[S2-5]

Role of Citrate Synthase in Acetate Utilization and Protection from Stress-Induced Apoptosis

Yong Joo Lee, Hong Yong Kang, and Pil Jae Maeng*

Department of Microbiology, School of Bioscience and Biotechnology, Chungnam National University, Daejeon 305-764, e-mail: pjmaeng@cnu.ac.kr

Abstract

The yeast *Saccharomyces cerevisiae* has been shown to contain three isoforms of citrate synthase (CS). The mitochondrial CS, Cit1, catalyzes the first reaction of the TCA cycle, i.e., condensation of acetyl-CoA and oxaloacetate to form citrate [1]. The peroxisomal CS, Cit2, participates in the glyoxylate cycle [2]. The third CS is a minor mitochondrial isofunctional enzyme, Cit3, and related to glycerol metabolism. However, the level of its intracellular activity is low and insufficient for metabolic needs of cells [3]. It has been reported that Δ*cit1* strain is not able to grow with acetate as a sole carbon source on either rich or minimal medium and that it shows a lag in attaining parental growth rates on nonfermentable carbon sources [2, 4, 5]. Cells of Δ*cit2*, on the other hand, have similar growth phenotype as wild-type on various carbon sources.

Thus, the biochemical basis of carbon metabolism in the yeast cells with deletion of *CIT1* or *CIT2* gene has not been clearly addressed yet. In the present study, we focused our efforts on understanding the function of Cit2 in utilizing C_2 carbon sources and then found that Δ*cit1* cells can grow on minimal medium containing C_2 carbon sources, such as acetate. We also analyzed that the characteristics of mutant strains defective in each of the genes encoding the enzymes involved in TCA and glyoxylate cycles and membrane carriers for metabolite transport. Our results suggest that citrate produced by peroxisomal CS can be utilized via glyoxylate cycle, and moreover that the glyoxylate cycle by itself functions as a fully competent metabolic pathway for acetate utilization in *S. cerevisiae*.

We also studied the relationship between Cit1 and apoptosis in *S. cerevisiae* [6]. In multicellular organisms, apoptosis is a highly regulated process of cell death that allows a cell to self-degrade in order for the body to eliminate potentially threatening or undesired cells, and thus is a crucial event for common defense mechanisms and in development [7]. The process of cellular suicide is also present in unicellular

organisms such as yeast *Saccharomyces cerevisiae* [8]. When unicellular organisms are exposed to harsh conditions, apoptosis may serve as a defense mechanism for the preservation of cell populations through the sacrifice of some members of a population to promote the survival of others [9]. Apoptosis in *S. cerevisiae* shows some typical features of mammalian apoptosis such as flipping of phosphatidylserine, membrane blebbing, chromatin condensation and margination, and DNA cleavage [10].

Yeast cells with Δ*cit1* deletion showed a temperature-sensitive growth phenotype, and displayed a rapid loss in viability associated with typical apoptotic hallmarks, *i.e.*, ROS accumulation, nuclear fragmentation, DNA breakage, and phosphatidylserine translocation, when exposed to heat stress. Upon long-term cultivation, Δ*cit1* cells showed increased potentials for both aging-induced apoptosis and adaptive regrowth. Activation of the metacaspase Yca1 was detected during heat- or aging-induced apoptosis in Δ*cit1* cells, and accordingly, deletion of *YCA1* suppressed the apoptotic phenotype caused by Δ*cit1* mutation. Cells with Δ*cit1* deletion showed higher tendency toward glutathione (GSH) depletion and subsequent ROS accumulation than the wild-type, which was rescued by exogenous GSH, glutamate, or glutathione disulfide (GSSG). Beside Cit1, other enzymes of TCA cycle and glutamate dehydrogenases (GDHs) were found to be involved in stress-induced apoptosis. Deletion of the genes encoding the TCA cycle enzymes and one of the three GDHs, Gdh3, caused increased sensitivity to heat stress. These results lead us to conclude that GSH deficiency in Δ*cit1* cells is caused by an insufficient supply of glutamate necessary for biosynthesis of GSH rather than the depletion of reducing power required for reduction of GSSG to GSH.

References

1. Suissa, M., K. Suda, and G. Schatz (1984) Isolation of the nuclear yeast genes for citrate synthase and fifteen other mitochondrial proteins by a new screening method. EMBO J. **3**(8): 1773-1781.
2. Kim, K.S., M.S. Rosenkrantz, and L. Guarente (1986) *Saccharomyces cerevisiae* contains two functional citrate synthase genes. Mol. Cell. Biol. **6**(6): 1936-1942.
3. Jia, Y.K., A.M. Becam, and C.J. Herbert (1997) The *CIT3* gene of *Saccharomyces cerevisiae* encodes a second mitochondrial isoform of citrate synthase. Mol. Microbiol. **24**(1): 53-59.
4. Kispal, G., M. Rosenkrantz, L. Guarente, and P.A. Srere (1988) Metabolic changes in *Saccharomyces cerevisiae* strains lacking citrate synthases. J. Biol. Chem. **263**(23): 11145-11149.
5. Lee, J.G., Y.J. Lee, C.H. Lee, and P.J. Maeng (2006) Mutational and functional analysis of the cryptic N-terminal targeting signal for both mitochondria and peroxisomes in yeast peroxisomal citrate synthase Cit2p. J. Biochem. (Tokyo) **140**(1): 121-133.
6. Lee, Y.J., K.L. Hoe, and P.J. Maeng (2007) Yeast cells lacking the *CIT1*-encoded mitochondrial citrate synthase are hypersusceptible to heat- or aging-induced apoptosis. Mol. Biol. Cell **18**(9):

3556-3567.

7. Lam, E., D. Pontier, and O. del Pozo (1999) Die and let live - programmed cell death in plants. Curr. Opin. Plant Biol. **2**(6): 502-507.
8. Madeo, F., E. Frohlich, and K.U. Frohlich (1997) A yeast mutant showing diagnostic markers of early and late apoptosis. J. Cell Biol. **139**(3): 729-734.
9. Frohlich, K.U. and F. Madeo (2000) Apoptosis in yeast--a monocellular organism exhibits altruistic behaviour. FEBS Lett. **473**(1): 6-9.
10. Ligr, M., F. Madeo, E. Frohlich, W. Hilt, K.U. Frohlich, and D.H. Wolf (1998) Mammalian Bax triggers apoptotic changes in yeast. FEBS Lett. **438**(1-2): 61-65.