Chapter | 10 |

Comparative Genetics of Aging

George L. Sutphin[1,2], Matt Kaeberlein[1]

[1]Department of Pathology and [2]Molecular and Cellular Biology Program, University of Washington, Seattle, Washington, USA

CHAPTER CONTENTS

INTRODUCTION

Aging is a degenerative process affecting virtually all known organisms that is characterized by progressive deterioration of cellular components and deregulation of cellular processes, resulting in mortality. Investigation toward understanding the molecular processes and environmental factors that influence the rate of aging is a primary focus of research related to the basic biology of aging. The identification and characterization of genetic pathways that interact with these processes to modulate longevity is paramount to understanding why we age and will probably facilitate the development of therapeutic strategies for combating aging and age-related disease.

Life span is the primary endpoint to consider when studying aging; however, directly measuring life span in mammals is both difficult and expensive because of the relatively high longevity of most mammalian species. For this reason, research into mammalian aging has often been limited to looking at secondary endpoints that tend to correlate with longevity, such as stress-resistance and metabolic parameters. The cost and labor associated with maintaining relatively large cohorts of long-lived animals in a laboratory environment is particularly prohibitive to large-scale approaches looking for longevity phenotypes in mammals, such as high-throughput genetic or chemical screens. An alternative approach has been to develop model systems with characteristics that lend themselves to the types of studies common in aging research. These characteristics include short life span, rapid reproduction resulting in a large number of offspring, ease of maintenance in the laboratory environment, well-characterized biology including fully sequenced genomes, and availability of powerful tools for genetic manipulation. Three

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nonmammalian organisms have emerged as particularly prominent models of aging: the budding yeast *Saccharomyces cerevisiae,* the nematode *Caenorhabditis elegans,* and the fruit fly *Drosophila melanogaster.*

We are ultimately interested in interventions that can be applied to reduce mortality and fight age-related disease in humans. The use of nonmammalian models in aging-related research raises an important question: are findings in relatively simple eukaryotes applicable to human aging? From a genetic standpoint the relevant question is whether genetic pathways that play a role in controlling aging and longevity in one species are common among evolutionarily divergent species or unique to that particular lineage. If genetic mechanisms can be identified that modulate life span across evolutionarily divergent simple eukaryotes, it is reasonable to expect that at least a subset of these mechanisms will be conserved in humans (Kaeberlein, 2004).

The best-studied example of a conserved longevity intervention is dietary restriction (DR), which has been defined as a reduction in food consumption in the absence of malnutrition (Kennedy et al., 2007; Masoro, 2005; Spindler, 2009; Weindruch & Walford, 1988). DR has long been known to increase life span in many different species, including yeast, worms, flies, and rodents. DR has also been reported to increase life span and health span in a nonhuman primate, the rhesus macaque, with the caveat that significance was achieved only when more than two-thirds of the deaths were censored as non-age-related (Colman et al., 2009). Several factors have been proposed to contribute to the health and longevity benefits of DR. To date, while DR is effective at extending life span in a variety of species, it remains unknown whether DR acts via similar mechanisms in different species (discussed further below), let alone whether DR can significantly improve longevity or health span in humans.

The rationale that conserved longevity interventions are more likely to be relevant for human health has spurred the hunt for "conserved aging genes," which for the purposes of this chapter will be defined as genes that function to modulate aging in multiple evolutionarily divergent species (Kaeberlein, 2004). Work from several groups has led to the identification of more than two dozen conserved aging genes, and comparative genetic analyses are beginning to place these genes into known aging pathways (Table 10.1). In this chapter we describe the current state of knowledge in this area. We first discuss how aging is studied in each of the common invertebrate model systems and then describe major classes of conserved aging genes. We also describe how genome-scale longevity studies in yeast and nematodes have accelerated progress in the comparative genetics of aging and are leading to mechanistic insights for how these genes may be influencing longevity across multiple species. Finally, we present an overview of the complex relationships between known conserved pathways that influence aging and how they interact with the response to environmental nutrients.

COMMON NONMAMMALIAN MODELS OF AGING

Aging has been studied in a wide variety of model organisms, both mammalian and nonmammalian. Among nonmammalian model systems, three species are widely used in aging-related studies: *S. cerevisiae, C. elegans,* and *D. melanogaster.* Several other nonmammalian organisms are also actively being used to study aging on a smaller scale, including bacteria (Ackermann et al., 2003; Nystrom, 2007; Stewart et al., 2005), fission yeast (Barker & Walmsley, 1999; Roux et al., 2006), other nematode and fly species (Carey et al., 2002; Davies et al., 2005; Sutphin & Kaeberlein, 2008), and fish (Terzibasi et al., 2007; Valenzano et al., 2006). For the purposes of this chapter, we focus primarily on comparative genetics of aging in the three most common models.

S. cerevisiae

The budding yeast *S. cerevisiae* has been used as a model organism for aging research for more than 50 years (Mortimer & Johnston, 1959). Two distinct paradigms have been defined for yeast aging: chronological and replicative. Chronological life span refers to the length of time that a yeast cell can retain viability in a nondividing state, while replicative life span refers to the number of viable daughter cells produced by a mother cell during vegetative growth (Fabrizio et al., 2001; Kaeberlein, 2006; Mortimer & Johnston, 1959).

Yeast Replicative Aging

Replicative life span is the older of the two yeast aging models and has been studied in greater detail. To date, nearly 100 genes are reported to modulate yeast replicative aging (Bitterman et al., 2003; Jazwinski, 2000; Steinkraus et al., 2008). Replicative life span is measured by microdissection of daughter cells away from mother cells while tallying the number of daughters produced at each age point. Replicative longevity varies widely among different laboratory strains, with the most strains having an average replicative life span between 18 and 26 generations (Kaeberlein, 2006). The most extensively studied yeast strains are the parental strains of the yeast open reading frame (ORF) deletion collection, which are closely related to the *S. cerevisiae* wild-type strain S288C (Kaeberlein et al., 2005a; Mortimer & Johnston, 1986). This collection has been used for genome-wide screens for single-gene

Table 10.1 Conserved aging genes

LONGEVITY PATHWAY	KNOWN OR PREDICTED PROTEIN FUNCTION	ENCODING GENE				REFERENCES
		YEAST	WORMS	FLIES	MICE	
Insulin/IGF-1-like signaling	AKT/protein kinase B	*SCH9*[a]	*akt-1, akt-2*[b]			Fabrizio et al., 2001; Hamilton et al., 2005; Hertweck et al., 2004
	FoxO family transcription factor	n/a	*daf-16*	*dFOXO*		Henderson & Johnson, 2001; Giannakou et al., 2004; Hwangbo et al., 2004
	Insulin receptor substrate (IRS)	n/a		*Chico*	*Irs1, Irs2*	Clancy et al., 2001; Selman et al., 2008; Taguchi et al., 2007
	Insulin/IGF-1-like receptor	n/a	*daf-2*	*InR*	*Insr, Igf1r*	Kenyon et al., 1993; Tatar et ssal., 2001; Bluher et al., 2003; Holzenberger et al., 2003
	Phosphoinositide 3-kinase (PI3K)	n/a	*age-1, aap-1*		*PI3K*$_\gamma$	Klass, 1983; Dorman et al., 1995; Wolkow et al., 2002; Barber et al., 2006
Sirtuins	Histone Deacetylase	*SIR2*	*sir-2.1*	*dSir2*		Kaeberlein et al., 1999; Tissenbaum & Guarente, 2001; Rogina & Helfand, 2004
	Histone Deacetylase	*RPD3*		*Rpd3*		Kim et al., 1999; Rogina et al., 2002
mRNA translation/TOR signaling	Large subunit ribosomal protein	*RPL19A*	*rpl-19*			Smith et al., 2008a,b; Hansen et al., 2007
	Large subunit ribosomal protein	*RPL6B*	*rpl-6*			Smith et al., 2008a,b; Hansen et al., 2007
	Large subunit ribosomal protein	*RPL9A*	*rpl-9*			Smith et al., 2008a,b; Hansen et al., 2007
	S6 kinase	*SCH9*[a]	*rsks-1*	*dS6K*	*S6K1*	Fabrizio et al., 2001, 2004; Urban et al., 2007; Hansen et al., 2007; Pan et al., 2007; Kapahi et al., 2004; Selman et al., 2009
	Small subunit ribosomal protein	*RPS6B*	*rps-6*			Kaeberlein et al., 2005a,b,c; Hansen et al., 2007

(*Continued*)

Table 10.1 (Continued)

LONGEVITY PATHWAY	KNOWN OR PREDICTED PROTEIN FUNCTION	ENCODING GENE				REFERENCES
		YEAST	WORMS	FLIES	MICE	
	Target of rapamycin kinase	*TOR1*	*let-363*	*dTOR*	*mTOR*[c]	Kaeberlein et al., 2005a,b,c; Powers et al., 2006; Vellai et al., 2003; Kapahi et al., 2004; Harrison et al., 2009
	Translation initiation factor	*TIF1, TIF2*	*inf-1*			Smith et al., 2008a,b; Curran & Ruvkun, 2007
	Translation initiation factor	*TIF4631*	*ifg-1*			Smith et al., 2008a,b; Henderson et al., 2006; Curran & Ruvkun, 2007; Pan et al., 2007
Stress resistance	Catalase			*Cat*	*Cat*[d]	Orr & Sohal, 1994; Schriner et al., 2005
	Heat shock protein		*hsp-6*	*Hsp70*		Yokoyama et al., 2002; Tatar et al., 1997
	Superoxide dismutase	*SOD1*		*Sod1*		Fabrizio et al., 2003; Orr & Sohal, 1994
Unknown	3-Phosphoinositide-dependent kinase	*PKH2*	*pdk-1*			Smith et al., 2008a,b; Paradis et al., 1999
	α-Mannosyltransferase	*ALG12*	*T27F7.3*			Smith et al., 2008a,b; Curran & Ruvkun, 2007
	Ammonium transporter	*MEP1, MEP2*	*amt-2*			Powers et al., 2006; Kim & Sun, 2007
	CCCH-type Zn-finger protein	*TIS11*	*pos-1*			Smith et al., 2008a,b; Curran & Ruvkun, 2007
	Ceramide synthase component	*LAG1*	*hyl-1*			D'Mello et al., 1994; Tedesco et al., 2008; Menuz et al., 2009
	Coenzyme Q7 homolog		*clk-1*		*Coq7*	Lakowski & Hekimi, 1998; Liu et al., 2005
	Cytoskeletal linker protein	*YGR130C*	*erm-1*			Smith et al., 2008a,b; Curran & Ruvkun, 2007
	DEAD-box helicase	*DBP3*	*B0511.6*			Smith et al., 2008a,b; Curran & Ruvkun, 2007

Table 10.1 (Continued)

LONGEVITY PATHWAY	KNOWN OR PREDICTED PROTEIN FUNCTION	ENCODING GENE				REFERENCES
		YEAST	WORMS	FLIES	MICE	
	Dehydrogenase	*ADH1*	*W09H1.5*			Smith et al., 2008a,b; Hamilton et al., 2005
	Endosomal complex adaptor protein	*HSE1*	*sem-5*			Smith et al., 2008a,b; Curran & Ruvkun, 2007
	G protein, α subunit	*GPA2*	*gpa-1, gpa-5, odr-3*			Lin et al., 2000; Lans & Jansen, 2007
	Golgi membrane ATPase ion transporter	*PMR1*	*eat-6*			Smith et al., 2008a,b; Lakowski & Hekimi, 1998
	Isocitrate dehydrogenase	*IDH1, IDH2*	*F43G9.1*			Smith et al., 2008a,b; Hamilton et al., 2005
	Metalloprotease	*AFG3*	*spg-7*			Smith et al., 2008a,b; Curran & Ruvkun, 2007
	Methionine sulfoxide reductase A	*MXR1*		*Eip71CD*		Koc et al., 2004; Ruan et al., 2002
	Polyphosphoinositide phosphatase	*INP51, INP53*	*unc-26*			Smith et al., 2008a,b; Lakowski & Hekimi, 1998
	Protein phosphatase regulatory subunit	*SIS2*	*Y46H3C.6*			Smith et al., 2008a,b; Hamilton et al., 2005
	RAB-family GTPase	*YPT6*	*rab-10*			Smith et al., 2008a,b; Hansen et al., 2005
	S-adenosylmethionine synthetase	*SAM1*	*sams-3*			Smith et al., 2008a,b; Curran & Ruvkun, 2007
	Surfeit gene 1			*Surf1*	*Surf1*	Zordan et al., 2006; Dell'Agnello et al., 2007
	Thioredoxin	*TrxT*			*Txn1*[d]	Umeda-Kameyama et al., 2007; Mitsui et al., 2002
	Transcription elongation factor	*SPT4*	*spt-4*			Smith et al., 2008a,b; Hamilton et al., 2005

Homologous genes for which altered expression or activity is reported to extend life span in two or more evolutionarily divergent organisms are shown.
[a]*SCH9* has been suggested as a yeast homolog to both mammalian Akt/PKB and mammalian S6K and shows S6K activity.
[b]*akt-1(RNAi) akt-2(ok393)* is longer lived than wild type, *akt-1(ok525)*, and *akt-2(ok393)*.
[c]Predicted based on life-span extension from treatment with the TOR inhibitor rapamycin.
[d]Mouse life-span extension shown by overexpressing the human version of the gene.

deletions that increase either chronological life span or replicative life span (Kaeberlein et al., 2005c; Powers et al., 2006) (described in more detail below). Environmental parameters such as temperature and medium composition are also known to influence replicative life span. One primary molecular cause of replicative aging in yeast is thought to be the mother-cell-specific accumulation of extrachromosomal rDNA circles (ERCs) (Defossez et al., 1999; Sinclair & Guarente, 1997), although additional uncharacterized factors are also known to contribute to replicative aging. Evidence suggests that these factors may include age-associated genomic instability, mitochondrial retrograde signaling, accumulation of oxidatively damaged proteins in the mother cell, and altered histone acetylation near telomeres (Aguilaniu et al., 2003; Dang et al., 2009; Kaeberlein et al., 1999; Kirchman et al., 1999; McMurray & Gottschling, 2003).

DR has been studied in the context of replicative life span by reducing either the amino acid or, more commonly, the glucose content of the growth medium (Jiang et al., 2000; Lin et al., 2000). Replicative life span extension has been reported at multiple glucose concentrations ranging from 0.5 to 0.005% glucose (M. Kaeberlein et al., 2006; Lin et al., 2002) compared to the standard concentration in yeast medium of 2%. The glucose concentration at which replicative life span is maximally extended is dependent on the genetic background of the strain, and there has been substantial debate regarding whether the mechanism by which DR extends life span is similar at differing glucose concentrations (Kaeberlein & Powers, 2007).

Yeast Chronological Aging

Chronological aging has typically been measured by culturing cells into a postdiauxic quiescent-like state in synthetically defined growth medium and monitoring the viability of cells over time, where viability is defined by the ability of cells to reenter the cell cycle and resume vegetative growth in the presence of a nutrient-rich medium (Fabrizio & Longo, 2003). Alternative growth conditions for monitoring chronological aging have been described but not widely used, including maintaining cells in water at a high temperature after 2–3 days of standard culture and aging cells in rich growth medium rather than synthetic defined medium (Harris et al., 2001; Piper et al., 2006). Like replicative life span, chronological life span varies among different laboratory strains and is robustly influenced by the composition of the growth medium (Fabrizio et al., 2005; Murakami et al., 2008; Smith et al., 2007). Chronological senescence is correlated with an accumulation of oxidatively damaged proteins, mitochondrial dysfunction, and induction of the yeast apoptotic-like response (Aerts et al., 2009; Fabrizio et al., 2003; Herker et al., 2004). Recently, acetic acid toxicity associated with acidification of the growth medium has been identified as a primary molecular cause of chronological senescence under standard conditions (Burtner et al., 2009). Although the molecular causes of chronological senescence appear to be distinct from replicative senescence, chronologically aged cells show a reduced replicative life span, suggesting that underlying similarities may exist (Ashrafi et al., 1999).

Similar to the case for replicative aging, life-span extension from DR in the yeast chronological aging paradigm can be accomplished by reducing the glucose concentration of the growth medium from 2 to 0.5% or lower (Murakami et al., 2008). A form of extreme DR has also been described in which cells are transferred from expired growth medium to water after 2–4 days of aging (Fabrizio et al., 2005).

C. elegans

C. elegans has arguably become the most informative model organism for genetic studies of basic mechanisms of aging. When measured under standard conditions (20°C on solid nematode growth medium), the life span of the common lab strain (N2) is about 3 weeks. *C. elegans* are typically fed a diet of *Escherichia coli* OP50 bacteria grown as a lawn on the surface of the agar plate and viability is determined by the ability of adult animals to move in response to touch (Sutphin & Kaeberlein, 2009). The *C. elegans* life cycle takes around 3 days and consists of externally laid eggs, four larval stages, and a reproductively active adult stage. The majority of adult animals are hermaphrodites and self-fertilize to produce several hundred offspring per individual. Rare male worms arise spontaneously and mate with hermaphrodites to produce broods that are half male and half hermaphrodite. Cells in adult animals are postmitotic with the exception of the germ line.

Studies in *C. elegans* have identified more than 300 genes that are associated with increased life span when their function is diminished (Braeckman & Vanfleteren, 2007; Smith et al., 2008b). Most of these genes were identified from large-scale RNA interference (RNAi) screens carried out using libraries that cover roughly 90% of the known ORFs in the nematode genome (Arum & Johnson, 2007; Chen et al., 2007; Curran & Ruvkun, 2007; Dillin et al., 2002; Hamilton et al., 2005; Hansen et al., 2005; Lee et al., 2003). RNAi is particularly powerful in *C. elegans,* as efficient gene knockdown can be achieved by simply feeding animals bacteria expressing double-stranded RNA with sequence corresponding to the gene of interest. Many of the currently known *C. elegans* aging genes can be broadly classified based on epistasis grouping and known or predicted function into one or more of the following classes: (1) insulin/IGF-1-like signaling, (2) mitochondrial function, (3) protein synthesis/mRNA translation, (4) chemosensory function, (5) dietary restriction, or (6) hypoxic response.

The molecular mechanisms that cause *C. elegans* to age are not known, but analysis of tissue-specific aging has led to the conclusion that neuronal cells largely retain function in old animals, while muscle cells in many animals show a gradual decline in function beginning near the transition to the postreproductive stage of adulthood (Herndon et al., 2002). Associated with this general decline in muscle function is a decrease in pharyngeal pumping, resulting in reduced food consumption (Huang et al., 2004; Kenyon et al., 1993; Smith et al., 2008a), and an accumulation of autofluorescent age pigment throughout the body (Gerstbrein et al., 2005; Klass, 1977). If a live food source is used, bacterial colonization of the gut can also contribute to senescence; however, the relevance of this to normal aging is unclear, as animals fed a killed bacterial food source show a similar progression of age-associated phenotypes with life span extended by only a few days (Garigan et al., 2002; Garsin et al., 2003).

DR in *C. elegans* has been studied using a variety of methods and there is currently little consensus regarding which methods are most appropriate (Greer & Brunet, 2009; Mair et al., 2009). Most methods of DR in *C. elegans* involve reducing the amount of bacterial food provided to the worms, but differ in whether the food is alive or killed, whether the growth environment is solid agar-based or liquid, and whether the amount of food is constant or varied (akin to feeding/fasting cycles in rodents) over the course of the experiment (Greer & Brunet, 2009). Under at least some conditions on agar-based medium, complete removal of the bacterial food during adulthood has been observed to increase life span maximally, a DR regimen referred to as bacterial deprivation (T. L. Kaeberlein et al., 2006; Lee et al., 2006). Age at onset of DR also varies from study to study and may influence the resulting life span; however, at least in the case of bacterial deprivation, similar median and maximal life-span extension has been demonstrated for DR initiated between day 4 and day 14, with similar maximal life-span extension achieved for DR initiated as late as day 24 (Smith et al., 2008a).

Drosophila

The fruit fly *D. melanogaster* is the earliest invertebrate player in aging research, with studies of life span dating back to 1916 (Loeb & Northrop, 1916). The fly life cycle lasts 1 to 2 weeks and consists of three easily distinguishable growth stages (embryo, larva, and pupae) followed by the reproductively active adult stage. Similar to *C. elegans,* the majority of the cells in the adult fruit fly are postmitotic, with exceptions in the germ line and a subset of gut cells. Flies are typically maintained in vials with a cornmeal–sugar–yeast or sugar–yeast agar-based food source. Unlike yeast and worms, flies cannot be frozen and must be actively maintained. Wild-type *D. melanogaster* has a median life span between 1 and 2 months when maintained at 25°C.

The fruit fly has been used extensively to explore nongenetic environmental manipulations that extend life span. DR can be accomplished by diluting yeast or other components in the food source (Bass et al., 2007a; Chapman & Partridge, 1996; Good & Tatar, 2001). Fruit flies also experience a strong inverse relationship between environmental temperature and life span (Helfand & Rogina, 2003; Miquel et al., 1976), and brief exposure to mild stressors such as high temperature or low-level radiation can result in increased life span (Hercus et al., 2003; Le Bourg et al., 2004; Vaiserman et al., 2003). Flies generally have a strong inverse correlation between reproduction and longevity. Strains bred for longevity by selecting offspring from late life reproduction show reduced egg laying early in life relative to ancestral strains (Luckinbill et al., 1984; Rose, 1984). In *Drosophila subobscura,* life-span extension observed in response to DR is accompanied by a reduction in egg production (Marden et al., 2003). Preventing mating can also double female life span (Smith, 1958), though, in *D. melanogaster,* seminal factors have been implicated in shortening female life span as opposed to some intrinsic cost associated with reproduction (Ueyama & Fuyama, 2003).

As a model system, *Drosophila* offers a variety of powerful genetic techniques for studying aging at a genetic level. While high-throughput methodology for studying life span has yet to be employed, gene- and pathway-specific approaches as well as smaller-scale candidate gene and random mutation studies have been useful in testing a variety of aging theories and in identifying new players in fly aging. *Drosophila* genes that play a role in modifying aging have been identified in a variety of pathways, including insulin/IGF-1-like signaling, mitochondrial function, oxidative stress resistance, sirtuins, and TOR signaling.

CONSERVED LONGEVITY INTERVENTIONS

The growing body of aging research using multiple divergent species has led to the discovery and characterization of several aspects of longevity control that have been evolutionarily conserved, of which DR is the most studied. Three (at least partially) distinct genetic pathways have been found to modulate aging in evolutionarily divergent organisms: insulin/IGF-1-like signaling (IIS), sirtuins, and the nutrient-responsive target of rapamycin (TOR) kinase. Each of these genetic pathways has also been proposed to play a role in life-span extension from DR. This section describes the current state of knowledge surrounding DR and each of these conserved longevity pathways with respect to aging. The relationship between each pathway and DR is

discussed in a later section, and additional discussion of DR is provided in Chapters 1, 9, and 21. Additional details regarding TOR signaling are provided in Chapter 9, and sirtuins in Chapter 11.

Dietary Restriction

The effects of DR on longevity are clearly shared among diverse organisms, but it remains an open question as to whether the underlying molecular mechanisms are also shared. Several hypotheses for how DR might mediate a reduced rate of aging have been proposed, including reduced inflammation, reduced damage from reactive oxygen species, improved glucose homeostasis, and enhanced resistance to a variety of stresses (Spindler, 2009). To date none of these hypotheses has been definitively shown to play a primary role in mediating the effects of DR. In addition to enhanced longevity and reduced age-associated disease, two DR-associated phenotypes that seem to be shared between different organisms are a reduction in reproductive rate and an increase in broad-spectrum stress resistance. This observation has led to the hypothesis that life-span extension in response to DR is an evolutionarily conserved mechanism for maintaining reproductive potential in response to transient environmental fluctuations in food availability (Harrison & Archer, 1988; Holliday, 1989).

One unresolved question regarding DR is whether the longevity and health benefits are solely due to reduced caloric consumption, as was initially assumed, or whether other dietary factors may also be involved. In support of a more general view of DR, simply restricting the dietary abundance of methionine in both mice and rats is sufficient to increase life span (Miller, et al., 2005; Orentreich et al., 1993). Similar observations have been made with respect to tryptophan in rats (Ooka et al., 1988; Segall & Timiras, 1976; Timiras et al., 1984). An alternative way to interpret these results is that the standard laboratory mouse diet does not contain an ideal balance of amino acids and that a subset, including methionine and tryptophan, is overly abundant. Indeed, Masoro et al. (1989) found that methionine restriction did not contribute to the life-span extension resulting from a 40% decrease in food intake in rats. The most convincing evidence for a model of DR that is not limited to restriction of caloric intake is the finding that food sensing can modulate longevity independent of food consumption in both nematodes and flies (Libert & Pletcher, 2007; Smith et al., 2008a). Whether food sensing modulates longevity in mammals is unknown.

Genetic Manipulation of Insulin/IGF-1-like Signaling

Among multicellular eukaryotes, IIS pathways mediate growth, stress resistance, and longevity in response to environmental conditions. The longevity-related IIS pathways share a core set of similar features in divergent organisms, including insulin-like molecules, one or more insulin/IGF-1-like receptors, a phosphatidylinositol 3-kinase (PI3K), an Akt kinase, and a FoxO-family transcription factor (Figure 10.1). Downstream genetic targets of IIS are regulated by controlling nuclear localization of the FoxO-family transcription factor, with increased IIS resulting in decreased transcription factor activity.

Worms and flies each possess a single IIS receptor that mediates signals from multiple insulin-like ligands (at least 30 in worms and 8 in flies; Bartke, 2008; Toivonen & Partridge, 2008). The *C. elegans* insulin/IGF-1-like receptor, PI3K, Akt kinase, and FoxO-family transcription factor are encoded by *daf-2*, *age-1*, *akt-1/2*, and *daf-16*, respectively (Kimura et al., 1997; Lin et al., 1997; Morris et al., 1996; Ogg et al., 1997). Worms with reduced IIS caused by mutations that decrease activity of either *daf-2* or *age-1* have a life span increased in a nonadditive, *daf-16*-dependent manner (Dorman et al., 1995; Kenyon et al., 1993). Life-span extension by reduced IIS therefore requires DAF-16, which regulates a diverse set of processes including fat storage, metabolism, development, fertility, and resistance to heat and oxidative stress (Finch & Ruvkun, 2001; Gems et al., 1998; Larsen, 1993). Extension of life span via mutation of *daf-2* also requires AAK-2, the catalytic subunit of the adenosine monophosphate-activated protein (AMP) kinase, and overexpression of *aak-2* is sufficient to increase life span (Apfeld et al., 2004).

Reduced IIS is thought to increase life span in worms, at least in part, by upregulating stress-response proteins. Long-lived worms with reduced IIS are resistant to multiple forms of environmental stress including reactive oxygen species, exposure to UV, and increased temperature (Martin et al., 1996; Murakami & Johnson, 1996). Transient heat shock is sufficient to extend life span (Butov et al., 2001; Lithgow et al., 1995; Michalski et al., 2001; Yashin et al., 2002) and causes nuclear localization of DAF-16 (Henderson & Johnson, 2001; Lin et al., 2001). Overexpression of the *C. elegans* heat-shock factor-1 (HSF-1) is sufficient to increase life span, and deletion of the *hsf*-1 blocks life-span extension from *daf-2* knockdown (Hsu et al., 2003; Morley & Morimoto, 2004). HSF-1 also activates multiple longevity genes including several that encode small heat-shock proteins (Hsu et al., 2003). Reducing IIS probably does not optimally activate the heat-shock response for life-span extension, however, as heat shock produces a further increase in life span and upregulation of small heat-shock protein genes in long-lived *age-1* mutants (Walker et al., 2001).

In addition to their role in aging, *daf-2* and *daf-16* regulate entry into the dauer larval stage, a long-lived alternate development pathway. Dauer larvae are sexually immature and characterized by a thick cuticle,

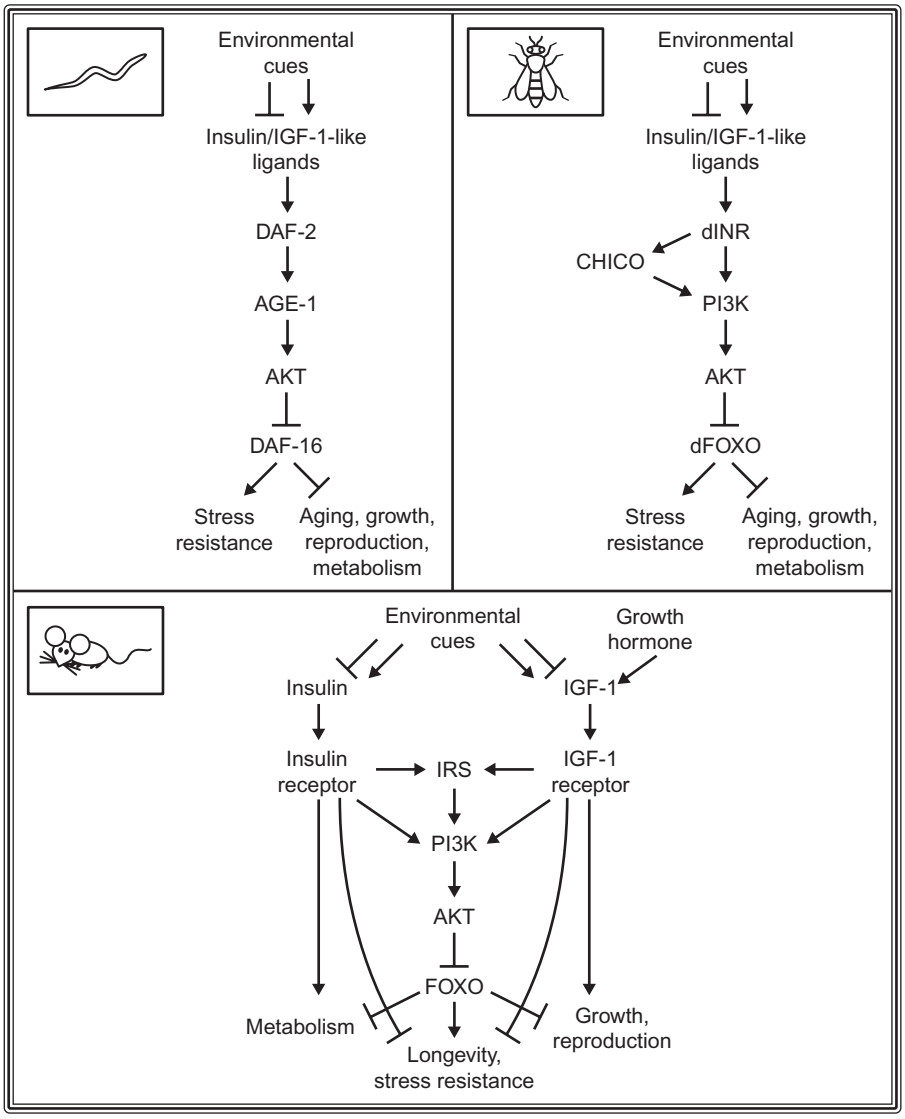

Figure 10.1 Insulin and IGF-1-like signaling pathways play a conserved role in aging in nematodes, flies, and mice.

constricted pharynx, and sealed buccal and anal cavities, resulting in an inability to eat or defecate and an increased resistance to environmental stresses such as harsh chemical treatment and desiccation (Cassada & Russell, 1975; Riddle, 1988). At least three environmental factors contribute to the decision to enter the dauer larval stage: population density, temperature, and food availability (Golden & Riddle, 1982, 1984). Dauer larvae exposed to favorable environmental conditions (i.e., low population density, reduced temperature, and abundant food) resume development and proceed to become reproductively active adults. Complete inhibition of *daf-2* results in constitutive entry into the dauer larva stage regardless of environmental signals, and worms with mutations in *daf-16* fail to enter the dauer larva stage or do so inefficiently (Gottlieb & Ruvkun, 1994). As might be expected, dauer larvae share many similarities with worms that have reduced (but not abolished) IIS, including enhanced longevity and stress resistance, suggesting that the benefits of reduced IIS may represent an adult dauer-like state and may potentially be subject to the same trade-offs, such as reduced reproduction. Notably, the dauer response can be decoupled from the prolongevity effects of reduced IIS, as RNAi knockdown of *daf-2* starting well into adulthood—even postreproductively—dramatically increases life span without altering development or influencing reproductive potential (Dillin et al., 2002; Smith et al., 2008a).

Similar to worms, reduction of IIS signaling in flies via mutations in *InR*, the gene encoding the insulin/IGF-1-like receptor, or *Chico*, the gene encoding the insulin receptor substrate (IRS), increases stress resistance and longevity (Clancy et al., 2001; Tatar et al., 2001; Tu et al., 2002). The influence of IIS on life span appears to be partially gender specific in flies, as mutation of *InR* extends only the female life span (Tatar et al., 2001). In the *Chico* mutants, both heterozygous and homozygous female flies displayed increased life span, whereas only heterozygous males displayed increased life span, relative to wild type (Clancy et al., 2001; Tu et al., 2002). This is in contrast to worms, in which mutation of *daf-2* increases hermaphrodite and male life spans to similar degrees (Gems & Riddle, 2000). Transient heat shock also extends fly life span (Hercus et al., 2003). Furthermore, partial genetic ablation of the median neurosecretory cells (MNCs) both reduces expression of MNC-specific *dilp* (*Drosophila* insulin-like peptide) genes and increases life span (Broughton et al., 2005). This also suggests that the role of IIS in aging is cell nonautonomous. While it is not currently known whether these phenotypes are dependent on dFOXO, the *D. melanogaster* FoxO-family transcription factor (Junger et al., 2003; Kramer et al., 2003; Puig et al., 2003), there is some evidence pointing in that direction. Reduced cell division caused by mutations that decrease IIS require dFOXO (Junger et al., 2003), and adult fat-body-specific overexpression of dFOXO is sufficient to extend life span (Giannakou et al., 2004, 2007; Hwangbo et al., 2004).

Unlike invertebrates, mammalian IIS involves only three insulin-like peptides (insulin, IGF-1, and IGF-2), but three receptor peptides (one insulin receptor and two IGF-1 receptors) dimerize to form five types of dimeric receptors. These include separate receptors for insulin and IGF-1 ligands (Taguchi & White, 2008), both of which appear to play a role in determining life span. Female mice with a heterozygous IGF-1 receptor knockout live ~30% longer than wild-type mice (Holzenberger et al., 2003), while both male and female fat-specific insulin receptor knockout mice with an adipose-specific insulin receptor knockout live ~18% longer than wild type (Bluher et al., 2003). Growth hormone, which is not present in invertebrates, also appears to interact with IIS in modulating life span in mice. IGF-1 production is promoted by increased growth hormone activity and mice with mutations in the growth hormone receptor or defects in the pituitary gland (Ames and Snell dwarf mice) show reduced growth hormone and IGF-1 levels and increased life span relative to controls (Brown-Borg et al., 1996; Coschigano et al., 2003; Flurkey et al., 2002). As with flies, the role of FoxO proteins in mouse life-span extension from reduced IIS is unknown. However, FoxO proteins are known to function in IIS pathways that affect metabolism (Burgering & Kops, 2002) and have been implicated in the increased stress resistance of certain long-lived mouse strains (Nemoto & Finkel, 2002).

Interestingly, while insulin sensitivity is typically associated with longevity in mice, there are several examples of mutations that both increase insulin resistance and extend life span. These include *KLOTHO* overexpression (Kurosu et al., 2005), $IRS1^{-/-}$ knockout (Selman et al., 2008), and brain-specific IRS2 knockout (Taguchi et al., 2007). These findings are difficult to interpret in light of the potential for pleiotropic effects, as resistance to both insulin and IGF-1 was observed in all cases.

The evolution of multiple IIS pathways in mammals has several implications for the role of IIS in aging. Functions performed by the single IIS pathway in invertebrates that are related to life-span extension may be divided between the insulin and the IGF-1 branches of IIS in mammals. Indeed, while there is evidence for overlapping function, insulin signaling is primarily involved in regulating metabolism, while IGF-1 modulates growth and development (Kim & Accili, 2002; Rincon et al., 2005). Multiple pathways would also have eased pleiotropic evolutionary restrictions and allowed the insulin and IGF-1 branches to specialize further and/or acquire new functions.

Overexpression of Sirtuins

Sir2 orthologs (sirtuins) are present in organisms from yeast to humans and function as NAD-dependent protein deacetylases (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000; Tanner et al., 2000). Sir2 is a histone deacetylase that promotes transcriptional silencing at three specific loci in the yeast genome: the ribosomal DNA (rDNA), the silent mating (HM) loci, and regions near the telomeres (Aparicio et al., 1991; Bryk et al., 1997; Gottschling et al., 1990; Ivy et al., 1986; Rine & Herskowitz, 1987; Smith & Boeke, 1997). Unlike yeast, the reported substrates of Sir2 orthologs in multicellular eukaryotes appear to be primarily nonhistone and include endoplasmic reticulum-stress response factors (Viswanathan et al., 2005), FoxO-family transcription factors (Brunet et al., 2004; Motta et al., 2004; van der Horst et al., 2004), peroxisome proliferator-activated receptor γ coactivator 1α (Gerhart-Hines et al., 2007; Rodgers et al., 2005), p53 (Luo et al., 2001; Vaziri et al., 2001), and several others (Dali-Youcef et al., 2007; Finkel et al., 2009).

A role for Sir2 orthologs in aging was first demonstrated by the observation that overexpression of Sir2 is sufficient to increase yeast replicative life span (Kaeberlein et al., 1999). Subsequent studies demonstrated a similar longevity-enhancing effect associated with overexpression of *sir-2.1* in worms and dSir2 in flies (Rogina & Helfand, 2004; Tissenbaum & Guarente, 2001). To date, there has been no report showing that increased expression of SIRT1 in

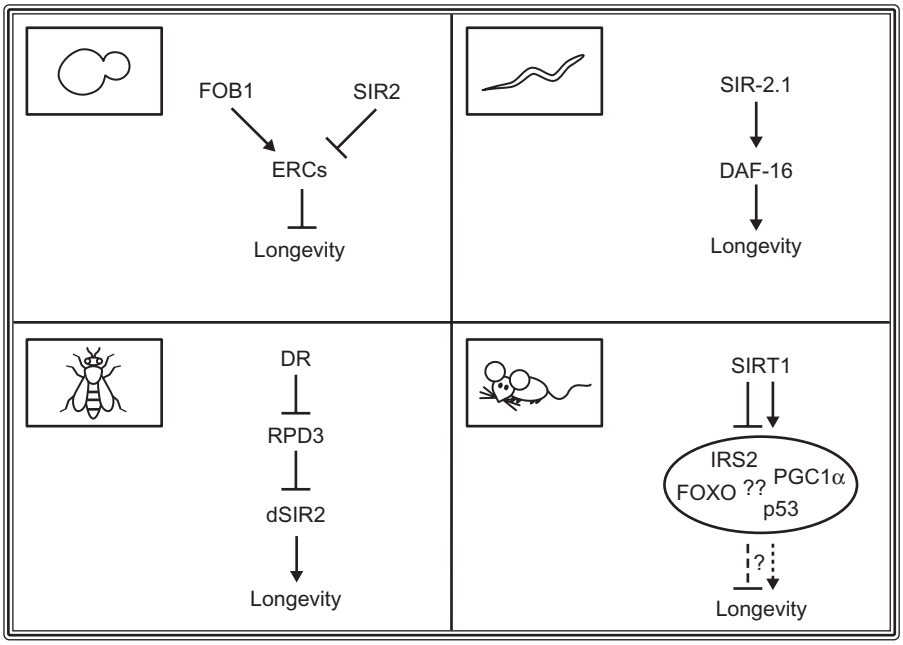

Figure 10.2 Sir2 orthologs promote longevity in yeast, nematodes, and flies by distinct mechanisms. The ability of SIRT1 overexpression to increase mouse life span has yet to be established, but SIRT1 influences a variety of age-associated phenotypes in mice, possibly via multiple substrate targets.

mammals is sufficient to increase life span, although SIRT1 transgenic mice are reported to have improved metabolic profiles (Banks et al., 2008; Bordone et al., 2007) and show resistance to colon cancer (Firestein et al., 2008).

One surprising feature of the longevity-promoting functions of sirtuins is the apparently distinct mechanisms by which they act in different organisms (Figure 10.2). In yeast, Sir2 is thought to slow replicative aging by promoting genomic stability in the rDNA and repressing the formation of extrachromosomal rDNA circles (Kaeberlein et al., 1999), one cause of replicative senescence in yeast cells (Sinclair & Guarente, 1997). Unlike yeast, there is no evidence that Sir2 orthologs modulate the formation of extrachromosomal rDNA circles in multicellular eukaryotes, nor are their data suggesting that rDNA circles cause aging in higher organisms. Instead, sirtuins appear to have evolved different prolongevity functions in these organisms. For example, in *C. elegans,* evidence suggests that *sir-2.1* modulates the downstream targets of IIS by interacting with *daf-16* in a 14-3-3-dependent manner (Berdichevsky et al., 2006; Wang et al., 2006). In flies, the relevant downstream targets of dSir2 have yet to be described, but it has been proposed that dSir2 acts in a longevity-promoting pathway with the Rpd3 histone deacetylase (Rogina & Helfand, 2004). Whether Sir2 orthologs really function to slow aging by different mechanisms in different organisms, or whether there exist as yet uncharacterized conserved sirtuin functions, is a question of continuing interest.

In contrast to the prolongevity effects associated with sirtuins, recent studies have suggested that sirtuin proteins may also promote aging in some systems or tissues. For example, yeast chronological life span is limited by Sir2 activity (Fabrizio et al., 2005; Kennedy et al., 2005). SIRT1-deficient mouse embryonic fibroblasts are highly resistant to replicative senescence and have increased replicative potential under chronic oxidative stress (Chua et al., 2005), in stark contrast to the observed decrease in replicative life span of yeast lacking Sir2 (Kennedy et al., 1995). A recent study found reduced IIS and Ras/ERK signaling in mice lacking SIRT1 (Li et al., 2008). Li et al. (2008) also found that SIRT1 knockdown enhanced oxidative stress resistance in mouse neuronal cell culture and that SIRT1 knockout mice had reduced oxidation of proteins and lipids in the brain, in contrast to the finding in flies that neuron-specific overexpression of dSir2 is sufficient for life-span extension (Rogina & Helfand, 2004), suggesting that both increasing and decreasing sirtuin activity may have neuroprotective consequences. These studies reinforce the idea that sirtuins perform different functions in different organisms and imply that the biology of sirtuins is more complex than initially suspected. Further effort will be required to unravel the intricacies of the action of sirtuins on longevity and to determine what similarities and differences exist between evolutionarily divergent species.

Reduced TOR Signaling

The TOR kinase is a highly conserved nutrient- and growth factor-responsive protein that is essential for viability in eukaryotic species (Stanfel et al., 2009). TOR was first identified as the molecular target of an antifungal compound (rapamycin) produced by the bacterium *Streptomyces hygroscopicus* (Vezina et al., 1975). Rapamycin was subsequently shown to inhibit the activity of protein products of two partially redundant yeast genes: TOR1 and TOR2 (Heitman et al., 1991). TOR proteins have since been identified in a variety of species, including humans, and have been shown to act in two distinct complexes: TOR complex 1 (TORC1) and TOR complex 2 (TORC2) (De Virgilio & Loewith, 2006; Martin & Hall, 2005). Although both TOR complexes are essential for viability (Guertin et al., 2006; Helliwell et al., 1998), only TORC1 is sensitive to rapamycin. It is currently thought that TOR-mediated longevity control occurs exclusively via altered TORC1 activity. TORC1 serves as a key regulatory nexus important for mounting an appropriate response to nutrients, growth cues, and cellular energy status (Wullschleger et al., 2006). TORC1 is activated by environmental nutrient availability in the form of both amino acids and glucose and is also responsive to IIS (through Akt) as well as the energy-sensing AMP-activated protein kinase (AMPK) (Arsham & Neufeld, 2006; Bhaskar & Hay, 2007).

The link between TOR and aging has been definitively demonstrated in four different organisms (Stanfel et al., 2009). Reduced TOR signaling is sufficient to increase life span in mice (Harrison et al., 2009), worms (Jia et al., 2004; Vellai et al., 2003), flies (Kapahi et al., 2004), and both yeast aging paradigms (Kaeberlein et al., 2005c; Powers et al., 2006). Aside from DR, TOR inhibition is the only intervention known to slow aging in each of these model systems (Kaeberlein & Kennedy, 2009). The importance of TOR signaling in yeast replicative and chronological life-span determination was uncovered from independent, unbiased longevity screens of the yeast ORF deletion collection. Deletion of *TOR1* was found to increase both replicative and chronological life span, as did pharmacological inhibition of TOR using rapamycin (Kaeberlein et al., 2005c; Powers et al., 2006). RNAi knockdown of the gene coding for TOR (*let-363*) or the TORC1 component raptor (*daf-15*) is sufficient to increase life span in worms (Jia et al., 2004; Vellai et al., 2003) and transgenic expression of a dominant-negative allele of TOR increases life span in flies (Kapahi et al., 2004). The effect of reduced TOR signaling on life span in a mammalian system was recently demonstrated by a study in which mice were fed a diet supplemented with rapamycin. Supplementation with rapamycin beginning at 600 days of age resulted in a significant increase in life span (Harrison et al., 2009).

The precise molecular mechanisms by which TOR signaling modulates aging in evolutionarily divergent organisms have yet to be completely characterized. Unlike the sirtuin pathway, components of TOR signaling are highly conserved both upstream and downstream of TORC1, including several TOR-regulated processes that have been suggested to play a role in longevity determination such as regulation of mRNA translation, autophagy, stress response, and mitochondrial metabolism. For example, autophagy is induced in a TOR-dependent manner by both DR and reduced IIS in *C. elegans* and is required for life-span extension in both cases (Hansen et al., 2008; Jia & Levine, 2007; Melendez et al., 2003). Altered TOR signaling is thought to be partially responsible for the beneficial effects of DR, which is discussed further below.

QUANTITATIVE EVIDENCE FOR CONSERVED MECHANISMS OF LONGEVITY CONTROL

A handful of conserved longevity factors have been known to exist for some time, but the degree to which mechanisms that control longevity are generally conserved between evolutionarily disparate organisms has only recently begun to be addressed. Unbiased genome-wide analyses of longevity in yeast and worms have afforded the first opportunities to assess the overlap between genes that modulate longevity in these two evolutionarily divergent organisms on a genomic level.

Demonstration of Conservation between Yeast and Worms

In a study published in 2008 in *Genome Research*, Smith et al. (2008b) took advantage of the large number of known longevity-associated genes in *C. elegans* to address the question of whether genetic control of aging has been conserved between yeast and worms. Underlying this analysis was the rationale that if genetic control of aging has been conserved, then yeast homologs of worm longevity-associated genes should have a greater likelihood of influencing longevity than randomly selected yeast genes. Since a majority of the known longevity-associated genes in *C. elegans* were derived from RNAi screens, Smith et al. (2008b) restricted their study to a set of 276 *C. elegans* genes reported to increase life span when expression or function is decreased. Yeast homologs of these genes could then be examined as deletion alleles and the corresponding effect on life span determined.

To identify orthologous gene pairs between worms and yeast, a two-tiered approach was taken. A high-stringency set of ortholog pairs was defined based on a modified reciprocal BLASTp best-match criterion.

Mapping of two yeast orthologs to one worm gene was allowed in cases in which BLASTp scores for yeast paralogs were within 10% of each other. A low-stringency set of homologs included all cases in which one or more yeast proteins could be identified with at least 20% sequence identity and 10% amino acid alignment to the worm aging protein, with a maximum of 6 yeast homologs allowed per worm gene. From 276 worm aging genes, 264 nonessential yeast genes (viable as single-gene deletions) were identified in the low-stringency homolog set, of which 78 also met the high-stringency ortholog criterion (Smith et al., 2008b).

Replicative life-span analysis was performed on each of the 264 single-gene deletion strains contained in the low-stringency homolog set. Using a rigorous iterative procedure for large-scale life-span analysis in yeast (Kaeberlein & Kennedy, 2005; Kaeberlein et al., 2005c), 25 single-gene deletions (9.5%) from this set were determined to be significantly long lived, of which 11 (14.1%) were also in the high-stringency ortholog set. In both sets, the frequency of long replicative life span significantly exceeded the frequency of long replicative life span observed in a study of 564 randomly chosen deletion strains (2.3%) (Kaeberlein et al., 2005c).

The results of Smith et al. allow for the conclusion that genetic control of longevity has been evolutionarily conserved between yeast and worms (Smith et al., 2008b). This study provides the first quantitative evidence for conservation of genetic determinants of aging. The nature of the aging models used in the comparison makes this finding particularly striking. Yeast replicative life span is a measure of mitotic aging. In contrast, cells in the adult *C. elegans* are completely postmitotic with the exception of the germ line. Such genetic conservation between mitotic and nonmitotic aging is not intuitively obvious. Demonstration of aging conservation also has important implications for human aging. On an evolutionary time scale, yeast and worms are separated by approximately 1.5 billion years, while worms and humans are separated by only approximately 1.0 billion years (Wang et al., 1999). We can thus speculate that a subset of genes that play a conserved role in aging in yeast and worms is likely to play a similar role in humans.

TOR Signaling Accounts for Many Conserved Longevity Factors

The most notable feature of the conserved longevity factors identified by Smith et al. (2008b) is the substantial enrichment for genes that code for proteins involved in regulating mRNA translation. Among the 25 ortholog pairs, only 2 were previously known to modulate aging in both yeast and worms: *TOR1/let-363* and *SCH9/rsks-1*. *SCH9* and *rsks-1* are homologs of mammalian ribosomal S6 kinase, which functions downstream of TOR signaling to modulate mRNA translation initiation (Pan et al., 2007; Urban et al., 2007). Excluding *TOR1/let-363* itself, 6 of the 10 remaining ortholog pairs in the high-stringency set can be definitively assigned functions related to mRNA translation: three ribosomal proteins of the large subunit (*RPL19A/rpl-19*, *RPL6B/rpl-6*, *RPL9A/rpl-9*) and three translation initiation factors (*TIF1/inf-1*, *TIF2/inf-1*, *TIF4631/ifg-1*). Given that TOR and S6 kinase are known to regulate positively both ribosome biogenesis and translation initiation factor activity, it is reasonable to speculate that all of these factors act in a single conserved longevity pathway (Figure 10.3).

The best evidence supporting the hypothesis that TOR signaling modulates longevity via regulation of mRNA translation comes from yeast replicative aging studies. Epistasis analysis clearly places *TOR1*, *SCH9*, and genes encoding ribosomal proteins of the large subunit (RPLs) into a single pathway (Stanfel et al., 2009). This is evidenced by nonadditivity of life-span extension when deletion of *TOR1* or *SCH9* is combined with a life-span-extending deletion of an RPL and Sir2-independent life-span extension from deletion of *TOR1*, *SCH9*, or RPLs (Kaeberlein et al., 2005c; Steffen et al., 2008).

Further support for this hypothesis has recently been provided by the identification of the nutritionally regulated Gcn4 transcription factor as a potential downstream mediator of life-span extension in response to reduced TOR signaling and altered mRNA translation (Kaeberlein et al., 2005c; Steffen et al., 2008). Cellular levels of Gcn4 are primarily controlled by translation and protein degradation (as opposed to transcription; Hinnebusch, 2005) and both RPL mutations and reduced TOR signaling have been shown to induce Gcn4 activity (Cherkasova & Hinnebusch, 2003; Foiani et al., 1991; Kubota et al., 2003; Martin-Marcos et al., 2007; Valenzuela et al., 2001). Steffen et al. (2008) showed that deletion of *GCN4* partially blocks replicative life-span extension in yeast lacking either an RPL or *TOR1*. Furthermore, Gcn4 activity was specifically upregulated in two long-lived RPL mutants (*rpl20bΔ* and *rpl31aΔ*) but not in mutants lacking paralogs of these genes that are not long lived (*rpl20aΔ* and *rpl31bΔ*), demonstrating that the increase in Gcn4 activity is linked to the increase in life span (Steffen et al., 2008).

A model placing TOR, S6 kinase, protein synthesis, and a Gcn4-like transcription factor in a linear pathway controlling life span may be overly simplistic on the broader stage of public mechanisms of longevity control. While both TOR signaling and TOR-regulated protein synthesis factors modulate life span in both yeast and worms (Smith et al., 2008b), longevity epistasis studies in *C. elegans* map *let-363* (TOR), but not *rsks-1* (S6 kinase) and translation initiation factors, to the same pathway as DR (Hansen et al., 2007). The results of Hansen et al. (2007) suggest that DR extends life span by reduced protein

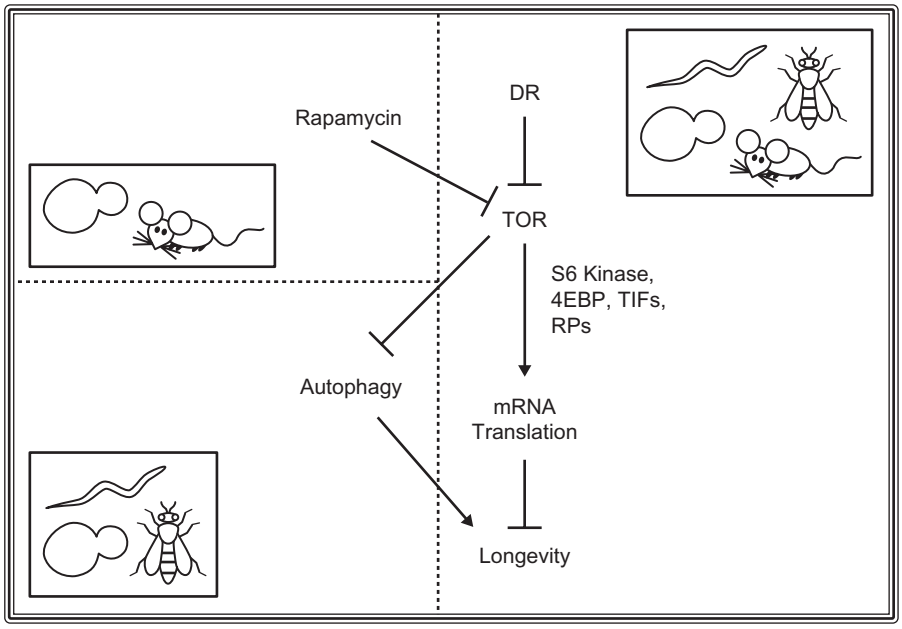

Figure 10.3 A reduction in TOR signaling extends life span in evolutionarily divergent organisms. Mutation of S6 kinase increases life span in yeast, nematodes, flies, and mice; the TOR inhibitor rapamycin increases life span in yeast and mice; and autophagy has been implicated in life-span extension from reduced TOR signaling in yeast, nematodes, and flies.

translation via TOR signaling, while knockdown of S6 kinase and other protein synthesis factors may act through a different mechanism.

Notably absent from the set of 25 conserved longevity ortholog pairs, not to mention any the genome-wide longevity screens in worms, are genes known to function in the same pathway as *SIR2*/*sir-2.1* (Curran & Ruvkun, 2007; Dillin et al., 2002; Hamilton et al., 2005; Hansen et al., 2005; Lee et al., 2003; Smith et al., 2008b). This lack of evidence for Sir2-related conservation of longevity control may reflect the limited understanding of upstream and downstream factors involved in *SIR2*/*sir-2.1*-mediated longevity control. It may also be due to the apparently dissimilar mechanisms by which Sir2 orthologs modulate longevity in different organisms, as discussed above.

INTERACTION BETWEEN DIETARY RESTRICTION AND CONSERVED LONGEVITY PATHWAYS

Life extension in response to DR was first observed in rats in 1934 (McCay & Crowell, 1934) and has since been demonstrated in a wide range of model systems. DR alters a multitude of physiological processes, and each of the conserved aging pathways discussed under Conserved Longevity Interventions—IIS, TOR signaling, and sirtuins—has been independently proposed to mediate the response to DR. In this section we discuss the relationship between DR and each of these pathways.

IIS: A Partial Interaction with DR with Respect to Secondary Aging Phenotypes

DR and mutations that reduce IIS have many phenotypic similarities, including enhanced longevity, stress resistance, reduced TOR signaling, and increased autophagic protein degradation. This is not surprising, since one of the major environmental factors to modulate IIS is nutrient availability. Thus, reduced IIS is a natural candidate for mediating the beneficial effects of DR. Interestingly, while DR and IIS clearly overlap, genetic studies have indicated that they also act through at least partially distinct mechanisms to control longevity.

The relationship between IIS and DR has been studied most extensively in *C. elegans.* Life-span extension from multiple approaches to DR, including bacterial dilution in liquid culture, axenic growth in liquid culture, bacterial deprivation, and mutation of *eat-2* (a genetic model resulting in reduced food intake due to decreased pharyngeal pumping), has been repeatedly shown to extend life span by a mechanism different from mutations that reduce IIS (Houthoofd et al., 2003; T. L. Kaeberlein et al., 2006; Lakowski & Hekimi, 1998; Lee et al., 2006). Specifically, all of

these DR methods increase life span in animals lacking DAF-16. In contrast, one study found both DAF-16 and AAK-2 to be required for a specific method of DR, termed sDR, involving maintenance of worms on solid agar plates in the presence of diluted bacterial food (Greer et al., 2007). Another study found that mutations in *daf-2* produced increased growth and stress resistance in *eat-2* mutants (Iser & Wolkow, 2007). Similarly, growth impairment normally observed in response to DR was suppressed in *daf-2* mutants (Iser & Wolkow, 2007). Thus, IIS and most forms of DR are thought to act in parallel pathways to mediate longevity in worms, but have potential to interact downstream by influencing AAK-2 and DAF-16 activity under some circumstances.

In flies, the life span on a range of food concentrations of long-lived *Chico* mutants, which have reduced IIS, is right-shifted relative to wild type, meaning that *Chico* mutants are shorter lived than controls on low food concentrations and longer lived on normal to high food concentrations (Clancy et al., 2002). This was initially taken as an indication that DR requires IIS to extend life span. However, a 2008 study found that deletion of dFOXO did not block the ability of DR to extend life span (Giannakou et al., 2008). Overexpression of dFOXO in the adult fat body partially mimicked the long-lived *Chico* mutants in that the mutant flies were longer lived at normal to high food concentrations (Giannakou et al., 2008). Thus IIS and DR interact when IIS is active, but IIS is not required for DR to extend life span. One possible explanation is that *Drosophila* IIS does not act entirely through dFOXO, but influences life span through a different mediator.

IIS shares a complex relationship with DR in mice as well. Growth hormone receptor knockout (GHRKO) mice are longer lived than wild-type controls and have reduced levels of both insulin and IGF-1 (Coschigano et al., 2000; Liu et al., 2004; Zhou et al., 1997). GHRKO mice are not longer lived than wild-type mice subject to DR, nor do GHRKO mice show increased longevity or improved insulin sensitivity when subjected to DR, with the exception of an increase in maximum life span in females (Al-Regaiey et al., 2007; Bonkowski et al., 2006). In contrast, DR extends the lives of mice with pituitary mutations, which are defective for production of several hormones, including growth hormone (Bartke et al., 2001). This suggests that DR and IIS may act via partially distinct pathways, although it is also possible that they act via a similar mechanism, but that neither intervention optimally activates that mechanism with respect to longevity.

Sirtuins: A Complex and Unresolved Connection to DR

The connection between Sir2 and DR in yeast has been a source of controversy (Guarente, 2005; Kaeberlein & Powers, 2007; M. Kaeberlein et al., 2006; Kennedy et al., 2005; Lamming et al., 2005). Sirtuins were first proposed as mediators of DR based on the known role of Sir2 in yeast replicative aging and the discovery that Sir2 is activated in yeast in a NAD-dependent manner (Guarente, 2000). This hypothesis was supported by early evidence that reducing glucose in the medium did not extend the replicative life span of short-lived yeast lacking *SIR2* (Lin et al., 2000). An alternative interpretation of this result is that accumulation of ERCs in the *sir2Δ* strain causes enough damage that cells die before they can respond to DR, masking the life-span extension normally observed. Indeed, subsequent studies from independent labs found that Sir2 is not required for life-span extension by DR under conditions where ERC accumulation is reduced (Jiang et al., 2000; Kaeberlein et al., 2004; Kaeberlein & Powers, 2007; M. Kaeberlein et al., 2006; Lamming et al., 2005). More specifically, while DR does not extend life span of *sir2Δ* strains (Kaeberlein et al., 2004; Lin et al., 2000), suppression of the short-lived *sir2Δ* phenotype by deletion of *FOB1* allows robust life-span extension by DR (Kaeberlein et al., 2004). Combining DR with overexpression of Sir2 or deletion of *FOB1* also results in an additive life-span extension (Kaeberlein et al., 2005a). DR has therefore been shown to control longevity via at least one Sir2-independent mechanism in yeast, and two studies have reinforced this model that DR does not act through Sir2 by showing that Sir2 activity is not enhanced in vivo by DR (Riesen & Morgan, 2009; Smith et al., 2009).

In multicellular eukaryotes the interaction between DR and sirtuins is unresolved. Reports concerning *sir-2.1* and DR in worms are conflicting, but, with the exception of one study (Wang & Tissenbaum, 2006), support the idea that DR by a variety of methods does not require *sir-2.1* for life-span extension (Greer & Brunet, 2009; Hansen et al., 2007; T. L. Kaeberlein et al., 2006; Lee et al., 2006; Mair et al., 2009). Consistent with a model in which *sir-2.1* and DR act via distinct mechanisms, life-span extension by *sir-2.1* overexpression requires *daf-16* (Tissenbaum & Guarente, 2001), while life-span extension by DR does not (Lakowski & Hekimi, 1998). Thus, while the majority of evidence supports a model in which DR and *sir-2.1* act in parallel, further work will be required to determine definitively how *sir-2.1* interacts with DR in worms, if at all.

The situation in the published literature is less complicated in flies. Epistasis maps dSir2 to the same pathway as both DR and the histone deacetylase Rpd3 with respect to longevity (Rogina & Helfand, 2004), and both DR and reduced Rdp3 activity increase transcription of *dSir2* (Rogina et al., 2002). Unlike the case in other organisms, the role of dSir2 in the response to DR in flies has not been studied extensively, however, and would benefit from additional characterization.

The relationship between SIRT1 and DR in mice is complex. SIRT1 has been linked to both stress resistance and the regulation of metabolic processes, including hormone levels and fat storage, providing a potential connection to DR via diet and nutrient sensing (Guarente & Picard, 2005). While the effect of DR on longevity in mice with elevated SIRT1 levels is not known, knocking out SIRT1 in mice represses the increase in physical activity normally observed in response to DR (Chen et al., 2005) and prevents life-span extension from DR (Li et al., 2008). SirT1 mRNA and protein levels are reported to be increased in some tissues in response to DR, but there is evidence that DR also downregulates SIRT1 in some tissues. One study in mice looking specifically at the liver found that SIRT1 activity is decreased in response to DR and increased in response to high-fat diet (Chen et al., 2008). Liver-specific SIRT1 knockout mice are also partially protected from fat accumulation and have improved metabolic characteristics on a high-fat diet relative to wild-type animals with similar food intake (Chen et al., 2008).

A common approach used to study the interaction between sirtuins and diet is to look at the response to pharmacological activators of sirtuins. The most common is resveratrol, a potent small-molecule activator of Sir2 found in the skin of grapes and other plants (Howitz et al., 2003). Resveratrol has been reported to increase life span in yeast (Howitz et al., 2003), worms (Viswanathan et al., 2005; Wood et al., 2004), flies (Bauer et al., 2004; Wood et al., 2004), and one short-lived species of fish (Valenzano et al., 2006), though the findings in yeast, worms, and flies have proven difficult to replicate (Bass et al., 2007b; Kaeberlein et al., 2005b). In mice, resveratrol was protective against the health consequences of a high-fat diet (Baur et al., 2006; Lagouge et al., 2006). A potential confounding factor in studies using resveratrol is specificity. Resveratrol activates AMPK in addition to SIRT1, raising the question as to which effects are caused by increased SIRT1 activity and which are caused by increased AMPK activity (Baur et al., 2006). SIRT1720, a small-molecule activator of SIRT1 that does not activate AMPK and has improved potency relative to resveratrol, was identified in a small-molecule screen (Milne et al., 2007). Like resveratrol, SIRT1720 was found to protect mice fed a high-fat diet from developing obesity and insulin resistance by promoting oxidative metabolism in metabolic tissues (Feige et al., 2008). While feeding mice a high-fat diet cannot exactly be considered the opposite of DR, these studies do provide a clear link between diet and sirtuins, and high-fat diet may indeed be a more appropriate model for modern human societies. Notably, Feige et al. (2008) also observed transcriptional changes typically associated with low energy levels in response to treatment with SIRT1720, suggesting a potential link between sirtuins and DR.

TOR Signaling: A Conserved Mediator of Life-Span Extension by DR

Among genetic pathways that regulate life span, the evidence is most consistent for TOR signaling as a mediator of the response to DR (Stanfel et al., 2009). As noted above, reduced TOR signaling is the only intervention aside from DR that extends life span in mice, flies, worms, and both yeast paradigms. The TOR signaling pathway is also a conserved nutrient-responsive pathway (Kapahi & Zid, 2004) that has been observed to be inhibited, as measured through a reduction in autophagy and S6 kinase (S6K) activity, in response to DR in a variety of model organisms (Arsham & Neufeld, 2006; Bhaskar & Hay, 2007; De Virgilio & Loewith, 2006).

Findings in invertebrate models point strongly toward TOR signaling as a mediator of dietary restriction. In yeast, replicative life-span extension by DR and *TOR1* deletion is nonadditive (Kaeberlein et al., 2005c). Replicative life-span extension by DR, deletion of *TOR1*, and deletion of *SCH9* (yeast S6K) is additive with deletion of *FOB1* and independent of *SIR2* (Kaeberlein et al., 2004; Kaeberlein et al., 2005c; M. Kaeberlein et al., 2006; Tsuchiya et al., 2006), placing DR and TOR in a common pathway that is distinct from Sir2 and Fob1. Life-span extension from reduced TOR signaling and DR is similarly nonadditive in worms (Hansen et al., 2007). Studies have also found that autophagy induced by reduced TOR signaling is required for DR life-span extension in both worms (Hansen et al., 2008; Jia & Levine, 2007; Toth et al., 2008) and flies (Juhasz et al., 2007). A connection between DR and TOR signaling has not been tested directly in the yeast chronological paradigm, though one group has linked chronological life-span extension by deletion of *TOR1* to mitochondrial respiration (Bonawitz et al., 2007). Bonawitz et al. (2007) proposed a model in which DR derepresses respiration by inhibiting TOR signaling, leading to increased mitochondrial oxygen consumption and resulting in decreased damage from reactive oxygen species and extension of chronological life span.

As with IIS and sirtuins, a role for TOR signaling in mammalian response to DR has yet to be definitively demonstrated, though indirect evidence from several studies examining phenotypes in mice treated with rapamycin provides some cause for optimism about a connection between aging, DR, and TOR signaling. For example, treatment with rapamycin prevents weight gain in both humans and rats (Rovira et al., 2008) and improves resistance to cancer,

neurodegeneration, and cardiac disease in mice (Gao et al., 2006; Wullschleger et al., 2006). DR has long been known to reduce the occurrence of cancer in rodents (Ross & Bras, 1965; Tannenbaum, 1942; Weindruch & Walford, 1982; Yu et al., 1982), is currently in clinical trials for treatment of cancer in humans (Weil, 2008), and has been found to suppress proteotoxicity in models of neurodegenerative diseases in nematodes (Steinkraus et al., 2008b).

Several studies have also examined the role of components of the TOR signaling pathway in the context of high-fat diet. A 2008 study found that mice with an adipose-specific knockout of raptor, an essential gene and specific component of the mammalian TORC1 (mTORC1) complex, were lean, had less adipose tissue, exhibited improved insulin sensitivity, and were resistant to diet-induced obesity relative to control mice (Polak et al., 2008). Polak et al. (2008) also found increased expression of genes encoding mitochondrial uncoupling proteins and heightened energy expenditure caused by an increase in uncoupled respiration, suggesting that mTORC1 regulates energy homeostasis by controlling adipose metabolism. Whole-body knockout of S6K, which is positively regulated by mTORC1, results in mice that are lean and have improved insulin sensitivity and resistance to age- and diet-induced obesity because of increased energy expenditure (Pende et al., 2000; Um et al., 2004). Consistent with these findings, whole-body knockout of 4E-BP1 and 4E-BP2, which are negatively regulated by mTORC1, results in obese mice that are hypersensitive to diet-induced obesity (Le Bacquer et al., 2007). These studies indicate that reduced TOR signaling is protective against the damaging effects of eating a high-fat diet, which is consistent with a model in which DR action on longevity is mediated by reduced TOR signaling. Chapter 2 provides further discussion of the relationship between TOR and DR.

CONCLUSIONS

The past few decades have seen the emergence of IIS, TOR signaling, sirtuins, and DR as important and evolutionarily conserved regulators of aging and longevity. Pharmacological agents that target components of these pathways, such as resveratrol and rapamycin, are being developed and tested for aging-related activities in model organisms. Clinical trials for some of these agents are already under way for treatment of cancer and diabetes and will probably be expanded to other age-related disorders. These trials mark the first clinical benefits derived from comparative genetics of aging in model organisms.

As key players in these conserved aging pathways continue to be uncovered and characterized using model systems, we will also gain a better understanding of how they function and interact to integrate environmental signals into cellular responses that modulate aging. It has become apparent that, although longevity interventions can be mapped to genetically distinct pathways through epistasis and other types of studies, in reality most (or all) of these conserved longevity modifiers interact within cells as part of a complex network. For example, TOR activity both modulates and is modulated by insulin-like signaling, while DR alters signaling through both pathways. In future studies, it will be important to consider not only which proteins play a conserved longevity role, but which interactions between longevity factors have also been conserved. Such an approach should make it possible to develop a more comprehensive picture of the overarching longevity network and may resolve lingering questions and controversies in the field while providing more effective routes toward therapies for improving human health span and longevity.

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