ELSEVIER

Experimental Gerontology 36 (2001) 1035–1047

Experimental Gerontology

www.elsevier.nl/locate/expgero

St. Petersburg Proceedings

Critical assessment of paradigms in aging research

Harriet Gershon[a], David Gershon[b,*]

[a]*Department of Immunology, Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

[b]*Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel*

Received 25 September 2000; received in revised form 13 December 2000; accepted 14 December 2000

1. Introduction

With the increase in human longevity observed in many areas of the world during the 20th century, research into the basic causes and mechanisms of aging is rapidly becoming a social and economic as well as a scientific challenge. The tools and methodology of developmental and cellular biology and molecular genetics have been rapidly integrated into this challenging field. In order to progress effectively in our understanding of Gerontology, appropriate and relevant models have to be established. The accelerating pace of research in modern biology has inevitably caused an intensive search for models that are both simple and provide rapid results. At times this trend to simplify does not well consider the biology of the whole multi-cellular organism with its inter-dependent networks of cellular communications. Inbreeding and rearing of experimental organisms under strict laboratory conditions for generation after generation have ignored interactions with the environment as inevitably encountered in a natural habitat. Inadequate consideration of the relevance of the models under study to the natural biology of the ultimate organism of interest, the human, may lead us to incomplete and often erroneous conclusions while expending the limited resources available for the study of this increasingly important subject.

Here we critically review some of the currently used models in gerontology research and evaluate both their shortcomings and contributions to advancing our understanding of the phenomenon of senescence.

2. Criteria for acceptable paradigms of aging

Biological aging entails the gradual decline in the capacity to respond to environmental

* Corresponding author. Tel.: +972-4-8294211/2; fax: +972-4-8221106.
E-mail address: dgershon@tx.technion.ac.il (D. Gershon).

PII: S0531-5565(01)00115-2

challenges and concomitant compromise of the survival capacity of the organism. The following criteria ought to be considered in the selection of the paradigms used to study aging.

1. Simplicity of the model and its suitability for experimental manipulation. Ample understanding of the developmental program of the organism under study is an important factor in the selection of a model system.
2. Adequate mimicry by laboratory conditions of the prevailing conditions in the natural habitat of the organisms (or cell cultures) under study. Parameters to be considered here include nutrition, temperature, humidity, oxygen tension, photoperiodism, population density and possible exposure to viruses, bacteria and other invasive organisms.
3. Relevance of the model system to mammals in general and humans in particular should be evaluated. Caution should be exercised in extrapolating results and their interpretation from lower to higher eukaryotes.

As will become clear in the following discussion, these criteria have not always been rigorously considered in the studies of certain purported paradigms of aging research.

3. The paradigm of the budding yeast *Saccharomyces cereviseae*

The budding yeast is a unicellular eukaryotic fungus that can be grown in large quantities on simple media and also be plated on agar plates as single cells and observed for clonal growth. They can be manipulated genetically with ease and mother cells can be separated from progeny by micromanipulation. *S. cereviceae* can proliferate in both haploid and diploid states, with abundant nutrients proliferation occurring in the diploid state. Upon starvation, the cells are driven into meiosis and spore formation. Under conditions of deprivation, spores can survive for extremely long periods of time. These haploid spores germinate and start propagating upon improvement of the nutritional conditions.

In the budding yeast, the cell divides asymmetrically into a mother cell and a diminutive cell, the bud, which eventually grows to the full size of the mother cell. Mortimer and Johnston were the first to measure the rate of bud production from single cells (Mortimer and Johnston, 1959). Such measurement requires observation of individual cells and separation of the mothers from progeny buds. There are contradictory findings regarding the number of progeny produced by early and late buds removed from the same mother cell (Kennedy et al., 1994; Egilmez and Jazwinski, 1989).

The number of buds produced by a cell varies considerably depending on genotype, nutritional conditions and perhaps temperature. Two major approaches have been used to determine ‘senescence’ and ‘life span’ in yeast. The first, initiated by Mortimer and Johnston (1959) and used extensively by others (Kennedy et al., 1994; Kim et al., 1999a), observes the budding capacity of individual cells as a function of time in culture. The finite number of buds produced by a mother cell is determined and is designated *budding life span*. To prevent over-crowding, which may drive the cells into stationary state (Werner-Washburne et al., 1996), each daughter cell is separated from the mother

cell by micromanipulation throughout the 'budding life span'. Mortimer and Johnston cautioned that local nutrient depletion around the individually cloned cells presents a problem in this protocol. In addition to depletion by cellular consumption, medium that is kept in the incubator at 25° C for a number of days, and at times several weeks, undergoes a decline in nutritional value. In this system, the fate of the post-mitotic cell is not well defined. Death verification is not always the endpoint, but rather it is implicitly accepted that post-mitotic cells are dead cells. It is quite possible that a fraction of these cells are quiescent rather than dead.

The second experimental protocol for studying aging in yeast is the *stationary phase* system in which whole populations are maintained in liquid medium until cell number reaches a plateau (Longo, 1999). The cells are then maintained for an additional period on either the expired medium or distilled water (Liou et al., 2000). Viability is determined by the ability of individual cells plated from aliquots that are removed periodically from the cultures to form colonies on agar plates. In the *stationary phase* approach, the cells initially grow rapidly in glucose-rich medium where energy is primarily derived from fermentation. With medium depletion, growth slows down, the cells enter a stationary phase, mitochondria begin to use stored substrates and there is a switch from fermentation to mitochondrial respiration. The population of cells maintained in the expired medium with very limited growth shows increased mortality after about five days (Longo, 1999). Cells maintained in the expired medium show increased mortality attributed primarily to the production of reactive oxygen species generated by the increasingly active mitochondria (Longo, 1999). In an alternative protocol, after depletion of glucose in the medium the population is transferred to water in which it can be kept for weeks in a non-dividing but viable state presumably due to a 100-fold reduction in the rate of metabolism with no switch to oxidative respiration (Longo, 1999). A variation of the *stationary phase system* for the study of chronological, rather than budding life span involves growing the cells to a stationary phase on minimal glucose medium. The mean survival time, with the particular strains used in this system, studied from aliquots serially withdrawn from the culture, is 7–8 days (Longo et al., 1997). In this experimental protocol, the cells maintain high rates of metabolism throughout the life span (Liou et al., 2000). A disturbing point in either variation of this approach is that the major observation recorded is actually the effect of nutrient deprivation and possibly of accumulation of toxic compounds in the medium.

The discrepancies in determination of life span with the two methodologies (*stationary phase* liquid culture and the *budding life span*) are often difficult to reconcile and raise the fundamental question: How do we define life span in yeast? Longo suggests that the stationary phase system reveals 'chronological age' rather than budding life span (Longo, 1999). Ashrafi et al. showed that the mean replicative life span gradually decreases with the length of time the cells are maintained in stationary phase (Ashrafi et al., 1999). However, it has been reported by Jazwinski's group that daughter cells of old and young mother cells have the same reproductive capacity (Egilmez and Jazwinski, 1989). Survival under minimal medium conditions, which is closer to what the yeast cell encounters in its natural habitat, may be more meaningful. In both systems it is very difficult to distinguish the difference between the ability to survive under various environmental conditions and true life span determinants.

While there is a superficial resemblance to aging mechanisms of multi-cellular

organisms, yeast, like bacteria, are basically immortal at the population level. There is no evidence from multi-cellular organisms that the number of cell divisions is a clock that determines the onset of programmed cell death. Cell death in unicellular organisms does occur and is inevitably organismal. Barring environmental disasters, populations of unicellular organisms go on multiplying indefinitely even though a proportion of cells die. Standardization of conditions such as constant replenishment of growth medium will circumvent the problem of the effect of nutrient depletion and accumulation of harmful breakdown compounds in the medium on the survival of yeast cells. Critical chemostat studies will yield a deeper insight on the rate and the causes of mortality in yeast cells.

The strains of yeast used for the study of aging have been both diploid and haploid. Using a haploid strain, it has been claimed that genome instability is a major determinant of the life span (Park et al., 1999). Genomic instability in a haploid strain cannot effectively represent the situation in diploid yeast nor in higher eukaryotes.

For the purpose of facilitating laboratory research, yeast strains have been adapted to efficient growth under the artificial conditions of constant nutrition and temperature rather than the variable conditions encountered in nature. Some of these genetic variants that are auxotrophs for several nutrients have been used as 'wild type'. Under various conditions these mutations can have a significant effect on the expression of other genes (Padilla et al., 1998). This demonstrates that genotypic modifications can have complex phenotypic ramifications. Many genes become unessential under these conditions because the environment is rich and supplies all the necessities of the cell. It is likely that a large number of apparently redundant yeast genes are designed to deal with physiological challenges that are not encountered in the laboratory environment but that commonly occur in the natural habitat (Goffeau et al., 1996). Most of the mutations that affect longevity under laboratory conditions are part of the process of transition from genotypes that are adapted to natural habitat to those that are better adapted to the artificial laboratory environment. All wild type strains used in laboratory models are auxotrophs that survive well in the laboratory. These strains would be feeble, if at all viable, under the much more demanding conditions in nature. It should be noted that the laboratory environment also eliminates many of the conditions of stress that prevail in nature such as extreme temperatures, deprivation of nutrients, and fluctuations in humidity. The result is that cells are selected for and maintained in the laboratory that may be carrying allelic forms of genes that are not viable in nature.

There have been several conflicting claims for individual yeast genes as life span or longevity modulators. Amongst these are Ras-2 as both an extender of life span (Liou et al., 2000) and a cause of death (Longo, 1999). Sir2, histone deacetylases, gene silencing and rDNA circles have all been purported to play roles in the determination of yeast life span and aging (Guarente, 2000; Kim et al., 1999b). Gene silencing involves chromatin acetylation in both yeast and higher eukaryotes. In higher eukaryotes but not in yeast it is associated with DNA methylation. Since the mechanism of gene silencing differs in important details between yeast and higher eukaryotes simple extrapolation from yeast to higher metazoans is, at best, risky.

It is a rare situation in nature that yeast grow as solitary clones, such as in the *budding life span* experimental protocol, rather than non-clonal populations composed of presumably mixed genotypes. In *suspension culture* populations, mutant genotypes that have

better adaptation for artificial laboratory conditions will have selective advantage over genotypes that are more adapted to the natural habitat. They may also show extended longevity in single cell cultures where they are not required to compete with other genotypes. It is likely that 'longevity' mutations carry pleiotropic effects on metabolic functions, cell cycle progression and fecundity.

The following problems should be resolved in order to validate yeast as a model in aging research:

(a) Do individual yeast cells truly have a life span? Cellular changes that occur in post-budding yeast cells and the mechanisms of cell death should be characterized.
(b) What does budding life span mean in the context of chronological life span? Does the finite number of buds a mother cell produces have any relation to its chronological age? This controversial point is particularly relevant since it has become obvious that mammalian cells in culture do not exhibit a correlation between donor age and number of population doublings (Cristofalo et al., 1998).
(c) Which yeast strains should serve as wild type? Consideration should be given to the problem of haploid vs. diploid strains. In order to standardize the results, wild type strains, which do not carry auxotrophic mutations, should be used to study longevity in yeast.
(d) What is the relationship between survival under adverse conditions and longevity in yeast? Chemostat studies, which avoid starvation and lengthy deprivation of nutrients, and have been used in the study of yeast for other purposes (van Hoek et al., 2000) should be helpful in this respect.
(e) Are the mutations (or deletions) of genes that appear to increase life span such as Ras2 really relevant to aging? It has to be demonstrated that yeast which carry mutations in these 'life span assuring genes' are not at a selective disadvantage during exponential growth when mixed in a population of wild type cells. If they are eliminated in a population consisting of various genotypes because of poor competitive capacity (and yeast in nature do not grow under solitary conditions), then the genes in question have dubious relevance to the determination of cellular life span.

4. The paradigm of the nematode *Caenorhabditis elegans*

4.1. Merits of the nematode system for aging research

C. elegans is a ubiquitous, soil dwelling free-living nematode. Free-living nematodes (not necessarily *C. elegans*) have been deemed suitable for aging research for the following reasons (see detailed discussion in Reznick and Gershon (1998) and Gershon (1970), and see the excellent reviews by Klass (1977) and Johnson (1984)):

(a) Free-living nematodes of the Rhabditis family are relatively simple multi-cellular organisms, with a small number of cells and well-defined cell types. These nematodes possess distinct somatic and germ cells. The somatic cells are essentially post-mitotic like most somatic cell systems in the mammal.

(b) These nematodes can be easily raised and maintained in large populations under relatively cheap and simple laboratory conditions both axenically on defined media (Dougherty et al., 1959; Rothstein and Cook, 1966) and monoxenically on *E. coli* (Sulston and Hodgkin, 1988).

(c) An analyzable genetic program precisely controls development. The developmental lineage of all *C. elegans* cells has been determined (Sulston, 1988; Horvitz, 1988).

(d) These nematodes have a very short generation time of a few days and short mean and maximal life spans.

(e) These organisms, particularly *C. elegans*, are highly suitable for molecular genetic analysis as their genome has been completely sequenced ((1999)).

4.2. Problematic points in the study of C. elegans

(a) In the soil this organism encounters considerable environmental fluctuations in availability of food, temperatures, humidity and probably oxygen tension. Because of the harsh conditions in its habitat, it has developed remarkable mechanisms of survival some of which are dispensable under controlled laboratory conditions. Growth for thousands of generations (~100 generations/year for ~30 years) in the laboratory under conditions far removed from the natural habitat has most probably preferentially selected for genotypes adapted to non-fluctuating culture conditions and made the genes required for coping with various environmental stressors redundant. For instance, in the soil *C. elegans* feeds on microorganisms and decaying organic matter (which supplies plant sterols and not cholesterol, an obligatory additive to laboratory cultures that feed on *E. coli*). In nature *C. elegans* does not encounter *E. coli* yet, in the laboratory, it has been forced to feed on this bacterium for a few thousands of generations. Inevitably, we are observing a mini-evolutionary process of adaptation to ad libitum feeding on a foreign diet (and other artifactual conditions). Appropriate examples of such evolution are the so-called 'gerontogenes', that when mutated 'extend' the life span under laboratory conditions (see Braeckman et al., 2000). Many of these genes belong to the *Age*-1, *Clk* (clock) and *eat* gene families. However, like most mutations these mutant gerontogenes must exert lower fitness on the mutated organism when it is exposed to conditions of its natural habitat. A fitting example is the Age-1 mutant that is selectively eliminated when grown in a mixture with wild type under mild starvation stress (Walker et al., 2000). We predict that the same will apply to the clk and eat mutations and certainly for the mutants with defective sense organ development described by Apfeld and Kenyon (1999). It is therefore proposed that the undeservedly popular concept of gerontogenes is a mistaken interpretation of survival adaptation under a narrow set of artificial laboratory conditions and does not apply to senescence per se.

(b) *C. elegans* is primarily hermaphroditic. Thus experimental populations are composed of clones of organisms that are essentially homozygous. Dioecious nematodes, such as *Tubatrix aceti* (e.g. Gershon and Gershon, 1970) must be included in aging studies to verify the universality of the aging processes described for *C. elegans*.

(c) *C. elegans* has four larval stages. Under unfavorable environmental conditions development is attenuated and *dauer larvae* are formed at the third larval stage. This

developmental diversion is controlled by a considerable number of genes. Dauer larvae do not feed and exhibit an extremely low level of metabolism (Riddle and Albert, 1997) and yet their survival is several times longer than that achieved under conditions that promote normal development. When conditions change, the Dauer larvae revert to the normal developmental program and exhibit the same post-larval life span as the well-fed unstressed animals. Many of the gerontogenes play a role in the control of this unusual developmental pathway. This stress-induced pathway is not universal. Particular caution must, therefore, be practiced in the design of experiments and interpretation of results when using *C. elegans*.

4.3. Contributions of the nematode model to the understanding of the aging process

(a) Significance of oxidative damage to the aging process. Earlier we showed that such damage occurs and plays a role in the determination of nematode life span (Epstein et al., 1972; Epstein and Gershon, 1972). Very importantly, this work demonstrated that prevention of damage during the developmental stages of the life cycle exerted the maximal effect on the life span.
(b) Accumulation of inactive enzyme molecules as a function of age was first demonstrated by our work with nematodes (Gershon and Gershon, 1970). We then described the same phenomenon for mammalian tissues (Gershon and Gershon, 1973). We then showed that this accumulation was a result of a great reduction in protein degradation in nematodes with age (Zeelon et al., 1973). A similar attenuation of degradation was later described by us for mammalian systems (Reznick et al., 1981; Lavie et al., 1982; Reznick et al,. 1985). The advantage of using the nematode system was that we could use simple means to show that the degradation system of older organisms could not cope with amino acid analog-induced increase in faulty proteins as efficiently as the young system. Analogous work in mammalian systems was much more complex and costly. Thus the nematode system led the way in the discovery of this important age-associated phenomenon.
(c) The pioneering work on genetic analysis of senescence in nematodes (Johnson, 1984) has shown the high degree of suitability of the nematode system for molecular genetic analysis of senescence. While we are critical of the roles claimed for the gerontogenes described to date, it is predicted that complicated multi-gene systems will have to be studied to analyze the underlying processes of aging. Thus complex analyses will be required that will necessitate large populations that are easily afforded by the nematode model.

5. The paradigms of mammalian cells in culture and the significance of telomeres

Hayflick first described the finite replicative capacity of fetal fibroblasts in culture (Hayflick and Moorhead, 1961; Hayflick 1965) and interpreted these findings as demonstrating cellular aging in vitro (Linskens et al., 1995). It was claimed that cessation of growth in culture is relevant to senescence in vivo. This interpretation led to the major

misconception that post-mitotic cells are dead cells. Another misconception derived from these studies is that the number of cell divisions is a limiting factor that serves as a clock, which determines the life span of a multi-cellular organism. A corollary to this is the suggestion in recent years that the gradual shortening of average telomere length as a function of time and number of population doublings in culture functions as a clock. Once the average shortening of telomere length reaches a certain value it purportedly terminates the capacity of the cell to divide (Harley et al., 1990).

The paradigm of mammalian cells in culture as used for the study of aging is fraught with serious faults.

(a) Most cells derived from any tissue do not survive the early period of cultivation. The prevailing culture conditions are potentially very damaging to primary cultured cells. Culture conditions are even detrimental to cell lines that are more adapted to culture conditions due to selection of cells with the appropriate genomic modifications.

(i) Repeated trypsinization is damaging and non-physiological. Trypsin degrades receptors, ion channels, extracellular matrix (ECM)-binding proteins and other surface proteins in a variety of cell types with a damaging effect on responsiveness to environmental cues and cell survival (e.g. Deman and Bruyneel, 1977; Mersel et al., 1983; Motwani et al., 1980; Reddy et al., 1989; Reiners et al., 2000; Smets et al., 1979; Winand and Kohn, 1975; McKeehan, 1977; Yamanaka and Deamer, 1974; el Achkar et al., 1989; Rasilo and Yamagata, 1989; Curtis and Hill, 1979; Huggins et al., 1976).

(ii) Culture under atmospheric oxygen, with its oxygen tension which is much higher (up to 10 fold) than cells encounter in vivo, can also exert very damaging effect on cell functions and viability (e.g. Colombatto et al., 1994; Eppig and Wigglesworth, 1995; Khaliq et al., 1995; Krieger et al., 1996; LaIuppa et al., 1998; Ono and Alter, 1995; Reykdal et al., 1999; Saito et al., 1995). Indeed, growth at low oxygen tension has been shown to allow better proliferation of primary ketatinocytes (Horikoshi et al., 1986) and melanocytes (Horikoshi et al., 1991) in culture.

(iii) Under in vivo homeostasis pH control is very stringent. Despite the buffers included in media, fluctuation of pH in culture (Eagle, 1973) is much greater than in vivo with damaging consequences.

(iv) Probably the most crucial negative effect of cell culturing is the deprivation of communication with other cell types. Inter-cellular signaling in the form of hormones, growth factors and ECM components is essential for controlled cell proliferation, differentiation and in many cases programmed cell death. The cultured cell is deprived of all of these important cues delivered by other cell types.

(b) There is no evidence for a 'Hayflick limit' in proliferative capacity of cells in vivo. On the contrary, there is substantial evidence that points to lack of proliferative limits in vivo. A number of investigators have shown this for a variety of cell types. Here we present a few examples.

(i) Serial skin transplants. In the late 1950s and early 1960s Krohn (prior to Hayflick's papers of 1961 and 1965) conducted a pioneering series of experiments addressing the question of whether or not there is a limit to cell proliferation in vivo (Krohn, 1966). Serial skin transplants from two mouse strains and their F_1 hybrids as well as transplantations of grafts from young and old donors into young and old hosts in all possible permutations

were performed. The proliferation observed far exceeded that necessary for one mouse life span. Also the capacity for proliferation in skin transplants did not change significantly between young and old grafts, at least in CBA and CBA × A F1 hybrids.

(ii) Serial mammary tissue transplants into murine mammary fat pad was carried out by Daniel (1977). These extensive experiments indicated that mammary epithelium cells exhibit a proliferative capacity that far exceeds that required for a single life span.

(iii) Harrison conducted an elegant series of experiments in which he serially transplanted bone marrow cells that rescued lethally irradiated mice (Harrison, 1985). Here again proliferative capacity exceeded that required for the life span of a mouse. Moreover, in most experiments cells of old individuals proliferated as well as young individuals when transplanted into young recipients.

(iv) Maintenance of stable hematocrit throughout the lifespan of healthy rats and humans has been demonstrated by us (Glass et al., 1983; Glass et al., 1985). The supply of erythrocytes, that have a mean survival of the blood of 60 and 120 days in rats and humans, respectively, does not diminish with age. Moreover, we demonstrated that in rats the rate of turnover of erythrocytes doubles with age. Considering that the hematocrit does not change with age our findings mean that the rate of supply of erythrocytes to the circulation doubles and yet the system remains stable. Thus there is no proliferative limit in this system in vivo.

(v) The lack of correlation between donor age and the number of PDLs of skin fibroblasts grown in vitro was unequivocally demonstrated by Cristofalo et al. (1998). They showed that fibroblasts from healthy individuals (not from cadavers) have the same proliferative capacity regardless of donor age. More convincingly, they showed that cells of the same individual at different ages showed the same proliferative capacity. The point made in this paper is of cardinal importance: if one carefully defines the source of cells and standardizes the experimental conditions one can minimize errors of interpretation which lead to the formation of unwarranted dogmas.

The above five examples demonstrate that what is called 'proliferative senescence' cannot and should not be used as a model of in vivo aging. In fact we propose that the 'Hayflick phenomenon' is a result of cell abuse in vitro. Without such abuse in vivo systems have a proliferative capacity that far exceeds needs for the maintenance of cellular proliferation throughout the life span of the organism.

(c) The maintenance of telomere length is a fascinating problem for chromosome stability and may be pertinent to maintenance of tumor cell proliferation. However, the whole vast literature of the past decade on relating it to 'cellular senescence' is inappropriate in view of what was discussed above. In view of all the evidence outlined above, that refutes the relevance of in vitro culture systems to organismal senescence and the lack of a relevant proliferative clock, it is suggested that the telomere theory of aging be considered irrelevant.

6. Conclusions and perspectives

For the sake of brevity, we have considered in this review only three popular paradigms out of 8–10 main models that are currently being used in aging research. As is obvious

from the above, the yeast and tissue culture systems are difficult to accept as paradigms for aging research. The acceptance of the tissue culture system into the pantheon of gerontology and its constant maintenance as a major paradigm is baffling. One must take into account the biological relevance of the system. Much of the evidence we presented here that emphatically shows no Hayflick limit in vivo has been in the literature for decades yet disregarded. The myriad of papers devoted to studying various aspects of in vitro 'cell senescence' is rather a study of cellular response to severe abuse. In the case of the yeast, there has been a disregard of the specific biology of the yeast, in particular, and unicellular organisms, in general. The ease with which the yeast cell can be genetically manipulated is fascinating and constitutes a magnificent tool for the study of the molecular biology of the cell. However, as explained above, the yeast cell cannot serve as a paradigm for the study of such a complex phenomenon as aging. We have published a detailed review of the yeast model (Gershon and Gershon, 2000a). The nematode system is a much more appropriate paradigm for aging research. It offers a multi-cellular yet simple organism with well-differentiated specialized post-mitotic cells. Differentiation and cell specialization are a major factor in aging (Gershon and Gershon, 2000b). However, in all experimental work one must take into account that one is dealing with laboratory-modified organisms that are the result of selection for growth under laboratory conditions which have considerably reduced environmental stresses. If this is ignored one can cause mutations that 'extend' the life span while reducing the fitness of the organism for life other than under these extremely protective conditions. This is the basis of the unfortunate term of gerontogenes. It is very unlikely that there are individual genes that dramatically affect the complex phenotype of the life span under conditions that include stresses like starvation, extreme temperatures, reactive oxygen species and others. It is, therefore, suggested that biology of the organism and its laboratory history be considered when one intends to devote much time and effort to work on a particular paradigm.

Future research should focus on multi-cellular organisms in search of the multi-genic systems involved in the determination of the life span. A major effort should be addressed to understanding the control of the activities of gene combinations that pleiotropically (indirectly) play a role in the determination of the life span. Also, work must include models of populations of organisms comprising heterogeneous genotypes that are raised under conditions that are as close to those encountered in the animal's habitat as possible.

Acknowledgements

We wish to thank Dr Vincent Cristofalo and the Lankenau Medical Research Center for providing the facilities for writing this review.

References

el Achkar, P., Langley, O.K., Mersel, M., 1989. Effect of cell surface trypsinization on ethanolamine base exchange enzymatic activity of astrocyte primary cultures and derived spontaneously transformed cell lines. Biochem. Biophys. Res. Commun. 159, 1055–1064.

Apfeld, J., Kenyon, C., 1999. Regulation of lifespan by sensory perception in *Caenorhabditis elegans* [see comments]. Nature 402, 804–809.

Ashrafi, K., Sinclair, D., Gordon, J.I., 1999. Passage through stationary phase advances replicative aging in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 96, 9100–9105.

Braeckman, B.P., Houthoofd, K., Vanfleteren, J.R., 2000. Patterns of metabolic activity during aging of the wild type and longevity mutants of *Caenorhabditis elegans*. Age 23, 51–69.

Colombatto, S., Giribaldi, G., Vargiu, C., Grillo, M.A., 1994. Modulation of ornithine aminotransferase activity by oxygen in rat hepatocyte cultures. Biochim. Biophys. Acta 1224, 329–332.

Cristofalo, V.J., Allen, R.G., Pignolo, R.J., Martin, B.G., Beck, J.C., 1998. Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation. Proc. Natl. Acad. Sci. USA 95, 10614–10619.

Curtis, A.S., Hill, O., 1979. Cell surface lipids and adhesion. IV. The effects of trypsin on lipid turnover by the plasmalemma. J. Cell Sci. 38, 283–292.

Daniel, C.W., 1977. Cell longevity: in vivo. In: Finch, C.E., Hayflick, L. (Eds.). Handbook of The Biology of Aging. Van Nostrand Reinhold Co, New York.

Deman, J.J., Bruyneel, E.A., 1977. Thermal transitions in the adhesiveness of HeLa cells: effects of cell growth, trypsin treatment and calcium. J. Cell Sci. 27, 167–181.

Dougherty, E.C., Hansen, E.L., Nicholas, W.L., Mollett, J.A., Yarwood, E.A., 1959. Axenic cultivation of *Caenorhabditis briggsae* with unsupplemented and supplemented chemically deined media. Annals New York Academy of Sciences 77, 176.

Eagle, H., 1973. The effect of environmental pH on the growth of normal and malignant cells. J. Cell Physiol. 82, 1–8.

Egilmez, N.K., Jazwinski, S.M., 1989. Evidence for the involvement of a cytoplasmic factor in the aging of the yeast *Saccharomyces cerevisiae*. J. Bacteriol 171, 37–42.

Eppig, J.J., Wigglesworth, K., 1995. Factors affecting the developmental competence of mouse oocytes grown in vitro: oxygen concentration. Mol. Reprod. Dev. 42, 447–456.

Epstein, J., Gershon, D., 1972. Studies on aging in nematodes. IV. The effect of antioxidants on cellular damage and life span. Mech. Ageing Dev., 257–264.

Epstein, J., Himmelhoch, S., Gershon, D., 1972. Studies on aging in nematodes. III. Electromicroscopical studies on age associated cellular damage. Mech. Ageing Dev. 1, 245–255.

1999. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. The *C. elegans* sequencing consortium[published errata in science 1999 Jan 1; 283 (5398): 35 and 1999 Mar 26; 283 (5410): 2103 and 1999 Sep 3;285(5433): 1493]. Science. 282 1998 2012–2018.

Gershon, D., 1970. Studies on aging in nematodes. I. The nematode as a model organism for aging research. Exp. Gerontol. 5, 7–12.

Gershon, H., Gershon, D., 1970. Detection of inactive enzyme molecules in ageing organisms. Nature 227, 1214–1217.

Gershon, H., Gershon, D., 1973. Altered enzyme molecules in senescent organisms: mouse muscle aldolase. Mech. Ageing Dev. 2, 33–41.

Gershon, H., Gershon, D., 2000a. The budding yeast, *Saccharomyces cerevisiae*, as a model for aging research. Mech. Ageing Dev. 120, 1–22.

Gershon, H., Gershon, D., 2000b. Paradigms in aging research: a critical review and assessment. Mech. Ageing Dev. 117, 21–28.

Glass, G.A., Gershon, H., Gershon, D., 1983. The effect of donor and cell age on several characteristics of rat erythrocytes. Exp. Hematol. 11, 987–995.

Glass, G.A., Gershon, D., Gershon, H., 1985. Some characteristics of the human erythrocyte as a function of donor and cell age. Exp. Hematol. 13, 1122–1126.

Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J.D., Jacq, C., 1996. Life with 6000 genes [see comments]. Science 274, 563–567.

Guarente, L., 2000. Sir2 links chromatin silencing, metabolism, and aging [In Process Citation]. Genes Dev. 14, 1021–1026.

Harley, C.B., Futcher, A.B., Greider, C.W., 1990. Telomeres shorten during ageing of human fibroblasts. Nature 345, 458–460.

Harrison, D.E., 1985. Cell and tissue transplantation: a means of studying the aging process. In: Finch, C.E., Schneider, E.L. (Eds.). Handbook of the Biology of Aging. Van Nostrand Reinhold Co, New York.

Hayflick, L., Moorhead, P.S., 1961. The serial cutivation of human diploid cell strains. Exp. Cell Res. 25, 585–621.

Hayflick, L., 1965. The limited in vitro lifetime of human diploid cell strains. Exp. Cell Res. 37, 614–620.

van Hoek, P., van Dijken, J.P., Pronk, J.T., 2000. Regulation of fermentative capacity and levels of glycolytic enzymes in chemostat cultures of *Saccharomyces cerevisiae*. Enzyme Microb. Technol. 26, 724–736.

Horikoshi, T., Balin, A.K., Carter, D.M., 1986. Effect of oxygen on the growth of human epidermal keratinocytes. J. Invest Dermatol. 86, 424–427.

Horikoshi, T., Balin, A.K., Carter, D.M., 1991. Effects of oxygen tension on the growth and pigmentation of normal human melanocytes. J. Invest Dermatol. 96, 841–844.

Horvitz, H.R., 1988. Genetics of cell lineage. In: Wood, W.B. (Ed.). The nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Huggins, J.W., Chesnut, R.W., Durham, N.N., Carraway, K., 1976. Molecular changes in cell surface membranes resulting from trypsinization of sarcoma 180 tumor cells. Biochim. Biophys. Acta 426, 630–637.

Johnson, T.E., 1984. Analysis of the biological basis of aging in the nematode, with special emphasis on *Caenorhabditis elegans*. In: Mitchel, D.H., Johnson, T.E. (Eds.). Invertebrate Models in Aging Research. CRC Press, Boca Raton, FL.

Kennedy, B.K., Austriaco Jr, N.R., Guarente, L., 1994. Daughter cells of *Saccharomyces cerevisiae* from old mothers display a reduced life span. J. Cell Biol. 127, 1985–1993.

Khaliq, A., Patel, B., Jarvis-Evans, J., Moriarty, P., McLeod, D., Boulton, M., 1995. Oxygen modulates production of bFGF and TGF-beta by retinal cells in vitro. Exp. Eye Res. 60, 415–423.

Kim, S., Kirchman, P.A., Benguria, A., Jazwinski, S.M., 1999. Experimentation with the yeast model. In: Yu, B.P. (Ed.). Methods in Aging Research. CRC Press, Boca Raton.

Kim, S., Benguria, A., Lai, C.Y., Jazwinski, S.M., 1999. Modulation of life-span by histone deacetylase genes in *Saccharomyces cerevisiae*. Mol. Biol. Cell 10, 3125–3136.

Klass, M.R., 1977. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. Mech. Ageing Dev. 6, 413–429.

Krieger, J.A., Landsiedel, J.C., Lawrence, D.A., 1996. Differential in vitro effects of physiological and atmospheric oxygen tension on normal human peripheral blood mononuclear cell proliferation, cytokine and immunoglobulin production. Int. J. Immunopharmacol. 18, 545–552.

Krohn, P.L., 1966. Transplantation and aging. In: Krohn, P.L. (Ed.). Topics of the Biology of Aging. Wiley, New York.

LaIuppa, J.A., Papoutsakis, E.T., Miller, W.M., 1998. Oxygen tension alters the effects of cytokines on the megakaryocyte, erythrocyte, and granulocyte lineages. Exp. Hematol. 26, 835–843.

Lavie, L., Reznick, A.Z., Gershon, D., 1982. Decreased protein and puromycinyl-peptide degradation in livers of senescent mice. Biochem. J. 202, 47–51.

Linskens, M.H., Harley, C.B., West, M.D., Campisi, J., Hayflick, L., 1995. Replicative senescence and cell death [letter; comment]. Science 267, 17.

Liou, L-L, Fabrizio, P., Moy, V.N., Vaupel, J.W., Valentine, J.S., Gralla, E.B., Longo, V.D. Ras2 and Superoxide Regulate Survival in Yeast. Submitted for Publication. 2000.

Longo, V.D., 1999. Mutations in signal transduction proteins increase stress resistance and longevity in yeast, nematodes, fruit flies, and mammalian neuronal cells. Neurobiol. Aging 20, 479–486.

Longo, V.D., Ellerby, L.M., Bredesen, D.E., Valentine, J.S., Gralla, E.B., 1997. Human Bcl-2 reverses survival defects in yeast lacking superoxide dismutase and delays death of wild-type yeast. J. Cell Biol. 137, 1581–1588.

McKeehan, W.L., 1977. The effect of temperature during trypsin treatment on viability and multiplication potential of single normal human and chicken fibroblasts. Cell Biol. Int. Rep. 1, 335–343.

Mersel, M., Benenson, A., Delaunoy, J.P., Devilliers, G., Mandel, P., 1983. Long-term effects of brain trypsinization before cell seeding on cell morphology and surface composition. Neurochem. Res. 8, 449–463.

Mortimer, R.K., Johnston, J.R., 1959. Life Span of Individual Yeast Cells. Nature 183, 1751–1752.

Motwani, N.M., Unakar, N.J., Roy, A.K., 1980. Multiple hormone requirement for the synthesis of alpha 2u-globulin by monolayers of rat hepatocytes in long term primary culture. Endocrinology 107, 1606–1613.

Ono, K., Alter, B.P., 1995. Effects of low oxygen tension and antioxidants on human erythropoiesis in vitro. Exp. Hematol. 23, 1372–1377.

Padilla, P.A., Fuge, E.K., Crawford, M.E., Errett, A., Werner-Washburne, M., 1998. The highly conserved,

coregulated SNO and SNZ gene families in *Saccharomyces cerevisiae* respond to nutrient limitation [published erratum appears in J. Bacteriol 1998 Dec; 810 (24): 6794]. J. Bacteriol 180, 5718–5726.

Park, P.U., Defossez, P.A., Guarente, L., 1999. Effects of mutations in DNA repair genes on formation of ribosomal DNA circles and life span in *Saccharomyces cerevisiae*. Mol. Cell Biol. 19, 3848–3856.

Rasilo, M.L., Yamagata, T., 1989. The glucose polymer of PC12 cells is susceptible to trypsinization. Biochem. Biophys. Res. Commun. 159, 1–6.

Reddy, N.M., Stevenson, A.F., Lange, C.S., 1989. Trypsinization and the radiosensitivity of mitotic and log phase Chinese hamster V79 cells exposed to 250 kVp X-rays. Int. J. Radiat. Biol. 55, 105–117.

Reiners Jr, J.J., Mathieu, P., Okafor, C., Putt, D.A., Lash, L.H., 2000. Depletion of cellular glutathione by conditions used for the passaging of adherent cultured cells. Toxicol. Lett. 115, 153–163.

Reykdal, S., Abboud, C., Liesveld, J., 1999. Effect of nitric oxide production and oxygen tension on progenitor preservation in ex vivo culture. Exp. Hematol. 27, 441–450.

Reznick, A., Gershon, D., 1998. Experimentation with nematodes. In: Yu, B.P. (Ed.). Methods in Aging Research. CBC Press.

Reznick, A.Z., Lavie, L., Gershon, H.E., Gershon, D., 1981. Age-associated accumulation of altered FDP aldolase B in mice. Conditions of detection and determination of aldolase half life in young and old animals. FEBS Lett. 128, 221–224.

Reznick, A.Z., Rosenfelder, L., Shpund, S., Gershon, D., 1985. Identification of intracellular degradation intermediates of aldolase B by antiserum to the denatured enzyme. Proc. Natl. Acad. Sci. USA 82, 6114–6118.

Riddle, D.L., Albert, P.S., 1997. Genetic and environmental regulation of Dauer larva development. In: Riddle, D.L., Blumenthal, T.R., Meyer, B.J., Priess, J.R. (Eds.). *C. Elegans* II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Rothstein, M., Cook, E., 1966. Nematode biochemistry. VI. Conditions for axenic culture of *Turbatrix aceti*, *Panagrellus redivivus*, *Rhabditis anomala* and *Caenorhabditis briggsae*. Comp. Biochem. Physiol. 17, 683–692.

Saito, H., Hammond, A.T., Moses, R.E., 1995. The effect of low oxygen tension on the in vitro replicative life span of human diploid fibroblast cells and their transformed derivatives. Exp. Cell Res. 217, 272–279.

Smets, L.A., Homburg, C., Van Rooy, H., 1979. Selective effects of trypsinization on established and tumour-derived mouse 3T3 cells. Cell Biol. Int. Rep. 3, 107–111.

Sulston, J., 1988. Cell Lineage. In: Wood, W.B. (Ed.). The nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sulston, J., Hodgkin, J., 1988. Methods. In: Wood, W.B. (Ed.). The nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Walker, D.W., McColl, G., Jenkins, N.L., Harris, J., Lithgow, G.J., 2000. Evolution of lifespan in *C. elegans*. Nature 405, 296–297.

Werner-Washburne, M., Braun, E.L., Crawford, M.E., Peck, V.M., 1996. Stationary phase in *Saccharomyces cerevisiae*. Mol. Microbiol. 19, 1159–1166.

Winand, R.J., Kohn, L.D., 1975. Stimulation of adenylate cyclase activity in retro-orbital tissue membranes by thyrotropin and an exophthalmogenic factor derived from thyrotropin. J. Biol. Chem. 250, 6522–6526.

Yamanaka, N., Deamer, D., 1974. Superoxide dismutase activity in WI-38 cell cultures: effects of age, trypsinization and SV-40 transformation. Physiol. Chem. Phys. 6, 95–106.

Zeelon, P., Gershon, H., Gershon, D., 1973. Inactive enzyme molecules in aging organisms. Nematode fructose-1,6-diphosphate aldolase. Biochemistry 12, 1743–1750.