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Systems biology of aging in four species
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Using DNA microarrays to generate transcriptional profiles of the aging process is a powerful tool for identifying biomarkers of aging. In *Caenorhabditis elegans*, a number of whole-genome profiling studies identified genes that change expression levels with age. High-throughput RNAi screens in worms determined a number of genes that modulate lifespan when silenced. Transcriptional profiling of the fly head identified a molecular pathway, the 'response to light' gene set, that increases expression with age and could be directly related to the tendency for a reduction in light levels to extend fly's lifespan. In mouse, comparing the gene expression profiles of several drugs to the gene expression profile of caloric restriction identified metformin as a drug whose action could potentially mimic caloric restriction *in vivo*. Finally, genes in the mitochondrial electron transport chain group decrease expression with age in the human, mouse, fly, and worm.

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Introduction
Aging is a process in which there is a progressive loss of function in multiple tissues resulting in an increasing probability of death. There are a large variety of genetic and environmental ways in which lifespan can be extended in model organisms. These include mutation of genes in the insulin-like signaling pathway [1], caloric restriction [2], overexpression of sirtuins such as *Sir2* [3], or even by inducing mild heat shock in worms [4]. Since lifespan can be extended in many ways, it seems that aging is a complex process and can be influenced independently by a relatively large number of age-associated pathways [5]. Rather than focusing on one or even several pathways in studying the molecular processes of aging, an increasingly powerful approach has been to screen the entire genome for age-associated genes and genetic path-

ways. DNA microarrays can be used to screen the genomes of several organisms for genes and pathways that either increase or decrease expression levels with age. In the past several years, DNA microarray studies of aging gene expression have been vigorously pursued in *Caenorhabditis elegans*, *Drosophila melanogaster*, mice, and humans.

A major aim of these studies is the identification of novel biomarkers of aging. For several decades, the aging field has searched for measurable biological parameters that can accurately predict the physiological decline of an aging organism [6]. Specific aging biomarkers are apparent at the level of the whole organism and also at the level of tissues. Given the complex nature of aging, it is hoped that transcriptional profiling studies will be able to identify molecular biomarkers of aging that are more sensitive and more specific than cellular or tissue biomarkers (Figure 1). In this review, we will examine the results of studies using transcriptional profiling and other systems biology techniques to study the effects of aging in worms, flies, mice, and humans. Here, we mainly restrict our focus to studies completed within the past 2 years. For a more complete review of systems biology of aging see [7].

Transcriptional profiles of aging in *C. elegans*
The roundworm *C. elegans* is a model system particularly suited to studies of aging, exhibiting a short lifespan of approximately 2 weeks and offering the availability of a robust genetic toolset. Previously, whole-genome transcriptional profiles of worm populations of varying ages were generated using DNA microarrays [8,9]. Lund *et al.* used cDNA microarrays to profile worms at six time points after reaching adulthood and discovered a transcriptional profile consisting of 164 genes that either increased or decreased expression with age. Transcriptional profiles have also been generated comparing mutant worms with altered longevity (such as *sir-2.1*, *daf-2*, *daf-16*, and *age-1*) with wild-type worms in order to identify downstream genes involved in lifespan [10•,11–13]. For instance, Viswanathan *et al.* tested the effects of resveratrol, a compound known to extend lifespan of worms, in several mutant backgrounds [10•]. They found that resveratrol failed to extend lifespan of *sir-2.1* mutant worms, suggesting that resveratrol acts through the *sir-2.1* pathway. Viswanathan *et al.* also generated a transcriptional profile of resveratrol-treated worms and observed that genes involved in the endoplasmic reticulum (ER) stress response displayed increased gene expression after resveratrol treatment. Silencing of *abu-11*, an ER stress response gene, by RNAi abolished lifespan extension by reveratrol treatment.

Figure 1
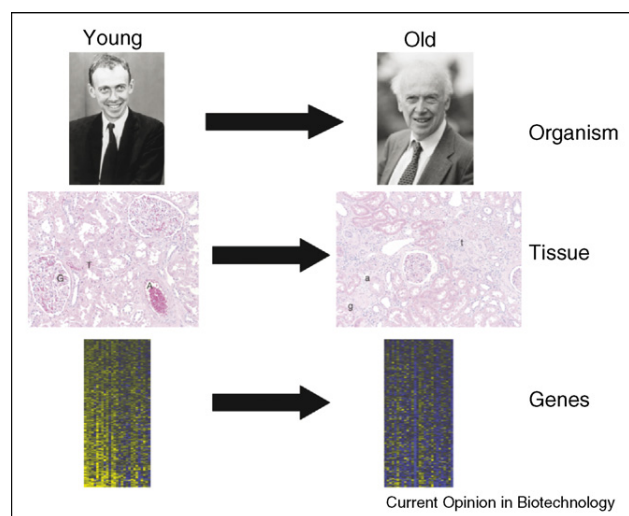
Biomarkers of aging. Examples of biomarkers of aging in young (left) and old (right) individuals. Top: at the level of the whole organism, biomarkers such as wrinkled skin and whitened hair are apparent in old individuals. Center: examples of histology in human kidney cortex. A, G, and T refer to arterioles, glomeruli, and tubules, respectively. Each of these histological features displays very different morphologies in old versus young individuals. Bottom: heat map of genes that decrease expression level in elderly human kidneys. These genes are molecular biomarkers of aging.

A concept of interest is to use biomarkers of aging to predict the lifespan of individuals within a population with an identical age and sharing the same environment. Levels of lipofuscin, a pigment thought to correlate with physiological age, were found to predict the remaining lifespan of worms [14]. Golden *et al.* performed DNA microarray analyses on individual worms, with the aim of discovering genes that vary stochastically among individuals within an age-matched population [15,16].

RNAi libraries may be used to systematically silence essentially every gene within the *C. elegans* genome. RNAi-silenced worms can then be examined for longevity phenotypes to uncover genes and pathways involved in aging. A number of genome-wide RNAi screens for longevity have been recently completed in *C. elegans* [17•,18•,19,20]. These studies found that a large number of genes expressing components of the electron transport chain promoted longevity when silenced, and these genes typically produced the largest increases in lifespan [17•,18•,19,20]. This supports the importance of the oxidative phosphorylation system in the determination of lifespan. Genetic epistasis experiments suggest that some of the genes could potentially act in the *daf-2* insulin receptor pathway whereas other genes might act in the caloric restriction pathway [17•]. Although these studies use epistasis to assign genes to pathways, further work remains to be accomplished at the molecular and

biochemical levels in order to definitively show the functional relationships between these longevity genes and mechanisms with which they interact. Notably, the results of these studies did not show a high degree of overlap, suggesting that future RNAi screens will continue to find additional longevity genes and that the total number of genes that can affect longevity is quite high.

Gene expression studies of aging in *D. melanogaster*

The fruit fly *D. melanogaster* is relatively short-lived, and aging in the fly has been studied using gene expression profiling. A benefit to working in the fly is that it can easily be dissected into component body parts and organs, allowing for the study of aging in specific tissues. Pletcher *et al.* used DNA microarrays to measure transcriptional changes in young and old flies, under normal and calorically restricted dietary conditions, and discovered 885 age regulated and 827 calorically restricted transcripts [21]. The rate of age-related change in these genes was ameliorated under calorically restricted conditions, suggesting that these genes are physiological biomarkers of aging in the fly. Landis *et al.* examined the transcriptional profiles of flies that were raised in the presence of 100% oxygen and compared it with a transcriptional profile of aging in flies, discovering that several pathways were similarly regulated between the two conditions [22]. This result supports the oxidative damage theory of aging in the fly.

Girardot *et al.* recently compared the transcriptional profiles of 3-day-old flies with 40-day-old flies not only at the level of the whole organism but also on dissected heads and thoraxes using Affymetrix DNA chips [23•]. Another group also profiled the effects of aging on the fly head using cDNA microarrays [24]. Both groups agreed substantively on which genes are age regulated in the fly head and found that energy pathways and particularly proton transporters significantly decrease expression with age. Interestingly, Girardot *et al.* found that this decrease in expression with age occurred less in the thorax than in the head, arguing that the dynamics of age regulation vary from tissue to tissue in the fly. Both groups found that the genes involved in the response to light decreased with age in the fly head. It has been known for some time that flies grown in darkness have increased median lifespan [25,26] when compared with flies grown in dim or bright light. The effect of reduced light on lifespan may be related to reduced expression of the genes involved in the response to light in old age.

DNA microarray studies of aging in mice

The common mouse *M. musculus* is a popular model for aging, despite its relatively long lifespan when compared with simple organisms such as worms or flies. The first transcriptional profiles of aging were generated in a limited number of mouse tissues such as muscle [27] and brain [28]. Recently, transcriptional profiles of such

diverse organs as adipose tissue [29], heart [30], liver [31••], and macrophages [32] have been studied. A recent study by Edwards *et al.* used an empirical Bayes approach to identify 712 transcripts that are age regulated in mouse muscle that were enriched for genes active in the *p53* pro-apoptotic pathway in addition to genes encoding mitochondrial proteins involved in energy generation [33]. The *p53*-related genes increase expression level as age progresses, suggesting that age-associated DNA damage may lead to a *p53* response in the muscle of older mice. The p53-related genes also exhibited a reduced expression level in mice undergoing caloric restriction, suggesting that they are physiological biomarkers of aging in the mouse.

There is a widespread interest in discovering biomarkers of aging for their ability to act as surrogate endpoints in screening for pharmaceuticals that extend longevity. Dhahbi *et al.* used the molecular profile of caloric restriction generated by DNA microarrays in mouse liver as such a surrogate endpoint. Drugs that mimic the transcriptional response to caloric restriction may also act to delay or ameliorate the effects of aging [31••]. Dhahbi *et al.* compared the transcriptional profile of caloric restriction with profiles of the livers from mice given anti-diabetic medications metformin, glipizide, and rosiglitazone. They found that the effects of metformin on liver expression showed a highly significant overlap with the molecular profile for caloric restriction.

A novel experiment by Bahar *et al.* assayed the robustness of the transcriptional network with age in mouse cardiomyocytes [34••]. Bahar *et al.* used RT-PCR to measure the variance in expression of a number of genes in cardiomyocytes sampled from young and old mice and found that cardiomyocytes from old mice displayed a larger variance in gene expression than the cardiomyocytes from younger mice. This suggests a mechanism of aging in which the entire transcriptome degrades with age, leading to noisier gene expression and a general loss of transcriptional control. Small increases in levels of gene dysregulation in old mice could be catastrophic to the entire genetic network when amplified across all genes expressed in a cell and result in cellular dysfunction and senescence.

Expression profiles of aging in humans
Despite its great relevance, the study of aging in humans is difficult because of the wide variability in genetic backgrounds and environmental influences in different individuals, in addition to the scarcity of human tissue samples for study. Nonetheless, transcriptional profiles of many human tissues, including brain [35,36], blood [37], eye [38], kidney [39,40], muscle [41,42,43••], and skin [44] have been generated. Several studies have used large sample sizes to overcome the genetic and environmental variabilities inherent in human populations, per-

mitting these studies to achieve a higher level of statistical significance [35,39,43••]. In recent years, there has been a trend toward comparing the transcriptional profiles of several different tissues to each other, aiming to determine genes and genetic pathways that show common patterns of age regulation in different tissues. In a study of aging in the kidney, Rodwell *et al.* [39] compared the transcriptional profile of the medulla to that of the cortex and found that two sections of kidney composed of very different cell types shared similar patterns of age-regulated gene expression. Fraser *et al.* [36] compared areas of the frontal cortex to the cerebellum and failed to find any significant overlap in age regulation. Finally, in a comparison of the molecular profiles of aging muscle, kidney, and brain, Zahn *et al.* discovered a common signature of aging in these three tissues consisting of six genetic pathways, including the mitochondrial electron transport chain, which decreases expression with age [43••]. These results suggest that while certain tissues may age very similarly to one another, such as the kidney, cortex and medulla, for other tissues, particularly tissues with widely divergent function, there exist only small numbers of common aging pathways.

To what degree do different species display similar patterns of age regulation? To date, only a few studies have compared transcriptional profiles of aging to detect the genes and pathways that are publicly age regulated between multiple species and those that are privately age regulated within a single species. Recently, Zahn *et al.* have compared transcriptional profiles of aging in worms, flies, mice, and humans to search for genetic pathways that show similar age regulation in all four species. They found that one genetic pathway, the electron transport chain, that decreases expression with age in all organisms investigated [43••]. The electron transport chain may prove to be a particularly useful biomarker for aging studies because it shows an overall two-fold decrease in expression over the course of life, regardless of the average lifespan of the organism.

Conclusion
A large number of transcriptional profiling studies of aging have been completed and the gene expression data have been made publicly available. These molecular profiles explore the effects of aging in a multitude of experimental conditions, among many tissues and many relevant species. In nearly all studies, only those results generated by that study are analyzed and results from other studies of gene expression profiles of aging are not examined. In future, it will become important to integrate the results from each of these studies in order to determine whether age-related effects are reproducible. Data integration could be used to classify tissues into different aging pathways according to similarities in their expression profiles. Data integration could also show which

genes and pathways are age regulated in a tissues-specific fashion or organism wide. Finally, data integration between species will continue to classify age-related changes as either private to one species or public across different species. Results from meta-analysis of existing data from several publications will help uncover significant, novel insights about the aging process, as well as identify key biomarkers of physiological aging. Beyond biomarkers, identifying the genes that specify and control, rather than merely correlate with lifespan will broaden our understanding of what aging is and how aging occurs in various organisms. To this end, the identification of promoter motifs in age-regulated genes as well as the transcription factors that bind them will aid in elucidating the molecular mechanisms of aging. In the future, as systems biology techniques progress, it may become possible to study aging at a multitude of levels within the cell, from transcriptional changes to changes in protein quality or quantity, to changes in metabolic flux through genetic pathways. Collating this information will provide a global fingerprint of aging, enabling one to use systems level analysis to dissect apart complex changes involved in aging.

Acknowledgements

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