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Common aging pathways in worms, flies, mice and humans

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Summary

Development of functional genomics tools has made it possible to define the aging process by performing genome-wide scans for transcriptional differences between the young and the old. Global screens for age regulation have been performed for worms and flies, as well as many tissues in mice and humans. Recent work has begun to analyze the similarities and differences in transcriptional changes in aging among different species. Most age-related expression changes are specific for a given species, but genes in one pathway (the electron transport chain pathway) show common age regulation in species from worms to humans. Evolutionary theories of aging provide a basis to understand how age regulation of a genetic pathway might be preserved between distantly related species.

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Key words: age regulation, gene expression profile, electron transport chain, gene set enrichment analysis, aging, evolution, genomics.

Introduction

Aging is a complex process driven by diverse molecular pathways and biochemical events. The gradual decline in cellular functions associated with aging is not caused by changes in the expression or activity of just a few individual genes, but rather by the cumulative changes from many genes. To elucidate molecular differences associated with aging, an attractive approach is to use DNA microarrays to scan the entire genome for genes that change expression with age. The set of age-regulated genes provides a comprehensive and unbiased view of molecular changes associated with age. The identities of particular age-regulated genes may suggest specific mechanisms for aging; e.g. decreased expression of the electron transport chain genes in old age suggests changes in energy generation and oxidative damage at the end of life. Furthermore, analysis of the entire set of age-regulated genes (a gene expression profile) can reveal emergent themes about the aging process; e.g. evaluation of all age-regulated genes in normal and calorically restricted mice shows that caloric restriction may slow down age-related expression changes (Park and Prolla, 2005).

Nearly all organisms age, and yet lifespan can be very different between species. For example, among major model organisms, the worm *Caenorhabditis elegans* lives for 2 weeks, the fly *Drosophila melanogaster* lives for 2 months, the mouse *Mus musculus* lives for 2 years and humans lives for ~80 years. A great deal might be learned by comparing the aging process in different species, revealing why humans age so slowly compared with worms. For example, one could ask whether human cells are exceptionally well protected against mitochondrial oxidative damage, DNA damage or telomerase shortening compared with worm cells. Several recent papers have compiled gene expression profiles for aging from multiple species and then compared them to each other to distinguish aspects of aging that are species specific and those that are shared.

There is a rich set of literature on using DNA microarray experiments to profile gene expression differences for aging in worms, flies, mice and humans (Kim, in press). Transcriptional profiles for aging contain quantitative data on age-related changes in expression for a large fraction of the genome. However, relatively few studies have integrated aging transcriptional profiles from different studies in a systematic way to find similarities and differences in aging among different species. Genes that show age-related transcriptional differences in multiple species are exceptionally interesting as biomarkers for age. Their age-related decline scales with lifespan, such that age-related changes occur relatively quickly in short-lived animals but slowly in long-lived ones. By contrast, genes that show age regulation in mice but not humans may help identify pathways and mechanisms that account for much longer lifespan in humans.

Early work by McCarroll and colleagues compared transcriptional changes in flies and worms (McCarroll et al., 2004). This work introduced the concept of treating quantitative changes in gene expression as a molecular phenotype and then comparing different expression profiles to each other to reveal overlaps in expression changes between two experiments. One of the results from this early work was an apparent similarity between aging in flies and worms. However, there were statistical flaws used in the calculation, and it is unclear whether the overlap between flies and worms was statistically significant (Melov and Hubbard, 2004). Furthermore, the work did not really measure aging, as the greatest changes in gene expression occurred in young adulthood and not in old age (McCarroll et al., 2004). Nevertheless, this paper introduced key concepts about using gene expression profiles as molecular phenotypes and set the stage for later papers to generate expression data on aging and statistical methods to analyze the data.

Fraser and colleagues compared the effects of aging on gene expression in the brains of humans and chimpanzees (Fraser et al., 2005). They analyzed previously published data on aging in the human brain, including five different areas of the cerebral cortex (Evans et al., 2003; Khaitovich et al., 2004; Lu et al., 2004). All five areas of the cortex showed similar patterns of age-related expression changes. Next, they measured changes in expression in the brain cortex as a function of age in chimpanzees. They found no correlation between age-related changes in the cortex of humans and chimpanzees, indicating that aging in humans and chimpanzees is very different.

A series of recent papers has compared age-related expression profiles in worms, mice, flies and humans. For worms and flies, DNA microarrays have been used on whole animals over the entire lifespan to profile transcriptional changes of aging (Landis et al., 2004; Lund et al., 2002; Pletcher et al., 2002). For mice, Zahn et al. used data from AGEMAP, which is a large database of expression changes as a function of age in 16 mouse tissues (J. Zahn, unpublished data). For humans, Zahn et al. measured age-related transcriptional changes in muscle and compared them with aging changes in the kidney and the brain (Lu et al., 2004; Rodwell et al., 2004; Zahn et al., 2006).

To compare transcriptional profiles between these four species, Zahn et al. developed new statistical methods for gene set enrichment analysis and empirical meta-analysis (Zahn et al., 2006). These new statistical methods were needed to overcome a pervasive methodological challenge in genomics studies called multiple hypothesis testing. The main strength of DNA microarrays – simultaneous readings of expression from thousands or tens of thousands of genes – is also a major statistical hurdle because the thousands of gene expression measurements make it possible for random events to occur that are very rare (i.e. the problem of multiple hypothesis testing). In a standard experiment testing only one gene, a P value of 0.05 is convincing because there is only a 1 in 19 chance that the result could occur by chance. However, with 1000 genes, a P value threshold of 0.05 is not convincing because about 50 of the 1000 genes can meet this threshold by chance. Hence, DNA microarray experiments nearly always use more stringent P values (e.g. <0.001) in order to screen out most of the data that can occur by chance.

One approach to overcome the statistical hurdles posed by multiple hypothesis testing is to use gene set enrichment analysis (Subramanian et al., 2005; Zahn et al., 2006). To use this method, one first categorizes all of the genes into pathways or gene sets; typically, one uses the gene sets defined by the Gene Ontology consortium (http://www.geneontology.org/), such as the set of ribosomal genes or the set of genes that encode components of the electron transport chain. Then, one looks at the expression of every gene in the gene set to see if there is an overall trend in expression. Do most of the genes increase or decrease with age? This method is both powerful and sensitive because: (1) there are only about ~600 gene sets *versus* ~20 000 genes in the genome, which reduces the problem of multiple hypothesis testing and (2) small changes in expression in many genes in a pathway can become statistically significant when considered as a group. For example, there are 95 genes that encode components of the electron transport pathway. Using gene set enrichment analysis, Zahn et al. showed that there is an overall trend of decreasing expression with age for this set of electron transport genes in worms, flies, mice and humans (Zahn et al., 2006), even though only a small number of these genes show very strong age-related changes by themselves (Fig. 1).

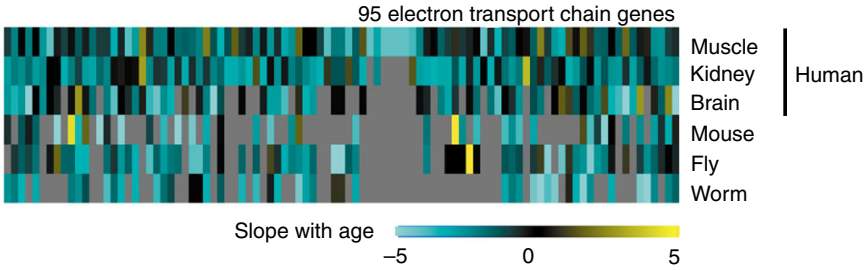

Fig. 1. The electron transport chain decreases expression with age in humans, mice, flies and worms. Rows represent either human tissues or model organisms. Columns correspond to 95 genes in the electron transport chain gene set. Scale represents the normalized slope of the change in $\log_2$ expression level with age. Grey indicates that genes were not present in that species. Original data from Zahn et al. (Zahn et al., 2006).

Empirical meta-analysis is a method to combine gene expression results from different experiments (Zahn et al., 2006). Zahn et al. compiled data on age-related changes in expression from worms, flies, mouse kidneys and human brain, kidney and muscle. The experimental designs used in each of these studies were quite different. The human study used patients with varying ages whereas the worm, fly and mouse studies used staged animals at discrete ages. The human and fly experiments used Affymetrix GeneChips (Santa Clara, CA, USA), the mouse experiment used spotted cDNA filters, and the worm experiment used DNA microarrays. Each gene expression study used different numbers of samples (e.g. 26 worm samples, 40 mouse samples, 81 human muscle samples). Despite these differences in experimental design, Zahn et al. were able to use empirical meta-analysis to search for gene sets that showed a general increase or decrease in expression with age in different tissues and species. From each experiment, one can calculate a *P* value that a gene or gene set changes expression with age. Empirical meta-analysis combines the *P* values from each individual experiment and then calculates an overall *P* value for an age-related trend in multiple experiments.

Only one gene group, the electron transport chain gene set, was similarly age regulated in worms, flies, mice and humans (Fig. 1) (Zahn et al., 2006). The overall level of expression in this set of genes decreased about twofold in old age for all four species. The electron transport chain genes are located in the nuclear DNA and encode components of a mitochondrial enzyme complex that is the primary source of generation of free radicals in the cell. Free radicals are highly reactive side-products that non-specifically damage cell components such as proteins and DNA. Oxidative damage from the mitochondrial free radicals may accumulate with time and thereby decrease overall cell function and ultimately limit organismal lifespan (Golden et al., 2002). The lifespan of worms and humans differ by ~2000-fold (2 weeks *versus* 80 years), and the slope of age-related changes in expression for this pathway scales with lifespan such that old worms and old humans showed a similar overall decrease in expression levels. Because this genetic pathway showed similar age regulation in diverse species, it may be an exceptionally good biomarker of age.

Do changes in the electron transport chain genes in old age promote longevity or hasten senescence? In mammals, the functional significance of age-related changes in this pathway is unclear. However, in *C. elegans*, reduction of gene activity of the electron transport chain genes by RNAi has a strong effect on extending lifespan (Lee et al., 2003). This observation suggests that decreased expression of genes in the electron transport chain pathway in old age may help prolong life in worms, and possibly other species as well.

Genes that encode proteins in the lysosome show a common increasing trend in expression in humans, mice and flies but not worms (J. Zahn, unpublished data). The lysosome is responsible for degeneration of cell surface receptors, and increased expression of lysosomal genes may mark increased receptor turnover in old age.

Aging – a universal process that falls outside the force of natural selection

These results show clear evidence for shared age-related transcriptional changes in diverse species. To understand how these age-related transcriptional changes might be preserved in diverse species, it is important to consider current theories of the evolutionary basis of aging. There is an extensive and sophisticated literature on evolutionary theories of aging that provides key concepts to understand and interpret inter-species aging differences (Campisi, 2005; Golden et al., 2002; Kenyon, 2005; Kirkwood, 2005; Kirkwood and Austad, 2000; Martin, 2002).

A key consideration is that old animals do not normally constitute a significant fraction of the population in the wild. Wild populations die from predation and disease, and thus the principle determinant of natural longevity is extrinsic mortality (Fig. 2) (Kirkwood and Austad, 2000). For example, mice live 2–3 years under laboratory conditions, but 90% of mice die in their first year in the wild (Phelan and Austad, 1989). During human evolutionary history, the effective human population size was a mere 10 000 individuals, and life expectancy was around 20 years. Human life expectancy increased to 28 years in ancient Greece and Rome and to 46 years by the year 1900 (Martin, 2002). Currently, life expectancy is ~77 years in North America. Thus, only in modern human history do old individuals constitute a significant fraction of human society. In ancient times, most individuals died before the onset of white hair, wrinkled skin, muscle deterioration, loss of joint fluidity and other signs of old age. The effects of aging such as these are processes observed only under laboratory conditions (for animals) or in modern society (for humans).

Because senescent individuals are rare in the wild, changes that occur in old animals or people do not either help or hurt the fitness of their populations. Extrinsic mortality from predation and disease, not aging, has the strongest effect on overall fitness of a population. This realization led Medawar to develop a powerful theory of aging called the mutation accumulation theory. This theory states that old age is not under selective pressure *per se*, and there is no evolutionary mechanism to rid a population of mutations that cause detrimental effects only in old animals (Medawar, 1952).

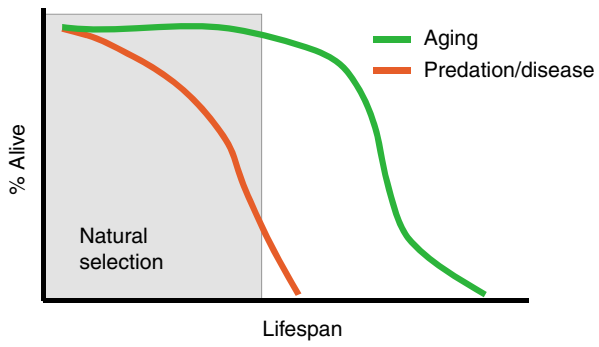

Fig. 2. Animals in natural populations die of predation and disease, not old age.

Mutations with late-acting phenotypes accumulate in the population over evolutionary time, and the cumulative effect of all of these late-acting mutations causes organismal senescence and limits lifespan.

Evolution would select for aging only insofar as that aging must occur more slowly than extrinsic mortality (i.e. one year for mice and 20 years for humans). For animals that lack predators, such as humans, bats and birds, the aging process is relatively slow because these animals survive many years in the wild. For animals that die rapidly from predation, such as worms, flies and mice, the aging process is correspondingly rapid as there is no evolutionary pressure to select for long life. In a classic experiment on the evolution of aging, Austad assessed the effect on aging due to a rapid decline in predation (Austad, 1993). He found that mainland opossums have a high rate of predation from carnivores. He found a small section of land that had become isolated from the mainland by a river, and which also happened to lack predators of the opossum. The opossums on the island had a significantly longer lifespan than those on the mainland, indicating rapid evolution of a longer lifespan. This result suggests that the lack of predation of island opossums made it possible for those individuals with slower rates of aging to come under natural selection and for the population to then evolve a longer lifespan. By analogy to the opossum example, the human genome is capable of supporting a longer lifespan than the mouse genome because there is less death from predation and disease in primitive humans than in mice.

Three categories of aging

What sorts of causes could explain declining organismal function in old age, and would one expect to see similar or different aging expression patterns in different species? The mutation accumulation theory suggests that mechanisms that limit lifespan may fall into three categories (Fig. 3). The first category includes all aging mechanisms that involve a gradual deterioration of cellular and metabolic processes with age. There is a strong selection for optimal function of cellular and biochemical pathways until young adulthood. After that, these pathways are not well maintained due to lack of selective pressure, and cellular function gradually declines, eventually resulting in cellular dysfunction and organismal senescence. Examples of aging mechanisms in this class include oxidative damage (Harmon, 1972), DNA damage (Hasty et al., 2003), telomere shortening (Shay and Wright, 2001), protein glycation (Schmidt et al., 1994) and transcriptional noise (Bahar et al., 2006), which are all mechanisms that could lead to cellular degradation and dysfunction in old age.

According to this view, aging represents the detrimental effect of cellular pathways that are not well maintained after young adulthood. The specific pathways that degenerate may be different for each species. For example, DNA damage may accumulate in humans but not worms because humans have about 10^{14} cells and *C. elegans* has only 959 cells (Sulston and Horvitz, 1977; Sulston et al., 1983). Telomere shortening may have a stronger effect on lifespan of humans than mice because mice have much longer telomeres than humans (Hemann et al., 2000). It could be that mutations that have accumulated in mice and that lead to rapid decline in three years may be entirely different from those that have accumulated in humans and that result in slow decline over 80 years. For mechanisms that fall in this category, genetic causes of aging are likely to be specific for each species.

The second category of aging mechanism involves pathways that are linked to a process that occurs during development or young adulthood. Some genetic pathways may exhibit antagonistic pleiotropy, which means that the same pathway is beneficial in young adulthood but detrimental in old age (Williams, 1957). For example, cell senescence is a mechanism to limit cell division that may be beneficial early in life to help prevent cancer but may be detrimental late in life because it limits cell divisions and eventually prevents cellular regeneration in aging tissues (Campisi, 2005). Caloric restriction falls into this category of aging because it can extend life in old age but is clearly linked to beneficial effects in young adults. Caloric restriction extends lifespan in yeast, worms, flies, mice and probably primates (Kenyon, 2005). All animals

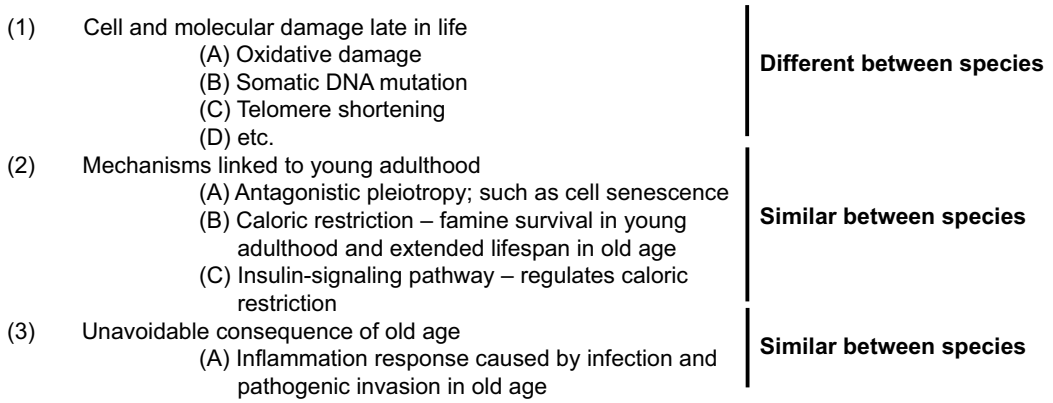

Fig. 3. Three categories of aging predicted by the mutation accumulation theory.

face times of starvation and drought, and a common strategy to improve fitness during harsh times is to extend lifespan and fertility in order to propagate the species when plentiful times return.

Because cell senescence and famine survival are key processes affecting young animals, they are under strong selective pressure and are evolutionarily conserved. The effects of cell senescence and caloric restriction on aging in late life may occur repeatedly for different animals, not because their effects on aging are conserved but rather because these aging mechanisms are tightly linked to functions in young animals.

Caloric restriction appears to be under control of the insulin-like growth factor signaling pathway (Guarente and Kenyon, 2000). Like caloric restriction, this signaling pathway has a role in famine survival and lifespan extension in many species. Specifically, mutations in the insulin-like signaling pathway have been shown to increase lifespan in worms, flies and possibly mice (Guarente and Kenyon, 2000).

The third category of aging includes external mechanisms that are unavoidably associated with old age. One example might be infection and inflammation that occurs in old age (Franceschi et al., 2000). As any animal grows old and begins to show physiological decline, there is an increased opportunity for pathogenic invasion. Thus, pathogenic invasion from external sources may occur in all species as they grow old, not because it is evolutionarily conserved but rather because it is an inescapable condition of old age.

What do the transcriptional profiles of aging in multiple species inform us about these three categories of aging? First, the vast majority of age-related transcriptional changes are different between different species (Fraser et al., 2005; Zahn et al., 2006). This observation suggests that most of the aging process falls under the first category – species-specific degeneration of cellular and metabolic pathways in old age.

Second, age regulation of only one pathway, the electron transport pathway, was observed in worms, flies, mice and humans (Zahn et al., 2006). The mechanisms responsible for decreased expression of the electron transport chain in old age are not known. It is unlikely that this pathway is commonly age regulated because of evolutionary selective pressure *per se*. Rather, common age regulation is likely the result of an unavoidable consequence of old age (aging category three). One possibility is that cellular damage from mitochondria in old age is unavoidable, which would suggest that oxidative damage may be a ubiquitous source of damage that limits lifespan in all animals. However, this possibility seems unlikely because mitochondrial function is highly conserved in all metazoans, and it seems unlikely that mitochondria in mice would cause extensive oxidative damage that severely limits life span (2 years) whereas mitochondria in humans would cause minimal damage and permit a long lifespan (80 years). Second, it is not obvious why mitochondria in lower animals would not function as efficiently as human mitochondria, and hence permit lifespans approaching that of humans. Another possibility is that expression of the electron transport chain scales with overall metabolic activity of the cell and that aging lowers cellular metabolic activity in all animals.

In summary, comparison of aging transcriptional profiles in worms, flies, mice and humans provides a quantitative, global view of the overall relatedness of the aging process across different species. These results provide a view of the relative proportion of the aging process that is specific to humans (private) rather than shared across animals (public). The vast majority of age-related transcriptional changes are private to humans, and these are likely the result of cell degeneration pathways that are species specific (aging category one) (Fig. 3). The emerging view from the genomics experiments is that the aging process is quite different in mice and humans, emphasizing the need for research using human samples to uncover aging mechanisms relevant to human longevity. A small amount of age-related changes in expression are public across species. These public aging pathways may be linked to functions in young adults (aging category two) or may be unavoidable consequences of growing old (aging category three). Identification of these public pathways is key because they highlight specific aging pathways that can be dissected apart in model organisms to elucidate general principles of aging.

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