyzes the conversion of superoxide anion ($O_2^{\bullet-}$) to H_2O_2 . Knocking out the gene encoding MnSOD is developmentally lethal, illustrating the importance of this function, but reducing MnSOD in mouse models does not lead to accelerated aging (Huang et al., 1999). However, inhibiting mitochondrial function by reducing the mitochondrial specific RNA polymerase does lead to accelerated aging pathology (Kujoth et al., 2005; Trifunovic et al., 2004) which can be suppressed by exercise (Safdar et al., 2011). In invertebrates certain forms of reduced mitochondrial function, such

as RNAi of mitochondrial subunits or certain mutations in mitochondrial functions, lead to a lifespan extension (Feng et al., 2001; Lee et al., 2003). However, other mutations such as in the *gas-1* gene (complex I subunit) and *mev-1* (complex II subunit), shorten lifespan (Adachi et al., 1998; Hartman et al., 2001; Ishii et al., 1990). Considerable follow up research on these results has not lead to a complete understanding of the mechanisms at play. One explanation for the disparate outcomes comes from the observation that the degree of loss of function for a given gene influences whether lifespan will be lengthened or shortened (Rea et al., 2007). In addition, there is possibility of a hormetic mechanism where a given alteration in electron transport increases oxidative stress, hence would be predicted to reduce lifespan, but instead results in extended lifespan due to the induction of antioxidants and repair processes (Ristow et al., 2009; Schulz et al., 2007). This complex interplay of oxidative stress and stress responses requires further description before we can make predicable pharmacological interventions.

3.2 Role of protein homeostasis in promoting longevity

The formation of molecular aggregates is a long-studied phenomenon, shared among diverse human diseases. It is especially well studied in neurodegenerative conditions; here aberrant forms of proteins such as α -synuclein (in Parkinson's), β -amyloid (in Alzheimer's) and huntingtin (in Huntington's) may contribute to disease progression (Selkoe, 2003). Aggregate formation is also observed in non-neurological systemic diseases like type II diabetes and several myopathies. Indeed it has become clear over the last few decades that protein aggregate formation is a phenotypic hallmark of ageing. As organisms age their protein homeostasis networks degrade; the decrease in its fidelity with age has now been reported in many systems and is likely a major component of the aging of organisms.

It is unclear whether the increase of protein aggregation with age is due to additive stress on the system over time, or a developmentally programmed decrease in resource investment towards protein homeostasis. While it is not clear why protein aggregation occurs, alterations in the balance of protein synthesis, protein folding and protein degradation all likely contribute to aggregate formation. Numerous *C. elegans* aging studies provide ample evidence that these processes are critical longevity determinants. For example, genes encoding members of the translational machinery (Rogers et al., 2011), molecular chaperones (Walker and Lithgow, 2003), autophagy (Hansen et al., 2008), the ER unfolded protein response (Henis-Korenblit et al., 2010) and proteosomal functions (Ghazi et al., 2007) can directly modulate lifespan or are required for lifespan extension in some long-lived mutants. It follows that pharmacologically targeting age-related decline in protein homeostasis could reduce and/or postpone age-related disease pathology and extend lifespan.

Worms have been genetically engineered to express human disease-associated proteins such as β -amyloid (Link, 1995; Link et al., 2003), polyglutamine (Gidalevitz et al., 2006), α -synuclein (Hamamichi et al., 2008) or aggregating forms of SOD-1 (Gidalevitz et al., 2009). Similar models have been generated in *D. melanogaster* (Lu and Vogel, 2009). For a comprehensive description of these models see "Invertebrate Models of Age Related Neurodegenerative Disorders" in this issue.

3.3 Dietary Restriction and its effect on lifespan

Dietary restriction (DR) is a robust means of extending the lifespan of model organisms. In invertebrates the beneficial effects of DR are not merely restricted to lifespan extension, but also alleviate many of the age-related declines in motor function, stress resistance and protein homeostasis (Cohen et al., 2006; Kastman et al., 2010; Steinkraus et al., 2008; Valdez et al., 2010). The benefits derived from reduced food intake are the result of the

modulation of several complex and only partially understood pathways. Despite their complexity, DR pathways appear conserved across species and therefore represent attractive pharmacological targets. By manipulating nutritional conditions, research performed in yeast, worms and flies have targeted signaling components that trigger the DR response (Katewa and Kapahi, 2010). For additional details about this pathway please see “Dietary Restriction and Aging” in this issue. In particular, the TOR pathway (Target of Rapamycin), Sirtuins and the forkhead box type transcription factors, have all been implicated in mediating DR. They are likely independent pathways and are conserved (to some extent) across metazoans (Kapahi et al., 2010; Lin et al., 2000; Panowski et al., 2007).

While it is quite clear that DR extends the lifespan of laboratory animals and is robust in invertebrate models, there is some doubt whether this holds true for certain genetic backgrounds including wild populations. A recent study which used 41 distinct lines of recombinant inbred mice has suggested that the effect of DR on animals can be more diverse and bidirectional than experiments on standard laboratory animals have previously indicated (Liao et al., 2010). However, this may be due to a failure to optimize the DR to each genetic background. It is currently unclear whether a DR regime tailored to and executed in humans would carry the beneficial effects we observe in model organisms. Despite this pending question of DR effects in non-laboratory organisms, the conservation of these major pathways, and their consistent role in modulating lifespan and ageing related phenotypes in model organisms, make DR pathways highly attractive targets for pharmaceutical interventions. They are also potentially more amenable to pharmacological manipulation compared to other lifespan modulating pathways, since they have an intrinsic ability to alter their action in response to external nutrient signals. This may allow for tapping into, or blocking, this innate exogenously modulated plasticity; pharmacological agents may mimic the signal(s) that indicate low nutrients (even though dietary conditions are in fact normal) and thereby induce an increased stress response in the animal, and ultimately cause it to live longer.

3.4 Insulin/IGF like signaling and its effects on lifespan

Insulin/IGF like signaling (IIS) has been shown to be important for modulating lifespan. In fact, *C. elegans* genes in this pathway were the first to be shown to dramatically influence metazoan lifespan (Johnson and Wood, 1982; Kenyon et al., 1993; Kimura et al., 1997; Klass, 1983). Homologous pathways have been shown to influence lifespan in vertebrates as well, including human beings (Baba et al., 2005; Bluher et al., 2003; Brown-Borg et al., 1996; Holzenberger et al., 2003; Kurosu et al., 2005; Migliaccio et al., 1999).

In *C. elegans* IIS pathway involves insulin and insulin like ligands that control insulin/IGF receptor activity. Inhibition of the receptor (DAF-2) activates the downstream FOXO type transcription factor, DAF-16 in *C. elegans*. Activated DAF-16 translocates into the nucleus where it activates the transcription of pro-longevity genes (Murphy et al., 2003; Oh et al., 2006). This triggers important morphological and metabolic changes, including an increase in fat and glycogen storage in intestinal and hypodermal cells (Kimura et al., 1997), as well as the induction of stress resistant genes, which confer thermotolerance and increased resistance to oxidative damage (Gems et al., 1998). For a comprehensive description of this pathway please see “Cell Signaling Pathways and Aging” in this issue. Due to the importance of this pathway in modulating aging, it is not surprising that many pro-longevity chemicals require the function of IIS; many reports have described chemicals that extend *C. elegans*’ lifespan in an IIS dependent manner.

4. Identification of Compounds Capable of Extending Lifespan

4.1 Compounds that extend lifespan via regulation of oxidative stress

There are many published and unpublished accounts concerning the effects of antioxidant treatment on lifespan. One of the first carefully conducted demonstrations of lifespan extensions as a consequence of compound treatment was provided in *D. melanogaster* (Brack et al., 1997) where it was shown that dietary uptake of the antioxidant and glutathione precursor N-acetylcysteine (NAC) results in a dose-dependent increase in median and maximum life span of up to 27%. The notion of lifespan extension by reducing oxidative stress in *C. elegans* first arose with the demonstration that long-lived *age-1* mutant worms (Friedman and Johnson, 1988a, b) conferred resistance to reactive oxygen species (ROS) including the herbicide paraquat (Vanfleteren, 1993) a superoxide anion generator. Indeed *age-1* mutants are also resistant to hydrogen peroxide (H₂O₂) (Larsen, 1993) and a range of other stresses (Lithgow, 2000).

As long-lived mutants in *C. elegans* exhibit resistance to oxidative stress we reasoned that oxidative damage was at least partially causative for aging, we therefore tested chemical antioxidants for effects on lifespan. We demonstrated that *C. elegans* longevity can be significantly increased by treatment with the small molecule synthetic catalytic mimetics (sometimes called “SCMs”) EUK-134 and EUK-8 that exhibit superoxide dismutase and catalase activities (Melov et al., 2000). This lifespan extension was achieved without affecting development rate or fertility. We went on to show that just like the *age-1* mutation, EUKs confer paraquat resistance (Sampayo et al., 2003). It is also tempting to think that the role of EUKs in protecting mitochondrial function in mouse models of oxidative stress (Melov et al., 2001) but this has not been demonstrated. Another group has failed to observe a lifespan increase with EUK-8 under apparently similar conditions (Keaney et al., 2004), one of the first examples of discrepancies between laboratories that suggest the importance of unknown environmental factors. Interestingly manganese, the catalytic constituent of EUKs, increases *C. elegans* thermotolerance and also increases lifespan of oxygen-sensitive *mev-1* mutant worms (Lin et al., 2006). The ability for antioxidants to increase lifespan remains controversial, since many studies report that antioxidants have no effect on longevity. It is likely that this controversy partially stems from the varied response from different species. For instance, α-lipoic acid, has been shown to have no effect on mouse lifespan (Lee et al., 2004), but does increase the lifespans of female *Drosophila* (Bauer et al., 2004) and *C. elegans* (Benedetti et al., 2008; Brown et al., 2006). Other notable accounts involving antioxidants and their effects on invertebrate lifespan include studies on vitamin E (Harrington and Harley, 1988; Zou et al., 2007), tocotrienol (Adachi and Ishii, 2000), coenzyme Q₁₀ (Ishii et al., 2004), and melatonin (Izmaylov and Obukhova, 1999; Thomas and Smith-Sonneborn, 1997). We will now discuss some more recent studies where prevention of oxidative stress may be playing a role on longevity.

Ferulic acid is a sesquiterpene coumarin present in genus *Ferula* a popular component in Chinese herbal medical preparations. This compound increased lifespan of wildtype *C. elegans* and although the mechanism of action was not thoroughly explored it provided oxidative and thermal protection suggesting that stress response plays a central role in the activity of this compound (Sayed, 2011). 4,4'-diaminodiphenylsulfone (DDS or dapsone) is a synthetic compound used in clinic to treat several skin diseases, particularly leprosy, that has been shown to increase lifespan in *C. elegans* (Cho et al., 2010). This effect is IIS and caloric restriction independent but the treatment with DDS decreases ROS production, oxygen consumption, mitochondrial V level and ATP levels (Cho et al., 2010). These results suggest that antioxidant activity could be a determinant in this compound's mechanism of action; however, these experiments do not rule out the possibility that alternative mechanisms could account for DDS's effect on lifespan. Interestingly another natural

product Cocoa, which is derived from *Theobroma cacao* increased lifespan in *D. melanogaster* potentially due to its antioxidant properties and its ability to chelate heavy metals (Bahadorani and Hilliker, 2008).

Another factor which may contribute to the controversy surrounding antioxidant induced lifespan extension, is the fact that oxygen radicals may themselves slow aging due to their induction of stress response mechanisms. For example, reduced glucose availability by treatment with 2-deoxy-D-glucose results in mitochondrial associated stress; restricting glucose promotes formation of reactive oxygen species (ROS), induces catalase activity, and increases oxidative stress resistance and survival rates (Schulz et al., 2007). This effect has been termed *mitohormesis* and treatment of worms with antioxidants prevents lifespan extension in this model. A number of small molecules are thought to extend lifespan by such a mechanism. Mitohormesis most likely represents part of a more general mechanism of *hormesis*, in which treatment with 'harmful' chemicals conversely leads to the activation of stress response(s) and other protective factors.

In summary, a robust extension of lifespan by an antioxidant across many species and in different conditions has not yet been demonstrated. It is clear that, in some experiments, antioxidants are capable of extending lifespan and although it is often assumed that they do so by reducing oxidative stress, such studies often fail to provide detailed biochemical evidence verifying this assumption.

4.2 Compounds that modulate protein homeostasis

Aggregation models, particularly those generated in *C. elegans*, have recently been used to assay the effects of pro-longevity compounds. Interestingly, many of the compounds that extend lifespan also cause a decrease in protein aggregation. For instance, celecoxib, which increases lifespan in worms through modulation of IIS, also decreases polyglutamine accumulation and improves the paralysis associated with aggregation of this peptide (Ching et al., 2011). Several natural products that act on the IIS pathway to increase lifespan, epigallocatechin gallate, trehalose and the polysaccharide from astragalus membranaceus (astragalin), also decrease the aggregation of A β peptide₃₋₄₂ (Abbas and Wink, 2010) and polyglutamine accumulation (Honda et al., 2010; Zhang et al., 2012). EGb 761 and lipoic acid, two compounds found in Ginkgo biloba, seem to increase lifespan through their antioxidant properties (Benedetti et al., 2008; Wu et al., 2002) and have also been reported to decrease pathological conditions induced by the overexpression of the A β peptide₃₋₄₂ (Wu et al., 2006).

Additionally, the ability of a stress response mimetic, derived from hydroxylamine (NG-094), to decrease protein aggregation in a worm model of Huntington's disease and increase lifespan has been reported to be HSF-1 dependent (Haldimann et al., 2011). HSF-1 (Heat Shock transcription Factor) is a master regulator of heatshock and non heatshock induced chaperone transcription. Furthermore, reserpine, which increases lifespan and improves stress resistance in a DAF-16 independent fashion (Srivastava et al., 2008), is also able to improve locomotion and decrease A β peptide₃₋₄₂ aggregation (Arya et al., 2009). It is interesting to note that the polyamine spermidine, one of the few compounds that has shown antiaging properties in yeast, *C. elegans* and *D. melanogaster* (Eisenberg et al., 2009) seems to work through increased autophagy suggesting that protein homeostasis could play a role in the increased lifespan elicited by this compound.

We previously noted that lithium, a drug frequently used in the treatment of bipolar disorder, extends lifespan in *C. elegans* potentially through an epigenetic mechanism (McColl et al., 2008). Interestingly, a recent study on the effects of lithium on human mortality from the Ristow group demonstrated that naturally occurring lithium levels in the tap water of distinct

Japanese municipalities were inversely associated with overall mortality rates (Zarse et al., 2011). In line with these results we have recently tested lithium in several heterologous aggregation models in *C. elegans* including a strain expressing human A β peptide_{3–42} under the control of a muscle specific (*myo-3*) promoter and found that it effectively suppressed the paralysis associated with A β aggregation (unpublished data). Another recent report, demonstrated that lithium suppress α -synuclein aggregation in a cell line derived from human neurons (Kim et al., 2011). Moreover, lithium has been shown to decrease the aggregation of polyQ in *Drosophila* (Berger et al., 2005) and rescues from polyglutamine induced cell death in neuronal and non-neuronal cell lines (Carmichael et al., 2002). The large amount of data demonstrating the beneficial effect of lithium suggests it has potential as a therapeutic for age related disease. However, it is important to keep in mind that the therapeutic concentration of lithium for mood disorders is close to the toxic range. For its potential use as an attenuator of age-related disease it will be important to determine any differences in effectiveness between the high doses used in acute treatments and the chronic use of lithium at low dose. The mouse appears an attractive model to test these treatment regimes. Alternatively, appropriate screens could be designed aimed at finding small molecules that mimic certain aspects of lithium action by targeting downstream mechanism.

We recently tested whether compounds, known to directly bind aggregated proteins, could affect aging (Alavez et al., 2011). Indeed, we found that some of these compounds, most notably Thioflavin T, extended lifespan and significantly decreased the paralysis phenotype associated with protein aggregation. We found that Thioflavin T's increased lifespan depends on HSF-1, (Hsu et al., 2003) and the longevity transcription factor involved in detoxification and stress resistance SKN-1 (Tullet et al., 2008), suggesting that the compound's extension of lifespan was dependent on its effects on protein aggregation and supporting the notion that protein homeostasis plays a major role in aging and age related pathology. In this paper we also reported that curcumin, the main component of *Curcuma longa* increases lifespan and prevents protein aggregation in *C. elegans*. This is in line with a report showing that curcumin is also able to increase lifespan in two different strains of *D. melanogaster*, perhaps through increasing its stress resistance (Lee et al., 2010). Collectively, these results indicate that modulators of protein homeostasis influence lifespan and demonstrate that these modulators likely act as common targets for multiple pro-longevity pathways. Due to the demonstrated importance of protein homeostasis pathways in modulating longevity and age-related phenotypes, chemical interventions, which target these pathways, represent some of the most promising candidates for treating age related disease in humans. For a comprehensive review regarding the ability of anti-aging compounds to prevent protein aggregation please see our recent review (Alavez and Lithgow, 2012)

4.3 Compounds that effect DR pathways and mediate lifespan extension

Reports of lifespan extension in multiple model organisms by the TOR pathway inhibitor rapamycin, has provided motivation to identify more such pharmacological modulators of DR pathways (Harrison et al., 2009). However, to date there are few chemicals that have been clearly demonstrated to induce DR effects in model organisms, and fewer still that have been extensively characterized in terms of their mechanism of action. One of the few chemicals to be implicated in DR type pathways is resveratrol which is an activator of sirtuins (Howitz et al., 2003) and appears to induce many of the metabolic changes associated with DR, however the absence of a lifespan extension in mice on normal diets (Baur et al., 2006) suggests that it does not mimic important aspects of DR. Recent work with resveratrol analogs that are more robust activators of sirtuins show more promise as potential human drugs. In *C. elegans*, the anti-depressant mianserin (Petrasccheck et al., 2007) and the anti-diabetic metformin (Onken and Driscoll, 2010) have both been shown to

extend lifespan through DR type pathways and represent some of the few demonstrated examples of drug interventions that can modulate these pathways. This is remarkable since there are a large number of genes contributing to these processes. These studies will be further discussed, later in this review, in the section on human drugs that have been found to modulate invertebrate lifespan.

It is likely that there remain many as yet unidentified chemicals capable of influencing the physiological state of animals by way of altering the activity of DR type pathways. A recent example is provided by the recent identification of a set of endogenous chemicals (n-acylethanolamines, including the endocannabinoid, anandamide) which exert control over the DR response in worms (Lucanic et al., 2011). The Gill laboratory, in collaboration with ourselves, found that the dietary state of the worm is in part controlled by internal levels of n-acylethanolamines; levels of these compounds increased with increased food intake and when levels were decreased, by over-expression of a hydrolytic enzyme that degrades them, animals displayed a DR phenotype. Moreover, several phenotypes of DR, were found to be blocked by treating animals with one of these endogenous compounds (EPEA; eicosapentanoylethanolamine). These results suggest that n-acylethanolamines are used by the worm to communicate nutrient levels systemically. In the context of pharmacological interventions, our results indicate that in addition to DR pathways, endogenous small molecule signaling cascades represent excellent pharmaceutical targets. However, as with other pathways, the extent of conservation between organisms is an important factor contributing to the potential significance for drug screening in model organisms.

4.4 Compounds that effect IIS pathways and mediate lifespan extension

Many of the chemicals shown to modulate lifespan through IIS are known drugs or natural compounds that have been used in traditional healing practices and supplements associated with alternative medicine. Natural products represent an untapped reservoir filled with great therapeutic potential. Natural compounds may hold particular promise because they are likely to have been subject to evolutionary pressure to sustain biological activity, and therefore have a high probability of influencing animal physiology. Several such compounds have been found to affect the lifespan of *C. elegans* in an IIS dependent manner.

Flavonoids are a class of plant derived polyphenols that share a common sub-structure, and have been widely reported to affect mammalian physiology; among these are quercetin and catechin, both of which seem to impact the IIS signaling pathway. Quercetin is widely distributed in edible fruits, vegetables and plants and may increase *C. elegans* lifespan by increasing the worms' resistance to several kinds of cellular stresses (Kampkotter et al., 2008; Pietsch et al., 2009). Interestingly, the effects of quercetin depend on two critical genes of the IIS pathway, but dependence on the transcription factor DAF-16 remains controversial (Saul et al., 2008). Another abundant flavonoid with multiple reported health benefits, is catechin, which may act at least partly in parallel to IIS (Saul et al., 2009). However, one form of catechin, epigallocatechin gallate ([*(2R,3R)*-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl] 3,4,5-trihydroxybenzoate) increases oxidative stress resistance and decreases A β peptide₃₋₄₂ aggregation through a mechanisms that correlates with DAF-16 nuclear localization (Abbas and Wink, 2009, 2010). Other polyphenols, namely tannic acid and ellagic acid, also extend worm lifespan (Saul et al., 2010). Although none of these compounds in isolation have been shown to increase the lifespan of other model organisms, in *D. melanogaster*, extracts of green (Li et al., 2007) and black teas (Peng et al., 2009), which contain catechins and unique compounds known as theaflavins, result in increased lifespan.

Icariin is a flavonol and appears to be the active ingredient in several traditional remedies which make use of the *epimedium* plant family. Shen and colleagues found that icariin

extends the lifespan of *C. elegans* grown in liquid media, and that the active form of icariin is likely an endogenously generated metabolite known as icariside II (Cai et al., 2011). They showed that icariin extends median lifespan, promotes thermotolerance and resistance to oxidative stress. However, it does not extend the lifespan of insulin/IGF like signaling pathway mutants and it promotes expression of transcriptional targets of DAF-16. Collectively these results demonstrate that icariin extends lifespan by activating the IIS pathway in *C. elegans*.

Trehalose is a disaccharide naturally found in *C. elegans*. It is thought that it confers resistance to dehydration as well as other stresses, perhaps by replacing water content in proteins which thereby increases their structural stability. Honda and colleagues tested the ability of trehalose to extend the lifespan of *C. elegans* and found that it was unique among disaccharides in promoting a robust extension of lifespan (Honda et al., 2010). Interestingly, the authors found that trehalose was effective at extending lifespan even when treating late-middle age worms. Treatment also extended the reproductive period and increased thermotolerance in wildtype animals. In a series of elegant experiments, Honda and colleagues found that a degree of the lifespan extension exhibited by IIS signaling mutants is due to an increase in trehalose. For example, trehalose levels are up in *daf-2* mutants and decreasing their levels (by RNAi of trehalose biosynthetic enzymes) dramatically suppresses the lifespan enhancement phenotype found in *daf-2* mutants. Trehalose has no additional longevity effect on long-lived *daf-2* mutants, but does extend the lifespan of *daf-16* mutants. This data suggests that trehalose normally acts downstream of DAF-16 and that its levels are regulated by IIS.

In a recent study that echoes the early studies in *C. elegans*, Honda and colleagues investigated the properties of royal jelly and its effect on the lifespan of *C. elegans* (Honda et al., 2011). Royal jelly is a complex mixture of biological proteins, sugars and fatty acids, which is secreted by bees and is fed to larvae and the queen bee. Interestingly, the authors found robust lifespan extension from protease treated and fractionated royal jelly. The authors' experiments suggest that different components of royal jelly lead to lifespan extension through multiple distinct pathways, including those both dependent and independent of insulin/IGF like signals.

The Krebs cycle intermediate oxaloacetic acid is able to increase lifespan in *C. elegans* (Williams et al., 2009). Mechanistic information on this is limited, but it was shown that the lifespan increase was abolished in *daf-16* mutants suggesting a critical role of IIS in controlling metabolism to promote longevity. Interestingly, a building block of fatty acids, acetate, also increases lifespan in worms (Chuang et al., 2009). Acetate prolongevity activity also depends on DAF-16 and seems to be modulated at the DAF-2 (receptor) level.

The finding that natural compounds can modulate activity of IIS pathway is of particular interest since it raises the possibility that nutraceutical interventions can be useful for targeting one of the major pathways involved in the control of lifespan, IIS. From invertebrates to mammals, the IIS pathway is a highly studied modulator of lifespan and presents multiple options for drug interventions. A particular challenge will be to identify compounds which target signaling components downstream of DAF-16. Regardless of their source, chemicals which target the IIS pathway may lead to the discovery of important drugs that will help us cope with the detrimental physiological changes associated with aging.

4.5 Compounds that affect lifespan extension by as yet undetermined means

Many other natural products have recently been tested for their ability to modulate *C. elegans*' lifespan. While several of these have been reported to require IIS pathways, some of these compounds appear to work in an IIS independent manner. A series of experiments

in *Drosophila* have shown that extracts from the Damask rose, *Rosa damascena* have lifespan extending effects in *Drosophila* with no obvious detriment on quality of life measures (Jafari et al., 2008; Schriner et al., 2011). Blueberry extracts which contain complex mixtures of polyphenols have also been shown to extend the lifespan of both *C. elegans*, in a DAF-16 independent manner, and *D. melanogaster* (Peng et al., 2012; Wilson et al., 2006). Recent results from Alfred Fisher's Lab have shown that the garlic constituent diallyl trisulfide increases the lifespan of *C. elegans* by enhancing the activity of the pro-longevity transcription factor, SKN-1 (Powolny et al., 2011). This compound has to be added to the growing list of compounds that require this transcription factor to increase lifespan.

5 Translating Chemical Biology of Aging Studies from Invertebrates to Humans

5.1 General Challenges That Impede the Discovery of Drugs Capable of Extending Human Lifespan

If the motivation for discovering compounds capable of extending the lifespan of invertebrates is ultimately to improve human health, then we must address how the basic experiments described here are to be translated into clinical research. Many investigators choose to study natural products, possibly because they wish to understand the mechanistic action of these products, but also perhaps due to the fact that FDA approval of edible natural product is considerably less complex and costly than approval of synthetic compounds. Others often begin even one step closer, by studying already approved human drugs, and examining whether they have an effect on invertebrate lifespan. Still, one of the biggest obstacles to stand between pharmacological research in invertebrates and ultimate human drug development is the intermediary step of testing compounds in mammalian models. Testing the ability of drugs to extend the lifespan of mice is expensive, especially when one considers its relatively long lifespan. This points to the need to choose compounds to be tested in mammals in a directed manner, so that a limited number of promising candidates can be tested in a cost effective manner, and further highlights the importance of invertebrate models in meeting such challenges.

6 Ways to Circumvent Challenges

6.1 Working Backwards: Testing Human Drugs for Lifespan Extension in Invertebrates

One way to expedite the discovery of drugs capable of extending the lifespan of humans is to begin by examining existing human drugs for their ability to extend the lifespan of invertebrates. Once these drugs have been verified to extend invertebrate lifespan, and the mechanism(s) by which they extend lifespan is determined, the likelihood that they might extend mammalian lifespan can be assessed. Ultimately such existing drugs could be reexamined in humans, this time for their ability to treat age-related diseases. A number of studies have tested the ability of human drugs to extend the lifespan of invertebrates, and their findings are discussed here.

6.2 Anticonvulsants

In 2005, Kerry Kornfeld and colleagues described a small chemical screen of drugs with known influence on human physiology for effects on *C. elegans* lifespan (Evason et al., 2005). The authors found that the anticonvulsant ethosuximide had a potent effect on the lifespan of worms. The authors demonstrated that two structurally related anticonvulsants (trimethadiazone and 3,3-diethyl-2-pyrrolidinone) also had lifespan extending effects. A variety of pharmacological and genetic experiments allowed the experimenters to determine that ethosuximide is likely to function through hyper-activation of the *C. elegans* nervous

system. They found that ethosuximide and/or trimethadione increased egg-laying, motility and hypersensitivity to a cholinesterase inhibitor. This suggested that the lifespan effects may result from the drugs modulation of nervous system activities.

Follow-up studies proved that ethosuximide lifespan extension indeed occurred through modulation of neuronal signaling (Collins et al., 2008). The authors found that high doses of ethosuximide caused larval lethality and used this as the basis for a forward genetic screen. This classically designed and elegant approach led to the identification and mapping of mutant alleles with resistance to ethosuximide. They focused on the identification of *che-3*, a gene which encodes a dynein heavy chain important for the formation and maintenance of cilia. A mutant of *che-3* was tested and found to be resistant to the lethal effects of ethosuximide. A number of new alleles of *che-3*, as well as alleles of *osm-3*, a Kinesin-2 family member involved in intraflagellar transport (IFT) and which is essential for the construction and maintenance of sensory cilia, were identified that confer ethosuximide resistance. This suggested ethosuximide treatment altered ciliated neuron function and indeed the compound has been found to mimic many of the phenotypes found in mutants with defective ciliated neurons, including chemotaxis and dauer entry defects. Importantly, the authors showed that ethosuximide did not induce ablation of the ciliated neurons but rather perturbed their function in some other manner. Collectively these experiments effectively demonstrate that the lifespan extension effect of ethosuximide results from the inhibition of sensory neurons that are responsible for detecting aspects of the environment, including the presence of external nutrients. It remains to be seen whether other compounds disused here also act through effects on these neurons.

The structurally distinct anticonvulsant, valproic acid, another FDA approved drug, has also been investigated for effects on aging (Evason et al., 2008). Valproic acid is a commonly prescribed anti-epileptic with a simple carboxylic acid structure (2-propylpentanoic acid). The authors indicate that valproic acid is likely to extend the lifespan of worms through a mechanism distinct from the anticonvulsants ethosuximide and trimethadione. They found that DAF-16 is required for the lifespan effects of valproic acid and that treatment increases nuclear localization of a DAF-16::GFP fusion in transgenic worms. (Nuclear localization of DAF-16 has previously been shown to be associated with activation of DAF-16 and thereby extends lifespan (Henderson and Johnson, 2001; Lin et al., 2001)). Additionally, the authors demonstrate that the lifespan extension effects of valproic acid and trimethadione are additive. This result was used to argue that valproic acid is likely to act in an independent pathway from trimethadione.

A recent study on the lifespan and health effects of another anticonvulsant, lamotrigine, was performed in *D. melanogaster* by Jafari and colleagues. Hundreds of flies, both male and female, were assayed for survival allowing for the calculation of the mortality rate. The study showed that lamotrigine lowered mortality rates in *Drosophila*. However, the authors also analyzed complex behavioral traits in the fly, and showed that treatment with the compound suppressed locomotion. The authors interpreted this lack a mobility as an indicator of poor health (Avanesian et al., 2010). So while the compound extended lifespan and reduced mortality rates, it had adverse effects on putative measures of quality of life such as motor activity and metabolic function. This study raises the important point, that if a chemical treatment decreases the overall health of the animal it is unlikely to be a potential therapeutic. However, it is conceivable that some treatments exhibiting extended lifespan but decreased health may have separable mechanisms. An otherwise strong chemical candidate should warrant further study to identify the actual linkage between the two effects. The decrease in health could feasibly be attributable to off target effects and this may be worth teasing out, by testing other models or chemical derivatives of the candidate.

In another candidate screen examining drugs known to regulate nervous system function in humans, Subramanian and colleagues identified reserpine as a compound capable of extending the lifespan of *C. elegans* (Srivastava et al., 2008). Reserpine is a natural alkaloid that blocks the vesicular monoamine transporter and has been used as an antipsychotic and antihypertensive. Pharmacogenetics strategies have indicated that the mechanism by which reserpine extends lifespan is at least partly independent of insulin/IGF like and serotonin signaling. A remaining question from this initial study is whether the action of reserpine is related to dietary restriction (DR) lifespan extension. The authors do possibility by examining the effect of treating an *eat-2* mutant with reserpine. *eat-2* encodes an acetylcholine binding ion channel that controls feeding behavior; mutants carrying defective copies of this gene are poor feeders and because of this are constitutively under DR. While the authors do observe a lifespan extension after reserpine treatment it is not clear that this is independent of DR. In particular, because *eat-2* alleles are hypomorphic and often do not lead to very strong DR, and so only weakly extend lifespan. This can be an issue since a hypomorph can be enhanced by a treatment that acts even in the same pathway. In the report from Srivastava and colleagues the *eat-2* allele they use does not appear to be significantly long-lived compared to the wild-type (Srivastava et al., 2008). This perhaps suggests that its lifespan could still be enhanced by a further DR treatment. In a subsequent study, this group demonstrated the ability of reserpine to ameliorate the effects of A β induced toxicity (Arya et al., 2009) in a *C. elegans* disease model for Alzheimer's (Link, 1995) and importantly showed that the drug did not merely decrease A β levels. In a recent follow-up study, they demonstrated that the modulation of lifespan and A β toxicity is related to acetylcholine signaling, as several mutants in acetylcholine signaling either do not respond to reserpine treatment or respond in a negative manner (Saharia et al., 2012). Since acetylcholine signaling is important for feeding behaviors, it seems worthwhile to further evaluate whether reserpine is acting via a dietary restriction mechanism to extend lifespan. Regardless, further work is needed to detail the specific mechanism of reserpine mediated lifespan extension.

6.3 Anti-depressants

In search of small molecules that extend the lifespan of *C. elegans*, Petrascheck and colleagues discovered a lifespan extending compound that bears structural similarity to known antidepressants of the serotonin receptor antagonist type (Petrascheck et al., 2007). They found that several of these known human drugs, also extend lifespan. They went on to show that mianserin and the related methiothepin indeed act as antagonists for *C. elegans*' serotonin receptors; using calcium imaging experiments in heterologously transfected cell cultures, the authors elegantly demonstrated that mianserin directly targets the serotonin type receptors SER-3 and SER-4. They also showed genetically that serotonin signaling is required for the lifespan extending effects of mianserin and that the effect specifically requires the *ser-3* and *ser-4* genes.

While the Petrascheck study suggests that serotonin signaling modulates *C. elegans*' lifespan, there remain major unanswered questions about the effect of serotonin receptor signaling on lifespan in *C. elegans*. The authors suggest that mianserin acts through a dietary restriction (DR) type pathway(s), since the drug had little additional lifespan extending effect on either animals undergoing DR or on a genetic model of DR. This is consistent with processes known to be regulated by serotonin signaling in *C. elegans*. Intriguingly, drug treatment was most effective if given to adults at an early stage, and was not nearly as effective when given at both larval and adult stages. This demonstration of a strict timing requirement for the activity suggests a complexity in the mechanism of action. Indeed, removal of either of the drug targets alone (SER-3 or SER-4) did not phenocopy mianserin treatment, though this may be explained by redundancy of the two receptors. Moreover, it was later shown by another group that the lifespan extending effects of mianserin do not

work under all standard culture conditions, but instead only work in liquid culture environments; this group went on to show that under other laboratory conditions mianserin actually shortens the lifespan of *C. elegans* (Zarse and Ristow, 2008). This differing behavioral response to chemicals under distinct environmental conditions has been demonstrated for other compounds as well. Zarse and Ristow go on to argue that this shortened lifespan is in fact the expected result for a serotonin type receptor antagonist in *C. elegans*, a statement supported by an earlier report which showed that a serotonin receptor knockout (*ser-4*) has a shortened lifespan (Murakami and Murakami, 2007). Collectively, all of these results demonstrate that serotonin type receptors and serotonin signaling modulate *C. elegans* lifespan in a complex manner that is influenced by the environment of the animal and is likely to be related to dietary restriction type pathways.

The number of nervous system modulators that have been shown to extend the lifespan of invertebrates is remarkable. Interestingly they seem to operate through distinct mechanisms to alter lifespan. While there is no apparent conserved mechanism between these chemicals it is perhaps plausible that due to the conservation of nervous system function across phyla, chemicals that alter these signaling cascades are highly likely to be effective across species. One question to ask is; why do these modulators of the nervous system all extend the lifespan of *C. elegans*? Likely this is due to the influence of the nervous system on the lifespan of the organism. Many behaviors and pathways controlled by the nervous system influence lifespan; these include perception of the environment, control of feeding behaviors and the release of insulin like peptides. The nervous system is intricately tied to longevity in the worm.

6.4 Anti-inflammatories

Celecoxib is a non-steroidal anti-inflammatory (NSAID), originally developed as a cyclooxygenase 2 inhibitor. However, it was suspected of having additional targets including PDK-1, a known component of IIS (Hsu et al., 2000; Zhu et al., 2004). Hsu and colleagues tested for aging effects of celecoxib in *C. elegans* and found that it extended the lifespan of the worms in a dose dependent manner, and that it had no effect on the growth of the worms' bacterial food (Ching et al., 2011). They then demonstrated that it also robustly extends the lifespan of a genetic model for DR, as well as a mutant with a defect in mitochondrial function, suggesting that the drug does not act in these known lifespan modulating pathways. The drug also failed to extend the lifespan of IIS pathway mutants, *daf-2* and *daf-16*. Since the authors suspected that the drug was acting through a non-cyclooxygenase target, they next tested the activity of a structurally related chemical that does not have activity against mammalian cyclooxygenase-2 (at least not at low doses). Remarkably the authors found that this compound exhibited similar properties as celecoxib, in that it extends lifespan to a similar extent and displays the same pharmacogenetic interactions. Consistent with a role in inhibiting the IIS pathway, the authors found that both chemicals decreased the phosphorylation of PGK-1 (a downstream target of PDK-1), indicating that the drugs act either on PDK-1 itself or some target upstream of PDK-1 in the IIS pathway. Further supporting the idea that these drugs function in the IIS pathway, the authors found that the drugs induced activation of DAF-16 (Ching et al., 2011). This meticulous study identifies two of the more potent chemical activators of DAF-16 ever reported. Due to the extensive knowledge of their mechanism of action and the conservation of their target, these drugs represent particularly attractive candidates for the further testing in other invertebrate and vertebrate models.

6.5 Anti-diabetics

The anti-diabetic drug metformin, a chemical of the biguanide class, has been found to extend the lifespan of *C. elegans* through a DR type mechanism (Onken and Driscoll, 2010).

Previously this drug had been reported to have no effect on mortality rates in flies (Jafari et al., 2007) but had been reported to extend lifespan of female mice through AMPK (adenosine mono-phosphate activated kinase) activation (Anisimov et al., 2008). In mammals metformin lowers blood glucose levels through activation of AMPK. Since previous reports suggested that metformin positively influences health and aging, Onken and Driscoll tested its effects on *C. elegans* and found that it extended the median lifespan of wildtype and insulin/IGF like signaling mutants (Onken and Driscoll, 2010). Moreover, they presented evidence that metformin acts by extending lifespan through a mechanism related to DR; the drug induced a DR-specific fluorometric property in worms (Gerstbrein et al., 2005), and failed to extend the lifespan of a DR mutant. They then identified two conserved kinases that mediate metformin signaling in vertebrates (PAR-4 and AAK-2) and found that they are required in *C. elegans* for the lifespan extending properties of the drug. Finally, the authors went on to show that metformin mediated lifespan extension requires *skn-1*, the *C. elegans* homologue of NRF2, a transcription factor generally required for stress response and previously implicated in DR (Bishop and Guarente, 2007). It remains ambiguous at which step the drug impinges on the DR pathway in *C. elegans*.

6.6 Start out in Mice: Identification of Compounds that Extend the Lifespan of Mice

The current excitement surrounding the development of drugs that target aging processes, is partly attributable to the realization that mammalian lifespan can be extended with small molecules. For example, male, but not female lifespan can be increased by feeding mice nordihydroguaiaretic acid and aspirin (2-acetoxybenzoic acid) (Strong et al., 2008). In addition, the immunosuppressant rapamycin inhibits mTOR signaling and is able to increase lifespan in both male and female mice when administered late in life (Harrison et al., 2009; Miller et al., 2011). This discovery has received significant attention, in part because it resulted from collaboration between three centers with expertise in mouse aging, and was funded by the National Institute on Aging, Interventional Testing Program. The study design, with experiments being conducted at three discrete sites, suggests that the effect of rapamycin on aging is robust, even if the mechanism(s) by which it extends lifespan remains obscure for now. Beneficial effects of rapamycin have also been reported in *Drosophila* by Linda Partridge's group who showed that rapamycin affects particularly the TORC1 branch of the TOR pathway (Bjedov et al., 2010). The beneficial effects of rapamycin and rapamycin-derived compounds seem to span several age related pathologies and it has shown particular promise in the treatment of cancer, having gone so far as to be tested in clinical trials in humans (Zaytseva et al., 2012). In fact, rapamycin has been approved by the U.S. Food and Drug Administration for the treatment of pancreatic cancer. As ongoing studies investigate the effectiveness of rapamycin in extending lifespan of various species, there is considerable speculation about whether this discovery will usher in a new era of pharmacological treatments for age related diseases such as Alzheimer's, Parkinson's, cardiovascular disease and adult cancers.

In addition to extending the lifespan of mammals, targeting aging pathways may identify compounds that improve health under specific diets or in particular circumstances. For example, the polyphenol resveratrol (3,5,4'-trihydroxy-trans-stilbene), originally suggested to be an activator of NAD⁺-dependent protein deacetylases of the sirtuin family, improves some health indicators and increases the lifespan of mice on a high-calorie diet, but has failed to increase the lifespan of mice on a standard diet, leading to controversy as to whether this compound slows normal aging (Baur et al., 2006; Miller et al., 2011). Results in invertebrate models add to the controversy about resveratrol effect on aging. While some groups reported an increase in lifespan elicited by resveratrol in yeast, worms and fruit flies (Gruber et al., 2007; Wood et al., 2004) other groups have been unable to reproduce these results (Bass et al., 2007).

6.7 Choosing Lifespan Extending Candidates from Invertebrate Models to be Tested in Mammalian Systems

While initially testing compounds in mice means that any discoveries are more likely to be relevant for drug development in humans, the prohibitive cost of mouse aging studies make it extremely impractical to carryout large scale chemical screens in mice. It is difficult to select candidates *a priori* based on their perceived likelihood of success. However, several criteria can be described that, if completely fulfilled, should immediately promote an effective invertebrate treatment into testing on mammals. First of all, the mechanism should be defined and found to be non-idiosyncratic for the model. That is, the chemical treatment should have a known mechanism that targets a conserved pathway important for aging in multiple models. Additionally, the response should be robust, should show a dose response and should be effective at multiple points during the lifespan. A chemical that does not give a strong and consistently effective response is not likely to be worth following up on in other organisms. If it does not show a dose response it is unlikely to be acting on a single target and if it only works at a specific time in development (such as a larval stage) it is likely an idiosyncratic response of the organism. Additionally, the compound should not be deleterious to the general health of the organism and finally, the chemical candidate should be effective across multiple invertebrate species.

Further investigation into the responsiveness of *C. elegans* to a multitude of chemicals, continues to demonstrate novel applications of the system, and suggest that research will be useful for refining candidates, to select those that hold the most therapeutic promise. For example, Peter Roy's group has investigated the idiosyncrasies of *C. elegans*' drug response and defined several parameters which help broadly determine drug efficacy in the model organism (Burns et al., 2010). They tested the likelihood of accumulation vs. metabolism of more than 1,000 commercially available drug-like small molecules. Only one in ten molecules accumulated to concentrations greater than 50% of the external concentration. With this information, the authors were able to determine general pharmacological characteristics which facilitated small-molecule accumulation in the worm. This study highlights the fact that an understanding of the shared structural features that generally promote drug efficacy can enhance predictive power, and in principle should allow for more targeted screening.

7 The Challenge of Translation

If one of the goals of pharmacological lifespan extension in invertebrates is to improve human health then we must address how the basic experiments described here are to be translated into clinical research or healthy dietary choices. It is clear that many investigators choose to study natural products. This in part may come from the need to develop a mechanistic understanding of the action of natural products but also may be because the regulatory path to human use is considerably less complex and costly.

One of the biggest hurdles is the testing of compounds in mammalian models. The cost to individual investigators of a mouse aging study makes it unlikely that large numbers of compounds will be tested in the near future. It is difficult to select the best high value candidate compounds, which highlights the need for an ever more detailed understanding of the mechanisms at play. The invertebrate models can provide this information. Most critical, is the issue of whether the mechanism of action is likely to be conserved between invertebrates and mammals. By determining the mechanisms at play and uncovering which factors (intracellular signaling pathways, transcription factors, etc.) are required for compound action, research in the invertebrates could provide high value compounds for mammalian studies.

One area of small molecule research in invertebrate aging has received little attention; the role of metabolism in compound action. Future studies should take standard pharmacokinetics into account and consider the role of modifications to compounds on the observed biological action. Classical pharmacokinetics is difficult in invertebrates mainly because of the lack of circulatory systems and the difficulty in dissecting individual tissues; animals usually have to be pulled to provide enough material for analysis. However, this should not be an insurmountable barrier to basic metabolic studies.

The need to develop therapies and preventions for age-related disease is great. Recent demonstration of lifespan extension with rapamycin in the mouse (Harrison et al., 2009), as well as the demonstration that resveratrol induces metabolic changes in obese humans that mimic the beneficial effect of DR (Timmers et al., 2011) provide some very encouraging examples of how discoveries in invertebrates can be translated into pre-clinical and clinical research. There is a bright future for this approach and a clear role to be played by the invertebrate models.

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References

- Abbas S, Wink M. Epigallocatechin gallate from green tea (*Camellia sinensis*) increases lifespan and stress resistance in *Caenorhabditis elegans*. *Planta Med.* 2009; 75:216–221. [PubMed: 19085685]
- Abbas S, Wink M. Epigallocatechin gallate inhibits beta amyloid oligomerization in *Caenorhabditis elegans* and affects the daf-2/insulin-like signaling pathway. *Phytomedicine.* 2010; 17:902–909. [PubMed: 20382008]
- Adachi H, Fujiwara Y, Ishii N. Effects of oxygen on protein carbonyl and aging in *Caenorhabditis elegans* mutants with long (age-1) and short (mev-1) life spans. *J Gerontol A Biol Sci Med Sci.* 1998; 53A:B240–B244. [PubMed: 18314552]
- Adachi H, Ishii N. Effects of tocotrienols on life span and protein carbonylation in *Caenorhabditis elegans*. *J Gerontol A Biol Sci Med Sci.* 2000; 55:B280–285. [PubMed: 10843344]
- Alavez S, Lithgow GJ. Pharmacological maintenance of protein homeostasis could postpone age-related disease. *Aging cell.* 2012; 11:187–191. [PubMed: 22226190]
- Alavez S, Vantipalli MC, Zucker DJ, Klang IM, Lithgow GJ. Amyloid-binding compounds maintain protein homeostasis during ageing and extend lifespan. *Nature.* 2011; 472:226–229. [PubMed: 21451522]
- Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, Zabezhinski MA, Tyndyk ML, Yurova MV, Kovalenko IG, Poroshina TE, Semchenko AV. Metformin slows down aging and extends life span of female SHR mice. *Cell Cycle.* 2008; 7:2769–2773. [PubMed: 18728386]
- Arya U, Dwivedi H, Subramaniam JR. Reserpine ameliorates Abeta toxicity in the Alzheimer's disease model in *Caenorhabditis elegans*. *Experimental gerontology.* 2009; 44:462–466. [PubMed: 19264117]
- Avanesian A, Khodayari B, Felgner JS, Jafari M. Lamotrigine extends lifespan but compromises health span in *Drosophila melanogaster*. *Biogerontology.* 2010; 11:45–52. [PubMed: 19430925]
- Baba T, Shimizu T, Suzuki Y, Ogawara M, Isono K, Koseki H, Kurosawa H, Shirasawa T. Estrogen, insulin, and dietary signals cooperatively regulate longevity signals to enhance resistance to oxidative stress in mice. *J Biol Chem.* 2005; 280:16417–16426. [PubMed: 15713666]
- Bahadorani S, Hilliker AJ. Cocoa confers life span extension in *Drosophila melanogaster*. *Nutr Res.* 2008; 28:377–382. [PubMed: 19083435]

- Bass TM, Weinkove D, Houthoofd K, Gems D, Partridge L. Effects of resveratrol on lifespan in *Drosophila melanogaster* and *Caenorhabditis elegans*. *Mech Ageing Dev.* 2007; 128:546–552. [PubMed: 17875315]
- Bauer JH, Goupil S, Garber GB, Helfand SL. An accelerated assay for the identification of lifespan-extending interventions in *Drosophila melanogaster*. *Proc Natl Acad Sci U S A.* 2004; 101:12980–12985. [PubMed: 15328413]
- Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature.* 2006; 444:337–342. [PubMed: 17086191]
- Beckman, KB.; Ames, BN.; Scandalios, JG. Oxidants, antioxidants and aging, Oxidative stress and the molecular biology of antioxidant defences. CSHL Press; New York: 1997. p. 201-246.
- Benedetti MG, Foster AL, Vantipalli MC, White MP, Sampayo JN, Gill MS, Olsen A, Lithgow GJ. Compounds that confer thermal stress resistance and extended lifespan. *Experimental gerontology.* 2008; 43:882–891. [PubMed: 18755260]
- Berger Z, Ttofi EK, Michel CH, Pasco MY, Tenant S, Rubinsztein DC, O’Kane CJ. Lithium rescues toxicity of aggregate-prone proteins in *Drosophila* by perturbing Wnt pathway. *Hum Mol Genet.* 2005; 14:3003–3011. [PubMed: 16141285]
- Bishop NA, Guarente L. Two neurons mediate diet-restriction-induced longevity in *C. elegans*. *Nature.* 2007; 447:545–549. [PubMed: 17538612]
- Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, Partridge L. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab.* 2010; 11:35–46. [PubMed: 20074526]
- Blüher M, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science.* 2003; 299:572–574. [PubMed: 12543978]
- Bokov A, Chaudhuri A, Richardson A. The role of oxidative damage and stress in aging. *Mech Ageing Dev.* 2004; 125:811–826. [PubMed: 15541775]
- Brack C, Bechter-Thuring E, Labuhn M. N-acetylcysteine slows down ageing and increases the life span of *Drosophila melanogaster*. *Cell Mol Life Sci.* 1997; 53:960–966. [PubMed: 9447249]
- Breger J, Fuchs BB, Aperis G, Moy TI, Ausubel FM, Mylonakis E. Antifungal Chemical Compounds Identified Using a *C. elegans* Pathogenicity Assay. *PLoS Pathog.* 2007; 3:e18. [PubMed: 17274686]
- Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. *Nature.* 1996; 384:33. [PubMed: 8900272]
- Brown MK, Evans JL, Luo Y. Beneficial effects of natural antioxidants EGCG and alpha-lipoic acid on life span and age-dependent behavioral declines in *Caenorhabditis elegans*. *Pharmacol Biochem Behav.* 2006; 85:620–628. [PubMed: 17156833]
- Burns AR, Wallace IM, Wildenhain J, Tyers M, Giaever G, Bader GD, Nislow C, Cutler SR, Roy PJ. A predictive model for drug bioaccumulation and bioactivity in *Caenorhabditis elegans*. *Nat Chem Biol.* 2010; 6:549–557. [PubMed: 20512140]
- Cai WJ, Huang JH, Zhang SQ, Wu B, Kapahi P, Zhang XM, Shen ZY. Icaritin and its Derivative Icariside II Extend Healthspan via Insulin/IGF-1 Pathway in *C. elegans*. *PLoS One.* 2011; 6:e28835. [PubMed: 22216122]
- Cao S, Hewett JW, Yokoi F, Lu J, Buckley AC, Burdette AJ, Chen P, Nery FC, Li Y, Breakefield XO, Caldwell GA, Caldwell KA. Chemical enhancement of torsinA function in cell and animal models of torsion dystonia. *Dis Model Mech.* 2010; 3:386–396. [PubMed: 20223934]
- Carmichael J, Sugars KL, Bao YP, Rubinsztein DC. Glycogen synthase kinase-3beta inhibitors prevent cellular polyglutamine toxicity caused by the Huntington’s disease mutation. *J Biol Chem.* 2002; 277:33791–33798. [PubMed: 12097329]
- Chang S, Bray SM, Li Z, Zarnescu DC, He C, Jin P, Warren ST. Identification of small molecules rescuing fragile X syndrome phenotypes in *Drosophila*. *Nat Chem Biol.* 2008; 4:256–263. [PubMed: 18327252]

- Chen X, Longgood JC, Michnoff C, Wei S, Frantz DE, Bezprozvanny L. High-throughput screen for small molecule inhibitors of Mint1-PDZ domains. *Assay Drug Dev Technol.* 2007; 5:769–783. [PubMed: 18078379]
- Ching TT, Chiang WC, Chen CS, Hsu AL. Celecoxib extends *C. elegans* lifespan via inhibition of insulin-like signaling but not cyclooxygenase-2 activity. *Aging cell.* 2011; 10:506–519. [PubMed: 21348927]
- Cho SC, Park MC, Keam B, Choi JM, Cho Y, Hyun S, Park SC, Lee J. DDS, 4,4'-diaminodiphenylsulfone, extends organismic lifespan. *Proc Natl Acad Sci U S A.* 2010; 107:19326–19331. [PubMed: 20974969]
- Chuang MH, Chiou SH, Huang CH, Yang WB, Wong CH. The lifespan-promoting effect of acetic acid and Reishi polysaccharide. *Bioorg Med Chem.* 2009; 17:7831–7840. [PubMed: 19837596]
- Cohen E, Bieschke J, Perciavalle RM, Kelly JW, Dillin A. Opposing activities protect against age-onset proteotoxicity. *Science.* 2006; 313:1604–1610. [PubMed: 16902091]
- Collins JJ, Evason K, Pickett CL, Schneider DL, Kornfeld K. The anticonvulsant ethosuximide disrupts sensory function to extend *C. elegans* lifespan. *PLoS Genet.* 2008; 4:e1000230. [PubMed: 18949032]
- Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, Fussi H, Deszcz L, Hartl R, Schraml E, Criollo A, Megalou E, Weiskopf D, Laun P, Heeren G, Breitenbach M, Grubeck-Loebenstien B, Herker E, Fahrenkrog B, Frohlich KU, Sinner F, Tavernarakis N, Minois N, Kroemer G, Madeo F. Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol.* 2009; 11:1305–1314. [PubMed: 19801973]
- Evason K, Collins JJ, Huang C, Hughes S, Kornfeld K. Valproic acid extends *Caenorhabditis elegans* lifespan. *Aging Cell.* 2008
- Evason K, Huang C, Yamben I, Covey DF, Kornfeld K. Anticonvulsant medications extend worm lifespan. *Science.* 2005; 307:258–262. [PubMed: 15653505]
- Feng J, Bussiere F, Hekimi S. Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. *Dev Cell.* 2001; 1:633–644. [PubMed: 11709184]
- Fitzgerald K, Tertyshnikova S, Moore L, Bjerke L, Burley B, Cao J, Carroll P, Choy R, Doberstein S, Dubaquié Y, Franke Y, Kopczynski J, Korswagen H, Krystek SR, Lodge NJ, Plasterk R, Starrett J, Stouch T, Thalody G, Wayne H, van der Linden A, Zhang Y, Walker SG, Cockett M, Wardwell-Swanson J, Ross-Macdonald P, Kindt RM. Chemical genetics reveals an RGS/G-protein role in the action of a compound. *PLoS Genet.* 2006; 2:e57. [PubMed: 16683034]
- Friedman DB, Johnson TE. A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics.* 1988a; 118:75–86. [PubMed: 8608934]
- Friedman DB, Johnson TE. Three mutants that extend both mean and maximum life span of the nematode, *Caenorhabditis elegans*, define the age-1 gene. *J Gerontol.* 1988b; 43:B102–109. [PubMed: 3385139]
- Gardner TS. The use of *Drosophila melanogaster* as a screening agent for longevity factors; pantothenic acid as a longevity factor in royal jelly. *J Gerontol.* 1948a; 3:1–8. [PubMed: 18856647]
- Gardner TS. The use of *Drosophila melanogaster* as a screening agent for longevity factors; the effects of biotin, pyridoxine, sodium yeast nucleate, and pantothenic acid on the life span of the fruit fly. *J Gerontol.* 1948b; 3:9–13. [PubMed: 18856648]
- Gems D, Sutton AJ, Sundermeyer ML, Albert PS, King KV, Edgley ML, Larsen PL, Riddle DL. Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics.* 1998; 150:129–155. [PubMed: 9725835]
- Gerstbrein B, Stamatatos G, Kollias N, Driscoll M. In vivo spectrofluorimetry reveals endogenous biomarkers that report healthspan and dietary restriction in *Caenorhabditis elegans*. *Aging cell.* 2005; 4:127–137. [PubMed: 15924569]
- Ghazi A, Henis-Korenblit S, Kenyon C. Regulation of *Caenorhabditis elegans* lifespan by a proteasomal E3 ligase complex. *Proc Natl Acad Sci U S A.* 2007; 104:5947–5952. [PubMed: 17392428]

- Gidalevitz T, Ben-Zvi A, Ho KH, Brignull HR, Morimoto RI. Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science*. 2006; 311:1471–1474. [PubMed: 16469881]
- Gidalevitz T, Krupinski T, Garcia S, Morimoto RI. Destabilizing protein polymorphisms in the genetic background direct phenotypic expression of mutant SOD1 toxicity. *PLoS Genet*. 2009; 5:e1000399. [PubMed: 19266020]
- Gruber J, Ng LF, Poovathingal SK, Halliwell B. Deceptively simple but simply deceptive--*Caenorhabditis elegans* lifespan studies: considerations for aging and antioxidant effects. *FEBS letters*. 2009; 583:3377–3387. [PubMed: 19815017]
- Gruber J, Tang SY, Halliwell B. Evidence for a trade-off between survival and fitness caused by resveratrol treatment of *Caenorhabditis elegans*. *Annals of the New York Academy of Sciences*. 2007; 1100:530–542. [PubMed: 17460219]
- Haldimann P, Muriset M, Vigh L, Goloubinoff P. The Novel Hydroxylamine Derivative NG-094 Suppresses Polyglutamine Protein Toxicity in *Caenorhabditis elegans*. *J Biol Chem*. 2011; 286:18784–18794. [PubMed: 21471208]
- Hamamichi S, Rivas RN, Knight AL, Cao S, Caldwell KA, Caldwell GA. Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model. *Proc Natl Acad Sci U S A*. 2008; 105:728–733. [PubMed: 18182484]
- Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C. A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. *PLoS Genet*. 2008; 4:e24. [PubMed: 18282106]
- Harrington LA, Harley CB. Effect of vitamin E on lifespan and reproduction in *Caenorhabditis elegans*. *MechAgeing Dev*. 1988; 43:71–78.
- Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, Miller RA. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*. 2009; 460:392–395. [PubMed: 19587680]
- Hartman PS, Ishii N, Kayser EB, Morgan PG, Sedensky MM. Mitochondrial mutations differentially affect aging, mutability and anesthetic sensitivity in *Caenorhabditis elegans*. *Mech Ageing Dev*. 2001; 122:1187–1201. [PubMed: 11389932]
- Henderson ST, Johnson TE. daf-16 integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Curr Biol*. 2001; 11:1975–1980. [PubMed: 11747825]
- Henis-Korenblit S, Zhang P, Hansen M, McCormick M, Lee SJ, Cary M, Kenyon C. Insulin/IGF-1 signaling mutants reprogram ER stress response regulators to promote longevity. *Proc Natl Acad Sci U S A*. 2010; 107:9730–9735. [PubMed: 20460307]
- Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloën A, Even PC, Cervera P, Le Bouc Y. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature*. 2003; 421:182–187. [PubMed: 12483226]
- Honda Y, Fujita Y, Maruyama H, Araki Y, Ichihara K, Sato A, Kojima T, Tanaka M, Nozawa Y, Ito M, Honda S. Lifespan-extending effects of royal jelly and its related substances on the nematode *Caenorhabditis elegans*. *PLoS One*. 2011; 6:e23527. [PubMed: 21858156]
- Honda Y, Tanaka M, Honda S. Trehalose extends longevity in the nematode *Caenorhabditis elegans*. *Aging cell*. 2010; 9:558–569. [PubMed: 20477758]
- Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*. 2003; 425:191–196. [PubMed: 12939617]
- Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. *The Journal of biological chemistry*. 2000; 275:11397–11403. [PubMed: 10753955]
- Hsu AL, Murphy CT, Kenyon C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science*. 2003; 300:1142–1145. [PubMed: 12750521]

- Huang TT, Carlson EJ, Raineri I, Gillespie AM, Kozy H, Epstein CJ. The use of transgenic and mutant mice to study oxygen free radical metabolism. *Ann NY Acad Sci.* 1999; 893:95–112. [PubMed: 10672232]
- Ishii N, Senoo-Matsuda N, Miyake K, Yasuda K, Ishii T, Hartman PS, Furukawa S. Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress. *Mech Ageing Dev.* 2004; 125:41–46. [PubMed: 14706236]
- Ishii N, Takahashi K, Tomita S, Keino T, Honda S, Yoshino K, Suzuki K. A methyl viologen-sensitive mutant of the nematode *Caenorhabditis elegans*. *Mutat Res.* 1990; 237:165–171. [PubMed: 2233820]
- Izmaylov DM, Obukhova LK. Geroprotector effectiveness of melatonin: investigation of lifespan of *Drosophila melanogaster*. *Mech Ageing Dev.* 1999; 106:233–240. [PubMed: 10100152]
- Jafari M. *Drosophila melanogaster* as a model system for the evaluation of anti-aging compounds. *Fly (Austin).* 2010;4.
- Jafari M, Khodayari B, Felgner J, Bussel II, Rose MR, Mueller LD. Pioglitazone: an anti-diabetic compound with anti-aging properties. *Biogerontology.* 2007; 8:639–651. [PubMed: 17628757]
- Jafari M, Zarban A, Pham S, Wang T. Rosa damascena decreased mortality in adult *Drosophila*. *J Med Food.* 2008; 11:9–13. [PubMed: 18361732]
- Johnson TE, Wood WB. Genetic analysis of life-span in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA.* 1982; 79:6603–6607. [PubMed: 6959141]
- Kampkotter A, Timpel C, Zurawski RF, Ruhl S, Chovolou Y, Proksch P, Watjen W. Increase of stress resistance and lifespan of *Caenorhabditis elegans* by quercetin. *Comp Biochem Physiol B Biochem Mol Biol.* 2008; 149:314–323. [PubMed: 18024103]
- Kapahi P, Chen D, Rogers AN, Katewa SD, Li PW, Thomas EL, Kockel L. With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. *Cell Metab.* 2010; 11:453–465. [PubMed: 20519118]
- Kastman EK, Willette AA, Coe CL, Bendlin BB, Kosmatka KJ, McLaren DG, Xu G, Canu E, Field AS, Alexander AL, Voytko ML, Beasley TM, Colman RJ, Weindruch RH, Johnson SC. A calorie-restricted diet decreases brain iron accumulation and preserves motor performance in old rhesus monkeys. *J Neurosci.* 2010; 30:7940–7947. [PubMed: 20534842]
- Katewa SD, Kapahi P. Dietary restriction and aging, 2009. *Aging cell.* 2010; 9:105–112. [PubMed: 20096035]
- Keaney M, Matthijssens F, Sharpe M, Vanfleteren J, Gems D. Superoxide dismutase mimetics elevate superoxide dismutase activity in vivo but do not retard aging in the nematode *Caenorhabditis elegans*. *Free radical biology & medicine.* 2004; 37:239–250. [PubMed: 15203195]
- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A *C. elegans* mutant that lives twice as long as wild type. *Nature.* 1993; 366:461–464. [PubMed: 8247153]
- Kim YH, Rane A, Lussier S, Andersen JK. Lithium protects against oxidative stress-mediated cell death in alpha-synuclein-overexpressing in vitro and in vivo models of Parkinson's disease. *J Neurosci Res.* 2011; 89:1666–1675. [PubMed: 21710541]
- Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science.* 1997; 277:942–946. [PubMed: 9252323]
- Klass MR. A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. *Mech Ageing Dev.* 1983; 22:279–286. [PubMed: 6632998]
- Kokel D, Li Y, Qin J, Xue D. The nongenotoxic carcinogens naphthalene and paradichlorobenzene suppress apoptosis in *Caenorhabditis elegans*. *Nature chemical biology.* 2006; 2:338–345.
- Kokel D, Xue D. A class of benzenoid chemicals suppresses apoptosis in *C. elegans*. *Chembiochem.* 2006; 7:2010–2015. [PubMed: 17051661]
- Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasoba T, Tanokura M, Weindruch R, Leeuwenburgh C, Prolla TA. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science.* 2005; 309:481–484. [PubMed: 16020738]
- Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, Shimomura I, Takayama Y, Herz J, Kahn CR, Rosenblatt KP,

- Kuro-o M. Suppression of aging in mice by the hormone Klotho. *Science*. 2005; 309:1829–1833. [PubMed: 16123266]
- Kwok TC, Ricker N, Fraser R, Chan AW, Burns A, Stanley EF, McCourt P, Cutler SR, Roy PJ. A small-molecule screen in *C. elegans* yields a new calcium channel antagonist. *Nature*. 2006; 441:91–95. [PubMed: 16672971]
- Larsen PL. Aging and resistance to oxidative damage in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A*. 1993; 90:8905–8909. [PubMed: 8415630]
- Lee CK, Pugh TD, Klopp RG, Edwards J, Allison DB, Weindruch R, Prolla TA. The impact of alpha-lipoic acid, coenzyme Q10 and caloric restriction on life span and gene expression patterns in mice. *Free radical biology & medicine*. 2004; 36:1043–1057. [PubMed: 15059645]
- Lee KS, Lee BS, Semnani S, Avanesian A, Um CY, Jeon HJ, Seong KM, Yu K, Min KJ, Jafari M. Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in *Drosophila melanogaster*. *Rejuvenation Res*. 2010; 13:561–570. [PubMed: 20645870]
- Lee SS, Lee RY, Fraser AG, Kamath RS, Ahringer J, Ruvkun G. A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. *Nat Genet*. 2003; 33:40–48. [PubMed: 12447374]
- Li YM, Chan HY, Huang Y, Chen ZY. Green tea catechins upregulate superoxide dismutase and catalase in fruit flies. *Mol Nutr Food Res*. 2007; 51:546–554. [PubMed: 17440995]
- Liao CY, Rikke BA, Johnson TE, Diaz V, Nelson JF. Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. *Aging Cell*. 2010; 9:92–95. [PubMed: 19878144]
- Lin K, Hsin H, Libina N, Kenyon C. Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet*. 2001; 28:139–145. [PubMed: 11381260]
- Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science*. 2000; 289:2126–2128. [PubMed: 11000115]
- Lin YT, Hoang H, Hsieh SI, Rangel N, Foster AL, Sampayo JN, Lithgow GJ, Srinivasan C. Manganous ion supplementation accelerates wild type development, enhances stress resistance, and rescues the life span of a short-lived *Caenorhabditis elegans* mutant. *Free Radic Biol Med*. 2006; 40:1185–1193. [PubMed: 16545686]
- Link CD. Expression of human beta-amyloid peptide in transgenic *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A*. 1995; 92:9368–9372. [PubMed: 7568134]
- Link CD, Taft A, Kapulkin V, Duke K, Kim S, Fei Q, Wood DE, Sahagan BG. Gene expression analysis in a transgenic *Caenorhabditis elegans* Alzheimer's disease model. *Neurobiology of aging*. 2003; 24:397–413. [PubMed: 12600716]
- Lithgow GJ. Stress response and aging in *Caenorhabditis elegans*. *Results Probl Cell Differ*. 2000; 29:131–148. [PubMed: 10838698]
- Lu B, Vogel H. *Drosophila* models of neurodegenerative diseases. *Annu Rev Pathol*. 2009; 4:315–342. [PubMed: 18842101]
- Lucanic M, Held JM, Vantipalli MC, Klang IM, Graham JB, Gibson BW, Lithgow GJ, Gill MS. N-acylethanolamine signalling mediates the effect of diet on lifespan in *Caenorhabditis elegans*. *Nature*. 2011; 473:226–229. [PubMed: 21562563]
- McColl G, Killilea DW, Hubbard AE, Vantipalli MC, Melov S, Lithgow GJ. Pharmacogenetic analysis of lithium-induced delayed aging in *Caenorhabditis elegans*. *J Biol Chem*. 2008; 283:350–357. [PubMed: 17959600]
- Melov S, Doctrow SR, Schneider JA, Haberson J, Patel M, Coskun PE, Huffman K, Wallace DC, Malfroy B. Lifespan extension and rescue of spongiform encephalopathy in superoxide dismutase 2 nullizygous mice treated with superoxide dismutase-catalase mimetics. *J Neurosci*. 2001; 21:8348–8353. [PubMed: 11606622]
- Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ. Extension of life-span with superoxide dismutase/catalase mimetics. *Science*. 2000; 289:1567–1569. [PubMed: 10968795]

- Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfranccone L, Pelicci PG. The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature*. 1999; 402:309–313. [PubMed: 10580504]
- Miller RA, Harrison DE, Astle CM, Baur JA, Boyd AR, de Cabo R, Fernandez E, Flurkey K, Javors MA, Nelson JF, Orihuela CJ, Pletcher S, Sharp ZD, Sinclair D, Starnes JW, Wilkinson JE, Nadon NL, Strong R. Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. *J Gerontol A Biol Sci Med Sci*. 2011; 66:191–201. [PubMed: 20974732]
- Moy TI, Ball AR, Anklesaria Z, Casadei G, Lewis K, Ausubel FM. Identification of novel antimicrobials using a live-animal infection model. *Proc Natl Acad Sci U S A*. 2006; 103:10414–10419. [PubMed: 16801562]
- Murakami H, Murakami S. Serotonin receptors antagonistically modulate *Caenorhabditis elegans* longevity. *Aging Cell*. 2007; 6:483–488. [PubMed: 17559503]
- Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature*. 2003; 424:277–283. [PubMed: 12845331]
- Oh SW, Mukhopadhyay A, Dixit BL, Raha T, Green MR, Tissenbaum HA. Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. *Nat Genet*. 2006; 38:251–257. [PubMed: 16380712]
- Onken B, Driscoll M. 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*. 2010; 5:e8758. [PubMed: 20090912]
- Panowski SH, Wolff S, Aguilaniu H, Durieux J, Dillin A. PHA-4/Foxa mediates diet-restriction-induced longevity of *C. elegans*. *Nature*. 2007; 447:550–555. [PubMed: 17476212]
- Peng C, Chan HY, Li YM, Huang Y, Chen ZY. Black tea theaflavins extend the lifespan of fruit flies. *Experimental gerontology*. 2009; 44:773–783. [PubMed: 19770032]
- Peng C, Zuo Y, Kwan KM, Liang Y, Ma KY, Chan HY, Huang Y, Yu H, Chen ZY. Blueberry extract prolongs lifespan of *Drosophila melanogaster*. *Experimental gerontology*. 2012; 47:170–178. [PubMed: 22197903]
- Perez VI, Van Remmen H, Bokov A, Epstein CJ, Vijg J, Richardson A. The overexpression of major antioxidant enzymes does not extend the lifespan of mice. *Aging Cell*. 2009; 8:73–75. [PubMed: 19077044]
- Petrasccheck M, Ye X, Buck LB. An antidepressant that extends lifespan in adult *Caenorhabditis elegans*. *Nature*. 2007; 450:553–556. [PubMed: 18033297]
- Petrasccheck M, Ye X, Buck LB. A high-throughput screen for chemicals that increase the lifespan of *Caenorhabditis elegans*. *Annals of the New York Academy of Sciences*. 2009; 1170:698–701. [PubMed: 19686215]
- Pietsch K, Saul N, Menzel R, Sturzenbaum SR, Steinberg CE. Quercetin mediated lifespan extension in *Caenorhabditis elegans* is modulated by age-1, daf-2, sek-1 and unc-43. *Biogerontology*. 2009; 10:565–578. [PubMed: 19043800]
- Powolny AA, Singh SV, Melov S, Hubbard A, Fisher AL. The garlic constituent diallyl trisulfide increases the lifespan of *C. elegans* via skn-1 activation. *Experimental gerontology*. 2011; 46:441–452. [PubMed: 21296648]
- Rea SL, Ventura N, Johnson TE. Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in *Caenorhabditis elegans*. *PLoS Biol*. 2007; 5:e259. [PubMed: 17914900]
- Ristow M, Zarse K, Oberbach A, Klötting N, Birringer M, Kiehnopf M, Stumvoll M, Kahn CR, Bluher M. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A*. 2009; 106:8665–8670. [PubMed: 19433800]
- Rogers AN, Chen D, McColl G, Czerwieniec G, Felkey K, Gibson BW, Hubbard A, Melov S, Lithgow GJ, Kapahi P. Life span extension via eIF4G inhibition is mediated by posttranscriptional remodeling of stress response gene expression in *C. elegans*. *Cell Metab*. 2011; 14:55–66. [PubMed: 21723504]

- Safdar A, Bourgeois JM, Ogborn DI, Little JP, Hettinga BP, Akhtar M, Thompson JE, Melov S, Mocellin NJ, Kujoth GC, Prolla TA, Tarnopolsky MA. Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. *Proc Natl Acad Sci U S A*. 2011; 108:4135–4140. [PubMed: 21368114]
- Saharia K, Arya U, Kumar R, Sahu R, Das CK, Gupta K, Dwivedi H, Subramaniam JR. Reserpine modulates neurotransmitter release to extend lifespan and alleviate age-dependent Abeta proteotoxicity in *Caenorhabditis elegans*. *Experimental gerontology*. 2012; 47:188–197. [PubMed: 22212533]
- Samara C, Rohde CB, Gilleland CL, Norton S, Haggarty SJ, Yanik MF. Large-scale in vivo femtosecond laser neurosurgery screen reveals small-molecule enhancer of regeneration. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:18342–18347. [PubMed: 20937901]
- Sampayo JN, Olsen A, Lithgow GJ. Oxidative stress in *Caenorhabditis elegans*: protective effects of superoxide dismutase/catalase mimetics. *Aging cell*. 2003; 2:319–326. [PubMed: 14677634]
- Saul N, Pietsch K, Menzel R, Steinberg CE. Quercetin-mediated longevity in *Caenorhabditis elegans*: is DAF-16 involved? *Mech Ageing Dev*. 2008; 129:611–613. [PubMed: 18692520]
- Saul N, Pietsch K, Menzel R, Sturzenbaum SR, Steinberg CE. Catechin induced longevity in *C. elegans*: from key regulator genes to disposable soma. *Mech Ageing Dev*. 2009; 130:477–486. [PubMed: 19501612]
- Saul N, Pietsch K, Menzel R, Sturzenbaum SR, Steinberg CE. The longevity effect of tannic acid in *Caenorhabditis elegans*: Disposable Soma meets hormesis. *The journals of gerontology*. 2010; 65:626–635. [PubMed: 20413530]
- Sayed AA. Ferulsinic acid attenuation of advanced glycation end products extends the lifespan of *Caenorhabditis elegans*. *J Pharm Pharmacol*. 2011; 63:423–428. [PubMed: 21749391]
- Schriner SE, Katoozi NS, Pham KQ, Gazarian M, Zarban A, Jafari M. Extension of *Drosophila* lifespan by *Rosa damascena* associated with an increased sensitivity to heat. *Biogerontology*. 2011
- Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab*. 2007; 6:280–293. [PubMed: 17908557]
- Selkoe DJ. Folding proteins in fatal ways. *Nature*. 2003; 426:900–904. [PubMed: 14685251]
- Sperling RA, Jack CR Jr, Aisen PS. Testing the right target and right drug at the right stage. *Sci Transl Med*. 2011; 3:111cm133.
- Srivastava D, Arya U, Soundara Rajan T, Dwivedi H, Kumar S, Subramaniam JR. Reserpine can confer stress tolerance and lifespan extension in the nematode *C. elegans*. *Biogerontology*. 2008; 9:309–316. [PubMed: 18409080]
- Steinkraus KA, Smith ED, Davis C, Carr D, Pendergrass WR, Sutphin GL, Kennedy BK, Kaerberlein M. Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in *Caenorhabditis elegans*. *Aging cell*. 2008; 7:394–404. [PubMed: 18331616]
- Strong R, Miller RA, Astle CM, Floyd RA, Flurkey K, Hensley KL, Javors MA, Leeuwenburgh C, Nelson JF, Ongini E, Nadon NL, Warner HR, Harrison DE. Nordihydroguaiaretic acid and aspirin increase lifespan of genetically heterogeneous male mice. *Aging cell*. 2008; 7:641–650. [PubMed: 18631321]
- Thomas JN, Smith-Sonneborn J. Supplemental melatonin increases clonal lifespan in the protozoan *Paramecium tetraurelia*. *J Pineal Res*. 1997; 23:123–130. [PubMed: 9406982]
- Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, Hoeks J, van der Krieken S, Ryu D, Kersten S, Moonen-Kornips E, Hesselink MK, Kunz I, Schrauwen-Hinderling VB, Blaak EE, Auwerx J, Schrauwen P. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab*. 2011; 14:612–622. [PubMed: 22055504]
- Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly YM, Gidlof S, Oldfors A, Wibom R, Tornell J, Jacobs HT, Larsson NG. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature*. 2004; 429:417–423. [PubMed: 15164064]

- Tullet JM, Hertweck M, An JH, Baker J, Hwang JY, Liu S, Oliveira RP, Baumeister R, Blackwell TK. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. *Cell*. 2008; 132:1025–1038. [PubMed: 18358814]
- Valdez G, Tapia JC, Kang H, Clemenson GD Jr, Gage FH, Lichtman JW, Sanes JR. Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. *Proc Natl Acad Sci U S A*. 2010; 107:14863–14868. [PubMed: 20679195]
- Vanfleteren JR. Oxidative stress and ageing in *Caenorhabditis elegans*. *Biochem J*. 1993; 292 (Pt 2): 605–608. [PubMed: 8389142]
- Walker GA, Lithgow GJ. Lifespan extension in *C. elegans* by a molecular chaperone dependent upon insulin-like signals. *Aging cell*. 2003; 2:131–139. [PubMed: 12882326]
- Williams DS, Cash A, Hamadani L, Diemer T. Oxaloacetate supplementation increases lifespan in *Caenorhabditis elegans* through an AMPK/FOXO-dependent pathway. *Aging cell*. 2009; 8:765–768. [PubMed: 19793063]
- Wilson MA, Shukitt-Hale B, Kalt W, Ingram DK, Joseph JA, Wolkow CA. Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. *Aging cell*. 2006; 5:59–68. [PubMed: 16441844]
- Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature*. 2004; 430:686–689. [PubMed: 15254550]
- Wu Y, Wu Z, Butko P, Christen Y, Lambert MP, Klein WL, Link CD, Luo Y. Amyloid-beta-induced pathological behaviors are suppressed by Ginkgo biloba extract EGb 761 and ginkgolides in transgenic *Caenorhabditis elegans*. *J Neurosci*. 2006; 26:13102–13113. [PubMed: 17167099]
- Wu Z, Smith JV, Paramasivam V, Butko P, Khan I, Cypser JR, Luo Y. Ginkgo biloba extract EGb 761 increases stress resistance and extends life span of *Caenorhabditis elegans*. *Cell Mol Biol (Noisy-le-grand)*. 2002; 48:725–731. [PubMed: 12396085]
- Zarse K, Ristow M. Antidepressants of the serotonin-antagonist type increase body fat and decrease lifespan of adult *Caenorhabditis elegans*. *PLoS One*. 2008; 3:e4062. [PubMed: 19112515]
- Zarse K, Terao T, Tian J, Iwata N, Ishii N, Ristow M. Low-dose lithium uptake promotes longevity in humans and metazoans. *Eur J Nutr*. 2011; 50:387–389. [PubMed: 21301855]
- Zaytseva YY, Valentino JD, Gulhati P, Evers BM. mTOR inhibitors in cancer therapy. *Cancer Lett*. 2012; 319:1–7. [PubMed: 22261336]
- Zhang H, Pan N, Xiong S, Zou S, Li H, Xiao L, Cao Z, Tunnacliffe A, Huang Z. Inhibition of polyglutamine-mediated proteotoxicity by Astragalus membranaceus polysaccharide through the DAF-16/FOXO transcription factor in *Caenorhabditis elegans*. *Biochem J*. 2012; 441:417–424. [PubMed: 21892924]
- Zhu J, Huang JW, Tseng PH, Yang YT, Fowble J, Shiau CW, Shaw YJ, Kulp SK, Chen CS. From the cyclooxygenase-2 inhibitor celecoxib to a novel class of 3-phosphoinositide-dependent protein kinase-1 inhibitors. *Cancer research*. 2004; 64:4309–4318. [PubMed: 15205346]
- Zou S, Sinclair J, Wilson MA, Carey JR, Liedo P, Oropeza A, Kalra A, de Cabo R, Ingram DK, Longo DL, Wolkow CA. Comparative approaches to facilitate the discovery of longevity interventions: effects of tocopherols on lifespan of three invertebrate species. *Mech Ageing Dev*. 2007; 128:222–226. [PubMed: 17169403]

Highlights

- Chemical screening for lifespan extending compounds
- Focused candidate based chemical screens and natural products
- Natural Products that act through DR and Insulin/IGF pathways
- Chemicals that may act as anti-oxidants
- Chemicals which improve protein homeostasis
- Potential as human treatments

Table 1

Compounds that increase lifespan in *C. elegans* and *D. melanogaster* described in this paper.

Compound	IMLS	Model	Type of compound	Potential Mechanism	References
Vitamin E	17–23%	C	Natural product	Antioxidant?	Harrington et al. 1998
Tocotrienol	20%	C	Natural product	Antioxidant?	Adachi et al. 2000
Co Q10	18% *	C	Metabolite, Natural product	Antioxidant?	Ishii et al. 2004
EUK-8/EUK134 †	54%	C	Synthetic	Stress resistance-Antioxidant?	Melov et al. 2000; Keany et al. 2003
Tamarixetin	25%	C	Natural product, Flavonoid	Stress resistance	Wu et al. 2002
EGb761	8%	C	Natural product	Stress resistance	Wu et al. 2002, 2006
Resveratrol †	10–18%	C	Natural product	Stress resistance, autophagy, TOR?	Wood et al. 2004; Bass et al. 2007
Ethosuximide/trimethadione/DEABL	17–47%	C	Synthetic, anticonvulsants	Serotonin dependent DR?	Evason et al. 2005
Blueberry extract	28%	C, D	Natural product	Increased HSR, osmotic pathway	Wilson et al. 2006; Peng et al. 2007
Cocoa	10%	D	Natural product	Antioxidant, metal chelator	Bahadorani and Hilliker, 2007
Damask rose extract	10%	D	Natural product	Antioxidant?	Jafari et al. 2008; Shriner et al. 2011
Lipoic acid	24%	C	Metabolite, Natural product	Stress resistance	Brown et al. 2006; Benedetti et al. 2008
LiCl	25%	C	Chemical, mood stabilizer	GSK-3 β and LSD-1 dependent	McColl et al. 2008
2-deoxy-D-glucose	13–17%	C	Glycolysis inhibitor	Mitohormesis	Schulz et al. 2007
Valproic acid	35%	C	Synthetic, psychoactive	DAF-16 dependent	Evason et al. 2008
Mianserine †/methiothepin/others	20–33%	C	Synthetic, psychoactive	Serotonin signaling activation	Petrasccheck et al. 2007; Zarse and Ristow 2008
Reserpine	31%	C	Natural product, antihypertensive	Stress resistance, requires Ser	Srivastava et al. 2008
Trolox/Propyl gallate	10–14%	C	Synthetic/food additive	Antioxidant	Benedetti et al. 2008
Quercetin	11–18%	C	Natural product, flavonoid	Stress resistance, DAF-16 dependent	Pietsch et al. 2009
Oxaloacetate	23%	C	Krebs Cycle metabolite	DAF-16/AMPK/dependent	Williams et al. 2009
Acetic acid/Reishi Polysaccharide	20–30%	C	Chemical reagent/natural product	DAF-16 dependent	Chuang et al. 2009
Catechin	13%	C	Natural product, flavonoid	DAF-2, NHR-8 and MEV-1 dependent	Saul et al. 2009
Spermidine	15%	C, D	Natural product, polyamine	Becline-1 dependent	Eisenberg et al. 2009
Rapamycin	6–15%	D	Natural product, immunosuppressant	TOR	Bjedov et al. 2010
Trehalose	30%	C	Natural product, disaccharide	Stress resistance, DAF-16 dependent	Honda et al. 2010
Metformin	40%	C	Biguanide, antidiabetic	DR mimetic, SKN-1 dependent	Onken et al. 2010

Compound	IMLS	Model	Type of compound	Potential Mechanism	References
DDS	22%	C	Synthetic, antibiotic	Antioxidant?	Cho et al. 2010
Lamotrigine	51–17%	D	Synthetic, psychoactive	Unknown	Avanesian et al. 2010
Thioflavin T	45%	C	Synthetic, benzothiazole	Protein homeostasis HSF-1 and SKN-1	Alavez et al. 2011
Curcumin	30%	C, D	Natural product	Protein homeostasis	Alavez et al. 2011, Lee et al. 2010
Diallyl trisulfide	12.6%	C	Natural product	SKN-1 dependent	Powolny 2011
Celecoxib	10–20%	C	Synthetic, COX2 inhibitor	DAF-16 dependent	Ching et al. 2011
Ferulic acid	18–22%	C	Natural product	Stress resistance	Sayed, 2011
NG-094	20% ^{**}	C	Synthetic	HSF-1 dependent	Haldimann et al. 2011
Royal jelly	7–18%	C, D	Natural product	DAF-2/DAF-16 dependent	Honda et al. 2011; Gardner, 1948
Astragalin	30.0%	C	Natural product	DAF-16 dependent	Zhang et al. 2012

IMLS, Increase in median lifespan;

^{*} Just in *mev-1* mutants;

^{**} Just in PolyQ strain;

C, *C. elegans*; D, *D. melanogaster*;

[†] Controversial