Cell Cycle News & Views

Chronological and replicative lifespan in yeast: Do they meet in the middle?

Comment on: Murakami C, et al. Cell Cycle 2012; 11:3087–96; PMID:22871733; http://dx.doi.org/10.4161/cc.21465

Michael Polymenis1 and Brian K. Kennedy2,3; 1Department of Biochemistry and Biophysics; Texas A&M University; College Station, TX USA; 2Buck Institute for Research on Aging; Novato, CA USA; 3Aging Research Institute; Guangdong Medical College; Dongguan, China; Email: polymenis@tamu.edu and bkennedy@buckinstitute.org; http://dx.doi.org/10.4161/cc.22041

Budding yeast is a preeminent model organism in studies of cellular aging pathways that are conserved in eukaryotes, including humans. There are two primary ways to query the lifespan of this organism.1 If one asks how many times a cell can divide, the answer will be its replicative lifespan (RLS). If, on the other hand, one asks how long a cell can stay alive without dividing, the answer will be its chronological lifespan (CLS).

Budding yeast is a facultative aerobe with exceptional genetic tractability. Hence, many environmental and genetic factors are known to affect replicative and chronological lifespan. Since the context of the RLS and CLS assays is different, with dividing vs. non-dividing cells, it is not immediately obvious whether these factors should be overlapping. The results to date are ambiguous. Some well-studied interventions like dietary restriction as well as reduced TOR and protein kinase A signaling, extend both replicative and chronological lifespan.1 However, in a quantitative comparison of gene deletions that extend lifespan in both assays, no significant overlap was observed.2

Further complicating the issue is the differences in methodology regarding chronological aging. When performed in synthetic-defined complete (SDC) media, it was recently shown that acidification of the medium during the growth phase accelerates mortality.1 Lifespan is extended by buffering the culture medium to pH 6.0 or performing the experiment in rich YEPD medium, which is more refractory to acidification. Interestingly, a recent report indicates that media acidification may be a limiting component to long-term survival of non-proliferative mammalian cells as well.3 To what extent acidification accelerates normal CLS aging mechanisms and whether it relates at all to replicative aging remain unknown.

Instead of focusing on the factors that are shared, or not, between CLS and RLS pathways, a different way to probe the relationship between CLS and RLS is to examine how one aging process affects the other. More than a decade ago, it was reported that the longer cells age chronologically, the fewer times they can divide when nutrients are restored.3

A new study by Murakami et al.4 found that chronologically aged cells had a reduced replicative lifespan, confirming the earlier report.4 In addition to replicating the initial study, in which the CLS portion of the assay was performed in YEPD, this study compared three CLS conditions: YEPD, SDC and buffered SDC, finding that replicative lifespan is dramatically shortened in the SDC conditions associated with acidification. These findings indicate that conditions associated with acidification and rapid chronological aging impact the replicative lifespan of the cells, suggesting that the consequences of acidification are related to those of slower aging in YPD and possibly replicative aging as well.

Murakami et al. went further. The CLS to RLS transition is essentially a transition from a non-dividing state, to a dividing one. Hence, querying parameters associated with cell cycle progression ought to be pertinent for the CLS to RLS transition. Indeed, Murakami et al. found that cells with the greatest replicative potential after quiescence were smaller and arrested properly in the G1 phase of the cell cycle, before DNA replication. These results further support the significance of G1 control mechanisms in aging.5,6 Why would cells that are chronologically aged have a reduced replicative lifespan? All cells in a quiescent population would be exposed to damage, either due to acidification or other causes. However, the authors note that once the population reenters a proliferative state, this damage may stay with the mother cells. This would “free up” the daughters, maximizing the fitness of the population as a whole. This model is appealing and far-reaching. Cycles of quiescent and proliferative states are the norm not only for single-celled organisms in the wild, but also for cells in animal tissues.

Many questions remain. For example, what is the mechanistic basis for the interventions...
Acetic acid and acidification accelerate chronological and replicative aging in yeast

Comment on: Murakami C, et al. Cell Cycle 2012; 11:3087–96; PMID:22871733; http://dx.doi.org/10.4161/cc.21465

Yeast is widely regarded as one of the most valuable model systems to study aging and particularly the genetics of aging. Researchers have established two different methods to study yeast aging known as the replicative lifespan (RLS) and the chronological lifespan (CLS). These have led to the identification of many mammalian genes that affect aging suggesting that they will continue to shed light on the fundamental biology of aging. In spite of the clear differences underpinning the mitotic cellular potential (RLS) and the survival in the non-dividing mode (CLS), the two models are clearly regulated by partly overlapping regulatory mechanisms. This idea is supported by the observation that chronologically aged diploid cells show decreased replicative lifespan proportional to the duration of the chronological aging. Even though this is generally agreed to be true, very few attempts have been made to integrate both models in a comprehensive manner. Furthermore, while mutations that affect Ras-cAMP-PKA or TOR/Sch9 signaling increase both the replicative and chronological lifespan, other genes appear to affect lifespan in only one of the two models indicating that partially distinct mechanisms affect the two aging processes.

In the August 15 issue of Cell Cycle, Matt Kaeberlein and coworkers1 present very interesting data, which help to fill the gap between the two aging model systems. They confirm1 that diploid chronologically aged yeast cells have a reduced replicative lifespan with respect to chronologically younger cells and show that pH and media composition (YPD or SDC) during the chronological aging phase, play a role in this phenomenon. S. cerevisiae, grown in 2% dextrose and excess amino acids, the media used in most chronological lifespan experiments, produces both ethanol and acetic acid as a normal end product of alcoholic fermentation which is accompanied by a drop in media pH to below 4.4 It has also been demonstrated that the level of protein oxidation may be acetic acid-dependent and not simply pH-dependent.1 In addition, intracellular acidification increases Ras signaling as well as ROS production, linking acidification to nutrient signaling pathways. These results are paralleled by the observations that mammalian tumor cells, maintained in stationary culture, lose viability by lactate media acidification4 indicating that acidification may have a conserved role in accelerating cellular aging. Together with previous studies, the work by Murakami et al. support two important conclusions; (1) acidification accelerates chronological aging, an effect which may be conserved in higher eukaryotes and that acetic acid does not simply function as a molecule with a toxic and “private” effect but as a carbon source that causes an expected pro-aging effect. In agreement with this conclusion the authors here speculate that asymmetric segregation of chronologically aged cellular components. Asymmetric inheritance during cell division is of general interest and has long been debated. In budding yeast, buds show the same mitotic potential with no respect to the mother cell age. It has also been demonstrated that carbonylated proteins, DNA circles and old mitochondrial aconitase remain confined to the aging mother cell. Mechanisms implying the involvement of septin, nuclear pore segregation and the involvement of Sir2 have been postulated.9

5. Ashrafi K, et al. Proc Natl Acad Sci USA 1999; 96:9100-5; PMID:10430902; http://dx.doi.org/10.1073/pnas.96.9100.
6. Murakami C, et al. Cell Cycle 2012; 11:3087-96; PMID:22871733; http://dx.doi.org/10.4161/cc.21465.
7. Delaney JR, et al. Cell Cycle 2011; 10:156-65; PMID:21191185; http://dx.doi.org/10.4161/cc.10.1.14457.
8. Yang J, et al. Cell Cycle 2011; 10:144-55; PMID:21248481; http://dx.doi.org/10.4161/cc.10.1.14455.
9. Wei M, et al. PLoS Genet 2009; 5:e1000467; http://dx.doi.org/10.1371/journal.pgen.1000467; PMID:19424415.

References

1. Longo vD, et al. Cell Metab 2012; 16:18-31; http://dx.doi.org/10.1016/j.cmet.2012.06.002; PMID:22768386.
2. Burntner, C. R., et al. Cell Cycle 2011; 10:1385-96.
3. Burntner, C. R., et al. Cell Cycle 2009; 8:1256-1270.
4. Leonieva OV, et al. Aging (Albany NY) 2011; 3:1078-91; PMID:22156391.
states and the damage accumulated in the non-dividing mode may be altruistically confined to mother cells when the cell starts dividing again.

In summary, this is a valuable study solidifying the overlap between yeast replicative and chronological aging and providing strong evidence for the role of acetic acid and acidification as accelerators of the yeast aging process, which may be more relevant to mammalian aging than expected.

References

1. Ashrafi K, et al. Proc Natl Acad Sci USA 1999; 96:9100-5; PMID:10430902; http://dx.doi.org/10.1073/pnas.96.16.9100.
2. Murakami C, et al. Cell Cycle 2012; 11.
3. Burtner CR, et al. Cell Cycle 2009; 8:1256-70; PMID:19305133; http://dx.doi.org/10.4161/cc.8.8.2287.
4. Fabrizio P, et al. J Cell Biol 2004; 166:1055-67; PMID:15452146; http://dx.doi.org/10.1083/jcb.200404002.
5. Semchyshyn HM, et al. Redox Rep 2011; 16:15-23; PMID:21605494; http://dx.doi.org/10.1179/174329211X12968219310954.
6. Colombo S, et al. EMBO J 1998; 17:3326-41; PMID:9628870; http://dx.doi.org/10.1093/emboj/17.12.3326.
7. Burhans WC, et al. Cell Cycle 2009; 8:2300-2; PMID:19502784; http://dx.doi.org/10.4161/cc.8.14.8852.
8. Leontieva, et al. Aging (Albany, NY Online) 2011; 3:1078-91.
9. Orlandi I, et al. Biochim Biophys Acta 2010; 1803:630-8; PMID:20211662; http://dx.doi.org/10.1016/j.jbba.2010.02.009.
10. Barbosa C, et al. J Biosci Bioeng 2009; 108:99-104; PMID:19619854; http://dx.doi.org/10.1016/j.jbiosc.2009.02.017.
DNA double strand breaks (DSBs) are the most deleterious form of DNA damage as unrepaird or misprocessed DSBs can lead to genomic instability and ultimately cancer. To counter the deleterious nature of DSBs, cells have developed a number of pathways which repair DSBs with the two most prominent being non-homologous end-joining (NHEJ) and homologous recombination (HR). The cellular response to DSBs requires efficient recognition of the damaged DNA, signal transduction pathways, activation of cell cycle checkpoint controls and repair pathway selection. At the heart of the cellular response to DSBs is the DNA damage response (DDR), which mediates DNA damage-induced signal transduction via activation of a large number of protein kinases. Phosphorylation mediated by these protein kinases is required for the transmission, and coordination of the DDR and the direct targets include the sensors of the DSBs, DDR signaling mediators, checkpoint control transducers and effectors, repair proteins, histones and chromatin modifiers.

While the role of protein kinases on the repair process have been well-documented, the role of serine/threonine protein phosphatases (PPs) in the DDR and the DSB repair process have only recently started to be uncovered. A number of PPs have been implicated in regulating the phosphorylation status of DDR proteins and HR, including PP1, PP2A, PP4, PP6 and Wip1. Direct substrates include initial sensors of the DSB (Ku70/Ku80), DDR signaling mediators (BRCA1), checkpoint control transducers and effectors (CHK1, CHK2 and p53), repair proteins (RPA), histones (γH2AX) and the DSB activated kinases (ATM, DNA-PKcs and ATR) implicating that reversible phosphorylation mediated by PPs plays an important role in the cellular response to DSBs.

In a report by Li et al. in Cell Cycle, the Xu group continued their studies on the role that protein phosphatase 4 (PP4) plays in DSB repair as they had previously shown that PP4 is required for HR. As a number of NHEJ factors are phosphorylated in response to DSBs, in this study the Xu group determined if PP4 played a role in NHEJ. Using established in vivo NHEJ assays, the authors showed that the PP4 catalytic subunit (PP4c) and its catalytic activity are required for NHEJ. The PP4 holoenzyme occurs in different assemblies of the catalytic subunit with one or more regulatory subunits. Knockdown of each of the PP4 regulatory subunits with specific siRNAs showed that PP4R2, but not PP4R3 or PP4R3β-containing, holoenzymes are involved in the regulation of NHEJ. Phosphorylation of KAP1 at S824 impacts repair of DSBs within heterochromatin by promoting chromatin relaxation to allow repair proteins access to the DSB. The authors then assessed if KAP1 was a substrate of PP4. Knockdown of PP4C or PP4R2 resulted in an increase in KAP1 phosphorylation at S824 following DNA damage. PP4 dephosphorylated KAP1 in vitro further implicating that KAP1 is a PP4 substrate. Finally, knockdown of KAP1 resulted in a decrease in NHEJ but co-depletion of PP4 and KAP1 did not have a synergistic effect on NHEJ suggesting that PP4 and KAP1 are in the same NHEJ epistasis group. Together, the data implicates that PP4’s ability to regulate NHEJ is through its ability to regulate KAP1 phosphorylation.

Many new interesting questions are raised by this study: (1) does PP4 play a role in all end-joining pathways or a specific one; (2) KAP1 dephosphorylation by PP4 should result in chromatin condensation, is this important for NHEJ; (3) a recent manuscript also found that PP4 dephosphorylates KAP1 at S824 but this was due to a holoenzyme with PP4R3β, is dephosphorylation of KAP1 by different PP4 holoenzymes regulated; (4) PP4c knockdown results in a greater decrease in NHEJ than KAP1 implicating that PP4 may have other NHEJ substrate(s), what are these substrates and what role do they play in regulating NHEJ. Together, it will be of great interest to continue to identify the role(s) that PP4 plays in regulating NHEJ.
References

1. Hoeijmakers JH. Nature 2001; 411:366-74; PMID:11357144; http://dx.doi.org/10.1038/35077232.

2. Polo SE, et al. Genes Dev 2011; 25:409-33; PMID:21363960; http://dx.doi.org/10.1101/gad.2021311.

3. Peng A, et al. Oncogene 2010; 29:5977-88; PMID:20838380; http://dx.doi.org/10.1038/onc.2010.371.

4. Liu J, et al. Cell Cycle 2012; 11:2643-9; PMID:22732494; http://dx.doi.org/10.4161/cc.20957.

5. Chowdhury D, et al. Mol Cell 2008; 31:33-46; PMID:18614045; http://dx.doi.org/10.1016/j.molcel.2008.05.016.

6. Ziv Y, et al. Nat Cell Biol 2006; 8:870-6; PMID:16862143; http://dx.doi.org/10.1038/ncb1446.

7. Goodarzi AA, et al. DNA Repair (Amst) 2010; 9:1273-82; PMID:21036673; http://dx.doi.org/10.1016/j.dnarep.2010.09.013.

8. Lee DH, et al. EMBO J 2012; 31:2403-15; PMID:22491012; http://dx.doi.org/10.1038/emboj.2012.86.