

Is aging an evolved developmental program?

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Abstract

What is aging? Evolutionary theory posits that it is the consequence of the accumulation of damage after the attainment of reproductive maturity, due to a weakening of the strength of natural selection in late life. This construct implies that aging represents disordered biology that is superimposed upon the living state. In this review, data collected across multiple species, including plants, strongly suggest that aging represents an evolved species-specific developmental program. Such a construct would account for the varied aging trajectories that can be observed across different species, each of which has its own characteristic pattern of aging. However, aging is a plastic developmental program and this plasticity accounts for the marked variation in aging trajectories observed across individuals within a single species; the result of environmentally induced epigenetic changes. Interventions directed at the plasticity of the aging process are the ones most likely to be successful in modifying this process.

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Introduction

The biology of the aging process has generally been considered as distinct from the biology underlying 'normal' physiological functions. Within the evolutionary framework, animal resources are preferentially allocated for growth, sexual maturation and reproduction in the early life cycle stages, thereby maximizing Darwinian fitness. As the animal ages, the strength of natural selection weakens and fewer resources are devoted to repair and maintenance processes. With aging, the weakening of natural selection means that genomic variants with beneficial phenotypic effects in the early stages of the life cycle can be associated with deleterious phenotypic effects in late post-reproductive life cycle stages [1, 2]. Within this construct, aging begins in the post-reproductive period and is viewed as an age-related decline in the physiological function of organs due to the accumulation of deleterious mutations [1].

Other investigators have proposed a more mechanistic framework for the aging process. Lopez-Otin et al. [3] described a number of physiological processes which, when disordered, are associated with aging. They categorized these disordered biological processes as the 'Hallmarks of Aging'. Within this mechanistic framework, aging is viewed as the end phenotype of multiple disordered physiological and metabolic processes. The implication is that by 'correcting' the underlying biological abnormalities, the aging process can be significantly modified.

An implication of both the evolutionary and mechanistic frameworks is that the biology of aging can be considered as separate and distinct from the biology of the living state; in fact, aging is now considered in the context of a risk factor for so-called chronic degenerative diseases [4]. This perspective will set a certain research agenda; indeed researchers using model organisms are already studying chemicals

such as rapamycin as potential anti-aging drugs for use in humans [5].

However, contrary to both the evolutionary and mechanistic perspectives, it is impossible to distinguish the biological processes underlying aging from those underlying the living state [6]. There are no biological processes specific to the aging process and there is, in fact, considerable evidence linking aging to developmental programs.

Studies in species

Jones *et al.* [7] graphed parameters of aging across 23 vertebrate, 10 invertebrate and 12 plant species. The investigators used a standardized age span, which began at the mean age of reproductive maturity for a given species and ended at a terminal age when only 5% of adults of that species were still alive. The parameters collected included relative mortality and relative fertility as well as survivorship, and these parameters were plotted against the standardized age span. It was found that each species has its own characteristic pattern for these parameters across its life span. For humans, the fertility trajectory is bell-shaped, with peak fertility occurring at a young age. Killer whales, lions and chimpanzees also show a bell-shaped fertility pattern, but for these mammals the fertility curves are less peaked, and are spread over a larger portion of the life span. Whereas humans are characterized by a relatively short fertility period followed by a long post-reproductive time span, some species such as the tundra vole remain fertile until the very end of the life cycle.

Humans show a sharp rise in relative mortality over the later stages of the life cycle, and a similar age-dependent mortality and fertility pattern were found in both modern post-industrial humans and in pre-industrial hunter-gatherers. However, other vertebrate species show very different age-dependent mortality patterns, varying from a relatively flat mortality trajectory with advancing age, to an actual decline in relative mortality with age - as observed in the desert tortoise.

The data of Jones *et al.* [7] underscore the extreme variation in age-dependent fertility and mortality observed across species, which does not easily fit into the evolutionary framework for aging. The patterns

predicted by the evolutionary theory of aging are ones of increasing age-dependent mortality and decreasing age-dependent fertility in the time period following attainment of reproductive maturity. With respect to the mechanistic framework for aging, to accommodate the extreme variation seen across species within this framework would necessitate that the aging of each species has its own specific mix of 'disordered' biological processes. A simpler construct is that the age-dependent aging and longevity pattern of a species is a function of its specific developmental program. The biological development (ontogeny) of each species is unique and establishes its aging and longevity trajectories.

De Magalhaes *et al.* [8] examined the relationships between maximum longevity and developmental variables across a number of mammalian species. Maximum longevity and mortality rate doubling time were used as proxies of aging rate. The developmental parameters included: time from conception to sexual maturity (time to maturity), post-natal growth rate, body mass, and adult life span (maximum longevity minus age at sexual maturity). De Magalhaes *et al.* [8] found a direct positive correlation between body mass and maximum longevity, and between body mass and time from conception to sexual maturity - hence larger animals take longer to reach adulthood, and also live longer. In addition, adult life span was directly correlated with time to maturity independently of body mass. This association indicates that for each mammalian species, the length of time required to reach sexual maturity directly correlates with the adult life span of that species. The implication is that this developmental/maturation process sets life span.

A similar set of relationships has been described for lizards and snakes by Shine and Charnov [9]. For females of the different species, there was an inverse relationship between age at reproductive maturity and instantaneous adult mortality rate; hence, a longer developmental time was associated with a lower mortality rate and therefore a longer adult life span. This finding is similar to the observations of de Magalhaes *et al.* [8] for mammalian species.

De Magalhaes and Church [1] and de Magalhaes [11] have expanded their descriptions of the developmental theory of aging. In this theory, aging is at least partly programmed. De Magalhaes [11] reviewed the

evidence that aging was the result of sequential changes in gene expression and epigenetic marks, which were all components of regulated processes. Aging has a programmatic component that originates in developmental mechanisms.

In summary, across vertebrate species, there is a strong correlation between programs regulating a species' development to the stage of reproductive maturity, and the aging and longevity trajectories of that species. How might a developmental program regulate senescence and longevity? Probably the best described model for examining this regulatory relationship comes from the field of plant biology: the programmed development and senescence of leaves.

Leaf development and senescence

The phenomenon of leaf senescence is a well-described biological process. In fully grown cells the process occurs post-mitotically: cellular components are degraded and the products are recycled from senescing leaves to other parts of the plant [12, 13]. The final result is death of the leaf. The recruitment of 'nutrients' from the senescing leaf for use by the remainder of the plant has been considered part of a gene program for optimizing reproductive function [10].

Breeze *et al.* [14] recently measured the transcriptome in developing and senescing leaves of the model plant *Arabidopsis thaliana*. Plants were grown under standardized conditions and the same whole leaf harvested from each plant at 22 different time points over a 21-day period. This time period covered growth of the leaf into an active organ performing maximum photosynthesis with carbon fixation, through to a senescing leaf in which cellular macromolecules were degraded and recycled. Sampled leaves reached full length by 23 days after sowing (DAS), and senescence began by 31 DAS as measured by a decrease in chlorophyll levels. Levels of the carbon-fixation enzyme Rubisco began to decline after 25 DAS; another marker of the onset of senescence.

The investigators identified 6,323 differentially expressed genes over the 21-day period. A major time point, in terms of gene expression, was at 29 to 33 DAS. At this time juncture, a major switch in gene

expression occurred with about half the genes being upregulated, and half being downregulated. These 6,323 genes were categorized into 48 clusters based upon time of expression changes, with genes in clusters 1 to 24 showing downregulation, genes in clusters 27 to 48 showing upregulation, and genes in clusters 24 to 26 showing a more complex pattern. However, the major time switch in gene expression occurred at the time the leaf entered the senescent stage. Downregulated genes were enriched in functions related to metabolic processes: amino acid and carbohydrate metabolism, photosynthesis, chlorophyll biosynthesis, and carbon fixation. Upregulated genes were enriched in functions related to macromolecular degradation and mobilization, autophagy transport, defense responses to reactive oxygen species, synthesis of senescence-promoting hormones, cell wall degradation, and synthesis of cytoskeleton proteins. Analysis of transcription factor (TF)-binding motifs and the expression of specific families of transcription factors showed a complex arrangement for the regulation of co-expressed genes. Certain TF-binding motifs were selectively enriched in gene clusters exhibiting similar expression patterns. Specific TF families were differentially expressed at particular times during senescence.

The genomic data of Breeze *et al.* [14] confirm that leaf senescence represents a developmental program, as defined by Jansson and Thomas [12]. Leaf death becomes the result of this programmed senescence.

Gene expression studies have been reported in a few model animals across their entire life cycles, and these can be considered analogous to the leaf gene expression study of Breeze *et al.* [14] In these studies, developmental genes are defined as those showing a significant change in expression during one or more life cycle stages.

Developmental gene expression studies in animal models

Drosophila melanogaster

Arbeitman *et al.* [15] measured gene expression in *Drosophila* across its life cycle (generally about 40 days). The life cycle was divided into four stages: embryos, larvae, pupae, and adults. From whole animal samples, they identified 3,219 genes that

displayed at least a four-fold difference in expression over this 40-day time span. Gene expression showed several types of patterns: some were induced during early embryogenesis and were then maintained during the entire life cycle, while other genes showed one or several peaks in expression at different developmental stages. Comparing expression patterns, a similarity was observed between the patterns seen in both the pupal and adult stages. The transcript levels of hundreds of genes changed at least four-fold during five developmental periods: beginning, middle and end of embryogenesis; the larval-to-pupal transition; and the pupal-to-adult transition. Genes with similar functions tended to be expressed at similar times. Cell cycle genes were expressed at high levels during early embryogenesis and were downregulated thereafter. 'Metabolic' genes were highly expressed before and during the larval stage, and at the beginning of adulthood. Cell adhesion genes showed very low expression during adulthood.

Graveley *et al.* [16] updated the developmental transcriptome of *Drosophila melanogaster*. These investigators assayed protein-coding genes, noncoding RNA (ncRNA) genes, and alternative splicing events using whole animals. The life cycle stages examined were the same as in the study of Arbeitman *et al.* [15]: embryos, larvae, pupae and adults. Twenty gene clusters with similar expression patterns were identified, each containing between 113 and 2,573 genes. Clusters 18 to 20 showed low expression during early developmental stages, with an increase in expression beginning at the larval-to-pupal transition, and terminating with adult males; however there was no significant expression in adult females. The investigators measured the variation in exon splicing across developmental stages, and identified groups of exons with co-ordinated splicing patterns. For example, some exons showed little splice variation across developmental stages, whereas others showed greater splicing variation in early or late developmental stages.

The developmental gene expression data from *Drosophila* describe a pattern of co-ordinated clusters of genes co-expressed at particular developmental stages. These observations are consistent with the notion that senescence is a developmental process. The gene expression data are certainly not as dramatic as that observed in the life cycle of the leaf. In the

leaf, a significant and well-defined change in gene expression occurs abruptly at the onset of the senescent stage. However, senescence in animals does not usually have as well a defined beginning as is observed in the leaf. This observation probably accounts for why developmental gene expression changes associated with senescence are more gradual in animals compared to plants.

Ciona intestinalis

For four months, Azumi *et al.* [17] measured gene expression during the life cycle of *Ciona intestinalis* (sea squirt); an invertebrate chordate. The developmental stages of this animal consist of 13 embryonic stages, larval and juvenile stages, as well as four adult stages. The investigators assayed the expression of 10,415 genes from whole animal samples. Overall, two large gene groups were evident: an upregulated group and a downregulated group. The primary time point at which these expression changes occurred was at the late embryonic period. The data were categorized into five major gene clusters and 49 sub-clusters. Cluster B was labeled the 'embryonic and adult' gene cluster. It contained 2,070 genes, which were highly expressed during the middle of the embryonic stage and maintained expression during adulthood. Cluster C was denoted the 'adult' gene cluster. It contained 1,646 genes, which were highly expressed in adulthood but showed low expression at other developmental stages. A sub-group of genes (287) within cluster C showed a significant increase in expression in late adulthood; the investigators referred to this sub-group as the 'aging-related' gene cluster. Other groups of genes within cluster C showed expression peaks at other times of the adult stage, or during the juvenile stage. In terms of functional groupings, most cell cycle genes were highly expressed in the egg and during embryonic stages, characterized by rapid cell division. Most genes involved in protein biosynthesis showed high expression during the middle embryonic stages and larval and adult stages.

The sea squirt displays a clear arrangement of co-expressed developmental genes, with a number of these genes linked to senescence.

Caenorhabditis elegans

Hill *et al.* [18] measured the expression of 18,791 genes across six developmental stages in the nematode worm *C. elegans*, from embryos to aged worms near the end of their two-week life span. Whole nematodes were used as the sample source for gene expression studies. Genes with a significant change in expression during the life cycle were considered ‘developmental genes’ and these were classified into 36 clusters on the basis of similarities in expression patterns. One cluster contained 104 genes whose expression increased throughout development, peaking in 60-hour old worms, followed by a progressive decline in expression, terminating in two-week old worms. Genes that were downregulated in two-week old worms were associated with muscle function, metabolic activity and the synthesis of extracellular matrix.

Jiang *et al.* [19] assayed developmental genes across life cycle stages from embryos, through larval stages and young adults. Whole animals were used for the analysis. They ordered genes into 25 clusters on the basis of similarities in expression profiles; a number of these clusters were downregulated in young adults. These investigators also examined groups of developmental genes sharing common functions. For example, cyclin genes showed high expression in life cycle stages characterized by cell proliferation; embryos, late larval stage and young adults. In summary, the data of Hill *et al.* [18] and Jiang *et al.* [19] showed an association between developmental gene expression and aging.

As a species, *C. elegans* displays considerable developmental plasticity in its life cycle. Under favorable environmental conditions, it progresses rapidly from embryo through four larval stages to adulthood [20]. Under unfavorable environmental conditions, *C. elegans* undergoes developmental transition to a specialized third larval stage called the dauer diapause. The dauer stage involves both morphological and metabolic changes. During this stage, worms are often immobile and non-feeding. Aerobic metabolism is suppressed in favor of anaerobic metabolism, senescence is negligible and the life span of this stage can last for several months. The changes associated with formation of dauer larvae are reversible, and upon return of favorable

environmental conditions, they revert to reproductive adults with a ‘normal’ life span of about two weeks. Dauer larvae represent a form of reproductive arrest with both arrested aging and an extended lifespan. The changes in gene expression associated with this plastic developmental transition have been examined.

Jones *et al.* [21] measured changes in gene expression associated with development of the dauer larval stage in whole animal samples. The investigators interrogated the expression of 11,130 of the predicted 19,100 protein-coding genes. Two-thousand-and-sixteen genes were expressed only in the dauer stage, and 2,681 different genes were expressed only in non-dauer growing worms. These differentially expressed genes included those encoding histone protein variants, and those encoding proteins involved in cell cycle events and maintenance of DNA structural integrity. In addition, the investigators detected a limited number of lineage-specific transcripts: 358 and 533 for the dauer and non-dauer stages respectively. In essence, the genomic differences between *C. elegans* dauer and non-dauer stages involved the differential expression of about 25% of *C. elegans*’ protein-coding genes, as well as the differential expression of a small number of lineage-specific genes.

The gene expression patterns across the life cycle of the leaf and the model animals reviewed in previous sections are consistent with the notion that aging is an evolved developmental program, with death the inevitable consequence of the aging trajectory. The aging program involves expression changes in large numbers of functionally related gene clusters. In addition, the data comparing the dauer and non-dauer stages in *C. elegans* suggest that there are a smaller number of species-specific or - in the case of the dauer - lineage-specific genes involved.

As suggested by de Magalhaes and Church [10], senescence probably evolved as an adaptation to enhance reproductive success. This scenario is most apparent in the case of the leaf: during its senescence, cellular components are degraded and recycled to other parts of the plant. This transfer of resources promotes reproductive success in the plant as a whole. In humans, senescence is characterized by a long post-reproductive time period, during which grandparents can transfer resources such as materials, labor, and

child-caring to their offspring. This transfer of resources promotes the reproductive success of their offspring; the so-called ‘grandparent hypothesis’.

Each species has evolved its own set of developmental programs, including aging, and these are encoded in the zygote’s genome and epigenome. The implication of this construct is that aging actually begins at the time of conception. These programs are established through the process of speciation. As a new species evolves, developmental programs encoding the life cycle stages of the new species will be established through modifications of the programs present in the ancestral lineage from which it diverged. A comparison of species sharing a common ancestor affords another window into the nature of developmental programs such as aging. An example of such a window is the genomic landscape underlying the unusual phenotype of the naked mole rat.

Naked mole rat (Heterocephalus glaber)

The naked mole rat (*Heterocephalus glaber*) diverged from its common ancestor with the mouse and rat about 73 million years ago. This species is characterized by an exceedingly long lifespan of about 30 years, negligible senescence, resistance to cancer and a long reproductive life span. Kim *et al.* sequenced the naked mole rat genome (tissue source for DNA not given) and found that most (93%) of it showed synteny with human, mouse and rat genomes, and there were 96 species-specific gene families. One-hundred-and-forty-one genes in the naked mole rat showed evidence of positive selection and this set included genes involved in telomere function [22].

Keane *et al.* [23] updated genome sequencing in the naked mole rat (DNA extracted from several non-specified tissues) and found a signature of positive selection for the proline-rich domain of the tumor suppressor protein p53, encoded by the gene *TP53*. This domain had four more proline motifs than that of the guinea pig domain.

Keane *et al.* also found evidence of positive selection for the gene encoding the hyaluronan (HA) receptor CD44. The naked mole rat’s resistance to cancer has been attributed to early contact inhibition (ECI). Contact inhibition is a process by which cell growth is

arrested when cells come into contact with each other, or the extracellular matrix; naked mole rat cells arrest at a much lower density than mouse cells [24]. Naked mole rat fibroblasts secrete a high molecular mass hyaluronan (HMM-HA), which is over five times larger than that of human or mouse [24]. This HMM-HA accumulates in the tissues and interacts with the CD44 receptor, which in turn interacts with the protein NF2 (Merlin) on the cytoplasmic face of the cell. The CD44 receptor in the naked mole rat has a higher affinity for HA than in mouse or human cells [24]. This HMM-HA-CD44-NF2 pathway is the trigger for ECI, and appears to be the mechanism responsible for cancer resistance in the naked mole rat.

Yu *et al.* [25] compared gene expression profiles of naked mole rat liver tissue to those of wild mice. The animals used in these experiments were young adult naked mole rats aged 2 to 3 years, and wild-derived mice aged 6.5 months. The methodology used by the investigators was such that over-expressed genes could be detected in the naked mole rat but not under-expressed genes. One-thousand-and-eighty-one genes were identified that showed at least a five-fold increase in expression in the naked mole rat compared to wild-derived mice. The functional categories associated with these over-expressed genes included: mitochondria, acetylation, oxidation-reduction, and fatty acid metabolism to name a few. Presumably, these over-expressed genes in naked mole rat liver (compared to wild-derived mice) are a piece of the genomic landscape puzzle accounting for its unusual phenotypic features, including its negligible aging and extended life span.

Genomic data from the naked mole rat are consistent with gene expression data from the leaf and model animals. The naked mole rat’s propensity to negligible aging and extended life span are rooted in the differential expression of many functionally related clusters of genes [25], which include genes that have undergone positive selection [23, 24] during the evolution of this species. These features indicate that senescence in the naked mole rat is a developmental program and, as such, the unusual phenotype of this species reflects a regulated polygenic process - it is not simply due to the effects of a limited number of specific ‘longevity’ genes.

Humans

Obviously gene expression studies similar to those done in model organisms cannot be done in humans. Suitable model animals have a short life span and can be used for whole animal gene expression measurements. In humans, one must rely on indirect observations to support the notion that aging is an evolved developmental program. However, evolution is a conservative process that generally builds upon successful designs. Since the human lineage is a product of evolution, evidence in model animals and plants would predict that aging is an evolved developmental program in humans as well.

Brain structure

A prevalent hypothesis in neuroscience is that 'healthy' age-related brain decline mirrors developmental maturation [26]. Douaud *et al.* [26] examined this hypothesis using structural MRI. The subjects of this study were 484 healthy participants aged between eight and 85 years. Using a linked independent component analysis, the MRI structural brain images were decomposed into 70 components. Only two of these components showed a significant association with age. The second of these was associated with a network of mainly transmodal brain areas encompassing the heteromodal cortex and limbic and paralimbic regions. The strength of this component had an inverted U relationship with age, displaying an increasing maturational (developmental) trajectory during childhood, adolescence and young adulthood, which peaked at age 40 years. Thereafter, the strength of this component showed an accelerated decline through to age 85 years, the last time point sampled. The inverted U component showed differences between males and females, with females having a significantly higher peak strength, and slightly later age of occurrence of this peak than males. The investigators found a positive correlation between the strength of the inverted U component and episodic memory and intellectual ability. In summary, component two described a network of brain regions in which development and aging mirrored each other, and the inverted U-shaped relationship with age also matched age-related changes in intellectual ability and episodic memory.

Intellectual ability

An interesting study reviewed by Underwood [27] was designed to examine the determinants of cognitive functioning in the elderly. In Scotland in 1947, 70,000 11-year old children took a comprehensive intelligence test known as the Scottish Mental Survey. An earlier version of the survey had been administered in 1932. In 2003, Ian Deary and colleagues from the University of Edinburgh collected more than 1,000 participants from the 1947 survey, and more than 500 participants from the 1932 survey. These investigators administered cognitive tests to these now elderly cohorts to determine what had happened to their cognitive abilities over their lifetime. A striking observation based on this study was that the cohorts' mean scores at age 11 accounted for 50% of the variance in their mean scores at age 77. Another finding was that diet, body mass index and caffeine consumption appeared to have little effect on the age-related trajectory of cognitive functioning when childhood intelligence was taken into account. These results, based upon averages, pointed to important trends determining later-life cognitive abilities; however, averages masked the extensive divergence in individual test scores that occurred as the individuals making up these cohorts aged.

This Scottish study [27], and the observations of Douaud *et al.* [26], suggest that cognitive functioning is rooted in a development program that largely sets the course of age-related changes in this function. Maturation of the brain, both structurally and functionally, sets age-related changes in both brain structure and functioning. However, the Scottish study also underscores the plasticity of this maturation program at the level of the individual, given the wide divergence in age-related cognitive functioning that was observed across individuals.

Evolution of the human life cycle

The human lineage diverged from its common ancestor with the chimpanzee and bonobo about 6-8 million years ago. Caspari and Lee [28] used dentition samples from four ancient hominin species to assess longevity during different stages of human lineage evolution. These investigators used fossil dentition patterns to classify individuals as either young, about

15 years of age, or old, about 30 years of age. They observed a progressive increase in longevity over the course of evolution of the human lineage. However, a significant increase in longevity occurred with the appearance of early modern humans in the upper Paleolithic period, between 40,000 and 11,000 years ago, the end of which merges with the appearance of agriculture; the Neolithic revolution. Caspari and Lee's data [28] showed that this was probably the first time there were a greater number of older adults than younger ones. At this stage in human evolution, this significant increase in longevity made it possible for social groupings to contain more than one generation, since an adult of 30 years or older could theoretically be a grandparent. Multigenerational social groups allowed for the transfer of resources, including childcare of grandchildren, between parents and children. Such intergenerational resource transfers would promote the reproductive success of children and would favor selection for a lengthening of the post-reproductive aging period. Hence, this cultural practice could have been the force selecting for a long post-reproductive period. Through such a process, evolution would shape the developmental program for aging. In fact, gene-culture co-evolution is considered the primary process driving human evolution.

According to Gurven and Kaplan [29], humans in the Paleolithic period prior to the introduction of agriculture had a mean life expectancy at birth of about 20 years, and a mean life expectancy at age 45 of five years. By the pre-industrial period (5,000 to about 300 years ago), humans had evolved their 'modern day' life cycle pattern [29]. In these societies (hunter-gatherers and agricultural societies), mortality rate was very high in the first ten years of life. Between the ages of ten and 40 years, mortality rate was significantly lower, followed by a progressive increase in mortality rate between ages 40 and 70 years. These data can be expressed as follows: life expectancy at birth was 31 years, at age 15 about 50 years, at age 45 about 21 years, and at age 65 about ten years. The most significant difference in the life cycle pattern between pre-industrialized societies and modern societies is the much lower infant mortality rate in modern societies. In summary, pre-industrialized societies had evolved the distinctive characteristics of the modern human aging and mortality pattern: lengthy period of prime adulthood,

long post-reproductive lifespan, delayed senescence decline, and an extended life span. Among early agricultural and hunter-gatherer societies, survivorship to grandparental age was quite common.

Gurven and Kaplan [29] argued that the long post-reproductive life span characteristic of the human lineage was adaptive by virtue of intergenerational transfers of resources and provisions. Older individuals in these societies increased their inclusive fitness by enhancing the fertility of their offspring, and the survivorship of their grand-offspring, through these intergenerational transfers. This 'grandparent' hypothesis was previously noted, and most likely accounts for the adaptive benefit of the human lineage pattern of aging.

Summary of species data

The data presented, based upon plants, model organisms and humans, strongly support the construct that aging is an evolved developmental program. Death is the inevitable terminal event of species-specific aging trajectories, and hence life span has not been the primary target of natural selection. Aging as a developmental program is encoded in the genome and epigenome of the zygote and, as such, can be considered to begin at conception. Aging is a polygenic process involving the differential expression of clusters of functionally related genes, some of which are lineage-specific. Although the regulation of developmental programs involves various DNA sequence motifs (e.g. promoters and enhancers), DNA binding proteins, non-protein coding RNAs, and post-translational histone modifications, the deeper question remains as to what confers species specificity to these programs. A human zygote develops into a human, and a giraffe zygote develops into a giraffe, even though the developmental programs for these two mammals share most genomic and regulatory elements. Berg *et al.* [30] looked at one small aspect of this question.

In the mammal, the blastocyst contains a cluster of enclosed cells called the inner cell mass (ICM), and outer cells forming the structure of the sphere called the trophectoderm (TE). The TE differentiates into the extra-embryonic components of the placenta, while the ICM differentiates into the definitive structures of

the fetus. Berg *et al.* [30] examined the time course of TE and ICM cell fate commitment in cattle and mouse. By the early blastocyst stages, cattle and mouse embryos are characterized by two morphologically distinct cell populations: the ICM, and outer trophectoderm. In the mouse, these two lineages are committed to their fate by the mid blastocyst stage, whereas in cattle, TE cells of the late blastocyst are still not fully committed to their fate. A number of regulatory factors are involved in cell fate decisions, but two homeodomain DNA binding motif-containing transcription factors were particularly prominent: Oct4 and Cdx2.

In the mouse, Cdx2 is located exclusively in the TE, while Oct4 can initially be found in both ICM and TE. However, by the late blastocyst stage, Oct4 is found exclusively in the ICM. Cdx2 appears to be essential to TE cell fate commitment, while Oct4 regulates ICM cell fate commitment. In the mouse, continued expression of Oct4 in TE prevents Cdx2-directed fate commitment of this tissue, and one of the functions of Cdx2 is to downregulate Oct4. This allows Cdx2 to direct TE cells along a committed pathway. In cattle, Oct4 persists in the TE into the late blastocyst; a stage beyond that observed in the mouse. Only in later blastocyst stages do levels of Oct4 in the TE fall in cattle, such that these TE cells can become committed to their fate. Berg *et al.* [30] noted that human and pig blastocysts behave in a similar fashion to those of cattle, whereas the mouse appears to be the outlier. Hence, in mice the Oct4/Cdx2 regulatory network has been modified to allow earlier TE commitment and differentiation, and therefore earlier blastocyst implantation than in other mammals.

The experiments of Berg *et al.* [30] open up a very small window on the complexities underlying differences in the regulation of cell fate decisions and developmental programs between species.

Studies in individual animals

At the end of their paper, Jones *et al.* [7] plot graphs of within-species mortality trajectories for laboratory rats and laboratory mice. These animals are highly inbred and will therefore be genetically similar. Each line in the family of trajectories plotted for rats and mice represents a different strain, sex, or population.

The individual lines all have the same general shape, but the family of lines for each species (rat and mouse) clearly underscores the marked variation - in this case for age-related mortality - which can be found within a single species. However, there is a limit to the extent of variation that can occur within a given species, and this limit is set by the developmental programs specific to that species. Although laboratory rats and mice show great variation in individual age-related mortality, one does not find a rat or mouse that lives as long as an elephant or whale.

As a species, humans age differently to other species; even closely related ones such as chimpanzees [29]. Yet within the human species there is obvious marked variation in the aging trajectories across individuals. Variation in the phenotypes generated by developmental aging programs across individual organisms is characterized under the rubric of developmental plasticity.

Examples of developmental plasticity

Daphnia water fleas display a remarkable ability to express specific phenotypic changes in response to environmental cues. For example, the presence of chemical signals released by predators can induce the growth of neck teeth, protective tail spines, and helmets. The genome of *Daphnia pulex* has been sequenced [31], and about 36% of its genes have no detectable homologs in other animals. The genome is characterized by a high rate of gene duplication with the accumulation of many tandem repeats. It is thought that the over-representation of duplicated genes in the Daphnia genome underlies its remarkable phenotypic plasticity.

Freund *et al.* [32] looked at behavioral plasticity in genetically identical mice. Forty young female inbred mice were kept in a large complex and enriched enclosure for three months. The mice's exploratory behavior was monitored over the three-month period and quantitated using a derived measure. Adult neurogenesis was also measured at the end of the experiment using bromodeoxyuridine (administered three weeks previously) to label new neurons. The investigators noted that by the end of the experiment there had been a massive magnification of baseline

individual differences in exploratory behavior among these genetically identical mice. There was a significant positive correlation between exploratory behavior and hippocampal neurogenesis. Mice who explored their habitat more broadly also grew more new hippocampal neurons. In summary, genetically identical mice exposed to a stimulating environment showed a progressive increase in their range of exploratory behavior with length of exposure to the enriched environment, but the magnitude of the increase showed large differences between individual animals. These individual differences were related to individual differences in the extent of hippocampal neurogenesis.

The developmental trajectory of honeybee larvae can be altered by the nature of the food given to them [33]. When fed with royal jelly, their developmental program is modified and leads to the creation of queens, whereas the feeding of less sophisticated food to larvae results in the formation of sterile worker bees. Kucharski *et al.* [33] investigated the genetic basis of this developmental plasticity and found that the underlying mechanism was related to changes in the DNA methylation status of specific genes (e.g. that encoding dynactin p62) primarily in the head region of the animal. The methyl group represents an example of an epigenetic mark, and addition or removal of methyl groups to DNA is associated with changes in gene expression.

Perhaps the most dramatic example of developmental plasticity was an experiment reported by Storrs and Williams [34]. The nine-banded armadillo normally gives birth to monozygous quadruplets. A single egg is fertilized, but implantation is delayed for several months. Once the zygote does implant it splits into four genetically identical embryos. Storrs and Williams [34] collected 16 sets of newborn quadruplets, and measured 20 parameters including body weights, organ weights, and neurotransmitter tissue levels. These investigators made the fascinating observation that within the sets of quadruplets there were always differences in the measured parameters. For example, the maximum variation in body weight observed within a set of quadruplets was almost three-fold, and for brain weight almost two-fold. Clearly, individual members of a set of quadruplets do not experience the same intrauterine environment; there may be differences in blood flow between the

individual embryos, or some other environmental factor may be operative. Regardless, the four embryos, genetically identical at conception, are not phenotypically identical at the time of birth following intrauterine development.

This observation underscores the role that the intrauterine environment plays in altering developmental programs in the embryo, including the aging program. The intrauterine environment appears to modify embryonic developmental programs through epigenetic changes, and several recent papers based on studies in monozygotic human twins support this proposal.

Human monozygotic twin studies

Although monozygotic twins are genetically identical at conception, members of the same twin set display various degrees of phenotypic divergence with age [35]. This divergence cannot be due to differences in DNA sequences, except in the case of a *de novo* mutation in one of the twins, rather it is due to environmentally induced epigenetic changes. These epigenetic changes can occur during intrauterine development.

Ollikainen *et al.* [36] studied four tissues (cord blood-derived mononuclear cells, human umbilical vein endothelial cells, buccal epithelial cells, and placental tissue) collected at birth from 56 monozygotic (MZ) and 35 dizygotic (DZ) twin pairs. The investigators studied the methylation status of four differentially methylated regions (DMRs) associated with the *IGF2/H19* locus. *IGF2* and *H19* are imprinted genes on chromosome 11 that exhibit parent-of-origin expression; *H19* is expressed from the maternal allele and *IGF2* from the paternal allele. DNA methylation involves the addition of a methyl group to a cytosine nucleotide, which is generally part of a CpG dinucleotide site. Ollikainen *et al.* [36] found small median within-pair methylation differences at individual CpG sites for all DMRs: 3-4% absolute difference for MZ twins, and 4-7% absolute difference for DZ twins. Within-pair methylation differences for MZ twins also varied between different tissues. However, when the investigators looked at within-pair variation for individual twin pairs, the discordance approached a value as high as 54% in some MZ twin

pairs. There were also striking tissue-specific variations for within-pair methylation discordance in some MZ twins, which highlights the potential for differences in intrauterine environment between individuals of the same MZ twin set.

Gordon *et al.* [37] collected cord blood mononuclear cells, human umbilical endothelial vascular cells and placenta cells from 22 MZ and 12 DZ twin pairs at the time of delivery. The three tissues were subjected to genome-wide DNA methylation analysis. In general, median within-pair discordance was higher in DZ than in MZ twin pairs. Although the median discordance within twin pairs was low overall, all twin pairs had specific CpG sites that displayed high levels of within-pair methylation discordance; in excess of 20%. Genes associated with these sites were enriched in functions related to development, morphogenesis, and cell cycle events. The investigators also found a correlation between birth weight and extent of within-pair methylation discordance for a small set of genes; seven in DZ cord blood mononuclear cells, and one in MZ human umbilical endothelial vascular cells. The investigators concluded that the within-pair discordance in DNA methylation in MZ and DZ twins was the result of a combination of stochastic and intrauterine environmental influences.

These studies indicate that the two individuals of a genetically identical twin pair can display significant divergence in epigenetic marks (DNA methylation), and this divergence occurs following conception, during development within the intrauterine environment. Changes in epigenetic marks can also occur in the post-natal period as a result of environmental influences (cues) that the individual experiences [2]. Presumably, there are critical time windows during which an individual is most sensitive to environmental alterations of epigenetic marks. Two such sensitive time windows appear to be the intrauterine development period, and the early post-natal period. It is well established that epigenetic mechanisms play a key role in regulating gene expression patterns.

Conclusions

In this review, the question is posed: what is aging? I believe current evidence supports the notion that

aging is an evolved species-specific developmental program. Within a given species, at the level of the individual, the developmental program displays considerable plasticity. As a species, our aging trajectory is constrained by our evolved developmental program, but the plasticity of this program allows for extensive variation between individual humans in this trajectory. However, there are limits to this variation; the species-specific program sets these.

There are a number of implications related to this construct of the aging process. As a developmental program, aging is encoded in the zygote genome and epigenome, so it can be considered to begin at conception rather than being restricted to the life cycle stage following attainment of reproductive maturity. Aging should not be considered a ‘risk factor’ for so-called chronic degenerative diseases since aging is an integral part of the fabric of life. Since aging is a developmental program, meaningful interventions need to be “targeted” to its plasticity. The most successful interventions will be those that modify environmental factors responsible for ‘detrimental’ epigenetic changes [38]. Pharmacological agents will probably not be successful, since these will no doubt have both unwanted and unpredicted effects; there are no specific aging genes or aging pathways that can be directly targeted. Finally, as presented in two recent publications [2, 6], current molecular biological data support the notion that chronic degenerative diseases are not ‘diseases’ but rather variants of the aging process.

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