Genomic approaches for the understanding of aging in model organisms

*Sang-Kyu Park**

Department of Medical Biotechnology, College of Medical Science, Soonchunhyang University, Asan 336-745, Korea

Aging is one of the most complicated biological processes in all species. A number of different model organisms from yeast to monkeys have been studied to understand the aging process. Until recently, many different age-related genes and age-regulating cellular pathways, such as insulin/IGF-1-like signal, mitochondrial dysfunction, Sir2 pathway, have been identified through classical genetic studies. Parallel to genetic approaches, genome-wide approaches have provided valuable insights for the understanding of molecular mechanisms occurring during aging. Gene expression profiling analysis can measure the transcriptional alteration of multiple genes in a genome simultaneously and is widely used to elucidate the mechanisms of complex biological pathways. Here, current global gene expression profiling studies on normal aging and age-related genetic/environmental interventions in widely-used model organisms are briefly reviewed. [BMB reports 2011; 44(5): 291-297]

INTRODUCTION

People have defined "aging" in many different ways. From a biological point of view, aging can be defined as a universal, progressive, and irreversible decline of function over time, resulting in reduced function of cells and eventually death of an individual. To explain the underlying mechanisms of aging, numerous theories have been suggested. The free radical theory suggests that accumulated cellular damage caused by free radicals is the major causal factor of aging (1). Free radicals are produced as a byproduct of metabolic pathways, mainly from mitochondria. The mitochondrial decline theory emphasizes the role of mitochondria in aging (2, 3). The genetic control theory suggests that the rate of aging and the time of death of each individual is already encoded in our genome (4). The telomerase theory proposes that the length of telomeres in cells

*Corresponding author. Tel: 82-41-530-3094; Fax: 82-41-530-3085; E-mail: skpark@sch.ac.kr

determines the lifespan of each cell (5).

Previous attempts to reveal the mechanisms of aging have focused on the identification of genes involved in aging using various model organisms, from yeast to mouse. So far, dozens of genes that extend the adult lifespan have been found, and many of these genes are involved in insulin/IGF-1-like signaling. Reduced activity of this signaling pathway can extend one's lifespan and delay many age-related phenotypes. In *C. elegans*, mutation of *daf-2* or *age-1*, which encode the receptor for insulin/IGF-1-like peptide and PI_3 kinase, respectively, confers longevity dependent on the stress resistance transcription factor DAF-16 (6, 7). Conserved genes in *Drosophila melanogaster* also mediate lifespan extension. Loss of InR (insulin-like receptor) or CHICO encoding insulin receptor substrate in *Drosophila melanogaster* extends lifespan significantly (8, 9). In mice, homozygous mutations in *Prop-1* or *Pit-1* increase lifespan up to 65% (10). *Prop-1* or *Pit-1* mutants cannot release GH (growth hormone) in response to GHRH (growth hormone releasing hormone) and consequently show a dwarf phenotype.

The development of DNA microarray introduced the concept of genomics in biology and presented a valuable and robust tool for the global measurement of gene expression. It is especially useful for the study of complex biological pathways such as aging. In addition, genomic sequence analysis of various model organisms makes it possible to produce DNA chips covering the whole genome of each organism. Transcriptional profiling of aging using DNA microarray expression studies has been reported in nematode, flies, mice, and monkeys and has identified a group of genes that alter the expression levels of genes associated with aging (11-15). These genomic approaches to the understanding of aging have allowed us to identify the underlying mechanisms of aging at the molecular level. Here, the transcriptional profile of normal aging in several model organisms and a possible correlation with known age-related interventions will be presented.

GLOBAL GENE EXPRESSION OF AGING IN *C. elegans*

The nematode *Caenorhabditis elegans* is a good model organism for aging research since it has a relatively short lifespan and produces a large number of progeny. As a result, it is very easy to make knockout or transgenic worms for specific genes

using genetic tools. *C. elegans* is the first multi-cellular organism whose whole genome has been fully sequenced, leading to the development of a DNA microarray covering the whole genome sequence of *C. elegans*. Global gene expression analysis using DNA microarray provides a transcriptional profile of aging in *C. elegans*.

Time-course gene expression profiling of *C. elegans* has revealed several groups of genes that are coordinately changed in expression during aging (Table 1). Genes involved in dauer-regulation and insulin/IGF-1-like signaling are up-regulated during aging (11). Expression of *ins-17* and *ins-18* is significantly increased in aged worms. SIR-2.1, the repressor of insulin/IGF-1-like signaling, is decreased in expression in old worms. Among genes that are down-regulated during aging are heat shock genes. Most heat shock genes follow a similar expression pattern as aging; increased expression in young adult worms and decreased expression in aged animals (11). HSP-16.2 is a stress-sensitive reporter that can predict longevity in *C. elegans* and shows decreased expression in later life according to microarray data (11, 16).

Another gene expression study on aging identified 1,254 genes that are altered in expression during aging. The tissue-specificity of these genes revealed that intestine- and oocyte-enriched genes are age-dependent, whereas neuronal, muscle, and pharyngeal genes are not (17). Promoter-binding motif analysis for the upstream region of age-related genes indicated that GATA transcription factor ELT-3 may regulate transcriptional changes involved in aging. The expression of *elt-3* is reduced in a tissue-specific manner with aging, and two repressors of ELT-3, *elt-5* and *elt-6*, are induced in expression during aging. These findings suggest that an *elt3/elt-5/elt-6* GATA transcription circuit plays a pivotal role in normal aging of *C. elegans* (17). Golden and Melov reported the transcriptional profile of individual aging nematodes. They analyzed the gene expression of 4-5 individual worms at four different ages with a cDNA array containing 921 stress-related genes. Among them, 40 genes were significantly changed in expression as a function of time, including several *hsp-16* family genes (18).

Table 1. Global view of transcriptional changes with aging in *C. elegans*

Genes	Direction
Heat shock proteins	
hsp-16 familiy, hsp-70	↓
Insulin homologs	
ins-17, ins-18	↓
sir-2.1	↓
Transposases	
Tn3, Mariner	↑
Insulin/IGF-1-like signal	
daf-2, age-1	No change
Antioxidant genes	No consistent pattern
Mitochondrial genes	No consistent pattern

Reduced insulin/IGF-1-like signaling leads to increased resistance to various stresses and extends both the mean and maximum lifespan of *C. elegans* (19). DAF-16 is a FOXO-family transcription factor mediating longevity in response to reduced insulin/IGF-1-like signaling. A gene expression profile study using DNA microarray revealed that DAF-16 regulates the expression of many age-related genes (20, 21). Genes involved in cellular metabolism, stress responses, and anti-microbial responses are up-regulated by DAF-16 during aging. DAF-16 also down-regulates several genes known to decrease the lifespan of *C. elegans*. Furthermore, knockdown of individual genes induced by DAF-16 during aging results in a shortened lifespan (21). Dauer is a developmentally arrested larval stage of *C. elegans* and confers long-term survival in unfavorable environments. Comparison of the transcriptional profiles of the dauer stage with that of long-lived *daf-2* showed that many DAF-16-dependent genes are common in both transcriptional outputs (22). Hierarchical clustering of genes with altered expression during aging in wild-type N2 and long-lived *daf-2* identified one functional group of genes that is common to both strains as well as one that is specific for the N2 strain (20, 22, 23). Heat shock genes are induced until mid-age and then reduced in later life in both strains. However, the expression of genes encoding proteases is significantly inhibited over time in the N2 strain. Further, changes in the transcription of protease-encoding genes do not occur in long-lived daf-2. These data suggest that maintenance of high expression of proteases throughout the lifespan is one of the underlying mechanisms of long-term reduced insulin/IGF-1 signaling, and genes involved in the heat shock response are not related to the increased lifespan of *daf-2*.

The other well-known life-extending mechanism in *C. elegans* is reduced activity of the mitochondrial electron transport chain (ETC). Mutations in genes involved in the activity of mitochondrial ETC, such as *isp-1* and *clk-1*, significantly increase lifespan (24, 25). Genomic screening of *C. elegans* chromosome I using a RNAi library showed that RNAi knockdown of genes encoding mitochondrial ETC components and mitochondrial ATP synthase can induce a longevity phenotype (26). Worms grown on bacteria expressing double-stranded RNA of these genes are smaller and move more slowly than normal. The lifespan extension by reduced expression of mitochondrial ETC-related genes is not dependent on the transcription factor DAF-16, which is necessary for the longevity effect of reduced insulin/IGF-1-like signaling. A recent study on the underlying mechanisms of mitochondrial ETC-mediated lifespan extension revealed that reduced activity of mitochondrial ETC in specific tissues is essential for longevity effect; ETC knockdown in intestinal and neuronal tissues is required for lifespan extension, whereas that in muscle tissue has no effect on longevity (27).

In nearly every species tested so far, dietary restriction (DR, also known as caloric restriction) increases lifespan and retards many age-related physiological changes. However, the under-

lying mechanisms involved in DR-induced longevity are not fully understood. Several transcription factors mediating DR-induced longevity in *C. elegans* have been identified: PHA-4, SKN-1, HIF-1, and AMPK-1 (28-31). Among them, the screening of downstream targets using DNA microarray has been performed with SKN-1. SKN-1 is involved in the development of the digestive system during embryogenesis and is required for the response to oxidative stress in adult worms (32, 33). A recent study showed that neuronal activation of SKN-1 mediates DR-induced lifespan extension (28). Genome-wide gene expression study revealed 211 genes whose expression is dependent on SKN-1 under oxidative stress (34). These include antioxidant genes (*ctl-1, sod-1,* and *gst-4*) as well as genes involved in the detoxification process (*cyp-14A2*). However, the induction of heat shock genes by oxidative stress is not SKN-1-dependent. Surprisingly, two SKN-1-dependent oxidative-stress-responsive genes, *nlp-7* and *cup-4*, are specifically required for lifespan extension by DR (35). NLP-7 is a neuropeptide-like protein expressed in neurons while CUP-4 is a coelomocyte-specific ion channel essential for endocytosis by coelomocytes (36, 37). Further genome-wide gene expression profiling of the DR response will identify novel pathways mediating DR-induced longevity in *C. elegans.*

TRANSCRIPTIONAL PROFILING OF AGING IN *Drosophila melanogaster*

Gene expression profiling studies in *Drosophila melanogaster* have identified several functional groups of genes regulated by aging (Table 2). Genes associated with the immune response are increased in expression during aging, including *Attacin A, Attacin B, Attacin C,* and *Attacin D* (they are involved in the antibacterial response) (38). Genes encoding proteins involved in energy metabolism and free radical metabolism are also up-regulated under normal aging process (14, 38). Among down-regulated genes during aging, genes related to mating behavior and reproduction are enriched, such as *Acp29AB, Acp62F, Cpl6,* and *Vm26Aa* (38). Genes encoding various chaperones and involved in the detoxification process are also decreased in expression during aging (15). To determine the tissue-specific transcriptional pattern during aging, DNA microarray analysis of specific body parts from *Drosophila* has

been conducted. In *Drosophila* head, genes involved in energy metabolism and neuronal function are decreased in expression with aging (39). Interestingly, these age-related transcriptional changes in the head mainly occur before day 13 of the adult stage and show little changes thereafter. Another study on gene expression patterns in the head during aging also reported down-regulation of genes related to neuronal function, including the transmission of nerve impulse (seven genes encoding accessory peptide family and three genes encoding male specific transcripts Mst57D protein family), synaptic transmission (*Cha, Ddc,* and *Dat*), and neurotransmitter secretion (*unc13, comatose, Csp,* and *AP-50*) (40). Age-related up-regulated genes in *Drosophila* head include genes involved in the immune response and amino acid metabolism. Aging in another important tissue in *Drosophila melanogaster,* the thorax, also results in the induction of genes involved in the immune response (40). In addition, genes linked to cellular morphogenesis and the proteasome complex are induced in the aged thorax. Down-regulated genes upon aging in the thorax are enriched with genes associated with cellular components of muscle fibers and mitochondrial membranes, suggesting increased stress and dysfunction of mitochondria in aged muscle.

Comparison of the transcriptional profiling of the oxidative stress response with that of aging revealed that one-third of genes that are significantly altered in expression by aging show similar changes in response to oxidative stress (41). DNA microarray analysis of aging and oxidative stress revealed that up-regulation of heat shock genes, antioxidant genes, and innate immune response genes are common under both conditions. On the other hand, aging and oxidative stress responses share the down-regulation of proteasome subunits, alkaline phosphatases, and triacylglycerol lipases (41). These studies support a role for oxidative stress in the regulation of normal aging. The genome-wide transcriptional profile of life-extending DR has also been compared to the gene expression patterns of normal aging in *Drosophila melanogaster* (14). DR can oppose age-related transcriptional alterations in many functional groups of genes, especially genes encoding defense/immunity proteins and stress response proteins.

A recent study compared the transcriptional profiles of aging between *C. elegans* and *Drosophila melanogaster* and reported shared genomic expression programs between them. In this study, aging in both *Drosophila melanogaster* and *C. elegans* was characterized by the repression of genes functioning in the mitochondrial ETC, the ATP synthase complex, and the citric acid cycle (42). Genes involved in DNA repair, oxidative stress responses, heat stress responses, and cellular transport also overlapped with each other in age-associated transcriptional profiles of both organisms. These findings suggest that there might be shared mechanisms of aging between various organisms, and genomic approaches could be used to identify conserved mechanisms of aging.

Table 2. Functional classification of genes involved in aging in *Drosophila melanogaster*

Up-regulation	Down-regulation
Immune response	Reproduction
Heat shock genes	Mitochondrial genes
Antioxidant genes	Muscle contraction
Energy metabolism	Neuronal function
Amino acid metabolism	Metabolism

LESSONS LEARNED FROM GENOMIC STUDIES OF AGING IN MICE

In long-living mammals, a large number of factors affect the aging process and the probability of death, including neoplasia, sepsis, and organ-specific failure. Therefore, gene expression profiling of each organ is more appropriate for understanding of aging than measurement of aging in the whole body. The characterization of genomic expression patterns in different tissues was previously carried out using Affymetrix oligonucleotide DNA microarrays in mice. Aging in gastrocnemius muscle resulted in up-regulation of genes involved in the stress response, such as heat shock genes, antioxidant genes, and DNA damage inducible genes, and neuronal injury related to reinnervation and neurite extension and sprouting (43). However, genes involved in energy metabolism, especially those required for glycolysis and mitochondrial function, were decreased in expression (Table 3). Comparison of the transcriptional profiles of the neocortex and cerebellum tissues obtained from young and old mice revealed differential gene expression patterns (44). In both brain-specific tissues, induction of genes encoding complement cascade components and major histocompatibility complex molecules was observed. Heat shock genes and antioxidant genes were also increased upon aging in the brain. Among the down-regulated genes, two major classes of genes were those involved in protein turnover and those encoding growth and trophic factors (Table 3). Taken together, aging in skeletal muscle and brain commonly induces stress response pathways, suggesting the age-related accumulation of free radicals in these post-mitotic tissues. The gene expression profile associated with heart aging is characterized by increased expression of structural proteins, such as collagen molecules, cell adhesion molecules, and extracellular matrix components (45). Aging of the heart is also associated with repressed expression of genes involved in fatty acid beta-oxidation (Table 3). These results indicate that the aged heart undergoes structural alteration, leading to cardiomyocyte hypertrophy and a metabolic shift from fatty acid to carbohydrate metabolism.

In addition to research focusing on the elucidation of mechanisms relating to aging, studies for discovering a possible way to retard the aging process and extend lifespan have been performed by many scientists. In many model organisms, DR shows anti-aging and life-extending effects as previously mentioned. Transcriptional profiles of tissues from diet-restricted mice were compared to those from mice fed a normal diet. The results showed that most transcriptional alterations during aging are either completely or partially prevented by DR. Analysis of gene expression patterns specific to DR mice suggest that DR exhibits anti-aging effects by reducing endogenous damage and by inducing metabolic shifts specific to individual tissues (43-45). Although the effect of DR on aging is promising, it is very hard to follow the DR regimen throughout an individual's lifetime. Hence, many have searched for possible molecules that can mimic the effect of DR, with the most widely studied being antioxidants. Middle-aged onset dietary supplementation with alpha-lipoic acid (LA) or coenzyme Q10 (CQ) exhibits a partial effect on age-related transcriptional changes (46). Up-regulation of cellular structural proteins in the aged heart is significantly prevented by LA or CQ supplementation, but the effect of LA or CQ is smaller than that of DR. Genes associated with the metabolic shift observed during heart aging are not affected by LA or CQ, whereas DR significantly inhibits age-related transcriptional changes in this

Table 3. Tissue-specific gene expression patterns during aging in mice

	Up-regulated genes	Down-regulated genes
Skeletal muscle	Stress response 　Heat shock genes 　Antioxidant genes 　Dna damage inducible genes Neuronal injury 　Reinnervation 　Neurite extension and sprouting	Energy metabolism 　Glycolysis 　Mitochondrial function
Brain	Inflammatory response 　Complement cascade 　MHC molecules Stress Response 　Heat shock genes 　Antioxidant genes 　Lysosomal proteases	Protein turnover 　Ubiquitin-proteasome pathway Growth and trophic factors 　Developmentally regulated genes 　Neuronal plasticity
Heart	Cellular structural proteins 　Extracellular matrix components 　Collagen deposition 　Cell adhesion Immune and inflammatory response 　Complement cascade 　MHC molecules	Fatty acid metabolism 　Beta-oxidation Protein synthesis 　Initiation factors

class of genes. Moreover, unlike DR, LA and CQ do not impact longevity or tumor incidence. The other well-known antioxidant, vitamin E, also showed a partial preventive effect on age-related transcriptional changes in the aged heart in a previous study (47). Similar to LA or CQ, the global transcriptional profile of the vitamin E-supplemented mouse revealed a preventive effect on the transcription of genes encoding cellular structural proteins and no effect the transcription of genes functioning in the metabolic shift in the aged heart. In the brain, induction of genes involved in ATP biosynthesis was significantly inhibited, whereas age-related up-regulation of genes involved in the immune response was not affected by dietary vitamin E. Interestingly, in both the heart and brain, vitamin E induced expression of anti-apoptotic genes and repressed pro-apoptotic genes, along with up-regulation of BCL2-interacting proteins and down-regulation of caspases and programmed cell death proteins (47). These results suggest that vitamin E supplementation may prevent some aspects of aging through the prevention of apoptosis in normal aged tissues. Recent gene expression profiling of aging identified transcriptional biomarkers of aging in the mouse heart and brain and tested the effects of eight different antioxidants (LA, CQ, resveratrol, curcumin, lycopene, acetyl-L-carnitine, astaxanthin, and tempol) as well as DR on the expression of these aging biomarkers (48). The results indicated that the ability of dietary antioxidants to oppose age-related expressional changes in the biomarkers is tissue-specific. In the heart, it was found that lycopene, resveratrol, acetyl-L-carnitine, and tempol are as effective as DR, whereas LA and CQ show similar effects as DR in cerebellum (48). These findings support the free radical theory of aging, emphasizing the role of oxidative stress in aging, and suggest that the anti-aging effect of each antioxidant might be tissue-specific.

CONCLUDING REMARKS

Aging is universal in all species and is one of the most complicated biological processes. Global gene expression profiling is very useful for elucidating the molecular basis of aging. Current microarray studies on aging have shown coordinated expression patterns during aging in many model organisms and have provided insights into the underlying mechanisms of aging that are both common in various organisms and specific to individual species. In addition, genes whose expression is regulated by aging could serve as biomarkers of aging. If so, these biomarkers could be used to determine the effects of genetic or nutritional intervention on aging and possibly estimate an individual's physiological age and predict time of death.
However, there are some limitations to gene expression profiling. The major limitation of DNA microarray data is that they do not exactly reflect the expression level of each gene since the final gene expression product is a protein and not nucleic acid. Although the mRNA level is usually well correlated with the amount of protein encoded by each gene, alterations

in mRNA levels may not parallel the protein levels of some genes. Translation efficiency, rate of mRNA degradation, and alternative splicing patterns could affect the relationship between mRNA and protein expression. There is also a technical limitation. To obtain a reliable data set, at least three replicates are needed. However, the DNA chip used in gene expression profiling is very expensive, and in many cases, it is very hard to obtain sufficient aged samples for replication of the experiments.

Genomic approaches in aging studies are a powerful method of elucidating the molecular mechanisms of aging and determining the effects of lifespan-modulating genetic mutations and environmental interventions, including DR, on aging. Further studies on aging using genome-wide data analysis will facilitate our understanding of aging and provide a possible way of retarding the aging process and extending our lifespan in the future.

REFERENCES

1. Herman, D. (1956) Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298-300.
2. Boffoli, D., Scacco, S. C., Vergari, R., Solarino, G., Santacroce, G. and Papa, S. (1994) Decline with age of the respiratory chain activity in human skeletal muscle. *Biochim. Biophys. Acta.* **1226**, 73-82.
3. Short, K. R., Bigelow, M. L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S. and Nair, K. S. (2005) Decline in skeletal muscle mitochondrial function with aging in humans. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5618-5623.
4. Best, B. P. (2009) Nuclear DNA damage as a direct cause of aging. *Rejuvenation Res*. **12**, 199-208.
5. Shay, J. W. and Wright, W. E. (2005) Senescence and immortalization: role of telomeres and telomerase. *Carcinogenesis* **26**, 867-874.
6. Johnson, T. E. (1990) Increased life-span of age-1 mutants in *Caenorhabditis elegans* and lower Gompertz rate of aging. *Science* **249**, 908-912.
7. Kenyon, C., Chang, J., Gensch, E., Rudner, A. and Tabtiang, R. (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461-464.
8. Clancy, D. J., Gems, D., Harshman, L. G., Oldham, S., Stocker, H., Hafen, E., Leevers, S. J. and Partridge, L. (2001) Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science* **292**, 104-106.
9. Tatar, M., Kopelman, A., Epstein, D., Tu, M. P., Yin, C. M. and Garofalo, R. S. (2001) A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**, 107-110.
10. Brown-Borg, H. M., Borg, K. E., Meliska, C. J. and Bartke, A. (1996) Dwarf mice and the ageing process. *Nature* **384**, 33.
11. Lund, J., Tedesco, P., Duke, K., Wang, J., Kim, S. K. and Johnson, T. E. (2002) Transcriptional profile of aging in *C. elegans*. *Curr. Biol.* **12**, 1566-1573.
12. Park, S. K. and Prolla, T. A. (2005) Gene expression profil-

ing studies of aging in cardiac and skeletal muscles. *Cardiovasc. Res.* **66**, 205-212.

13. Park, S. K. and Prolla, T. A. (2005) Lessons learned from gene expression profile studies of aging and caloric restriction. *Ageing Res. Rev.* **4**, 55-65.

14. Pletcher, S. D., Macdonald, S. J., Marguerie, R., Certa, U., Stearns, S. C., Goldstein, D. B. and Partridge, L. (2002) Genome-wide transcript profiles in aging and calorically restricted *Drosophila melanogaster. Curr. Biol.* **12**, 712-723.

15. Zou, S., Meadows, S., Sharp, L., Jan, L. Y. and Jan, Y. N. (2000) Genome-wide study of aging and oxidative stress response in *Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A.* **97**, 13726-13731.

16. Rea, S. L., Wu, D., Cypser, J. R., Vaupel, J. W. and Johnson, T. E. (2005) A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans. Nat. Genet.* **37**, 894-898.

17. Budovskaya, Y. V., Wu, K., Southworth, L. K., Jiang, M., Tedesco, P., Johnson, T. E. and Kim, S. K. (2008) An elt-3/elt-5/elt-6 GATA transcription circuit guides aging in *C. elegans. Cell* **134**, 291-303.

18. Golden, T. R. and Melov, S. (2004) Microarray analysis of gene expression with age in individual nematodes. *Aging Cell* **3**, 111-124.

19. Johnson, T. E., Henderson, S., Murakami, S., de Castro, E., de Castro, S. H., Cypser, J., Rikke, B., Tedesco, P. and Link, C. (2002) Longevity genes in the nematode *Caenorhabditis elegans* also mediate increased resistance to stress and prevent disease. *J. Inherit. Metab. Dis.* **25**, 197-206.

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

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

22. McElwee, J. J., Schuster, E., Blanc, E., Thomas, J. H. and Gems, D. (2004) Shared transcriptional signature in *Caenorhabditis elegans* Dauer larvae and long-lived daf-2 mutants implicates detoxification system in longevity assurance. *J. Biol. Chem.* **279**, 44533-44543.

23. Halaschek-Wiener, J., Khattra, J. S., McKay, S., Pouzyrev, A., Stott, J. M., Yang, G. S., Holt, R. A., Jones, S. J., Marra, M. A., Brooks-Wilson, A. R. and Riddle, D. L. (2005) Analysis of long-lived *C. elegans* daf-2 mutants using serial analysis of gene expression. *Genome Res.* **15**, 603-615.

24. Feng, J., Bussiere, F. and Hekimi, S. (2001) Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans. Dev. Cell* **1**, 633-644.

25. Lakowski, B. and Hekimi, S. (1996) Determination of life-span in *Caenorhabditis elegans* by four clock genes. *Science* **272**, 1010-1013.

26. Dillin, A., Hsu, A. L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A. G., Kamath, R. S., Ahringer, J. and Kenyon, C. (2002) Rates of behavior and aging specified by mitochondrial function during development. *Science* **298**, 2398-2401.

27. Durieux, J., Wolff, S. and Dillin, A. (2011) The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* **144**, 79-91.

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

29. Chen, D., Thomas, E. L. and Kapahi, P. (2009) HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in *Caenorhabditis elegans. PLoS Genet* **5**, e1000486.

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

31. Panowski, S. H., Wolff, S., Aguilaniu, H., Durieux, J. and Dillin, A. (2007) PHA-4/Foxa mediates diet-restriction- induced longevity of *C. elegans. Nature* **447**, 550-555.

32. An, J. H. and Blackwell, T. K. (2003) SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. *Genes Dev.* **17**, 1882-1893.

33. An, J. H., Vranas, K., Lucke, M., Inoue, H., Hisamoto, N., Matsumoto, K. and Blackwell, T. K. (2005) Regulation of the *Caenorhabditis elegans* oxidative stress defense protein SKN-1 by glycogen synthase kinase-3. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 16275-16280.

34. Park, S. K., Tedesco, P. M. and Johnson, T. E. (2009) Oxidative stress and longevity in *Caenorhabditis elegans* as mediated by SKN-1. *Aging Cell* **8**, 258-269.

35. Park, S. K., Link, C. D. and Johnson, T. E. (2010) Life-span extension by dietary restriction is mediated by NLP-7 signaling and coelomocyte endocytosis in *C. elegans. FASEB J.* **24**, 383-392.

36. Fares, H. and Greenwald, I. (2001) Genetic analysis of endocytosis in *Caenorhabditis elegans*: coelomocyte uptake defective mutants. *Genetics* **159**, 133-145.

37. Nathoo, A. N., Moeller, R. A., Westlund, B. A. and Hart, A. C. (2001) Identification of neuropeptide-like protein gene families in Caenorhabditiselegans and other species. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14000-14005.

38. Lai, C. Q., Parnell, L. D., Lyman, R. F., Ordovas, J. M. and Mackay, T. F. (2007) Candidate genes affecting Drosophila life span identified by integrating microarray gene expression analysis and QTL mapping. *Mech. Ageing Dev.* **128**, 237-249.

39. Kim, S. N., Rhee, J. H., Song, Y. H., Park, D. Y., Hwang, M., Lee, S. L., Kim, J. E., Gim, B. S., Yoon, J. H., Kim, Y. J. and Kim-Ha, J. (2005) Age-dependent changes of gene expression in the Drosophila head. *Neurobiol. Aging* **26**, 1083-1091.

40. Girardot, F., Lasbleiz, C., Monnier, V. and Tricoire, H. (2006) Specific age-related signatures in Drosophila body parts transcriptome. *BMC Genomics* **7**, 69.

41. Landis, G. N., Abdueva, D., Skvortsov, D., Yang, J., Rabin, B. E., Carrick, J., Tavare, S. and Tower, J. (2004) Similar gene expression patterns characterize aging and oxidative stress in *Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A.* **101**, 7663-7668.

42. McCarroll, S. A., Murphy, C. T., Zou, S., Pletcher, S. D., Chin, C. S., Jan, Y. N., Kenyon, C., Bargmann, C. I. and Li, H. (2004) Comparing genomic expression patterns across

species identifies shared transcriptional profile in aging. *Nat. Genet.* **36**, 197-204.

43. Lee, C. K., Klopp, R. G., Weindruch, R. and Prolla, T. A. (1999) Gene expression profile of aging and its retardation by caloric restriction. *Science* **285**, 1390-1393.

44. Lee, C. K., Weindruch, R. and Prolla, T. A. (2000) Gene-expression profile of the ageing brain in mice. *Nat. Genet* **25**, 294-297.

45. Lee, C. K., Allison, D. B., Brand, J., Weindruch, R. and Prolla, T. A. (2002) Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 14988-14993.

46. Lee, C. K., Pugh, T. D., Klopp, R. G., Edwards, J., Allison, D. B., Weindruch, R. and Prolla, T. A. (2004) The impact of alpha-lipoic acid, coenzyme Q10 and caloric restriction on life span and gene expression patterns in mice. *Free Radic. Biol. Med.* **36**, 1043-1057.

47. Park, S. K., Page, G. P., Kim, K., Allison, D. B., Meydani, M., Weindruch, R. and Prolla, T. A. (2008) alpha- and gamma-Tocopherol prevent age-related transcriptional alterations in the heart and brain of mice. *J. Nutr.* **138**, 1010-1018.

48. Park, S. K., Kim, K., Page, G. P., Allison, D. B., Weindruch, R. and Prolla, T. A. (2009) Gene expression profiling of aging in multiple mouse strains: identification of aging biomarkers and impact of dietary antioxidants. *Aging Cell* **8**, 484-495.