restriction (or *dietary restriction*) increases lifespan and delays aging in many animals, probably as a nonadaptive side effect of this famine response. Many of the genes and pathways that regulate the way that cells respond to nutritional undersupply have been identified, initially in yeast and *C. elegans*. In general, manipulation of these pathways (through genetic knockout or overexpression or pharmacologic agonists and antagonists) alters the aging benefits of caloric restriction and, in some cases, the lifespan of animals on normal diets. These pathways are all very influential cellular "switches" that control a wide range of key functions including protein translation, autophagy, mitochondrial function and bioenergetics, and the cellular metabolism of fats, proteins, and carbohydrates. The discovery of these nutrient-sensing pathways has led to targets for pharmacologic extension of lifespan. The main nutrient-sensing pathways that influence aging and responses to caloric restriction include the following:

- *SIRT1*. The sirtuins are a class of histone deacetylases that inhibit gene expression. The key nutrient-sensing member of this class in mammals is SIRT1. The activity of SIRT1 is regulated by levels of reduced nicotinamide adenine dinucleotide (NAD^+), which are increased when cellular energy stores are depleted. Important downstream targets include PGC1a and NRF2, which act on mitochondrial biogenesis.
- *Target of rapamycin (TOR, or mTOR in mammals)*. mTOR is activated by branched-chain amino acids, providing a link to dietary protein intake. It is a complex that orchestrates two pathways (TORC1 and TORC2). Key downstream targets of mTOR of relevance to aging include the tuberous sclerosis protein (TSC) and 4EBP1, which influence protein production.
- *5′ Adenosine monophosphate–activated protein kinase (AMPK)*. AMPK is activated by increased levels of AMP, which reflect cellular energy status.
- *Insulin signaling and IGF-I/growth hormone*. These two pathways are usually considered together because they are the same in lower animals and have diverged only in higher animals. Insulin responds to carbohydrate intake. An important downstream target for this pathway is a transcription factor called daf16 in worms and FOXO in mammals and the fruit fly.

Mitochondrial Genes Mitochondrial function is influenced by genes located both in the mitochondria (mtDNA) and the nucleus. mtDNA is considered to have a prokaryotic origin and is highly conserved across taxa. It forms a circular loop of 16,569 nucleotides in humans. Aging is associated with increased frequency of mutations in mtDNA as a consequence of its high exposure to oxygen-derived free radicals and relatively inefficient DNA repair machinery. Nuclear DNA encodes approximately 1000–1500 genes for mitochondrial function, including genes involved with oxidative phosphorylation, mitochondrial metabolic pathways, and enzymes required for biogenesis. These genes are thought to have originated in mtDNA but subsequently translocated to the nucleus, and unlike mtDNA genes, their sequence is stable with aging.

Genetic manipulation of mitochondrial genes in animals influences aging and lifespan. In *C. elegans*, many mutants with defective electron transfer chain function have increased lifespan. The mtDNA "mutator" mice, which lack the mtDNA proofreading enzyme, have increased mtDNA mutations and premature aging, whereas overexpression of mitochondrial uncoupling proteins leads to longer lifespan. In humans, hereditary variability in mtDNA is associated with diseases (mitochondriopathies such as Leigh's disease) and aging. For example, in Europeans, mitochondrial DNA haplogroup J (haplogroups are combinations of genetic variants that exist in specific populations) is associated with longevity, and haplogroup D is overrepresented in Asian centenarians.

STRATEGIES THAT INCREASE HEALTHSPAN AND DELAY AGING

Aging is an intrinsic feature of human life whose manipulation has fascinated humans ever since becoming conscious of their own existence. Recent reports and scientific literature are shaping a picture where different dietary restriction regimes and exercise interventions may improve healthy aging in laboratory animals. Several long-term experimental interventions (e.g., resveratrol, rapamycin, spermidine, metformin) may open doors for corresponding pharmacologic strategies. Surprisingly, most of the effective aging interventions proposed converge on only a few molecular pathways: nutrient signaling, mitochondrial proteostasis, and the autophagic machinery.

Lifespan is inevitably accompanied by functional decline, a steady increase in a plethora of chronic diseases, and ultimately death. For millennia, it has been a dream of mankind to prolong both lifespan and healthspan. Developed countries have profited from the medical improvements and their transfer to public health care systems, as well as from better living conditions derived from their socioeconomic power, to achieve remarkable increases in life expectancy during the last century. In the United States, the percentage of the population age 65 years or older is projected to increase from 13% in 2010 to 19.3% in 2030. However, old age remains the main risk factor for major life-threatening disorders, and the number of people suffering from age-related diseases is anticipated to almost double over the next two decades. The prevalence of age-related pathologies represents a major threat as well as an economic burden that urgently needs effective interventions.

Molecules, drugs, and other interventions that might decelerate aging processes continue to raise interest among both the general public and scientists of all biologic and medical fields. Over the past two decades, this interest has taken root in the fact that many of the molecular mechanisms underlying aging are interconnected and linked with pathways that cause disease, including cancer and cardiovascular and neurodegenerative disorders. Unfortunately, among the many proposed aging interventions, only a few have reached a certain age themselves. Results often lack reproducibility because of a simple inherent problem: interventions in aging research take a lifetime. Experiments lasting the lifetime of animal models are prone to develop artifacts, increasing the possibilities and time windows for experimental discrepancies. Some inconsistencies in the field arise from overinterpreting lifespan-shortening models and scenarios as being related to accelerated aging.

Many substances and interventions have been claimed to be antiaging throughout history and into the present. In the following sections, interventions will be restricted to those that meet the following highly selective criteria: (1) promotion of lifespan and/or healthspan, (2) validation in at least three model organisms, and (3) confirmation by at least three different laboratories. These interventions include (1) caloric restriction and fasting regimens, (2) some pharmacotherapies (resveratrol, rapamycin, spermidine, metformin), and (3) exercise.

Caloric Restriction One of the most important and robust interventions that delays aging is caloric restriction. This outcome has been recorded in rodents, dogs, worms, flies, yeasts, monkeys, and prokaryotes. Calorie restriction is defined as a reduction in the total caloric intake, usually of about 30% and without malnutrition. Caloric restriction reduces the release of growth factors such as growth hormone, insulin, and IGF-I, which are activated by nutrients and have been shown to accelerate aging and enhance the probability for mortality in many organisms. Yet the effects of caloric restriction on aging were first discovered by McCay in 1935 long before the effects of such hormones and growth factors on aging were recognized. The cellular pathways that mediate this remarkable response have been explored in many experimental models. These include the nutrient-sensing pathways (TOR, AMPK, insulin/IGF-I, sirtuins) and transcription factors (FOXO in *D. melanogaster* and daf16 in *C. elegans*). The transcription factor Nrf2 appears to confer most of the anticancer properties of caloric restriction in mice, even though it is dispensable for lifespan extension.

Two studies have reported the effects of caloric restriction in monkeys with different outcomes: one study observed prolonged life, while the other did not. However, both studies confirmed that caloric restriction increases healthspan by reducing the risk for diabetes, cardiovascular