Chapter 20
Aging: Evolutionary Theory Meets Genomic Approaches

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Abstract Modern evolutionary theory describes aging as the result of an accumulation of late-acting, deleterious genes caused by reduced force of natural selection late in life, combined with selection for genes that are beneficial early in life but damaging late in life. Theories based on this logic predict that organisms will be optimized for overall fitness as opposed to maximum longevity. Recent advances in genomics combined with large-scale methods for single gene knockout in several common aging models have allowed the first genome-wide studies of life span. These studies provide insight into several aspects of the biology of aging that relate to evolution, including the scope of cellular processes that influence longevity and the conservation of longevity determinants between organisms. Here we review the evolution of the aging field over the past several years and the implications of the move toward genomics. We also highlight key results and discuss their importance and relation to evolutionary theories of aging.

20.1 Introduction

"When one or more individuals have provided a sufficient number of successors they themselves, as consumers of nourishment in a constantly increasing degree, are an injury to those successors. Natural selection therefore weeds them out, and in many cases favors such races as die almost immediately after they have left successors" (Alfred Russel Wallace, 1865–1870).

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Aging is commonly defined as a degenerative process characterized by a progressive decline in fitness resulting in mortality. How does a process that ultimately results in the death of each individual in a species evolve by natural selection? The first attempts by evolutionary biologists to answer this question followed closely on the heels of the theory of natural selection itself. The earliest written argument was made by Alfred Russel Wallace in an informal note, the essence of which is captured in the above quote (Wallace 1889). August Weismann, a German biologist and evolutionary theorist, expanded Wallace's ideas into a theory describing aging as a programmed process to end an organism's life in the absence of accident or predation in order to make way for succeeding generations (Weismann 1889). In modern evolutionary biology, aging is viewed not as an advantageous trait that is selected for directly but as a side-effect of a decline in the force of natural selection with increasing age combined with selection for other traits. Although the early "programmed aging" theories have lost favor, they illustrate the magnitude of the problem aging posed to evolutionary theory.

Invertebrate organisms have emerged as the preeminent model systems in aging research. The most prominent are the budding yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, which share a number of characteristics that make them ideal for aging research: relatively short life span, rapid production of large numbers of offspring, ease of maintenance and manipulation in the laboratory environment, well characterized biology, fully sequenced genomes and the availability of powerful genetic tools. A great deal of effort has gone into the identification and characterization of interventions and genetic pathways that influence longevity in these systems. Much of this work has been accomplished by looking at secondary age-associated phenotypes, such as stress resistance and fecundity, or by looking for genes associated with pathways already known to influence aging. This approach has yielded valuable insight and an understanding of specific pathways and processes that influence life span but does not inform with respect to the total number of genes and pathways that affect aging. Are there only a few aging genes or many? The past decade has seen the creation of an open reading frame (ORF) deletion collection in yeast and RNAi libraries in nematodes and fruit flies, allowing for the first time the development of unbiased methods for looking at life span on a genomic-scale.

The desire to apply findings from studies in diverse organisms to human aging raises another central question: to what degree are the molecular mechanisms involved in the determination of life span conserved between evolutionarily divergent organisms? A number of interventions have been identified that extend life span in divergent organisms, including dietary restriction (Chapman and Partridge 1996; Fabrizio et al. 2004; Good and Tatar 2001; Jiang et al. 2000; Lakowski and Hekimi 1998; Lin et al. 2000; McCay et al. 1935), reduced insulin/IGF-1-like signaling (IIS) (Bluher et al. 2003; Holzenberger et al. 2003; Kenyon et al. 1993; Tatar et al. 2001), increased sirtuin activity (Kaeberlein et al. 1999; Rogina and Helfand 2004; Tissenbaum and Guarente 2001) and reduced target of rapamycin (TOR) signaling (Kaeberlein et al. 2005; Kapahi et al. 2004; Powers et al. 2006;

Vellai et al. 2003). Results from the first genome-wide longevity studies indicate that a large number of genes are likely to play a role in longevity and provide the first quantitative evidence for evolutionary conservation of longevity determinants. Here we discuss the transition of the aging field into genome-scale research and the implications of recent findings in the context of evolutionary aging theory.

20.2 The Evolution of Aging: Why Not Immortality?

The models proposed by Wallace and Weismann describe aging as a programmed process that evolved through direct selection on senescence as a beneficial trait (Weisman 1889). These models suffer from a reliance on group theory and do not provide an explanation for why an individual with a mutation that confers increased life span—and thus an increased opportunity to produce offspring—would not be selected over individuals without such a mutation (Kirkwood 2005). This view of aging was challenged in 1952, when Peter Medawar proposed the "mutation accumulation" theory, revolutionizing the way most biologists think about the evolution of aging (Medawar 1952). The underlying reasoning is based on the observation that, even in the absence of aging or other intrinsic decline, most species experience a substantial rate of mortality from external forces such as accident, disease, or predation. For a given species, fewer individuals live to progressively older ages, diminishing the force of natural selection in an age-dependent manner and resulting in stronger selection against genes that are deleterious early in life relative to genes that are deleterious late in life (Fisher 1930; Haldane 1941). The mutation accumulation theory states that genes with late-acting deleterious effects will accumulate in the germline, resulting in an increase in mortality with age (Medawar 1946, 1952).

George C. Williams refined Medawar's reasoning by incorporating the concept of pleiotropy. In the "antagonistic pleiotropy" model of aging, age-dependent increase in mortality is caused by an accumulation of genes that function to the benefit of the organism early in life but become deleterious with advanced age, thus providing a means by which senescence can be selected for indirectly (Williams 1957). A third related theory, proposed by Thomas Kirkwood and termed "disposable soma," states that natural selection will favor genes that promote redirection of resources from maintenance of soma to reproduction, resulting in an accumulation of damage that increases with age (Kirkwood 1977).

The theories of mutation accumulation, antagonistic pleiotropy and disposable soma all represent aging as a result of negligible natural selection with advanced age rather than a programmed process. An important extension of these arguments is that natural selection is concerned with overall fitness, which does not necessarily correlate with enhanced longevity (Kirkwood and Holliday 1979). Organisms should therefore possess genes that optimize fitness and not maximize life span.

20.3 Measuring and Interpreting Life Span Phenotypes

Overall fitness may provide an explanation for why we age, but to develop an understanding of how we age, longevity is the most relevant characteristic. However, life span is a complex phenotype and interpretation can be complicated. From a technical standpoint, one issue arises from variation in the definition of life span in different organisms. At what point do we say something has died? When dealing with macroscopic animals the answer to this question is fairly straight forward. An animal is considered dead at the point of failure of the majority of its macro-scale systems (e.g. the respiratory system or the circulatory system). Individual cells that are technically still alive will only remain so for a period of time that is short relative to the life span of the animal. To an extent this is also true for smaller animals that are on the verge between the micro- and macroscopic worlds, such as worms and flies. While the failure of major systems is more difficult to judge, by general acceptance these animals are considered dead at the point when they fail to respond to external stimuli.

The path is less clear for single cell systems. *S. cerevisiae* is one of the most prominent organisms in aging research, but at what point can you say a yeast cell is dead? Two models of aging have been developed in yeast, one mitotic and one non-mitotic (Steinkraus et al. 2008) (Fig. 20.1). The first, termed replicative life span (RLS), measures the number of cell divisions an individual cell completes before undergoing replicative senescence (Mortimer and Johnston 1959). The second, termed chronological life span (CLS), measures the duration of time that a cell remains viable in a growth arrested state (Fabrizio and Longo 2003). RLS might be considered analogous to aging in mitotic tissue, such as skin and blood and CLS to aging in non-mitotic tissue, such as heart or brain, although it is unclear if this analogy, based on the proliferative potential of mammalian tissues, truly holds.

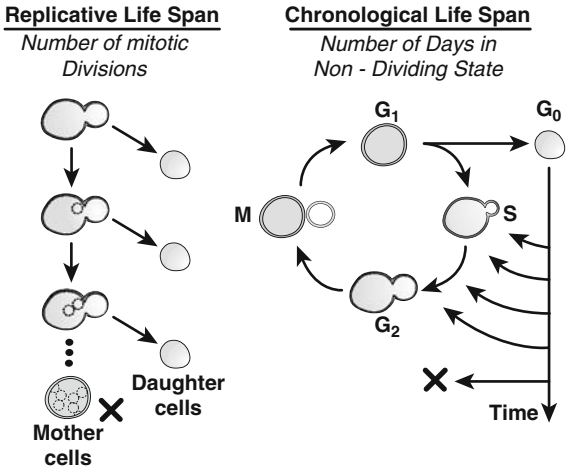

Fig. 20.1 Yeast aging models. Two models of aging are used in yeast. Replicative life span (*left*) is a measure of the number of cell divisions a cell undergoes before undergoing replicative senescence. Chronological life span (*right*) is a measure of the number of days a cell remains viable in a post-replicative state

Notably, accumulating evidence suggests a considerable degree of conservation in factors that influence aging, even between mitotic models, such as yeast replicative aging, and primarily non-mitotic models, such as *C. elegans*, in which only the germline is mitotically active during adulthood. Inter-organism conservation of longevity determinants is discussed in detail below.

A second complexity in interpreting life span relates to the inherent interdependence of the genetic pathways that converge on longevity. At least three pathways are known to modulate life span in diverse organisms: IIS, sirtuins and TOR signaling. While these pathways are at least partially independent, they interact both upstream, by responding to similar environmental queues and downstream, by influencing overlapping sets of downstream targets to regulate complex processes such as metabolism or growth. Modifying the action of even a single gene that only directly plays a role in a single pathway can potentially alter the contributions to the mortality of many other pathways (Kennedy 2008). By measuring life span, we are effectively looking at the integrated contributions from each pathway. Because of these complexities, simple analyses, such as traditional epistasis, may not always be straight forward to interpret. Take the controversial role of the yeast histone deacetylase Sir2 in DR for example. Sir2 was originally proposed as a mediator of DR based on the observations that overexpression of *SIR2* extends RLS (Kaeberlein et al. 1999) and that Sir2 is activated in a NAD-dependent manner (Lin et al. 2000). Indeed, DR was shown to be ineffective at extending RLS in yeast lacking *SIR2* (Lin et al. 2000). By classical interpretation, this result led to the conclusion that DR requires Sir2 in order to extend life span. However, it was subsequently shown that DR robustly extends RLS in strains where both *SIR2* and *FOB1* are deleted (Kaeberlein et al. 2004; Lamming et al. 2005) and in long-lived strains that either lack *FOB1* or overexpress *SIR2* (Kaeberlein et al. 2004). Thus Sir2 is not necessary for extension of life span by DR and likely controls life span via a separate mechanism. Importantly, while *SIR2* and DR provide an illustrative counterexample, classical epistasis analysis can work with respect to life span and probably does in most cases. In *C. elegans* for example, *daf-2*, the gene encoding the IIS receptor, negatively regulates the activity of *daf-16*, the gene encoding the IIS FOXO-family transcription factor. Mutations in *daf-2* dramatically increase life span and mutations in *daf-16* shorten life span relative to wild type. Epistasis analysis correctly predicts that life span extension via reduced activity of DAF-2 is blocked by mutations in DAF-16 (Kenyon et al. 1993).

A final caution applies to interpreting life span phenotypes in the context of natural selection. As discussed in Section 20.2, longevity should not be equated to fitness. In fact, in a system optimized for fitness, mutations that increase longevity will generally incur a fitness cost. However, the cost/benefit analysis is not always obvious or straightforward. For example, wild-type guppies are short-lived, grow quickly and reproduce early in the wild where predation is high. Place the same guppies in a predator-free environment and they both live longer and produce more progeny (Reznick et al. 2004). The combined benefit associated with longer life and increased total reproduction is outweighed by the ability to grow and reproduce rapidly in a high mortality environment. In *C. elegans*, *age-1* (PI-3 kinase) mutants

are outcompeted by wild type worms when subject to periods of starvation (Walker et al. 2000) despite increased survival when exposed to increased temperature and longer life span (Lithgow et al. 1995). Some long-lived fruit fly mutants, such as those lacking *indy,* an amino acid transporter, actually have more progeny than wild type (Rogina et al. 2000). Long-lived mutants commonly studied in aging research are not found in nature, which implies that there will be some cost associated with the beneficial phenotypes. These examples demonstrate that longevity phenotypes are often context dependent and the strictly-defined, low-variability environment of the laboratory can sometimes obscure what is really going on.

Our goal in this section is to highlight a few of the practical challenges faced when studying aging and working with phenotypes with complex origins, such as longevity. Life span remains the most relevant phenotype to aging and epistasis in the context of longevity will remain at the forefront of aging research. However, we encourage vigilance when analyzing and interpreting results.

20.4 Conservation of Longevity Control

One of the primary goals of aging research is the development of interventions to impede the aging process in an effort to fight age-associated pathologies in humans. It would be theoretically optimal to investigate interventions in mammalian systems. Unfortunately, studying longevity directly is difficult in mammals due to their long life spans. For example, life span experiments require approximately 3 years in mice, 25 years in rhesus monkeys and are ethically and functionally impractical in humans.

Invertebrate models offer many powerful advantages in the context of aging research (discussed in Section 20.1) and a substantial portion of the knowledge we possess about how and why organisms age has been generated using these models. An important consideration when interpreting evidence from invertebrate systems is relevance to human aging. Several environmental interventions, such as DR and transient heat shock, are known to influence longevity in multiple evolutionarily divergent organisms suggesting that they may do so in humans as well (Table 20.1). DR in particular has been shown to extend life span in yeast, worms, flies, mice, spiders, rats, dogs and hamsters (Kennedy et al. 2007; Masoro 2005; Weindruch and Walford 1988). There are likely a set of key environmental conditions that induce a similar set of responses—increased longevity, enhanced resistance to stress, reduced fecundity—in a wide range of organisms. However, this does not necessarily guarantee that the molecular mechanisms that mediate these responses are the same in different organisms and there are cases where it appears that certain age-associated responses, including increased life span, are mechanistically implemented in different ways in different organisms. Thus we have another key question in aging research: are the genetic pathways involved in the determination of life span evolutionarily conserved among diverse organisms? In the past several decades three pathways have emerged as conserved regulators of longevity: IIS,

Table 20.1 Conserved environmental and genetic interventions known to influence life span. Arrows indicate whether the intervention increases (↑), decreases (↓), is not applicable to (n/a) or has an unknown effect on (?) life span in each aging model

		Yeast		Worms	Flies	Mice
		Replicative	Chronological			
Environmental interventions	Dietary restriction	↑	↑	↑	↑	↑
	Transient exposure to stress	↑	?	↑	↑	↑
	Antioxidants	?	↑	?	↑	↑
Genetic interventions	Reduced IIS	n/a	n/a	↑	↑	↑
	Increased sirtuin activity	↑	↓	↑	↑	?
	Reduced TOR signaling	↑	↑	↑	↑	?

sirtuins and TOR signaling (Table 20.1). Each pathway is at least partially distinct, though there is evidence for some interaction between pathways through both influence from environmental conditions and action on downstream targets. Each pathway has been independently proposed as a potential mediator of the beneficial effects of DR.

20.4.1 IIS Promotes Aging

IIS pathways are conserved among multicellular eukaryotes and share a set of core components, including insulin-like proteins, insulin/IGF-1-like receptors, a phosphatidylinositol-3 (PI-3) kinase, an Akt kinase and a FOXO-family transcription factor. IIS inhibits activity of the FOXO-family transcription factors by preventing their nuclear localization, controlling expression of downstream target genes in response to environmental queues. Worms and flies each possess a single insulin/IGF-1-like receptor and numerous insulin-like signaling molecules (Bartke 2008; Toivonen and Partridge 2008). Reducing IIS extends life span and increases stress resistance in both species (Clancy et al. 2001; Dorman et al. 1995; Hercus et al. 2003; Kenyon et al. 1993; Martin et al. 1996; Murakami and Johnson 1996; Tatar et al. 2001; Tu et al. 2002). Unlike invertebrates, mammals possess only three insulin-like ligands—insulin, IGF-1 and IGF-2—and five dimeric insulin/IGF-1-like receptors, including different receptors for insulin and IGF-1, which form from different combinations of one insulin receptor and two IGF-1-receptor monomer subtypes (Taguchi and White 2008). Increased longevity has been linked to reduced activity of both the insulin receptor (Bluher et al. 2003) and the IGF-1 receptor (Holzenberger et al. 2003), as well as the related growth hormone signaling pathway (Brown-Borg et al. 1996; Coschigano et al. 2003; Flurkey et al. 2002).

20.4.2 Sirtuins: Playing Both Sides?

Sir2-orthologs (sirtuins) are NAD-dependent protein deacetylases that have been identified in eukaryotic species from yeast to humans (Imai et al. 2000; Landry et al. 2000; Smith et al. 2000; Tanner et al. 2000). The first evidence for a role in aging for sirtuins came with the discovery that overexpression of Sir2 is sufficient to extend replicative life span in yeast (Kaeberlein et al. 1999) and life span extension has subsequently been demonstrated for overexpression of *sir-2.1* in worms (Tissenbaum and Guarente 2001) and dSir2 in flies (Rogina and Helfand 2004). A role for sirtuins in mammalian longevity has yet to be definitely demonstrated, though transgenic mice overexpressing SIRT1 have several phenotypes that are associated with increased life span, including improved metabolic profiles (Banks et al. 2008; Bordone et al. 2007), delayed disease progression in neurodegenerative disease models (Kim et al. 2007) and reduced incidence of colon cancer (Firestein et al. 2008).

The role of sirtuins in aging has an intriguing feature. While sirtuins appear to modulate longevity in a diverse set of eukaryotic organisms, current data suggests that they do so through at least partially disparate mechanisms. In yeast, Sir2 deacetylase activity primarily targets histones and promotes silencing specifically at 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 and Herskowitz 1987; Smith and Boeke 1997). One cause of replicative senescence in yeast is the accumulation of extrachromosomal rDNA circles (ERCs) with age (Sinclair and Guarente 1997). Sir2 is thought to regulate replicative life span primarily by repressing ERC formation via promotion of rDNA genomic stability (Kaeberlein et al. 1999). Sirt1, the mammalian counterpart of yeast Sir2, may also affect chromatin stability through chromatin interactions (Oberdoerffer et al. 2008). However, they have a variety of other targets as well, including stress response factors and FOXO-family transcription factors, among others (Brunet et al. 2004; Gerhart-Hines et al. 2007; Luo et al. 2001; Motta et al. 2004; Rodgers et al. 2008; van der Horst et al. 2004; Vaziri et al. 2001; Viswanathan et al. 2005). There is no evidence that ERCs contribute to aging, interact with sirtuins, or even accumulate with age in multicellular eukaryotes. In worms, *sir-2.1* may modulate life span by interacting with *daf-16*, the IIS FOXO-family transcription factor, in a 14-3-3 dependent manner (Berdichevsky et al. 2006; Wang et al. 2006), while in flies there is evidence suggesting a longevity-related interaction between dSir2 and Rpd3 histone deacetylase (Rogina and Helfand 2004).

Recent evidence suggests that sirtuins may both promote and antagonize the aging process. For example, in contrast to the protective roll Sir2 plays in yeast replicative aging overexpression of Sir2 limits yeast chronological life span (Fabrizio et al. 2005; Kennedy et al. 2005) and SIRT1 deficiency in mouse embryonic fibroblasts (MEFs) promotes resistance to replicative senescence and increases replicative potential under chronic oxidative stress (Chua et al. 2005). Furthermore, studies in mice and flies suggest that both increasing and decreasing

sirtuin activity may be neuroprotective (Kim et al. 2007; Li et al. 2008; Rogina and Helfand 2004). Thus, while sirtuins are known to play a role in determining longevity, further work will be necessary to understand the full range of sirtuin interactions and how they influence mortality.

20.4.3 Reduced TOR Signaling Provides Consistent Life Span Extension

TOR is a nutrient-responsive kinase with high evolutionary conservation among eukaryotes. TOR acts as part of two complexes, TOR complex 1 (TORC1) and TOR complex 2 (TORC2) (De Virgilio and Loewith 2006; Martin and Hall 2005), though only TORC1 is thought to be involved in aging. TORC1 is a central regulator of response to nutrients, growth signals and energy status (Wullschleger et al. 2006) and both TORC1 and TORC2 are required for viability (Guertin et al. 2006; Helliwell et al. 1998). Aside from DR, reducing TOR signaling is the only intervention known to extend life span in worms (Jia et al. 2004; Vellai et al. 2003), flies (Kapahi et al. 2004) and both yeast paradigms (Kaeberlein et al. 2005; Powers et al. 2006). The role of TOR signaling in mammalian aging is unknown, though a longevity study of mice fed a diet supplemented with rapamycin, a pharmacological inhibitor of TORC1, is currently underway at the National Institute on Aging Interventions Testing Program (Miller et al. 2007). As a counterexample to sirtuins, the TOR signaling pathways are conserved both upstream and downstream of TORC1, including an S6 kinase and processes regulated downstream such as mRNA translation, autophagy, stress response and mitochondria metabolism.

20.4.4 DR and the Search for a Mechanism

DR is defined as a decrease in dietary intake without malnutrition and is the most widely effective and intensely studied intervention known to extend life span. DR has been shown to enhance longevity and increase stress resistance in eukaryotic species from yeast to mice and intense effort has gone into uncovering the underlying molecular mechanisms. Each of the pathways discussed above have independently been proposed as a key mediator of life span extension via DR. The best and most consistent evidence is for TOR signaling. Life span extension from reduced TOR signaling and DR are non-additive in yeast replicative aging, worms and flies (Hansen et al. 2007; Juhasz et al. 2007; Kaeberlein et al. 2005). A recent study in yeast found that the starvation-responsive *GCN4* transcription factor attenuates life span extension by deletion of *TOR1* (Steffen et al. 2008). Interestingly, *GCN4* and TOR signaling influence many of the same cellular processes and *GCN4* expression is primarily regulated by translation, suggesting a model in

which TOR signaling or DR influences *GCN4* target genes by translationally regulating *GCN4* expression (Steffen et al. 2008; Valenzuela et al. 2001; Yang et al. 2000). TOR has yet to be definitively linked to DR with respect to aging in mice or the yeast chronological paradigm.

IIS appears to influence life span by mechanisms at least partially distinct from DR in worms, flies and mice (Bartke et al. 2001; Giannakou et al. 2008; Kaeberlein et al. 2006), though there are potentially conflicting findings in flies (Clancy et al. 2002) and evidence for interaction with respect to other age-associated phenotypes in all three organisms (Bluher et al. 2003; Gershman et al. 2007; Greer et al. 2007; Iser and Wolkow 2007; Libert et al. 2007).

The interaction between sirtuins and DR is unresolved. The role of sirtuins in mediating life extension by DR is a source of ongoing controversy in yeast aging (Guarente and Picard 2005; Kaeberlein 2006; Kaeberlein et al. 2007; Kennedy et al. 2005; Lamming et al. 2005) though the majority of evidence suggests that they influence life span via independent mechanisms (Kaeberlein and Powers 2007). DR and *sir-2.1* appear to act in parallel in worms to extend life span (Lee et al. 2006; Tsuchiya et al. 2006), while the opposite is supported by evidence in flies (Rogina and Helfand 2004; Rogina et al. 2002). The relationship between DR and SIRT1 has not been determined in mice. Thus, while the available evidence supports a hypothesis placing TOR signaling downstream of DR with respect to longevity, it is not known for certain what other pathways are involved. The quest for a mechanism continues.

20.5 Aging Genomics

The ongoing research surrounding DR, IIS, sirtuins and TOR signaling demonstrates that conserved aging factors exist but does not provide any indication as to how common genes involved in determination of longevity are in the genome. To investigate the number of longevity genes in a given organism or the degree of conservation of longevity control between evolutionarily diverse organisms we must follow the way of the modern geneticist: genomics.

Aging has often been studied indirectly by looking at secondary phenotypes associated with longevity, such as enhanced stress resistance or decreased fecundity, or by looking at genes that interact with specific pathways known to play a role in aging. While these types of studies are valuable and help to improve our understanding of the biological systems that influence aging, they cannot provide information about global age-associated changes, an unbiased estimate of the number of genes in a given organism that act to modulate life span, or an estimate as to what degree these genes are conserved among divergent organisms. The past decade has seen a response to these issues in the form of aging genomics. To date two approaches have been used to look at aging on the genome scale: microarrays and large-scale genetic screens for longevity phenotypes (Kaeberlein 2004; Steinkraus et al. 2008).

20.5.1 Microarrays Uncover Age-Associated Gene Expression Patterns

Two strategies have been used in the application of microarrays to aging. The most common is comparison of gene expression patterns between young and old individuals to find changes that correlate with age. Studies have found that factors involved in oxidative stress response are upregulated with age in flies (Landis et al. 2004; Pletcher et al. 2002; Zou et al. 2000), mice (Weindruch et al. 2001) and monkeys (Kayo et al. 2001), which is consistent with an observed increase in expression of oxidative stress genes in young individuals from long-lived *C. elegans* strains (McElwee et al. 2003; Murphy et al. 2003). One group used microarray analysis to compare age-associated gene expression changes between *C. elegans* and *D. melanogaster* (McCarroll et al. 2004) and found a similar age-related gene expression program involving mitochondrial metabolism and DNA repair, among others. Further studies of this type will be of interest, particularly involving comparison of gene expression patterns between invertebrates and mammals.

The second microarray strategy compares gene expression patterns between young, age-matched individuals with different longevity phenotypes (resulting from differences in environmental exposure, genotype, or both) with the goal of identifying genetic programs that contribute to increased life span when activated early in life. For example, this strategy was used to demonstrate that gene expression for Ames dwarf mice is different from wild-type mice subject to DR and that changes in gene expression in response to DR are different for wild-type and Ames dwarf mice (Masternak et al. 2004). This is in agreement with the independent action of DR and IIS on life span in *C. elegans* (Houthoofd et al. 2003; Kaeberlein et al. 2006; Lakowski and Hekimi 1998; Lee et al. 2006). Similar studies have linked DR to osmotic stress and increased respiration in yeast (Kaeberlein et al. 2002; Lin et al. 2002) and to growth hormone signaling in mice (Miller et al. 2002). Microarrays have also been used to demonstrate that gene expression changes associated with DR occur quickly relative to life span in mice (Dhahbi et al. 2004), which is consistent with a rapid decrease in mortality in response to DR in flies (Mair et al. 2003) and mice (Dhahbi et al. 2004) and the observation that DR extends worm life span even when initiated late in life (Smith et al. 2008). The ability to identify short-term changes in gene expression with potential long-term consequences on life span opens the possibility of screening for pharmacological agents that mimic the beneficial effects of DR (Kaeberlein 2004).

A study combining the two microarray strategies compared the age-associated changes between mice fed a control diet and mice subject to DR and found that DR reversed a subset of the age-related gene expression changes (Weindruch et al. 2001). The findings from aging microarray studies to date demonstrate their potential for identifying global changes associated with advanced age or enhanced longevity. Notably, early attempts have suffered from a myriad of technical and analytical problems, such as limited sample size or lack of rigorous statistical analysis.

Nevertheless, the field remains optimistic that microarrays will be prevalent in the future of aging research and addressing the technical challenges and discussion of novel approaches to using microarrays in aging has been the topic of many reviews (Becker 2002; Golden et al. 2006; Han et al. 2004; Melov and Hubbard 2004; Nair et al. 2003; Werner 2007). Technical issues aside, microarrays are inherently limited in that they are observational in nature. Another method is needed to identify genes mechanistically involved in aging.

20.5.2 Genome-Scale Life Span Screens Identify a Large Number of Longevity Genes

The methods and findings discussed so far demonstrate that life span is under genetic control and that the mechanisms of control are conserved across divergent species, at least to a degree. The next task is to determine whether the majority of genes involved in life span control are already known, or whether there are still a substantial number of longevity genes yet to be discovered. This requires the ability to look at a large fraction of the genes in a particular genome. To reiterate an earlier point, looking at life span phenotypes is the only way to directly identify genes involved in longevity control. The search for genes that influence life span went genome-wide with the creation of large-scale genetic libraries for *S. cerevisiae* and *C. elegans*. These libraries are being used to screen for mutations that extend life span. Looking specifically for increased life span is particularly important when screening at the genomic level, where the potential for false positives from mutations that shorten life span independent of aging is vast.

In *C. elegans*, RNA interference (RNAi) can be used to knock down the expression of any given open reading frame (ORF) by feeding animals bacteria expressing double-stranded RNA corresponding to that ORF (Timmons and Fire 1998). Two RNAi libraries were created using *Escherichia coli* that together cover more than 90% of the ORFs in the *C. elegans* genome (Kamath et al. 2003; Rual et al. 2004). Two large-scale longevity screens (Hamilton et al. 2005; Hansen et al. 2005) and several screens targeting specific subsets of genes (Chen et al. 2007a; Curran and Ruvkun 2007; Dillin et al. 2002; Kim and Sun 2007; Lee et al. 2003) were performed using these libraries, resulting in the identification of 276 genes that extend life span when knocked down. The two genome-wide screens represent the first unbiased approach to the discovery of novel aging genes.

The implication of the discovery of a large number of genes that influence life span in independent studies is that a substantial fraction of the genes in the *C. elegans* genome are likely to play a role in aging. The question, "why so many?," brings us back to evolutionary theory. One implication of post-Medawar aging theory is that organisms will be selected for overall fitness and not for maximum longevity. Under this model for selection you might expect to find a large number of genes that increase life span when their expression is altered. A further extension of

the relationship between fitness and longevity is that mutations that increase longevity should also have a detrimental effect on fitness. Indeed, long-lived *C. elegans* mutants were found to have reduced fitness relative to wild type in both a demographic survival analysis (Chen et al. 2007b) and in direct competition assays (Jenkins et al. 2004; Walker et al. 2000). Mutations resulting in enhanced longevity are also associated with reduced performance in other areas reproduction in particular in worms (Apfeld and Kenyon 1999; Van Voorhies and Ward 1999) and flies (Buck et al. 2000; Burger et al. 2007; Marden et al. 2003; Mockett and Sohal 2006).

The *S. cerevisiae* library consists of approximately 4,800 yeast strains in a common strain background, each with a single non-essential gene deletion (Winzeler et al. 1999). The strategy of completely knocking out a gene removes the problems associated with variability in the efficiency of gene knock down by RNAi experienced in the *C. elegans* RNAi screens but has the disadvantage of excluding all essential genes. The yeast deletion collection was used to screen for long-lived mutants in both the replicative and chronological paradigms. Measurement of replicative life span is manual labor intensive and the screen is still ongoing. In an initial report for 564 of the single gene mutants 13 (2.3%) were found to be long-lived, five of which are known to function in the TOR signaling pathway (Kaeberlein et al. 2005). The chronological life span screen used a high-throughput method to measure life span for all ~4,800 genes in the deletion collection (Powers et al. 2006). Of the 90 longest-lived strains, 16 have been implicated in TOR signaling and nutrient uptake (Powers et al. 2006). Thus the first unbiased longevity screens in yeast strongly implicate TOR signaling as a central regulator of aging and longevity.

20.6 The Search for Conserved Longevity Determinants: The Genome-Wide Multi-organism Approach

Early applications of genomics to longevity have provided the first glimpse of the true scope of the aging landscape and identified potential key players in the aging process. Large-scale, unbiased screens for life span in different organisms also provide the first opportunity to address in a quantitative manner the degree of conservation of aging determinants between these organisms. This directly impacts the question of relevance to human aging. On the evolutionary timeline, yeast and nematodes are separated by approximately 1.5 billion years, while nematodes and humans are separated by only approximately 1 billion years (Wang et al. 1999). Thus, if we can identify genes that play a conserved role in modulating life span between yeast and nematodes, a subset is likely to play a similar role in mammalian aging as well (Fig. 20.2).

A recent study provided the first quantitative evidence for conservation of longevity control between *S. cerevisiae* and *C. elegans* (Smith et al. 2008).

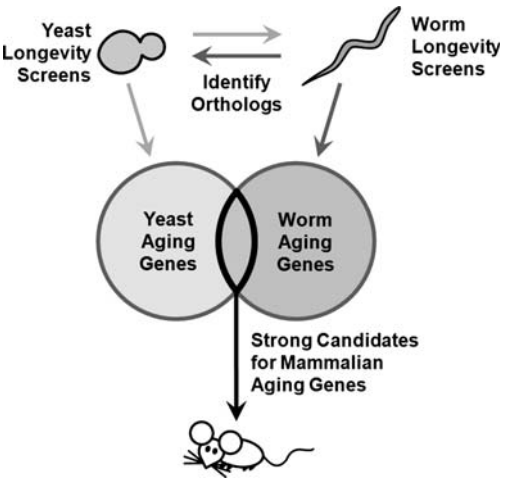

Fig. 20.2 A genome-wide, multi-organism approach to studying genes involved in aging. Cross-examining orthologs of longevity genes between evolutionarily divergent organisms, such as yeast and worms, allows the identification of genes that play a conserved role in longevity determination. These genes are strong candidates for aging genes in diverse organisms, such as mammals

Smith et al. (2008) measured replicative life span for single gene deletion strains corresponding to each of the 276 worm genes identified in the RNAi longevity screens (see Section 20.5.2). First, yeast genes were selected based on protein homology using a two-tiered approach. Yeast orthologs for each worm gene were identified using a high-stringency modified BLASTp reciprocal best match criterion, allowing selection of two yeast genes for a single worm gene when two yeast paralogs had BLASTp scores within 10% of each other. Up to 6 yeast homologs were then selected for each worm gene, requiring at least 20% protein sequence identity and at least 10% amino acid alignment. In total, 264 yeast homologs were selected for analysis, of which 76 met the high-stringency requirements.

Replicative life span analysis identified 25 (9.5%) long-lived mutants from the set of 264 analyzed (Smith et al. 2008). Of the 76 orthologs that met the high-stringency requirement, 11 (14.5%) were long-lived. This is a substantial enrichment for longevity determinants as compared to the expected 2.3% (3.4% if only yeast genes with worm potential orthologs are considered) as estimated by the unbiased screen of 564 genes (Kaeberlein et al. 2005), demonstrating conservation of genes that control aging between yeast and worms. Notably, 15 of the 25 genes identified have clear human orthologs (Smith et al. 2008).

20.7 Uncovering the Mechanisms Behind Conserved Longevity Factors: A Central Role for Translation?

If aging is not under direct evolutionary selection, why should genes influencing longevity be conserved among disparate species? A clue came from the long-lived high-stringency ortholog pairs identified by Smith et al. (2008), which were

significantly enriched (6 out of 11) for genes involved in TOR signaling and more specifically, mRNA translation. Apart from TOR itself, these factors include S6 kinase, translation initiation factors and ribosomal subunits. Combining these findings with the evidence for TOR signaling as a conserved pro-aging pathway and strong candidate for mediation of the beneficial effects of DR (see Section 20.4) suggests mRNA translation as a central process in the modulation of life span. Unlike aging, the organismal response to environmental nutrient levels is critical to organismal fitness. We propose that longevity is conserved primarily because altered responses to nutrient levels profoundly impact longevity. In low nutrient environments (DR or mutations that impair nutrient signaling), organisms devote energy to somatic maintenance and live longer (Kirkwood 1977). Therefore, the conserved effects of certain pathways on longevity in disparate species reflect a longstanding impact of the proper response to environmental nutrient levels on fitness and longevity is tightly coupled to this response.

Several mechanisms have been suggested for how reduced translation might enhance longevity, including altered resource allocation (potentially away from investment in reproduction, in agreement with the disposable soma theory of aging), differential translation of subsets of mRNA and improved protein homeostasis (Kaeberlein and Kennedy 2008). We expect translation to be a central topic in aging research over the next few years and anticipate further clarification of its mechanistic role in longevity determination.

20.8 Conclusion

From the evolutionary insights by Medawar in the 1950s, our understanding of the aging process progressed steadily for roughly the next 30 years through the study of interventions that influence life span, such as DR and secondary age-associated phenotypes (e.g. stress resistance). The advances in genetics in the 1990s led to rapid identification of several key pathways and processes involved in the determination of life span and the demonstration that these factors play a conserved role in aging across multiple species. The genomics-revolution of the past decade and the development of methodology for studying aging and life span determinants on the genomic-scale have provided the first glimpse of the large number of factors involved in controlling aging and the degree of conservation of these factors between evolutionarily divergent organisms.

Our understanding of the aging process and its interaction with disease has also started to yield its first clinically application in humans. Pharmacological agents targeting sirtuins and TOR signaling are now in clinical trials for treatment of age-associated pathologies such as cancer and metabolic disease. The discovery of new longevity genes and novel aspects of known aging pathways has accelerated at a break-neck pace and we expect that this knowledge will rapidly be translated to clinical practice as well.

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