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Rescue of Oxidative Stress by Molecular Chaperones in Yeast

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Abstract

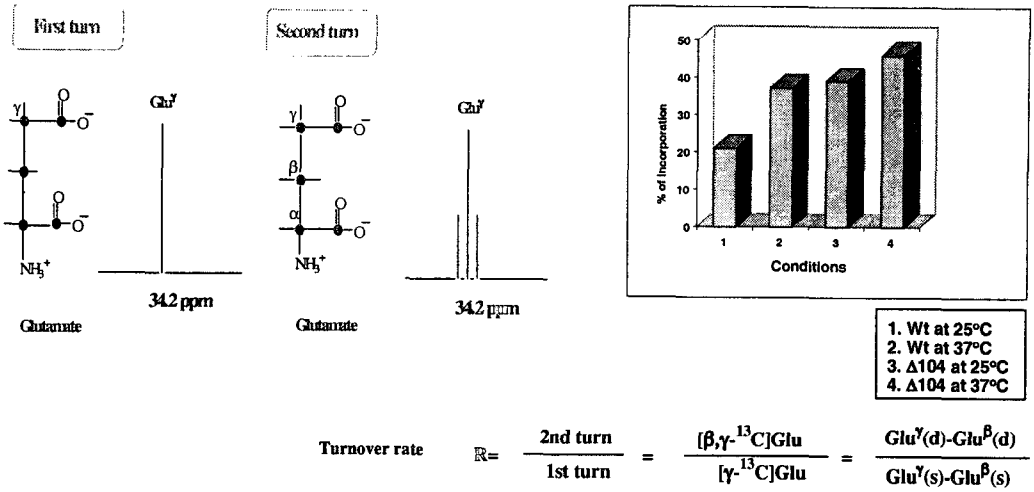
Heat shock proteins (HSPs) are induced in most living cells by mild heat treatment, ethanol, heavy metal ions and hypoxia. In yeast *Saccharomyces cerevisiae*, mild heat pretreatment strongly induces Hsp104 and thus provide acquired thermotolerance. The ability of hsp104 deleted mutant (△hsp104) to acquire tolerance to extreme temperature is severely impaired. In providing thermotolerance, two ATP binding domains are indispensible, as demonstrated in ClpA and ClpB proteases of *E. coli*.

The mechanisms by which Hsp104 protects cells from severe heat stress are not yet completely elucidated. We have investigated regulation of mitochondrial metabolic pathways controlled by the functional Hsp104 protein using ^{13}C-NMR spectroscopy and observed that the turnover rate of TCA cycle was enhanced in the absence of Hsp104. Production of ROS, which are toxic to kill cells radiply via oxidative stress, was also examined by fluorescence assay. Mitochondrial dysfunction was manifested in increased ROS levels and higher sensitivity for oxidative stress in the absence of Hsp104 protein expressed. Finally, we have identified mitochondrial complex I and Ferritin as binding protein(s) of Hsp104 by yeast two hybrid experiment. Based on these observations, we suggest that Hsp104 protein functions as a protector of oxidative stress via either keeping mitochondrial integrity, direct binding to mitochonrial components or regulating metal-catalyzed redox chemistry.

Results

(1) ^{13}C-acetate incorporation by ^{13}C-NMR spectroscopy.

Oxidation of ^{13}C-acetate in yeast was studied using ^{13}C-NMR spectroscopy for the purpose of discovering a role of HSP104 protein in yeast respiration. ^{13}C-NMR spectra were obtained for perchloric acid extracts of yeast cells grown in the presence of [2-^{13}C]acetate as a sole carbon source (Figure 1). Data on ^{13}C enrichment of glutamate carbons from ^{13}C-NMR peaks were examined to quantify the incorporation of [2-^{13}C]acetate. ^{13}C-acetate is presumably incorporated into citrate, which in turn gets into α -ketoglutarate in tricarboxylic acid (TCA) cycle. By comparing the intensities of the singlet and doublet peak at the [4-^{13}C]glutamate, we were able to correlate the turnover rate of TCA cycle to the efficiency of respiration. In the △104 strain, the ratio between the doublet and the singlet increased up to 70% compared to that of wild type. The higher rate of incorporation observed at C4 glutmate peak implies that the rate of TCA cycle is faster in the mutant than in the wild type.



(Figure 1)

(2) Mitochondrial dysfunction and Sensitivity to OS in the absence of Hsp104

In order to examine the mitochondrial integrity influenced by the presence of Hsp104, mitochondrial fractions from both wt and △hsp104 were purified and checked for the membrane integrity. Both the mitochondrial protein concentration and membrane potential decreased in the absence of Hsp104 by 53% and 15%, respectively. Each strain was then exposed to various kinds of oxidative stress, such as H_2O_2 and menadione. Consistent with data of mitochondrial dysfunction, survival rate against oxidative stress was diminished and ROS production was significantly enhanced in the absence of Hsp104 (Table 1).

	Strains	Exposure to H_2O_2(%)(1)	Exposure to menadione (%)(1)
(A) Survival Test	W303	67	75
	Δ104	29	21
(B) ROS(2) production	W303	145	134
	Δ104	224	300

(1) Cells were treated with 1.0mM H_2O_2 for 10 min or 0.1mM menadione for 20 min.

(2) ROS was measured by H2DCFDA fluorescence.

(Table 1)

(3) Identification of binding partners of Hsp104 protein by yeast two hybrid

We have used the yeast two hybrid system (pLexA) to search for putative binding protein(s) of Hsp104. We have constructed the bait plasmids (pLexA/hsp104 and pLexA/hsp104-pKT218/620) and cotransformed the yeast host (EGY48) with human brain cDNA library. Three proteins including NADH dehydrogenase were identified with high specificity. This study suggests that Hsp104 protects cells from oxidative stress by directly binding and probably stabilizing mitochondrial protein(s) involved in electron transport complexes, threrby regulating ROS levels.

References

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