

## Hypothesis: cell volume limits cell divisions

Tomasz Biliński and Grzegorz Bartosz✉

Department of Biochemistry and Cell Biology, University of Rzeszów, Rzeszów, Poland;

✉e-mail: bilinski@univ.rzeszow.pl

Received: 10 June, 2006; revised: 25 September, 2006; accepted: 09 October, 2006

available on-line: 14 November, 2006

Mammalian somatic cells and also cells of the yeast *Saccharomyces cerevisiae* are capable of undergoing a limited number of divisions. Reaching the division limit is referred to, apparently not very fortunately, as replicative aging. A common feature of *S. cerevisiae* cells and fibroblasts approaching the limit of cell divisions *in vitro* is attaining giant volumes. In yeast cells this phenomenon is an inevitable consequence of budding so it is not causally related to aging. Therefore, reaching a critically large cell volume may underlie the limit of cell divisions. A similar phenomenon may limit the number of cell divisions of cultured mammalian cells. The term replicative (generative) aging may be therefore illegitimate.

**Keywords:** yeast, *Saccharomyces cerevisiae*, replicative aging, cell volume

The existence of a limit of the number of cellular divisions, referred to as generative aging, is common to somatic cells of Metazoa and budding yeasts. Studies of this phenomenon revealed fundamental differences between these cell types. In the case of somatic metazoan cells, the whole population dies (though not synchronically). In the budding yeast, only a mother cell dies eventually while the whole population is immortal. Current views ascribe the limits of divisions of these cell types to different mechanisms: telomere shortening in somatic cells and accumulation of a senescence factor in the yeast (Guarente, 2000). In spite of the different mechanisms underlying generative aging, the yeast has been assumed to be a valid model for the aging of Metazoa.

It is worthwhile to consider whether the limitation of cell divisions is ubiquitous and inevitable in the living world or is only accidental in some groups of organisms. If it was truly universal, the life on our planet would have to cease; therefore, it must be specific to some cell types and some situations, e.g. being a side effect of a chosen developmental strategy.

If one were to assume that the Hayflick limit of mammalian cells is due to the inactivation of telomerase and is beneficial to the organism, the

biological advantage of limitations of yeast cell divisions is difficult to see. Why does this feature persist? Cells which have lost their ability to divide constitute a negligible fraction of yeast population so they do not affect the growth of the population. Non-separated yeast populations contain slightly more than 50% of virgin cells, about 25% of cells which have accomplished one division, about 12% of cells which have divided two times, etc., so that 99% of the population are cells which have accomplished not more than 5 divisions, i.e. they still have about 30 divisions to reach the division limit.

One may wonder whether the limited number of yeast cell divisions warrants employing the budding yeast as a model organism to explain human aging. There is an obvious danger that studies of the mechanisms limiting the number of yeast cell divisions will bring explanations valid only for the yeast!

A common feature of senescent fibroblasts cultured *in vitro* and an old yeast mother cell is the enormous increase in cell volume, exceeding several times the initial volume. This may indicate that the giant volume is either a symptom or a consequence of the process of replicative aging of both cell types. However, an opposite relationship is also possible: the excessive cell volume may be the reason for the

limitation of cell divisions. This hen-end-egg problem could be resolved if one could prove that attaining giant cell volumes is due to mechanisms not related to aging, a process which may require much longer time to be revealed.

What are the proposed reasons for the limit of cell divisions of the budding yeast? Yeast has an active telomerase and its telomeres do not shorten with cell divisions. The accumulation of a "senescence factor" in the yeast mother cell has been therefore postulated. This factor has been identified with extrachromosomal rDNA circles (Sinclair & Guarente, 1997) or oxidized proteins (Aguilaniu *et al.*, 2003). However, yeast cells may also reach the division limit without accumulation of rDNA circles (Ashrafi *et al.*, 1999) while the number of attainable cell divisions does not increase under anoxia (Wawryn *et al.*, 2002) where oxidative damage does not occur. The accumulation of a "senescence factor" is attributed to the asymmetry of cell divisions (Jazwinski *et al.*, 1989) or a mechanism retaining oxidized proteins in the mother cell (Aguilaniu *et al.*, 2003).

The asymmetry of cell division is thought to be the main factor deciding whether cells of a given type age (Gillespie *et al.*, 2004). In most cases, cells divide into two approximately equal progenitor cells during the M phase of the cell cycle. Cells of the fission yeast *Schizosaccharomyces pombe* divide symmetrically at the beginning of the G1 phase; but cases of asymmetrical divisions have been reported, which result in a finite ability to divide (Barker & Walmsley, 1999). In the budding yeast, the smaller daughter cell emerges from the mother cell, as an independent compartment, already in the beginning of the S phase. At the end of the cell cycle, only separation of these two independent compartments takes place. This difference between true cell divisions and budding is crucial for the understanding of the reason of the inevitable increase of the volume of the mother cell. Due to intensive synthesis in the G1 phase, cell growth must lead to the increase in the volume of the mother cell (Hartwell & Unger, 1977) since the daughter cell does not exist yet! An increase in the volume of the mother cell on each cell division is inevitable because there is no mechanism of its reduction in the M phase. This increase in cell volume is therefore due to its mode of multiplication and not to aging.

In fibroblasts, "unbalanced growth" resulting from inhibition of cell divisions without inhibition of cell growth has been demonstrated to decrease the cell division potential *in vitro*, accompanied by attaining an excessive volume (Angello *et al.*, 1989; Sumikawa *et al.*, 2005). A similar situation takes place in yeast cells treated with pheromone where the number of cell divisions after release of pheromone action is a linear function of the duration of

pheromone treatment (Zadrag *et al.*, 2005; 2006). We postulate that the only common reason for the loss of the division capacity of both cell types is attaining excessive cell volume, although the causes for reaching their excessive volume are quite different in both systems. It is noteworthy that fibroblasts taken from even very old healthy donors have a normal size and their division potential is unexpectedly high (Cristofalo *et al.*, 1998), unlike that of cells cultured *in vitro*. Cardiac fibroblasts grown under 5% oxygen tension are phenotypically closer to cells freshly isolated *ex vivo* than fibroblasts grown under 20% oxygen tension; the latter reaching a much bigger size (Roy *et al.*, 2003). Perhaps we still cannot cultivate the cells properly *in vitro*? If so, we should ask whether the authors are not right who:

- postulate that one of the main reasons for the existence of a limit of cellular divisions is due to attaining excessive cell volume (Angello *et al.*, 1989; Zadrag *et al.*, 2005; 2006)
- question the *in vivo* relevance of the Hayflick limit (Rubin, 2002)
- are skeptical as to the role of replicative cell aging in the aging of vertebrates (Campisi, 2001).

## REFERENCES

- Aguilaniu H, Gustafsson L, Rigoulet M, Nystrom T (2003) Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* **299**: 1751–1753.
- Angello JC, Pendergrass WR, Norwood TH, Prothero J (1989) Cell enlargement: one possible mechanism underlying cellular senescence. *J Cell Physiol* **140**: 288–294.
- Ashrafi K, Sinclair D, Gordon JL, Guarente L (1999) Passage through stationary phase advances replicative aging in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **96**: 9100–9105.
- Barker MG, Walmsley RM (1999) Replicative aging in the fission yeast *Schizosaccharomyces pombe*. *Yeast* **15**: 1511–1518.
- Campisi J (2001) From cells to organisms: can we learn about aging from cells in culture? *Exp Gerontol* **36**: 607–618.
- Cristofalo VJ, Allen RG, Pignolo RJ, Martin BG, Beck JC (1998) Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation. *Proc Natl Acad Sci USA* **95**: 10614–10619.
- Gillespie CS, Proctor CJ, Boys RJ, Shanley DP, Wilkinson DJ, Kirkwood TB (2004) A mathematical model of ageing in yeast. *J Theor Biol* **229**: 189–196.
- Guarente L (2000) Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev* **14**: 1021–1026.
- Hartwell L, Unger MW (1977) Unequal division in *Saccharomyces cerevisiae* and its implications for the control of cell division. *J Cell Biol* **75**: 422–435.
- Jazwinski SM, Egilmez NK, Chen JB (1989) Replication control and cellular life span. *Exp Gerontol* **24**: 423–436.
- Roy S, Khanna S, Wallace WA, Lappalainen J, Rink C, Cardounel AJ, Zweier JL, Sen CK (2003) Characterization of perceived hyperoxia in isolated primary cardiac fibroblasts and in the reoxygenated heart. *J Biol Chem* **278**: 47129–47135.

- Rubin H (2002) Promise and problems in relating cellular senescence *in vitro* to aging *in vivo*. *Arch Gerontol Geriatr* **34**: 275–286.
- Sinclair DA, Guarente L (1997) Extrachromosomal rDNA circles — a cause of aging in yeast. *Cell* **91**: 1033–1042.
- Sumikawa E, Matsumoto Y, Sakemura R, Fujii M, Ayusawa D (2005) Prolonged unbalanced growth induces cellular senescence markers linked with mechano transduction in normal and tumor cells. *Biochem Biophys Res Commun* **335**: 558–565.
- Wawryn J, Swiecilo A, Bartosz G, Bilinski T (2002) Effect of superoxide dismutase deficiency on the life span of the yeast *Saccharomyces cerevisiae*. An oxygen-independent role of Cu,Zn-superoxide dismutase. *Biochim Biophys Acta* **1570**: 199–202.
- Zadrag R, Bartosz G, Bilinski T (2005) Replicative aging of the yeast does not require DNA replication. *Biochem Biophys Res Commun* **333**: 138–141.
- Zadrag R, Kwolek-Mirek M, Bartosz G, Bilinski T (2006) Relationship between the replicative age and cell volume in *Saccharomyces cerevisiae*. *Acta Biochim Polon* **53**: 747–751.