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Mechanisms of Aging in the Naked Mole-Rat: The Case For Programmed Aging

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Abstract—Maximum lifespan is an important species trait that generally scales with body size. Never the less there are many mammals that deviate from this allometric relationship and live more than double the expected lifespan predicted on the basis of body size. These exceptionally long-lived species appear to defy the expected rate of aging and provide powerful tools with which to elucidate if maximum species lifespan is indeed encoded in the genome; determine possible biological clocks and their downstream molecular mechanisms that may abrogate or regulate rates of aging. One such mammal is the naked mole-rat [NMR]. This, the longest-lived rodent known, lives 8.6-times longer than similar-sized mice, yet it maintains cancer-free, good health for more than 85% of its astonishing 30-year lifespan. NMRs, like other long-lived species, show pronounced resistance to most cellular stressors. This may be due to enhanced cellular protection and/or better maintenance of somatic integrity. In contrast, short-lived species generally direct many of their resources into rapid growth and early reproduction rather than fend off threats to their soma. Although the mechanisms facilitating this species divergence in somatic maintenance are poorly understood, these most likely represent an evolutionary trade-off between partitioning energy and resources into somatic maintenance (thereby contributing to the survival of the individual) versus investments in growth and reproduction and ensuring the rapid attainment of sexual maturity and the long-termsurvival of the species. This species-specific difference in resource and somatic management must be encoded in the genome, thus enabling cells and the organism to mount the appropriate level of cytoprotection, commensurate with their expected longevity. We explore this premise by reevaluating the various theories of aging in the light of what is known from the biology of the longest-living rodent, the naked mole-rat.

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INTRODUCTION

With the increase in human longevity observed in the last century, research into the basic causes and mechanisms of aging is becoming a social, economic and scientific challenge. Advances in the tools of cell biology and molecular genetics have been rapidly integrated into this most interesting and challenging field, yet aging remains one of the most poorly understood biological phenomena. At a glance, aging research suffers from the lack of a precise definition of fundamental terms, such as "aging," and a "programmed" model of how an organism ages. Furthermore, gerontological research has been fraught with studies terminating in correlative, not causative relationships. A true functional understanding of any biological process requires knowledge of the underlying biochemical reactions and regulatory circuits. Here we will review popular aging theories and examine this in the light of data obtained from traditional short-lived animal models of aging research and non- traditional long-lived animal models, particularly the naked mole-rat (NMR; Fig. 1).

What is "Programmed" Aging?

Most evolutionary biologists argue that aging cannot be selected for because it does not increase fitness of the individual (for review, see [1]). In fact, they hypothesize that aging is a by-product of maintaining life processes (e.g. somatic maintenance and/or prevention of the accumulation of deleterious mutations) and the toll that reproduction costs somatic

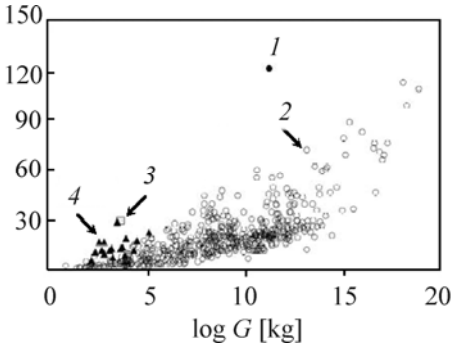

Fig. 1. Relationship between body size and maximum species longevity in mammals. Note that mole-rats, bats and humans conspicuously stand out as being exceptionally long-lived for their respective body sizes. (*1*) Humans, (*2*) others organisns, (*3*) naked mole-rat, and (*4*) bats.

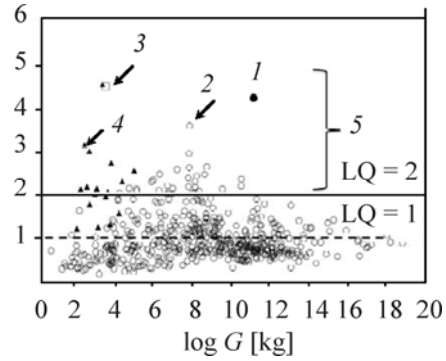

Fig. 2. Mammalian longevity quotients. The ratio of the observed maximum lifespan to that predicted on the basis of body size is known as the longevity quotient (LQ). Animals that are considered long-living are those that live at least twice as long as predicted on the basis of body size. This feature has evolved repeatedly among mammals, however NMRs and humans conspicuously stand out as being exceptionally long-lived. (*1*) Humans, (*2*) others organisns, (*3*) naked mole-rat, (*4*) bats, (*5*) long-lived organisms.

tissue maintenance [1]. Recently, however, an abandoned set of theories has been resurrected [2]. Previously termed "adaptive aging," "aging by design" or "programmed death," these theories are primarily based on the observation that each species has a unique maximum longevity. The central hypothesis posits that an organism's maximal lifespan potential is encoded by the species genome. Rather than ask whether the deleterious manifestations of aging are genetically encoded (i.e. "programmed"), we ask whether there is a broader frame encompassing longevity to determine the potential for maximum lifespan.

Pronounced species differences in longevity and age-associated variables exist across the animal kingdom. Reported maximum life span potential (MLSP) varies more than 40.000 fold and is generally associated with the degree of somatic maintenance and reproductive strategy. The species-specific difference in longevity, resource and somatic management must be encoded in the genome, thereby enabling cells of the organism to mount the appropriate level of cytoprotection commensurate with their predicted MLSP.

Understanding what mechanisms the evolutionary forces of nature use to create species with disparate longevity may provide important insights into the mechanisms of aging and how it may be influenced by the genome.

Maximum life span is a species-specific trait that varies as a function of the size of the organism (Fig. 2), such that larger organisms both develop and age more slowly than smaller species. This is illustrated by the elephant, which has a predicted life span of 80 years, while the 6 gram large-eared tenrec (*Geogale aurita*) lives 2.2 years [3]. Long-lived species, defined as those species that live at least twice as long as expected on the basis of their body size, have evolved repeatedly in mammals (Fig. 3). For example, both the mouse-sized NMR and human conspicuously stand out as being unusually long-lived for their body-size, living five times longer than predicted by allometry (Figs. 2 and 3). The observation that maximum lifespan is subject to allometric scaling and that different species, such as humans, bats, and NMRs defy this allometric relationship and live longer than predicted, even after the elimination of extrinsic mortality factors (e.g., predation), indicates that life span is subject to genetic control [4–6].

The Naked Mole-Rat Model Ecology

Our laboratory has focused on aging in the longest-living rodent, the NMR. NMRs are strictly subterranean, highly social rodents found in the arid regions of north east Africa. These hairless mammals (Fig. 1) live in an extensive maze of underground burrows and meet all their nutrient and water requirements from the roots, corms and tubers they encounter below ground [7, 8]. They have lived in this milieu since the early Miocene [9], and have evolved a suite of physiological and morphological traits well

Fig. 3. The unusual looking naked mole-rat is a highly social rodent, that lives in large colonies underground. Many of their morphological features including their fusiform cylindrical shape, short limbs and lack of external ear pinnae and poor eyesight are common traits of subterranean rodents.

suited to life underground [10]. Their evolved longevity most likely is due to ecological constraints that influence their extrinsic mortality [11, 12]. By living in a thermally buffered underground milieu, NMRs are protected from climate extremes and against predators; as such their extrinsic mortality is low and concurs with a key tenet of the evolutionary theory of aging.

Eusociality

Enhanced fitness and prolonged longevity is also associated with social living; NMRs can live in large colonies of up to 295 members with one actively breeding female, "the queen," and 1–3 actively breeding males [13]. Animals remain in their natal colony until they die and while subordinates have the ability to become active breeders they remain reproductively quiescent until the active queen dies [14]. Similar to bats that live in large roosts, eusocial insects (e.g., bees) and social primates, NMRs also show extended longevity [9, 15, 16]. This may reflect the benefits of the intergenerational information transfer, communal living and shared foraging.

Unlike the eusocial insects, NMR breeding individuals in captivity do not live longer than their non-breeding counterparts. In their natural milieu, breeders live ~4-fold longer than non-breeders and this is attributed to the fact they do not perform chores such as foraging that may expose them to factors that influence extrinsic mortality. In captivity, most deaths in the colony occur during the first few weeks of life. After six weeks of age, chances of survival into very old age (>25 years) are high.

Characterization of Aging

NMRs share many phenotypic traits with experimental mouse models of extended longevity (e.g., dietary restriction, dwarf, and klotho- over-expressing mice) in that they have a lower metabolic rate (0.75×), lower resting body temperature (32–34°C), lower fasting blood glucose levels (0.75×) and lower hormone levels (e.g., thyroxine) than those of wildtype mice [17–19]. In captivity, breeding females continue to reproduce well into their third decade and show no age-associated decline in fertility. NMRs also show attenuated agerelated deteriorations in activity, body composition and physiology until near the end of their life, thereby delaying the aging process and markedly compressing the period of generalized frailty [20]. In keeping with their prolonged good health, 2- and 26-year old NMRs show similar levels of protein expression for a diverse range of enzymes, structural and signaling proteins indicating that genomic integrity and protein homeostasis are rigorously maintained with age [21, 22]. Although we still do not know with certainty the common causes of mortality, NMRs show remarkable resistance to neoplasia. No malignant tumors have been observed in our large colony that has been maintained for over 28 years. These long-lived rodents may yield novel insights into the mechanisms of aging and if aging is under genetic control as well as whether or not it is programmed.

Negligible Senescence

NMRs show negligible senescence in that the probability of death does not increase with an individual's chronological age. Furthermore, NMRs show attenuated rates of decline in all physiological, morphological and biochemical traits and compress the typical pattern of aging to the last 15% of their lifespan, equivalent to humans maintaining constant functionality until more than 100 years old. It should be emphasized that negligible aging does not mean immortality. In addition to the NMR, some turtles, rockfish and deep oceandwelling organisms appear to fit under the category of animals that have negligible

aging and maintain their soma for the majority (>85%) of their life spans. With the exception of the NMR, most of the putative negligible aging organisms have not been well characterized [20, 23–28].

The patterns of gene evolution and expression are subject to the environment in which an organism evolves. The mole-rats, having evolved in a dark, dank milieu for millennia provide a useful model system to test the somatic theory of aging as well. For example Nevo and others have reported that blind subterranean mole rats (*Spalax ehrenbergi*) show multiple sequence differences in genes (e.g., DNA binding domain of p53, VEGF, myoglobin, and HIF1α) that play an important role in stress resistance and these may provide important adaptive mechanisms to survive and indeed thrive in this extreme hypoxic and hypercapnic environment [29–33]. Certain of those genetic changes may be private to *Spalax ehrenbergi* or more conserved in species living in similar habitats. Our laboratory has compared changes in the p53 DNA binding domain of *Spalax ehrenbergi* and NMRs to several other rodent species and humans (unpublished data) and found that this genetic polymorphism is not widely conserved in mole rats and may be a uniquely private mechanism of *Spalax* to the stressors encountered in their milieu.

Species that have evolved to live in unpredictable environments usually employ a *r*selection life strategy: they attain sexual maturity early and reproduce quickly, furthermore they have large litters and show low levels of parental investment. In contrast, *K*-selected species, which have evolved to live in stable conditions (low predation included) reach sexual maturity later, have longer gestation, fewer offspring per litter and increased care for their young. Although litter size is extremely large by rodent standards (up to 30 pups), for the most part NMRs are *K*-selected. Most members in the colony never attain sexual maturity and gestation is extremely long (11 weeks). The cost of reproduction to the dominant female is extremely high; she more than doubles her mass during pregnancy, has more than 3-fold higher metabolic requirements and spends more than a month lactating while she is already pregnant with the next litter [34] yet she continues to breed throughout her long life [20]. However, if one looks at NMRs at the colony level, clearly partition less of their energy into reproduction and more into repair and maintenance of the soma thereby protecting tissues and internal processes from cellular stressors.

Stress Resistance

Enhanced longevity is associated with increased stress resistance by cells of a phylogenetically diverse bestiary of long-lived species [35–41]. Stress resistance, as measured by the viability of an easily isolated cell type (fibroblasts) treated with a variety of noxious agents [heat, paraquat, UV radiation, and heavy metals) has been repeatedly shown to correlate with species lifespan, overexpression of antioxidant genes and single gene mutations impacting the IIS [37, 39, 40, 42–45]. Data collected in our laboratory for the relative resistance/susceptibility of NMR skin fibroblasts to a broad array of cellular stressors, including oxidative stress, alkylating agents, heavy metals and specific DNA damaging agents when compared with mouse dermal fibroblasts concur. We find that there is a two-fold difference in LD50 of the two species in response to heavy metals and even more pronounced NMR resilience when certain other cellular stressors are used. When we compare the NMR data with those from similar-sized mice, it is clear that body size is not the determinant of the observed differences in cellular resistance. This generalized toxin resistance suggests that NMR cell responses are not due to mechanisms that counteract the toxicity of specific agents. Instead, broad toxin resistance may be a feature of generalized better cellular protection and maintenance of somatic integrity, though a mechanism is yet to be proposed for these findings and is currently under investigation in our laboratory.

The Naked Mole-Rat and Other Theories of Aging

Oxidative Stress Theory and Aging

According to the oxidative stress theory, damage initiated by reactive oxygen species is a major contributor to the functional decline that is characteristic of aging [46]. Reactive oxygen species (ROS) are continuously produced predominantly in the mitochondria of biological tissues, and under homeostatic conditions play a role in various signaling pathways [47–49]. Abnormally high ROS concentrations cause oxidative stress and are associated with tissue damage and this is thought to drive the physiological decline known as aging [49]. However, it is now becoming accepted that the role of oxidative and other damage as a consequence of aerobic metabolism over time as a driver for the aging process is an oversimplification. There is no simple linear relationship between total mitochondrial metabolism

and oxidative damage [50–54]. More importantly, while ample supporting evidence for the oxidative stress theory exists, both within and across phyla, longevity associations with lower ROS production or lower ROS-induced macromolecular damage [46, 55–58] are countered by numerous examples [22, 59–62]. In fact, our laboratory has shown that the NMR does not have elevated antioxidant defenses and has significantly higher levels of oxidative modified DNA and proteins than shorter-lived rodents [22, 59, 60].

Mutations as a Driving Force for Aging

Mutations in the nuclear and mitochondrial genomes are associated with several ageassociated diseases including cancer and various degenerative diseases [63–65]. The role of somatic mutations as a driving force of aging is controversial [66, 67]. Mutations can facilitate the formation of destabilizing molecules including oxidants and incorrectly coded proteins that may drive pathological conditions and aging [68–70]. "Mutator" phenotypes are one hallmark of cancer in humans and rodent models [71].

The key question for longevity research is whether or not mutation frequencies become elevated enough to drive aging in a mammalian system. Researchers have focused on frequencies of mitochondrial DNA mutations based on the premise that mitochondria are the main organelles from which ROS are generated. Vermulst et al. [72] examined the frequency of mitochondrial point mutations in mitochondrial DNA in "mitochondrial DNA mutator mice" and normal wild-type mice during aging [72]. Mitochondrial mutation frequency increased 11 fold during normal aging in the wild type mice. In contrast, the "mutator" mice had a 500-fold increase in mitochondrial DNA mutation frequency without displaying signs of premature aging. Thus, point mutation burden alone does not appear to drive the aging process. We have not examined mutation frequency in the nuclear or mitochondrial genomes of the NMR. However, there is no reported case of tumor burden, used as an indirect measure of the effects of the culmination harmful mutations, in the NMR.

Insulin or IGF-1 Signaling and Aging

The insulin or IGF-1 signaling pathway's (IIS) has been shown to be an important, functionally conserved pathway involved in the determination of lifespan (Fig. 4). Genetic control of life span has been shown by forward genetic approaches as the identification of

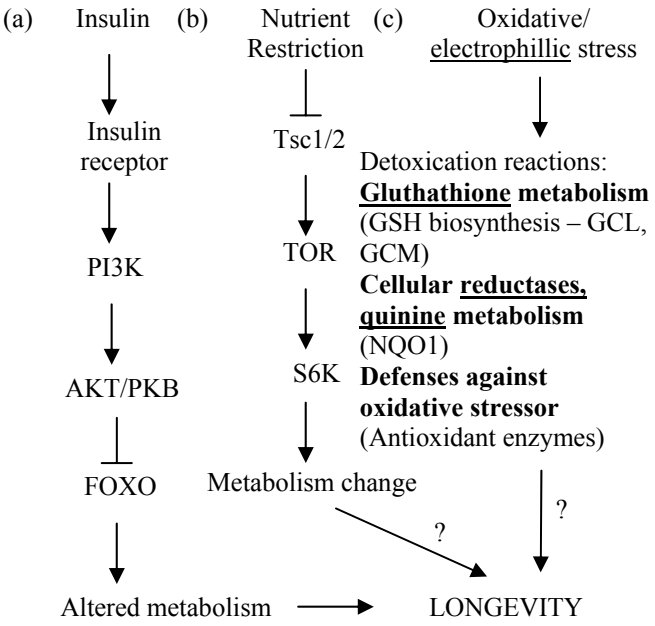

Fig. 4. Longevity mechanisms act to attenuate insulin signaling pathway and activate pathways involved in stress resistance. The insulin and TOR pathways are two major nutrient and stress sensing pathways that appear to modulate longevity several model systems. (a) Following activation of the insulin receptor, the signal is transduced either directly by IRS (insulin receptor substrate) in mammals, to PI3K (phosphatidyl inositol 3-kinase), leading to the activation of AKT/PKB (AKT/protein phosphatase B) and dephosphorylation of FOXO nuclear translocation of FOXO to the nucleus and activation of genes involved in growth. Reduced insulin signaling, from caloric restriction or single gene mutations, leads to greater FOXO activity, leading to altered metabolism and transcription of genes regulating stress response and promotes longevity. (b) TOR controls cell growth and proliferation through regulation of translation. TOR is a highly conserved serine/threonine protein kinase. TOR mediated activation of S6K has been shown to mediate altered protein synthesis in flies, leading to enhanced longevity; It is unclear if TOR regulates life span in mammals. (c) In response to oxidative/electrophilic stressors modify intracellular thiols are modified, particularly GSH, as well as other detoxification enzymes.

single gene mutations that can extend life span up to 10 fold in some model organisms [36]. However, in most mammalian systems, single gene alterations or nutrient manipulations (CR, methionine restriction) have only modest impact upon life span (30–50%). This observation demonstrates that there are many open avenues of research potential to explore using comparative approaches for mammalian systems, in which different mammalian species may have longevities varying as much as 40 fold [73, 74]. IIS

putative roles in aging of lower eukaryotic models (e.g., *C. elegans* and *S. cerevisiae*) and the insulin-signaling mutant rodent have been extensively reviewed elsewhere [75–78].

The IIS pathway as an underlying mechanism of CR and key control mechanism of aging remains to be irrefutably proven, although a wealth of empirical data exists to support this premise. A decline in insulin sensitivity and concomitant higher levels of blood glucose are commonly associated with aging and correlated with many deleterious effects [79–83].

Conversely, reduced insulin and low fasting blood glucose concentrations are features correlated with extended longevity in CR animals and many long-lived mutant models (Fig. 4; [84–86]). Our laboratory has demonstrated that the NMR, like other rodent insulin signaling pathway mutant models, displays an impaired glucose tolerance response similar to profiles observed in insulin resistance or deficiency [87]. Thus, NMRs may share similar mechanisms as these mutant models to achieve their remarkable longevity. Circulating insulin levels in the NMR cannot currently be assayed using RIA methods available in commercial kits, most likely due to an inability of antibodies to recognize NMR insulin. We have sequenced NMR insulin and have found several polymorphisms common to the hystriconath suborder (unpublished data) [88, 89].

NMRs are hyper-responsive to a dose of human insulin, displaying a prolonged depression in blood glucose levels following treatment with human insulin. These data suggest that NMR may be naturally deficient in insulin and show depressed insulin signaling associated with longevity. Several types of cellular defense mechanisms are hypothesized to mediate the effects of IIS on life span, including xenobiotic detoxification [35, 90, 91], alterations in protein turnover/synthesis [92–94], autophagy [95, 96] and enhancements in immune function [97, 98]. The rate-limiting pathway is currently unknown.

Programmed Aging and the Naked Mole-Rat

In this review we have attempted to address two key questions: (1) Are conventional aging theories sufficient to explain the incredible longevity of the NMR; and (2) Is programmed longevity applicable to the NMR? Traditionally, evolutionary theories strongly oppose explaining the process of aging as programmable (1). We have attempted to fit the ecological and physiological parameters known for the NMR into conventional theories of aging (see the table). Clearly, the NMR fits into some (perhaps several) but certainly not all of the popular mechanistic theories to explain aging/longevity. A refinement on the programmed longevity theory [99] posits that species achieve a particular longevity due to the stability of their life-strategies, based on genetic programs, that provide a framework for maximal lifespan. Given the opportunity to enhance longevity while maintaining a stable life strategy would therefore increase lifespan. They argue that such is the case with all major life extension interventions such as DR and reduction of IGF1 (and their invertebrate analogs). These data are supported by life extension in worms, flies and mice (for review see [99]). To date, NMR longevity has not been experimentally enhanced in any way.

However, this species has a longer lifespan in captivity than in nature and holds the record for the longest living rodent [9, 20]. As such, when we review its biology under the conditions outlined by Longo et al. 2005 [99], each species has evolved a stable balance between physiological activities (e.g. metabolism, reproduction) and lifespan. They argue that lifespan extension interventions (DR and genetic manipulations) push the organism into a low maintenance mode associated with low fertility and this predominantlym hypometabolic state leads to enhanced lifespan. If we apply this premise to NMRs, one could hypothesize that a stable "maintenance mode" of programmed aging could account for their unusually long life. Most NMRs do not breed and NMRs have low resting metabolic rates, 66–75% of that of mice. The breeders however have strikingly similar longevity to non-breeders and although resting metabolic rate in NMRs is low, these rodents like most subterranean rodents, have a high metabolic scope increasing metabolic rate at least 3–5 times while burrowing [100], during non-shivering thermogenesis [101] and during pregnancy and lactation [102]. Therefore one cannot conclude that their extraordinary longevity is simply due to hypometabolism, leading to the partitioning of more of their resources into somatic maintenance and cellular resistance to stress. Longo et al. [99] also introduced the concept of programmed altruistic aging, stating that at the group level aging evolved as a form of lifespan management to benefit the individuals within a particular group. The requirements that the authors list in order to check the validity of this theory are "identification of mutations

Naked mole-rat data and whether or not they appear to support popular theories of aging

Aging theory	Hypothesis	Evidence from naked mole-rats	Support
Antagonistic pleitropy	Genes that may be beneficial earlier in life also code for traits that may manifest as deleterious	No evidence	Maybe
Disposable soma	Energy is allocated to body maintenance of reproduction	Breeders and non0breeders have similar lifespan although breeding females double their mass, have large litters and breed until death	No
Mutation accumulation	Deleterious mutations accumulate with age causing a decline in form and function	No neoplasm; attenuated decline in physiological systems with age	No
Oxidative stress (modified from free radical)	Reactive oxygen species (or free radicals) bombard the cells causing damage	High steady state levels of ixidative stress/damage; no increase in these levels with age	No/maybe
Prigrammed longevity	Species evolved a genetically programmed (stable) balance between metabolic activities and lifespan	Regardless of their reproductive status, all individuals share similar lifespan	No/maybe
Programmed and altruistic aging)	Aging benefits group< but not necessarily the organism	Unique eusosial structure of colony (1 breeding female and 1–3 breeding ,ales). High levels of naturally-occurring inbreeding	Maybe

that can significantly increase lifespan, similarities between normal aging and mammalian apoptosis, evidence for the benefit that is provided by the aging program, identification of a sequence of molecular processes that are required to cause aging and death, and the demonstration that the program occurs both under conditions that mimic those encountered in natural environments and in organisms that are isolated from natural environments" [99]. The majority of these requirements have not been studied; as to date no interventions for lifespan manipulation have been done (though some important pathways which may regulate lifespan are currently under study). A strictly deterministic genetic aging program is unlikely for several reasons: (1) such a rigid program would be maladaptive to an negatively affected individual, making it impossible to undergo natural Darwinian selective pressures; (2) unlike development, which is a very precisely integrated genetic program, there are several fold variances in the life spans of among individuals belonging to a uniformly genetic cohort maintained in identical environments; (3) mutations which disrupt a pro-aging program would result in immortal individuals, an observation that has not been recorded outside of the realms of pure science fiction; (4) the evolutionary argument that the vast majority of individuals in a population do not live long enough to "age" does not support the concept of programmed aging. Natural selection acts only on allele carriers that improve the reproductive fitness of an individual thus increasing the likelihood that the gene will be passed on to progeny. If only a small fraction of a wild population survives to an age where the hypothetical aging program becomes active or limiting, then such a program would experience weak selective pressure and be unlikely to prevent the loss of genes through random mutations. Thus a deterministic aging program is unlikely to have evolved in a natural population subject to selective pressures of a harsh, unprotected environment. Nevertheless, the genome of an organism may play a pivotal role in aging, influencing the efficacy of the cellular mechanisms available for combating stochastic causes of damage accrual. Possibly a species specific biological clock or master regulator influences cellular resistance to stress and controls cellular homeostasis through repair and removal of damaged components. This biological clock may gradually become less effective with age, thus resulting in less efficient repair and maintenance and a generalized deterioration of the soma. The challenge ahead is to determine the elements of commonality among long-lived species that differ from

those of shorter-lived species and assess if there are agents that may emulate these so as to simultaneously enhance somatic maintenance and stress resistance and thereby affect the multiple and diverse features associated with aging.

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