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TOWARD A CONTROL THEORY ANALYSIS OF AGING

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Abstract

Aging is due to the accumulation of damage over time that affects the function and survival of the organism; however, it has proven difficult to infer the relative importance of the many processes that contribute to aging. To address this, here we outline an approach that may prove useful in analyzing aging. In this approach, the function of the organism is described as a set of interacting physiological systems. Degradation of their outputs leads to functional decline and death as a result of aging. In turn, degradation of the system outputs is attributable to changes at the next hierarchical level down, the cell, through changes in cell number or function, which are in turn a consequence of the metabolic history of the cell. Within this framework, we then adapt the methods of metabolic control analysis (MCA) to determine which modifications are important for aging. This combination of a hierarchical framework and the methodologies of MCA may prove useful both for thinking about aging and for analyzing it experimentally.

Keywords

cell metabolic history; metabolic control analysis; nonspecific damage

INTRODUCTION

Extensive biochemical, organismal, population, and comparative studies on aging have focused on qualitative and, sometimes, quantitative assessment of traits that contribute to normal aging. It is hence evident that aging is caused by accumulation of damage, resulting from a lack of capacity to protect, maintain, and repair somatic tissues over time (1–7). However, attempts to determine which particular types of aging-related damage are key to loss of function have been largely unsuccessful because the diversity of sources and types of damage is great and can vary with tissue, organism, and age (2, 8–10). It could, reasonably, be argued that the development of a general description of aging is premature because we lack both detailed descriptive data and a sufficiently mature understanding of aging to produce realistic models of the process. However, we believe that there is value in the development of conceptual frameworks that help direct attention to the kinds of data and experiments that could move toward a more quantitative description and analysis of the aging process.

Before we outline our approach, we first consider the properties of normal aging that it must accommodate. In nature, avoiding death often depends on finding food, avoiding predators, keeping warm, and surviving infections. However, when these extrinsic hazards are largely eliminated, the intrinsic aging process generally still leads to loss of function and death (1, 4, 11, 12), although some organisms age slowly and, in some cases, seem hardly to age at all (13, 14). Average and maximal life span under controlled conditions are broadly predictable for a given species. This is a familiar fact but, nevertheless, both intriguing and informative. Aging reduces the genetic contribution of an individual to the next generation and is hence disadvantageous; no genes have evolved to cause death. Instead, aging occurs through wear and tear that leads to the progressive accumulation of damage. However, different kinds of organisms evidently avoid, repair or withstand damage to different extents and hence differ in their rates of aging. This biodiversity can be due to different genomes. For instance, maximum life spans can vary by orders of magnitude among different species of mammals and birds (4, 15–17). In addition, mutations in single genes can greatly increase life span in the budding yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the mouse (18–20). How the genome is expressed within a species can also dramatically affect life span. For example, queens of social insects, such as ants, bees, and wasps, tend to age much more slowly than genetically identical worker castes (21). Thus, the genome and how it is expressed constrain mortality and life span.

However, genetic constraints on life span are relatively loose because, even for genetically identical animals in a standardized environment, there is considerable variability in life span (12, 22–25), possibly in part owing to heterogeneity in robustness among individuals from stochastic events at the level of the cell, tissue, and organism, despite their similar genotypes and environments (12, 22, 23, 25, 26). Finally, several environmental interventions, such as decreased temperature (27), lowered oxygen tension (28), and dietary restriction (DR) (29, 30), can also increase life span.

A combination of genetic determination, environmental variation, and stochastic events thus contribute to the probability of dying at each age, the agespecific mortality, $P(t)$. There is no a priori reason for $P(t)$ to have any particular dependence on age or to be similar for different organisms. However, as first noted for humans by Gompertz (31), in many organisms, including the standard laboratory model organisms, nematode, fruit fly, and mouse, mortality increases roughly exponentially over the main part of adulthood (6, 12, 13). Thus, a Gompertz plot of $\ln P(t)$ against time is approximately linear over this region, although there are many exceptions (13).

The challenges are to explain how normal wear and tear at a metabolic level can account for the general properties of organismal aging, how interventions act to alter life span, and why there is such variation in aging within and between species. One obstacle is that mutations and environmental interventions affect organisms at many levels of function, making it difficult to pinpoint how an intervention affects aging. To illustrate, consider how the poison cyanide leads to death. Is it due to inhibition of cytochrome oxidase? Loss of mitochondrial proton motive force? Defective ATP synthesis? Loss of control over ion gradients in the cell? Defective action of actinomyosin? Defective muscle cell contraction? Poor blood

pumping by the heart? Clearly, even in a simple, acute situation, it is not possible to say what killed an organism without first delineating the interacting biochemical and physiological entities and considering each independently to determine what precisely can lead to death. Similar complexities in biological processes at different levels of organization should be addressed to understand how normal aging occurs and to link biochemical alterations to changes in function and in mortality. A second major issue affecting our understanding of aging is to determine the relative significance or ranking of different types of damage and biochemical modification for normal aging. To address these two issues we have developed a hierarchical description of how the various levels of organization within an organism can contribute to aging. We then use this framework to show how it may be possible to quantify and rank the factors that contribute to aging by applying concepts derived from metabolic control analysis (MCA). Over the next few sections, the description of the aging organism as a hierarchical system with interacting levels of organization is developed.

A HIERARCHICAL FRAMEWORK FOR CONSIDERING ORGANISMAL AGING

We first place the physiological and metabolic processes of an organism into interacting hierarchies so that it is clear how biochemical alterations affect aging. In the top level of the hierarchy, all of an organism's functions are ascribed to a set of physiological systems that interact with each other and the environment (Figure 1). Each physiological system is considered to be a "black box" that only communicates with other systems and the environment through inputs and outputs. The outputs can occur independently of external inputs to the system, or they can be a function of inputs from other systems or from the environment (Figure 1). This leads to an interacting network of physiological systems that, in principle, gives a complete description of the organism's functions. Although each system is functionally discrete, interacting only through inputs and outputs, physical separation is not necessary. For example, the immune system is largely composed of individual cells that distribute throughout the body and that can infiltrate other tissues during inflammation and aging (32) but will still only connect with other systems through outputs such as cytokine release.

Each system can, in principle, be described by phenomenological models without any knowledge of its internal workings (Figure 1). System aging occurs over time through its output becoming inappropriate, thus impairing organismal function and elevating mortality rate. Damage within the system causes intrinsic dysfunction, leading to inappropriate system outputs. An undamaged system can also exhibit extrinsic dysfunction when its outputs are inappropriate solely because the inputs from other physiological systems are inappropriate. For this black box description, it is not necessary to understand how the damage that leads to intrinsic dysfunction arises.

The progressive dysfunction with aging of the organism is therefore due to dysfunctioning of some or all of its systems as defined by their outputs. Of course, the practical difficulties of defining a physiological system, of knowing all the interactions, and of accidentally omitting unknown or unsuspected interactions are enormous. Even so, thinking of aging in this way can be useful both as a heuristic exercise and to assist in identifying fruitful

avenues for experimental work. In the next section, we consider the types of system dysfunction that can contribute to aging.

DYSFUNCTION OF PHYSIOLOGICAL SYSTEMS DURING AGING

System function generally declines with aging; examples include human kidney glomerular filtration rate (35); muscle strength in mice, flies, and humans (36, 37); mammalian neurological function as measured by memory formation (38, 39); β -cell insulin secretion in humans (40); and the mammalian immune system (41, 42). Studies of functionally isolated systems, such as pancreatic islets (40), show that intrinsic system dysfunction can occur, but in vivo it is usually not clear if decline is due to intrinsic damage or defective inputs. For example, stem cell activity in mice declines on aging, but can be ameliorated by parabiotic pairings, whereby a young and an old mouse share a circulatory system (43), suggesting that at least part of the decline with age depends upon systemic factors from other physiological systems.

Important questions are whether all systems within an individual decline and whether the relative declines in different systems contribute similarly to mortality. From an evolutionary perspective, natural selection may adjust costly tissue maintenance to maximize reproductive success and thereby lead to similar rates of functional decline for all tissues (44). Many or all physiological systems within an organism would then wear out at similar rates, but with those rates determined at the level of the individual system. In addition, similar rates of functional decline of systems within an organism could occur because the outputs of a dysfunctioning system caused dysfunction in connected systems (Figure 1), even though their intrinsic rates of decline were different. An alternative explanation for parallel rates of aging in different tissues would be the existence of an aging process common to different physiological systems (45). Supporting this idea, mutations in single genes can extend healthy life span by ameliorating many forms of aging-related damage (e.g., 18–20), pointing to the existence of a single common aging process.

Furthermore, the evolutionary conservation of the effects on aging of some of these mutations between yeast, worms, flies, and mice raises the possibility of a similar underlying aging process in these very different organisms. Alternatively, the rate of aging of individual physiological systems could vary idiosyncratically, according to genetic susceptibility and environmental and stochastic events, with no single biological age attributable to an individual organism (46). Clearly, some aspects of aging can be organ specific and caused, for example, entirely by differences in environment as with skin aging and exposure to sunlight (47). Determining whether physiological systems within an individual organism decline in function at similar rates, and whether decline in all systems or in only a few key systems contributes to aging, is basic to understanding aging. However, multiple traits in single individuals are seldom investigated during aging, and even fewer studies have examined functional decline in different physiological systems. Markers of aging have been investigated, such as common changes in RNA transcript profiles during aging in different tissues (37, 48) and the accumulation of similar markers of oxidative damage (49). However, the relevance of these markers to system function is unclear (50).

Measurements are needed of the relative rates of decline in physiological systems within individual organisms over time to compare how they vary from individual to individual within a population and how they respond to interventions that extend life span. An even greater challenge is in comparing the relative importance of decline in the function of different systems to functional decline of the organism and probability of death. At the moment there are no methods to quantify, or even to rank, the relative roles of the functional declines of different physiological systems in the functioning and probability of death of the whole organism. Consequently, although aging can be ascribed to the relative decline in various systems, as is outlined in Figure 2, which shows that changes in system function lead to alterations in $P(t)$, considerable challenges remain in quantifying how the various systems contribute to normal aging. In addition, so far each system has been treated as a black box. To understand how dysfunction occurs within systems during aging, we look at the next level down in the hierarchy at the constituent cells of the systems.

CHANGES IN CELL NUMBER, FUNCTION, AND PHENOTYPE DURING AGING

Many kinds of changes to cells occur during aging, but only those that affect the functional outputs of physiological systems will influence aging (Figure 2). Most changes in the outputs of systems are due to alterations in their constituent cells. Even changes outside cells, such as in the extracellular matrix or exoskeleton, can often be ascribed to changes in cell function. For example, the accumulation of extracellular debris such as fatty plaques can be assigned to the dysfunction of macrophages. Bone is a dynamic system controlled by the activity of osteoclasts and osteoblasts. The form of structures that no longer contain living cells, such as tooth enamel or hair, are ascribable to the cells that constructed them. Even so, some age-related modifications, such as glycation-mediated loss of elasticity of blood vessel walls or damage to lens proteins, may be difficult to ascribe to cell function. However, these modifications occur at the same hierarchical level as cell changes; therefore, they affect system function in much the same way as changes to cell function. Thus, the functional declines of physiological systems during aging are caused by changes in cell number or function or by changes in noncellular components of the systems.

Changes in Cell Number During Aging

During aging many tissues undergo changes in cell number owing to cell death and to disruption of the mechanisms that maintain cell number (51). The change in cell content with aging varies tremendously with tissue and organism, with both cell loss and hyperplasia possible (51), as well as infiltration of some cell types, such as adipocytes or lymphocytes, into other tissues (32, 42, 52).

In postmitotic tissues, including most tissues in adult flies and all tissues in nematodes apart from the gonad, cells that are lost are not replaced (53–56). In mammals, therefore, the number of cells in many postmitotic tissues decreases with age, and this cell loss often correlates with a decline in system function. For example, the loss of functioning glomeruli in aging kidney correlates with decreased organ function with age (35). However, cell loss with aging is not general for all postmitotic tissues. One example is the mammalian brain

where there is no evidence for neuron loss with age (38, 39), even though in the adult brain there is very limited capacity to replace lost cells (57), and this is restricted to the dentate gyrus and hippocampus (58, 59).

In mitotic tissues when differentiated cells are lost, they can be replenished by division of other differentiated cells (e.g., mammalian liver) or from a pool of pluripotent, self-replenishing stem cells (e.g., mammalian gut endothelium, fly ovary) (60, 61). However, many differentiated cells in mitotic tissues divide less readily with age in vivo, with an increasing proportion entering replicative senescence (51, 62–64). For example, in old baboons, more than 15% of skin fibroblasts exhibit markers of senescence (62). Yet, the extent to which the accumulation of senescent cells contributes to diminished mitotic capacity and to decreased cell function with aging is uncertain. In addition, mammalian stem cells are less effective at replacing lost cells as the organism ages (57, 60, 65). However, it is unclear if decline in stem cell function causes decline in number of differentiated cells. Therefore in mitotic tissues, cell number can decline on aging through increased cell death, diminished replacement of lost cells, or both.

Cell number can also increase with age. For example, the number of adipocytes in human visceral adipose tissue increases (52), and the increased tissue mass can raise production of proinflammatory cytokines that influence the function of other systems (52). Cell hyperplasia also occurs in many tissues during aging, producing nodules that can disrupt system function (e.g., 66, 67) and that can also develop into cancers. Indeed, aging has been described as the most potent of all carcinogens. Metastatic cancers can undergo extensive genetic and epigenetic alterations, change their location in the body, and interfere in diverse ways with the function of other physiological systems. Similarly, some cells of the immune systems also infiltrate tissues, and during aging a proinflammatory state develops (e.g., 68–70) whereby several tissues are invaded by various classes of immune cells, which contribute to pathogenesis during aging (e.g., 71, 72).

Change in cell number during aging depends on physiological context and on the signals and contacts received in vivo and is hence difficult to study in vitro. More experiments are needed to measure change in cell number in tissues in different systems during aging as well as in different individuals and species. It is also important to determine whether changes are due to increased loss or defective replacement, which is often unclear. Most importantly, it is vital to determine how much cell loss or gain can occur before it impairs system functions and thereby determine how important change in cell number is for normal aging.

Changes in Cell Function and Phenotype During Aging

Changes in cell function with age could also affect system outputs. A decline in cell function with age is often found, for example, in the synaptic transmission in neurons (38) and the rates of contraction by musculoskeletal motor units (36). The finding that decline in neuronal function in the aging mammalian brain is associated with decreased numbers of synaptic connections and conduction, but not with decreased cell number, indicates the importance of loss of function independent of cell loss (38). Cell phenotype has been investigated more often than has cell function. For example, muscle fiber size decreases, with a decrease in myofilament numbers and poor packing of sarcomeres in both fly and

human muscle (73). Neurons shrink and have fewer spines and dendrites, lower synapse concentrations, and myelin dystrophy (38). Changes in gene expression, increased numbers of senescent cells, morphological changes, and accumulation of damage markers with age (e.g., 49) can also occur. With age, there is a gradual divergence of cell phenotypes within a tissue, perhaps because of stochastic events (24, 74, 75). For example, gene expression varies more between individual cardiomyocytes in aging mouse heart than in young cells (73, 76), and small changes in expression of many genes in the kidney cumulatively correlate with a small change in cell function (32). However, the relevance of these markers to loss of cell function is unclear. More importantly, the ways in which declines in cell function cause system dysfunction have not been investigated systematically. Changes in cell number and function within a system are probably intimately related. Loss or dysfunction of cells with age could have deleterious effects on the remaining cells in the system, because they are likely on average to be working harder, spending more time trying to restore themselves to homeostasis, and thus increasing the probability of cell death or dysfunction. In addition, senescent and other damaged cells can have a deleterious bystander effect by secreting factors that enhance local inflammation and tissue structure changes that may lead to more cell death (51). These mechanisms may explain why loss and dysfunction of cells in a system should accelerate with age. Therefore, changes in cell number, function, or phenotype can contribute to aging by altering the function of their physiological systems (Figure 2). However, there are considerable uncertainties as to how these alterations may contribute to system function and thus to mortality. To understand the biochemical processes that lead to the loss of cells and the change in function that occur during aging, we have to drop down to the next level in the hierarchy, that of the individual cell.

CELL METABOLIC HISTORY

Cell dysfunction and death are attributable to the cell's metabolic history, which is a combination of the initial state of the cell and subsequent cumulative changes. These will lead on to the changes in cell function and number that affect system function and thereby mortality (Figure 2). The initial state of the cell is due to its genome, developmental history, physical niche occupied within the organism, and epigenetic factors that affect genome expression (77). All these factors combine to lead to a particular state of its functional components. The cell's subsequent metabolic history can cause permanent DNA sequence modification, alter gene expression, and change the functional machinery of the cell by nonspecific damage, posttranslational modification, and environmental factors. These factors combine to determine the cell's intrinsic probability of dying, proliferating, or malfunctioning over time. Similar factors affect the function of noncellular components of systems, such as the extracellular matrix, that change their function on aging. Extrinsic factors from other cells and the environment will also alter the cell's chances of dying or malfunctioning. Factors clearly identified as important in the cell's metabolic history are nonspecific damage and changes in gene expression and signaling pathways.

Nonspecific Damage

Accumulation of diverse forms of nonspecific damage to biomolecules with age is a major contributor to cell loss and dysfunction. Oxidative damage has attracted the most interest

(49, 78, 79), but processes such as thermal denaturation, misincorporation of monomers into biopolymers, radiation, and inappropriate chemical reactions are also likely to be important. These damage processes are an inevitable consequence of carrying out thousands of chemical reactions in an enclosed space containing many reactive molecules, resulting in a range of damaged and poorly functioning biomolecules and subcellular structures during aging.

A range of processes reduces formation and duration of damaging agents, protects the cell against the damage, and repairs or degrades the altered target biomolecules. Increased steady-state levels of damaged biomolecules could reflect elevated generation of damage, decreased repair and turnover, or a combination of both. Some types of damage, such as a misfolded protein, can in principle be dealt with by the cell, whereas others cannot, such as fixation of a DNA mutation or accumulation of damaged material that can be neither broken down nor removed from the cell. With damage repair, there is a steady-state balance between impact of damage and its avoidance, repair, removal, or sequestration. This steady state could theoretically be set to prevent accumulation of non-specific damage to a level that affects function by devoting a sufficient amount of the cell's resources to maintain itself indefinitely. For irreversible damage, which cannot be repaired, the cell could also decrease damage by committing more resources to prevention. However, there may be no evolutionary advantage in devoting resources to maintain an undamaged somatic cell indefinitely at the expense of reproduction, and mechanisms that prevent, repair, or degrade damage are hence limiting, leading to cell dysfunction during aging.

Damage accumulation to cellular lipids, proteins, and nucleic acids during aging is abundantly documented (e.g., reviewed in 49, 80–83). Furthermore, a role for nonspecific damage in normal aging is supported by studies where life span is increased by reducing damage by, for example, overexpressing heat shock proteins in worms (84, 85) and increasing antioxidant defenses in the mouse (86). Autophagy is essential for some forms of life span extension in *C. elegans* (87). However, caution in interpretation is warranted because it is often unproven that these manipulations increase life span by decreasing damage accumulation, rather than by altering other processes such as signaling pathways.

Nonspecific damage, such as oxidative damage, can impair the activities of enzymes, the fluidity of membranes, or the activity of organelles (49), and thus impair cell function. However, to demonstrate a role in normal aging is less straightforward because we need to know whether damage affects cell survival or function in vivo sufficiently to affect the outputs of its physiological system and hence aging. For example, mutation to nuclear DNA is an important candidate contributor to aging because it is irreversible and it does occur during aging. However, its contribution to cellular systems and organismal aging is still debated (88). Mutations to mitochondrial DNA also accumulate with age (89), but their role in cell dysfunction and organismal aging is more questionable because the normal mutation load to mitochondrial DNA during aging may be insufficient to explain functional decline (90). Yet, there have been few detailed studies of the chain of events from accumulation of damage to biomolecules, to the effects on cell functions, and hence cell survival or function, through to the physiological system and aging. Cells may have considerable thresholds for the accumulation of damage before function or survival is impaired, and because a major

consequence for cells of damage accumulation is death, damage could have a major impact but leave no obvious trace among living cells in the organism.

Gene Expression and Cell Signaling

Changes in gene expression and cell signaling during normal aging could contribute to aging by affecting the rate of accumulation of cell nonspecific damage or by independently altering pathways that directly affect the ability of the cell to function or survive. These changes would then act to influence the outputs of physiological systems in such a way as to affect mortality (Figure 2).

There is an extensive literature showing changes in gene expression and cell signaling pathways in cells during aging (91–96). RNA transcript profiles have revealed a number of changes in gene expression in mouse muscle (91), including decreases in expression of genes encoding proteins involved in energy metabolism and increases in expression of stress response genes (91). In addition there is increased stochasticity and variability in expression between cells (73, 76). There are no systematic decreases in the expression of defense, protective, or repair pathways, and in fact, increases are often seen instead (91), consistent with a response to increased damage on aging. Thus, a systematic downregulation of protective pathways does not seem to account for aging, although it is possible that increased variability in gene expression may lead to stochastic decreases in protective systems in individual cells.

Cellular signaling pathways are intimately involved in extension of organismal life span by single gene mutations and environmental interventions. For example, DR, a moderate reduction in food intake while avoiding malnutrition, extends life span in diverse organisms, including budding yeast, nematodes, fruit flies, and rodents (97). Furthermore, intensive study of DR in rodents has shown that it delays or ameliorates the impacts of multiple forms of damage, dysfunction, and disease (29). Although it is unclear if the mechanisms by which DR extends life span are evolutionarily conserved, recent work has implicated several evolutionarily conserved signaling pathways in the response to DR, including the nutrient-sensing target of rapamycin signaling pathway (98–100) and the insulin/insulin-like growth factor (IGF) pathways (101, 102). Mutations in genes encoding components of these same signaling pathways can also extend healthy life span in yeast (e.g., 103–105), *C. elegans* (19), *Drosophila* (99, 106), and mouse (107, 108).

The implication is that the altered activity of these pathways ameliorates the kinds of damage that are normally limiting for organismal life span. For example, extension of life span by reduced insulin/IGF signaling is often associated with up-regulation of cellular pathways that increase the activity of stress resistance and cellular detoxification pathways in *C. elegans*, *Drosophila*, and mouse (e.g., 109–111). However, interventions such as DR are associated with decreased damage accumulation, but this does not seem to be caused by an increase in defensive pathways, many of which actually decline during DR (112–114). These interventions might also alter the threshold for the amount of cell damage required to cause cell death or dysfunction (115, 116).

In normal aging, a large number of changes in gene expression and cell signaling pathways occur, but their significance in the aging process is likely to vary tremendously with tissue [for instance, between postmitotic and mitotically competent tissues (109)] and organism. These changes will influence aging only by affecting cell number or function in a system, so that system output is altered. Consequently, more studies are needed to determine how changes in cell signaling and gene expression affect cell number and function during normal aging and how their contribution can be quantified or ranked.

Consequences of Cell Metabolic History for Cell Survival, Replication, and Function

We have divided the cell's metabolic history into effects of nonspecific damage and of changes in cell signaling and gene expression, but in reality, these factors are interrelated and interact to alter a wide range of cell functions during aging. The importance of these for system function and therefore aging will vary with cell type and context. However, some types of cell function may be of pervasive significance for aging, for example, energy metabolism, protein and RNA synthesis, and ion homeostasis. Among these, energy metabolism is often considered particularly important because it affects a wide range of processes, and any defect will have widespread consequences for other metabolic systems within the cell. The ability of mitochondria to generate ATP diminishes with age (e.g., 117), and declines in the expression of genes involved with the mitochondrial electron transport chain are seen during aging in humans, mice, and flies (37, 48, 118). Defects in energy metabolism and their effects on aging are widely reported (49, 78, 119, 120), and they lead to aging phenotypes (121, 122). For example, it is vital for cells to rapidly restore and maintain homeostasis after exposure to environmental challenges, including muscle contraction and nerve conduction, by pumping out ions such as calcium. Defects in oxidative phosphorylation could impair the cell's ability to maintain its cytosolic ATP/ADP ratio under challenge and thus increase the time spent in dyshomeostasis. During these periods, the cell may function less well and may be more vulnerable to death by apoptosis or necrosis (123).

A cell's metabolic history also contributes to decreased replicative ability and vulnerability to death, and this history impairs tissue regeneration and maintenance during aging. Cellular senescence and apoptosis prevent cell division in damaged cells that could go on to become neoplastic or to malfunction in other ways with serious consequences for the organism (51). Apoptosis is also used to remove cells selectively during development and to ensure that a damaged cell dies cleanly rather than by necrosis (124, 125). Nonspecific damage, particularly to nuclear DNA, causes many cells either to enter senescence or activate apoptosis. Many cells become senescent owing to alterations to their telomeres (126). In extreme cases, excessive cell division greatly shortens the telomeres. However, in many cases, cells probably undergo senescence without a large number of cell divisions, and telomeres may then act as damage sensors or sentinels of nonspecific DNA damage, particularly oxidative damage (63, 126, 127). The situation is similar in apoptosis, where cell damage leads to activation of factors, such as p53, that cause exit from the cell cycle, providing an opportunity to repair damage within the cell and then reenter the cell cycle or if the damage is too great to undergo apoptosis (128, 129). Thus, apoptosis and senescence fill a vital role by preventing cells from becoming neoplastic. Consequently, many tumors arise

from mutations in genes, such as p53, that are involved in inducing apoptosis. Apoptosis/senescence and cancer in response to DNA damage are finely balanced: too much and the organism loses cells too rapidly and undergoes premature aging while too little leads to increased tumor load (51).

The relationship between cell damage, function, and survival and their effects on aging are complex. For example, although oxidative damage is often assumed to be a major contributor to aging, in some models such as in mice heterozygous for the mitochondrial antioxidant enzyme manganese superoxide dismutase (130) and in the long-lived rodent the naked mole rat (131), the relationship between oxidative damage and life span is the opposite of that predicted. Thus, what is required is a way of linking types of damage and changes in cell signaling and gene expression within cells to changes in cell survival and cell function. In turn, these alterations need to be linked to changes in system functions that affect mortality (Figure 2). It seems probable that, for example, large amounts of damage to some cell types may be unimportant for mortality because they can withstand higher levels of damage or because the damage does not affect critical cell functions and system outputs that affect aging. In contrast, small amounts of damage in other cells may be critical for aging. More studies are required to measure how factors such as damage affect outputs and how these in turn affect aging.

OVERVIEW OF THE HIERARCHICAL DESCRIPTION OF AGING

The hierarchical description of aging is summarized in Figure 2. The increase in $P(t)$ of the organism with time is due to system dysfunctions that lead to changes over time in their functional outputs. These changes have a greater or lesser influence on the $P(t)$, as indicated by the variable width of the arrows linking to mortality. System dysfunction is, in turn, due to either changes in the number or in the function of its constituent cells or equivalent components, owing to metabolic history. Interventions can only affect aging by changing system functions that alter the $P(t)$ of that organism, and many changes that occur during aging will have no impact on mortality.

The hierarchical approach helps clarify the contribution of various factors to aging, provides a framework for discussing aging that is internally consistent, and accommodates the fact that different tissues and organisms may age through quite different pathways. Even so, considerable challenges remain, particularly in determining which factors within a hierarchy are most critical for aging and thus where we should focus interventions and experimental effort. Approaches that can quantify, or at least rank, the contributions of various factors to aging at various levels of the hierarchies are required. How the hierarchical description can be extended to be quantified in ways that are useful for experimentalists investigating aging is the topic of the next section.

QUANTIFICATION OF THE FACTORS CONTRIBUTING TO AGING

The hierarchical description of aging provides a framework that helps us pose appropriate questions about the kinds of processes that contribute to aging and clarifies how biochemical alterations within cells impact on aging through their effects on physiological systems (Figure 2). However, even when considering the changes that occur during aging in a

hierarchical context, we continually come up against the problem of how to determine whether a process contributes to aging or not. Even if it does, the challenges are then to quantify or rank the relative contributions of different processes, at different levels in the hierarchy, to aging within an organism and determine how these contributions vary within and between species and are affected by interventions that affect life span. There are many questions central to aging that require a quantitative answer. For example, we would like to be able to assess the relative importance of different physiological systems and system outputs for aging and to know whether cell dysfunction or loss of cells during aging is more important. For cell loss, is accumulation of senescent cells, loss of stem cells, or the increased rate of cell loss most important? Which processes within a cell contribute most to its loss of function or inability to replicate as it ages? What forms of nonspecific damage are of most importance to the decline in cell function during normal aging? What is required is a way of incorporating quantification, or at least ranking, into the hierarchical approach we have described to determine which system or process does contribute to aging, which is most important, and, hence, where interventions are likely to have most impact on aging.

If they existed, robust, quantifiable models of aging might be useful to determine the processes of greatest significance. However, modeling aging is problematic at several levels. An immediate hurdle is that our lack of detailed knowledge of the processes involved makes modeling of aging premature. A further challenge is the dynamic aspect; during aging, the systems themselves change with time, and any modeling approach has to accommodate this. In addition, time lags are likely to be important. Processes such as cell loss may occur decades before they affect function or probability of death. Therefore, although there are a number of interesting approaches under development to model aging (e.g., 2, 8–10), robust and quantifiable models of aging that can rank the importance of systems, cells, and cellular components to aging are not yet on the horizon.

Therefore, we require an empirical approach that would enable us to identify the factors that contribute to aging and to determine the relative importance of these factors to aging in experimental animals, despite our incomplete knowledge of the system and limited means of intervening to alter the rate of aging. We think a promising way to do this is to adapt aspects of metabolic control analysis (MCA). In the following sections, we describe MCA and show how it may be used to design and interpret experiments, using current technologies to answer important questions about aging.

Metabolic Control Analysis and Aging

MCA was initially developed independently by Kacser & Burns (132, 133) and Heinrich & Rapoport (134) and has since been developed and used to describe the control and regulation of a range of metabolic pathways and networks (135–139). In considering the control of a metabolic pathway by MCA, the first step is to develop an explicit definition of the limits of the system and of the measurable variables, such as metabolic intermediates and pathway fluxes. Importantly, apart from clearly defining its limits, there is no requirement for a complete description of the system, and sections of it can be treated as black boxes to accommodate measurable variables. Once these measurable variables and their interactions

are defined, the system is manipulated in small ways, and the changing relationships between the variables reveal the extent to which each step is controlling.

Consider the analysis by MCA of a simple metabolic pathway of intermediates connected by enzyme-catalyzed reactions to address which enzymatic steps exert control over the overall pathway flux. To do this, the activity of each enzymatic step in the pathway is varied very slightly, independently of changes in other components of the pathway, and the effect of this on the overall flux is determined. This simple example yields several interesting conclusions. A major one is a simple definition of control, where the greater the change in the overall flux on altering the activity of an enzyme, then the greater the control of that enzyme over flux. However, this change in overall flux will be the result of the change in enzyme activity in the context of the system, as altering its activity impacts on the overall flux by changing the concentrations of the metabolic intermediates that link it to the rest of the pathway. Thus, the control over flux is a property of the pathway, not of the enzyme in isolation. An important consequence of control being a system property is that several steps in a pathway can share control, and the distribution of control among these varies as metabolic conditions are altered. This contrasts with views of metabolic pathways having a single rate-limiting step. Another important aspect is that overall flux changes are measured in response to relatively minor alterations in enzyme activity. This is because all pathway components would become controlling over flux if their activity was decreased by 99%, but this is not physiologically relevant. Finally, many changes in enzyme activity may change levels of metabolic intermediates, but if there is no overall change in flux, then that step is not controlling. MCA has developed a powerful mathematical formalism to enable the extent of control to be quantitated, to determine whether all the important controlling sites have been uncovered, and to indicate how controlling steps act on overall flux by influencing other steps in the system (135–139).

There are many similarities between the control of metabolic pathways and the impact of changes in biochemical factors on aging. Just as the overall effect of an enzyme's activity on pathway flux is a property of the whole system, so the effect of any metabolic change on mortality is dependent on its biological context. A similar metabolic change may have no impact on aging in one organism, tissue, or situation but may be critical in another. Another important parallel is that, just as enzymes are only controlling over a pathway's flux if small changes in their activity impact on flux, a factor can only control aging if a relatively minor alteration affects overall mortality. A corollary is that large changes in many biochemical pathways and processes are known that can increase or decrease life span, but this is not evidence that these processes influence normal aging. These parallels between aging and the regulation of metabolic pathways, and that MCA can determine information on steps important in controlling a process in complicated systems of which we have incomplete knowledge, suggest that adapting approaches from MCA to aging may be fruitful. In the next section, we outline some ways in which MCA may be used to answer questions about aging.

Application of MCA to Aging

Applying the concepts of MCA to aging organisms requires a measurable mortality readout indicative of organismal aging, which we assess as we make small manipulations to factors that may control aging and record the quantifiable results. The mortality readout could be anything that correlated with aging, but the $P(t)$, the rate of change of the $P(t)$ with time, or the median life span are the most direct measures of aging. Alterations in this mortality readout are then measured in response to small changes in factors that may control aging. A factor can only be said to affect aging if it alters the chosen mortality readout, and the greater its impact on this, the larger its contribution to aging. The factors changed could include increasing and decreasing the expression level by small increments of a critical enzyme or receptor to assess the effects on mortality. Alternatively, the effect upon mortality readout of small increases and decreases of levels of damage to cellular components could be assessed by the differential expression of protective pathways, or by exposure to toxins and protective agents.

These experiments can be carried out with populations of flies, worms, or mice, and the mortality readout can be measured in a range of populations in which the variable has been slightly increased and decreased. The mortality readout is then plotted on the y-axis against the change in the variable on the x-axis (Figure 3). There are a number of possible outcomes, but three curves illustrate the most important. Most factors will not affect aging, as illustrated by curve A in Figure 3. The flat area in the center shows that altering this variable around its normal value does not influence mortality. Of course, with many biological components, it is possible to increase or decrease it to an extent that will affect mortality, as illustrated by the upward trends at either end of curve A. However, as this increase in mortality occurs outside the range of values for that variable during normal aging, illustrated by the central shaded area, this factor does not contribute to normal aging. The effect of a variable that is harmful to the organism is shown in curve B. Decreasing the amount of this variable increases longevity, whereas increasing it will lead to increased mortality. Most importantly, the changes in the amount of the variable that affect aging occur within the normal range of the variable during aging. At some point, decreasing this variable further will impact on mortality, leading to increasing mortality, but this occurs outside the normal range of variation during aging. The effect of a variable that protects against aging is shown in curve C. Decreasing the amount of the variable is harmful, but increasing it is protective, and most importantly, the changes in the amount of the variable that are sufficient to affect the mortality readout occur during normal aging. Many combinations of these three curves are possible, but these illustrate the critical aspects. The most important point is that if a factor controls aging then the curve of mortality readout against the variable of interest has a measurable slope as it passes through the region of the normally aging population.

Figure 3 indicates how ideas from MCA can be used to determine whether or not a process contributes to aging. There is a well-developed mathematical apparatus for MCA that can be used to quantify and rank the contribution of various factors to the control of metabolic fluxes (135–139). It is clear that the greater the control of a process over aging the steeper the slope of curves such as B and C as they pass through the point of normal aging in Figure

3. For example, it is clear that allowing for appropriate normalization, the process in curve B has more control over aging than that in curve C. Quantification and comparison of the relative control of different factors in MCA are done by comparing the normalized fractional changes in an output, such as a flux, with the normalized fractional changes in the factors being varied. This leads to dimensionless quantities called control coefficients that enable the proportion of control over a flux to be assigned to each process. Analysis of the control of aging requires similar careful normalization of the changes relative to the endogenous levels of the variable, requiring a detailed development of the parallels between the mathematical formalism of MCA and the control of aging that will be described in future publications. Even so, the approach outlined in Figure 3 shows how MCA can be used to determine if an intervention affects normal aging and to rank and quantify the contributions of factors to aging. In the next section, the practical aspects of carrying out these experiments are considered.

Practical Considerations for Applying MCA to Aging

Here we consider how studies, such as those described in Figure 3, could be done using currently available animal models and technologies. This approach requires (a) factors that may impact on aging to be modulated by a series of small increments and decrements in different populations of experimental animals and (b) the effects of these changes on a mortality readout for each population to be determined. In the first instance, such experiments can be done with populations of nematodes or *Drosophila* because these are already routinely used in aging research and have the advantages of a short life span as well as ease of genetic manipulation and measurement of mortality.

A number of mortality readouts could be chosen, but measuring the slope of a plot of $\ln P(t)$ against time for a population has a number of attractions. Over much of the life span, it is linear; consequently, a large number of individuals in a given population contribute to the readout, it is already routinely measured in aging research in worms and flies, and it generates a single number for each population. Nevertheless, other readouts of aging, such as median life span or the intercept of curves of $\ln P(t)$ against time with the y-axis—or death within or by a certain time interval, may also prove useful.

The variable whose effect on aging is being investigated will have to be increased and decreased very slightly (perhaps by as little as a few percent) and incrementally. The mortality readout would be determined in a series of populations of flies or nematodes where the variable was modified slightly. In addition, the range of values of the variable during normal aging would have to be measured. If the variable was a protein, then in nematodes and *Drosophila*, its expression level could be modulated downward by standard RNAi approaches and modified so as to decrease expression of the protein by only a few percent. For a small increase in expression of the same protein, a number of current approaches can be adapted to generate strains with slightly increased expression levels of the target protein. After generating several different populations, each with a slight variation in the expression level of the protein of interest, their mortality readouts would then be measured and plotted against the level of the protein of interest. If a type of damage, such as oxidative damage or accumulation of misfolded protein was of interest, then this could be

increased slightly by addition of pharmacological or environmental stressors or by decreasing expression of protective enzymes. Damage could be decreased by addition of protective agents or by increasing expression of protective proteins. These approaches can be extended by selectively expressing the proteins in only one tissue or physiological system or by only changing expression at different stages during the subjects' life spans. Similarly, the effect of cell number in a system could be addressed by increasing or decreasing the expression of toxic or protective proteins to modulate slightly the number of cells in a tissue.

Consider some examples: the DAF2 receptor is known to impact on the life span of nematodes, and its activity is thought to correlate inversely with life span. If the amount of this receptor was altered and compared with a mortality readout, we might predict a result, similar to curve B in Figure 3. However, it could be that the effects of changing the amounts of these receptors on life span only occur with large-scale changes, and the curve might look more like curve A. With increased antioxidant defenses we might predict a curve such as C in Figure 3; however, it could be that the thresholds for effects on mortality are such that there is no change relative to the normal range over aging. Another interesting type of damage is increased mtDNA mutation load, where high levels clearly lead to an aging phenotype, but it is unclear if this only occurs because the dependence of mortality on mtDNA damage is a curve of type A.

The approaches outlined should help determine whether a single factor can impact on aging. Many interesting questions in aging research arise from environmental interventions, such as DR, that alter life span, but it is difficult to determine which of the many changes that occur during DR are important for aging and which are not. This issue can be addressed by the MCA approach by selecting plausible factors that change in DR and manipulating these independently to see if they contribute to the changes in aging seen during DR.

Thus, by developing the MCA approaches and applying them to currently available experimental models of aging using experimental approaches that are already developed, we should be able to address a number of critical questions about the factors that control aging.

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SUMMARY POINTS

1. Aging arises from the accumulation of damage resulting from a lack of capacity to protect, maintain, and repair somatic tissues over time. Accumulation of damage leads to loss of function and, ultimately, death.
2. The rate of aging of individuals can vary as a result of genetic, epigenetic, and environmental variation as well as of stochastic events.
3. The accumulation of damage during aging occurs at multiple levels, from the physiological system, through organs, to cells, and individual biomolecules. Not all of the changes that occur with age are likely to be causal in loss of function and increased likelihood of death, and it is often difficult to determine which factors are important for aging.
4. One way of clarifying causality with events occurring at multiple levels during aging is to make explicit the hierarchical level under consideration and its relationship to other levels. The mortality of the individual is ultimately due to the change in function of its physiological systems. These system changes are caused by changes in the number or function of its component cells. Cell changes are themselves due to the metabolic history of the cell and to its impact on the ability of the cell to function and survive. The aspects of the metabolic history of the cell that are important for aging are the accumulation of nonspecific damage and changes in cell signaling and gene expression.
5. Even when the hierarchical description has been adapted, the remaining critical question in aging research is to develop methodologies that will enable the relative contributions of various metabolic changes to aging to be quantified and related to each other. What is required is a methodology that can be used to address these questions in the experimental animal systems currently in use using technologies now available. We suggest that adaptation of MCA to aging will enable significant progress in determining the relative importance of the factors that contribute most to aging.

FUTURE ISSUES

1. Can we successfully adapt the methodologies of MCA to aging in model organisms such as worms, flies, and mice so as to determine the relative contribution of various factors to aging? In doing so, is it possible to use current approaches such as RNAi to manipulate the levels of factors that are thought to contribute to aging? Is it also possible to use this approach to determine the pathways through which changes during interventions such as DR occur?
2. If the MCA approach proves fruitful in aging research, is it able to contribute toward answering critical questions, including: Can we quantify or rank the relative importance of the functional outputs of different physiological systems that are important for aging? Can we quantify the contribution of changes in cell number and function to the alteration in a system's functional outputs over aging? Is it possible to quantify or rank the importance of nonspecific damage and changes in gene expression and cell signaling pathways as well as to determine how they affect cell function and survival in vivo?
3. If quantification of the contribution of various processes to aging proves feasible, then are the critical factors for aging similar or different for individuals within a population and also between different species? Is the hierarchical description and application of the MCA approach helpful in developing new insights into aging and in suggesting novel interventions that may affect aging? Can this approach be usefully extended to aging-associated degenerative diseases and to other complex, multifactorial diseases?

Figure 1.

Interacting physiological systems are shown schematically as two interacting systems. These systems are affected by inputs from the environment and by inputs from other physiological systems. System outputs can originate within the system independently of other inputs or can be dependent on the inputs from the environment and/or other systems. Organisms can be divided into physiological systems in a number of different ways; for example, human physiology can be described using 11 systems: skin, respiratory system, circulatory system, central nervous system, endocrine system, reproductive system, lymphoid system, musculoskeletal system, urinary system, digestive system, and special sense organs (33, 34). The increased mortality of the organism as it ages is due to the decline over time of various systems outputs. These can be intrinsic where the system outputs become inappropriate during aging because of alterations within the system. Dysfunction in system outputs can also occur for undamaged systems when the inputs are inappropriate.

Figure 2.

Aging of an organism is due to the decline in function of the top-level physiological systems into which the organism has been divided. This dysfunction leads to changes in system outputs that have a greater or lesser influence on the age specific mortality [$P(t)$], as indicated by the variable width of the arrows linking to mortality. Dysfunction of each system is in turn due to changes in either the number or function of its constituent cells. The changes to cells are caused by their metabolic history and are due to nonspecific damage and to changes in signaling pathways and gene expression. These in turn lead to effects on cell function and on cell number.

Figure 3.

Analysis of aging by metabolic control analysis. Here a generic indicator of mortality, the mortality readout, is plotted against a variable that is both decreased and increased relative to its level in normal populations. Each value of the mortality readout is determined for a separate population in which the value of the variable is altered. The central shaded area indicates how the variable alters in normal aging. Three scenarios are shown. In curve A, the variable has no impact on aging as varying it over the range that occurs in normal aging does not affect mortality. At high and low levels, it does impact on mortality, but because these occur outside the range found in normal aging, they do not contribute to aging. Curve B shows a variable that contributes to aging; increasing it in the central shaded area raises the level of mortality, whereas decreasing it lowers mortality. In contrast, in curve C, increasing the variable in the central shaded area decreases mortality, and decreasing it increases mortality, as might happen if this variable were protective. For both curves B and C, the steepness of the slope as the curve passes through the point of unmodulated aging gives an indication of how controlling the two processes are over aging. In this example, the process described by curve B is more controlling over aging than that described by curve C.