# Prospects for postponing human aging

MICHAEL R. ROSE[1] AND THEODORE J. NUSBAUM
Department of Ecology and Evolutionary Biology, School of Biological Sciences, University of California, Irvine, California 92717, USA

ABSTRACT **The postponement of human aging is a long-standing aspiration in many cultures, particularly non-Western ones. Renewed interest in this goal is now being shown within the biomedical research context. This interest has probably arisen because several different approaches have yielded dramatic increases in mean and maximum life span in laboratory organisms. Among the cases where aging has been postponed in animal models are the nematode, *Caenorhabditis elegans,* the common lab fruit fly, *Drosophila melanogaster,* and the dietarily restricted rodent, both *Mus* and *Rattus.* These successes raise the question of whether similar postponement of aging might be achieved in humans. Among the broad research strategies available, five can be delineated: *1)* random testing of favorite interventions; *2)* searching for genes that can postpone aging among all or most species; *3)* study of in vitro mammalian cell cultures; *4)* study of dietarily restricted mammals; and *5)* selecting mammals for postponed aging. Although all these methods could conceivably lead to the postponement of human aging, they are very different from each other with respect to their degree of uncertainty, cost, and delay.— Rose, M. R., Nusbaum, T. J. Prospects for postponing human aging. *FASEB J.* 8: 925–928; 1994.**

*Key Words: genetics of senescence* • Drosophila • *nematode* • *genetic engineering*

ATTEMPTS TO POSTPONE HUMAN AGING ARE common among human civilizations, reaching far back into history. This aspiration has also been a persistent feature of Western thought, among both alchemists and scientists. More striking is the role of postponed aging in non-Western cultures, particularly in Taoist thought. Until modern times, however, it has not been clear that scientists could postpone aging at all, despite some success in modulating animal life spans by environmental means. Since the 1980s, this situation has changed radically. The genetic postponement of aging has been achieved among some invertebrates, and rodent aging has been postponed by reduction of adult caloric intake. Therefore, we can now legitimately address the question of the postponement of human aging in a practical manner.

## POSTPONED AGING IN *DROSOPHILA* AND *CAENORHABDITIS*

The animals that play the leading role in the genetic postponement of biological aging are *Drosophila melanogaster* and *Caenorhabditis elegans,* the common laboratory fruit fly and nematode, respectively. This will not be surprising for anyone who knows the history of 20th century biology. The *Drosophila* work came first, but we will discuss the nematode research initially.

Nematodes have had their aging postponed primarily by mutagenesis, but also by the creation and selection of recombinant inbred lines (1–3). This has been possible because nematodes normally are self-fertilizing, and therefore are both homozygous and free of most recessive, deleterious alleles. Thus they can be screened in large numbers, either as mutant stocks or as recombinant inbred lines, without inbreeding depression (3). The absence of inbreeding depression is particularly important for the study of longevity and related characters, because these characters are substantially impaired by inbreeding in other species. The best-known longer-lived stocks are those of the *age-1* mutant (e.g., ref 4; see also refs 5, 6 for other mutants that extend life span).

The *Drosophila* work proceeded along entirely different lines because fruit flies are outbreeding, and thus are sensitive to inbreeding depression, especially where characteristics such as longevity are concerned (7). Instead of mutagenesis or the like, fruit fly stocks with postponed aging have been created by selection for later fertility in several different labs (8–11). At present, the longest-lived lines have had their mean and maximum longevities increased by about twofold. Associated with these increases in survival are a number of physiological or functional enhancements, e.g., increased later fecundity, increased stress resistance, increased locomotor capacity at later ages (12). Accordingly, there is little reason to doubt that these fruit flies have postponed aging.

## FROM FLY TO HUMAN: FIVE STRATEGIES

The fruit fly and nematode work makes it clear that aging can be dramatically postponed by different types of genetic intervention. This, in turn, has the implication that human aging might eventually be postponed by the determined application of some combination of biomedical research strategies. Five major strategies can be delimited, strategies that probably subsume almost all the best prospects for progress. These strategies are: *1)* random testing of favorite interventions; *2)* searching for genes that can postpone aging among all or most species; *3)* study of in vitro mammalian cell cultures; *4)* study of dietarily restricted mammals; and *5)* selecting mammals for postponed aging. Each of these will be discussed in turn.

### 1. Random testing

Most molecular and cell biologists have their favorite genes or mechanisms they propose to control the aging process. Some favorites have been annihilated by decisive tests, such as the error catastrophe theory of aging, which is now about as dead as any such mechanism (13). Other mechanisms of

[1]To whom correspondence and requests for reprints should be addressed.

this kind have received growing experimental support, one example being the free radical theory of aging (14, 15).

What is to be done with such findings, where postponing human aging is concerned? One approach would be to test various drugs or other interventions that are generally related to the favored aging mechanism. Such testing might begin directly with humans, as illustrated by the now-commonplace use of antioxidant substances as anti-aging substances. Another approach would be to test such favored substances first in an infrahuman mammalian system, an example being the spin-trap compound, phenyl-butyl nitrone (PBN), which appears to rejuvenate the nervous system of tested rodents (16).

The problem that such immediate testing raises is that it is not clear that any of these substances is likely to have an overall effect that is beneficial. A particular substance may indeed enhance free radical detoxification, but have more severe secondary effects that make it, on balance, life-shortening.

The piecemeal use of one or another substance may well work. We may happen upon substances that have great benefits. But this entire approach is essentially adventitious, rather than systematic.

### 2. Seeking universal genes

An approach that promises great experimental power, at first sight, is the search for genes that postpone aging in non-mammalian model systems. The ease with which such genes can be identified in systems like yeast (17), nematodes (18), or *Drosophila* (19), compared with any mammalian model, argues for their use in the identification of genes that can postpone aging across most animals.

Note the sleight of hand in this conclusion. It is far more feasible to identify genes that postpone aging in simple invertebrate systems. But there is no guarantee that the practically universal "aging genes" will exist. It might seem as if this cautionary note is overdrawn, given the great phylogenetic conservation of numerous important loci, from Cu,Zn superoxide dismutase to EF-1$\alpha$, and the fact that just such loci have been implicated in the control of aging in *Drosophila* (20). Yet the mere retention of a locus does not ensure that *all* its functions are retained. Moreover, it does not ensure that alleles of similar effect on a protein's biochemistry will then have a similar effect on the whole organism's physiology. As aging is ultimately an attribute of the whole organism, the extrapolation from one system's genetically postponed aging to another phylogenetically distant system will be laden with material uncertainties.

However, there are reasons for hoping that some loci that postpone aging in, say, *Drosophila,* could also have the same effect in mammals, including humans. Thus the universal genes approach involves an additional, and useful, step beyond the random testing approach. With the universal genes strategy, there is at least screening in a model organism. This should greatly reduce the total number of candidate genes that need to be tested.

### 3. In vitro mammalian cells

For two decades, the only well-attested, generally important experimental system in aging research was in vitro culture of mammalian cells. Hayflick (21, 22) showed that all normal mammalian cell cultures have limited proliferative capacity. For 30 years investigators have studied this system with a view to connecting it to whole organism aging. In that time, the best connection that has been provided is between the number of culture doublings and maximum species life span (13). Unfortunately, that correlation is apparently confounded with body size, numerically, because the longer-lived species are also the largest species. Larger species may be expected to require more cell division for organismal development, growth, and tissue replenishment. Thus all evidence linking cell proliferation limits to life span is uncertain. After decades of work, it remains unclear whether in vitro culture proliferation limits are related to whole organism aging.

The obvious starting point that might be used to connect in vitro research to the postponement of human aging is the discovery of the controls of in vitro proliferation limits. Recently there have been breakthroughs connecting the loss of telomeric sequence in vitro to the proliferation limits (23). Assuming, for the sake of discussion, the validity of this mechanism as the control for cell proliferation, the field is still left with the missing connection to whole organism aging. Furthermore, if cells are not restricted in their proliferation, then they may be at greater risk of becoming tumorous. Thus, interventions that enhance in vitro proliferation may be of little net value for postponing organismal aging.

Overall, it seems as if strategies based on in vitro cell systems face severe problems in their application to postponing human aging. One basic feature of such research that limits any claim to relevance to postponing human aging is that its findings have never led to the postponement of organismal aging in any system, human or otherwise. This is an appreciable contrast to strategies two and four, both of which have involved the postponement of organismal aging.

### 4. Dietary restriction of mammals

Before the 1980s, it was known that reduced nutrition in rodents would prolong development and total life span, usually in conjunction with reduced fertility (e.g., ref 24). These results were ambiguous with respect to their relevance to aging per se, in that they may have primarily reflected developmental "stretching," whereby the juvenile phase is prolonged but aging is not postponed. However, beginning in the 1980s, it was shown that reduced nutrition confined to the adult stage could significantly postpone rodent aging (25). By a wide variety of measures and using a variety of stocks, it has been established that moderate nutritional restriction under germ-free conditions can significantly increase mean life span, maximum life span, and a range of physiological functions (25). These findings are among the most important ever obtained in gerontology, because they constitute some of the best evidence available concerning the postponement of aging in mammals. Clearly, mammalian aging can be postponed, too.

There remain, however, many problems of interpretation where this result is concerned. A variety of hypotheses have been put forward concerning the functional interpretation of the dietary restriction effects (26). One interpretation is that normal laboratory feeding of rodents is overfeeding, compared with rodent feeding in the wild, so that the improvement in rodent life span is a result of being spared a pathological environment (25). Against this view is the fact that environmental modulation of invertebrate life span and aging is ubiquitous (13, 27, 28). Thus, such modulation is probably a result of general selection pressures for varying reproductive and survival attributes in the face of a varying nutritional environment. The problem where humans, in particular, are concerned is that we may have had control of our evolutionary environment to such an extent that we have lost the kind of response exhibited by rodents. That is, whereas rodents may be able to postpone their aging in response to dietary restriction under certain limited condi-

tions, humans may have evolutionarily lost the mechanisms that underlie this particular response. We may not have the same nutritional controls upon aging that rodents possess. If not, then this research strategy will not lead to successful interventions in human aging.

On the other hand, even with such an unpromising assumption it may still be possible for dietary research with rodents to reveal prospects for postponing human aging. The key would be the mediating mechanisms that connect the rodent dietary controls to the specific aging processes that are postponed. It is possible that those mechanisms could be triggered in humans by interventions other than dietary restriction, even if in rodents such mechanisms are uncovered by dietary intervention.

### 5. Selecting on aging in mammalian models

Unfortunately, we are not phylogenetically close to fruit flies or nematodes, so that extrapolation from the molecular biology or physiology of genetically postponed aging in those organisms may not be a reliable guide to the postponement of aging in humans. That is, genetic information concerning the postponement of aging in invertebrates may not provide any information immediately useful for achieving the goal in humans.

Thus we may need to genetically postpone aging in mammalian model systems. The problem is how. It will not be possible to postpone aging in mice or the like using the methods applied to the nematode, *C. elegans*. Mice, too, are subject to inbreeding depression along with most other mammals. It is the lack of such inbreeding depression that has been the key to successful screening for postponed aging in mutated or recombinant *C. elegans* (3). But it would be possible to apply the same approaches developed with *Drosophila:* selection followed by physiological and molecular analysis of postponed aging in lines with substantially postponed aging (e.g., ref 28).

Briefly, this kind of research would begin with outbred populations of mice. Mice are the system of choice because they are the best-known mammals from the standpoint of experimental genetics. These populations would then be subjected to selection for postponed aging. The use of typical inbred mouse lines would be pointless, due to their lack of genetic variance with which to obtain a response to selection. Hybrid stocks of mice also would not be useful because they would be subject to pronounced linkage disequilibrium, making genetic analysis of such stocks extremely difficult. Several different selection protocols will accomplish this end. Once mice with significantly postponed aging have been obtained, they will be excellent material for comparison with normal mice. One important aspect of this strategy is the extensive replication of control and selected lines. Only with replication will it be possible to distinguish differentiation that is specifically associated with the response to selection as opposed to differentiation caused by accidental drift.

## COMPARISON OF THE ALTERNATIVES

Nothing is more appealing than the prospect of picking some favorite substance or gene and using it to postpone human aging immediately. However, humans have been trying out such substances since far back in historical time, for two millennia and more. Modern biology, with all its reductionist power, has yet to produce substances that significantly postpone human aging. Clearly, the problem is not easily solved. Although it may be that the random choice of some favored intervention will occasionally postpone human aging, this does not constitute a very powerful strategy.

The universal genes strategy is one step better, in that it is based on loci that have shown efficacy in the postponement of aging in model systems. But the strategy is somewhat weakened by the lack of certainty about the very assumption of phylogenetic universality that underlies the entire approach. It is often the case that as phylogenetic distance increases, homology of sequence and function decreases, and thus we expect diminished biomedical significance from systems that are phylogenetically more distant. Though not a powerful approach in principle, starting from other genetic systems will probably yield some successful interventions—more so than random testing in all likelihood.

The in vitro cell culture approach has little scientific claim to credibility as a means of postponing human aging. It may, of course, serendipitously yield some interventions, but for now it seems similar to the random testing approach in strategic soundness.

Dietary restriction research may not apply to humans quite as straightforwardly as some would hope. However, it may have potential as an indirect means of probing some mechanisms that can effect mammalian postponed aging. Despite being a well-established field of investigation, this research strategy is associated with a great deal of uncertainty. We do not even know whether humans have the capacity to respond to dietary restriction with postponed aging.

Selection for rodents with postponed aging would cast a broad net with respect to the mechanisms that control mammalian aging. It is the only systematic approach to aging research so far proposed; that is, it does not require serendipity. Modern molecular techniques can be used to screen large numbers of loci to identify genetic differences between postponed-aging mice and their controls, in contrast to other strategies that might identify a single locus. Furthermore, to the extent that the behavior of the products of these loci is understood, particular physiological systems will be implicated in postponing aging. The creation of postponed-aging lines and their controls also enhances the prospects for identifying physiological differences underlying postponed aging, independently of genetic studies, simply because there is a standard of comparison. Moreover, there will be far fewer problems of failed homology than with the universal genes strategy.

A major problem facing this strategy is the time required to produce such rodents, at least with the typical selection protocols (28). A key to advancing this strategy will lie in the development of more rapid selection procedures. However, selecting on large numbers of replicate populations mitigates this problem to some extent because of the concomitant increase in the power of statistical inference under such conditions; even small differences produced by the initial rounds of selection can be distinguished statistically. An additional problem of this strategy is the sheer cost of selecting on large numbers of rodents. Oddly enough, given the many physiological and genetic differences that could be revealed by selection on rodents, this will be the most cost-effective strategy, compared with strategies that are essentially adventitious. This is not to say that this is an inexpensive strategy, only that it is the cheapest available. Although it is true that there will be major technical problems analyzing the specific genetic and physiological mechanisms that give rise to rodent aging, such as the difficulty of identifying genes that are differentiated in longer-lived lines and discovering their physiological role, it is the best possible system in its combination of experimental power and phylogenetic proximity. It

would be ideal to have the experimental power of yeast genetics in an organism as close to humans as the chimpanzee, but that will never be the case. In any case, once rodents with selectively postponed aging are created, their scientific and commercial value will be substantial.

In conclusion, it seems as if strategies two, four, and five each has merit. Significantly, these are all strategies that use whole organisms, and have a strong genetic or physiological component. By itself, selection on rodent aging is the most powerful in terms of being able to identify many genes and physiological processes involved in normal and postponed mammalian aging. Combined, particularly by coupling *Drosophila* and rodent physiological genetics, these three strategies offer humankind the best hope of significantly postponed aging. FJ

Our research on aging is supported by grants from the National Institute on Aging, US-PHS AG06346 and US-PHS AG09970.

## REFERENCES


1. Johnson, T. E. (1987) Aging can be genetically dissected into component processes using long-lived lines of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **84**, 3771-3781
2. Johnson, T. E. (1990) The increased life span in *Caenorhabditis elegans* results from lowering the Gompertz rate of aging. *Science* **249**, 908-912
3. Johnson, T. E., and Wood, W. B. (1982) Genetic analysis of life span in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **79**, 6603-6607
4. Friedman, D. B., and Johnson, T. E. (1988) A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* **118**, 75-86
5. Van Voorhies, W. A. (1992) Production of sperm reduces nematode lifespan. *Nature (London)* **360**, 456-458
6. Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtlang, R. (1993) A C. elegans mutant that lives twice as long as wild type. *Nature (London)* **366**, 461-464
7. Clarke, J. M., and Smith, J. M. (1955) The genetics and cytology of *Drosophila subobscura*. XI. Hybrid vigor and longevity. *J. Genet.* **53**, 72-80
8. Rose, M., and Charlesworth, B. (1980) A test of evolutionary theories of senescence. *Nature (London)* **287**, 141-142
9. Rose, M. R. (1984) Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* **38**, 1004-1010
10. Luckinbill, L. S., Arking, R., Clare, M. J., Cirocco, W. C., and Buck, S. A. (1985) Selection for delayed senescence in *Drosophila melanogaster*. *Evolution* **38**, 996-1003
11. Partridge, L., and Fowler, K. (1992) Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Evolution* **46**, 76-91
12. Rose, M. R. (1989) Genetics of increased life span in *Drosophila*. *Bioessays* **11**, 132-135
13. Rose, M. R. (1991) *Evolutionary Biology of Aging*, Oxford University Press, Oxford
14. Harman, D. (1956) Aging—a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298-300
15. Tyler, R. H., Brar, H., Singh, M., Latorre, A., Graves, J. L., Mueller, L. D., Rose, M. R., and Ayala, F. J. (1993) The effects of superoxide dismutase alleles on aging in *Drosophila*. *Genetica* **91**, 143-149
16. Floyd, R. (1991) Oxidative damage to behavior during aging. *Science* **254**, 1597
17. Jazwinski, M. S. (1993) The genetics of aging in the yeast *Saccharomyces cervisiae*. *Genetica* **91**, 35-51
18. Johnson, T. E., Tedesco, P. M., and Lithgow, G. J. (1993) Comparing mutants, selective breeding, and transgenics in the dissection of aging processes of *Caenorhabditis elegans*. *Genetica* **91**, 65-77
19. Fleming, J. E., Spicer, G. S., Garrison, R. C., and Rose, M. R. (1993) Two-dimensional protein electrophoretic analysis of postponed aging in *Drosophila melanogaster*. *Genetica* **91**, 183-198
20. Nusbaum, T. J., and Rose, M. R. (1994) Aging in *Drosophila*. *Comp. Biochem. Physiol.* In press
21. Hayflick, L., and Moorhead, P. S. (1961) The serial cultivation of human diploid cell strains. *Exp. Cell. Res.* **25**, 585-621
22. Hayflick, L. (1965) The limited in vitro lifetime of human diploid cell strains. *Exp. Cell. Res.* **327**, 614-636
23. Harley, C. B., Futcher, B. A., and Greider, C. W. (1990) Telomeres shorten during ageing of human fibroblasts. *Nature (London)* **345**, 458-460
24. McCay, C. M., Pope, F., and Lunsford, W. (1956) Experimental prolongation of the life span. *Bull. N. Y. Acad. Med.* **32**, 91-101
25. Masoro, E. J. (1988) Food restriction in rodents: an evaluation of its role in the study of aging. *J. Gerontol.* **43**, B59-B64
26. Graves, J. L. (1993) The costs of reproduction and dietary restriction: parallels between insects and mammals. *Growth Dev. Aging* **57**, 233-249
27. Comfort, A. (1979) *The Biology of Senescence*, 3rd Ed, Churchill Livingston, Edinburgh
28. Rose, M. R. (1990) Should mice be selected for postponed aging? A workshop summary. *Growth Dev. Aging* **54**, 7-17