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The chronological life span of *Saccharomyces cerevisiae* to study mitochondrial dysfunction and disease

Edoardo Parrella, Valter D. Longo *

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

Saccharomyces cerevisiae has played an important role as a model system to understand the biochemistry and molecular biology of mammalian cells. The genetic tools available and the short life span have also made *S. cerevisiae* a powerful system to study aging. The yeast chronological life span (CLS) is a measure of the survival of a non-dividing population of cells, and thus can model aging of mammalian non-dividing cells but also of higher eukaryotic organisms. The parallel description of the pro-aging role of homologs of Akt, S6 kinase, adenylate cyclase, and Tor in yeast and in higher eukaryotes, suggests that findings in the *S. cerevisiae* will be valuable to understand human aging and diseases. Moreover, the similarities between mitochondria and age-dependent mitochondrial damage in yeast and mammalian cells indicate that *S. cerevisiae* is a valuable model to study mitochondrial dysfunction and diseases that involve this organelle. Here, we describe the use of *S. cerevisiae* CLS in combination with three methods to quantify age-dependent mitochondrial damage and the accumulation of mitochondrial DNA mutations.

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1. Introduction

1.1. Saccharomyces cerevisiae as a model organism to study aging

As a unicellular eukaryote, *S. cerevisiae* has the advantage of being the simplest and shortest-lived organism among the major aging model systems (chronological mean life span is 6–15 days depending on the genetic background used; see section below). Because of the extensive genetic and molecular biology techniques available and wide characterization and often-conserved roles of its genes, baker's yeast is an ideal organism in which to model aging and diseases [1].

Increasing evidence points to similarities in age-dependent phenotypes including mitochondrial damage in yeast and mammalian cells. When limited to a non-fermentable carbon source, yeast is forced to obtain energy exclusively from mitochondrial respiration. The time yeast take to form a visible colony on a non-fermentable carbon source plate increases with age. In addition, the percentage of cells that can use non-fermentable carbon sources and therefore respiration for growth decreases with age [2]. Also, inactivation of the mitochondrial aconitase by superoxide increases with age in yeast [3,4] and in higher organisms [5]. Furthermore, both nuclear and mitochondrial DNA (mtDNA) mutations increase with aging in mammalian dividing cells [6] and post-mitotic cells [7] and preliminary evidences suggest that mtDNA mutations also increase in aging yeast (Longo et al., unpublished results).

Several methods are available to identify and quantify specific nuclear and mitochondrial DNA mutations on a variety of experimental settings in higher organisms [8–11]. Although the mouse is a very valuable organism to model diseases and study the effect of mutations on cancer and aging, its life spans is long, and the procedures to measure DNA mutations are complex. Therefore, yeast represents a powerful and simple system to complement more sophisticated models in the study of the role of DNA mutations in aging and diseases.

1.2. Chronological life span

Our laboratory introduced a method to measure life span in yeast based on the survival of populations of non-dividing yeast cells in a mostly high-metabolism non-dividing state, i.e., the chronological life span (CLS) [3,12,13]. This method differs from the replicative life span, which measures the reproductive potential of individual yeast mother cells [14], and better resembles the methods used to measure life span in higher eukaryotes. Experimentally, the organisms are grown in synthetic complete glucose medium (SDC), and kept in the same culture until at least 90% of the population has died. During the growth phase (approximately 10 h), energy is produced mainly by the fermentative catabolism of glucose. When most of the glucose is exhausted, the population switches to a prevalently respiratory metabolism which utilizes in part the ethanol produced during the growth phase. Yeast cells stop dividing after 24–48 h and survive for 5–7 days while

* Corresponding author. Fax: +1 213 821 5714.
E-mail address: vlongo@usc.edu (V.D. Longo).

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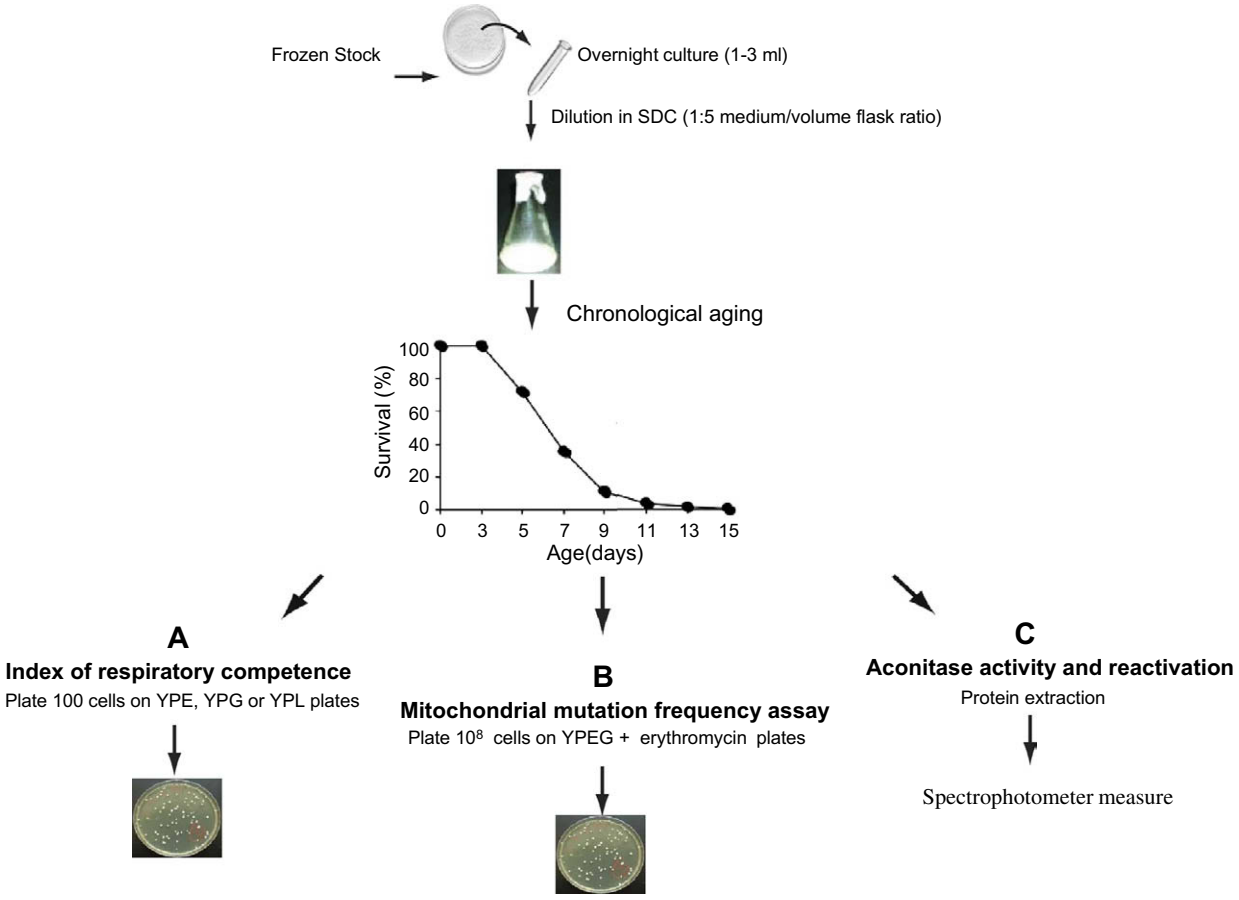

Fig. 1. Scheme representing the use of chronological life span to monitor mitochondrial damage. Few colonies are inoculated from a fresh plate in 1–3 ml of SDC. The overnight culture is diluted in 10–50 ml of SDC. Cell survival is determined every 2 days by diluting the yeast cultures and plating them onto rich-medium (YPD) plates to measure the colony forming units (CFUs). CFUs are counted after 2–3 days. Alternately the cells are switched to water and the cultures are washed every 2 days. (A) IRC. The same number of cells is harvested from the yeast culture, diluted and plated onto YPE, YPG or YPL. CFUs are counted after 2–3 days. The IRC is calculated based on the number of viable cells as measured by the CFUs assay. (B) Detection of mtDNA point mutations. yeast cells (10^8) are harvested from the SDC solution, washed and plated onto YPEG + erythromycin plates. The erythromycin resistant CFUs are scored after 10 days. Mutation frequency is calculated as the number of erythromycin resistant colonies/total number of colonies scored on YPD plates. (C) Aconitase activity. Aliquots of cells are harvested from the SDC cell culture and subjected to cellular lysis. The whole protein extract is used to measure spectrophotometrically the enzyme activity.

maintaining high metabolic rates for the first 3–4 days [15]. The aging organisms are maintained at 30 °C with shaking, and their viability is monitored by harvesting aliquots of cells at regular times and plating them onto rich-medium plates. The viability is scored by counting the number of cells able to form colonies but can also be measured by live/dead staining (FUN-1) or by measuring the proteins released by cells into the medium [15]. An additional advantage of the CLS, is that it mimics environmental conditions encountered by microorganisms in their natural environment, where they survive as non-dividing populations in the presence of limited external nutrients [16].

In addition, CLS studies can be performed in water by switching the cells from SDC medium to water after 72 h [13]. These experimental conditions induce a low metabolism stationary phase and represent a form of severe caloric restriction (CR) that allows the yeast to survive much longer than when maintained in SDC. In fact, the mean life of wild-type strain DBY746 in water is approximately 2–3 times longer than in SDC (15–20 days). These conditions also mimic extreme starvation situations encountered by yeast in nature. Both the paradigms (SDC or water) can be used for mitochondrial damage analysis. However, incubation in water is particularly useful for studies of mitochondrial function during aging, since it extends the monitoring of the index of respiratory competence (IRC, described in the following paragraphs) for several weeks and allows a better correlation between IRC and cell death [2].

1.3. CLS to model human mitochondrial diseases

Chronological life span has been used to study mitochondria-related diseases such as Friedreich's ataxia, an hereditary neurodegenerative disease associated with cardiomyopathy and diabetes. Deficiency of frataxin, a mitochondrial protein involved in the assembly of the iron–sulfur clusters and cellular anti-oxidant protection, seems to underlie this disease [17]. Isaya and co-workers employed a method similar to the one described in this manuscript to study the activity of the yeast frataxin [18]. By studying mutations that specifically impair ferroxidation of yeast frataxin and reduce chronological life span, they described a primary role for frataxin in iron detoxification, a crucial function to take in consideration while designing therapeutic strategies to treat Friederich's ataxia.

Our laboratory combined the chronological life span with several techniques to quantify the age-dependent mitochondrial damage and the accumulation of mtDNA mutations. In the following paragraphs, we present and discuss these methods: index of respiratory competence, erythromycin resistance assay, aconitase activity assay. A broad view of the steps currently in use in our group to perform these assays can be observed in Fig. 1.

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