Acta Biochimica Polonica

Vol. 46 No. 2/1999

249–253

QUARTERLY

Communication

Deficiency in superoxide dismutases shortens life span of yeast cells*°

Jarosław Wawryn[1], Anna Krzepiłko[1], Agata Myszka[1] and Tomasz Biliński[2, ✉]

[1]*Zamość College of Agriculture, Zamość,* [2]*Pedagogical University of Rzeszów, Rzeszów, Poland*

Received: 12 April, 1999

Key words: yeast mutant, aging, superoxide dismutase, catalase

Deficiencies in superoxide dismutases (Cu,Zn-SOD or Mn-SOD) strongly shorten the life span of yeast cells. The effects of these deficiencies are additive. In contrast, deficiencies in catalases do not influence life span. Our results confirm that free radical processes may be involved in aging.

A wide variety of theories have been proposed to explain the aging process [1–5]. Although no single hypothesis fully explains all aging phenomena, the genome-based and free radical theories are supported by significant observational and experimental evidence. Numerous experimental results indicate that aging is caused by a progressive accumulation of defects initiated by free radical processes. The intensity of free radical processes may be modulated by changing endogenous generation of the reactive oxygen species (ROS) or by modifying the antioxidant capacity of the cell using mutants deficient in various elements of the antioxidant systems or transformants overproducing these enzymes. The results of studies concerning overexpression of cytosolic superoxide dismutase (Cu,Zn-SOD) and catalase genes in *Drosophila melanogaster* [6] show that the transformation amelio-

*This paper was presented in a preliminary form at the 4th Symposium "Free Radicals in Biology and Medicine", 8–10 June, 1998, Łódź, Poland.

°This work was supported by Grant 281/P04/96/11 from the State Committee for Scientific Research. The authors are also indebted to the Polish Science Foundation for supplying us with the scientific equipment indispensable in this research.

✉Corresponding author: Tomasz Biliński, Department of Biology, Pedagogical University of Rzeszów, T. Rejtana 16, 35-310 Rzeszów, Poland, tel./fax: (48 17) 853 3504; fax: (48 17) 852 2044; e-mail: bilinski@univ.rzeszow.pl

rates the age-related accumulation of molecular oxidative damage while prolonging the metabolic life of flies.

The yeast *Saccharomyces cerevisiae* has already been used as a model organism to study the mechanisms of aging [7–10] due to the limited number of cell divisions of the yeast cell [11]. This facultative aerobe also offers the possibility of testing the role of free radical processes in aging thanks to the availability of mutants deficient in various antioxidant enzymes.

The aim of this work was to test the influence of deficiencies in selected antioxidant cellular systems on the life span of yeast cells.

MATERIALS AND METHODS

Replicative life span (the ability of the cell to form buds) and the generation time of yeast cells were measured according to [7] but without placing samples in a refrigerator after each session, to avoid the stress connected with cooling and warming cycles.

Media and growth conditions. The studied strains were grown in the standard liquid YPGlucose medium containing Difco 1% Yeast Extract, 1% Difco Bacto-Peptone and 2% glucose overnight on a rotatory shaker and then transferred on the same medium solidified with 2% Difco Agar. After 12 h of growth the cells were transferred to fresh solid medium and placed in an incubation chamber under the microscope. In each experiment mutant strains were tested simultaneously with their wild type counterparts to minimize the possible influence of variations in experimental conditions.

Strains. SP-4 – *MATα leu1 arg4* – wild type [12], A50 – *MATa leu1 arg4 ctt1 cta1* – deficient in catalases A and T – isogenic to SP-4 [13], DSCD1-1C – *MATa leu1 arg4 sod1* – deficient in Cu,Zn-SOD, isogenic to SP-4 [14].

DL1 – *MATα leu2-3,112 his3-11,15 ura3-251,372,328 lac2* – wild type, parental strain of Mn-SOD$^-$, kindly supplied by Dr. G. Schatz, (Biozentrum, Basel, Switzerland) [15], Mn-SOD$^-$ – as above, *sod2 LEU2* – deficient in mitochondrial superoxide dismutase (Mn-SOD).

In order to study the additivity of the effects of mutations leading to deficiencies in Cu,Zn-SOD and Mn-SOD the strains bearing *sod1* and *sod2* mutations were crossed. The double mutant DSCD6-6B (*MATα ura3 sod1 sod2*) was isolated. Since the two parental strains differ in their life spans and have different genetic background none of them could be used as a control. Thus, the *sod1* mutation was spontaneously reverted in DSCD6-6B and the obtained strain DSCD6-6BR – *MATα ura3 SOD1 sod2* – deficient only in Mn-SOD served as an isogenic control for the double mutant.

In order to study whether the simultaneous deficiency in Cu,Zn-SOD and in catalases affects the life span of yeast cells the strain A50DSCD 1-9C3d 9C – *MATa leu1 arg4 ctt1 cta1 sod1* was isolated from the cross between A50 strain and its isogenic counterpart of the opposite mating type, bearing *sod1* mutation. The segregants from the same cross A50DSCD 1-9C19C1d – *MATa leu1 arg4 sod1* deficient only in Cu,Zn-SOD or catalases A50DSCD 1-95b9C5b – *MATa leu1 arg4 ctt1 cta1* served as internal controls.

Statistical methods. To compare mean values of generation times and life span the Student's *t*-test was used. This was justified by previous comparison of variancies, which proved their differences to be insignificant.

RESULTS AND DISCUSSION

The first two rows of Table 1 present data concerning basic aging parameters of the studied strains expressed as mean and maximal numbers of buds formed by a virgin cell. The chronological age of dividing cells was calculated by multiplying their mean replicative life span by the generation time. The results show that only deficiencies in superox-

ide dismutases result in significant shortening of the replicative life span and the chronological age of the mutant cells. The mutations leading to catalase deficiencies do not appear to strongly influence either parameter. Table 2 shows that the effects of mutations leading to superoxide dismutase deficiencies have additive effects, whereas catalase deficiency does not increase the negative effects of Cu,Zn-SOD deficiency.

Our results show that gene products which play the most crucial role in preventing free radical damage [14–16] also assure normal life span of the standard strains. Cytosolic superoxide dismutase is also essential for stationary phase survival of yeast cells [17]. These results are consistent with the results concerning consequences of overexpression of antioxidant enzymes in *Drosophila melanogaster* [6], except for the role of catalase. The difficulties in proving the protective roles of catalases in yeast are not surprising, because it is very difficult to clearly show negative consequences of catalase deficiencies in *S. cerevisiae* [14], except for hypersensitivity to millimolar concentrations of hydrogen peroxide. Yeast cells are well adapted to high levels of hydrogen peroxide and can tolerate treatment with high concentrations of this compound [14]. It is of note that simultaneous deficiencies in catalases and in cytochrome *c* peroxidase do not increase the sensitivity of the cell to oxidative stress, although the three enzymes together are responsible for the removal of more than 90% of extracellular hydrogen peroxide [18].

The aging phenomenon cannot be fully explained by a single hypothesis. It may be not a solely stochastic process. The complexity of the problem results from the fact that life span is determined by numerous genetic mechanisms. Genetic analysis of the crosses between various "standard" strains of yeast which differed in their life spans revealed that the rate of aging depends on a number of genes [19]. The nature of the mechanisms affecting the life span of yeast cells is not clear and it cannot be excluded that some of them could change the intensity of oxidative metabolism and in this way influence the rate of formation of reactive oxygen species. Others could change the capacity of the cell to cope

Table 1. The effects of deficiencies in basic antioxidant enzymes on the aging of yeast cells.

Numbers in parentheses show values obtained for mutant strains, expressed as percentage of values obtained for the corresponding standard strain. This presentation makes possible the comparison of the effects of particular mutations on the replicative life span and chronological age of cells, irrespective of the life span of the standard strains, because the strain DL1 which is a standard strain for the mutant deficient in Mn-SOD has unusually short life span. The vertical line separates groups of directly comparable isogenic strains. In each experiment a minimum of 20 cells of one strain were tested.

Strain	SP-4	A-50	DSCD1-1C	DL 1	Mn-SOD
Characteristics	Wild type	Catalase deficient	Cu,Zn-SOD deficient	Wild type	Mn-SOD deficient
Mean replicative life span	**34.3** ± 2.89 (100)	**31.4** ± 5.95 (91.5)	**12.9** ± 1.70 (37.6)	**22.4** ± 2.17 (100)	**8.3** ± 1.68 (37.1)
Maximal replicative life span	**51** (100)	**46** (90.2)	**27** (53)	**31** (100)	**15** (48.4)
Generation time (min)	**89.2** ± 1.87	**99.8** ± 4.70	**121.3** ± 4.67	**115.4** ± 4.54	**172.4** ± 13.43
Mean chronological life span (h)[1]	**51.00** (100)	**52.2** (102.3)	**26.1** (51.2)	**43.1** (100)	**23.9** (55.4)

[1]Mean replicative life span × generation time.

Table 2. Additivity of effects of mutations leading to superoxide dismutase deficiencies.

Numbers in parentheses show values obtained for mutant strains expressed as percentage of values obtained for the corresponding standard strain. The vertical line separates groups of directly comparable isogenic strains.

Strain	SP-4	A50DSCD 1-9C1d	A50DSCD 1-9C5b	A50DSCD 1-9C3d	DSCD6-6BR*	DSCD6-6B
Characteristics	Wild type	Cu,Zn-SOD deficient	Catalase deficient	Cu,Zn-SOD and catalase deficient	Mn-SOD deficient	Cu,Zn-SOD and Mn-SOD deficient
Mean replicative life span	**34.3** ± 2.89 (100)	**16.7** ± 2.73 (48.7)	**36.3** ± 5.67 (105.7)	**16.3** ± 2.54 (47.5)	**16.9** ± 2.44	**3.9** ± 0.74
Maximal replicative life span	**51** (100)	**29** (56.9)	**46** (90.2)	**30** (58.8)	**28**	**12**
Generation time (min)	**89.2** ± 1.87	**136.6** ± 6.10	**79.0** ± 3.11	**135.0** ± 4.07	**138.0** ± 4.53	**212.3** ± 14.23
Mean chronological life span (h)	**51.0** (100)	**38.0** (74.5)	**47.8** (93.6)	**36.7** (71.9)	**38.9**	**13.8**

**sod1* mutation reverted spontaneously in DSCD6-6B. In each experiment a minimum of 20 cells of one strain were tested.

with oxidative stress. It is of note that long-lived mutants of yeast were isolated by screening for stress resistant cells. A recent finding [20] shows that mild heat shock extends life span of yeast.

Therefore, some genetic determinants of longevity could create a link between purely stochastic and gene based explanations of the aging process.

REFERENCES

1. Harman, D. (1994) *Ann. N.Y. Acad. Sci.* **717**, 1–15.
2. Barnett, Y.A. (1994) *Br. J. Biomed. Sci.* **51**, 278–287.
3. Kowald, A. & Kirkwood, T.B. (1994) *J. Theor. Biol.* **168**, 75–94.
4. Zakian, V.A. (1995) *Science* **270**, 1601–1607.
5. Knight, J.A. (1995) *Ann. Clin. Lab. Sci.* **25**, 1–12.
6. Sohal, R.S., Agarwal, A., Agarwal, S. & Orr, W.C. (1995) *J. Biol. Chem.* **270**, 15671–15674.
7. Egilmez, N.K. & Jazwinski, M. (1989) *J. Bacteriol.* **171**, 37–42.
8. Jazwinski, S.M. (1996) *Science* **273**, 54–59.
9. Austriaco, N.R.J. (1996) *Yeast* **12**, 623–630.
10. Sinclair, D.A. & Guarente, L. (1997) *Cell* **91**, 1033–1042.
11. Mortimer, R.K. & Johnston, J.R. (1959) *Nature* **183**, 1751–1752.
12. Biliński, T., Łukaszkiewicz, J. & Śledziewski, A. (1978) *Biochem. Biophys. Res. Commun.* **83**, 1225–1233.
13. Traczyk, A., Biliński, T., Litwińska, J., Skoneczny, M. & Rytka, J. (1985) *Acta Microbiol. Polon.* **34**, 231–241.
14. Biliński, T., Krawiec, Z., Liczmański, A. & Litwińska, J. (1985) *Biochem. Biophys. Res. Commun.* **130**, 533–539.

15. VanLoon, A.P.G.M., Pesold-Hurt, B. & Schatz, G.A. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3820–3824.

16. Fridovich, I. (1975) *Annu. Rev. Biochem.* **44**, 147–159.

17. Longo, V.D., Gralla, E.B. & Valentine, J.S. (1996) *J. Biol. Chem.* **271**, 12275–12280.

18. Wiśnicka, R., Krzepiłko, A., Wawryn, J., Krawiec, Z. & Biliński, T. (1998) *Biochem. Mol. Biol. Int.* **44**, 635–641.

19. Kennedy, B.K., Austriaco, N.R.J., Zhang, J. & Guarente, L. (1995) *Cell* **80**, 485–496.

20. Shama, S., Lai, C.Y., Antoniazzi, J.M., Jiang, J.C. & Jazwinski, S.M. (1998) *Exp. Cell Res.* **245**, 379–388.