Biochemical Genetic Pathways that Modulate Aging in Multiple Species

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The mechanisms underlying biological aging have been extensively studied in the past 20 years with the avail of mainly four model organisms: the budding yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster*, and the domestic mouse *Mus musculus*. Extensive research in these four model organisms has identified a few conserved genetic pathways that affect longevity as well as metabolism and development. Here, we review how the mechanistic target of rapamycin (mTOR), sirtuins, adenosine monophosphate-activated protein kinase (AMPK), growth hormone/insulin-like growth factor 1 (IGF-1), and mitochondrial stress-signaling pathways influence aging and life span in the aforementioned models and their possible implications for delaying aging in humans. We also draw some connections between these biochemical pathways and comment on what new developments aging research will likely bring in the near future.

The science of aging has rapidly advanced in the past two decades owing to the use of evolutionarily divergent model organisms in experimental studies. The most common of these include the budding yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, the domestic mouse *Mus musculus*, and, to a lesser extent, the rat *Rattus norvegicus*. Studies of aging in nonhuman primates, such as rhesus monkeys and marmosets, have also had a large impact on the field in the last few years. Additionally, recent comparative biological approaches to aging have adopted less commonly used model organisms that feature exceptional longevity, such as the naked mole rat, short-lived fish, certain species of clams, or those with potent regenerative potential, such as hydra (Austad 2009; Valen-

zani et al. 2009; Ridgway et al. 2011; Petralia et al. 2014).

Historically, a major question in the field has been the degree to which studies of aging in nonhuman organisms will be informative about the mechanisms of human aging and the processes that contribute to age-related disease (Gershon and Gershon 2000; Warner 2003). Although it is still not possible to definitively answer this question, a large body of data exists supporting the idea that at least some basic principles of aging are broadly conserved, even in single-celled eukaryotes, such as budding yeast. In particular, several genetic and environmental factors that modulate longevity in two or more commonly used model organisms have been identified (Sutphin and Kaeberlein 2011). Manipulation of these conserved modifiers of lon-

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evity is sufficient to increase life span in evolutionarily divergent species, showing that they are likely to modulate the aging process itself. This, in turn, suggests that there are at least some fundamental aspects of aging that are shared between yeast, worms, flies, and mice. Given that the evolutionary distance between these species is much greater than the distance between mice and humans, it seems likely that at least a subset of the principles of aging gleaned from these model systems will also be applicable to people (Kaeberlein 2013).

A general principle of conserved modifiers of longevity is that they tend to regulate the relationship between growth and environmental cues, particularly nutrient status. The best studied of these is dietary restriction (DR), also referred to as caloric restriction or calorie restriction, which can be defined as a reduction in nutrient availability in the absence of malnutrition. DR has been shown to extend life span and improve health during aging in numerous species (Weindruch and Walford 1988). This may reflect a fundamental principle that rates of aging are linked to developmental pathways modulating growth and reproductive trajectories in response to variable environmental situations. For the remainder of this article, we will describe these conserved longevity pathways, their genetic and biochemical properties, and mechanisms by which they may act to modulate rates of aging and risk for age-related disease.

THE FOUR MOST COMMON MODEL ORGANISMS USED IN AGING RESEARCH

As mentioned above, the four major nonhuman model organisms used in aging-related research are budding yeast, nematode worms, fruit flies, and laboratory mice. Each of these species has its own strengths and weaknesses as a model for human aging, and it is important to consider the ways in which they are similar but also how they differ with respect to physiology, longevity, and aging traits. Notably, the shape of the survival curves in these organisms is superficially similar to each other and to human survival data as modeled by Gompertz–Makeham ki-

netics (Kaeberlein et al. 2001), although such similarity does not imply any conservation of aging mechanisms.

Yeast

Aging was first defined in budding yeast by Robert Mortimer and John Johnston more than 60 years ago, when it was discovered that yeast mother cells undergo a limited number of mitotic cell divisions before reaching a terminal replicative arrest (Mortimer and Johnston 1959). This form of aging in yeast has since been extensively studied and is referred to as replicative aging, with the corresponding longevity metric defined as replicative life span (RLS) (Steinkraus et al. 2008; Steffen et al. 2009; Kaeberlein 2010). A key feature of replicative aging in yeast is asymmetric division; mother cells retain and accumulate molecular damage during mitotic division, whereas daughter cells are generally spared from inheriting such damage and are born with a full RLS potential even when produced by aged mothers (Egilmez and Jazwinski 1989; Kennedy et al. 1994). There is good evidence for asymmetric inheritance of at least three different types of damage during yeast replicative aging: nuclear extrachromosomal ribosomal DNA (rDNA) circles (Sinclair and Guarente 1997; Defossez et al. 1998, 1999), oxidatively damaged or misfolded cytoplasmic proteins (Aguilaniu et al. 2003; Erjavec and Nystrom 2007; Erjavec et al. 2007), and dysfunctional mitochondria (Lai et al. 2002).

A second form of yeast aging has also been described, referred to as chronological aging. In contrast to replicative aging, chronological aging of yeast cells is accomplished by preventing mitotic cell division and maintaining cells in a nondividing state (Fabrizio and Longo 2007; Longo et al. 2012). The corresponding longevity metric, chronological life span (CLS), is defined as the length of time a yeast cell can survive in a nondividing state, with survival determined by the ability to reenter the cell cycle and resume vegetative growth on exposure to appropriate growth-promoting cues. Several different methods have been described for performing chronological aging experiments (Piper et al. 2006;

Murakami et al. 2008; Murakami and Kaeblerlein 2009; Longo and Fabrizio 2012). Similar to replicative aging, there is evidence for accumulation of oxidatively damaged proteins and mitochondrial dysfunction during chronological aging.

The relationship between chronological and replicative aging and the relevance of these two distinct types of aging in yeast to aging in multicellular eukaryotes remains an area of active study. Large-scale studies have detected significant overlap between genetic control of longevity in *C. elegans* with genetic control of RLS (Smith et al. 2008a), but not CLS (Burtner et al. 2011). This could be related to the fact that acidification of the culture medium limits CLS under the most commonly used conditions for chronological aging experiments (Burtner et al. 2009; Murakami et al. 2011), which is not the case for RLS (Wasko et al. 2013). Nonetheless, key regulators of CLS also modulate RLS as well as aging in worms, flies, and mice (described further below). In addition, chronologically aged cells also show a reduction in subsequent RLS, suggesting that similar forms of age-associated damage may contribute to both mitotic (RLS) and postmitotic (CLS) aging in yeast cells (Ashrafi et al. 1999; Murakami et al. 2012; Delaney et al. 2013).

Worms

The nematode worm *C. elegans* is a facultative hermaphrodite that hatches from an egg and undergoes four larval stages (L1–L4) before reaching reproductive adulthood. Once adulthood has been reached, a typical hermaphrodite maintained at the standard temperature of 20°C will lay about 200 eggs over a period of 3–5 days before depletion of sperm, followed by an extended postreproductive period of 2–3 wk. With the exception of the germ line, adult *C. elegans* are thought to be completely postmitotic.

Life span in worms is typically defined as the length of time from hatching until death, which is determined manually by the absence of movement on gentle prodding. There are several features to consider when designing *C. elegans* life

span studies, including the temperature (generally 15°C–25°C), the amount, strain, and metabolic state of bacterial food to provide, and whether to use the drug 5-fluorodeoxyuridine (FUDR) to prevent hatching of progeny (Sutphin and Kaeblerlein 2009). Nearly all studies of aging in *C. elegans* use the standard wild-type N2 control strain. Depending on the conditions chosen, N2 life span can range from about 15 days (25°C, live food) to 35 days (15°C, growth-arrested food) (Leiser et al. 2011). In addition to life span, *C. elegans* affords the ability to monitor age-associated measures of health span, such as maintenance of muscle function, tissue atrophy, and accumulation of autofluorescent pigment (Herndon et al. 2002; Huang et al. 2004). As in yeast, there is good evidence that maintenance of protein homeostasis and mitochondrial function play a central role in *C. elegans* aging.

Flies

The fruit fly *D. melanogaster* was the first invertebrate organisms to be widely used in aging research, with life-span studies dating back to 1916 (Loeb and Northrop 1916). The fly life cycle consists of three easily distinguishable growth stages (embryo, larva, and pupae) occurring over a span of 10 days at 25°C, followed by reproductive adulthood. Flies are typically maintained in the laboratory in vials with an agar-based cornmeal–sugar–yeast or sugar–yeast food source. Unlike yeast and worms, methods for creating long-term frozen stocks are not available in *Drosophila*, but stock centers into which researchers deposit published strains as well as large centers that maintain libraries of transgenic, RNAi, and mutant fly lines are a valuable tool to fly researchers.

Life-span studies in *Drosophila* measure the length of time between eclosion (emergence of an adult fly from its pupal case) and death, with a median life span of wild-type strains averaging ~2–3 mo when maintained at 25°C. Because of genetic drift often found in individual laboratory strains and the differences in food composition between laboratories, genetic background and food type need to be tightly controlled in fly

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life-span and behavioral studies (Tatar et al. 2014). The adult fly shows many structures homologous to mammalian organs, such as heart, lung, kidney, gut, and reproductive tract. Importantly, *Drosophila* have a relatively simple nervous system that contains the same basic neural circuitry as mammals, modulating complex behavior and allowing for measures of health span and cognitive function often seen in human aging and neurodegenerative disease. As seen in yeast and worms, strong evidence exists linking protein homeostasis and mitochondrial function to organismal aging and life span (Cho et al. 2011; Rera et al. 2011; Bai et al. 2013).

Mice

The laboratory mouse *M. musculus* has become the premier mammalian model organism for aging research, in large part owing to the availability of several well-characterized inbred strains and the relatively early development of methods for knocking out and transgenically expressing different genes. Although several different inbred strains have been commonly used for aging-related studies, C57BL/6 has become the strain of choice for most mouse aging and longevity studies. Median life span can vary greatly among strains, and there is wide variation in reported life span even for C57BL/6, with median life spans ranging from ~800 to >920 days (Coschigano et al. 2003; Perez et al. 2009). For many years, the National Institute on Aging has provided aged C57BL/6 mice to the research community, which has spurred numerous studies of aging-related traits in this background. In recent years, the importance of quantifying health span measures in addition to life span has become more widely recognized, and mice provide an opportunity to query numerous age-dependent measures of health that are shared with humans but not invertebrate species. The limitations of working in a single inbred strain background have also become better recognized, and the use of genetically heterogeneous mice for aging studies, such as the UM-HET3 four-way cross background, is becoming more common.

CONSERVED LONGEVITY PATHWAYS

Studies from model organisms have identified several orthologous genes that similarly modulate longevity across broad evolutionary distance (Kaeberlein 2007; Sutphin and Kaeberlein 2011). In some cases, the mechanisms by which the corresponding proteins affect aging are poorly understood, although in many cases they can be ascribed to one or more “conserved longevity pathways.” The remainder of this review will discuss the best characterized of these pathways and the growing evidence for complex interrelationships among them.

Mechanistic Target of Rapamycin (mTOR)

mTOR is a serine/threonine protein kinase of the phosphoinositide-3-kinase-related family that is highly conserved among eukaryotes (Keith and Schreiber 1995; Stanfel et al. 2009). mTOR activity generally promotes cellular growth and cell division in response to nutrient and growth factor cues through a complicated network of interactions (Laplanche and Sabatini 2012; Shimobayashi and Hall 2014; Johnson et al. 2015). The mTOR protein acts in at least two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), each of which has distinct components, upstream regulators, and downstream substrates (Weber and Gutmann 2012; Takahara and Maeda 2013; Huang and Fingar 2014). The mTORC1 complex has been characterized extensively and functions as a central regulator of longevity. mTORC1 is known to promote global messenger RNA (mRNA) translation, repress autophagy, and modulate mitochondrial metabolism, with each of these downstream functions implicated in its role in aging (Johnson et al. 2015). The mTORC2 complex, in contrast, is less well characterized. Evidence from *D. melanogaster*, *Dictyostelium discoideum*, and human cells suggest that mTORC2 responds to insulin and Ras signaling (Lee et al. 2005; Sarbassov et al. 2005; Charest et al. 2010), but its precise mechanism of activation remains elusive. mTORC2 regulates the activity of several substrates involved in cytoskeleton reorganization and cell polarity, but

also in cell growth and metabolism (Loewith et al. 2002; Sarbassov et al. 2004, 2005; García-Martínez and Alessi 2008; Ikenoue et al. 2008).

The first evidence that reduced mTORC1 signaling is sufficient to increase life span came from studies in yeast, in which mutation of the S6 kinase homolog Sch9 was shown to extend chronological life span (Fabrizio et al. 2001); although, at the time, the link between Sch9 and mTORC1 was not appreciated. A few years later, genetic inhibition of mTOR itself, as well as other components of the mTORC1 complex, was found to extend life span in worms (Vellai et al. 2003; Jia et al. 2004), flies (Kapahi et al. 2004), and, soon thereafter, replicative life span in yeast (Kaeberlein et al. 2005a).

The importance of mTORC1 in mammalian aging was first documented in studies performed by the National Institute on Aging's Interventions Testing Program (ITP). The ITP is a program designed to test the effect of interventions solicited from the scientific community on life span in the genetically heterogeneous mouse strain UM-HET3 background (Miller et al. 2007; Nadon et al. 2008). In 2009, the ITP reported that treating mice with the mTORC1 inhibitor rapamycin beginning at 600 days of age was sufficient to increase median and maximum life span in both male and female UM-HET3 mice (Harrison et al. 2009). As with genetic inhibition of mTOR, pharmacological inhibition of mTOR with rapamycin has also been found to increase life span in yeast (Powers et al. 2006; Medvedik et al. 2007), worms (Robida-Stubbs et al. 2012), and flies (Bjedov et al. 2010).

Since publication of the initial study showing life-span extension from rapamycin in UM-HET3 mice, the ITP has also reported life-span extension following rapamycin treatment initiated at 9 mo of age (Miller et al. 2011), as well as a partial dose-response trial testing three different doses of the drug, with the highest dose yielding the most robust increase in life span (Miller et al. 2014). Other groups have also reported life-span extension from rapamycin treatment in the C57BL/6 and 129/Sv mouse strain backgrounds (Anisimov et al. 2011; Neff et al. 2013; Zhang et al. 2014). In addition to extending life span, rapamycin also appears to

improve numerous age-related health span parameters, including cancer risk, cardiac function, cognitive function, and immune function (Chen et al. 2009; Halloran et al. 2012; Majumder et al. 2012; Wilkinson et al. 2012; Flynn et al. 2013; Neff et al. 2013; Dai et al. 2014).

In addition to rapamycin treatment, several genetic models of reduced mTORC1 signaling have improved health span and enhanced longevity in mice. Knockout of the *S6k1* gene encoding S6 kinase increases life span in female mice (Selman et al. 2009) and confers enhanced resistance to diet-induced obesity (Um et al. 2004). Mice heterozygous for both mTOR and mLST8, two components of the mTORC1 complex, are also long-lived (Lamming et al. 2012), as are mice expressing hypomorphic alleles of mTOR, which also show preservation of function in many organ systems during aging (Wu et al. 2013).

The complexity of mTOR signaling presents a challenge in untangling the mechanistic basis for the positive effects of mTORC1 inhibition on life span and health span. At a molecular level, there is accumulating evidence that inhibition of mTORC1 increases life span through a combination of differential translation of target mRNAs (for more details, see Zid et al. 2009; Carson et al. 2012), induction of autophagy, and altered mitochondrial metabolism (Fig. 1) (Kaeberlein 2013; Johnson et al. 2015). It remains unclear which of these mechanisms is more important for life-span extension or which tissues and cell types are most critical for mediating their effects on longevity. In mice, a general reduction in inflammation and reduced cancer incidence are also important effects of mTOR inhibition, which contribute to improved longevity and health span. Regardless of the mechanisms of action, the large number of studies documenting life-span extension and improved health span from mTORC1 inhibition provide some expectation that this pathway similarly modulates human aging (discussed below).

Sirtuins

Sirtuins are a family of nicotinamide adenine dinucleotide (NAD⁺)-dependent enzymes

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Figure 1. The growth hormone (GH)/insulin-like growth-factor (IGF)-1-signaling axis and its intracellular mediators. GH released from the pituitary gland stimulates IGF-1 production from the liver (systemic IGF-1) and peripheral tissues (local IGF-1). IGF-1 signals to several conserved players in aging research through the IGF-1 receptor, such as the transcription factor FoxO and the mechanistic target of rapamycin complex 1 (TORC1) kinase complex. These factors regulate cellular growth and homeostasis pathways, which can have a profound effect on longevity and aging phenotypes. GHRH, Growth-hormone-releasing hormone; IRS, insulin receptor substrate; PDK-1, phosphoinositide-dependent protein kinase 1; BP, binding protein; SREBP, sterol receptor element-binding protein.

that catalyze posttranslational modification of proteins—primarily deacetylation (Imai et al. 2000; Landry et al. 2000; Smith et al. 2000), but also adenosine diphosphate (ADP)-ribosylation (Haigis et al. 2006). In some cases, removal of succinyl, malonyl, glutaryl, and perhaps other moieties from lysine residues can also be catalyzed by sirtuins (Nakagawa et al. 2009; Du et al. 2011; Peng et al. 2011; Park et al. 2013; Rardin et al. 2013; Tan et al. 2014). The key biochemical feature that distinguishes sirtuins from other protein deacetylases is their

absolute requirement for NAD^+ , which gets consumed during these reactions. The term “sirtuin” is derived from the budding yeast Sir2 protein, which was the first member of this family of enzymes to be extensively characterized, and was initially identified as a factor required for transcriptional silencing at the heterothallic (*HM*)-mating loci and subsequently at subtelomeric regions and ribosomal DNA in *S. cerevisiae* (Klar et al. 1979; Ivy et al. 1986; Rine and Herskowitz 1987; Aparicio et al. 1991; Fritze et al. 1997; Smith and Boeke 1997). Yeast and flies

have five sirtuin proteins, *C. elegans* has four, and both mice and humans have seven sirtuins (SIRT1-7) (Frye 2000; Giblin et al. 2014).

The importance of sirtuins in aging was first defined in budding yeast, in which overexpression of Sir2 was shown to increase the RLS of mother cells (Kaeberlein et al. 1999). The primary mechanism by which Sir2 promotes replicative longevity in yeast appears to involve increased genomic stability within the rDNA (Gottlieb and Esposito 1989; Kaeberlein et al. 1999), which is composed of dozens of 9.1-kb DNA sequences arrayed in tandem (Petes and Botstein 1977; Philippsen et al. 1978). In addition to enhancing rDNA stability, yeast Sir2 has also been suggested to promote asymmetric retention of damaged proteins within the mother cell, which may also contribute to its prolongevity role (Aguilaniu et al. 2003).

Following the initial report that Sir2 overexpression extends life span in yeast, subsequent studies in both *C. elegans* and *D. melanogaster* reported a similar life-span extension from overexpression of the Sir2 orthologs in those organisms, *sir-2.1* and dSir2, respectively (Tissenbaum and Guarente 2001; Rogina and Helfand 2004). Thus far, no definitive mechanism of action has emerged for how worm and fly sirtuins enhance longevity, and there has been ample controversy over both of the initial reports (Burnett et al. 2011; Viswanathan and Guarente 2011). Although a comprehensive review of the literature on this topic is beyond the scope of this work, the emerging consensus appears to be that the importance of Sir2 orthologs in worm and fly aging is more modest than initially suggested, is likely dependent on specific experimental conditions and genetic background, and that the effects on life span from overexpression may be exquisitely sensitive to dosage (Lombard et al. 2011).

As mentioned above, there are seven mammalian sirtuins with distinct tissue and subcellular distributions, as well as differing biochemical activities (Finkel et al. 2009; Satoh et al. 2011; Giblin et al. 2014). SIRT1 is the mammalian ortholog of yeast Sir2 and has been implicated (both as a protective and as risk factor) in a wide array of age-associated disorders, including

heart failure and cardiovascular disease (Hsu et al. 2008; Luo et al. 2014), different forms of cancer (Firestein et al. 2008; Lim et al. 2010; Srisuttee et al. 2012; Yuan et al. 2013), metabolic syndrome (Li 2013; Chang and Guarente 2014), and various neurodegenerative diseases (Herskovits and Guarente 2014). Whole-body overexpression of SIRT1 does not appear to extend life span in mice, even though it improves some metabolic parameters that may be associated with health span (Bordone et al. 2007). Several different promoters, including a variety of tissue- and cell-type-specific drivers, have also failed to show increased life span from SIRT1 overexpression in mice, although in some cases improved health measures were detected, similar to whole body overexpression (Alcendor et al. 2007; Herranz et al. 2010; Jeong et al. 2012). Recently, a mouse overexpressing SIRT1 specifically in the hypothalamus (BRASTO mice—brain-specific SIRT1 overexpressing) was reported to be both long-lived and have improved health span, suggesting that SIRT1 activity in the brain may be particularly important for healthy aging in mice (Satoh et al. 2013).

Although SIRT1 has been studied most extensively, there is a growing body of literature suggesting that the other sirtuin family members, SIRT2-7, may also modulate healthy aging in mammals. SIRT6, in particular, seems to be important for mouse longevity, as *Sirt6*^{-/-} mice show phenotypes consistent with accelerated aging in some tissues (Mostoslavsky et al. 2006), and male but not female mice overexpressing SIRT6 have extended life span (Kanfi et al. 2012). Life-span extension has thus far not been reported for other sirtuins in wild-type mice; however, overexpression of SIRT2 in short-lived BUBR1 hypomorphic mice can improve cardiac function and partially suppress the life-span defect of male animals (North et al. 2014). SIRT3 is a mitochondrial sirtuin that has also been reported to influence age-related metabolic dysfunction and to promote improved mitochondrial function in a variety of contexts, including during DR (Shi et al. 2005; Nakagawa et al. 2009; Palacios et al. 2009; Schwer et al. 2009; Hirschey et al. 2010; Someya et al. 2010; Hallows et al. 2011).

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A large body of work links pharmacological activation of sirtuins with improved metabolic and health span parameters. The first so-called sirtuin-activating compound (STAC) to be studied was resveratrol, which was reported to increase replicative life span in yeast (Howitz et al. 2003). Resveratrol has also been reported to increase life span in worms and flies (Wood et al. 2004) and to increase survival in short-lived mice fed a high-fat diet (Baur et al. 2006). This work has been clouded, however, by reports that resveratrol does not directly activate Sir2 or SIRT1 (Borra et al. 2005; Kaeberlein et al. 2005b), and by studies that have failed to reproduce the reported life span extension from resveratrol in yeast (Kaeberlein et al. 2005b), worms, or flies (Bass et al. 2007). The ITP has also reported that resveratrol has no effect on life span or health span of UM-HET3 mice fed a normal diet (Miller et al. 2011). Additional studies using second-generation STACs have provided further evidence that pharmacological activation of sirtuins may improve some measures of health span during aging in mice, particularly metabolic deficits associated with normative aging or diet-induced obesity, although with only modest increase in life span (Minor et al. 2011; Mercken et al. 2014; Mitchell et al. 2014).

Adenosine Monophosphate-Activated Kinase

The 5'-AMP-activated protein kinase (AMPK) is an important regulator of cellular energy homeostasis. AMPK coordinates the regulation of metabolic pathways, including glucose uptake and utilization as well as oxidation of fats, in response to changes in the relative abundance of AMP and ATP. AMPK is activated by AMP and inhibited by ATP, making it directly responsive to the AMP/ATP ratio, an indicator of energy availability in the cell (Hardie 2007). Several cellular stresses activate AMPK by affecting the AMP/ATP ratio, including starvation, impaired mitochondrial respiration, and hypoxia. Other stresses, such as DNA damage, inhibit AMPK without affecting the AMP/ATP ratio (Budanov and Karin 2008).

Most studies examining the role of AMPK in aging suggest that activation of AMPK is associated with increased longevity and improved health span. Overexpression of one of two worm orthologs of the AMPK- α subunit, AAK-2, is sufficient to extend life span, and deletion of *aak-2* prevents life-span extension in response to certain forms of DR (Apfeld et al. 2004; Schulz et al. 2007; Greer and Brunet 2009). More recently, it has been shown in flies that activation of AMPK in neurons or intestine increases life span and slows aging in a non-cell-autonomous manner (Ulgherait et al. 2014). In mice, chronic activation of AMPK protects against diet-induced obesity (Yang et al. 2008), and two related AMPK activators, phenformin and metformin, have also been reported to enhance longevity in mice (Dilman and Anisimov 1980; Anisimov et al. 2003; Martin-Montalvo et al. 2013) and worms (Onken and Driscoll 2010). Metformin is the most widely prescribed antidiabetic drug in the world. It clearly enhances life expectancy for diabetic patients, and there is some suggestive evidence that diabetics taking metformin may live longer than nondiabetics (Bannister et al. 2014).

One of the challenges associated with interpreting the effects of metformin is that, unlike rapamycin, it is a relatively "dirty drug." The mechanism by which metformin activates AMPK is indirect and poorly understood, and it is unclear whether the effects of metformin on life span and health span are directly mediated solely through AMPK or also through "off-target" effects. For example, metformin is also an inhibitor of complex I of the mitochondrial electron transport chain (El-Mir et al. 2000; Owen et al. 2000) and, in worms, has been suggested to increase life span through reducing folate production by the gut microbiota (Cabreiro et al. 2013) and through activation of the peroxiredoxin PRDX-2 via a mitochondrial stress response (mitohormesis, discussed below) (De Haes et al. 2014).

Insulin-Like Growth Factor 1 (IGF-1) Signaling

The insulin/IGF-1-like signaling axis (IIS) is perhaps the best-studied longevity pathway,

and is known to regulate longevity in worms, flies, mice, and perhaps humans. The genetic and biochemical features of this pathway were first worked out in *C. elegans*, and later studies showed a similar overall structure in flies and mammals (Fontana et al. 2010; Kenyon 2011). Insulin and insulin-like peptides are the main determinants of development, growth, and body size in animals, with highly conserved genes across the evolutionary spectrum. In invertebrate animals, the insulin receptor regulates the response to nutrients as well as growth and development (Fernandez et al. 1995; Kimura et al. 1997; Edgar 2006). In vertebrates, insulin generally regulates nutrient consumption and storage, and IGF-1 promotes growth and proliferation (Upton et al. 1997; Reinecke and Collet 1998). IGF-1 is also responsive to nutrients, although indirectly: nutrients stimulate production of the hypothalamic growth-hormone-releasing hormone (GHRH) (Gelato and Merriam 1986) and Ghrelin, a hormone produced by the digestive system (Kojima et al. 1999). These two hormones signal to the somatotrophic cells of the anterior pituitary gland and promote the release of growth hormone (GH) (Forsyth and Wallis 2002), which, in turn, stimulates the production of IGF-1 in the liver and in peripheral tissues. Ghrelin, GHRH, and GH add a further level of complexity to growth, size, and aging regulation in vertebrates.

IGF-1 promotes cell growth, proliferation, and cell-cycle progression by engaging the IGF-1 receptor (IGF-1R). The IGF-1R activates the TOR pathway (Oldham and Hafen 2003), promotes cell survival via AKT (Song et al. 2005), curtails stress responses and cell-cycle arrest by inhibiting the transcription factor FoxO (Stitt et al. 2004), and can activate the mitogen-activated protein kinase (MAPK) pathway through p66^{Shc} (Migliaccio et al. 1999; Giorgio et al. 2012).

The IIS was first implicated in aging with the discovery that loss-of-function mutations in the *C. elegans* phosphatidylinositol-3-kinase gene *age-1* and the insulin-like receptor gene *daf-2* could double life span, and this life-span extension was dependent on the FOXO-family transcription factor DAF-16 (Friedman and Johnson 1988; Kenyon et al. 1993; Dorman et al.

1995). Both *daf-2* and *daf-16* had been previously implicated in control of entry into the *C. elegans* dauer, a stress-resistant developmentally arrested alternative developmental state (Vowels and Thomas 1992). At the time their role in longevity control was discovered, the genes for *age-1*, *daf-2*, and *daf-16* had not yet been cloned, and it was not until a few years later that the homologies to the mammalian proteins were appreciated (Morris et al. 1996; Kimura et al. 1997; Ogg et al. 1997). Additionally, components of the pathway were subsequently identified in worms (Tissenbaum and Ruvkun 1998), and similar effects were reported for mutations that reduce IIS in flies (Clancy et al. 2001; Tatar et al. 2001; Tu et al. 2002).

In mice, longer life span and delayed aging have been recorded in Ames dwarf, Snell dwarf, GH-receptor knockout (GHRKO), GHRHKO, GHRH receptor mutants (GHRH^{lit}), and, to a lesser extent, in IGF-1R^{+/-} mice (Brown-Borg et al. 1996; Coschigano et al. 2000; Flurkey et al. 2001; Holzenberger et al. 2003; Liou et al. 2013) and IGF-1-deficient mice (Lorenzini et al. 2013). Ames dwarf and Snell dwarf mice produce low amounts of GH, prolactin, and thyroid-stimulating hormone owing to mutations that impair the development of the anterior pituitary gland, and together with GHRKO mice have extremely reduced levels of circulating IGF-1 (Bartke 2005). Conversely, IGF-1 levels are elevated in IGF-1R^{+/-} mice, probably because of lack of negative feedback on GH release, but intracellular signaling through AKT and p66^{Shc} is dampened in these animals (Holzenberger et al. 2003). IIS mutant mice display higher levels and activity of antioxidant enzymes (Brown-Borg et al. 1999; Brown-Borg and Rakoczy 2000; Hauck and Bartke 2000) and their cells show increased resistance to oxidative stress in culture. In fact, reducing IIS in mice inhibits p66^{Shc}, activates the phase 2 antioxidant response via NFE2L2 (Nrf2) (Holzenberger et al. 2003; Murakami et al. 2003; Salmon et al. 2005; Leiser and Miller 2010; Sun et al. 2011), and possibly activates the FoxO transcription factor (Nemoto and Finkel 2002). Furthermore, reduced IIS protects mice against age-related pathologies, such as Alzheimer's disease (Cohen et al. 2009).

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In addition to increased stress resistance, animals with defective GH signaling have altered metabolism, as evidenced by increased insulin sensitivity and glucose tolerance, reduced fat accumulation, resistance to high-fat diets, and increased oxidative metabolism and fatty acid oxidation, especially at older ages (Bartke and Westbrook 2012). Some of these phenotypes are recapitulated in IGF-1R^{+/-} and IGF-1-deficient mice, such as resistance to high-fat diets and reduced fat accumulation, even though both of these models present slight impairments in glucose homeostasis (Holzenberger et al. 2003; Salmon et al. 2015). Because metabolism is regulated by a limited number of endocrine organs in metazoans, these observations suggest that IIS influences aging both via cell-autonomous mechanisms, such as stress resistance, and nonautonomous ones. This dual role for IIS is supported by several bodies of evidence. First, life-span extension and delayed aging are more prominent in pituitary-deficient and GHRKO mice, which have specific defects in the somatotrophic axis, than in the general, broad-targeting, IGF-1R^{+/-} and IGF-1-deficient mice, in which the beneficial effects of the mutations are reduced in magnitude and mostly restricted to female animals (Holzenberger et al. 2003; Lorenzini et al. 2013). Second, mutations in the IIS pathway can extend life span even when they occur only in select endocrine organs, such as adipose tissue in flies and mice, or intestine and neurons in *C. elegans*, thus suggesting that reducing specific endocrine signaling is as effective as a general suppression of growth-factor signaling (Kenyon 2005). This may explain the apparent dichotomy between the specific effects of GH and IGF-1 supplementation on one side, and the long-lived phenotype of reduced GH and IGF-1-signaling mutants on the other side. Although reducing systemic GH and IGF-1 signaling promotes longevity by improving metabolism and raising cellular defenses to stress, high local levels of IGF-1 could protect specific tissues against damage and apoptosis, which is especially detrimental in postmitotic tissues, such as muscle, heart, and brain (Bartke 2008). Interestingly, Ames mice show increased levels of IGF-1

in the brain (Sun et al. 2005a,b), and IGF-1 protects against age-related decline in heart function (Moellendorf et al. 2012), further supporting this hypothesis. This evidence notwithstanding, elevated local IGF-1 may still have detrimental effects for longevity, as indicated by the long-lived phenotype of mice lacking the pregnancy-associated plasma protein A (PAPP-A), a serum metalloprotease that increases peripheral activity of IGF-1 by cleaving IGF-1-binding proteins (IGF-BPs) (Conover and Bale 2007). The impact of GH and IGF-1 on longevity may thus be exquisitely tissue specific. GHR tissue-specific knockout mice have been developed, including fat, macrophage, liver, muscle, and β -cell-specific knockouts, and it will be of particular interest to see which of these animals have altered longevity and health span (Sun and Bartke 2013).

Mitochondrial Stress and Antioxidants

Mitochondrial function has long been thought to be important for healthy aging. Indeed, the first molecular theory of aging proposed that free radicals, produced in part through mitochondrial metabolic processes, were the primary cause of age-related damage and declines in cellular function (Harman 1956). In recent years, the idea that mitochondrial oxidative stress has a purely negative effect on age-related parameters has been revised, based on a growing body of evidence supporting the idea that mitochondrial stress and reactive oxygen species (ROS) can also induce protective mechanisms in addition to damage (Bennett and Kaeblerlein 2014), an idea referred to as “mitohormesis” (Ristow and Zarse 2010). In addition, several life-span-extending mutations have been identified that impair mitochondrial function, suggesting a complex relationship between longevity and mitochondria.

Loss-of-function alleles of the genes encoding the Rieske iron sulfur protein *isp-1* and the coenzyme Q biosynthetic gene *clk-1* increase life span in worms (Felkai et al. 1999; Feng et al. 2001). Furthermore, genome-wide RNAi studies in *C. elegans* provide direct evidence that decreasing mitochondrial respiration enhances

longevity (Hwang et al. 2012; Yanos et al. 2012). RNAi clones corresponding to numerous components of the mitochondrial electron transport chain were found to extend life span when knockdown was initiated during development (Dillin et al. 2002; Lee et al. 2003). Limited examples of life-span extension from inhibition of mitochondrial function have also been described in fruit flies (Copeland et al. 2009; Owusu-Ansah et al. 2013) and mice (Hughes and Hekimi 2011; Wang et al. 2012), although these appear to be far less common than in *C. elegans*. This may be partially explained by the lack of apoptosis in adult *C. elegans*, which allows worms to maintain somatic tissue in the presence of increased oxidative damage. In all cases, severe mitochondrial dysfunction leads to reduced survival, consistent with the central idea of mitohormesis that a little mitochondrial stress is protective, although a large degree of mitochondrial stress is detrimental. This concept has been further tested in studies showing that treatment with low doses of paraquat, which produce mitochondrial ROS in the form of superoxide, is sufficient to enhance longevity in worms (Yang and Hekimi 2010; Hwang et al. 2014).

Although inhibition of mitochondrial respiration appears to be a conserved pro-longevity strategy, the mechanistic basis for this life-span extension remains obscure, and the degree to which similar mechanisms are engaged in different organisms is unclear (Bennett et al. 2014a). It was initially suggested that mitochondrial inhibition extends life span in *C. elegans* through generation of a neuronal signal that is propagated to the intestine and other tissues to induce the mitochondrial unfolded protein response (UPR^{mt}), which up-regulates mitochondrial-specific chaperones and proteases (Durieux et al. 2011). The neuronal mitochondrial signal has not yet been identified; however, one study in flies suggests that mitochondrial dysfunction in muscle can also induce a pro-longevity signal in that organism (Owusu-Ansah et al. 2013). The model that the UPR^{mt} promotes longevity has since been weakened, however, with the finding that induction of the UPR^{mt} is neither necessary nor sufficient for

life-span extension in worms (Bennett et al. 2014b). Despite the lack of causal evidence that the UPR^{mt} mediates enhanced longevity, mitochondrial stress in long-lived flies and mice is capable of activating the UPR^{mt} in certain tissues, akin to *C. elegans* (Owusu-Ansah et al. 2013; Pulliam et al. 2014). Thus, the UPR^{mt} may be one response that is induced to regulate mitochondrial proteostasis in conjunction with others that directly promote longevity.

Several groups have attempted to define the mechanistic basis for life-span extension from electron transport chain (ETC) inhibition in *C. elegans*, resulting in the identification of multiple transcription factors that are required for enhanced longevity in a subset of cases. These include AHA-1, CEH-18, CEH-23, CEP-1, GCN-2, HIF-1, JUN-1, NHR-27, NHR-49, and TAF-4 (Ventura et al. 2009; Lee et al. 2010a; Walter et al. 2011; Baker et al. 2012; Khan et al. 2013). Of these, hypoxia-inducible factor 1 (HIF-1) is particularly interesting, as HIF-1 serves as a major transcriptional regulator of the hypoxic response, which is highly conserved in metazoans (Leiser and Kaerberlein 2010). HIF-1 is activated in response to low levels of ROS produced by some forms of mitochondrial inhibition (Lee et al. 2010a), and activation of HIF-1, either through genetic manipulation or hypoxic conditioning, is sufficient to extend the life span in *C. elegans* (Mehta et al. 2009; Muller et al. 2009; Zhang et al. 2009a; Leiser et al. 2011, 2013). In addition, HIF-1 can be activated in mammalian cells by metabolites that are enriched in *C. elegans* mitochondrial mutants, suggesting multiple mechanisms that may impinge on HIF-1 during mitochondrial dysfunction (Lu et al. 2005; Butler et al. 2013). Chronic activation of HIF-1 in humans causes Von Hippel–Lindau syndrome and is, therefore, unlikely to improve longevity or health span (Ivan and Kaelin 2001). However, it remains possible that tissue-specific activation of HIF-1 or activation of a subset of HIF-1 target genes could be beneficial for longevity in mammals. Recently, another mechanism for life-span extension from inhibition of respiration in *C. elegans* has been proposed involving the activation of the intrinsic apoptotic pathway

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(Yee et al. 2014), but whether this mechanism plays any role in aging in other organisms is yet to be determined.

Another aspect of mitohormesis that should be considered is the role of antioxidant systems in coping with oxidative stress. Studies implicating mitohormesis with increased antioxidant capacity were first described in *C. elegans* using both inhibitors of glycolysis and knockdown of *daf-2* (Schulz et al. 2007; Zarse et al. 2012). These interventions were found to increase mitochondrial respiration, cause a burst of ROS, and increase cellular antioxidant activity. This response and the associated life-span extensions were proposed to be mediated by SKN-1 and PMK-1. SKN-1 is the worm homolog of the NFE2L2 (Nrf2) transcription factor of the phase 2 detoxification response, which controls expression of antioxidant and glutathione biosynthesis genes, whereas PMK-1 is a p38 MAPK homolog that regulates SKN-1 nuclear localization and activity under oxidative stress conditions (Inoue et al. 2005). Overexpression of SKN-1 is sufficient to extend life span in *C. elegans* (Tullet et al. 2008). Additionally, flies with reduced expression of the Nrf2 negative regulator Keap1 show enhanced paraquat resistance and life-span extension (Sykiotis and Bohmann 2008).

The role of Nrf2/SKN-1 in aging of mammals is correlative but compelling. In rodents, Nrf2 activity decreases with age (Suh et al. 2004; Shih and Yen 2007), conceivably leading to increased oxidative damage. In addition, DR and many DR mimetics activate Nrf2 and increase antioxidant capacity of mice (Martin-Montalvo et al. 2011). However, Nrf2 KO mice still receive benefits from DR, such as increased insulin sensitivity and similar increased life span compared with wild-type controls (Pearson et al. 2008). Whether Nrf2 mediates any of the health benefits of the long-lived mitochondrial mutant mice models is still an open question. For example, mice lacking the ETC chaperone Surf1 show Nrf2 activation in cardiac tissue (Pulliam et al. 2014), but whether this is beneficial to cardiac aging or is just an indicator of mitochondrial dysfunction in this background is unknown. Nevertheless, activation of Nrf2 and

other antioxidant pathways is a common feature of several long-lived mouse models (Leiser and Miller 2010; Sun et al. 2011; Michael et al. 2012), and Nrf2 mediates at least some of the longevity effects of rapamycin in vitro (Lerner et al. 2013), suggesting that this transcription factor may actually play an active role in enhancing health span.

A similar case could be drawn for p66^{shc}, a mediator of GH/IGF-1 signaling (see above) that regulates oxidative stress responses. Although its ablation increases resistance to oxidative and genotoxic stress, and was shown to extend life span in laboratory mice (Migliaccio et al. 1999), more recent studies have brought into question the long-lived phenotypes of p66^{shc} knockout mice (Giorgio et al. 2012; Ramsey et al. 2014), suggesting a complex relationship between oxidative stress, stress resistance, and longevity.

For many years, it was conventional wisdom that antioxidants should have a beneficial effect on longevity and health. The mitohormesis model and supporting data would suggest this idea is overly simplistic, and the actual data showing a direct relationship between exogenous treatment with antioxidants and longevity or health span in any species is quite limited. In mammals, the strongest evidence in support of the idea that boosting antioxidant capacity can improve healthy aging comes from studies of mice transgenically expressing mitochondrial targeted human catalase (MCAT). Catalase catalyzes the breakdown of H₂O₂ and normally functions within the peroxisome. MCAT mice show increases in life span and decreases in cardiac pathology, inflammation, and tumor burden (Schriner et al. 2005; Dai et al. 2009; Lee et al. 2010b). Furthermore, MCAT mice maintain better muscle function with age owing to reduced oxidation of ryanodine receptor 1, the Ca²⁺ release channel of the sarcoplasmic reticulum involved in muscle contraction (Uman-skaya et al. 2014). Ectopic expression of other antioxidant enzymes, such as thioredoxin-1, thioredoxin reductase, Mn superoxide dismutase (MnSOD), copper zinc superoxide dismutase (CuZnSOD), or nonmitochondrial catalase, does not cause robust life-span extension in fruit

flies or mice, even in cases of combinatorial expression (Mitsui et al. 2002; Orr et al. 2003; Jang et al. 2009; Perez et al. 2009, 2011). In both mice and worms, deletion of antioxidant enzymes in many cases does not shorten life span, despite causing substantial increases in oxidative damage to cells and tissues and striking sensitivity to oxidative stress (Van Raamsdonk and Hekimi 2009; Zhang et al. 2009b). Taken together, the bulk of data would seem to suggest that antioxidant capacity does not seem to be generally limiting for life span of wild-type organisms. However, increased antioxidant capacity or improvements in redox homeostasis, perhaps in specific cellular compartments, such as mitochondria, can improve longevity and health during aging at least in some situations.

CONNECTIONS BETWEEN CONSERVED ANTIAGING PATHWAYS: TOWARD A NETWORK VIEW OF AGING

As mentioned in the introductory section, one of the general features of known conserved longevity pathways is that they tend to regulate the relationship between environmental cues and growth and reproduction. Therefore, it is not surprising that all of the major conserved longevity pathways have been proposed to play key roles in mediating the beneficial effects of DR. In nonmammalian models, the case can best be made for inhibition of mTOR acting downstream from DR, as mTOR is a direct sensor of amino acids (Bar-Peled and Sabatini 2014), and studies in yeast, worms, and flies have indicated that inhibition of mTOR, or downstream processes regulated by mTOR, such as autophagy, are both necessary and sufficient for life-span extension from DR (Kapahi et al. 2004; Kaeberlein et al. 2005a; Jia and Levine 2007; Morck and Pilon 2007; Zid et al. 2009). In mammals, the situation is probably more complex, and initial studies suggest that DR and rapamycin induce overlapping but distinct changes, suggesting that rapamycin treatment does not mimic every aspect of DR or vice versa (Fok et al. 2014).

IIS and DR increase life span through overlapping but also distinct mechanisms. DR and

reduced IIS affect growth, metabolism, endocrine signaling, and stress resistance in a similar way (Bartke et al. 2001; Yamaza et al. 2010), yet DR can further increase life span in Ames mice (Bartke et al. 2001), GHRHKO (Liou et al. 2013), and, to a lesser extent, in GHRKO mice (Bonkowski et al. 2006), suggesting that DR engages other pathways in addition to IIS. Similarly, DR increases life span in *dFoxO*^{-/-} flies, although not to the extent of wild-type animals. In worms, several studies have reported that different methods of DR are able to extend life span in a *daf-16* null background, although removal of food is sufficient to activate DAF-16 (Henderson and Johnson 2001), suggesting that the IIS pathway is engaged by DR in worms.

Whether rapamycin or other DR mimetics further extend the life span of IIS-mutant or dietary-restricted mammals is still unknown. Preliminary evidence suggests that DR inhibits mTOR to promote the function of at least one stem-cell niche, the Paneth cells of the intestine (Yilmaz et al. 2012). In addition, *S6K1*^{-/-} mice show several features typical of the IIS mutants or of mice under DR, such as reduced size, better insulin sensitivity, and improved glucose tolerance at old ages, and have a gene expression profile that overlap with *IRS1*^{-/-} and dietary-restricted mice (Selman et al. 2009). Together with the observation that Ames dwarf mice have reduced mTOR signaling (Sharp and Bartke 2005), this evidence further suggests that DR, IIS, and TOR extend longevity through overlapping pathways in mice too.

A role for sirtuins in DR was first suggested based on studies in yeast showing that deletion of Sir2 prevents replicative life-span extension from DR (Lin et al. 2000). Based on this observation, Guarente and colleagues proposed that DR might activate sirtuins by increasing the availability of NAD⁺, a substrate of sirtuin-mediated deacetylation (Guarente and Picard 2005). The generality of this model has been weakened by subsequent reports that neither Sir2 nor the other yeast sirtuins are absolutely required for replicative life-span extension from DR in yeast, and that overexpression of Sir2 further increases life span in combination with DR (Kaeberlein et al. 2004, 2006; Tsuchiya et al.

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2006). Likewise, Sir2 is not required for chronological life-span extension from DR in yeast, and *sir-2.1* is not required for life-span extension from several different methods of DR in *C. elegans* (Kaeberlein and Powers 2007). In contrast, studies in mice have generally supported the idea that sirtuins, particularly SIRT1 and SIRT3 are activated in some tissues by DR and may be required for some of the health benefits associated with this dietary regimen (Giblin et al. 2014).

There is abundant evidence that mitochondrial metabolism is altered in response to DR, although whether this is mechanistically related to the life-span extension seen in respiratory-deficient animals is unclear. In *C. elegans*, inhibition of electron transport chain components only extends life span when knockdown is accomplished during development (Rea et al. 2007), whereas DR extends life span in *C. elegans* when initiated during adulthood (Smith et al. 2008b), suggesting that these are distinct longevity pathways. In yeast, mitochondrial biogenesis and mitochondrial respiration are increased in response to DR (Lin et al. 2002), and similar effects have been reported in some tissues of mice and in cultured mammalian cells (Nisoli et al. 2005; Lopez-Lluch et al. 2006), further supporting the idea that mitochondrial inhibition extends life span by a mechanism distinct from that of DR.

AMPK has also been implicated in mediating mitochondrial and metabolic adaptation to DR. In worms, most forms of DR are dependent on *aak-2* (Greer and Brunet 2009) and AMPK is necessary for the beneficial effects of DR on cardiac function in mouse (Chen et al. 2012). Furthermore, metformin, an AMPK activator, induces metabolic changes in mouse liver and skeletal muscle consistent with those seen under caloric restriction, although not completely overlapping (Martin-Montalvo et al. 2013). These observations indicate that DR may exert some of its beneficial effects through AMPK, although compelling evidence is still lacking, especially in mammals.

Importantly, all of the pathways described above interact with each other, making it difficult and perhaps unrealistic to pinpoint any

individual factor as the sole or even the major mediator of DR. For example, both IGF-1 and AMPK can modulate the TOR pathway, and all three pathways can influence mitochondrial function and metabolism independently and in concert with one another (see above). Similarly, cross talk between the TOR pathway and several sirtuins has been found in vitro (Csibi et al. 2013; Hong et al. 2014), several proteins of the IGF-1R-signaling pathway are subject to deacetylation by sirtuins in vitro (Zhang 2007; Sundaresan et al. 2011), and AMPK can promote sirtuin activity by increasing the levels of NAD⁺ in mouse skeletal muscle (Canto et al. 2009). In addition, mitochondrial dysfunction and ROS can activate TOR independent of growth factors and nutrients (Nacarelli et al. 2014). Together, these observations suggest that age-delaying interventions are likely to engage multiple pathways to some extent and that, although single gene perturbations may have sizable effects in term of health and longevity, no single factor is solely responsible for long-lived and healthy aging phenotypes. Importantly, all of the above age-related pathways increase the activity of homeostatic mechanisms, such as autophagy and stress-resistance and detoxification enzymes. The importance of these mechanisms for aging phenotypes, as well as the cells and tissue most affected by their activity, are currently object of intense research and will likely help fill in the blanks on the network of interaction that affect aging and age-related diseases.

CONCLUSIONS AND FUTURE DIRECTIONS

Over the past few decades, there have been numerous successes at identifying genetic factors that appear to modulate the rate of aging in evolutionarily divergent organisms. We now know of several conserved pathways and gene families that are key modulators of aging, and a picture of the network in which they interact is beginning to emerge. In the case of rapamycin, these advances have progressed to the point where we have a pharmacological intervention with the ability to extend life span and delay age-related declines in function in organisms

from yeast to worms. Going forward, we anticipate these trends to continue, with the identification of additional longevity-promoting interventions and further advances in understanding the molecular mechanisms of aging.

A key challenge, however, will be developing strategies to successfully translate these discoveries to improve human health span. One approach is to attempt to validate the efficacy of interventions, such as rapamycin, to treat age-related diseases in patients. Although this type of approach may be successful, it is unclear whether any benefits will be obtained from delaying aging in individuals whose health has already deteriorated to the point where clinical diagnosis has occurred. A more promising approach is to test these interventions as preventative, or even rejuvenating, therapies in people. One good example of this is a recent placebo controlled study in which short-term treatment with low-dose rapamycin was found to improve the immune response to an influenza vaccine of otherwise healthy elderly people (Mannick et al. 2014). Although it remains unclear whether these effects are mechanistically related to the life-span extension seen in mice following rapamycin treatment, this parallels prior work showing a similar rejuvenation of immune function in aged mice (Chen et al. 2009). Although this study does not show that the other positive effects of rapamycin on longevity and health span in mice will also be seen in humans, it is highly suggestive.

In addition to examining the potential benefits of rapamycin as a preventative measure for select age-related phenotypes in humans, we have recently proposed that a more comprehensive evaluation of rapamycin in a large mammal can be accomplished using the domestic dog *Canis lupus familiaris* (Check Hayden 2014). Companion dogs, in particular, have several advantages as a model for human aging over laboratory organisms. These include a breadth of phenotypic and genetic diversity across breeds, a high quality of veterinary care and expertise, exposure to most of the same environmental conditions as people, susceptibility to many of the same diseases of aging as people, and life spans that are amenable to experimental time

frames. For example, if rapamycin treatment initiated in middle age extends life span and health span in dogs similarly to the effects seen in mice, we might expect the average life span of a cohort of large-size dogs to increase from about 9 years to about 11 years if treatment were initiated at 6–7 years of age. Thus, a 5-year study would be more than sufficient to document these effects if sufficiently powered.

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