Contents

Part V

Organ Systems and Diseases 1113

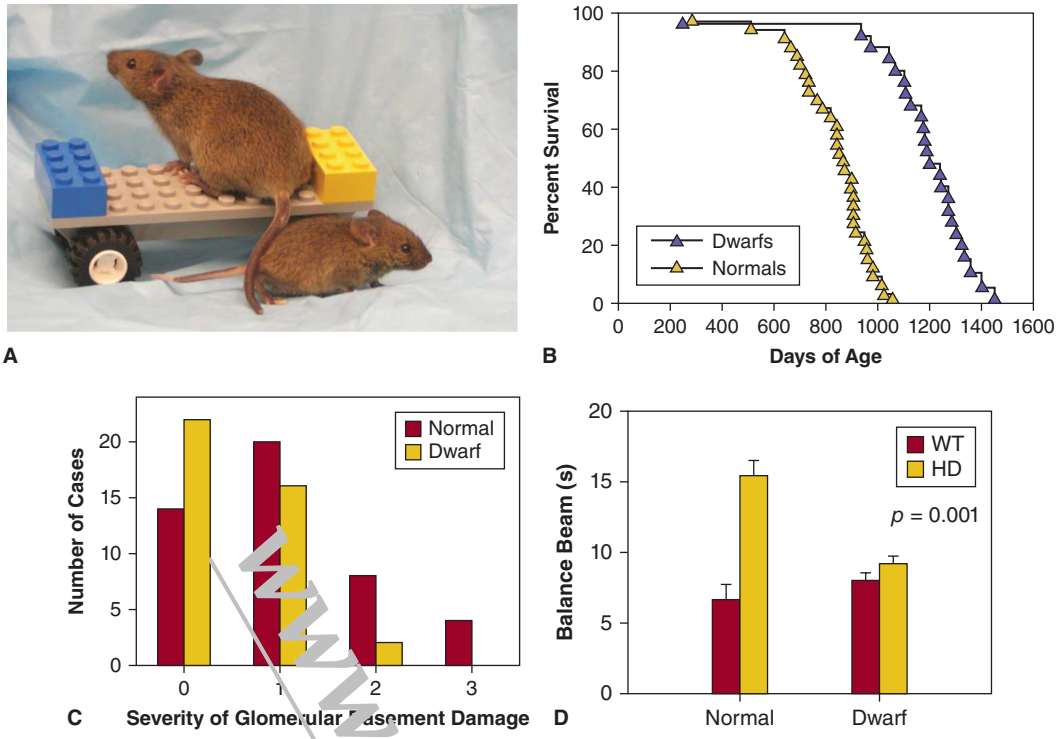

FIGURE 1-3. Mice carrying the Snell dwarf mutation are one-third of the size of their normal littermates, because the mutation diminishes production of growth hormone by the pituitary (**A**). This mutation increases mean and maximum lifespan by about 40% (**B**), and reduces the severity of kidney lesions at death among many other forms of late-life illness (**C**). Mice carrying a dominant mutation for Huntington disease (HD) perform less well than wild-type (WT) control mice on a balance beam task (**D**), but the HD gene does not produce corresponding symptoms when it is placed on a Snell dwarf background. (A, Reproduced with permission from Richard Miller; B, Reproduced with permission from Flurkey K, Papaconstantinou J, Miller RA, Harrison DE. Life span extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc Natl Acad Sci U S A*. 2001;98[12]:6736–6741; C, Data from Vergara MM, Smith-Wheelock JM, Harper R, et al. Hormone-treated Snell dwarf mice regain fertility but remain long-lived and disease resistant. *J Gerontol A Biol Sci Med Sci*. 2004;59[12]:1244–1250; D, Data from Tallaksen-Greene SJ, Sadagurski M, Zeng L, et al. Differential effects of delayed aging on phenotype and striatal pathology in a murine model of Huntington disease. *J Neurosci*. 2014;34[47]:15658–15668.)

Building on interest in TOR signaling and its effects on protein translation, work in invertebrates and increasingly in mammals has revealed fundamental links between the maintenance of proteostasis and longevity. Proteostasis refers globally to the processes that improve the quality and function of cellular proteins, collectively termed the proteome. It includes regulation of protein translation, folding, damage repair, and degradation. Autophagy is a process by which damaged cellular proteins and even whole organelles such as mitochondria are degraded in lysosomes. In invertebrates, intact autophagy systems are required for longevity extension by essentially all interventions that increase lifespan. Increased expression of the autophagy protein ATG5 extends mouse lifespan and improves neuromuscular function and glucose tolerance in old mice. Rapamycin, which extends lifespan in many different organisms, decreases translation of many cellular proteins, while promoting increased autophagic function. Snell and GHR-KO mice show augmented levels of a specific form of autophagy, "chaperone-mediated autophagy," which can affect levels of proteins involved in cell cycling, fat synthesis and mRNA translation. Collectively, these findings indicate that maintenance of proteostasis is a key requirement of longevity.

Sirtuins

Sirtuin proteins, which play a major role in aging in yeast, have captured the public interest and sparked robust debate in aging circles. The sirtuins are a family of protein deacylases that consume the metabolic cofactor NAD+ during catalysis. Cellular NAD+ levels increase under conditions of fasting or DR, in a tissue-type specific manner. In light of their NAD+ requirement, sirtuins have been implicated as energy sensors and potentially as mediators of some of the benefits of DR. In yeast, worms, and flies, sirtuin overexpression extends longevity, albeit more modestly in worms and flies than initial reports suggested. Mammals possess seven distinct sirtuins; these are a diverse protein family, from the standpoint of biochemical activity, protein targets, and localization. Mammalian sirtuins target their protein

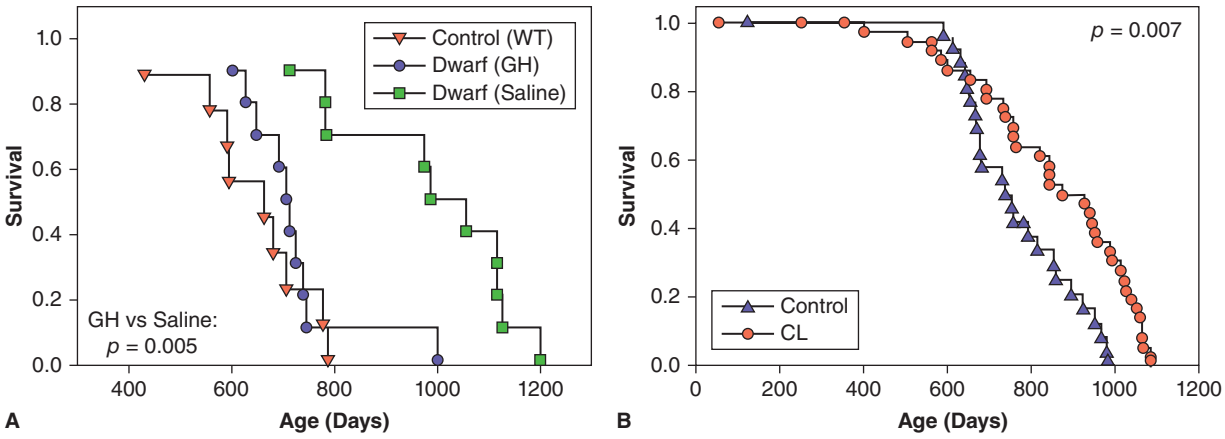

FIGURE 1-4. Early-life hormonal and nutritional status can have major effects on late-life survival and disease. The left panel shows an experiment in which Ames dwarf mice were given injections of growth hormone (GH) for an 8-week period beginning at 2 weeks of age. The hormone-injected mice had survival patterns similar to that of normal mice (WT controls), and therefore much shorter than Ames mutant mice (green) that had only saline injections at early ages. The right panel shows a complementary experiment, in which genetically normal mice were partially deprived of milk ("CL" for "crowded litter") by adding extra pups during the suckling phase, from birth to 3 weeks. This transient milk deprivation led to a substantial increase in lifespan in the CL mice. (A, Reproduced with permission from Panici JA, Harper JM, Miller RA, et al. Early life growth hormone treatment shortens longevity and decreases cellular stress resistance in long-lived mutant mice. *FASEB J.* 2010;24[12]:5073–5079; B, Adapted with permission from Sun L, Sadighi Akha AA, Miller RA, et al. Lifespan extension in mice by preweaning food restriction and by methionine restriction in middle age. *J Gerontol A Biol Sci Med Sci.* 2009;64[7]:711–722.)

substrates via deacetylation, or removal of other posttranslational modifications, to regulate a wide range of proteins involved in gene expression, DNA repair, metabolism, and many other processes. Three sirtuins are present in the mitochondrial matrix, where they regulate intermediary metabolism and other aspects of mitochondrial function.

In mammals, most studies have focused on SIRT1, the closest homolog of the yeast SIR2 protein. Whole-body overexpression of SIRT1 does not increase overall lifespan in mice, although it is protective against age-associated metabolic dysfunction and certain forms of neoplasia. Overexpression of SIRT1 in the brain only, however, does extend mouse lifespan. Overexpression of the sirtuin SIRT6 throughout the body also extends lifespan in male mice. SIRT6 promotes metabolic homeostasis and enhances genomic stability; it will be important to elucidate which of SIRT6's many functions are most important in its pro-longevity role. For example, sequence variations among SIRT6 homologs across different mammalian species may contribute to altered DNA repair efficiencies, and even to differences in lifespan.

Beginning with resveratrol, a series of small molecule activators of SIRT1 have been developed. In mice, these drugs show some beneficial effects, particularly in the context of dietary challenge such as high-fat feeding. There are continued controversies regarding these agents, particularly focused on the extent to which they target SIRT1 directly, versus exerting indirect effects through other signaling molecules, such as AMPK. Nevertheless, these or similar agents could conceivably find utility in treating age-associated metabolic dysfunction in humans.

Stress Response

Mutations that extend lifespan in invertebrates typically render the animals resistant to multiple forms of lethal injury, whether the threat comes from oxidative agents, heat, heavy metals, or irradiation. Genetic dissection of the relevant pathways—which must have evolved very early in the evolutionary tree, that is, prior to the common ancestor shared by worms, flies, and humans—has shown, surprisingly, that in normal, non-mutant worms, the levels of stress resistance, and thus resistance to aging, are relatively diminished by inhibition of specific DNA-binding transcription factors, members of the FoxO family (see **Figure 1-5**). These pathways are retained by evolutionary pressures because they provide reproductive advantages in the natural environment, in which animals must be able to quickly take advantage of transient access to nutrients. Activation of FoxO proteins in the laboratory produces mutant animals that are not ideally suited for natural conditions, but which are resistant to many kinds of stress and which age more slowly than normal. Studies of gene expression patterns in the long-lived mutant worms have shown that the FoxO proteins can regulate transcription of over 100 genes that together protect against many different forms of cellular damage. The list includes enzymes that destroy free radicals, heat shock proteins, and other chaperones that guard against misfolded proteins, proteins that protect against infection, and chelating agents that bind toxic metal ions, among others.

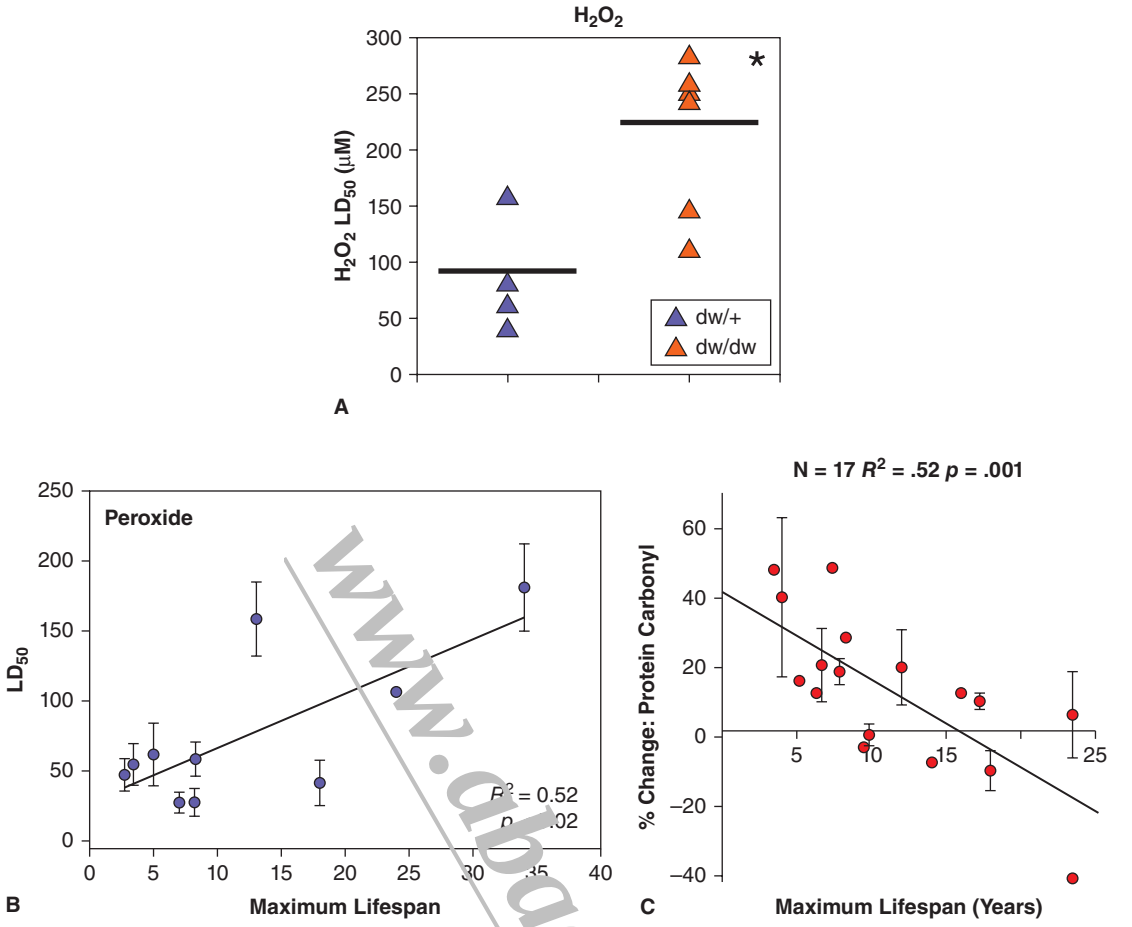

FIGURE 1-6. Links between exceptional longevity and resistance to stress. (**A**) Skin-derived fibroblasts from long-lived mutant (red symbols, dw/dw, Snell dwarf) mice are resistant to the lethal effects of hydrogen peroxide, H_2O_2. Each symbol is an individual mouse, and the Y-axis shows the amount of H_2O_2 needed to kill 50% of the cells. (**B**) Skin-derived fibroblasts from long-lived species are relatively resistant to lethal injury induced by H_2O_2. Each symbol represents a different rodent or bat species with the indicated maximum lifespan. Species, left to right, are laboratory mouse, wild-caught mouse, rat, red squirrel, white-footed mouse, deer mouse, fox squirrel, porcupine, beaver, and little brown bat. LD_{50} is the amount of hydrogen peroxide that kills 50% of the cells. (**C**) Cells from long-lived rodent species are resistant to changes in oxidized proteins ("protein carbonyl" on Y-axis) induced by 1-h exposure to H_2O_2. Each symbol is a different species, with the species' lifespan indicated on the X-axis. (A, Adapted with permission from Murakami S, Salmon A, Miller RA. Multiplex stress resistance in cells from long-lived dwarf mice. *FASEB J.* 2003;17[11]:1565–1566; B, Reproduced with permission from Harper JM, Salmon AB, Leiser SF et al. Skin-derived fibroblasts from long-lived species are resistant to some, but not all, lethal stresses and to the mitochondrial inhibitor rotenone. *Aging Cell.* 2007;6[1]:1–13; C, Reproduced with permission from Pickering AM, Lehr M, Kohler WJ, et al. Fibroblasts from longer-lived species of primates, rodents, bats, carnivores, and birds resist protein damage. *J Gerontol A Biol Sci Med Sci.* 2015;70[7]:791–799.)

idea, DNA lesions, mutations, and aneuploid cells (cells with incorrect chromosomal number or rearranged chromosomes) accumulate with age in mammalian tissues. Because such events occur stochastically on a cell-by-cell basis, it has proven difficult to rigorously identify and quantitate such events. Aging leads, in mice and humans, to increased incidence of aneuploidy. Although evidence that accumulation of DNA damage contributes to aspects of aging other than neoplasia is currently modest, elevated levels of a protein involved in repair of DNA damage, SIRT6, can increase mouse lifespan in males and delay cancer and metabolic dysfunction. Further work in this and similar models may help to clarify the possible role of genome maintenance in aging and nonneoplastic diseases.

The role of ROS in inducing age-associated genetic damage, or in aging more generally, remains controversial. Resistance to many forms of stress, including oxidative injury, is a common feature of long-lived mutants, as described above. However, many mouse mutations that impair resistance to ROS damage have no detectable effect on lifespan and show no obvious acceleration of age-related pathology. Furthermore, administration of antioxidants to mice or people does not extend lifespan and

may under some circumstances actually increase mortality. Conversely, for the most part mouse strains with increased activity of ROS defense systems show protection from specific toxins that cause oxidative injury, but do not show extended lifespan. The only current exception is a mouse strain overexpressing the antioxidant enzyme catalase in mitochondria. These animals show increased lifespan, and, by some measures, improved health in old age.

Cellular Senescence, Telomeres, and Cancer

The famous observation of Hayflick that human diploid fibroblasts cease to grow in culture after a limited number of population doublings sparked a line of experimentation that continues to yield important insights into the molecular control of cell growth, differentiation, and neoplastic transformation. Human fibroblasts placed in tissue culture will divide until approximately 50 cell doublings have occurred, after which the remaining cells survive indefinitely in a healthy but nondividing state. In the 1970s and 1980s, this "clonal senescence" model seemed to be an attractive approach to study the genetics and cell biology of aging. It is now clear that growth cessation of continuously passaged human fibroblasts is caused principally by the progressive loss of telomeric DNA at the ends of each chromosome at each mitosis, which occurs in cells that lack a specialized enzyme complex called telomerase. Critically shortened telomeres induce a DNA damage response in the cell, leading to senescence or cell death. Short telomeres can influence intracellular signaling to hamper mitochondrial function, thereby impairing cellular metabolism, suggesting that damaged telomeres might exert effects even in tissues such as the liver that are largely postmitotic.

Telomeres and cancer (also see Chapter 82) Telomere-dependent clonal senescence clearly plays a critical role in the protection of humans from many forms of cancer. Telomerase is turned on, and telomere attrition prevented, in approximately 90% of malignant tumors in humans. Genomic sequencing efforts have revealed that individuals with mutations that chronically elevate telomerase activity, or increase the accessibility of telomeres to telomerase, are at increased risk for a variety of cancers.

Studies of the role of telomeres and telomerase in the biology of aging in mice have produced controversial findings. Mice have much longer telomeres than humans, but live much shorter lives. The rate at which telomeres shorten, however, is more rapid in mice than in humans, and mouse strains engineered to have elevated telomerase show increased cancer rates, consistent with a role for telomere length as a contributor to malignancy in mice, as in humans. Paradoxically, elevated telomerase function may improve some aspects of health in mice carrying other alleles that block many forms of neoplasia, though with no effect on maximum lifespan, a provocative observation worth further exploration. As indicated in **Figure 1-7**, telomere erosion can, in different settings, lead to cellular senescence, or to changes in cell properties related to DNA damage, genomic instability, or neoplastic transformation. The potential role of these changes in late-life human disease is an area of active investigation. Genetic disruption of telomerase can also produce lines of mice with shortened telomeres. These mice are short-lived, but the spectrum of pathology, featuring skin ulceration, infertility, increased frequencies of specific forms of neoplasia, and frequently lethal gastrointestinal lesions, does not closely resemble the pattern of illnesses seen in normal aging mice or humans. Thus telomere attrition, except for its major role in oncogenesis, does not seem likely to represent a major contributor to aging in rodents.

Senescent Cells Early work based on histologic assays suggested that senescent cells were rare (< 0.1%), even in biopsies from very old donors, although new methods for detecting other aspects of the senescent phenotype have led to upward revisions of this estimate. Senescent cells in vitro express a suite of secreted enzymes and inflammatory cytokines (not produced by dividing fibroblasts), which may facilitate the growth of cancer cells and could in principle contribute to other aspects of aging at the tissue or organ level. The *p16/INK4a* protein serves to maintain a balance between senescence and oncogenic transformation. P16 is induced as part of the process during which cellular senescence halts cell proliferation. An increase in levels of P16 is observed in many tissues of aging mice, but particularly in stem cells, suggesting that senescent stem cells may indeed accumulate as a consequence of normal aging and may represent an evolved mechanism to prevent early-life neoplasia. P16 accumulation leads to diminished stem cell function in the aging bone marrow, brain, and pancreas. Mice engineered to have reduced levels of p16 retain stem cell activities at ages at which these cells proliferate poorly in normal animals, though these mice are somewhat more cancer-prone than normal controls. In humans, genetic polymorphisms near the *p16/INK4a* gene have been linked to age-associated conditions such as type 2 diabetes, coronary artery disease, and frailty. Interventions that prevent age-related induction of p16 might be an attractive approach to diminishing many forms of late-life illness in parallel, if the intervention did not simultaneously increase cancer risk.

Several research groups have developed genetic methods to delete senescent cells from adult mice, and there is current interest in the possible therapeutic potential of pharmacologically targeting senescent cells. However, thus far there is little evidence for beneficial effects of these drugs, termed senolytics, in humans and little data on possible negative effects of such treatment. A series of ongoing clinical trials will evaluate the possible efficacy of senolytic drugs in the context of many age-associated diseases in humans, including osteoarthritis, Alzheimer disease, and others.