Aging seems to reflect a patterned program of decline in functional reserves and a reduced ability to maintain homeostasis. Advances in molecular biology have provided new insights into the process of aging at the cellular level. In this review, a few selected models of aging that illustrate the molecular methods and techniques used in aging research are discussed.

The characteristic changes that accompany aging seem to follow a regulated patterned program. From an evolutionary selection perspective, the reasons why a regulated terminal program leading to an organism's demise was chosen over a program of immortality are puzzling. An evolutionary conjecture for the selection bias toward a terminal program might be that in the postreproductive phase of an organism's life cycle, the selection pressure is lessened or no longer present. The lessening of selection pressure is the result of mortality caused by extrinsic harm in early life; in the wild, most organisms do not live long enough to reach old age. The absence of selection pressure leads to the formation of an organ system that permits increasing entropic deregulation of cellular mechanism and default death. However, to reach the reproductive phase, it is likely that cellular mechanisms evolved to slow or regulate aging, thus increasing the chances of an organism attaining reproductive phase at the opportune moment. These age-regulating processes would counter harmful internal and environmental effects and contribute to a selection bias. Therefore, the extent to which these life-sustaining processes can repair and maintain an organism from increasing entropy and environmental harm constitutes the lifespan of an organism. Under this light, aging is a process that can be described at the cellular level.

Aging and death are intimately linked. Aging can be defined as an equation with the probability of death as one of its variables. A second variable is changes in the phenotype, which occur because of degradation of age-regulating cellular processes. The phenotypic changes of aging, however, are distinct from the diseases of aging in that phenotypic changes affect all individuals, whereas diseases of aging affect only a subpopulation. This is highlighted, for example, by the fact that the maximum human lifespan has not been extended substantially despite the quantum improvements in medical sciences that have been made in this century. The medical advances have affected the diseases of aging, but not the aging process itself.

Recent advances in human genetics and molecular biology have greatly increased our understanding of aging. These advances include identification of genes important in aging, genetic studies of germline diseases that cause the appearance of premature aging, an improved molecular understanding of the diseases of aging, and others. In this review, a few selected models of aging that illustrate the molecular methods and techniques used in current research in aging are discussed. These models include cellular damages caused by reactive oxygen species (ROS), the genetic program, and genomic instability.

**REACTIVE OXYGEN SPECIES**

The idea of cellular oxidative damage caused by ROS, free radicals generated during metabolic processes, contributing...
to the aging process has been a popular model, first proposed in the 1950s. Reactive oxygen species include the superoxide anion, hydrogen peroxide, and hydroxyl radicals, which are formed when oxygen is reduced. The organelle damages caused by ROS are indiscriminate, but constant, over an organism's lifetime. The model proposes that the cumulative damaging effects of ROS lead to eventual cellular breakdown and death. The extent to which an organism may survive depends on the countermeasures programmed within a cell to decrease the damaging effects of ROS. The cellular solutions to ROS are the genes that encode for antioxidants.

Advances in molecular biology have allowed the creation of transgenic animals, in which targeted genes can be either overexpressed or deleted. Such animals allow analysis of targeted genes at the molecular level in a milieu of living macroscopic environment. Direct support for the free radical hypothesis of aging was substantiated with the creation of transgenic Drosophila flies (small 2-winged flies used in genetic research) overexpressing the antioxidants copper-zinc superoxide dismutase (SOD) and catalase. Superoxide dismutases are enzymes that regulate intracellular superoxide anion levels. These transgenic flies lived one third longer and displayed a delayed loss in physical performance compared with wild-type control flies. A second transgenic Drosophila designed to express the human SOD1 gene within fly motor neurons lived 40% longer than the wild-type flies. This study exemplified the striking evidence that overexpression of a single gene within a single cell type (motor neuron) could produce profound changes in the lifespan of these flies. The remarkable results obtained from these flies have raised the suggestion that regulation of aging may be a relatively simple process, at least for these flies. Similar experiments using mice, a more complex animal model, were performed by other molecular biologists; transgenic knockout mice (target genes are deleted) for SOD1, SOD3, and glutathione peroxidase were created. Interestingly, no rapid aging process was found in the knockout mice, although the knockout mice were more susceptible to motor neuron loss after an injury. These results have suggested that SODs may not be essential regulators of aging in more complex animals, but they are necessary under extreme conditions for the organism to survive injuries.

Despite the disappointing results obtained from SOD transgenic knockout mice, studies of other molecular biologists further strengthened the notion that genomic DNA is liable to free radical attack, resulting in cumulative genetic defects with aging. Mutant mice with a single targeted mutation in the gene encoding the p66sc protein lived one-third longer than wild-type animals. These mutant mice displayed normal development and body weight. The p66sc protein is a cytoplasmic signal transducer that mediates signals from mitogenic cell surface receptors to the Ras protein. Mutant mice had increased cellular resistance to agents that cause oxidative damage, consistent with the results of in vitro experiments, suggesting that increased resistance to oxidative damage was responsible for their longevity.

Other evidence supporting the connection between ROS and aging was found in genetic manipulation experiments using Caenorhabditis elegans (C elegans is a 1-mm-long soil nematode found in temperate regions). For example, a mutation of the age-1 gene, a negative regulator of SOD, caused the mutant C elegans to live 2 times longer than the wild-type controls. Consistent with the expected effect of the age-1 mutation, they expressed increased SOD/catalase levels and a higher resistance to oxidative stress compared with nonmutant controls. These findings support the ROS hypothesis of aging, as suggested by the Drosophila model. Taken together with the SOD and p66sc protein knockout mouse findings, it is probable that genes responsible for reducing ROS are only part of counterbalancing mechanisms that forestall aging to a limited extent. It is also probable that ROS would have the most harmful age-promoting effect on postmitotic cells (ie, cells that do not regenerate, such as neurons and cardiomyocytes) because the harmful effects are cumulative. However, for cells that replicate rapidly, such as enterocytes that endure the reduced effects of ROS during their short lifetime, regulation of aging by genes encoding for antioxidants may play a minimal role. For the premiotic cells, a second model proposes that genetic programs that are responsible for an organism’s development are more important in regulating aging.

GENETIC PROGRAMS

Many experiments involving genetic programs and pathways relevant to aging have been performed on C elegans. Caenorhabditis elegans proceed through 4 larval stages before reaching adulthood, the reproductive stage. Under adverse environmental conditions, such as starvation, they enter a dormant state called dauer larva stage. With improvement in environmental conditions, adult development is resumed. As an adult, C elegans live for a few weeks, compared with 6 months in the dauer diapause stage. To understand the genetic developmental control of dauer transition, hence regulation of aging that leads to longevity in the dauer stage, the genes that determine the transition into the dauer stage have been studied extensively.

The dauer formation (daf) gene and age-1 are examples of genes involved in the transition into the dauer stage. When mutations in the daf-2, daf-23, or age-1 genes are made, mutant larvae proceed to adult development and concurrently double their adult lifespan. Mutations of the daf or age gene presumably turn on a pattern of inherent gene expression that transduces dauer physiological features in the adult state, thereby allowing the organism to live longer. Analyses of cloned daf-2 and age-1 genes have shown that daf-2 encodes for nematode insulin-like growth factor 1 receptor (IGF-1R) and that age-1 codes for the mammalian homologue of the p110 catalytic subunit of phosphatidylinositol 3-kinase that lies downstream of the daf-2 receptor (ie, IGF-1R). These findings have suggested that phosphatidylinositol 3-kinase (age-1) signaling controls lifespan and the dauer diapause decision. Interestingly, the phosphatidylinositol 3-kinase pathway has received much recent attention in human molecular biology, because it has been linked to the cellular survival pathway and is associated with various diseases, including diabetes mellitus and cancer.
During the past several years, many data have been generated to suggest that growth factor pathways, in particular the growth hormone–IGF–insulin pathway, are implicated in the aging process. In the mammalian system, the insulin receptor is involved in energy metabolism, whereas IGF-1R, activated by its ligand IGF-1, which is secreted by liver cells in response to growth hormone stimulation, promotes growth. Growth hormone–insulin–IGF-1R mutant invertebrates not only live longer but they tend to have a smaller body size and an increased resistance to oxidative stress. Specifically, whether IGF-1R is involved in the aging process in mammals has been evaluated by construction of Igf-1r gene knockout mice. The Igf-1r homozygous knockout mice were not viable, but heterozygous mice (mice that have one normal copy of Igf-1r) were viable, and lived 26% longer than the wild-type mice. In addition, Igf-1r heterozygous mice were more resistant to externally applied systemic oxidative stress compared with control mice. Correlation of decreased IGF-1/IGF-1R and longevity suggests an adaptive evolutionary acquirement, whereby reduction in growth hormone/IGF-1 contributes to the organism’s ability to survive during states of reduced metabolism by increasing resistance to oxidative stress.

The possibilities of manipulating developmental genes to extend the lifespans of C. elegans and regulating genes involved in endocrine metabolism to prolong longevity in mice suggest that the rate of aging may be under genetic control and that expression of the right patterns of genetic survival signals induces longevity. However, even under the influences of cellular survival signals, death is only delayed for a limited period. As the next hypothesis postulates, this may be because of an intrinsic cellular inability to maintain a stable genome indefinitely.

**GENOMIC INSTABILITY**

The idea of genome instability contributing to the aging process is not a new one; the lifetime accumulation of somatic gene mutations ultimately causing cellular dysfunction and aging is plausible. This notion was corroborated in a transgenic mice model in which a marker transgene was analyzed for rearrangement in aged mice. Rapid rearrangement was found in the liver, but not in the postmitotic brain cells, of old mice. Although random genetic mutations contributing to aging are possible, haphazard genetic rearrangement and somatic mutations contributing to the aging process are not compatible with a recognizable progressive pattern of aging and a predictable lifespan of different animals. A predictable lifespan and aging pace suggest more controlled genetic breakdown. Various candidate causes of controlled genetic breakdown have been proposed. Among them is a suggestion of an internal molecular clock that counts the number of allowed cellular replications before reaching senescence. One such candidate molecular clock is telomeres.

Telomeres are tandemly repeating DNA sequences found at the physical ends of linear eukaryotic chromosomes. Linear chromosomal ends cannot be replicated by DNA polymerases, and without corrective measures, cell divisions would result in chromosomal loss. This is prevented by actions of telomerase, a reverse transcriptase. Telomerase prevents shortening of a chromosome by extending the telomeric repeat sequences. The notion that shortening of telomeres leads to cell senescence has been supported by the findings that primary human culture cells with low telomerase activity have shortened telomeres and a limited replicative capacity. Conversely, activation of telomerase was shown to extend the lifespan of cultured human cells. Other studies, however, have challenged the idea that telomere shortening causes aging. One such study was based on the construction of telomerase knockout mice. No phenotypic changes suggestive of rapid aging were found in these mice. Although there are conflicting findings that argue for and against telomeres as a possible molecular clock, it is clear that telomeres play a critical role in protection against genomic instability.

The idea of wear and tear within a cell causing eventual cellular defects in genomic maintenance machinery, contributing to aging, was bolstered by the recent observations made from mutant Xpd knockout mice. The Xpd gene encodes a DNA helicase protein subunit of core transcription factor IIH. Transcription factor IIH is involved in the unwinding of DNA and is required for transcription initiation by RNA polymerase II. It is also involved in repairing genetic lesions by nucleotide excision. Mutations in Xpd lead to accumulation of DNA damages, and compromise transcriptions of critical genes. The mutant mice had a reduced lifespan and showed signs of premature aging, such as osteoporosis, osteosclerosis, cachexia, and early graying. The results from Xpd knockout mice have suggested that continual DNA damages incurred during the cellular lifetime cause genomic instability and that the lifespan of an organism is dependent on the proficiency of cellular maintenance machinery to counterbalance intrinsic genomic instability.

**CONCLUSIONS**

In this brief review, selected models of aging that illustrate the methods of molecular biology have been discussed. As experimental models suggest, molecular processes that control aging in less complex organisms may involve relatively few regulatory processes. However, in more complex organisms, the processes that control the rate of aging may be numerous and interdependent. One major hurdle confronting research in aging is the difficulty in correlating gene expressions that regulate aging and the aging phenotype. This difficulty may be overcome with further advances in DNA array chip technology. By using this technology, it may become feasible to follow changes in gene expression patterns through an organism’s lifespan. The rate of aging and the aging phenotype, therefore, may be ultimately explained or correlated by changes in gene expression patterns. The completed human genome project will undoubtedly further advance research in aging. As technological improvements in genome analysis are made in the future, it will be possible to analyze efficiently the genetic polymorphism of select human ethnic groups who are known for their longevity. These advances will greatly accelerate our pace of understanding and deciphering molecular mechanisms that regulate aging.
Advances made in our understanding of nature are accompanied by the burden of responsibility to use the knowledge wisely. This is particularly true with respect to our increasing knowledge in the field of aging. Whether we should interfere with the aging process itself is a difficult question to answer given the fact that many parts of the world are afflicted with overpopulation and starvation. Who will be chosen to live longer while others face their inevitable default death? Perhaps, future foci of research should only be directed at studying diseases associated with aging; thereby, individuals may live out their predetermined lifespan with sustained health. However, this does not seem feasible given the likely postulate that process of aging and disease of aging are intertwined; understanding the diseases of aging mandates understanding the processes of aging. Answers to these philosophical questions are difficult to determine. It is clear, however, that these dilemmas behoove us to cultivate not only our scientific endeavors but also foster our humanity and justice to face squarely these difficult questions that await us in the future.

Accepted for publication June 7, 2003.

I thank Dai H. Chung, MD, for his discussion and comments on the manuscript; and Eileen Figueroa for manuscript preparation.

Corresponding author: Sunghoon Kim, MD, Department of Surgery, The University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-0536 (e-mail: kimsu@msnotes.wustl.edu).

REFERENCES