

Mutations in signal transduction proteins increase stress resistance and longevity in yeast, nematodes, fruit flies, and mammalian neuronal cells

Valter D. Longo*¹

Division of Neurogerontology Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, 3715 McClintock Avenue, Los Angeles, CA 90089-0191, USA

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Abstract

Mutations in Ras and other signal transduction proteins increase survival and resistance to oxidative stress and starvation in stationary phase yeast, nematodes, fruit flies, and in neuronal PC12 cells. The chronological life span of yeast, based on the survival of nondividing cells in stationary phase, has allowed the identification and characterization of long-lived strains with mutations in the G-protein Ras2. This paradigm was also used to identify the *in vivo* sources and targets of reactive oxygen species and to examine the role of antioxidant enzymes in the longevity of yeast. I will review this model system and discuss the striking phenotypic similarities between long-lived mutants ranging from yeast to mammalian neuronal cells. Taken together, the published studies suggest that survival may be regulated by similar fundamental mechanisms in many eukaryotes and that simple model systems will contribute to our understanding of the aging process in mammals. © 1999 Elsevier Science Inc. All rights reserved.

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1. Introduction

Oxidative damage to macromolecules has been implicated in aging and certain aging-related diseases [1,40,56] and is believed to result from stochastic microenvironmental fluctuations in the balance between oxidants, such as O_2^- , H_2O_2 , and $\cdot OH$, and antioxidants, including superoxide dismutases, peroxidases, and glutathione. However, the demonstrated ability of a single protein, such as Ras, to regulate the generation of reactive oxygen species, antioxidant defenses, and cell death in mammalian cells [11,44,61] raises the possibility that oxidative damage and aging may be regulated by a limited number of genes.

Caenorhabditis elegans (nematode), *Drosophila* (fruit fly), and mice are the three main model systems that are being genetically manipulated to experimentally address this topic [7]. *Saccharomyces cerevisiae* (yeast), thanks to straightforward genetic techniques and to the wealth of information available at the biochemical, molecular, and

cellular level, is emerging as a novel and powerful model system to study the genetics of aging [14,22,35].

2. Budding life span and stationary phase

Yeast is a simple, unicellular, eukaryote for which extensive genetic and molecular biology are known. The entire genome has been completely sequenced and contains 5885 potential genes [9,71]. The similarities of a large number of signal transduction and other housekeeping proteins between yeast and humans have enhanced our understanding of human systems, thanks in part to the ability of mammalian proteins to functionally substitute for their yeast analogs. Examples include the antioxidant superoxide dismutase [35], Ras [16], and heat shock proteins [45]. In contrast to mammalian systems, the simplicity of genetic manipulations in yeast allows the removal or over expression of one or multiple genes to study the function of a particular protein [15]. In addition, this small eukaryote can be grown to large, stationary-phase populations of billions of organisms that can be used to screen for longevity mutations or to identify novel genes involved in the long-term resistance to insults, such as oxidative and thermal stress.

* Corresponding author. Tel.: +1-213-740-4915; fax: +1-213-740-0853.

E-mail address: vlongo@usc.edu (V.D. Longo)

¹ VDL is a John Douglas French Alzheimer's Foundation fellow.

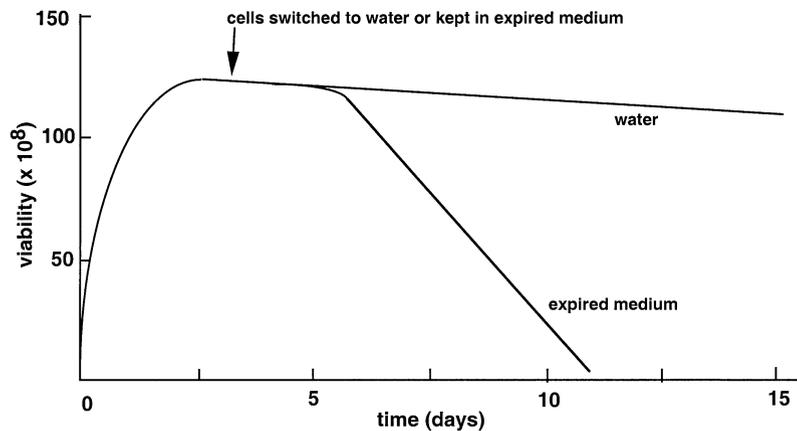


Fig. 1. The chronological life span of yeast. Typical survival curves for yeast stationary phase populations maintained in water or in expired medium. Cells are normally inoculated at a density of 1×10^6 cells/mL and grown in complete minimal medium at 30°C in a shaking incubator. After reaching a density of $2\text{--}4 \times 10^7$ cells/mL, external nutrients become scarce, growth slows down, and the population begins to store glycogen and other nutrients. By Day 4 (in strain EG103) cells enter the nondividing stationary phase characterized by decreased metabolic rates and increased protection against heat and oxidative stress. Mean survival, depending on the strain and the incubation medium (water vs. expired medium), ranges from 8 to 50 days. Viability in the culture is measured every 24 or 48 h by serially diluting aliquots of the population and plating onto rich medium plates. The ability of each live cell to form a colony is used to estimate viability in the culture.

Aging in yeast is often measured by counting the finite number of buds that can be generated by a single mother cell maintained in the growth phase (budding life span) [48]. Published studies that used this paradigm suggest that replicative aging is caused by the accumulation of rDNA circles resulting in nucleolar fragmentation [59]. During the budding life span, each daughter cell must be separated from the mother cell by micromanipulation to prevent overcrowding and entry of the population in stationary phase [69]. In this nondividing phase, the cells can survive for long periods without dividing. Although the relationship between the budding life span and stationary phase survival is not clear [58], a recent study demonstrated that yeast cells maintained in stationary phase show a decrease in replicative life span after reentering the cell cycle [2]. The mean replicative life span was 27 after 1 day, 20 after 13 days, and 16 after 33 days in stationary phase, suggesting that nondividing yeast undergo senescence. Furthermore, one of the mutants with increased budding life span, *sir4-42*, was isolated by screening for cells able to survive longer than the wild-type in stationary phase [26]. However, other studies demonstrate that the over expression of the G-protein Ras2, which causes rapid death in stationary phase, increases the budding life span [62], whereas the deletion of Ras2, which doubles stationary phase survival (Longo et al., unpublished results), decreases budding life span [62]. The effect of Ras2 on budding life span was proposed to involve retrograde regulation; a regulation of signaling from the mitochondrion to the nucleus [29]. In summary, although some of the genes and mechanisms that regulate budding potential can also affect survival in stationary phase, further studies are necessary to clarify the relationship between these two paradigms.

Stationary phase is relatively well-understood from de-

acades of study [70]. As the level of external nutrients decreases, yeast cells store glycogen and other nutrients intracellularly, decrease metabolic rates and protein synthesis, and develop increased thermotolerance and antioxidant defenses [70]. Cells can survive in stationary phase for days to months depending on the strain and on the incubation medium (Fig. 1) and can, under very low nutrient conditions, undergo meiosis and form spores able to survive for months to years, with metabolic rates lower than those of stationary phase cells [4].

Although the majority of multicellular eukaryotes, including the ones that can enter a hypometabolic state, such as nematodes, will spend most of their adult life with normal metabolic rates, yeast, however, either sporulate or unavoidably survive in the low metabolism stationary phase. In fact, "much of the microorganismal mass in the world is estimated to exist under nutrient-depleted conditions" [69]. Thus, it may be more correct to view the short growth phase of yeast as a hypermetabolic state aimed at quickly generating a large population and the long stationary phase as the normal metabolic state for long-term survival during which internal nutrient reserves are used slowly. The very low metabolism spore state, entered only by a minority of cells under extreme nutrient conditions, may be viewed as a true hypometabolic state, analogous to the nematode dauer larva (as recently pointed out by Kenyon and colleagues [5]).

3. Chronological life span of yeast

Most studies of yeast are performed by using logarithmically growing cells. However, the growth phase is not suited to study the accumulation of oxidative and other forms of macromolecular damage because individual cells

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