The Nature of Gerontogenes and Vitagenes

Antiaging Effects of Repeated Heat Shock on Human Fibroblasts

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ABSTRACT: Our survival and the physical quality of life depends upon an efficient functioning of various maintenance and repair processes. This complex network of the so-called longevity assurance processes is composed of several genes, termed vitagenes. The homeodynamic property of living systems is a function of such a vitagene network. Because aging is characterized by the failure of homeodynamics, a decreased efficiency and accuracy of the vitagene network can transmutate it into a gerontogene network. It is not clear how various components of the vitagene network operate and influence each other in a concordant or a discordant manner. Experimental strategies through which this transmutation of vitagenes into virtual gerontogenes may be elucidated include induction of molecular damage, antisense intervention, and genetic screening for varied efficiencies of the members of the vitagene family. A reversal of this approach by maintaining or recovering the activity of vitagenes will lead to a delay of aging, a decreased occurrence of age-related diseases, and a prolongation of a healthy life span.

Our survival and the physical quality of life depends upon an efficient functioning of various maintenance and repair processes. This complex network of the so-called longevity assurance processes is composed of several genes, which may be called *vitagenes*. The homeodynamic property of living systems is a function of such a vitagene network. Some of the main longevity assurance processes that constitute the vitagene network are listed in TABLE 1. These processes have to work in the presence of extrinsic and intrinsic sources of damage, such as environmental and nutritional agents, spontaneous errors of macromolecular synthesis, postsynthetic modifications of macromolecules making them inactive or abnormal, and other defects occurring during the course of normal metabolism. Apparently, this homeodynamic network of vitagenes appears to work optimally during the major part of life, allowing growth, development, differentiation, and maturation to occur. There are no obvious reasons why these maintenance, defense, and repair mechanisms could not operate forever and make an organism immortal. Yet, a progressive failure of maintenance underlines and typifies the process of aging.

Aging has many facets, and almost all the experimental data suggest that aging is an emergent, epigenetic, and meta-phenomenon, which is not controlled by a single mechanism.[1,2] Individually no tissue, organ, or system becomes functionally exhausted, even in very old organisms; yet it is their combined interaction and interdependence that determines the survival of the whole. Because it is necessary to look for genes as the ultimate controllers of all biological processes, the term *gerontogenes* has been suggested to refer

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TABLE 1. Major Components of the Homeodynamic Vitagene Network

Molecular level	Tissue and organ level
Maintenance and repair of the genome.	Neutralizing and removing toxic chemicals.
Fidelity of genetic information transfer.	Tissue regeneration and wound healing.
Turnover of macromolecules.	Cell death and cell replacement.
Stress-protein synthesis.	
Scavenging of free radicals.	
Cellular level	**Physiological level**
Maintaining the differentiated state.	Neuronal response.
Regulation of cell proliferation.	Hormonal response.
Stability of the cellular milieu (viscosity, ion balance, pH).	Immune response.
	Stress response.
Stability of the cell membranes.	Thermoregulation.

to any genetic elements that are involved in aging.[3,4] However, there is still no agreement as regards the nature of the gerontogene network and the number of genes involved in it.

GERONTOGENES—REAL OR VIRTUAL?

Evolutionary theories of aging and longevity discount the notion of the adaptive nature of aging. This is because Darwinian evolution, which works primarily through the process of natural selection for reproductive success, does not leave any margin for the selection of aging and death as an advantageous trait for the individual.[5–7] Evolutionary forces work only on life processes and do not select for death. Natural selection of what is recently termed a "selecton,"[8] is in the form of an efficient vitagene network to assure its longevity for fulfilling its purpose of life, that is, reproduction and continuation of generations. The so-called programmed death or apoptotic mechanisms are essential parts of the developmental processes (or may even be a component of the homeodynamic vitagene network) necessary for the making of a reproductively successful individual.

Once the Darwinian purpose of life is fulfilled, there is no reason (or selection pressure) left either for the maintenance of the body or for its destruction. Thus aging, as a failure of maintenance, occurs without a cause or a purpose. It just happens. Any search for genes that were selected specifically to cause aging is misdirected and ill-informed. Furthermore, the diversity of the forms and variations in which age-related alterations are manifested indicate that the progression of aging is not deterministic but stochastic in nature. An age-related increase in variability among individuals, in terms of any physiological, cellular, or biochemical parameter studied, is a reflection of the stochastic nature of aging. In the words of an anonymous poet, *We are born as copies, but we die as originals.*

Yet, aging appears to have a genetic component of some kind. The role of genes in aging is evident from an apparent practical limit to maximum life span within a species,[9,10] along with the evidence from studies on twins,[11,12] from the human genetic mutants of premature aging,[13] and from genetic linkage studies for the inheritance of life span and for genetic markers of exceptional longevity.[14] Thus, aging appears to be genetically regulated without involving any genes that may be held responsible as its cause. This paradoxical situation of the genetic aspects of aging and longevity on the one hand and the stochastic

nature of the progression of the aging phenotype can be resolved by developing radically novel views about the nature of gerontogenes.

If aging is seen as the failure of the homeodynamic vitagene network due to stochastic causes of damage and perturbations, the term gerontogenes refers to the functional transmutation of the vitagene network, due to progressive accumulation of random damage. Therefore, the term gerontogenes does not refer to a tangible physical reality of real genes for aging but refers to an altered state of vitagenes, which gives the appearance of being the genes for aging. Thus, gerontogenes are not real; they are virtual.[4] More importantly, the idea of virtual gerontogenes is in line with the evolutionary explanation of the aging process as being an emergent phenomenon caused by the absence of eternal maintenance and repair instead of being an active process caused by evolutionary adaptation.

Obviously, not every gene is potentially a virtual gerontogene, although, of about 100,000 genes in the human genome, potentially every gene can affect the survival of an organism. Therefore, a distinction must be made between immediate survival or death on the one hand and the process of aging on the other. The inactivation of any essential gene involved in fundamental metabolic processes will result in the death of an organism without having anything to do with the process of aging. This situation is similar to the toxic effects of various chemicals that may result in the immediate or somewhat delayed death of an organism without affecting its rate of aging, as determined by well-established parameters of survival and mortality kinetics.[9,15] Similarly, a single gene mutation may give rise to an apparently accelerated-aging phenotype without qualifying as being the gene that causes aging.[13,16] Therefore, the set of possible virtual gerontogenes can be narrowed down to those genes that fit those criteria of survival kinetics. In this sense, the sets of vitagenes involved in the maintenance and repair of the cellular and subcellular components best qualify as the candidates to become virtual gerontogenes.

Evidence for the hypothesis that candidate virtual gerontogenes operate through the vitagene network comes from experiments performed to retard aging and to increase the life span of organisms. For example, antiaging and life-prolonging effects of calorie restriction are seen to be accompanied by the stimulation of various maintenance mechanisms. These include increased efficiency of DNA repair, increased fidelity of genetic information transfer, more efficient protein synthesis, more efficient protein degradation, more effective cell replacement and regeneration, improved cellular responsiveness, fortification of the immune system, and enhanced protection from free radical– and oxidation-induced damage.[17,18] Similarly, antiaging effects of a wide variety of hormones, vitamins, a dipeptide carnosine,[19] modified amino acids, and nucleic acid bases on human cells, rodents, and insects[2,20–22] are also mainly due to the effects of these chemicals on maintaining or recovering the efficiency of vitagenes.

Genetic selection of *Drosophila* for longer life span also appears to work mainly through an increase in the efficiency of maintenance mechanisms, such as antioxidation potential.[5,23] An increase in life span of transgenic *Drosophila* containing extra copies of Cu-Zn superoxide dismutase (SOD) and catalase genes is due primarily to enhanced defenses against oxidative damage.[24] The identification of long-lived mutants of the nematode *Caenorhabditis elegans*, involving various genes, may provide other examples of virtual gerontogenes because in almost all these cases increased life span is accompanied by an increased resistance to oxidative damage, an increase in the activities of SOD and catalase enzymes, and an increase in thermotolerance.[25–28]

MODULATING THE NETWORK

Estimates of the number of genes that might qualify as being a part of the vitagene/gerontogene network of mammals run up to a few hundred out of about one hundred thousand genes, and their allelic variants. Direct gene therapy directed towards the overall aging process seems to hold little promise. This is because gene therapy for aging will require methods to improve upon the "genetic hand of cards" that determines the aging and longevity of a "genetically normal" individual.

The question of total gene therapy for aging is linked with the issue of defining what is a normal combination of genes in a so-called normal and healthy individual. It is in the very nature of genetic polymorphism and the interactive nature of the genome that each individual is unique and that the term "normal" is extremely wide ranging. Therefore, improving upon an already normal situation with respect to the genetic constitution related to total life span is a no-win situation.

Assuming that there are only 50 longevity-assuring vitagenes that constitute the network in which they interact with each other, this gives rise to 2^{50} or 10^{15} (a million billion) possibilities of their interacting and influencing each other. Not considering billions of cells in an adult, even at the level of a single cell zygote, interfering with such a complex network and improving upon what is already a normal combination for that particular individual (in the absence of any obvious genetic diseases) is a mission impossible.[29] What is more likely to be achieved in the not-so-distant future is that experimental manipulation of certain genes will fine-tune or retune the network and will prevent the onset of various age-related diseases and impairments by maintaining the efficiency of homeostatic processes.

The following lines of research can form the basis of a promising strategy to understand and modulate the aging process: (1) studying the extent of maintenance and repair of the genes involved in maintaining the stability of the nuclear and mitochondrial genome; (2) studying the efficiency of transcription of various vitagenes and posttranscriptional processing of their transcripts; (3) studying the accuracy and efficiency of translation of vitagenes and analyzing the specificity, stability, and turnover of vitagene products (vitaprins?), including their posttranslational modifications; (4) searching for natural or induced mutants (including transgenic and knockout organisms) with altered levels of maintenance and repair of the crucial vitagenes; (5) searching for age-specific and age-related disease-specific biomarkers for diagnostic purposes and for monitoring the effects of potential therapeutic agents; and (6) experimental modulation of various types of maintenance mechanisms and studying its effects on other levels, such as gene stability, gene product synthesis, and turnover. Some of the main defense processes that may provide a relatively easy access to experimentation include responsiveness to stress, efficiency of signal transduction pathways, and regulators of cell-cycle progression.

EXPERIMENTAL STUDIES ON UPREGULATING THE STRESS RESPONSE IN HUMAN FIBROBLASTS

An organism's ability to respond to stress is a major component of its homeodynamic vitagene network, and altered responsiveness is one of the most significant features of aging.[2,30] The so-called heat-shock response as an important mechanism of cellular defense is very well established.[31–33] It is also known that the extent of heat-shock

response decreases during aging.[32] Therefore, it has been hypothesized that if organisms are exposed to brief thermal treatment so that their stress response–induced gene expression is upregulated and this particular pathway of maintenance and repair is stimulated, one should observe antiaging and longevity-promoting effects. Such a phenomenon in which stimulatory responses to low doses of otherwise toxic substances improve health and enhance life span is known as hormesis.[34,35] Recently, antiaging and life-prolonging effects of heat shock have been reported for *Drosophila*[36] and the nematodes.[27,28]

I have tested the effects of mild but repetitive heat shock on various cellular and biochemical characteristics of human skin fibroblasts undergoing aging *in vitro*. I undertook a series of pilot experiments to determine suitable temperature conditions that fulfilled the following criteria: (1) the thermal treatment had no effects on immediate survival of the cells, as checked by a trypan blue exclusion test; (2) the cells responded to the thermal treatment by inducing the synthesis of major heat-shock proteins (hsp), as detected by metabolic labeling of cells with radioactive amino acids followed by SDS-polyacrylamide gel electrophoresis (PAGE); and (3) thermally treated cells could be subcultured normally without any effect on their attachment frequency.

All experiments were performed on a normal human adult female skin fibroblast line, designated ASS, which has been used previously to test for the antiaging effects of the cytokinin hormone kinetin.[20] At least three parallel cultures of control (series A1 to A3) and heat-shocked cells (series H1 to H3) were serially passaged at 1:4 or 1:2 split ratio until the end of their proliferative life span, using normal culture conditions of medium-containing antibiotics, 10% fetal calf serum, and incubation at 37°C with 95% humidity. The H-series cells were given a 30-min heat shock at 41°C by immersing the culture flasks in a fine-regulated water bath. The cultures were kept at 37°C for 60 min before changing the medium. Heat-shock treatment was repeated twice a week with following restrictions: cultures were not subcultivated within 24 h of heat shock, and heat shock was not given to newly subcultivated cultures for at least 24 hours. Growth rates, population doubling (PD) rates, cell yield, and cumulative population doubling levels (CPDL) achieved *in vitro* were determined. In addition, morphological characteristics, actin filament organization, and the senescence-specific β-galactosidase staining pattern of normal and heat-shocked cells were compared. The extent of heat-shock response in terms of hsp synthesis was also checked at various PD levels, by SDS-PAGE.

The present series of experiments have demonstrated that repetitive and mild heat shock has several positive and antiaging effects on human cells in culture. Briefly the results are summarized as follows. Human fibroblasts could be exposed to mild heat shock at 41°C repeatedly during their limited proliferative life span *in vitro* without any apparent negative effects on survival, attachment frequency, PD rates, and CPDL potential. Although the temperatures higher than this (up to 43°C) could stimulate a more intense heat-shock response, in terms of hsp synthesis, cells could not survive more than seven repeated thermal treatments. Continuous survival of human fibroblasts for 140 days, during which time they underwent about 30 PDs and received 35 repeated heat shocks at 41°C, is a novel effect not observed before (details are published elsewhere[37]).

Although there was no prolongation of the proliferative life span of human fibroblasts after repeated heat-shock treatment, several other antiaging effects were observed. Most dramatically, the age-related alteration in the morphology of cells, which is one of the most obvious changes during cellular aging, was significantly slowed down in heat-shocked cells. The control cultures showed the typical age-related increase in cell size, flattened appearance, increased morphological heterogeneity, loss of arrayed arrangement,

increased number of lysosomal residual bodies, increased number of actin filaments, and increased proportion of multinuclear cells during serial passaging. However, the heat-shocked cultures showed a highly reduced rate of these age-related alterations and maintained a relatively young morphology even at the end of their proliferative life span. These cells did not undergo significant enlargement, maintained to a large extent their spindle-shape and arrayed arrangement, did not accumulate many residual bodies, did not show many rod-like actin filaments, and had an almost complete absence of multinucleate cells. A reduced rate of cell enlargement was also evident from the analysis of cell yield per cm^2 of cell culture flasks, which was reduced from about 4×10^4 cells in young cultures to 1×10^4 cells in senescent-controlled cultures, but was maintained at a level two to three times higher (between 2.5 and 3×10^4 cells) in repeatedly heat-shocked cultures. Maintenance of young morphology and reduced cell size is a strong indication of antiaging effects of heat shock, as also observed for such antiaging treatments as carnosine and kinetin.

That there may be several other antiaging or hormesis-like effects of repeated mild heat shock on human cells is also evident from a comparison of the proportion of β-galactosidase positive cells in the culture. Recently, this marker has been suggested to be a good indicator of senescent cells in culture. Whereas more than 95% of cells in a high-passage control culture at the end of its proliferative life span were β-galactosidae positive, less than 5% of cells in heat-shocked cultures were detectable by this marker. We are now investigating what other cellular, physiological, biochemical, and molecular effects of repeated heat shock occur in human cells. Some of the characteristics to be tested are the rates and extent of transcription and translation of various genes, the extent of gene-specific and total DNA repair, including telomere length, the extent and rates of protein synthesis and degradation, and the accumulation of molecular damage, such as oxidative damage in DNA, lipid-peroxidation products, and abnormal proteins.

Most importantly, the above experiments show that it is possible to retune the vitagene network in such a way that its transmutation to a virtual gerontogene network is slowed down. Due to the highly complex nature of interactions within the vitagene network, it is most unlikely that a complete transmutation of vitagenes into gerontogenes can ever be prevented. Furthermore, retuning one or more vitagenes may or may not have significant effects on the final outcome in terms of reducing the rates of aging and increasing the life span. However, what can definitely be achieved by this approach is that increased levels and/or efficiency of one or more maintenance and repair pathways will have positive effects in terms of improving the physical quality of life and increasing its chances of survival, and ultimately, achieving a healthy old age.

REFERENCES

1. Holliday, R. 1995. Understanding Ageing. Cambridge University Press. Cambridge.
2. Rattan, S.I.S. 1995. Ageing—a biological perspective. Mol. Aspects Med. **16:** 439–508.
3. Rattan, S.I.S. 1985. Beyond the present crisis in gerontology. BioEssays **2:** 226–228.
4. Rattan, S.I.S. 1995. Gerontogenes: Real or virtual? FASEB J. **9:** 284–286.
5. Rose, M.R. 1991. Evolutionary Biology of Aging. Oxford University Press. New York.
6. Kirkwood, T.B.L. 1992. Biological origins of ageing. *In* Oxford Textbook of Geriatric Medicine. J.G. Evans & T.F. Williams, Eds.: 35–40. Oxford University Press. Oxford.
7. Charlesworth, B. & K.A. Hughes. 1996. Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence. Proc. Natl. Acad. Sci. USA **93:** 6140–6145.
8. Mayr, E. 1997. The object of selection. Proc. Natl. Acad. Sci. USA **94:** 2091–2094.
9. Finch, C.E. & M.C. Pike. 1996. Maximum life span predictions from the Gompertz mortality model. J. Gerontol. Biol. Sci. **51A:** B183–B194.

10. JOHNSON, T.E. 1997. Genetic influences on aging. Exp. Gerontol. **32:** 11–22.
11. MCGUE, M., J.W. VAUPEL, N. HOLM & B. HARVALD. 1993. Longevity is moderately heritable in a sample of Danish twins born 1870–1880. J. Gerontol. **48:** B237–B244.
12. YASHIN, A.I. & I. IACHINE. 1995. How long can humans live? Lower bound for biological limit of human longevity calculated from Danish twin data using correlated frailty model. Mech. Ageing Dev. **80:** 147–169.
13. MARTIN, G.M. 1985. Genetics and aging: The Werner syndrome as a segmented progeroid syndrome. Adv. Exp. Med. Biol. **190:** 161–170.
14. JAZWINSKI, S.M. 1996. Longevity, genes, and aging. Science **273:** 54–59.
15. PIANTANELLI, L., A. BASSO & G. ROSSOLINI. 1994. Modelling the link between aging rate and mortality rate. Ann. N.Y. Acad. Sci. **719:** 136–145.
16. YU, C.-E. *et al.* 1996. Positional cloning of the Werner's syndrome gene. Science **272:** 258–262.
17. MASORO, E.J. 1995. Dietary restriction. Exp. Gerontol. **30:** 291–298.
18. MASORO, E.J. & S.N. AUSTAD. 1996. The evolution of the antiaging action of dietary restriction: A hypothesis. J. Gerontol. Biol. Sci. **51A:** B387–B391.
19. MCFARLAND, G.A. & R. HOLLIDAY. 1994. Retardation of the senescence of cultured human diploid fibroblasts by carnosine. Exp. Cell Res. **212:** 167–175.
20. RATTAN, S.I.S. & B.F.C. CLARK. 1994. Kinetin delays the onset of ageing characteristics in human fibroblasts. Biochem. Biophys. Res. Commun. **201:** 665–672.
21. SHARMA, S.P., P. KAUR & S.I.S. RATTAN. 1995. Plant growth hormone kinetin delays ageing, prolongs the life span and slows down development of the fruitfly *Zaprionus paravittiger.* Biochem. Biophys. Res. Commun. **216:** 1067–1071.
22. SHARMA, S.P., J. KAUR & S.I.S. RATTAN. 1997. Increased longevity of kinetin-fed *Zaprionus* fruitflies is accompanied by their reduced fecundity and enhanced catalase activity. Biochem. Mol. Biol. Int. **41:** 869–875.
23. LUCKINBILL, L.S. 1993. Prospective and retrospective tests of evolutionary theories of senescence. Arch. Gerontol. Geriatr. **16:** 17–32.
24. ORR, W.C. & R.S. SOHAL. 1994. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster.* Science **263:** 1128–1130.
25. LAKOWSKI, B. & S. HEKIMI. 1996. Determination of life-span in *Caenorhabditis elegans* by four clock genes. Science **272:** 1010–1013.
26. LARSEN, P.L. 1993. Aging and resistance to oxidative damage in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **90:** 8905–8909.
27. LITHGOW, G.J., T.M. WHITE, S. MELOV & T.E. JOHNSON. 1995. Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. Proc. Natl. Acad. Sci. USA **92:** 7540–7544.
28. LITHGOW, G.J. 1996. Invertebrate gerontology: The age mutations of *Caenorhabditis elegans.* BioEssays **18:** 809–815.
29. RATTAN, S.I.S. 1997. Gene therapy for ageing: Mission impossible? Eur. J. Genet. Soc. **3**. 27–29.
30. RATTAN, S.I.S. & A. DERVENTZI. 1991. Altered cellular responsiveness during ageing. BioEssays **13:** 601–606.
31. HAYES, S.A. & J.F. DICE. 1996. Roles of molecular chaperones in protein degradation. J. Cell Biol. **132:** 255–258.
32. HOLBROOK, N.J. & R. UDELSMAN. 1994. Heat shock protein gene expression in response to physiologic stress and aging. *In* The Biology of Heat Shock Proteins and Molecular Chaperones. R.I. Morimoto, A. Tissières & C. Georgopoulos, Eds.: 577–593. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.
33. JINDAL, S. 1996. Heat shock proteins: Applications in health and disease. Trends Biotechnol. **14:** 17–20.
34. NEAFSEY, P.J. 1990. Longevity hormesis: A review. Mech. Ageing Dev. **51:** 1–31.
35. POLLYCOVE, M. 1995. The issue of the decade: Hormesis. Eur. J. Nucl. Med. **22:** 399–401.
36. KHAZAELI, A.A., M. TATAR, S.D. PLETCHER & J.W. CURTSINGER. 1997. Heat-induced longevity extension in *Drosophila*. I. Heat treatment, mortality, and thermotolerance. J. Gerontol. Biol. Sci. **52A:** B48–B52.
37. RATTAN, S.I.S. 1998. Repeated mild heat shock delays ageing in cultured human skin fibroblasts. Biochem. Mol. Biol. Int. **45:** 753–759.