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A simple model system for age-dependent DNA damage and cancer

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Summary

Aging is the major risk factor for many human cancers. However, the mechanisms responsible for the effect of aging on tumor incidence are poorly understood, in part because few model systems are available to study age-dependent genomic instability. Furthermore, the role of DNA mutations in “normal aging” and “life span extension” is unclear. Our laboratory has developed a novel method to study aging in yeast based on the survival of non-dividing populations (chronological life span). Two major pathways have been identified that control chronological aging: the Ras/PKA/Msn2/4 and the Sch9 pathways. The downregulation of either of them promotes life span extension. Importantly, similar pathways (insulin/IGF1-like), regulate longevity in higher eukaryotes suggesting a common evolutionary origin for the life span-regulatory mechanisms. Moreover, both Ras and Sch9 are functional homologs of two major mammalian oncogenes (Ras and Akt), which underlines the close link between cancer and aging. By combining chronological life span with simple assays for the detection of DNA mutations and dedifferentiation we have developed a powerful system to identify genes that regulate genomic instability and understand the fundamental mechanisms that may be responsible for age-dependent DNA mutations and cancer in mammals. Here we describe the use of this system to monitor the age-dependent accumulation of different types of DNA mutations including base substitutions, frame-shift mutations, and gross chromosomal rearrangements (GCRs).

1. Introduction

In developed countries cancer is the second-biggest cause of death and its occurrence increases greatly with age. Several mechanisms have been proposed to explain how aging promotes human cancer (Hasty and Vijg, 2002). Many of these rely on an age-dependent increase of DNA damage and/or reduction of genomic maintenance. However, a conclusive role for genomic instability in causing age-related cancer has yet to be established. Furthermore, the role of DNA mutations in the aging process is unclear and a role for DNA repair genes in life span extension has not been demonstrated. The understanding of the mechanisms that link age, genomic instability and cancer will be accelerated by the availability of simple experimental models to monitor DNA mutations during aging (Hasty and Vijg, 2002).

Our laboratory has developed a novel system to study aging in yeast based on measurements of the survival of populations of non-dividing yeast (chronological life span)(Fabrizio and

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Longo, 2003; Longo, 1997). This system has allowed us to identify two pathways whose downregulation extends the chronological life span: the Ras/PKA and Sch9 pathways. Notably, yeast Ras2 and Sch9 are functional homologs of mammalian Ras and Akt, which activate two of the major human oncogenic pathways. Furthermore, Ras and Akt are central components of signal transduction pathways activated by IGF-I, which accelerates aging in mice (Coschigano et al., 2000; Holzenberger et al., 2003). Thus, chronologically aging *S. cerevisiae* can serve as a simple and valuable model system to identify genes and pathways whose role in aging regulation may be conserved.

We have recently combined chronological life span with the analysis of different types of DNA mutations to start elucidating how aging may affect DNA stability. By monitoring the frequency of *CAN^r* mutants in chronologically aging yeast we demonstrated that both overexpression of antioxidant enzymes Sod1 and catalase and lack of Sch9 reduce age dependent genomic instability (Fabrizio et al., 2004). We have also shown that lack of both Sch9 and of the NAD-dependent histone deacetylase Sir2 promotes a further decrease in *CAN^r* mutants and a further extension in life span (Fabrizio et al., 2005). This was the first evidence for a pro-aging role of Sir2, in addition to its previously described anti-aging roles (Bitterman et al., 2003; Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001). Importantly, several similarities between mice lacking Sirt1, the closest mammalian Sir2 homolog, and the long-lived dwarf mice support the hypothesis that under certain conditions Sir2 may also play a pro-aging role in mammals (Longo and Kennedy, 2006). In analogy with yeast, Sirt1 might be contributing to genomic instability and cancer in mice.

The conservation of the aging pathways identified by using yeast chronological aging and the amenability of this aging paradigm to the measurement of specific types of DNA mutations provide a straightforward system to dissect the molecular mechanisms responsible for the age-dependent loss of DNA stability, which may be conserved and play a central role in human cancer. Hereafter, we describe the similarities between aging cells in yeast and mammals and the appearance of cancer-like phenotypes in chronologically aging yeast cultures. We also provide the detailed methods to monitor the accumulation of point mutations, small insertions/deletions, and GCRs in chronologically aging yeast.

2. Aging cells: similarities between yeast and mammals

Recent studies suggest that the regulation of longevity may be conserved in many organisms including mammals (Kenyon, 2001; Longo and Finch, 2003). Although aging itself may not have evolved, phases that minimize aging by maximizing protection against damage during starvation conditions may have evolved to optimize reproduction efficiency and accuracy. In fact, in the wild, microorganisms are commonly found in non-dividing phases and survive by slowly utilizing stored nutrients (Werner-Washburne et al., 1996). This might have been the origin of life span regulation in ancestral unicellular organisms. Thus, chronological life span, which is measured on yeast populations that have reached saturation after utilizing the limited amount of nutrients present in the incubation medium, represents a valuable paradigm to identify the molecular mechanisms that can slow down aging to overcome periods of starvation while protecting the germ-line (Fabrizio and Longo, 2003).

To further support the use of *S. cerevisiae* as a model for human aging is the fact that their cells are affected by similar damage during aging. In mammalian cells aging is associated with a decrease in function and with increased vulnerability to challenges. Age-dependent deteriorative changes and loss of cell function are also observed in non-dividing yeast: 1) the time to form a visible colony on a fermentable or non-fermentable carbon source plate, which forces yeast to obtain energy exclusively from respiration, increases with age (VL, unpublished results), 2) the percentage of cells that can utilize non-fermentable carbon sources and therefore

mitochondrial respiration for growth decreases with age (Longo et al., 1999) 3) the number of buds generated by a mother yeast cell (replicative life span) removed from stationary phase cultures decreases progressively with age in stationary phase (Ashrafi et al., 1999), 4) the inactivation of the superoxide target aconitase increases with age (Fabrizio and Longo, 2003; Fabrizio et al., 2001). 5) The number of mutations in the *CAN1* gene involved in arginine transport increases by up to five fold with chronological aging (Fabrizio et al., 2003). These data suggest that some of the age-dependent changes observed in yeast are analogous to those observed in mammalian cells. For example, human diploid fibroblasts and lymphocytes obtained from old individuals have a decreased replicative life span (Campisi, 1996) and mitochondrial damage accumulates in human post-mitotic cells (Yan et al., 1997). Furthermore, both nuclear and mitochondrial DNA mutations increase with aging in mammalian dividing cells (Arnheim and Cortopassi, 1992) and post-mitotic cells (Finch and Goodman, 1997).

3. Cancer-like age-dependent dedifferentiation in yeast

Analogously to the exponential increase of cancer incidence observed in human aging, cancer-like mutant cells are generated within aging *S. cerevisiae* cultures. The presence of such dedifferentiated mutants is normally easily detected since they acquire the ability to divide by metabolizing nutrients released by dead cells and viability counts can increase by up to 100 fold (Fabrizio et al., 2004). However, depending on either the genetic background or the specific mutants examined, the occurrence of age-dependent dedifferentiation can be more elusive. Usually, subpopulations of cells are considered to be dedifferentiated if the viability count during the high mortality phase (days 7-17) in the liquid culture, either increases by at least 50% or does not decrease in 3 consecutive counts (6 days) by day 17, according to the Gompertz formula (Finch, 1990). In our previous studies, we demonstrated that the frequency of this cancer-like phenotype doubles in cells lacking cytosolic superoxide dismutase and is greatly reduced in long-lived mutants or mutants overexpressing superoxide dismutases (Fabrizio et al., 2004). In fact, yeast lacking “oncogenes” Sch9 and Ras2 did not show any age-dependent dedifferentiation in a vast number of experiments (Fabrizio et al., 2004) suggesting that yeast regrowth represents a useful phenotype to study the age-dependent effect of mutations associated with cancer.

The appearance of dedifferentiated cells in the late phases of a chronological life span experiment is often associated with the presence of cells that are not properly G1-arrested in the early phases of aging (day 1-4). The percent of cells that fails to undergo G1-arrest might predict the appearance of the dedifferentiation phenotype. In fact, a higher number of G1-arrest deficient cells may correlate with a higher probability to observe dedifferentiation and be associated with mutations in check-point proteins and/or DNA-repair enzymes (FM, unpublished results). Furthermore, these budded cells, whose DNA is replicated or partially replicated, are likely to contribute to the accumulation of age-dependent DNA mutations in cells with defective DNA-repair systems.

4. Monitoring the age-dependent mutation frequency *S.cerevisiae*

Among the major model systems available to study aging, *S.cerevisiae* has the advantage to be the simplest and shortest-lived (mean life span is 6-15 days depending on the genetic background used). Furthermore, classic yeast genetics and newer molecular biology techniques are available to detect the age-dependent appearance of several types of spontaneous mutations. More complex systems commonly used for aging research are *C. elegans* and *Drosophila*. Although they have played a pivotal role in the identification of genes responsible for life span regulation and they can be used to study tumorigenesis (Pinkston et al., 2006), they have limited use in the identification and quantification of specific age-dependent DNA mutations since

their cells are post-mitotic. Although mice are clearly a powerful model system to study age-dependent cancer (Hasty and Vijg, 2002), they have a very long life span and the procedures to knock out and overexpress genes or measure DNA damage are complex and lengthy. Thus, *S. cerevisiae*'s chronological life span represents a powerful and simple system that can complement the mouse model system in the investigation of the role of DNA mutations in aging and cancer.

4.1 Detection of a wide spectrum of mutations by monitoring *CAN^r* mutations

The *CAN1* gene encodes for a high affinity arginine permease involved in the uptake of arginine but also of its analog canavanine, which is toxic to the cells at the concentration of 60 mg/L. Since canavanine is a competitive inhibitor, arginine must be excluded from the medium used to test the sensitivity to the drug (Guthrie and Fink, 1991). Any Arg⁺ yeast strain carrying a wild type *CAN1* gene is canavanine sensitive (Can^s) and can be used for this assay. When a cell acquires a mutation that inactivates *CAN1* it gains the ability to form a colony on minimal medium containing canavanine. This assay has been used extensively for studies of DNA-repair and the most frequent spontaneous *CAN1* mutations that occur in exponentially growing cells have been characterized (Chen et al., 1998). The majority of the *CAN1* mutations have been reported to be point mutations (65%) or frameshifts (25%). However, a number of complex mutational events have also been detected (10%) (Chen et al., 1998).

In a standard chronological life span study, yeast are diluted in minimal complete medium (SDC) from an overnight culture and incubated at 30°C with shaking. After 3 days, the majority of the population stops dividing and the number of viable cells reaches a plateau (100% survival). The viability of the culture is normally monitored starting from day 3 every 2 days by plating appropriate dilutions on rich medium plates (YPD) and counting the colony forming units (CFUs) (Fabrizio and Longo, 2003). To measure the frequency of Can^r resistant mutants in a chronologically aging culture ~2 10⁷ viable cells are harvested, washed with water twice, and plated on selective medium (SDC-ARG, 60 mg/l L-canavanine sulfate) every 2 days (Fig. 1-2). The mutation frequency is calculated on the number of viable cells, which is obtained as described above. Normally we observe a gradual age-dependent increase in *CAN1* mutations which reaches a 5-fold increase by the time wild type yeast (DBY746, BY4741) reaches ~10% survival (Fig.1).

4.2 Detection of base substitutions by monitoring Trp⁺ mutants

To limit the range of mutational events detected to single point mutations, the frequency of reversion of any amber or ochre mutation can be monitored. We normally use strain DBY746 that carries a *trp1*-289 amber mutation (C → T at residue 403 of the coding sequence) and measure the frequency of *trp1*-289 to Trp⁺ reversions (Capizzi and Jameson, 1973). The experiment is conducted similarly to that described above for the Can^r mutations. Starting from day 3 of a chronological longevity experiment, ~10⁸ viable cells are harvested, washed with water twice and plated on selective medium (SDC-TRP) (Fig.2). Viability is measured as described above and used to calculate the Trp⁺ mutation frequency. Trp⁺ mutants are usually an order of magnitude fewer than Can^r mutants and also increase ~5 fold by the time the majority of the wild type population has died.

4.3 Detection of frameshift mutations by monitoring Lys⁺ mutants

A few auxotrophic yeast strains have been engineered to allow the detection of frameshifts that reestablish prototrophy. Among these are strains carrying the *lys2ΔBgIII* allele, which was generated by filling-in a BglIII restriction site at the *LYS2* locus. This manipulation leads to a +4 shift in the coding sequence that confers lysine auxotrophy and was used to characterize the frameshift events that restore prototrophy during the cell cycle or in non-dividing cells (Steele and Jinks-Robertson, 1992). When the cells are G0 arrested the vast majority of the

frameshift mutations is due to small deletions in mononucleotide repeats (Heidenreich et al., 2003). Furthermore, 50% of the frameshifts is caused by the error-prone activity of the non-homologous end-joining (NHEJ) repair pathway (Heidenreich et al., 2003).

Our method to measure age-dependent frameshift mutations has been adapted from that originally described by Steele and Jinks-Robertson to study adaptive reversion (Steele and Jinks-Robertson, 1992). Yeast strain EH150 carrying the *lys2ΔBgIII* allele is inoculated in YPD and grown overnight. 10^8 cells are washed with water twice, plated on a selective SDC-LYS plate and incubated at 30°C for the mutation assay. Lys⁺ colonies that appear within 3 days from the plating on SDC-Lys are those originated from revertants appeared during the growing phase (Heidenreich et al., 2003). The Lys⁺ colonies that are scored from day 4 are those originated from the G0 arrested cells. The frequency of age-dependent frameshift reversions is measured every day by dividing the cumulative number of colonies present on the plate by the number of viable cells (Fig.2).

4.4. Age-dependent gross chromosomal rearrangements (GCRs)

To monitor the appearance of mutants that carry GCRs during chronological aging we have generated a DBY746 derivative in which we replaced the gene *HXT13* (coding for a highly redundant hexose transporter) with a *URA3* cassette, as described by Chen and Kolodner (Chen and Kolodner, 1999). *HXT13* is located ~7.5 kb telomeric to *CAN1* on chromosome V. The assay to detect GCRs is performed by washing ~1-2 10^8 cells with water twice and plating them on SDC-ARG containing canavanine (60mg/L) and 5FOA (1mg/L), which allows the growth of mutants that have lost both the *CAN1* and *URA3* genes (Can^r 5FOA^r) (Fig.2). In the majority of the cases examined these mutants arise from the loss of the whole DNA region containing *CAN1* and *URA3* that can be replaced by a telomeric sequence or regions from chromosome V or other chromosomes (Chen and Kolodner, 1999). The frequency of GCRs (calculated based on the viable cells) in chronologically aging yeast is also subject to an age-dependent increase and is dependent on the activity of several DNA-repair genes (FM, unpublished results).

5. Conclusions

In our previous work we have shown that the downregulation of the Sch9 pathway in yeast extends life span by 3 fold and is associated with a decrease of age-dependent mutation frequency and dedifferentiation (Fabrizio et al., 2004). The much reduced or delayed cancer-like phenotypes during chronological aging in *sch9* mutants and other long-lived mutants suggest that the regulation of life span, genomic instability, and dedifferentiation are tightly linked.

Here we have described how to use yeast carrying specific alleles to monitor different types of mutations, including frameshifts and GCRs, during aging. This system allows the study of the impact of any gene, which can be deleted, downregulated, or overexpressed, on age-dependent mutation accumulation and cancer-like dedifferentiation. This system is also useful for the understanding of the role of acute or chronic treatment with any drug or toxin on age-dependent genomic instability and cancer-like phenotypes. Further studies using this system should allow the identification of the fundamental molecular mechanisms that cause age-dependent genomic instability and of the network of genes that regulate it. The role of *SCH9* and *RAS* in the regulation of age-dependent cancer-like phenotypes in *S. cerevisiae* and of activated forms of homologs of *SCH9* and *RAS* in many human cancers, suggest that the simple yeast paradigm described here, may reveal novel and fundamental new insights on the mechanisms responsible for human cancers.

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References

- Arnheim N, Cortopassi G. Deleterious mitochondrial DNA mutations accumulate in aging human tissues. *Mutation Research* 1992;275:157–67. [PubMed: 1383758]
- Ashrafi K, Sinclair D, Gordon JI, Guarente L. Passage through stationary phase advances replicative aging in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 1999;96:9100–9105. [PubMed: 10430902]
- Bitterman KJ, Medvedik O, Sinclair DA. Longevity regulation in *Saccharomyces cerevisiae*: linking metabolism, genome stability, and heterochromatin. *Microbiol Mol Biol Rev* 2003;67:376–99. [PubMed: 12966141]table of contents
- Campisi J. Replicative senescence: an old lives' tale? *Cell* 1996;84:497–500. [PubMed: 8598035]
- Capizzi RL, Jameson JW. A table for the estimation of the spontaneous mutation rate of cells in culture. *Mutat Res* 1973;17:147–8. [PubMed: 4682600]
- Chen C, Kolodner RD. Gross chromosomal rearrangements in *Saccharomyces cerevisiae* replication and recombination defective mutants. *Nat Genet* 1999;23:81–5. [PubMed: 10471504]
- Chen C, Umez K, Kolodner RD. Chromosomal rearrangements occur in *S. cerevisiae* *rfa1* mutator mutants due to mutagenic lesions processed by double-strand-break repair. *Mol Cell* 1998;2:9–22. [PubMed: 9702187]
- Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology* 2000;141:2608–13. [PubMed: 10875265]
- Fabrizio P, Battistella L, Vardavas R, Gattazzo C, Liou LL, Diaspro A, Dossen JW, Gralla EB, Longo VD. Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*. *J Cell Biol* 2004;166:1055–67. [PubMed: 15452146]
- Fabrizio P, Gattazzo C, Battistella L, Wei M, Cheng C, McGrew K, Longo VD. Sir2 blocks extreme life-span extension. *Cell* 2005;123:655–67. [PubMed: 16286010]
- Fabrizio P, Liou LL, Moy VN, Diaspro A, SelverstoneValentine J, Gralla EB, Longo VD. SOD2 Functions Downstream of Sch9 to Extend Longevity in Yeast. *Genetics* 2003;163:35–46. [PubMed: 12586694]
- Fabrizio P, Longo VD. The chronological life span of *Saccharomyces cerevisiae*. *Aging Cell* 2003;2:73–81. [PubMed: 12882320]
- Fabrizio P, Pozza F, Pletcher SD, Gendron CM, Longo VD. Regulation of longevity and stress resistance by Sch9 in yeast. *Science* 2001;292:288–90. [PubMed: 11292860]
- Finch, CE. Longevity, Senescence, and the Genome. University Press; Chicago: 1990.
- Finch CE, Goodman MF. Relevance of 'adaptive' mutations arising in non-dividing cells of microorganisms to age-related changes in mutant phenotypes of neurons. *Trends Neurosci* 1997;20:501–7. [PubMed: 9364662]
- Guthrie, C.; Fink, GR. Guide to Yeast Genetics and Molecular Biology. In: Abelson, JN.; Simon, MI., editors. In *Meth. Enzymol.* 194. Academic Press, Inc.; San Diego: 1991. p. 931
- Hasty P, Vijg J. Aging. Genomic priorities in aging. *Science* 2002;296:1250–1. [PubMed: 11951000]
- Heidenreich E, Novotny R, Kneidinger B, Holzmann V, Wintersberger U. Non-homologous end joining as an important mutagenic process in cell cycle-arrested cells. *Embo J* 2003;22:2274–83. [PubMed: 12727893]
- Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloën A, Even PC, Cervera P, Le Bouc Y. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 2003;421:182–7. [PubMed: 12483226]
- Kenyon C. A conserved regulatory system for aging. *Cell* 2001;105:165–8. [PubMed: 11336665]
- Longo, V. Studies of superoxide dismutase, Ras and Bcl-2. University of California; Los Angeles: 1997. The chronological life span of *Saccharomyces cerevisiae*. Thesis

- Longo VD, Finch CE. Evolutionary Medicine: from Dwarf Model Systems to Healthy Centenarians. *Science* 2003;299:1342–1346. [PubMed: 12610293]
- Longo VD, Kennedy BK. Sirtuins in aging and age-related disease. *Cell* 2006;126:257–68. [PubMed: 16873059]
- Longo VD, Liou LL, Valentine JS, Gralla EB. Mitochondrial Superoxide Decreases Yeast Survival in Stationary Phase. *Arch. Biochem. Biophys* 1999;365:131–142. [PubMed: 10222047]
- Pinkston JM, Garigan D, Hansen M, Kenyon C. Mutations that increase the life span of *C. elegans* inhibit tumor growth. *Science* 2006;313:971–5. [PubMed: 16917064]
- Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci U S A* 2004;101:15998–6003. [PubMed: 15520384]
- Steele DF, Jinks-Robertson S. An examination of adaptive reversion in *Saccharomyces cerevisiae*. *Genetics* 1992;132:9–21. [PubMed: 1398066]
- Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* 2001;410:227–30. [PubMed: 11242085]
- Werner-Washburne M, Braun EL, Crawford ME, Peck VM. Stationary phase in *Saccharomyces cerevisiae*. *Mol. Microbiol* 1996;19:1159–66. [PubMed: 8730858]
- Yan LJ, Levine RL, Sohal RS. Oxidative damage during aging targets mitochondrial aconitase [published erratum appears in *Proc Natl Acad Sci U S A* 1998 Feb 17;95(4):1968]. *Proc. Natl. Acad. Sci. U S A* 1997;94:11168–72. [PubMed: 9326580]

Fig. 1. Age-dependent increase in *CAN1* mutation frequency. The appearance of Can^r mutants in chronologically aging yeast is monitored by plating 2×10^7 cells on SDCARG+canavanine (60mg/mL) every 2 days. Canavanine resistance is mostly caused by base substitutions or frameshift mutations in the *CAN1* gene.

Fig.2.

Scheme representing the use of chronological life span to monitor age-dependent mutation frequency. A. Detection of point mutations+frameshifts+complex mutational events: 2×10^7 cells are plated on SDC-ARG+canavanine every 2 days. Can⁺ mutation frequency is calculated based on the number of viable cells as measure by CFUs assay. B. Detection of point mutations: 10^8 cells are plated on SDC-TRP every 2 days. Trp⁺ colonies growth is caused by reversion of an amber stop codon (corresponding to residue 135 of the coding sequence). C. Detection of GCRs: 10^8 cells are plated on SDCARG+canavanine+5FOA every 2 days. Can⁺ 5FOA⁺ cells growth is mostly caused by loss of a large region of DNA that contains both the *CAN1* and the *URA3* genes (Chen and Kolodner, 1999).

D. Detection of frameshift mutations: 10^8 cells are plated starting from a YPD overnight inoculum on SDC-LYS. Lys+ colonies are scored every day and their growth is due mostly to small insertions (2 or 2+3n) or small deletions (1 or 1+3n). The frequency of frameshifts is calculated every day by dividing the cumulative number of colonies on the plate by the number of viable cells.