From the Department of Biosciences and Nutrition
Karolinska Institutet, Stockholm, Sweden

DISCOVERING AND TARGETING THE MECHANISMS THAT GOVERN AGE-RELATED DECLINE

Xin-Xuan Lin

Karolinska Institutet

Stockholm 2019

Published by Karolinska Institutet.
Printed by Universitetsservice-AB 2019

ISBN 978-91-7831-487-4

Discovering and targeting the mechanisms that govern age-related decline

THESIS FOR DOCTORAL DEGREE (Ph.D.)

Wednesday the 5th of June, 2019 at 08:30
Room Erna Möllersalen, NEO building, Blickagången 16, Flemingsberg

By

Xin-Xuan Lin

Principal Supervisor:
Dr. Christian Riedel
Karolinska Institutet
Department of Biosciences and Nutrition
Integrated Cardio Metabolic Centre

Co-supervisor(s):
Assoc. Prof. Peter Swoboda
Karolinska Institutet
Department of Biosciences and Nutrition

Prof. Maria Eriksson
Karolinska Institutet
Department of Biosciences and Nutrition

Opponent:
Asst. Prof. Sabrina Büttner
Stockholm University
Department of Molecular Biosciences
The Wenner-Gren Institute

Examination Board:
Dr. Andrea Hinas
Uppsala University
Department of Cell and Molecular Biology, Microbiology

Assoc. Prof. Sara Hägg
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Prof. Camilla Björkegren
Karolinska Institutet
Department of Biosciences and Nutrition

To my dearest family

ABSTRACT

Aging is recognized as a major risk factor for many diseases. Thus, the identification of a means to target aging would bear great potential to improve human health and quality of life. This thesis tries to help this identification by improving our understanding of how aging is regulated, and by developing and applying new strategies to identify aging-preventive pharmaceuticals.

The transcription factor (TF) FOXO, also known as DAF-16 in *Caenorhabditis elegans* (*C. elegans*), is considered a central nexus for aging regulation. It is located downstream of the insulin/insulin-like growth factor 1 signaling (IIS) pathway, and is activated by low IIS as well as many other stresses and aging-regulatory stimuli. Studies in many model organisms have shown that DAF-16/FOXO activation not only leads to longer lifespan, but also increased stress resistance. While the mechanisms leading to DAF-16 activation and its target genes have been extensively studied, the mechanism of how DAF-16 relays aging-regulatory stimuli to activate the transcriptional response remains unclear. In paper 1, we used *C. elegans* to determine that DAF-16 can form a complex with HLH-30 transcription factor, a master regulator in autophagy and lysosomal biogenesis, and known to be important for longevity in *C. elegans*. These two TFs require each other to mediate longevity in *daf-2* (low IIS mutant) and *glp-1* (a germline deficient mutant whose longevity also depends on DAF-16) animals. Moreover, they co-regulate many target genes and co-occupy many promoter regions, indicating that HLH-30 plays an important role in DAF-16-dependent transcriptional responses and longevity. Interestingly, even though both TFs are important for longevity and different types of stress resistance, we found that their genetic interaction is context-dependent. They function in the same genetic pathway to cope with oxidative stress, while they act through independent pathways to cope with heat stress. Finally, we observed that they have opposing roles for dauer formation (a developmental diapause state that enables worms to survive in harsh environments). By further mechanistic exploration, we provide a model whereby DAF-16 forms a transcriptional regulatory module with HLH-30 that relays diverse distress signals to stimulate the appropriate transcriptional responses, thereby ensuring the organism's survival.

Despite a growing understanding of how aging is regulated, we are still lacking suitable methods of screening for pharmacological interventions which, by targeting these pathways, would lead to aging-preventive effects in humans. In paper 2, we developed and applied an *in silico* screening method to identify aging-preventive pharmaceuticals, also known as geroprotectors. We started by building machine-learning-based algorithms using age-stratified human tissue transcriptomic data from the Genotype-Tissue Expression (GTEx) project and applied them for *in silico* screen from more than 1300 compounds in the Connectivity Map (CMap) database. These compounds were ranked by the likelihood of their geroprotective ability, and the best candidates were further validated in *C. elegans*. We found that two heat shock protein 90 (HSP90) inhibitors, monorden and tanespimycin, can improve the worms' lifespan and healthspan. We argue that HSP90 inhibitors activate the cytosolic unfolded protein response by elevating HSP expression through the TF HSF-1 (heat shock factor 1). This in turn improves protein homeostasis, leading to cytoprotection, and ultimately better health and a longer life.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which are referred to in the text by their roman numerals.

I. **X.-X. Lin***, I. Sen*, G. E. Janssens, X. Zhou, B. R. Fonslow, D. Edgar, N. Stroustrup, P. Swoboda, J. R. Yates, G. Ruvkun, and C. G. Riedel, "DAF-16/FOXO and HLH-30/TFEB function as combinatorial transcription factors to promote stress resistance and longevity," *Nat. Commun.*, vol. 9, no. 1, p. 4400, 2018.

II. G. E. Janssens*, **X.-X. Lin***, L. Millan-Arino*, I. Sen, A. Kavsek, R. I. Seinstra, N. Stroustrup, E. A. A. Nollen, and C. G. Riedel, "Transcriptomics-based screening identifies pharmacological inhibition of Hsp90 as a means to defer aging. *Cell Reports*, accepted for publication. (2019) http://doi.org/10.1016/j.celrep.2019.03.044

*: Equal contribution.

LIST OF PUBLICATIONS (not included in the thesis)

I. X. Zhou, I. Sen, **X.-X. Lin**, and C. G. Riedel, “Regulation of Age-related Decline by Transcription Factors and Their Crosstalk with the Epigenome,” *Curr. Genomics*, vol. 19, no. 6, pp. 464–482, Jul. 2018.

CONTENTS

LIST OF ABBREVIATIONS

AMPK	AMP-activated protein kinase
CAMKII	Ca2+/calmodulin-dependent kinase type II
ChIP-seq	Chromatin immunoprecipitation-sequencing
CITP	Caenorhabditis intervention testing program
CLEAR	Coordinated lysosomal expression and regulation
CMap	Connectivity Map
CREB	cAMP response element binding protein
CRTC-1	CREB-regulated transcriptional co-activator 1
DAE	DAF-16 associated element
DMSO	Dimethyl sulfoxide
DR	Dietary restriction
ER	Endoplasmic reticulum
FOXO	Forkhead box protein O
FUDR	5-fluoro-2'-deoxyuridine-5'-phosphate
GO	Gene ontology
GTEx	Genotype-tissue expression
HCF-1	Host cell factor 1
HSF-1	Heat shock factor 1
HSP	Heat shock protein
HSR	Heat shock response
IGF-1	Insulin-like growth factor 1
IIS	Insulin/IGF-1 signaling
Ins	Insulin
ITP	Intervention testing program
LM	Lifespan machine
mRNA-seq	mRNA-sequencing
mTOR	Mechanistic target of rapamycin
NGM	Nematode growth media
NIA’s ITP	National Institute on Aging’s Intervention Testing Program
OE	Overexpression
PI3K	Phosphoinositide 3 kinase

PIP	Phosphatidylinositol 3, 4, 5-triphosphate
PTM	Posttranslational modification
RNAi	RNA interference
SASP	Senescence-associated secretory phenotype
TF	Transcription factor
TFEB	Transcription factor EB
TORC1	mTOR complex 1
TORC2	mTOR complex 2
UPR	Unfolded protein response
UPR^{ER}	Endoplasmic reticulum unfolded protein response
UPR^{mt}	Mitochontrial unfolded protein response

1 INTRODUCTION

1.1 INTRODUCTION TO AGING

The advance of modern medication and the improvement of nutrition and public health have substantially increased the average human lifespan over the last century. Nevertheless, this has given rise to challenges associated with a burgeoning aging population and age-related diseases. These age-related diseases (such as cancer, cardiovascular disease, metabolic disorders, and neuronal degenerative disease) account for more than 80% of disease burden in high-income countries. A recent WHO analysis shows heart disease, stroke, and diabetes to have had associated costs of around US$83 billion between 2006 and 2015 in 23 countries; the 2010 World Alzheimer's Disease Report estimated the overall cost for dementia care alone in 2010 was more than US$600 billion worldwide. Such health-care burden is expected to increase further, given that the world population continues to grow and age rapidly. Finding solutions to combat age-related pathologies and improve the quality of later life has therefore become a pressing task.

Although advances in medical science allow us to treat many age-related diseases and slow physiological decline in these patients, the effectiveness of such treatments is limited because these diseases often are co-occurring (Goldman et al., 2013). Instead, a new concept of treating age-related disorders is emerging – that of targeting their major risk factor, the aging process itself. Aging is a progressive decline of overall function of an organism caused by damage accumulation and loss of homeostasis. This decline usually starts long before people are diagnosed with an age-related disease. Targeting the aging process therefore bears the advantage of delaying the onset of multiple age-related diseases and prolonging the healthy lifespan.

Following decades of research in aging biology, scientists have now characterized nine interconnected molecular and cellular processes as the hallmarks of aging that are most applicable to humans (López-Otín et al., 2013). These processes include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulation of nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Intervening in one or more of these processes is thought to reduce illness and disability and promote healthy aging.

1.2 *CAENORHABDITIS ELEGANS* AS A MODEL ORGANISM FOR AGING RESEARCH

The field of aging research has benefited from studies of model organisms including the yeast *Saccharomyces cerevisiae*, the worm *Caenorhabditis elegans* (*C. elegans*), the fly *Drosophila melanogaster*, and the mouse *Mus musculus*. Each model has its strengths: for instance, single-celled yeast provides a very simple and amenable model to study unique replicative lifespan and chronological lifespan; flies and worms are metazoans that have more complicated anatomy, with many tissues homologous to human organs, and they allow for measurement of healthspan and cognitive functions; finally, the mouse model is ideal to study the age-related physiological changes that are most relevant to humans. Importantly, all these model organisms

possess genes and aging-regulatory pathways that are evolutionarily conserved in humans, but at the same time with limited biological complexity that makes the exploration of these pathways much more amenable and feasible.

In this thesis work, we use *C. elegans* to address our research questions. *C. elegans* was introduced by Sydney Brenner in 1974 (Brenner, 1974) as a genetic model. Owing to its unique features, *C. elegans* has become an important organism for studying various biological processes and human diseases, including aging. First, its genome has been completely sequenced, and around 80% of *C. elegans*' genes have homologs in humans (Kaletta and Hengartner, 2006; Lai et al., 2000; The C. elegans Sequencing Consortium, 1998). Moreover, many of the signaling pathways involved in development, stress responses, and other physiological functions, are conserved between *C. elegans* and humans. Therefore, discoveries made in *C. elegans* can provide major insights into human biology.

Another advantage of *C. elegans* is its relatively short lifecycle. It takes about 3 days from an egg through four larval stages (L1 to L4) to reproductive adulthood at 20°C. Depending on the ambient temperature (15°C to 25°C), the average lifespan of *C. elegans* is only 2 to 3 weeks, which is advantageous for conducting lifespan assays in aging research. In addition, *C. elegans* is predominantly a hermaphrodite in its natural form, and can self-fertilize and reproduce large amounts of progeny (300 to 350 during its entire reproductive period). It feeds on bacteria, and is easy to cultivate using standard *Escherichia coli* bacteria on petri dishes in the lab. These features make *C. elegans* suitable for large-scale studies.

Furthermore, *C. elegans* is an excellent model for genetic manipulation and screening: mutants can be generated by chemical mutagenesis, or the genome can be edited using the CRISPR/Cas9 technique; genetic crosses with its male form allow for easy combination of genotypes; and transgenetic worms can be obtained by microinjection of plasmid. All strains can be easily kept at -80°C for long-term storage. Furthermore, RNA interference (RNAi) can be induced in animals simply by feeding them bacteria expressing specific double-stranded RNA of the target gene. Two large collections of *C. elegans* RNAi bacteria clones, made by the Ahringer lab (Kamath, 2003) and the Vidal lab (Rual, 2004), are now commercially available and allow for targeting of nearly all genes in the genome. Finally, *C. elegans* has a transparent body that allows for the detection of fluorescent proteins *in vivo*. These distinguishing characteristics of *C. elegans* make it an excellent tool for aging research.

In the introduction that follows we will focus on the current state of knowledge in *C. elegans* within the context of aging, including genetic manipulations in the nutrient sensing pathways, proteostasis regulation, and pharmacology of aging, to provide the relevant background information for this thesis work.

1.2.1 Lifespan vs. healthspan

Extending lifespan in animal models often leads to alleviated chronic diseases and improved stress resistance. In addition, studies have found that centenarians also has extended healthspan, indicating a correlation between lifespan and healthspan (Dillin and Cohen, 2011; Fontana et al., 2010; Hansen and Kennedy, 2016; Milman and Barzilai, 2016). However, modulating the

aging process does not always extend lifespan and healthspan in proportion (Bansal et al., 2015). Since the goal of delaying aging in humans is to have a longer healthy life, the notion of improving healthspan rather than solely prolonging lifespan has become more recognized in aging research.

Measuring healthspan however, remains a challenge (Tatar, 2009). First, healthspan is, in general, considered a living period that is free of chronic disease and disability. In this regard, healthspan cannot be assessed by a simple measurement like 'live vs. dead', but requires examination of a series of physiological function changes with age. Furthermore, since these changes progress gradually, the distinction between being 'healthy' and 'unhealthy' is less clear, and depends on the individual. It is also sometimes difficult to interpret the age-dependent changes as system degeneration or system adaptation. Finally, many model organism-specific healthspan measures are hard to translate to humans.

A common laboratory approach is to evaluate anthropomorphic function. In mice, these would be, for example, rotarod performance tests, oral glucose tolerance tests, or tests of cognitive function. Similarly, some anthropomorphic healthspan indices can be assessed in other model organisms; for example, one can examine sarcopenia in *C. elegans* (Herndon et al., 2002), or heart function (Ocorr et al., 2007; Wessells et al., 2004) and sleeping fragmentation in *Drosophila* (Shaw, 2000; Shaw et al., 2002).

1.2.2 Tools to study *C. elegans* lifespan

As mentioned previously, *C. elegans* genetic techniques and survival assays have helped in the discovery of genes important to a number of physiological processes, including aging-regulatory pathways. Conventionally, these survival assays were performed by gently touching worms growing on a bacteria-seeded agar surface in a petri dish. Despite the simplicity of this method, it is labor intensive, and thus limits the scale of experiments. Recently, different methods were developed to enable automated and high-throughput survival analyses in *C. elegans* – notably the 'Lifespan Machine' developed by Stroustrup et al. (2013), which was used in this thesis work. The device is built using conventional flatbed scanners that acquire plate images at intervals of 15 to 30 minutes; these time-lapse images are then processed by an image-analysis pipeline to determine the death time of all individual worms in the experiment. Further to this, Churgin et al. (2017) designed a customized microplate, named 'WorMotel', that allows examination of individual worm behavior throughout its life. Alternatively, *C. elegans* can also be cultured in liquid-based media, their survival determined by spontaneous movement; this can be conducted on a larger scale using multi-well plates, and, as such, is commonly used for genetic screening or compound screening. Similarly, automated liquid-based systems on microfluidic chips, such as the 'Worm Farm' (Xian et al., 2013), have been developed to improve lifespan assay handling.

1.2.3 Healthspan assessment in *C. elegans*

In addition to lifespan, other age-related physiology changes indicating the health state of animals have been described in *C. elegans* (Huang et al., 2004). For instance, body movement and thrashing are both kinds of locomotion that manifest deterioration with age. Body

movement is usually measured as the distance traveled on the plate over a period of time; younger animals in general exhibit fast and sinusoidal movement, whereas older animals show slow and discontinued movement patterns (which eventually stop). Thrashing, on the other hand, examines a worm's lateral movement rate in liquid, with frequency declining with age. Recently, Hahm et al. (2015) adapted the Short Physical Performance Battery assessment (Guralnik et al., 1994) to worms to allow the measurement of maximum velocity in short time. They found that this metric declines with age, and better reflects a worm's lifespan when compared to other mobility tests. In addition, in the pharynx, an organ that undergoes contraction to pump food from the animal's mouth into the intestine, contraction also declines gradually with age. Furthermore, both the number of eggs produced and the duration of the entire reproductive period can be considered as healthspan measures. Finally, autofluorescence accumulation and stress-resistance assays (e.g. assays of heat stress or oxidative stress) are also commonly used to evaluate health in *C. elegans* (Klass, 1977; Park et al., 2017). Comparison of healthspan changes in conjunction with the different aging interventions will provide insights into the impact of these intervenes on animal's health and healthspan.

1.3 THE PLASTICITY OF AGING: LESSONS FROM *C. ELEGANS*

A large body of work in model organisms reveals that the aging process is plastic. It can be regulated by dietary, genetic, and pharmacological approaches (Kennedy et al., 2014).

1.3.1 Dietary restriction

In the early part of the 20th century, it was discovered that a reduction in food intake without malnutrition was associated with extended lifespan in rodents (McCay et al., 1935; Osborne et al., 1917). Similarly, dietary restriction (DR) has been shown to increase lifespan (and even improve health) in worms and other species (Fontana et al., 2010). In *C. elegans*, there exists several DR methods, all of which increase lifespan; these include the *eat-2* mutation, which leads to low food intake (Lakowski and Hekimi, 1998); dilution of feeding bacteria in liquid culture (Bishop and Guarente, 2007; Klass, 1977); restricting the growth of the feeding bacteria on the plate by diluting the concentration of peptone (Hosono et al., 1989); limiting bacterial intake by providing restrictive amount of bacteria on plates (solid DR) (Greer et al., 2007); and the complete deprivation of bacteria from the plate (Kaeberlein et al., 2006; Lee et al., 2006). Worms cultured on various modified axenic media also exhibit DR phenotypes (Houthoofd et al., 2003; Lenaerts et al., 2008; Szewczyk et al., 2006). Interestingly, these DR regimens achieve longevity via different yet overlapping nutrient sensing pathways and transcription factors (TFs) such as DAF-16, PHA-4, SKN-1, or HSF-1 (Greer and Brunet, 2009). For instance, DAF-16 was found to be important for dilution of peptone in plate method (Greer and Brunet, 2009) and solid DR method (Greer et al., 2007), but not required for *eat-2* mutants (Lakowski and Hekimi, 1998) to extend lifespan. In contrast, PHA-4 is essential for *eat-2* mutants and the dilution of feeding bacteria method (Panowski et al., 2007), but not for solid DR to induce longevity (Greer and Brunet, 2009). SKN-1 and HSF-1 were reported to be activated by bacteria dilution (Bishop and Guarente, 2007) and bacteria deprivation (Steinkraus et al., 2008), respectively. In addition to TFs, another regulator called SMK-1 (the homolog of mammalian SMEK [Suppressor of MEK null] homolog) is found to be important for PHA-4-

mediated *eat-2* longevity in *C. elegans* (Panowski et al., 2007). Dissecting the underlying mechanism by which individual DR methods confer longevity would facilitate the search for DR mimetics that promote healthy aging.

1.3.2 Aging-regulatory networks

Studies have shown that longevity in response to DR is regulated by various evolutionarily conserved nutrient sensing pathways, including the insulin/IGF-1 signaling (IIS), mechanistic target of rapamycin (mTOR) signaling, AMPK signaling, and sirtuins pathways. Genetic manipulation of these pathways can also impact lifespan under well-fed conditions. Often, these pathways react to environmental or internal stresses, such as unsuitable temperature, toxins, and infertility. Integrating various signals, these pathways form an interconnected network and promote stress-responsive gene expression through downstream TFs, thereby slowing the aging process, promoting longevity, and ultimately ensuring the organism's survival (reviewed in C. J. Kenyon, 2010). Impairment of these signaling pathways and TFs will lead to reduced stress resistance and shorter lifespan, suggesting the vital roles of these TFs in the regulation of aging. For instance, reduction of IIS can more than double the lifespan of a worm by activating DAF-16, the *C. elegans* ortholog of FOXO TFs, which then translates this signal into gene expression changes. By contrast, loss of DAF-16 results in short-lived worms. Studying and improving our understanding of these aging-regulatory pathways, as well as the downstream transcriptional regulation, will be beneficial to elucidate the underlying causes of the aging process and help us to promote healthy aging.

1.3.2.1 The IIS pathway and TF DAF-16

An important axis in aging-regulatory pathways is the IIS pathway and its downstream effector, the forkhead TF DAF-16/FOXO (Brunet et al., 1999; Kenyon et al., 1993; Kimura et al., 1997; Kops and Burgering, 1999; Lin et al., 1997; Ogg et al., 1997). In *C. elegans, daf-2* encodes the insulin receptor, which is bound by insulin-like ligands and thereby activates phosphoinositide 3-kinase (PI3K) signaling under favorable conditions. Activated PI3K generates phosphatidylinositol 3, 4, 5-triphosphate (PIP3), which in turn activates AKT-1, AKT-2, and SGK-1 kinases by recruiting them and their activator PDK-1 to the plasma membrane where PDK-1 phosphorylates AKT-1, AKT-2, and SGK-1. Subsequently, the phosphorylated AKT-1–AKT-2–SGK-1 complex inhibits DAF-16 by phosphorylation, resulting in sequestration of DAF-16 by 14-3-3 proteins in the cytoplasm. However, PI3K signaling can be inhibited by phosphoinositide 3-phosphatase PTEN, which dephosphorylates PIP3 and inactivates its downstream cascade. As a result, DAF-16 dissociates from the 14-3-3 proteins and translocates into the nucleus to regulate the expression of its target genes that, as a consequence, confer organismal longevity (**Figure 1**). Disturbing the IIS pathway by introducing mutations in *daf-2* or *age-1* (which encodes the PI3K catalytic subunit) in *C. elegans* at least doubles the lifespan of this animal. This longevity is largely dependent on DAF-16 nuclear accumulation. Consistently, inactivation of DAF-16 in these long-lived mutants results in their inability to extend lifespan. In addition to DAF-16, other TFs were found to contribute to *daf-2* longevity, including the heat-shock TF HSF-1 (Hsu, 2003) and the Nrf-like TF SKN-1(Tullet et al., 2008).

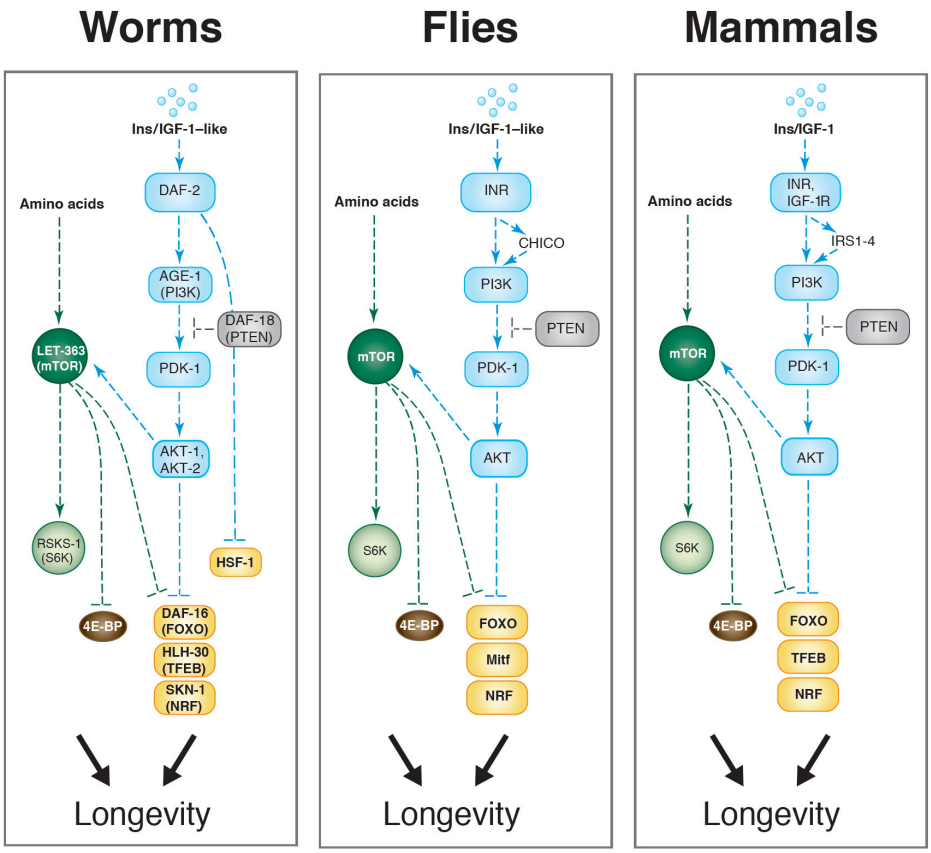

Figure 1: A simplified overview of the conserved IIS pathway and mTOR signaling pathway that regulate longevity in worms, flies, and mammals. Low levels of insulin/IGF-1 or disruption of the IIS pathway lead to the activation of TFs (e.g. DAF-16, HLH-30, SKN-1). These TFs are also regulated by the mTOR signaling pathway that is intertwined with IIS. Similarly, inhibition of the mTOR signaling pathway (e.g. by reduction of mTOR kinase or its target RSKS-1/S6K) can increase lifespan. Figure adapted from (Fontana et al., 2010).

There are four FOXO proteins in mammals, namely FOXO1, FOXO3, FOXO4, and FOXO6, playing a wide range of roles in various biological processes. *C. elegans* has only a single FOXO, DAF-16, to regulate longevity, stress resistance, development, and metabolism (Lee, 2003; McElwee et al., 2003; Murphy et al., 2003; Oh et al., 2006). So how does DAF-16 specify these diverse functional outputs? One way is by context-dependent alternative splicing that yields different isoforms with different biological functions. In addition to a known DAF-16a isoform that contributes to long lifespan, a study by Kwon et al. (2010) showed that the DAF-16d/f isoform is also required to promote longevity in the context of reduced IIS, suggesting that a combination of distinct DAF-16 isoforms can be used to fine-tune the regulation of its targets. Another mechanism for DAF-16 to exert its function is through collaboration with co-regulators. Studies in *C. elegans* showed that DAF-16 does not work alone, but requires joint

efforts from other proteins including HSF-1, SKN-1, and SMK-1 (Chiang et al., 2012a; Hsu, 2003; Seo et al., 2013; Tullet et al., 2008; Volovik et al., 2014; Wolff et al., 2006). Moreover, a recent study has identified several DAF-16 cofactors, and further demonstrated that DAF-16 recruits one of these cofactors, the chromatin remodeler SWI/SNF, to its target genes to open chromatin, facilitate transcription, and thereby promote longevity (Riedel et al., 2013). It's no surprise to learn that additional regulatory mechanisms to open or condense chromatin are vital for an organism's survival under environmental insults: because chromatin structure affects gene accessibility for TFs and other regulatory elements, any inadequate chromatin structure will lead to dysregulation of transcription, replication, and repair; the last two processes being particularly important for the maintenance of genome stability and prevention of accelerated aging.

DAF-16 is the crucial effector that extends lifespan in response to low IIS. Ever since its first description in the context of aging in *C. elegans*, studies have emerged supporting its conserved aging-regulatory function. It also contributes to aging-regulatory signaling pathways beyond IIS, such as in mTOR, AMPK, and sirtuin pathways, underscoring DAF-16's role as the central aging-regulatory nexus.

1.3.2.2 mTOR

Mechanistic target of rapamycin (mTOR) kinase is a nutrient and amino-acid sensor that mediates a wide range of biological functions, such as cell growth, autophagy, and longevity (Seah et al., 2016). It has been shown in many species that inhibition of mTOR by rapamycin prolong lifespan. In addition, loss of *C. elegans* mTOR kinase *let-363* expression by either RNAi or mutation leads to lifespan extension (Long et al., 2002; Vellai et al., 2003). Moreover, inhibition of other mTOR pathway components that leads to reduced mTOR signaling activity, such as the mTOR complex 1 (TORC1) activator DAF-15/raptor and the mTOR target RSKS-1/S6 kinase, also shows association with prolonged lifespan. These longevity phenotypes largely depends on the function of the PHA-4 and SKN-1 TFs (Hansen et al., 2007; Robida-Stubbs et al., 2012).

Another TF regulated by mTOR is the conserved helix-loop-helix TF HLH-30 (Roczniak-Ferguson et al., 2012). HLH-30, along with its closest mammalian homolog Transcription factor EB (TFEB), is known as a master regulator of lysosomal biogenesis and autophagy (Settembre and Ballabio, 2011; Settembre et al., 2011, 2013), which are important processes in the context of metabolism and the promotion of longevity (Lapierre et al., 2013; O'Rourke and Ruvkun, 2013). Similar to DAF-16, HLH-30/TFEB activity is mainly controlled by its cellular localization through posttranslational modification (PTM). TFEB is negatively regulated by TORC1 through phosphorylation, and subsequently sequestrated by 14-3-3 proteins in the cytoplasm. The phosphatase calcineurin, however, can activate TFEB by dephosphorylation under nutrient-deprived conditions or other stresses. Active TFEB then translocates into the nucleus, binds to target genes with specific consensus motifs called CLEAR (Coordinated Lysosomal Expression and Regulation), and drives the expression of responsive genes (**Figure 2**). Recent work by Lapierre et al. (2013) showed direct evidence that HLH-30 is required for

lifespan modulation in *C. elegans*. In addition, HLH-30 promotes longevity at least in part by upregulating autophagy gene expression.

To determine whether DAF-16 is involved in the mTOR signaling pathway, several lifespan assays were conducted, followed by epistatic analyses. Interestingly, loss of DAF-16 function suppresses longevity caused by DAF-15/raptor loss, but does not suppress the long-lived phenotype caused by inhibition of mTOR/*let-363*, S6 kinase/*rsks-1*, or mTOR complex 2 (TORC2). This indicates that lifespan extension by interrupting TORC1 depends on DAF-16, while lifespan extension by inhibition of TORC2 or both mTOR complexes does not require DAF-16 (Hansen et al., 2007; Robida-Stubbs et al., 2012; Vellai et al., 2003).

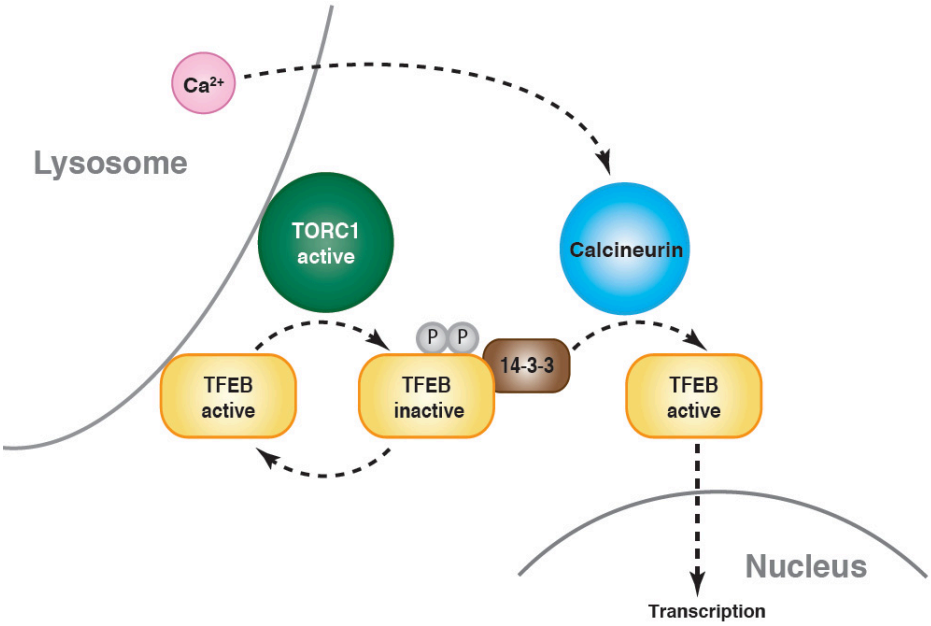

Figure 2: Model of TFEB activation. Under normal conditions, TFEB is phosphorylated by TORC1 and is sequestrated by 14-3-3 in the cytoplasm. Under stress, Ca^{2+} is released from the lysosome and leads to calcineurin activation, which subsequently dephosphorylates TFEB. Active TFEB can then translocate into the nucleus and drive transcription. Circles containing "P" indicate phosphoryl groups.

1.3.2.3 AMPK

AMP-activated protein kinase (AMPK) is an energy-level sensor that is activated in response to a high ratio of AMP to ATP in the cell. Its activity is essential for lifespan extension by some DR regimens. Moreover, genetic analyses indicate it is also required for extended lifespan in several long-lived mutants of *C. elegans,* including *daf-2*, *isp-1*, and *clk-1* mutants, as well as *sir-2.1* overexpression (OE) animals. OE of the AMPK catalytic α subunit (*aak-2*) or

constitutively activation of AMPK have been shown to increase lifespan and protect worms against oxidative stress in a DAF-16-dependent manner. In contrast, under low IIS (*daf-2* mutant), AAK-2 exerts its function in parallel to DAF-16 (Apfeld et al., 2004; Curtis et al., 2006; Greer et al., 2007).

How does AMPK genetically interact with DAF-16 and promote longevity? It has been shown that AMPK can controls DAF-16 activity directly by phosphorylation (Greer et al., 2007) and possibly indirectly by controlling calcineurin/CAMKII (Ca2+/calmodulin-dependent kinase type II) signaling and CRTC-1 (CREB-regulated transcriptional co-activator 1)/CREB (cAMP response element binding protein) signaling pathway. The phosphatase calcineurin targets DAF-16 and results in DAF-16 inactivation. This can be reversed by CAMKII which phosphorylates DAF-16 and thus restores its activity. Consistent with these observations, both constitutively activation of CAMKII and decreased calcineurin activity lead to lifespan extension in nematodes, whereas CAMKII mutation shortens the animal's lifespan (Tao et al., 2013). Furthermore, evidence showed that in mammalian cells, AMPK can also be activated by CAMKII, suggesting that AMPK and CAMKII together inhibit calcineurin activity and lead to DAF-16 activation (Hawley et al., 2005; Hurley et al., 2005; Tao et al., 2013; Woods et al., 2005). Under low nutrient conditions, AMPK can phosphorylate *C. elegans* CRTC-1 and prevent calcineurin from dephosphorylating CRTC-1, thus leading to CRTC-1 cytoplasmic retention and the TF CREB inactivation, and eventually a longer lifespan. Meta-analyses showed that genes downregulated upon AMKP and CRTC-1 activation are enriched for DAE (DAF-16 associated element) motif (Murphy et al., 2003) at their promoter regions, suggesting that DAF-16 might function downstream of CRTC-1 signaling, and be remotely controlled by neuronal CRTC-1 (Burkewitz et al., 2015; Mair et al., 2011).

1.3.2.4 Sirtuins

Sirtuins are NAD^+-dependent deacetylases first discovered in yeast (Rine and Herskowitz, 1987), where OE of *Sir2* was reported to increase replicative lifespan (Kaeberlein et al., 1999). Subsequent studies further confirmed that sirtuins also regulate aging in worms, flies, and mice (Haigis and Guarente, 2006; Kanfi et al., 2012; Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001).

In *C. elegans*, OE of *sir-2.1* gene leads to extended lifespan in a manner dependent on DAF-16. SIR-2.1 activates DAF-16 by means of scaffolding 14-3-3 proteins and by antagonizing the DAF-16 repressor HCF-1 (Host Cell Factor 1), a process that is independent from IIS-mediated longevity (Berdichevsky and Guarente, 2006; Rizki et al., 2011; Tissenbaum and Guarente, 2001; Wang and Tissenbaum, 2006). However, new evidence argues that this SIR-2.1-mediated longevity in *C. elegans* was largely contributed by an unlinked *dyf* (dye-filling) mutation in the initial strains (Burnett et al., 2011). Segregating away the *dyf* mutation results in a minor but significant effect of SIR-2.1 on longevity (Mouchiroud et al., 2013; Viswanathan and Guarente, 2011), but whether DAF-16 is still involved in SIR-2.1 lifespan modulation, and by which mechanism, needs to be re-examined.

Overexpression of another sirtuin, SIRT6, increases lifespan in male mice (Kanfi et al., 2012), but OE of the *C. elegans* homolog of SIRT6, SIR-2.4, does not extend worms' lifespan.

Nevertheless, lack of SIR-2.4 function in worms impaired DAF-16 nuclear accumulation and elevated the susceptibility to external stresses, indicating that DAF-16 is involved in SIR-2.4-mediated normal stress resistance (Chiang et al., 2012b; Jedrusik-Bode et al., 2013)

1.3.3 Proteostasis in aging

Maintaining protein homeostasis (proteostasis) is essential for proper cellular function and survival of an organism. Loss of proteostasis is one of the hallmarks of aging, and of the various age-related protein aggregation diseases that often lead to short lifespan. In order to maintain a healthy proteome in the cell, there are carefully orchestrated surveillance systems to balance protein synthesis, protein degradation/clearance, and protein quality control.

The cellular protein folding system is comprised of protein chaperones of the HSP70 system, the HSP90 system, the small chaperones, and chaperonins. (Frydman, 2001; Kim et al., 2002; Schumacher et al., 1994). Newly translated peptides are predominantly bound by HSP70 chaperones and chaperonins for proper folding and to protect these peptides from aberrant interactions and aggregation. However, chaperones not only help *de novo* peptide folding, they can also recognize misfolded proteins and help them to regain a natively folded state. In addition, by recognizing misfolded proteins, they can induce expression of additional chaperones, thereby trigger unfolded protein responses (UPRs) that counteract the misfolding by expression of additional chaperones. Meanwhile, the UPR can also decrease protein translation to reduce the burden of new aggregates building up, and send the aggregates for degradation via the ubiquitin–proteasome system or autophagy.

Unfolded protein response systems are highly conserved across metazoans. Besides responding to unfolded proteins in the cytoplasm and nucleus, such systems can also be triggered by protein aggregates in the mitochondria or the endoplasmic reticulum (ER), termed UPR^{mt} and UPR^{ER}, respectively. Moreover, other environmental insults (such as thermal or oxidative stress) tend also to activate these UPRs (reviewed in Åkerfelt, Morimoto, & Sistonen, 2010; R.I., 2008; Westerheide & Morimoto, 2005). UPR systems are mediated by various TFs and regulators. The TF HSF-1 is considered as a master regulator for the maintenance of protein homeostasis – in particular in the cytoplasm and nucleus. Under normal conditions, HSF-1 is bound to HSP90 chaperones in the cytoplasm, but when a proteotoxic stress is encountered, HSF-1 disassociates from HSP90, translocates into the nucleus, and activates a heat shock response (HSR) by upregulation of chaperone genes, thereby helping the cell to restore proteostasis (Åkerfelt et al., 2010; Hsu, 2003). The TF ATFS-1 is responsible for UPR^{mt} activation in *C. elegans* (Nargund et al., 2012). ATFS-1 is normally imported into the mitochondria; however, during mitochondrial-specific stress, the mitochondrial import efficiency of ATFS-1 is reduced, allowing some of the cytosolic ATFS-1 to translocate into the nucleus and regulate UPR^{mt}-related gene expression. Other regulators are involved in UPR^{ER} to help the cell to restore ER proteostasis. For example, ER-associated endoribonuclease IRE-1 becomes activated through autophosphorylation, which then splices its target, the TF XBP-1. The spliced XBP-1 is then translated, whereby it regulates target genes to enhance resistance to ER stress (**Figure 3**) (Henis-Korenblit et al., 2010; Taylor and Dillin, 2013).

These cellular activities in response to unfolded proteins are associated with longevity. For instance, HSF-1 activity is important for normal lifespan in wild type worms and long lifespan in *daf-2* long-lived mutants, and HSF-1 OE is associated with lifespan extension in *C. elegans* (Hsu, 2003). In addition, constitutively activated XBP-1 in neurons is sufficient to increase longevity in worms (Taylor and Dillin, 2013). Moreover, reduced protein translation increases lifespan in *C. elegans* (Hansen et al., 2007; Pan et al., 2007), with *daf-2* long-lived mutants also showing lower protein translation rates (Essers et al., 2015). Finally, studies in *C. elegans* and other models have indicated that autophagy is important for longevity (reviewed in Hansen, Rubinsztein, & Walker, 2018). Together, finding strategies to restore protein homeostasis may be an effective strategy to delay aging and combat age-related diseases.

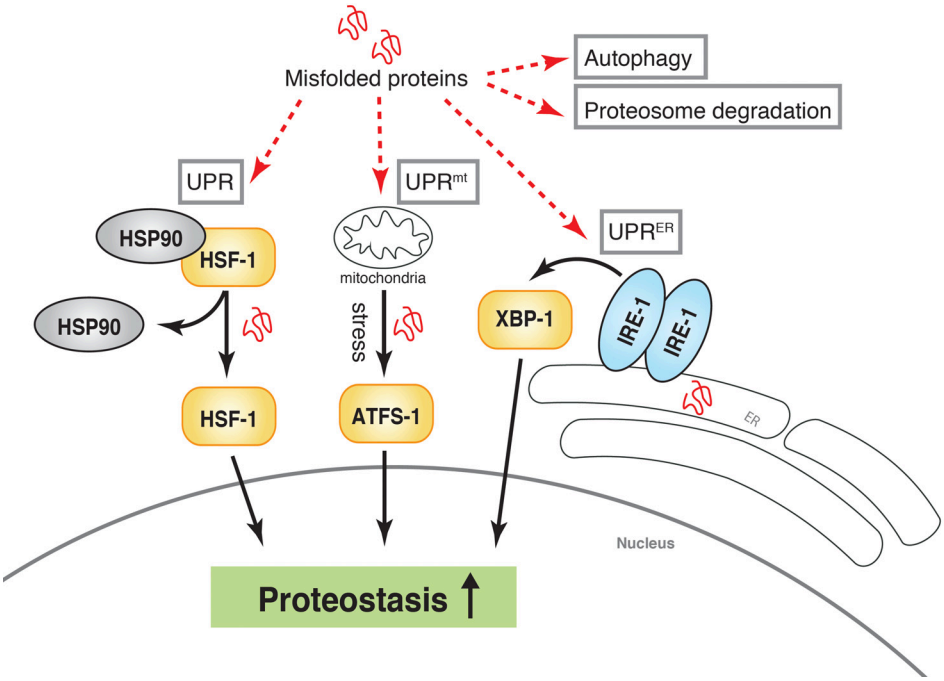

Figure 3: Misfolded proteins can activate the UPR and trigger proteolytic systems, including autophagy and proteasomal degradation, to restore proteostasis. Normally, HSF-1 is bound by HSP90 chaperones in the cytoplasm. However, when misfolded proteins accumulate, HSF-1 disassociates from HSP90, translocates into the nucleus, and upregulates heat-shock gene expression. Misfolded proteins also induce UPR in the mitochondria (UPR^{mt}) and the ER (UPR^{ER}). Under mitochondrial-specific stress, some of mitochondrial ATFS-1 accumulate in the cytosol, and can translocate into the nucleus to regulate gene expression that alleviates mitochondrial stress and recovers mitochondrial function. Likewise, under ER stress, endoribonuclease IRE-1 activates XBP-1, which results in gene expression changes that help to relieve ER stress and improve proteostasis.

1.3.4 Pharmacological intervention in aging

Drug interventions may be the most practical approach to target aging and delay the onset of age-related diseases in humans. Several promising geroprotective treatments have been investigated in mice as part of the National Institute on Aging's Intervention Testing Program (NIA's ITP) (Nadon et al., 2008). Although the use of mammalian models should yield results most applicable to humans, high costs limit their use for high-throughput drug screens. *C. elegans* provides an excellent alternative system for such drug screening, where preliminary drug hits can be re-validated and re-tested for toxicity, and the targeted pathways can be determined by genetic analyses with relative ease. Thus, the NIA also initiated a *Caenorhabditis* Intervention Testing Program (CITP) aiming to find compounds that can extend lifespan and healthspan (Lucanic et al., 2017). Despite an increasing number of screens conducted in *C. elegans*, there still exists discrepancies in the methodology between different research groups concerning, among other things, use of 5-fluoro-2'-deoxyuridine-5'-phosphate (FUDR), use of live or dead feeding bacteria, and the developmental stage at which worms are exposed to the drugs. Moreover, due to *C. elegans*' thick cuticles and the characteristics of tested drugs, it is hard to evaluate the delivery efficiency of a drug and the internal drug concentration. It is also worth noting the potential confounding effects between tested compound and bacteria or cultured media. Depending on the design and the handling of an experiment, tested drugs could exhibit variations of desired outcomes, indicating the reproducibility challenges when using *C. elegans* for compound screens. Therefore, one of the goals of CITP is also to standardize protocols and create reproducible data (Lithgow et al., 2017; Lucanic et al., 2017).

Efforts have been made to identify compounds that can prolong lifespan, or reproduce DR effects, as DR has been shown in many models to improve lifespan and healthspan (Calvert et al., 2016; Carretero et al., 2015). Resveratrol is considered as a DR mimetic that can prolong the life of yeast, flies, and worms (Baur and Sinclair, 2006; Baur et al., 2006; Howitz et al., 2003; Timmers et al., 2012); it has also been shown to protect against age-related physiological dysfunctions in mice (Baur et al., 2006; Lagouge et al., 2006). Even though its mechanism remains to be clarified, evidence suggests that resveratrol may exert its function by interacting with SIRT1 and by activating autophagy (Morselli et al., 2010).

Rapamycin is an immunosuppressive drug used to reduce graft vs. host disease in humans. Its anti-aging and lifespan-extending properties, reported in mice and other model organisms, were found to be associated with the inhibition of mTOR kinase and the promotion of autophagy (Bjedov et al., 2010; Harrison et al., 2009; Powers et al., 2006; Robida-Stubbs et al., 2012). Although studies found that rapamycin confers many aging-protective benefits, long-term consumption has been shown to lead to detrimental side effects on health, as observed in both animal models and human subjects (Lamming et al., 2012). In spite of the side effects, the geroprotective benefits of rapamycin have opened up the search for other existing drugs with potential antiaging properties.

Likewise, metformin which has a long history of being prescribed to treat type 2 diabetes, has also has been found to delay aging in many model organisms. Clinical data showed that

diabetes patients taking metformin outlived the patients taking another anti-diabetic drug, and even the control populations who did not take any medications (Anisimov et al., 2008; Bannister et al., 2014; Cabreiro et al., 2013; De Haes et al., 2014; Martin-Montalvo et al., 2013; Wu et al., 2016). Thus, a recently launched clinical trial called 'Targeting Aging with Metformin' hopes to find that metformin can delay the onset of multiple age-related diseases (Barzilai et al., 2016). Despite metformin's prominent anti-aging effect, its mechanism remains unclear, as it was found to target multiple conserved aging-regulatory pathways. For instance, it was found that metformin inhibits the mitochondrial electron transport chain complex 1 (El-Mir et al., 2000; Owen et al., 2000). Other studies have suggested that it mimics DR, activates AMPK, and reduces mTOR activity either dependent or independent of AMPK (Dowling et al., 2007; Gwinn et al., 2008; Howell et al., 2017; Kalender et al., 2010; Onken and Driscoll, 2010). Another work in *C. elegans* further indicated that metformin coordinates AMPK and mTOR activities via the lysosomal v-ATPase-Regulator complex (Campbell et al., 2017).

In addition to finding DR mimetics, the search for compounds that target the hallmarks of aging (such as cellular senescence) is another strategy. Cellular senescence is an irreversible cell-cycle arrest induced by various insults, including oncogene activation, telomere shortening, and other genotoxic stresses. Senescent cells contribute to age-related decline by secreting pro-inflammatory cytokines, chemokines, and other proteins (collectively termed the senescence-associated secretory phenotype [SASP]) which drive accumulation of senescent cells and contribute to tissue dysfunction and aging. Indeed, clearing senescent cells has been shown to increase the healthspan and lifespan of mice (Baar et al., 2017; Baker et al., 2011, 2016). Thus, interventions to reduce cellular senescence or remove senescent cells could ameliorate age-related dysfunctions. One study found HSP90 inhibitors to be a new class of senolytics that can selectively eliminate senescent cells and extend the healthspan of progeroid mice (Fuhrmann-Stroissnigg et al., 2017). The HSP90 chaperone is known to stabilize a large client-protein repertoire that includes TFs (e.g. HSF-1, NF-κB, p53) and kinases (e.g. AKT, RAF), among which are numerous oncoproteins. Cancerous cells harness these HSP90 clients to promote their own growth and survival; therefore HSP90 inhibition has initially been pursued as a strategy to treat cancers (reviewed in Trepel, Mollapour, Giaccone, & Neckers, 2010; Tukaj & Węgrzyn, 2016). A recent study suggests that as senolytics, HSP90 inhibitors could be used to delay aging. Consistently, HSP90-inhibitor treatment can also suppress chronic inflammation, another contributor of aging and age-related disease (Tukaj and Węgrzyn, 2016). In addition, HSP90 inhibitors have been shown to extend lifespan in *C. elegans* (Fuentealba et al., 2019). While the mechanisms by which HSP90 inhibitors exert these aging-preventive functions still needs to be explored, these findings underline the potential of re-purposing HSP90 inhibitors as geroprotectors.

1.3.4.1 Bioinformatics approach

To facilitate in the search for new geroprotectors, bioinformatic methods have been suggested to study aging processes and to direct drug and drug-target discovery (reviewed in Fabris, Magalhães, & Freitas, 2017). For example, Barardo et al. (2017b) analyzed the features of geroprotectors present in the DrugAge database (a database that contains information on compounds that have been shown to affect lifespan in model organisms [D. Barardo et al.,

2017]) to build predictive models using random forest algorithms. These features include Gene Ontology (GO) terms and chemical descriptors that describe a compound's chemical structure and its main properties. The model that had the best predictive accuracy was built based on both features, and was then applied to the DGIdb 2.0 database (The Drug Gene Interaction database [Wagner et al., 2016]) to screen for compounds that are likely to extend lifespan in *C. elegans*.

In addition, many of such bioinformatic approaches have taken advantage of analyzing transcriptome datasets. Because the transcriptome is dynamic, and reflects physiological conditions, information learned from transcriptome changes during chronological aging or age-related conditions can be used to predict biological age in humans (Peters et al., 2015; Sood et al., 2015); it can also be used to predict geroprotectors that reverse the aging process. For instance, Fortney et al. (2012) and Calvert et al. (2016) screened for compounds in Connectivity Map (CMap) database (a database of drug-induced gene expression profiles in multiple cell types [Lamb et al., 2006]) that induce transcriptional changes as seen in caloric restriction conditions in mice. Such compounds would likely be DR mimetics. Aliper et al. (2016) applied the computational tool GeroScope to transcription profiles of human hematopoietic and mesenchymal stem cells, and screened for drugs that mimic young transcriptional profiles in age-related pathways. Dönertaş et al. (2018) used human brain tissue transcriptomes from The Genotype-Tissue Expression (GTEx) project to look for drugs that target human brain aging in the CMap database (GTEx provides a dataset of human tissue transcriptomes from donors of various ages and both genders [GTEx Consortium, 2015]).

2 AIMS

Aging is a major risk factor of mortality and morbidity. Studies have shown that targeting the aging process by genetic manipulations or by pharmacological targeting of the pathways that regulate it (e.g. the IIS pathway and its downstream TF DAF-16) can increase both lifespan and healthspan in animal models. The objectives of this thesis are to understand the molecular mechanism of DAF-16-mediated transcription using the model organism *C. elegans*, and to develop and apply new strategies to identify compounds that defer aging.

Paper 1

In this study, we used *C. elegans* to investigate how DAF-16 exerts its prolongevity function by cooperating with another TF, HLH-30, and explored how the genetic interactions between DAF-16 and HLH-30 are different in response to diverse stresses.

Paper 2

In this study, we developed a novel screening method to search for aging-preventive compounds, also known as geroprotectors, by using human tissue transcriptomes and drug-induced gene expression profiles. Next, we tested the results from our screening in *C. elegans* to validate the candidates' geroprotective effects. Having thereby identified the HSP90 inhibitors monorden and tanespimycin, we explore the underlying mechanism by which they benefit lifespan and healthspan.

3 METHODOLOGICAL CONSIDERATIONS

Ethical permits were not required for this thesis work, given that we worked only in *C. elegans* or *in vitro*.

We used *C. elegans* as a model organism to investigate the mechanisms by which the TFs DAF-16 and HLH-30 confer longevity, and to evaluate the lifespan effects of the potential geroprotectors identified by our screening in paper 2. *C. elegans* serves as an excellent platform for aging research for several reasons: it has a short lifespan, a completely sequenced genome, and is easy to maintain and amenable for genetic manipulations. Most importantly, its signaling pathways and proteins are highly conserved in humans, thus the knowledge we gain from this simple animal model can shed light on the complex human physiology.

Lifespan is a commonly used assessment in aging research. The advantage of using *C. elegans* for lifespan assays is that it has a relatively short lifespan, thus allowing for fast assessment of many manipulations in parallel. Usually, synchronous worm populations are used for lifespan assays, which can be obtained by bleaching gravid adults and allowing the eggs to grow into starved L1, or by allowing gravid adults to lay eggs on the NGM (nematode growth media) plates. Both methods were used in this thesis work. The lifespan experiments were normally conducted at a temperature of 20°C, or at between 15°C to 25°C as desired. Animals' survival was determined by whether worms would respond to physical touch. Despite the simplicity of conducting lifespan assays by hand, it poses the limitation of being tedious, and is thus hard to carry out in large-scale assays; moreover, it is hard to produce high-resolution data, with timepoints only an hour apart, as it would require constant inspections. As such, for much of our work, we employed an existing method, the 'Lifespan Machine' (LM), for lifespan assay in addition to the manual method. The LM is a combination of a modified flatbed scanner and image processing software (Stroustrup et al., 2013). This automated system acquires time-lapse images of individual plates, and processes these images to identify worms and their death times, which will be used to construct survival curves. Moreover, the LM allows for a larger scale of lifespan experiments, and generates high-resolution survival data. Despite the advantages of using the LM, we observed that animals in general lived slightly shorter lives than with the 'physical touch' approach. It is possible that the constant exposure to scanner illumination has an impact on worm lifespan. We used Kaplan–Meier analysis to plot survival curves and determined differences between survival curves by log rank test.

Stress-resistance assays were conducted similarly to the lifespan assays. These assays evaluate how long can worms survive under different stresses such as heat, oxidant, or UV radiation. Usually worms die rapidly after introducing such stressors (within 1 or 2 days); this is where the LM becomes immensely valuable, because we can obtain survival data at a high resolution of ~1 hour, while by hand we would often reach only 4 to 6 hours.

Aging research is not only about prolonging lifespan, but, and more importantly, about extending healthy lifespan. Therefore, examining the effect of genetic or pharmacological manipulations on an organism's healthspan has become important in aging research. Common age-related physiological declines used to assess healthspan in *C. elegans* include reproductive

span, progeny size, body movement, mobility (distance or length a worm travels within a given timeframe), thrashing rate (lateral movement frequency in liquid), maximum velocity (Hahm et al., 2015), and pharyngeal pumping (Huang et al., 2004; Onken and Driscoll, 2010; Ryu et al., 2016). Here we looked at thrashing rate and maximum velocity, and used ImageJ plugin wrMTrck to perform the analyzes (Nussbaum-Krammer et al., 2015).

C. elegans is an excellent tool for *in vivo* drug screening owing to the same properties stated above. As *C. elegans* is typically cultured on NGM plates, many drugs have been tested under such plate-based culturing conditions. More recently, liquid-based culturing conditions were developed to facilitate high-throughput drug screening in *C. elegans*. The development of *C. elegans*-based drug screening and the challenges are reviewed in O'Reilly, Luke, Perlmutter, Silverman, & Pak (2014). One concern of using *C. elegans* for drug testing is the inefficient uptake of non-water-soluble drugs due to the worm's thick cuticle. However, a study by Zheng et al. (2013) showed that the concentration of Resveratrol (dissolved in DMSO solvent) in worms is comparable to the concentration reached in mice. In addition, how the drugs are delivered, the use of live or dead bacteria as a food source, and the use of solvent have great impacts on drug efficacy. Here, we took an approach similar to the 'spot dead method'(Zheng et al., 2013) with some modifications: we spot the drugs on top of the NGM plates using dead bacteria as food source. All drugged plates were prepared one day before use.

Since DMSO is known to increase lifespan of *C. elegans* in liquid (Frankowski et al., 2013; Wang et al., 2010), we used the recommended maximal DMSO concentration (0.33%) (Ye et al., 2014) in drug and control plates in this study. Nevertheless, we found that, while 0.33% DMSO had no impact on the lifespan of worms feeding on control RNAi (L4440), it still promoted longevity in *hsp-90* RNAi-treated worms. That is, *hsp-90* RNAi alone showed no significant lifespan changes, whereas in the presence of DMSO solvent in the plate, *hsp-90* RNAi led to lifespan extension. Maybe this could be due to DMSO inducing mild proteotoxic stress, under which *hsp-90* loss becomes beneficial. But this still needs to be carefully evaluated.

In order to facilitate the initial *in vivo* drug screening, we only tested a small numbers of candidate drugs at a single concentration of 50 μM, and worms were placed on the drugged plates from L4. It is possible that, at the concentration of 50 μM, some drugs might exhibit toxicity, or others might not be potent enough to manifest lifespan benefits. Moreover, since we administered drugs to worms only one time from the L4 stage, differences in degradation rates might affect the lifespan effect of different drugs. As such, it remains interesting to re-examine the drugs that failed to extend lifespan at different concentrations, as well as to investigate the remaining candidates from the list. Despite the foreseeable limitations, we were still able to confirm the lifespan extending effects of some known geroprotectors, and further found felbinac and two HSP90 inhibitors, monorden and tanespimycin, to extend lifespan in *C. elegans*. Finally, drugs identified in *C. elegans* may act differently in mammals due to their physiological differences, and therefore require further validation.

4 RESULTS

4.1 PAPER 1

The TFs DAF-16 and HLH-30 function as combinatorial TFs to promote longevity and stress resistance in *C. elegans*.

Transcriptional responses to environmental stimuli are the key to an organism's survival and longevity. Although DAF-16 is a known aging regulator conserved across species, its mechanisms of action are not fully understood. A previous study has shown that DAF-16 employs various binding partners to fulfil its function. In this paper we aim to continue the effort in investigating the functions of DAF-16 in relation to another binding partner, HLH-30. We explore their genetic interactions in promoting longevity and in response to stresses.

Using *C. elegans* as a model system, we first confirmed that DAF-16 and HLH-30 can form a complex, and the formation of complex is not mediate by DNA or RNA. The functions of DAF-16 and HLH-30 are tightly regulated by their PTMs and cellular localization. Under normal conditions, they are retained in the cytoplasm by binding to 14-3-3 proteins, and released to the nucleus upon stimulation to regulate gene expression. Therefore, we inspected which stimuli can trigger their activation and nuclear translocation. First, we showed that both DAF-16 and HLH-30 express ubiquitously in the animal and distribute homogeneously in the cell under normal conditions. When we subjected worms to various stresses, including heat, oxidant, UV radiation, pathogens, and starvation, we observed different degrees of nuclear accumulation of DAF-16 and HLH-30 depending on the given stimuli. Moreover, DAF-16 and HLH-30 can undergo nuclear translocation independently of one another, suggesting they perceive stimuli and become activated independently.

Next, we showed that both DAF-16 and HLH-30 are required for the long lifespan effects in *daf-2* and *glp-1* mutants. In addition, we found that combined loss of both DAF-16 and HLH-30 resulted in no additive lifespan effect, suggesting that they function in the same genetic pathway to promote longevity. Consistently, our mRNA-sequencing (mRNA-seq) data showed that both DAF-16 and HLH-30 are required for the majority of gene expression changes in *daf-2* and *glp-1* mutants, and that they co-regulate significant subsets of genes. Further characterization of these groups of co-regulated genes showed that they are enriched for aging-related genes.

As both DAF-16 and HLH-30 are TFs, we asked whether they also bind to the same promoter regions. To this end, we conducted chromatin immunoprecipitation-sequencing (ChIP-seq) to investigate genome-wide binding sites of DAF-16 and HLH-30 using *daf-2* mutant animals. Our data showed that DAF-16 and HLH-30 co-occupied various promoter regions, and that the immediate downstream genes of these promoters are enriched for aging-associated genes. Taken together, these findings support the notion that DAF-16 and HLH-30 co-occupy many target promoter regions, and promote longevity by regulating target-gene expression.

Moreover, we asked whether DAF-16 and HLH-30 also collaborate in stress resistance by investigating the survival of wild type, *daf-16*, *hlh-30* or *daf-16; hlh-30* animals under heat stress and oxidative stress. Interestingly, we found that although both DAF-16 and HLH-30 are important for heat-stress and oxidative-stress resistance, they function in the same pathway to cope with oxidative stress, while they work independently under heat stress. Our mRNA-seq data suggested that they co-regulate oxidative-responsive genes, while each individually controls different sets of heat-responsive genes.

Lastly, we asked whether HLH-30 is involved in dauer formation, a developmental diapause state that enables worms to survive harsh environments. We found that while DAF-16 is essential for dauer formation, HLH-30 inhibits dauer formation.

In summary, we provide a new transcription regulatory module of two important aging regulators, DAF-16 and HLH-30. Together they form a complex upon activation and co-localize at many promoter regions to regulate target-gene expression. The genetic interactions of DAF-16 and HLH-30 are dynamic and dependent on environmental stimuli: they function in the same pathway to promote longevity and increase resistance to oxidative stress, while they regulate heat-stress response independently. In contrast, during dauer development DAF-16 and HLH-30 oppose each other.

4.2 PAPER 2

Transcriptomics-based screening identified HSP90 inhibitors as geroprotectors.

Pharmacological intervention is one of the approaches to delay aging, and perhaps the most applicable means to humans. To identify compounds that may improve human health and longevity, so called 'geroprotectors', we employed a random forest algorithm to build age classifiers from human tissue transcriptomes (data obtained from the GTEx database), and applied them to drug-induced transcriptional profiles to predict 'youthful' states and thus geroprotectors (data obtained from CMap).

In total, 24 age-classification models were applied to more than one thousand drugs present in the CMap database, which gave rise to a significance-based ranking list of the top 31 compounds that passed our significance cutoff ($p<0.05$), and thus predicted as most likely to be geroprotective. Among these 31 candidates were several drugs known to increase lifespan in model organisms, including valproic acid, trichostatin A, LY-294002, estradiol, sirolimus/rapamycin, genistein, trifluoperazine, wortmannin, metformin, and acetylsalicylic acid.

Next, we took the top candidates for further consideration and asked whether they have longevity effects in *C. elegans*. To this end, 14 out of the 15 top-ranked drugs were tested in worms at the concentration of 50 μM, whereas one top-ranked drug, tanespimycin, was omitted in this validation due to its high cost and its redundancy with another candidate, monorden (both are HSP90 inhibitors). Further to this, we selected 15 other drugs that were of interest but not part of the 'top candidates' ranking, and evaluated their lifespan benefits in worms. We found five compounds, namely monorden, rapamycin, LY-294002, valproic acid, and felbinac,

to significantly increase lifespan by more than 10%. Four of these five (excluding felbinac) were candidates generated by our model prediction, underlining the efficacy of our prediction method.

Monorden, a HSP90 inhibitor that has not been described as a geroprotector in literature, showed the most lifespan extension in our lifespan assays. In addition to the lifespan benefit, we found that worms treated with monorden exhibited an extended period of active life, and a modest but significant improvement in mobility, suggesting monorden treatment can increase both lifespan and healthspan. These observations prompted us to evaluate whether inhibiting HSP90 in general leads to lifespan extension. As such, we targeted HSP-90 in *C. elegans* using another HSP90 inhibitor, tanespimycin, that was initially excluded from our lifespan assays due to its high cost. We also used RNAi to knockdown *hsp-90* homolog *daf-21* from the L4 larval stage in *C. elegans*. Indeed, we observed increased lifespan both by giving tanespimycin and by *daf-21* RNAi, suggesting that HSP90 inhibition represents a new means to target the aging process.

To understand by which mechanism HSP90 inhibition conferred longevity, we turned to the transcriptional response profile of monorden in CMap. Comparing it to transcriptomes of two other established geroprotectors, rapamycin and LY-294002, we observed that monorden induced a distinct upregulation of the cytosolic UPR, in particular an upregulation of HSPs. HSPs are primarily induced by HSF-1 to protect the organism against stressful conditions. Here we found that monorden-treated animals have better survival under heat stress, and that HSF-1 is required for monorden-induced longevity. Since the monorden-induced transcription profile is distinct from those induced by rapamycin or LY-294002, we reasoned that monorden may function via a different pathway than rapamycin and LY-294002, and thus give an additive beneficial effect on lifespan when combined with rapamycin or LY-294002. To test this, we administered additional monorden to rapamycin- or LY-294002-treated worms and assessed their lifespan. We found that monorden further extended lifespan in rapamycin- or LY-294002-treated animals, suggesting that monorden uses, at least in part, a different mechanism than rapamycin and LY-294002 to confer its benefits – namely that of upregulating HSPs through HSF-1.

In conclusion, we developed a transcriptome-based screening approach that was able to identify aging-preventive compounds, and further highlighted monorden as a geroprotector. We explored the mechanism of monorden to defer aging and found that it requires HSF-1, activating cytosolic UPR which then restores proteostasis – a pathway that is distinct from the mechanism triggered by many other geroprotectors, including rapamycin and LY-294002.

5 DISCUSSION

A large body of research revealed several signaling pathways that influence aging through a collection of TFs. One of the most central players is DAF-16, essential to promote longevity and stress resistance under many stimuli including low IIS or in germline-less animals. Another important TF is HLH-30, a known master regulator of autophagy which has been reported as essential for lifespan extension in many long-lived mutants (Lapierre et al., 2013), including *daf-2* and *glp-1* germline-deficient mutant animals (Hsin and Kenyon, 1999), indicating that HLH-30 may play an important role in DAF-16-dependent transcription responses. However, the nature of the relationship between these two TFs in aging-regulatory aspects has remained elusive.

Our work (paper 1) shows that HLH-30 physically and genetically interacts with DAF-16 for promotion of longevity and stress resistance. For DAF-16 to fulfill lifespan extension, it requires the presence of HLH-30, and vice versa. Interestingly, even though both DAF-16 and HLH-30 are activated by various stimuli and re-localize to the nucleus (Lapierre et al., 2013), our survival data indicated that they do not always function in the same genetic pathway; they require each other to promote longevity under low IIS and to protect against oxidative stress, but they function independently when encountering heat stress. By exploring the gene expression profiles under heat and oxidative conditions, we showed that DAF-16 and HLH-30 independently activate different subsets of heat-responsive genes, while predominantly co-activating oxidative-responsive genes. This genetic interaction seems also to be reflected in their physical interactions, as our size-exclusion chromatography data suggests that DAF-16, as well as HLH-30, shifts to a higher molecular weight under oxidative stress and low IIS, indicating DAF-16 incorporates into larger complexes, and may engage in the formation of the DAF-16-HLH-30 complex. On the contrary, such molecular weight shift was not observed under heat stress. Nevertheless, additional experiments, for instance by conducting co-immunoprecipitation under different stimuli, will be required to clarify their physical interactions under these stresses.

Previous work has shown that HSF-1, a master regulator of HSR required for *daf-2* longevity, functions by activating small HSPs together with DAF-16 (Hsu, 2003). In addition, both HSF-1 and HLH-30 are required to elevate autophagy gene expression under mild heat stress (Kumsta et al., 2017). This points to the possibility of HSF-1 being an alternative regulatory partner of DAF-16 and HLH-30 under heat stress. Indeed, we found heat responsive genes are activated either by DAF-16 or by HLH-30, and, given the nature of combinatorial controls of gene expression, it is likely that another TF, like HSF-1, is involved in activating these heat-responsive genes. As elevated HSR and autophagy generally lead to longevity, it will be interesting to determine how HSF-1 is placed in the context of the DAF-16–HLH-30 regulatory module. Further experiments to investigate the physical interactions of HSF-1 with DAF-16 and HLH-30, and the genome-wide binding profiles of HSF-1, DAF-16, and HLH-30 under heat-stress conditions, will help to answer this question.

Our large-scale purification data indicates a higher affinity of HLH-30 to DAF-16 when DAF-16 is predominantly in the nucleus (i.e. in the *daf-2* mutant background). Moreover, we found knocking down one does not affect the other's ability to enter the nucleus, suggesting they can translocate into nucleus independently, and that the DAF-16–HLH-30 complex only assembles in the nucleus. Nevertheless, it remains to be fully elucidated as to how different stimuli lead to the different genetic interactions between DAF-16 and HLH-30. PTMs not only determined TF's subcellular localizations, they also affect TF's structure, which can lead to a change of DNA binding activity, the affinity to other proteins, and the proteins' function (Filtz et al., 2014). Therefore, it is likely that different PTM landscapes, caused by different external signals, dictate the physical and genetic interactions of DAF-16 and HLH-30. Studies of the mammalian FOXO family have revealed various phosphorylation sites and other types of PTM under different conditions (reviewed in Calnan & Brunet, 2008). Likewise, mammalian TFEB, known to be regulated by mTOR, is also found to be directly regulated by AKT kinase (Palmieri et al., 2017), indicating that other TFEB/HLH-30 activation pathways may be unexplored, and thus different PTMs await to be revealed. As DAF-16 and HLH-30 compose an important aging-regulatory module, it will be interesting to investigate how their PTM landscape is shaped, which may in turn provide information for developing therapeutic interventions that target these PTMs to defer aging while avoiding undesirable side effects.

In contrast to a previous report (Lapierre et al., 2013), we found HLH-30, instead of promoting, slightly inhibits dauer formation by opposing some DAF-16-mediated dauer gene expression. This discrepancy might be due to the different experimental setup, in which we used *hlh-30* mutant and set the temperature at 22.5°C instead of *hlh-30* RNAi at 22°C. The dauer program is a complex process regulated by different signaling pathways, and only some dauer-related genes have been described. Further examinations will be required to clarify the role of HLH-30 in this dauer development.

Taken together, we showed that HLH-30 is required for DAF-16-dependent transcription and long lifespan. Their genetic interactions are dynamic and context-dependent, which ensures that only the accurate and essential transcriptional responses are being executed when encountering stresses and during phases of developmental decision-making. It remains to be determined whether there are similar regulatory modules under other aging-regulatory pathways, and how other known aging regulators may influence the DAF-16–HLH-30 module.

In paper 2, we took a bioinformatic approach to search for therapeutics that could potentially protect humans from aging. Our unbiased computational screen has yielded a ranking list of compounds based on their likelihood of inducing younger transcription profiles. On our short-list, we found several compounds known to increase lifespan in model organisms, as well as new candidates that were not described as geroprotectors previously. We then tested the top candidates in *C. elegans*, and found that the HSP90 inhibitor monorden increased lifespan the most among the tested drugs.

The mechanism of HSP90 inhibitors has largely been explored, and is likely how monorden confers longevity. In short, pharmacological inhibition of HSP90 leads HSF-1 to disassociate from the HSP-90–HSF-1 complex, and then translocate into the nucleus. Nuclear HSF-1

subsequently induces the expression of HSPs, which in turn restore proteostasis, protect the organism against deleterious conditions, and promote its survivals. Consistently, monorden-mediated longevity requires the presence of HSF-1; the transcriptional response to monorden shows clear upregulation of cytosolic UPR proteins (HSP-1, HSP-12.6, and HSP-70) orchestrated by HSF-1. In addition, monorden improves thermal tolerance in worms. Furthermore, we evaluated the effect of monorden in *hsp-90* knockdown animals and found monorden does not extend the lifespan of *hsp-90* RNAi-treated worms, which is in line with monorden being an HSP90 inhibitor. Altogether, it suggests that the geroprotective mechanism of monorden is conferred by inhibition of HSP90, subsequent HSF-1 activation, and the upregulation of HSP expression. Whether other HSP90 client proteins are involved in monorden-mediated longevity requires future investigation.

As many of HSP90 client proteins are pivotal to the survival and the growth of cancerous cells, HSP90 inhibitors have been extensively investigated as cancer therapeutics. However, the elevated HSR that follows HSP90 inhibition lowers the sensitivity of cancerous cells to the drug. To overcome this shortcoming, efforts are underway to minimize the HSR in order to enhance the efficacy of HSP90 inhibition in cancer treatment. For example, previous studies have shown that combined treatment with an HSP90 inhibitor and an mTOR or PI3K/mTOR inhibitor can overcome the side effects of an elevated HSR in cancer treatment (Acquaviva et al., 2014; Francis et al., 2006). Interestingly, we have found combining monorden with the mTOR inhibitor rapamycin or with the PI3K inhibitor LY-294002 (both drugs are known to extend lifespan in model organisms) to have mild additive effects on longevity. It will be interesting to investigate how monorden-transcriptome is altered by adding rapamycin or LY-294002, and to see if a unique transcriptional signature emerges. Furthermore, it would also be interesting to investigate whether other signaling pathways and TFs are involved in this combined treatment approach. A better understanding of these concepts will facilitate finding the best strategy when using HSP90 inhibition to target aging, and even when identifying or developing other geroprotectors.

In addition to pharmacological inhibition of HSP-90, we found that knockdown of *hsp-90* by RNAi from the L4 stage also leads to lifespan extension in worms. This observation contradicts a previous report whereby RNAi of *hsp-90* would shorten worm's lifespan (Somogyvári et al., 2018). While it requires further examination, we argued that this discrepancy might due to the use of different RNAi clones, different knockdown intensities, different timing of giving RNAi between the studies, and the presence of DMSO in plates in our study. Interestingly, a previous study has shown that treating a mammalian cell line with monorden overnight stimulates the synthesis of HSP90 (Schulte et al., 1998). Furthermore, we observed only a moderate overlap between monorden-induced and *hsp-90* RNAi-induced transcriptional changes, suggesting that the mechanism of *hsp-90* RNAi-mediated longevity may only overlap partially with monorden. The reasons for this limited overlap will require more research in the future.

6 MAIN CONCLUSIONS

The main conclusions of each study in this thesis are summarized below:

Paper 1: Our findings show that DAF-16 and HLH-30 can physically interact with each other, and form an aging-regulatory module that relays various stress signals into transcriptional responses. We demonstrate that DAF-16 and HLH-30 promote longevity and resistance to oxidative stress collaboratively and through the same genetic pathway by co-regulating the relevant target genes, while they function independently by controlling separate target-gene sets to confer heat-stress resistance.

Paper 2: We use human tissue transcriptomes to build age-classifier models that are capable of distinguishing 'younger' from 'older' transcriptional states. We use these models to identify compounds that can induce 'younger' transcriptomes, and thus could be geroprotectors. Next, we test the top candidates resulting from this analysis in *C. elegans,* and show that several compounds can extend the lifespan of the animals, including the HSP90 inhibitors monorden and tanespimycin. Focusing on HSP90 inhibitors, we found monorden-treated worms have better mobility and resistance to heat stress. We further show that HSP90 inhibitors confer geroprotective effects by activating HSF-1, which in turn upregulates HSPs, and thereby improves protein homeostasis. Such ability is unique to HSP90 inhibitors, and therefore they can further extend the lifespan of animals treated with other geroprotectors (e.g. rapamycin or LY-294002).

7 FUTURE PERSPECTIVES

Our work (paper 1) described the combinatorial regulation of stress resistance, development, and longevity by HLH-30 and DAF-16. However, their exact relationship with other known aging-regulatory TFs such as HSF-1 and SKN-1, and their modulation by upstream signals still awaits discovery (Chiang et al., 2012a; Hsu, 2003; Seo et al., 2013).

First, it will be interesting to conduct a comprehensive study on how these TFs interact with each other, and whether these interactions change under different conditions. For instance, performing ChIP-seq under different conditions may reveal different binding profiles and distinct overlapping peaks. This will contribute to better understanding of aging-regulatory networks.

Second, stress-responsive genes generally contribute to longevity. Thus, further characterizing the co-regulated targets of DAF-16 and HLH-30 under various scenarios will help to distinguish the stress protective and aging-regulatory target genes. Once candidate genes are revealed, these can be followed-up and validated individually by RNAi knockdown, by introducing mutations using the CRISPR/Cas9 system (Dickinson and Goldstein, 2016), or by using already available mutations in *C. elegans* strains (e.g. from The Million Mutation Project library [Thompson et al., 2013]), with the resulting phenotypes then being evaluated.

Third, as DAF-16 and HLH-30 are conserved across species (Eijkelenboom et al., 2013; Palmieri et al., 2011; Webb et al., 2016), we could consider a meta-analysis comparing our DAF-16 and HLH-30 ChIP-seq data with mammalian FOXO and TFEB ChIP-seq data, to strengthen our argument that their age-regulatory relationship is evolutionarily conserved. Further investigation in mammalian systems will provide insights into their aging-regulatory function in humans, and may lead us to potential strategies of combating age-related disorders.

Fourth, our second study, testing only a fairly small number of top-candidate geroprotective compounds, will serve as a pilot project for the overall approach. We have yet to determine the geroprotective ability of the remaining candidates, and re-test the compounds that failed to extend lifespan by evaluating different concentrations and refreshing the drugs at multiple times during life time. We intend to lay the foundation for future large-scale screens searching for compounds that are able to exert rejuvenating function in connection to the aging regulatory pathways, such as IIS pathway and its output effector, DAF-16/FOXO.

A recent study has found that HSP90 inhibitors function as senolytics (Fuhrmann-Stroissnigg et al., 2017), a class of anti-aging drugs that function by eliminating senescent cells. Together with our finding that HSP90 inhibitors target aging by improving protein homeostasis, this suggests HSP90 inhibitors could target different aging hallmarks. It is therefore of high interest to further explore the mechanisms used by HSP90 inhibitors. We can combine the use of HSP90 inhibitors with other geroprotectors targeting different pathways to maximize their benefits. Meanwhile, we could continue the efforts to evaluate the geroprotective effects of HSP90 inhibitors in mammalian models, as well as efforts to optimize the dosage and the administration schedule.

8 ACKNOWLEDGEMENTS

我最要感謝的是爸媽、彥君、京翰、季曄：因為有你們的支持與鼓勵，這本博士論文才得以完成。尤其是媽媽，非常感謝妳！

I would like to express my first and the biggest thanks to my parents and siblings, thank you for your love, support, and encouragement. This thesis would not have been possible without you! And to my family in Netherlands, Ellen, Stefan, Florien, Sanne, and Thijs, thank you for your hearty welcome and support over the years!

I would like to thank all my supervisors and collaborators for your feedback and dedicated scientific works present in this thesis; the opponent and examination committee for taking the time to review this thesis; all the financial and infrastructural supports from ERIBA/UMCG, ICMC, and Karolinska Institute.

To the Riedel lab and the members, I would like to thank Christian Riedel for accepting me as a PhD student, and every member of the team for helping me with the experiments and giving me feedback on my projects.

To the Swoboda lab and the members, thank you for taking me in, creating a great working environment, and having an exotic-snack corner that made me survive many lab meetings and lab work. Peter, you always make time for me even for a trivial matter, thank you for your kindness and guidance, I have learned so much from you! Debora, you are so positive and friendly, you never hesitate to give me a hand when I need one, thank you for helping me to settle in the group and helping me with all kinds of difficulties I ran into.

I would like to thank my mentor Rik Korswagen, who introduced me to *C. elegans* and inspired me to pursue PhD study, and Ellen Nollen, who not only gave me scientific advice but also shared life wisdom with me.

I also want to thank all my friends, I am truly grateful to have you by my side along this journey. All the great memories I have in Groningen and in Stockholm are because of you!

To my friends from Taiwan, thank you for comforting me when I miss home, and supporting me when I face challenges.

To Dr. Olga and Ale, you guys are full of positive energy and bring so much laughter to my life. Your enthusiasm for science made it really enjoyable to discuss science with you and work next to you, thank you.

To Julie and Hsiao-yin, you are my sisters in the Netherlands! You always take good care of me, bring me delicious food, listen to my dreams and worries, give me hugs and advices. I am so lucky, thank you!

To Myriam, JJ, and Byambaa, you are great scientists that I look up to, and great friends that I can turn to. Thank you for so many wonderful times we spent together, thank you for always having my back!

To Georges, you are so kind and you always full of ideas, and your passion in science is contagious! Thank you for helping me, showing me how much fun it is to do research.

To Emmy, thank you for helping me through difficult times and always believing in me.

To my housemates from Vårberg, Örnsberg, and Apelbergsgatan, you are my family in Stockholm! Thank you for giving me so many wonderful memories and being so amazing.

And finally, to Rik and Alex, you are the best things happened to me. Thank you for being in my life.

9 REFERENCES

Acquaviva, J., He, S., Sang, J., Smith, D.L., Sequeira, M., Zhang, C., Bates, R.C., and Proia, D.A. (2014). mTOR Inhibition Potentiates HSP90 Inhibitor Activity via Cessation of HSP Synthesis. Mol. Cancer Res. *12*, 703–713.

Åkerfelt, M., Morimoto, R.I., and Sistonen, L. (2010). Heat shock factors: Integrators of cell stress, development and lifespan. Nat. Rev. Mol. Cell Biol. *11*, 545–555.

Aliper, A., Belikov, A. V., Garazha, A., Jellen, L., Artemov, A., Suntsova, M., Ivanova, A., Venkova, L., Borisov, N., Buzdin, A., et al. (2016). In search for geroprotectors: In silico screening and in vitro validation of signalome-level mimetics of young healthy state. Aging (Albany. NY). *8*, 2127–2152.

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

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

Baar, M.P., Brandt, R.M.C., Putavet, D.A., Klein, J.D.D., Derks, K.W.J., Bourgeois, B.R.M., Stryeck, S., Rijksen, Y., van Willigenburg, H., Feijtel, D.A., et al. (2017). Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. Cell *169*, 132–147.e16.

Baker, D.J., Wijshake, T., Tchkonia, T., Lebrasseur, N.K., Childs, B.G., Van De Sluis, B., Kirkland, J.L., and Van Deursen, J.M. (2011). Clearance of p16 Ink4a-positive senescent cells delays ageing-associated disorders. Nature *479*, 232–236.

Baker, D.J., Childs, B.G., Durik, M., Wijers, M.E., Sieben, C.J., Zhong, J., A. Saltness, R., Jeganathan, K.B., Verzosa, G.C., Pezeshki, A., et al. (2016). Naturally occurring p16Ink4a-positive cells shorten healthy lifespan. Nature *530*, 184–189.

Bannister, C. a, Holden, S.E., Morgan, C.L., Halcox, J.P., and Schernthaner, G. (2014). Can people with type 2 diabetes live longer than those without ? sulphonylurea monotherapy and matched , non-diabetic controls. Diabetes, Obes. Metab. *16*, 1165–1173.

Bansal, A., Zhu, L.J., Yen, K., and Tissenbaum, H.A. (2015). Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants. Proc. Natl. Acad. Sci. *112*, E277–E286.

Barardo, D., Thornton, D., Thoppil, H., Walsh, M., Sharifi, S., Ferreira, S., Anžič, A., Fernandes, M., Monteiro, P., Grum, T., et al. (2017a). The DrugAge database of aging-related drugs. Aging Cell *16*, 594–597.

Barardo, D.G., Newby, D., Thornton, D., Ghafourian, T., de Magalhães, J.P., and Freitas, A.A. (2017b). Machine learning for predicting lifespan-extending chemical compounds. Aging (Albany. NY). *9*, 1721–1737.

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

Baur, J.A., and Sinclair, D.A. (2006). Therapeutic potential of resveratrol: The in vivo evidence. Nat. Rev. Drug Discov. *5*, 493–506.

Baur, J.A., Pearson, K.J., Price, N.L., Jamieson, H.A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J.S., Lopez-Lluch, G., Lewis, K., et al. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. Nature *444*, 337–342.

Berdichevsky, A., and Guarente, L. (2006). A Stress Response Pathway Involving Sirtuins, Forkhead and 14-3-3 Proteins. Cell Cycle *5*, 2588–2591.

Bishop, N.A., and Guarente, L. (2007). Two neurons mediate diet-restriction-induced longevity in C. elegans. Nature *447*, 545–549.

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

Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics.

Brunet, A., Bonni, A., Zigmond, M.J., Lin, M.Z., Juo, P., Hu, L.S., Anderson, M.J., Arden, K.C., Blenis, J., and Greenberg, M.E. (1999). Akt Promotes Cell Survival by Phosphorylating and Inhibiting a Forkhead Transcription Factor. Cell *96*, 857–868.

Burkewitz, K., Morantte, I., Weir, H.J.M., Yeo, R., Zhang, Y., Huynh, F.K., Ilkayeva, O.R., Hirschey, M.D., Grant, A.R., and Mair, W.B. (2015). Neuronal CRTC-1 Governs Systemic Mitochondrial Metabolism and Lifespan via a Catecholamine Signal. Cell *160*, 842–855.

Burnett, C., Valentini, S., Cabreiro, F., Goss, M., Somogyvári, M., Piper, M.D., Hoddinott, M., Sutphin, G.L., Leko, V., McElwee, J.J., et al. (2011). Absence of effects of Sir2 overexpression on lifespan in C. elegans and Drosophila. Nature *477*, 482–485.

Cabreiro, F., Au, C., Leung, K.-Y., Vergara-Irigaray, N., Cochemé, H.M., Noori, T., Weinkove, D., Schuster, E., Greene, N.D.E., and Gems, D. (2013). Metformin retards aging in C. elegans by altering microbial folate and methionine metabolism. Cell *153*, 228–239.

Calnan, D.R., and Brunet, A. (2008). The FoxO code. Oncogene *27*, 2276–2288.

Calvert, S., Tacutu, R., Sharifi, S., Teixeira, R., Ghosh, P., and de Magalhães, J.P. (2016). A network pharmacology approach reveals new candidate caloric restriction mimetics in C. elegans. Aging Cell *15*, 256–266.

Campbell, J.M., Bellman, S.M., Stephenson, M.D., and Lisy, K. (2017). Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: A systematic review and meta-analysis. Ageing Res. Rev. *40*, 31–44.

Carretero, M., Gomez-Amaro, R.L., and Petrascheck, M. (2015). Pharmacological classes that extend lifespan of Caenorhabditis elegans. Front. Genet. *6*, 77.

Chiang, W.-C., Ching, T.-T., Lee, H.C., Mousigian, C., and Hsu, A.-L. (2012a). HSF-1 Regulators DDL-1/2 Link Insulin-like Signaling to Heat-Shock Responses and Modulation of Longevity. Cell *148*, 322–334.

Chiang, W.-C., Tishkoff, D.X., Yang, B., Wilson-Grady, J., Yu, X., Mazer, T., Eckersdorff, M., Gygi, S.P., Lombard, D.B., and Hsu, A.-L. (2012b). C. elegans SIRT6/7 Homolog SIR-2.4 Promotes DAF-16 Relocalization and Function during Stress. PLoS Genet. *8*, e1002948.

Churgin, M.A., Jung, S.-K., Yu, C.-C., Chen, X., Raizen, D.M., and Fang-Yen, C. (2017). Longitudinal imaging of Caenorhabditis elegans in a microfabricated device reveals variation in behavioral decline during aging. Elife *6*, 333–345.e4.

Curtis, R., O'Connor, G., and DiStefano, P.S. (2006). Aging networks in Caenorhabditis

elegans: AMP-activated protein kinase (aak-2) links multiple aging and metabolism pathways. Aging Cell *5*, 119–126.

Dickinson, D.J., and Goldstein, B. (2016). CRISPR-based methods for caenorhabditis elegans genome engineering. Genetics *202*, 885–901.

Dillin, A., and Cohen, E. (2011). Ageing and protein aggregation-mediated disorders: from invertebrates to mammals. Philos. Trans. R. Soc. B Biol. Sci. *366*, 94–98.

Dönertaş, H.M., Fuentealba Valenzuela, M., Partridge, L., and Thornton, J.M. (2018). Gene expression-based drug repurposing to target aging. Aging Cell.

Dowling, R.J.O., Zakikhani, M., Fantus, I.G., Pollak, M., and Sonenberg, N. (2007). Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res. *67*, 10804–10812.

Eijkelenboom, A., Mokry, M., de Wit, E., Smits, L.M., Polderman, P.E., van Triest, M.H., van Boxtel, R., Schulze, A., de Laat, W., Cuppen, E., et al. (2013). Genome-wide analysis of FOXO3 mediated transcription regulation through RNA polymerase II profiling. Mol. Syst. Biol. *9*, 638–638.

El-Mir, M.Y., Nogueira, V., Fontaine, E., Avéret, N., Rigoulet, M., and Leverve, X. (2000). Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J. Biol. Chem. *275*, 223–228.

Essers, P.B., Nonnekens, J., Goos, Y.J., Betist, M.C., Viester, M.D., Mossink, B., Lansu, N., Korswagen, H.C., Jelier, R., Brenkman, A.B., et al. (2015). A Long Noncoding RNA on the Ribosome Is Required for Lifespan Extension. Cell Rep. *10*, 339–345.

Fabris, F., Magalhães, J.P. de, and Freitas, A.A. (2017). A review of supervised machine learning applied to ageing research. Biogerontology *18*, 171–188.

Filtz, T.M., Vogel, W.K., and Leid, M. (2014). Regulation of transcription factor activity by interconnected post-translational modifications. Trends Pharmacol. Sci. *35*, 76–85.

Fontana, L., Partridge, L., and Longo, V.D. (2010). Extending healthy life span--from yeast to humans. Science *328*, 321–326.

Fortney, K., Morgen, E.K., Kotlyar, M., and Jurisica, I. (2012). In Silico Drug Screen in Mouse Liver Identifies Candidate Calorie Restriction Mimetics . Rejuvenation Res. *15*, 148–152.

Francis, L.K., Alsayed, Y., Leleu, X., Jia, X., Singha, U.K., Anderson, J., Timm, M., Ngo, H., Lu, G., Huston, A., et al. (2006). Combination mammalian target of rapamycin inhibitor rapamycin and HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin has synergistic activity in multiple myeloma. Clin. Cancer Res. *12*, 6826–6835.

Frankowski, H., Alavez, S., Spilman, P., Mark, K.A., Nelson, J.D., Mollahan, P., Rao, R. V., Chen, S.F., Lithgow, G.J., and Ellerby, H.M. (2013). Dimethyl sulfoxide and dimethyl formamide increase lifespan of C. elegans in liquid. Mech. Ageing Dev. *134*, 69–78.

Frydman, J. (2001). Folding of Newly Translated Proteins In Vivo: The Role of Molecular Chaperones. Annu. Rev. Biochem. *70*, 603–647.

Fuentealba, M., Dönertaş, H.M., Williams, R., Labbadia, J., Thornton, J.M., and Partridge, L. (2019). Using the drug-protein interactome to identify anti-ageing compounds for humans. PLoS Comput. Biol. *15*, e1006639.

Fuhrmann-Stroissnigg, H., Ling, Y.Y., Zhao, J., McGowan, S.J., Zhu, Y., Brooks, R.W., Grassi, D., Gregg, S.Q., Stripay, J.L., Dorronsoro, A., et al. (2017). Identification of HSP90 inhibitors as a novel class of senolytics. Nat. Commun. *8*, 422.

Goldman, D.P., Cutler, D., Rowe, J.W., Michaud, P.-C., Sullivan, J., Peneva, D., and Olshansky, S.J. (2013). Substantial Health And Economic Returns From Delayed Aging May Warrant A New Focus For Medical Research. Health Aff. *32*, 1698–1705.

Greer, E.L., and Brunet, A. (2009). Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in C. elegans. Aging Cell *8*, 113–127.

Greer, E.L., Dowlatshahi, D., Banko, M.R., Villen, J., Hoang, K., Blanchard, D., Gygi, S.P., and Brunet, A. (2007). An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. Curr. Biol. *17*, 1646–1656.

GTEx Consortium (2015). The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene regulation in humans. Science (80-.). *348*, 648–660.

Guralnik, J.M., Simonsick, E.M., Ferrucci, L., Glynn, R.J., Berkman, L.F., Blazer, D.G., Scherr, P.A., and Wallace, R.B. (1994). A Short Physical Performance Battery Assessing Lower Extremity Function: Association With Self-Reported Disability and Prediction of Mortality and Nursing Home Admission. J. Gerontol. *49*, M85–M94.

Gwinn, D.M., Shackelford, D.B., Egan, D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E., and Shaw, R.J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol. Cell *30*, 214–226.

De Haes, W., Frooninckx, L., Van Assche, R., Smolders, A., Depuydt, G., Billen, J., Braeckman, B.P., Schoofs, L., and Temmerman, L. (2014). Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. Proc. Natl. Acad. Sci. U. S. A. *111*, E2501-9.

Hahm, J.-H., Kim, S., DiLoreto, R., Shi, C., Lee, S.-J. V, Murphy, C.T., and Nam, H.G. (2015). C. elegans maximum velocity correlates with healthspan and is maintained in worms with an insulin receptor mutation. Nat. Commun. *6*, 8919.

Haigis, M.C., and Guarente, L.P. (2006). Mammalian sirtuins - Emerging roles in physiology, aging, and calorie restriction. Genes Dev. *20*, 2913–2921.

Hansen, M., and Kennedy, B.K. (2016). Does Longer Lifespan Mean Longer Healthspan? Trends Cell Biol. *26*, 565–568.

Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S.-J., and Kenyon, C. (2007). Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. Aging Cell *6*, 95–110.

Hansen, M., Rubinsztein, D.C., and Walker, D.W. (2018). Autophagy as a promoter of longevity: insights from model organisms. Nat. Rev. Mol. Cell Biol. *19*, 579–593.

Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., Nadon, N.L., Wilkinson, J.E., Frenkel, K., Carter, C.S., et al. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature *460*, 392–395.

Hawley, S.A., Pan, D.A., Mustard, K.J., Ross, L., Bain, J., Edelman, A.M., Frenguelli, B.G., and Hardie, D.G. (2005). Calmodulin-dependent protein kinase kinase-β is an alternative upstream kinase for AMP-activated protein kinase. Cell Metab. *2*, 9–19.

Henis-Korenblit, S., Zhang, P., Hansen, M., McCormick, M., Lee, S., Cary, M., and Kenyon,

C. (2010). Insulin/IGF-1 signaling mutants reprogram ER stress response regulators to promote longevity. Proc. Natl. Acad. Sci. U. S. A. *107*, 9730–9735.

Herndon, L., Schmeissner, P., Dudaronek, J., Brown, P., Listner, K., Sakano, Y., Paupard, M., Hall, D., and Driscoll, M. (2002). Stochastic and genetic factors influence tissue-specific decline in agening C. elegans. Nature *419*, 808–814.

Hosono, R., Nishimoto, S., and Kuno, S. (1989). Alterations of life span in the nematode Caenorhabditis elegans under monoxenic culture conditions. Exp. Gerontol. *24*, 251–264.

Houthoofd, K., Braeckman, B.P., Johnson, T.E., and Vanfletere, J.R. (2003). Life extension via dietary restriction is independent of the Ins/IGF-1 signalling pathway in Caenorhabditis elegans. Exp. Gerontol. *38*, 947–954.

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

Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kisielewski, A., Zhang, L.L., et al. (2003). Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature *425*, 191–196.

Hsin, H., and Kenyon, C. (1999). Signals from the reproductive system regulate the lifespan of C. elegans. Nature *399*, 362–366.

Hsu, A.-L. (2003). Regulation of Aging and Age-Related Disease by DAF-16 and Heat-Shock Factor. Science (80-.). *300*, 1142–1145.

Huang, C., Xiong, C., and Kornfeld, K. (2004). Measurements of age-related changes of physiological processes that predict lifespan of Caenorhabditis elegans. Proc. Natl. Acad. Sci. *101*, 8084–8089.

Hurley, R.L., Anderson, K.A., Franzone, J.M., Kemp, B.E., Means, A.R., and Witters, L.A. (2005). The Ca 2+ /Calmodulin-dependent Protein Kinase Kinases Are AMP-activated Protein Kinase Kinases. J. Biol. Chem. *280*, 29060–29066.

Jedrusik-Bode, M., Studencka, M., Smolka, C., Baumann, T., Schmidt, H., Kampf, J., Paap, F., Martin, S., Tazi, J., Muller, K.M., et al. (2013). The sirtuin SIRT6 regulates stress granule formation in C. elegans and mammals. J. Cell Sci. *126*, 5166–5177.

Kaeberlein, M., Mcvey, M., and Guarente, L. (1999). Saccharomyces cerevisiae by two different mechanisms promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev. *13*, 2570–2580.

Kaeberlein, T.L., Smith, E.D., Tsuchiya, M., Welton, K.L., Thomas, J.H., Fields, S., Kennedy, B.K., and Kaeberlein, M. (2006). Lifespan extension in Caenorhabditis elegans by complete removal of food. Aging Cell *5*, 487–494.

Kalender, A., Selvaraj, A., Kim, S.Y., Gulati, P., Brûlé, S., Viollet, B., Kemp, B.E., Bardeesy, N., Dennis, P., Schlager, J.J., et al. (2010). Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. Cell Metab. *11*, 390–401.

Kaletta, T., and Hengartner, M.O. (2006). Finding function in novel targets: C. elegans as a model organism. Nat. Rev. Drug Discov. *5*, 387–399.

Kamath, R. (2003). Genome-wide RNAi screening in Caenorhabditis elegans. Methods *30*, 313–321.

Kanfi, Y., Naiman, S., Amir, G., Peshti, V., Zinman, G., Nahum, L., Bar-Joseph, Z., and Cohen, H.Y. (2012). The sirtuin SIRT6 regulates lifespan in male mice. Nature *483*, 218–221.

Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., Epel, E.S., Franceschi, C., Lithgow, G.J., Morimoto, R.I., Pessin, J.E., et al. (2014). Geroscience: Linking aging to chronic disease. Cell *159*, 709–713.

Kenyon, C.J. (2010). The genetics of ageing. Nature *464*, 504–512.

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

Kim, S., Schilke, B., Craig, E.A., and Horwich, A.L. (2002). Folding in vivo of a newly translated yeast cytosolic enzyme is mediated by the SSA class of cytosolic yeast Hsp70 proteins. Proc. Natl. Acad. Sci. *95*, 12860–12865.

Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science *277*, 942–946.

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

Kops, G.J.P.L., and Burgering, B.M.T. (1999). Forkhead transcription factors: new insights into protein kinase B (c-akt) signaling. J. Mol. Med. *77*, 656–665.

Kumsta, C., Chang, J.T., Schmalz, J., and Hansen, M. (2017). Hormetic heat stress and HSF-1 induce autophagy to improve survival and proteostasis in C. elegans. Nat. Commun. *8*, 14337.

Kwon, E.-S., Narasimhan, S.D., Yen, K., and Tissenbaum, H.A. (2010). A new DAF-16 isoform regulates longevity. Nature *466*, 498–502.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., et al. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell *127*, 1109–1122.

Lai, C.H., Chou, C.Y., Ch'ang, L.Y., Liu, C.S., and Lin, W. (2000). Identification of novel human genes evolutionarily conserved in Caenorhabditis elegans by comparative proteomics. Genome Res. *10*, 703–713.

Lakowski, B., and Hekimi, S. (1998). The genetics of caloric restriction in Caenorhabditis elegans. Proc. Natl. Acad. Sci. *95*, 13091–13096.

Lamb, J. (2006). The Connectivity Map: Using Gene-Expression Signatures to Connect Small Molecules, Genes, and Disease. Science (80-.). *313*, 1929–1935.

Lamming, D.W., Ye, L., Katajisto, P., Goncalves, M.D., Saitoh, M., Stevens, D.M., Davis, J.G., Salmon, A.B., Richardson, A., Ahima, R.S., et al. (2012). Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science (80-.). *335*, 1638–1643.

Lapierre, L.R., De Magalhaes Filho, C.D., McQuary, P.R., Chu, C.-C., Visvikis, O., Chang, J.T., Gelino, S., Ong, B., Davis, A.E., Irazoqui, J.E., et al. (2013). The TFEB orthologue HLH-30 regulates autophagy and modulates longevity in Caenorhabditis elegans. Nat. Commun. *4*, 2267.

Lee, S.S. (2003). DAF-16 Target Genes That Control C. elegans Life-Span and Metabolism. Science (80-.). *300*, 644–647.

Lee, G.D., Wilson, M.A., Zhu, M., Wolkow, C.A., De Cabo, R., Ingram, D.K., and Zou, S. (2006). Dietary deprivation extends lifespan in Caenorhabditis elegans. Aging Cell *5*, 515–524.

Lenaerts, I., Walker, G.A., Van Hoorebeke, L., Gems, D., and Vanfleteren, J.R. (2008). Dietary Restriction of Caenorhabditis elegans by Axenic Culture Reflects Nutritional Requirement for Constituents Provided by Metabolically Active Microbes. Journals Gerontol. Ser. A Biol. Sci. Med. Sci. *63*, 242–252.

Lin, K., Dorman, J.B., Rodan, A., and Kenyon, C. (1997). daf-16: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. Science *278*, 1319–1322.

Lithgow, G.J., Driscoll, M., and Phillips, P. (2017). A long journey to reproducible results. Nature *548*, 387–388.

Long, X., Spycher, C., Han, Z.S., Rose, A.M., Müller, F., and Avruch, J. (2002). TOR Deficiency in C. elegans Causes Developmental Arrest and Intestinal Atrophy by Inhibition of mRNA Translation. Curr. Biol. *12*, 1448–1461.

López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. Cell *153*.

Lucanic, M., Plummer, W.T., Chen, E., Harke, J., Foulger, A.C., Onken, B., Coleman-Hulbert, A.L., Dumas, K.J., Guo, S., Johnson, E., et al. (2017). Impact of genetic background and experimental reproducibility on identifying chemical compounds with robust longevity effects. Nat. Commun. *8*, 1–13.

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

Martin-Montalvo, A., Mercken, E.M., Mitchell, S.J., Palacios, H.H., Mote, P.L., Scheibye-Knudsen, M., Gomes, A.P., Ward, T.M., Minor, R.K., Blouin, M.-J., et al. (2013). Metformin improves healthspan and lifespan in mice. Nat. Commun. *4*, 2192.

McCay, C.M., Crowell, M.F., and Maynard, L.A. (1935). The Effect of Retarded Growth Upon the Length of Life Span and Upon the Ultimate Body Size. J. Nutr. *10*, 63–79.

McElwee, J., Bubb, K., and Thomas, J.H. (2003). Transcriptional outputs of the Caenorhabditis elegans forkhead protein DAF-16. Aging Cell *2*, 111–121.

Milman, S., and Barzilai, N. (2016). Dissecting the Mechanisms Underlying Unusually Successful Human Health Span and Life Span. Cold Spring Harb. Perspect. Med. *6*, a025098.

Morselli, E., Maiuri, M.C., Markaki, M., Megalou, E., Pasparaki, A., Palikaras, K., Criollo, A., Galluzzi, L., Malik, S.A., Vitale, I., et al. (2010). Caloric restriction and resveratrol promote longevity through the Sirtuin-1-dependent induction of autophagy. Cell Death Dis. *1*, e10–e10.

Mouchiroud, L., Houtkooper, R.H., Moullan, N., Katsyuba, E., Ryu, D., Cantó, C., Mottis, A., Jo, Y.-S., Viswanathan, M., Schoonjans, K., et al. (2013). The NAD(+)/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling. Cell *154*, 430–441.

Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., and Kenyon, C. (2003). Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature *424*, 277–283.

Nadon, N.L., Strong, R., Miller, R.A., Nelson, J., Javors, M., Sharp, Z.D., Peralba, J.M., and Harrison, D.E. (2008). Design of aging intervention studies: the NIA interventions testing program. Age (Omaha). *30*, 187–199.

Nargund, A.M., Pellegrino, M.W., Fiorese, C.J., Baker, B.M., and Haynes, C.M. (2012). Mitochondrial Import Efficiency of ATFS-1 Regulates Mitochondrial UPR Activation. Science (80-.). *337*, 587–590.

Nussbaum-Krammer, C.I., Neto, M.F., Brielmann, R.M., Pedersen, J.S., and Morimoto, R.I. (2015). Investigating the Spreading and Toxicity of Prion-like Proteins Using the Metazoan Model Organism C. elegans. J. Vis. Exp. 1–15.

O'Reilly, L.P., Luke, C.J., Perlmutter, D.H., Silverman, G.A., and Pak, S.C. (2014). C. elegans in high-throughput drug discovery. Adv. Drug Deliv. Rev. *69–70*, 247–253.

O'Rourke, E.J., and Ruvkun, G. (2013). MXL-3 and HLH-30 transcriptionally link lipolysis and autophagy to nutrient availability. Nat. Cell Biol. *15*, 668–676.

Ocorr, K., Akasaka, T., and Bodmer, R. (2007). Age-related cardiac disease model of Drosophila. Mech. Ageing Dev. *128*, 112–116.

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A., and Ruvkun, G. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature *389*, 994–999.

Oh, S.W., Mukhopadhyay, A., Dixit, B.L., Raha, T., Green, M.R., and Tissenbaum, H.A. (2006). Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. Nat. Genet. *38*, 251–257.

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

Osborne, T.B., Mendel, L.B., and Ferry, E.L. (1917). The effect of retardation of growth upon the breeding period and duration of life of rats. Science (80-.). *45*, 294–295.

Owen, M.R., Doran, E., and Halestrap, A.P. (2000). Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem. J. *348 Pt 3*, 607–614.

Palmieri, M., Impey, S., Kang, H., di Ronza, A., Pelz, C., Sardiello, M., and Ballabio, A. (2011). Characterization of the CLEAR network reveals an integrated control of cellular clearance pathways. Hum. Mol. Genet. *20*, 3852–3866.

Palmieri, M., Pal, R., Nelvagal, H.R., Lotfi, P., Stinnett, G.R., Seymour, M.L., Chaudhury, A., Bajaj, L., Bondar, V. V., Bremner, L., et al. (2017). Corrigendum: mTORC1-independent TFEB activation via Akt inhibition promotes cellular clearance in neurodegenerative storage diseases. Nat. Commun. *8*, 15793.

Pan, K.Z., Palter, J.E., Rogers, A.N., Olsen, A., Chen, D., Lithgow, G.J., and Kapahi, P. (2007). Inhibition of mRNA translation extends lifespan in Caenorhabditis elegans. Aging Cell *6*, 111–119.

Panowski, S.H., Wolff, S., Aguilaniu, H., Durieux, J., and Dillin, A. (2007). PHA-4/Foxa

mediates diet-restriction-induced longevity of C. elegans. Nature *447*, 550–555.

Park, H.-E.H., Jung, Y., and Lee, S.-J. V (2017). Survival assays using Caenorhabditis elegans. Mol. Cells *40*, 90–99.

Peters, M.J., Joehanes, R., Pilling, L.C., Schurmann, C., Conneely, K.N., Powell, J., Reinmaa, E., Sutphin, G.L., Zhernakova, A., Schramm, K., et al. (2015). The transcriptional landscape of age in human peripheral blood. Nat. Commun. *6*, 8570.

Powers, R.W., Kaeberlein, M., Caldwell, S.D., Kennedy, B.K., and Fields, S. (2006). Extension of chronological life span in yeast by decreased TOR pathway signaling. Genes Dev. *20*, 174–184.

R.I., M. (2008). Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. Genes Dev. *22*, 1427–1438.

Riedel, C.G., Dowen, R.H., Lourenco, G.F., Kirienko, N. V, Heimbucher, T., West, J. a, Bowman, S.K., Kingston, R.E., Dillin, A., Asara, J.M., et al. (2013). DAF-16 employs the chromatin remodeller SWI/SNF to promote stress resistance and longevity. Nat. Cell Biol. *15*, 491–501.

Rine, J., and Herskowitz, I. (1987). Four genes responsible for a position effect on expression from HML and HMR in Saccharomyces cerevisiae. Genetics *116*, 9–22.

Rizki, G., Iwata, T.N., Li, J., Riedel, C.G., Picard, C.L., Jan, M., Murphy, C.T., and Lee, S.S. (2011). The Evolutionarily Conserved Longevity Determinants HCF-1 and SIR-2.1/SIRT1 Collaborate to Regulate DAF-16/FOXO. PLoS Genet. *7*, e1002235.

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

Roczniak-Ferguson, A., Petit, C.S., Froehlich, F., Qian, S., Ky, J., Angarola, B., Walther, T.C., and Ferguson, S.M. (2012). The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. Sci. Signal. *5*, ra42-ra42.

Rogina, B., and Helfand, S.L. (2004). Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc. Natl. Acad. Sci.

Rual, J.-F. (2004). Toward Improving Caenorhabditis elegans Phenome Mapping With an ORFeome-Based RNAi Library. Genome Res. *14*, 2162–2168.

Ryu, D., Mouchiroud, L., Andreux, P.A., Katsyuba, E., Moullan, N., Nicolet-Dit-Félix, A.A., Williams, E.G., Jha, P., Lo Sasso, G., Huzard, D., et al. (2016). Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents. Nat. Med. *22*, 879–888.

Schulte, T.W., Akinaga, S., Soga, S., Sullivan, W., Stensgard, B., Toft, D., and Neckers, L.M. (1998). Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. Cell Stress Chaperones *3*, 100.

Schumacher, R.J., Hurst, R., Sullivan, W.P., McMahon, N.J., Toft, D.O., and Matts, R.L. (1994). ATP-dependent chaperoning activity of reticulocyte lysate. J. Biol. Chem. *269*, 9493–9499.

Seah, N.E., de Magalhaes Filho, C.D., Petrashen, A.P., Henderson, H.R., Laguer, J., Gonzalez, J., Dillin, A., Hansen, M., and Lapierre, L.R. (2016). Autophagy-mediated

longevity is modulated by lipoprotein biogenesis. Autophagy *12*, 261–272.

Seo, K., Choi, E., Lee, D., Jeong, D.-E., Jang, S.K., and Lee, S.-J. (2013). Heat shock factor 1 mediates the longevity conferred by inhibition of TOR and insulin/IGF-1 signaling pathways in C. elegans. Aging Cell *12*, 1073–1081.

Settembre, C., and Ballabio, A. (2011). TFEB regulates autophagy: an integrated coordination of cellular degradation and recycling processes. Autophagy *7*, 1379–1381.

Settembre, C., Di Malta, C., Polito, V.A., Garcia Arencibia, M., Vetrini, F., Erdin, S., Erdin, S.U., Huynh, T., Medina, D., Colella, P., et al. (2011). TFEB links autophagy to lysosomal biogenesis. Science *332*, 1429–1433.

Settembre, C., De Cegli, R., Mansueto, G., Saha, P.K., Vetrini, F., Visvikis, O., Huynh, T., Carissimo, A., Palmer, D., Klisch, T.J., et al. (2013). TFEB controls cellular lipid metabolism through a starvation-induced autoregulatory loop. Nat. Cell Biol. *15*, 647–658.

Shaw, P.J. (2000). Correlates of Sleep and Waking in Drosophila melanogaster. Science (80-.). *287*, 1834–1837.

Shaw, P.J., Tononi, G., Greenspan, R.J., and Robinson, D.F. (2002). Stress response genes protect against lethal effects of sleep deprivation in Drosophila. Nature *417*, 287–291.

Somogyvári, M., Gecse, E., and Sőti, C. (2018). DAF-21/Hsp90 is required for C. elegans longevity by ensuring DAF-16/FOXO isoform A function. Sci. Rep. *8*, 12048.

Sood, S., Gallagher, I.J., Lunnon, K., Rullman, E., Keohane, A., Crossland, H., Phillips, B.E., Cederholm, T., Jensen, T., van Loon, L.J., et al. (2015). A novel multi-tissue RNA diagnostic of healthy ageing relates to cognitive health status. Genome Biol. *16*, 185.

Steinkraus, K.A., Smith, E.D., Davis, C., Carr, D., Pendergrass, W.R., Sutphin, G.L., Kennedy, B.K., and Kaeberlein, M. (2008). Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in Caenorhabditis elegans. Aging Cell *7*, 394–404.

Stroustrup, N., Ulmschneider, B.E., Nash, Z.M., López-Moyado, I.F., Apfeld, J., and Fontana, W. (2013). The Caenorhabditis elegans Lifespan Machine. Nat. Methods *10*, 665–670.

Szewczyk, N.J., Udranszky, I.A., Kozak, E., Sunga, J., Kim, S.K., Jacobson, L.A., and Conley, C.A. (2006). Delayed development and lifespan extension as features of metabolic lifestyle alteration in C. elegans under dietary restriction. J. Exp. Biol. *209*, 4129–4139.

Tao, L., Xie, Q., Ding, Y.H., Li, S.T., Peng, S., Zhang, Y.P., Tan, D., Yuan, Z., and Dong, M.Q. (2013). CAMKII and calcineurin regulate the lifespan of caenorhabditis elegans through the FOXO transcription factor DAF-16. Elife *2013*, 1–23.

Tatar, M. (2009). Can we develop genetically tractable models to assess healthspan (rather than life span) in animal models? Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. *64*, 161–163.

Taylor, R.C., and Dillin, A. (2013). XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. Cell *153*, 1435–1447.

The C. elegans Sequencing Consortium (1998). Genome Sequence of the Nematode C. elegans: A Platform for Investigating Biology. Science (80-.). *282*, 2012–2018.

Thompson, O., Edgley, M., Strasbourger, P., Flibotte, S., Ewing, B., Adair, R., Au, V., Chaudhry, I., Fernando, L., Hutter, H., et al. (2013). The million mutation project: A new approach to genetics in Caenorhabditis elegans. Genome Res. *23*, 1749–1762.

Timmers, S., Auwerx, J., and Schrauwen, P. (2012). The journey of resveratrol from yeast to human. Aging (Albany. NY). *4*, 146–158.

Tissenbaum, H.A., and Guarente, L. (2001). Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature *410*, 227–230.

Trepel, J., Mollapour, M., Giaccone, G., and Neckers, L. (2010). Targeting the dynamic HSP90 complex in cancer. Nat. Rev. Cancer *10*, 537–549.

Tukaj, S., and Węgrzyn, G. (2016). Anti-Hsp90 therapy in autoimmune and inflammatory diseases: a review of preclinical studies. Cell Stress Chaperones *21*, 213–218.

Tullet, J.M.A., Hertweck, M., An, J.H., Baker, J., Hwang, J.Y., Liu, S., Oliveira, R.P., Baumeister, R., and Blackwell, T.K. (2008). Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. Cell *132*, 1025–1038.

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

Viswanathan, M., and Guarente, L. (2011). Regulation of Caenorhabditis elegans lifespan by sir-2.1 transgenes. Nature *477*, E1-2.

Volovik, Y., Moll, L., Marques, F.C., Maman, M., Bejerano-Sagie, M., and Cohen, E. (2014). Differential Regulation of the Heat Shock Factor 1 and DAF-16 by Neuronal nhl-1 in the Nematode C. elegans. Cell Rep. *9*, 2192–2205.

Wagner, A.H., Coffman, A.C., Ainscough, B.J., Spies, N.C., Skidmore, Z.L., Campbell, K.M., Krysiak, K., Pan, D., McMichael, J.F., Eldred, J.M., et al. (2016). DGIdb 2.0: mining clinically relevant drug–gene interactions. Nucleic Acids Res. *44*, D1036–D1044.

Wang, Y., and Tissenbaum, H.A. (2006). Overlapping and distinct functions for a Caenorhabditis elegans SIR2 and DAF-16/FOXO. Mech. Ageing Dev. *127*, 48–56.

Wang, X., Wang, X., Li, L., and Wang, D. (2010). Lifespan extension in Caenorhabditis elegans by DMSO is dependent on sir-2.1 and daf-16. Biochem. Biophys. Res. Commun. *400*, 613–618.

Webb, A.E., Kundaje, A., and Brunet, A. (2016). Characterization of the direct targets of FOXO transcription factors throughout evolution. Aging Cell *15*, 673–685.

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

Westerheide, S.D., and Morimoto, R.I. (2005). Heat Shock Response Modulators as Therapeutic. J. Biol. Chem. *280*, 33097–33100.

Wolff, S., Ma, H., Burch, D., Maciel, G.A., Hunter, T., and Dillin, A. (2006). SMK-1, an Essential Regulator of DAF-16-Mediated Longevity. Cell *124*, 1039–1053.

Woods, A., Dickerson, K., Heath, R., Hong, S.-P., Momcilovic, M., Johnstone, S.R., Carlson, M., and Carling, D. (2005). Ca2+/calmodulin-dependent protein kinase kinase-β acts upstream of AMP-activated protein kinase in mammalian cells. Cell Metab. *2*, 21–33.

Wu, L., Zhou, B., Oshiro-Rapley, N., Li, M., Paulo, J.A., Webster, C.M., Mou, F., Kacergis, M.C., Talkowski, M.E., Carr, C.E., et al. (2016). An Ancient, Unified Mechanism for

Metformin Growth Inhibition in C. elegans and Cancer. Cell *167*, 1705–1718.e13.

Xian, B., Shen, J., Chen, W., Sun, N., Qiao, N., Jiang, D., Yu, T., Men, Y., Han, Z., Pang, Y., et al. (2013). WormFarm: A quantitative control and measurement device toward automated Caenorhabditis elegans aging analysis. Aging Cell *12*, 398–409.

Ye, X., Linton, J.M., Schork, N.J., Buck, L.B., and Petrascheck, M. (2014). A pharmacological network for lifespan extension in Caenorhabditis elegans. Aging Cell *13*, 206–215.

Zheng, S.Q., Ding, A.J., Li, G.P., Wu, G.S., and Luo, H.R. (2013). Drug Absorption Efficiency in Caenorhbditis elegans Delivered by Different Methods. PLoS One *8*, 1–9.