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Subcellular Localization of Catalase Encoded by the *ctt1*$^+$ Gene in *Schizosaccharomyces pombe*

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The *ctt1*$^+$ gene in *Schizosaccharomyces pombe* encodes a catalase responsible for H_2O_2-resistance of this organism as judged by the H_2O_2-sensitive phenotype of the *ctt1*Δ mutant. In this study, we investigated the subcellular localization of the Ctt1 gene product. In wild type cells catalase activity was detected in the organelle fraction as well as in the cytosol. The *ctt1*Δ mutant contained no catalase activity, indicating that both cytosolic and organellar catalases are the products of a single *ctt1*$^+$ gene. Western blot analysis revealed two catalase bands, both of which disappeared in the *ctt1*Δ mutant. The major, faster-migrating band existed in the cytosol whereas the minor, slower-migrating band appeared to be located in organelles, most likely in peroxisomes. These results suggest that the *ctt1*$^+$ gene product targeted to the peroxisome is a modified form of the one in the cytosol.

Key words: catalase, *ctt1*$^+$, subcellular fractionation, peroxisome, *S. pombe*

Catalase is an antioxidant enzyme which decomposes H_2O_2 into O_2 and H_2O. It is present in virtually all aerobic organisms. In eukaryotes, it is a characteristic constituent of the peroxisome, but evidence for the existence of extra-peroxisomal catalase has been presented in a number of cases. The yeast *Sacharomyces cerevisiae* produces two catalase proteins, the peroxisomal catalase A encoded by the *CTA1* gene (2), and the cytosolic catalase T encoded by the *CTT1* gene (8).

Peroxisomal matrix proteins are synthesized on the free polysome, and are imported post-translationally into organelles of the cell (4). None of the peroxisomal matrix proteins studied to date are burdened by any modifications that are sometimes necessary for import into organelles (9).

For the fission yeast *Schizosaccharomyces pombe*, a catalase gene *ctt1*$^+$ was reported (7). In the *ctt1*Δ mutant, catalase activity was not detected. Here we report the presence of catalase in both the cytosol and peroxisome and demonstrate that the *ctt1*$^+$ gene encodes both cytosolic and peroxisomal catalases. Moreover, we show evidence that peroxiosomal catalase exists in a different form from the cytosolic one.

Materials and Methods

Strains and culture conditions

S. pombe strains used in this study were ED665 (wild type) and SI1-3B (*ctt1Δ* mutant) (5). For routine growth, YEPD (1% w/v yeast extract, 2% w/v glucose, 2% w/v peptone) medium was used. Cells containing the plasmid or disrputed gene were cultured in EMM with the appropriate supplements (5).

Disruption of the ctt1$^+$ *gene*

*Hin*dIII/*Bam*HI frament of the *ctt1*$^+$ gene (about 2.4 kb) was cloned into pTZ18R and the *Acc*I fragment containing the start codon was substituted by the *ura4*$^+$ gene. The resulting plasmid was digested with *Hin*dIII and *Bam*HI, and a 3.8 kb fragment containing the *ura4*$^+$ cassette was used to transform diploid cells to achieve gene replacement according to Moreno *et al.* (6). Ura$^+$ prototrophs were selected. The correct integration of the *ura4*$^+$ cassette was verified by PCR. Tetrads were analyzed on a YES plate and the *ctt1Δ* mutant was selected by the catalase assay.

Preparation of cell-free extracts

Harvested cells were washed in 10 mM potassium phosphate buffer and resuspended in the same buffer containing 1 mM PMSF. Cells were disrupted by abrasion with glass beads in Eppendorf tubes using a vortex mixer.

Catalase assay and staining

Catalase activity was assayed according to the method of Beers and Sizer (1) by measuring the decomposition of H_2O_2 at 240 nm. One unit of enzyme activity was defined as the activity that catalyzed the degradation of 1 μmol of H_2O_2 min^{-1}. The staining of catalase activity in the gel

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was done following 8% native PAGE. The gel was incubated with 4 mM H_2O_2 for 10 min, briefly rinsed, and soaked in 2% potassium ferricyanide and 2% ferric chloride solution under illumination until a green background color developed.

Subcellular fractionation

Washed cells were suspended in 0.1 M Tris-HCl buffer, pH 9, containing 2.5 mM dithiothreitol and incubated for 20 min at 30°C with shaking. To obtain protoplasts, cells were then pelleted, washed once with digestion buffer (1.3 M sorbitol, 1mM EDTA and 5 mM MOPS, pH 7.2) and suspended in the same buffer containing 0.1% lyticase (Sigma) and 0.1% mercaptoethanol. They were incubated at 33°C for 1 h with gentle shaking. Protoplasts were pelleted, washed twice with digestion buffer, and resuspended in chilled homogenization buffer (0.65 M sorbitol, 0.5 mM EDTA, 2.5 mM MOPS, pH 7.2). They were homogenized at 0°C with a homogenizer by 20 strokes of the pestle. The homogenate was centrifuged at 3000 × g for 10 min. The pellet was re-homogenized and centrifuged. The combined supernatants were centrifuged with a SW28 rotor at 27,000 rpm (131,000 × g) for 15 min. The pellet comprising the crude organelle fraction was suspended in homogenization buffer. Separation of organelles was carried out on a linear 0~30% gradient of Ficoll 400 in homogenization buffer. The crude organelle fraction was applied on top of the gradient. Centrifugation was carried out in a Beckman ultracentrifuge with a SW28 rotor at 22,000 rpm (87,000 × g) for 2 h. The gradient was unloaded from the top by collecting 1 ml fractions.

Preparation of antibodies against catalase and Western blot analysis

By PCR amplication with a mutagenic primer, the start codon of the *ctt1*$^+$ gene was changed to the *Nde*I site. The N-terminal 975-bp *Nde*I/*Nde*I fragment of the *ctt1*$^+$ gene was cloned into the *Nde*I site of plasmid pET3a. The resulting plasmid was used to transform *E. coli* BL21 (DE3) pLysS. A fresh colony was grown to mid-exponential phase and induced by 0.4 mM IPTG for 1 h. Harvested cells were resuspended in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) containing 0.1% Triton-X100, disrupted by sonication, and centrifuged at 12,000 rpm for 15 min. The truncated catalase protein was recovered from the insoluble fraction and purified by SDS-PAGE. The protein was eluted from the gel in phosphate buffered saline (PBS; 20 mM sodium phosphate, pH 7.5, 150 mM NaCl), and was used to immunize mice. Serums containing polyclonal antibodies were used at a 1 : 5000 dilution for Western blot analysis.

Results and Discussion

H_2O_2-sensitive phenotype of the ctt1Δ mutant

We tested the role of catalase in *S. pombe* by examining the phenotype of the *ctt1Δ* mutant. Both the wild type (ED665+pUR19N control plasmid) and *ctt1Δ* mutant (SI1-3B) were grown to the exponential phase and tested for sensitivity to H_2O_2. As demonstrated in Fig. 1, the *ctt1Δ* mutant was extremely sensitive to H_2O_2 compared with the wild type. Treatment with 4 mM H_2O_2 for 1 h reduced the number of surviving colonies to about 10% whereas the survival of the wild type was not affected sig-

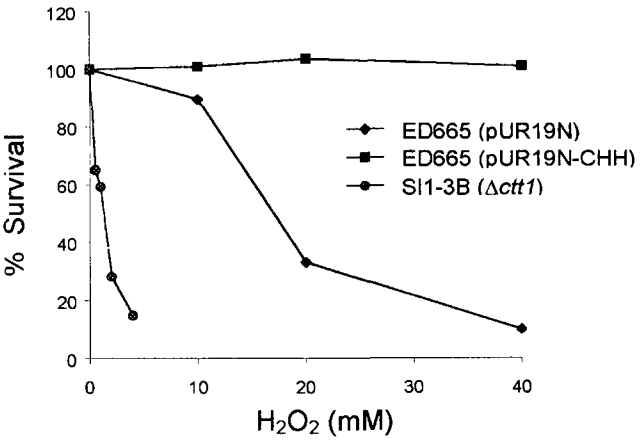

Fig. 1. Role of Ctt1 catalase in protecting *S. pombe* cells against H_2O_2. Exponentially growing *S. pombe* cells were treated with hydrogen peroxide at various concentrations for 1 h. Aliquots were taken to determine colony forming units on YES plates. ED665 cells containing the pUR19N plasmid and SI1-3B (*ctt1Δ* mutant) were tested as well as ED665 cells containing a multi-copy *ctt1*$^+$ gene on pUR19N (pUR19N-CHH).

A

	catalase activity (unit/mg)
Whole cell extract	124
Cytosol	110
Organelles	1.8

B

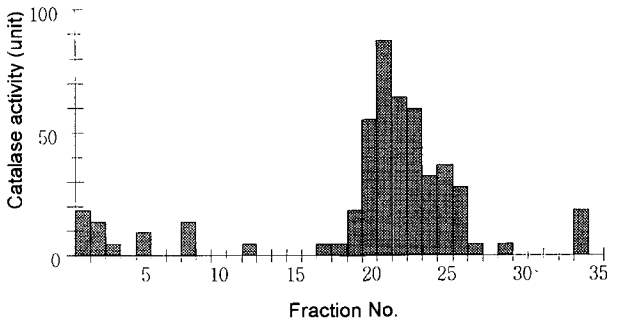

Fig. 2. Subcellular localization of catalase in *S. pombe*. (A) Wild type cells in the early stationary phase were fractionated as described in Materials and Methods. Catalase activity was assayed in each fraction. (B) Separation of organelles was carried out on a linear Ficoll gradient as described in Materials and Methods. Each fraction was assayed for catalase activity. The peak fractions detected in fractions 21-22 are thought to contain peroxisomes.

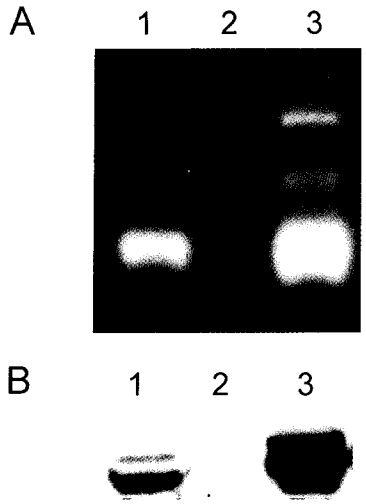

Fig. 3. The product of *ctt1*$^+$ determined by activity staining (A) and Western blot analysis (B). Extracts were prepared from ED665 (wild-type, lane 1), SI1-3B (*ctt1Δ* mutant) with pWH5 (lane 2), and SI1-3B with the *ctt1*$^+$ gene cloned in the multicopy pWH5 plasmid (lane 3). (A) Cell extracts were electrophoresed on an 8% native polyacrylamide gel and stained for catalase activity. (B) Cell extracts were electrophoresed on a 10% SDS-polyacrylamide gel, blotted and probed with antibodies against catalase.

nificantly. When the *ctt1*$^+$ gene was overproduced on a multicopy plasmid (pUR19N-CHH), the resistance against H_2O_2 increased drastically. These results indicate that the *ctt1*$^+$ gene is primarily responsible for H_2O_2-resistance of this organism.

Subcellular localization of catalase

To determine the subcellular localization of catalase in *S. pombe*, we fractionated wild-type cells in the stationary phase. Most of the catalase activity was detected in the cytosolic fraction (Fig. 2A). But, weak activity was also detected in the organelle fraction. To ascertain that this activity was indeed derived from organelles, the organelle fraction was subjected to Ficoll 400 density gradient ultracentrifugation. A peak of catalase activity appeared in the organelle fraction co-sedimenting closely with the mitochondrial fraction. Even though we do not have direct evidence that this fraction is peroxisomal, it is most likely that this catalase peak is derived from the peroxisome since catalase is known to be a characteristic constituent of peroxisomes in most eukaryotes. This result indicates that catalases exist in both the cytosol and organelles and most likely the peroxisome.

The ctt1$^+$ *gene encodes both cytosolic and organellar catalases*

To investigate whether two kinds of catalases are *ctt1*$^+$ products, the presence of both the catalase activity and the protein in the *ctt1Δ* mutant was examined (Fig. 3). No catalase activity was detected in the *ctt1Δ* mutant (Fig. 3A).

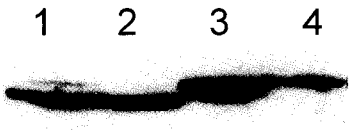

Fig. 4. Organellar catalase exists in a form different from the cytosolic one. Whole cell extract (lane 1), cytoplasmic (lane 2), whole organellar (lane 3), and the fractionated organelle (peroxisomal, lane 4) samples were analyzed by Western blot using antibodies against catalase.

When the cell extracts from the mutant were fractionated, catalase activity was not detected in either the cytosolic fraction or the organellar fraction, indicating that both cytosolic and peroxisomal catalases are *ctt1*$^+$ products. When the presence of the Ctt1 polypeptide was verified by Western blot analysis, no Ctt1 protein was detected in the mutant as expected (Fig. 3B).

Two forms of Ctt1 catalase

In Western blot analysis, we noticed that Ctt1 catalase existed as two forms. The faster-migrating form was the major one. To ascertain that these two bands are indeed *ctt1*$^+$ products, we transformed the *ctt1Δ* mutant with pWH5 with *ctt1*$^+$ gene. In this transformant overproducing catalase, two bands were distictly visible. This indicates that the *ctt1*$^+$ product exists in two forms. The heterogeneity in the observed molecular weight was more pronounced when catalase was overexpressed. A similar result has been reported in the case of Pex15p (3).

To determine the subcellular localization of these two forms of polypeptide, fractions used for the experiment shown in Fig. 2 were examined by Western analysis (Fig. 4). The faster-migrating band was mainly in the cytosolic fraction whereas the slower-migrating one was detected in the whole organelle fraction. Furthermore, the faster-migrating band was not detected at all in the Ficoll-fractionated peroxisomal fraction. Therefore, the faster-migrating band in the whole organellar fraction is thought to be derived from the contaminating cytosol and cell debris. These results indicate that the peroxisomal catalase exists in a different form from cytosolic catalase even though both are derived from the same gene.

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References

1. Beers, R.F. and I.W. Sizer. 1951. Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, *J. Biol. Chem.* 195, 133-137.
2. Cohen, G, F. Fessl, A. Traczyk, and H. Ruis. 1985. Isolation of

the catalase A gene of *Saccharomyces cerevisiae* by complementation of the *cta1* mutation. *Mol.Gen.Genet.* 200, 74-79.
3. Elgersma, Y., L. Kwast, M. van den Berg, W.B. Snyder, B. Distel, S. Subramani, and H.F. Tabak. 1997. Overexpression of Pex15p, a phosphorylated peroxisomal integral membrane protein required for peroxisome assembly in *S. cerevisiae*, causes proliferation of the endoplasmic reticulum membrane. *EMBO J.* 16, 7325-7341.
4. Lazarow, P.B. and Y. Fujiki. 1985. Biogenesis of peroxisomes. *Annu. Rev. Cell Biol.* 1, 489-530.
5. Lee, S. 1999. The role and subcellular localization of catalase in *Schizosaccharomyces pombe*. M.S. thesis, Seoul National University, Seoul.
6. Moreno, S., A. Klar, and P. Nurse. 1991. Molecular genetics analysis of fission yeast : *Schizosaccccharomyces pombe. Methods in Enzymol.* 104, 796-823.
7. Nakagawa, C.W., N. Mutoh, and Y. Hayashi. 1995. Transcriptional regulation of catalase gene in the fission yeast *Schizosaccharomyces pombe*: Molecular cloning of the catalase gene and northern blot analysis of the transcript. *J. Biochem.*118, 109-116.
8. Spevak, W., F. Fessl, J. Rutka, A. Traczyk, M. Skoneczny, and H. Ruis. 1983. Isolation of the catalase T structural gene of *Saccharomyces cerevisiae* by functional complementation. *Mol. Cell. Biol.* 3, 1545-1551.
9. Subramani, S. 1996. Convergence of model systems for peroxisome biogenesis. *Curr. Opion. Cell Biol.* 8, 513-518.