

Hypothesis: Interventions That Increase the Response to Stress Offer the Potential for Effective Life Prolongation and Increased Health

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*In the last decade it has become evident that many laboratory manipulations, both genetic and environmental, can lead to significant life extension. All or almost all of the observed life-extension phenotypes are associated with increased resistance and/or ability to respond to environmental stress. These observations show dramatically that life span is not maximized. We suggest that latent within many species — perhaps even humans — is the ability for large increases of life expectancy. The striking correlation between the increased stress resistance of all long-lived mutants in *C. elegans* and other species and the increased resistance of dietary restricted rodents to environmental toxins is consistent with an evolutionary conservation of a life-span maintenance/environmental stress resistance program. We suggest that it may be possible to develop methods for life extension in mammals, including humans, using relatively straightforward manipulations, such as drug treatments. It should be obvious that these findings have tremendous implications for human society at large, and we suggest that the implications of these findings should be explored.*

A REVOLUTION in our outlook on the aging process has occurred in the laboratory in the last decade, but that revolution remains to be appreciated outside of the relatively small group of researchers working in the field. Simply put, the findings are these: many laboratory manipulations, both genetic and environmental, can lead to significant life extension.

In this article we will first describe the interventions that have been successful in leading to extended life expectancy in the laboratory. Second, we will elucidate the fact that all the observed life-extension phenotypes are associated with increased resistance and/or ability to respond to environmental stress. Third, we will suggest that these interventions show dramatically, as suggested by the evolutionary theory of aging, that life span is not maximized. The crucial suggestion is that many species have a latent capability for increased life span that can be induced by relatively simple genetic or environmental manipulations. These observations have tremendous implications for our continued desire to increase human life and health. Most important is the concept that this capability for increased life span is latent within most, if not all, species. We suggest that numerous forms of exogenous treatments can be developed that will lead to significant life extension and increased health without heroic biomedical interventions.

The observations on which this hypothesis is based are simple. Organisms have been manipulated into achieving longer than normal life spans using several relatively straightforward interventions. (a) Generally known and recognized is the fact that dietary restriction (DR, the limitation of caloric intake while still maintaining a high nutrition diet) has been demonstrated to lead to life extensions in numerous rodent

studies and may be efficacious in primates, as well. (b) Genetic alterations leading to longer life have been demonstrated in yeast, the round worm, and the fruit fly, but have not yet been directly demonstrated in laboratory vertebrates (Johnson et al., 1996; Martin et al., 1996). Especially surprising are the findings that manipulation of one or two genes can double or even quadruple life expectancy (Jazwinski, 1996; Lithgow, 1996). (c) Exposure to low levels of toxic agents extends life span and leads to increased resistance to the toxin in numerous instances; this phenomenon is known as "hormesis" (Neafsey, 1990; Polycove, 1995).

Genetic Interventions Leading to Extended Life Span

The companion article by Masoro and Austad (1996) clearly describes the results for life extension mediated by DR, and that phenomenon will not be further reviewed here. We will instead focus on the genetic interventions that have been found to lead to life extension which can all be described as being due to increased stress resistance. There are now five examples of single-gene mutants in the round worm that lead to life extension of up to twofold. Mutants in *age-1* were the first to be identified (Klass, 1983; Friedman and Johnson, 1988). Until recently the mode of action of this gene was unclear, but it now seems likely that *age-1*, as well as *daf-2* and *daf-23*, two other gerontogenes that lead to extended life (Kenyon et al., 1993; Larsen et al., 1995), are in the same signal-transduction pathway. The Daf genes control the formation of dauer larvae (an alternative, long-lived developmental form of the nematode; Riddle, 1988). Mutants in two other gerontogenes — *spe-26* (Van Voorhies, 1992) and *clk-1* (Wong et al., 1995) — have been hypothesized to act through reduced fertility and an altered

biological clock, respectively. The life extension phenotype of mutants in all five genes is suppressed by *daf-16* (Murakami and Johnson, 1996), which again ties these genes into a general signal transduction pathway. Single-gene mutants have also been recovered in fungi that lead to increased survival of vegetative cells in both *Neurospora* (Munkres and Furtek, 1984) and in yeast, where mutations in any of four genes lead to a 30 to 50% extension of replicative ability (D'mello et al., 1994; Sun et al., 1994; Kennedy et al., 1995) and survival under nongrowing conditions (Kennedy et al., 1995). In the fruit fly, selective breeding in the laboratory has been shown to lead to life-span prolongation (Luckinbill et al., 1984; Rose, 1984; Partridge and Fowler, 1992; Zwaan et al., 1995). Genetic manipulation of superoxide dismutase and catalase levels has also been shown to lead to life extension (Fleming et al., 1992; Orr and Sohal, 1994).

Hormesis

Finally, low levels of environmental stress have been shown to increase life span. In the nematode, short exposure to either ionizing radiation (Johnson and Hartman, 1988) or elevated temperature (Lithgow et al., 1995) has been shown to lead to a modest but statistically very significant and very replicable life extension. Similarly, in *Drosophila*, short treatment at an elevated temperature (Maynard Smith, 1958; Khazaeli et al., in press) also leads to decreased mortality in the surviving population. These latter three observations are examples from the literature on life span of a phenomenon known as hormesis, in which low levels of a toxic substance (in these examples either radiation or heat) lead to increased subsequent survival capability. These hormesis effects on life span have been associated with an increased ability to withstand the toxin initiating the hormesis. The concept that small amounts of a toxic agent can lead to beneficial effects, although almost heretical to environmental activists and smacking of homeopathy, has firm foundations in the pharmacological literature of many drugs: alcohol, anesthetic gases, and many vitamins, to name a few (Furst, 1987). There are hundreds of examples of radiation hormesis in the literature (see reviews by Neafsey, 1990; Fritz-Niggli, 1995; van Wyngaarden and Pauwels, 1995) in which viability is enhanced following low levels of exposure to radiation.

Observations on Stress Response

All instances of life extension, whether by genetic intervention or environmental manipulation, are associated with increased ability to respond to environmental stress and decreased susceptibility to stress-induced damage. We wish to suggest that *the ability to respond to stress is the rate-determining factor leading to aging and senescence*. Following is a brief review of the findings, many still unpublished, that lead us to this hypothesis.

Many studies show that DR rodents have elevated ability to respond to both reactive oxidant species and to heat stress (Weindruch and Walford, 1988; Aly et al., 1993; Heydari et al., 1993), and these are further reviewed by Masoro and Austad (1996) in the companion article. Altered response to stress has been long thought to be part of the problem in aged animals (Harman, 1956; Pacifici and Davies, 1991).

So far, the evidence suggests that all genetic manipulations that result in effective life extension appear to do so as a result of increased resistance and/or increased repair capability and thus display an increased survival after many forms of environmental stress. All known life-extension mutants in the nematode have increased resistance to toxic agents (Martin et al., 1996; Murakami and Johnson, 1996). Mutants of the *age-1* gerontogene have been most intensively studied and are associated with increased resistance to reactive oxygen species (ROS; Larsen, 1993; Vanfleteren, 1993), elevated temperature (Lithgow et al., 1994, 1995) and UV irradiation (Duhon et al., 1996; Murakami and Johnson, 1996). Similarly, mutants in the two dauer genes (*daf-2* and *daf-23*) are also associated with increased resistance to some or all of the stresses just mentioned (Lithgow et al., 1995; Martin et al., 1996; Murakami and Johnson, 1996). Finally, even mutants in other genes like *spe-26* and *clk-1* that are implicated in a sperm production and a cell cycle program, respectively, probably act to lengthen life as a result of their increased resistance to environmental stress, because these mutants also show resistance to all stresses that have been tested (Lithgow et al., 1995; Martin et al., 1996; Murakami and Johnson, 1996). Interestingly, not all mutant alleles in these latter two genes cause extended life, and there is a perfect correlation between allelic effects on life span and on stress resistance; i.e., all alleles that extend life also show correlated increases in stress resistance (Murakami and Johnson, 1996). With *spe-26*, especially, it is clear that the stress response is a better predictor of life extension than is the reduced fertility (Murakami and Johnson, 1996). Finally, all nematode gerontogenes studied induce both the stress response and life extension by means of another gene, *daf-16* (Murakami and Johnson, 1996), which mediates dauer development (Riddle, 1988).

The story is similar for long-lived mutants in fungi and in long-lived lines of *Drosophila* in that these stocks show increased stress resistance. Kale and Jazwinski (1996) have shown that *ras-1* mutants are more resistant to UV irradiation than is the wild type. Kennedy et al. (1995) have even used increased stress resistance as a selective regimen for identifying long-lived strains that are more resistant to heat, starvation, and ethanol. In *Neurospora*, mutants that show longer survival of conidia (vegetative spores) were identified and shown to be associated with elevated ROS scavenging capability (Munkres et al., 1984). Long-lived lines of *Drosophila* are more resistant to desiccation, starvation, ethanol (Service et al., 1985), and ROS (Dudas and Arking, 1995). Increased activity of SOD and/or catalase has also been implicated in life extension through transgenic studies (Fleming et al., 1992; Orr and Sohal, 1994).

Implications

The striking correlation between the increased stress resistance of all long-lived mutants in *C. elegans* and other species and the increased resistance of dietary restricted rodents to environmental carcinogens (Feuers et al., 1993; Manjgaladze et al., 1993), heat (Heydari et al., 1993), and reactive oxidants (Koizumi et al., 1987) is consistent with an evolutionary conservation of a life-span maintenance/environmental stress resistance program. Conservation of

gene identity and function (McCombie et al., 1992) between nematodes and mammals also leads to optimism concerning the evolutionary conservation of the physiological and molecular underpinnings of such life-span prolongation mechanisms.

Life extension can only result from altering the rate-determining process(es) that determine life span. As originally proposed by Kirkwood and Cremer (1982), life is perched at a balance point between increased repair capability and increased evolutionary fitness. We must begin the critical analysis of these mechanisms in rodents and other mammals. It seems likely that the increased life span is due in part to increased repair capability and in part to increased initial resistance. The details of the mechanisms by which the increased resistance in invertebrates occurs are being elucidated, and it is likely that several systems are being simultaneously induced (Vanfleteren, 1993; Vanfleteren and De Vreese, 1995; Lithgow et al., submitted). Much future research must be undertaken to elucidate the details of these mechanisms. The observation that resistance to environmental stress can be increased to higher levels, although surprising at first thought, makes evolutionary sense after recognizing that repair need not be maximal to ensure optimal species survival.

These observations have tremendous implications for our continued desire to increase human life and human health. Foremost is the concept that this reserve capacity for repair is latent within us. Thus, it may not take heroic biomedical interventions such as gene therapy or massive surgical interventions to stimulate natural processes in the cell and in the body to work at higher, more effective levels of resistance and/or repair. Second, manifold forms of exogenous treatment may become available that will lead to significant life extension and health increase by stimulating increased repair capability. Multiple additional forms of intervention that target repair capabilities may thus offer hope for increasing life and health. As gerontologists, we need to be ready for the next revolution in health care of the elderly in which preventive measures at early ages and stimulation of latent capabilities, rather than amelioration, will come increasingly into play. We can expect interventions into aging to result in future cohorts of increasingly fit elderly. The development of such interventions to promote a longer and healthier life span will become a larger focus of legitimate aging research in the future.

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