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Ageing

Using whole-genome transcriptional analyses to identify molecular mechanisms of aging

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Whole-genome transcriptional analyses of aging organisms and tissues have revealed changes in many genes and biological processes. A comparison of these studies reveals common age-related changes, including the downregulation of metabolic and mitochondrial function, and the upregulation of stress and immune responses. This suggests that much of the aging process might be conserved across species, although tissue- and species-specific changes also occur. These comparisons will help identify the underlying mechanisms of aging.

Introduction

The development of DNA microarray technology has made it possible to compare the entire transcriptome of an organism or tissue in different states, allowing the inference of biological mechanisms at work [1]. A global perspective might be especially helpful in understanding complex biological phenomena, such as aging, where multiple genetic and biochemical pathways are expected to play a role. The key to understanding aging will be to identify all of the major regulatory pathways and targets, and then to experimentally determine the contribution of each factor to the aging process.

The transcriptional changes accompanying age have been analyzed in organisms ranging from yeast to humans. Here I will examine common biological themes that have emerged from such studies, discuss the relevance of the findings to current theories of aging, and highlight emerging methods that will push the field forward.

The aging transcriptome

Nonvertebrate model organism studies of aging

The short lifespans and genetic tractability of yeast, worms and flies have made them crucial in the study of longevity-regulating pathways [2]. Additionally, their genomes were sequenced relatively early, placing them at the forefront of whole-genome transcriptional analyses.

The number of daughter cells produced by an individual *Saccharomyces cerevisiae* mother defines its 'replicative lifespan' [2,3]. Old mother cells can be separated from daughter cells through elutriation centrifugation, and two groups used this separation technique to define the transcriptional profile of old mothers [4,5]. Genes involved in cell cycle control, lipid metabolism, mitochondrial function, apoptosis and DNA repair (the 'DNA damage signature' [6,7]), changed with age. Ribosomal components are downregulated with age, perhaps indicating an effort to conserve energy [8], whereas specific histone genes are upregulated. The fact that transcription was largely induced in old cells relative to young cells suggests that the aging profile reflects an active response to damage and genomic instability accompanying age, rather than a general downregulation of transcription.

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The replicative lifespan of yeast might provide a model for mitotically dividing cells in higher organisms, whereas *C. elegans*, whose adult cells are postmitotic, could serve as a model of nondividing cells and tissues. Worms develop to adulthood in 2 days, are reproductive for roughly 4 days, and have a relatively long (2–3 weeks) postreproductive lifespan. Hill *et al.* [9] included one 2-week-old sample in their analysis of gene expression changes throughout *C. elegans* development and observed reductions in transcripts of genes related to muscle function, extracellular matrix and metabolism. Lund *et al.* [10] carried out a timecourse over 19 days, which is beyond the median lifespan of wild-type worms [11], while other studies examined changes in adults through middle age (8 days) [12,13]. Like yeast, worms exhibit changes in genes associated with metabolism, mitochondrial function, stress-response (particularly heat shock proteins) [10,13] and histones [12], with age. New themes of aging emerge with the analysis of multicellular systems: collagens are downregulated [12,13] and insulins show gene-specific regulation with age [10,12]. Neither DNA damage signature or cell-cycle genes appear to be regulated with age in *C. elegans*, whose soma is entirely postmitotic.

Many of the same classes of genes observed in yeast and worm aging are also regulated in old flies: aged *Drosophila melanogaster* [13–17] also reduce transcripts associated with metabolism, oxidative phosphorylation, and mitochondrial function, while heat shock proteins, oxidative stress response, chaperones and detoxification genes are upregulated [14–17]. Additionally, genes associated with reproduction are downregulated, whereas immune response genes increase with age.

Mammalian studies: tissue-specific analyses

Surveys of transcriptional changes of select subsets of genes in mouse tissues were among the earliest microarray studies of aging [18]. Aging profiles of tissues include skeletal muscle [18,19], liver [20], various brain tissues [21–24], salivary gland [25], cardiac muscle [26,27] and adipose tissue [28]. Although there are some tissue-specific effects (e.g. downregulation of genes involved in synaptic transmission and neuronal structure, and upregulation of specific proteases in aged brain [24]), many of the reported changes are similar across tissues, with increased transcript levels of inflammatory and immune response genes, stress response genes (including heat shock, oxidative stress response and DNA damage response genes), and extracellular matrix components (collagens). This generally downregulated set includes genes associated with metabolism, mitochondrial function, protein synthesis and turnover and growth factors. A study of aged rhesus monkey skeletal muscle displayed a similar constellation of changes to those observed in mouse [29], suggesting that there might be a general mammalian aging signature.

Many human tissues, including kidney [30–32], muscle [33], brain [34,35] and eye [36,37], as well as a variety of human tissue-culture lines [38,39], have been examined for age-related changes. The kidney analyses [30–32] revealed increases in genes associated with immune/inflammation response and extracellular matrix turnover, and decreases in transcripts associated with mitochondrial function, metabolism and stress response. Changes in apoptosis, cell growth, genome stability and cell signaling genes were also observed [32]. Similar to a study of *Drosophila* heads [14], aged human brain exhibits decreases in genes associated with synaptic function and vesicle transport [34]. Mitochondrial and protein turnover genes are also downregulated in middle age, followed by an upregulation of stress response, immune response and DNA repair genes [34]. Loss of muscle (sarcopenia) with old age is associated with increased transcription of genes associated with apoptosis (including the FOXO3A transcription factor, which induces cell-death genes) and inflammation [33], as well as increases in histone H3, RNA-binding factors and the beta retionoid X receptor. Decreased transcripts include the Krueppel-related zinc finger protein, histone H2bd and mitochondrial and amino acid metabolism genes. Interestingly, the circadian cycle negative regulator period-2 is upregulated [33] in skeletal muscle, perhaps reflecting the breakdown of circadian rhythms with age.

Thus, it appears that many of classes of gene function that change with age in nonvertebrates (stress response, DNA damage response, immune function, mitochondrial function, histones, metabolism, ECM) represent an aging signature that is shared with higher organisms (Table 1), whereas other changes might be tissue- or organism-specific.

Altered aging profiles

The global transcriptional analysis of lifespan-extending conditions offers the opportunity to discover factors that positively contribute to longevity. Dietary restriction (DR) has been shown to extend lifespan in worms, flies, yeast and rodents [2]. The effects of DR on transcription were measured in several mouse tissues, including heart, skeletal muscle, brain and adipose tissue [23,40]. In general, the transcriptional changes that accompany age (increased inflammatory and stress response, decreased energy metabolism) are reversed in dietary-restricted mouse tissues, suggesting a 'metabolic reprogramming' [40] that shifts the transcription to a younger profile. A notable exception, however, is that skeletal muscle from middle-aged DR rhesus monkeys does not exhibit this reversal in the age profile, but instead appears to be in a lowered metabolic state [29]. Although these results might indicate that primates are not amenable to lifespan increases through dietary restriction, it is more probable that the reversal in aging profile can only be assessed at a time later in life because the middle-aged DR-treated monkeys do exhibit measurable positive changes in physiology [40].

Table 1. Age-related transcriptional changes

	Upregulated with age	Downregulated with age
***S. cerevisiae* [4,5]**	DNA damage response	Mitochondrial components
	Cell cycle regulation	Ribosomal genes
	Mitochondrial components	Metabolism
	Lipid metabolism	
	Cell wall synthesis	
	Histones	
***C. elegans* [9,10,12,13]**	Stress response	Mitochondrial function
	Histones	Oxidative phosphorylation
	Insulins	Muscle function
	Immune response	Extracellular matrix/collagens
		Metabolism
		Insulins
***Drosophila melanogaster* [14–17]**	Stress response	Mitochondrial function
	Immune response	Oxidative phosphorylation
		Reproductive function
		Metabolism
Mouse	Immune/inflammatory response	Metabolism
Cardiac muscle [26,27]	Stress response	Mitochondrial function
Skeletal muscle [18,19]	DNA damage response	Oxidative phosphorylation
Liver [20]	Extracellular matrix	Protein synthesis/turnover
Adipose tissue [28]	Apoptosis	Growth factors
Brain, neurons [21–24]		Fatty acid metabolism
		Biosynthetic enzymes
		Neural plasticity factors
Rhesus monkey	Immune/inflammation	Mitochondrial function
Skeletal muscle [29]	Stress response	Oxidative phosphorylation
	Neuronal death/remodeling/repair	Metabolism
		Cell growth
Human	Extracellular matrix	*mortalin*-2 HSP 70 (antiapoptosis)
Kidney [30–32]	Ribosomal proteins	Insulin-like growth factor
	Immune response	Mitochondrial function
	Apoptosis/cell death	
	Renal disease genes	
Skeletal muscle [33]	Immune response	Energy metabolism
	FOXO3A, FOXO1	Mitochondrial function
	H3 Histone, 3A	Histone 1, H2bd
	Ubiquitin-proteasome	Krueppel-related zinc finger
	Stress response/repair	
	Steroid hormone-related	
Brain [34,35]	Stress response/repair	Mitochondrial function
	Immune/inflammation	Synaptic function
		Neuronal survival

Dietary restriction might offer some hope in staving off the effects of aging, but due to the extreme nature of the DR regimen (a 30–40% reduction in caloric intake), exercise might offer a more realistic aging therapy. Heart muscle from mice selectively bred for high voluntary exercise levels was compared with sedentary mouse heart tissue, and like DR, suppressed the age-related changes in inflammation and stress response [27]. Voluntary exercise increases median, not maximum lifespan; but if the targets of exercise are similar to the targets of dietary restriction, why would only the latter increase maximum lifespan? The authors suggest that exercise might only provide short-term benefits, or might affect only a subset of tissues. In addition to physical activity, mental activity might also prove beneficial: an enriched environment improves memory and reverses transcriptional changes associated with brain aging [24].

Genetic mechanisms can also affect aging rates. The accelerated aging phenotypes of progeroid syndromes might reveal particularly crucial factors in the aging process. A comparison of human primary fibroblasts from young, old and Werner syndrome progeria patients [39] highlighted a shared change in the response to DNA-damage between the old and progeroid cells, perhaps unsurprising because *WRN* is a DNA helicase. Another progeria, Hutchinson–Gilford

Syndrome, is caused by a mutation in the gene for lamin A protein, a component of the nuclear lamina. When HGPS fibroblasts were compared to age-matched fibroblasts, transcription factors were the largest functional category found to be differentially expressed, and were primarily downregulated [41]. Extracellular matrix components, including genes known to be involved in arteriosclerosis (a primary cause of HGPS mortality) were also affected. Although some of the gene expression changes observed in progeroid syndromes (e.g. DNA damage response) are shared with natural aging, most appear to be either disease-specific or tissue-specific; this might not be helpful in the study of aging, but there might be specific targets that could be useful in the treatments of these diseases.

One of the best-studied genetic determinants of lifespan is the insulin-IGF-1 receptor/FOXO transcription factor pathway [2]. Several groups used microarrays [12,42–44] and later, SAGE [45] to identify the full set of transcriptional targets of the DAF-16 FOXO transcription factor of *C. elegans*, which is required for the long lifespan of *daf-2* insulin receptor mutants. Although some of the expected genes, such as those encoding superoxide dismutase, heat shock proteins, metabolic enzymes and detoxification factors, were upregulated, many novel genes and gene classes were also identified [12]. One study ranked the set of genes using a combination of expression magnitude, expression consistency over 70 arrays, and consistent regulation by *daf-2* and *daf-16*, then used RNAi to test the top 58 genes for their contributions to lifespan [12]. The vast majority of these genes, including several genes of unknown function, affects lifespan, though none fully phenocopy the *daf-16* null, suggesting that the coordination of DAF-16 of a broad spectrum of genes is crucial for the effects of insulin receptor on longevity.

The insulin receptor/FOXO pathway and its effects on longevity are conserved from worms to flies [46] through mammals [47]; in fact, heterozygous IGF-1 receptor knockout mice are long-lived [48], as is the fat-specific insulin receptor knockout (FIRKO) mouse [47]. It will be interesting to identify the targets of the mammalian and Drosophila FOXOs and compare them with the DAF-16 transcriptional targets; this should reveal whether FOXOs use a conserved set of gene functions to promote longevity in all organisms, or whether only the regulatory part of the signaling pathway is conserved.

What can we learn from these global analyses?

It is clear that certain changes are typical of aging tissues: the differential expression of genes involved in stress responses, immune function, metabolism, mitochondrial function, extracellular matrix formation and chromatin components is a common theme in aging profiles. The activation of stress response genes in both aging and with oxidative damage [16,17] suggests that the ROS response (the usual suspect in aging studies) plays a role in aging. However, the small change in *C. elegans* lifespan upon the reduction of superoxide dismutase and catalase [12] indicates that other genes are likely to be important in longevity, as well.

Although it has been commonly assumed that the transcriptome degrades with age [30], in fact there does not seem to be a systemic disregulation of expression [5,15]. Also, age-related changes in transcription do not appear to be localized to specific chromosomal regions [15]. However, the promoters of genes with reduced expression in aged frontal cortex show significant DNA damage [34], suggesting an interesting mechanism of vulnerability to oxidative stress. It is possible that epigenetic factors could contribute to differences in gene expression with age [49], and might also account for differences in final transcriptional output in related species [35], as well as the stochastic rate of aging in genetically identical animals [50].

Do similarities in expression profiles suggest that aging mechanisms are shared? McCarroll *et al.* [13] examined the expression of *C. elegans* and *Drosophila* orthologs pairs with age and found a highly significant shared set of transcriptional changes, including the repression of mitochondrial components. The timing of these changes is important; the fact that they occurred early in adulthood suggests that rather than a response to damage, these genes are part of a program of aging [13].

Regardless of whether existing transcriptional profiles can be used to address the underlying mechanisms, they can be used to find biomarkers of aging and to define a molecular signature of aging [30], both of which have practical applications. For example, the efficacy of treatments to affect health and longevity might be assessed through transcriptional profiling and comparison with normal aged profiles, and biomarkers can be used in model systems to screen for longevity genes and to assess lifespan effects [17,50]. Additionally, organs from older individuals could be profiled to determine the 'chronicity index' of the tissue [30], increasing the pool of eligible donors [31].

Future directions for global transcriptional analysis of aging

Although significant progress has been made in the identification of changes that occur with age, the challenge of understanding the molecular events that lead to aging is still ahead of us. One of the major limitations to interpreting the meaning of expression differences between old and young animals is the fact that cause and effect cannot be deconvoluted in single-point comparisons. In many studies the same result (e.g. downregulation) is attributed to either a cause or an effect of aging, depending exclusively on prior knowledge of the function of gene product in the cell. The lack of functional information about unknown genes, or even the extent of contribution of known genes to the aging process is

a severe limitation to the interpretation of transcriptional data and the discovery of new gerontogenes. Here I will discuss recent advances in methods and analyses that I believe will be especially helpful in addressing this challenge.

Transcriptional profiles of additional longevity mutants and treatments could be used to define (or to test the hypothesis of the existence of) a set of *common longevity targets*, for example, between the insulin pathway, DR and other longevity pathways [2]. Of course, given large enough gene sets, one would expect to find some random overlap in transcription between any two processes or treatments [43], regardless of a true shared mechanism. Therefore, estimating the rate of *false positives* [51] will be important when testing the theory of shared mechanisms [13].

Timecourses can be used to clearly define changes throughout age [15,30,34], to separate genetic programs from responses [13], and to distinguish true age-related changes from subtle developmental timing differences in longevity mutants [12]. For example, by examining prefrontal cortex from 30 adults aged 26–106 years [34], Lu *et al.* were able to distinguish two clusters of genes whose expression defines the young adult transcriptome ($\leq$40 years) and the aged transcriptome ($\geq$70 years); the former group includes genes necessary for brain function, whereas the latter primarily consists of damage response. These transcriptional studies will be enhanced by the use of *computational methods of timecourse analysis* [32] to reveal patterns of change across age, and to reveal biomarkers of chronological age [15].

Model systems have several advantages to offer in the study of aging; multiple longevity pathway mutants exist in yeast, worms and flies [2], and lifespan experiments on these mutants can be carried out rather quickly. Combining transcriptional timecourse data with careful *phenotypic characterizations* will make the model systems especially useful in addressing the cause versus effect problem. The ability to functionally analyze candidate genes is perhaps the most important attribute of model systems. In *C. elegans*, the high-throughput feeding-RNAi method allows one to quickly obtain functional data even for previously unknown genes [12]. As RNA interference methods are optimized in other systems, more functional analysis will accompany transcriptional profiling. The experimental determination of the contribution of each gene to longevity will be an important component of the global model of aging.

Defining *orthologs* [13,52] across species will enable the direct comparison of transcriptional results and will allow us to test the hypothesis of shared mechanisms of aging. McCarroll *et al.* [13] used this approach and identified a shared program between worms and flies in early aging. In another example, Fraser *et al.* [35] compared the pattern of age-associated gene expression in chimpanzee cortex with the human cortex aging pattern. (Because the sequence similarity is high between humans and chimps, both can be assessed on human arrays.) The authors suggest that the differences that they found might be due to differences in metabolism between the species and subsequent ROS damage rates. Another source of differences might be environmental; given the enriched-environment effects on aging mouse brain [24], this might be an interesting aspect to consider. Similar analyses across species will distinguish species-specific from shared mechanisms of aging.

The growing use of *Gene Ontology* (GO) terms [13,15] and EASE [53] is helping to identify biological processes at work. As more transcriptional analyses are deposited into public databases and the biological descriptions of gene function are augmented, all of this information can be integrated into large-scale data comparisons. These additions to our current knowledge will help test hypotheses of aging, and will define the underlying molecular mechanisms.

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