

Organelle Targeting of Citrate Synthases in *Saccharomyces cerevisiae*

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The intracellular distribution and trafficking of the two citrate synthases, CS1 and CS2, has been investigated in *Saccharomyces cerevisiae*. Antibodies against CS1 and CS2 were generated from rabbit and mouse, respectively, and were used in tracing the localities of the isoenzymes by immunoelectron microscopy, which showed that CS1 is targeted to mitochondria and CS2 to peroxisomes. The subcellular localization of CS1 and CS2 was also examined by cell fractionation on a Nycodenz stepped gradient, and CS1 was found to be cosedimented with mitochondria, and CS2 with peroxisomes, respectively. The *N*-terminal sequence of CS1 was determined to be S-S-A-S-E-Q-T, which indicates that 37 *N*-terminal amino acids of the CS1 precursor are removed by the cleavage as R(35)-H-Y↓S(38) in the process of mitochondrial targeting and that this protein contains a R-3 type cleavage motif in the mitochondrial targeting signal. The *N*-terminal amino acid sequence of CS2 was revealed to be L-Q-S-N-S-S, which suggests that the 15 *N*-terminal amino acids are cut off from the CS2 precursor by the cleavage as A(13)-S-Y↓L(16) during its import into peroxisomes. It is thus quite noticeable that the *N*-terminal 15 amino acids of CS2 are cut off notwithstanding the presence of *C*-terminal peroxisomal targeting signal (PTS1), S-K-L, because none of the proteins containing PTS1 has been reported to have *N*-terminal sequence that are cleaved upon translocation of the protein into peroxisomes yet.

KEY WORDS □ nonmitochondrial citrate synthase, mitochondrial citrate synthase, organelle targeting, *Saccharomyces cerevisiae*

Citrate synthase (EC 4.1.3.7) is a condensing enzyme that catalyzes the formation of citrate from acetyl-CoA and oxaloacetate (14). *Saccharomyces cerevisiae* contains two types of citrate synthase encoded by two distinct nuclear genes, *CIT1* and *CIT2*. *CIT1* first cloned by Suissa *et al.* (21) encodes mitochondrial citrate synthase (CS1) which is known to be involved in TCA cycle. On the other hand, *CIT2* cloned by Rosenkrantz *et al.* (12, 18) encodes nonmitochondrial citrate synthase (CS2) which is probably involved in one or both of the nonmitochondrial metabolic pathways, biosynthesis of glutamate and glyoxylate cycle (5). The amino acid sequences of the CS1 and the CS2 precursors deduced from the DNA sequences share a high degree of homology in amino acid (75%) as a whole. However, the *N*-terminal amino acid sequences of the two proteins that extend beyond the *N*-termini of the citrate synthase from *Escherichia coli* or pigs diverge strikingly (18). The predicted sequence of

the CS1 precursor has 39-amino acid-*N*-terminal extension containing a typical motif for a mitochondrial signal sequence. The sequence of the CS2 precursor, on the other hand, has a 20-residue-*N*-terminal extension with completely different amino acid sequence from that of the CS1 precursor, which provides a possible explanation for why the CS2 precursor, unlike the CS1 precursor, fails to be imported into mitochondria.

In this study, we made an effort to analyze the definite intracellular localization of the nonmitochondrial citrate synthase, CS2, in comparison with that of CS1. We prepared anti-CS1 and anti-CS2 antisera from rabbit and mouse, respectively, and traced the locality of the isoenzymes by immunoelectron microscopy and by differential centrifugation of intracellular organelles. We also determined the *N*-terminal amino acid sequences of the citrate synthases to reveal the sites of cleavage during the process of intracellular

organelle targeting.

MATERIALS AND METHODS

Organisms and media

All the experiments described were performed with *S. cerevisiae* PSY37 and its derivatives (Table 1). The parental strains, *S. cerevisiae* PSY37, PSY38 and PSY40, were grown on YEPD complete medium and the transformants, pLCS1/PSY40 and pUCS2/PSY37, on YNB minimal medium and stored at 4°C. YEPD and YNB media were prepared as described previously (19).

Purification of CS1 and CS2

Citrate synthases of yeast, CS1 and CS2, were purified from the cell lysates of *S. cerevisiae* pLCS1/PSY40 and pUCS2/PSY38 respectively through the procedures including affinity chromatography on a Reactive Blue 4 agarose gel and ion exchange chromatography on a Mono Q HR 5/5 FPLC column as described in the previous paper (3).

Enzyme assay

Citrate synthase was assayed by 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) method (20). The reaction mixture (1 ml) contained 100 mM Tris-HCl (pH 8.0), 0.25 mM DTNB, 0.2 mM oxaloacetate, 0.1 mM acetyl-CoA, and 10 μ l of enzyme solution. The reaction was followed by measuring the change in A_{412} at 30°C. One unit of enzyme activity was defined as the amount of enzyme producing 1 μ mole min^{-1} of CoA under the conditions of assay.

Preparation of anti-CS1 and anti-CS2 antisera

An anti-CS1 antiserum was prepared from rabbits immunized with the purified CS1. To immunize the rabbits, the purified CS1 (500 μ g/animal) mixed with complete Freund's adjuvant (CFA) was injected intramuscularly. Three weeks after initial immunization, booster shots were given with the same amount of antigen mixed with incomplete Freund's adjuvant (IFA). One week after the booster shots, the antibody titers were assayed by Ouchterlony immuno-double diffusion method, and then two more booster shots were given at an interval of 3 weeks. After one week from the last booster shots, the whole blood of the immunized rabbits were exsanguinated under anaesthesia by cardiac puncture. To prepare an anti-CS2 antiserum, six-week-old BALB/c female mice were immunized with the purified CS2 (200 μ g/mouse) mixed with CFA. Booster shots were given with the same amount of antigen mixed with IFA after 2 weeks. After one week from the first booster shots, the antibody production was measured by Ouchterlony immuno-double diffusion method, and the second booster shots were given after one week. Then the mice were sacrificed to collect the anti-CS2 antiserum after 3 days.

Table 1. Yeast strains and plasmids.

Strains and Plasmids	Descriptions
<i>S. cerevisiae</i> PSY38	<i>cit1::LEU2, CIT2, ura3</i>
<i>S. cerevisiae</i> PSY40	<i>cit2::URA3, CIT1, leu2</i>
pLCS1	<i>CIT1, LEU2</i>
pUCS2	<i>CIT2, URA3</i>

Western blot analysis

Western blot analysis was performed by the method of Towbin *et al.* (24). The proteins were separated by SDS-PAGE and transferred to a nitrocellulose membrane by electroblotting in the transfer buffer (25 mM Tris-HCl, 190 mM glycine, 20% methanol, pH 9.3) using a semi-dry transfer set. The nitrocellulose membrane was stained with 10% Ponceau S and treated with blocking solution containing 5% skim milk, 0.05% Tween 20, and 10 mM PBS (phosphate buffered saline, pH 7.4) for 2 hrs to prevent nonspecific adsorption of the immunological reagents. Then the membrane was soaked in the 2,000-fold diluted primary antiserum, *i.e.*, anti-CS1 or anti-CS2 antiserum, 16 hrs at 4°C and treated with 2,000-fold diluted secondary antiserum, *i.e.*, horseradish peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG for 2 hrs at room temperature. Peroxidase reaction was performed in 4-chloro-1-naphthol (4-CN) solution (3.6 mg 4-CN/1.2 ml CH_3OH , 18.8 ml PBS, and 80 μ l H_2O_2) (11).

Immunoelectron microscopy

Yeast cells were treated with a 0.1 M sodium phosphate buffer (pH 7.4) containing 2% para-formaldehyde and 0.4% glutaraldehyde for 2 hr at room temperature. Subsequently, cells were washed with PBS and fixed completely with a fixing solution (0.4% glutaraldehyde in 2% neutral buffered phosphate) for 12 hrs at 4°C. Fixed cells were washed again with PBS for 4 hrs at 4°C. The samples were dehydrated through a graded series of ethanol in water, 30, 50, 70, 95, 100%, and then infiltrated in a graded Lowicryl K4M in ethanol (50, 67% and 100%) for each change at 35°C. Polymerization was performed by UV irradiation in Lowicryl K4M at -35°C and at room temperature for 24 hrs, respectively. Ultra-thin sections were prepared using an ultramicrotome and mounted on a carbon-coated grids and dried.

For immunohistochemical staining, the samples were treated consecutively with PBS, 2% gelatin-PBS, and 0.02 M glycine-PBS, 10 min each treatment, and rinse in three changes of 1% BSA-PBS solution for 3 min each. The sections were pretreated with 1:20 dilution of normal goat serum and then incubated with a diluted anti-CS1 or anti-CS2 antiserum optimally diluted with PBS for 2 hrs at room temperature. On the sections treated with primary antiserum, a 1:20 dilution

of protein A-gold (20 nm, Zymed) was applied for 40 min. After washing in BSA-PBS, PBS and distilled water, the samples were counterstained with uranyl acetate and lead nitrate, and examined in the transmission electron microscope.

Fractionation of intracellular organelles

For the fractionation of intracellular organelles, 0.15 g (wet weight) of washed yeast cells were suspended in 1 ml of 0.1 M Tris- SO_4 buffer (pH 8.5) containing 10 mM dithiothreitol and incubated at 30°C for 30 min. After a rinse with 1.2 M sorbitol, the cells were resuspended in isotonic buffer (1.2 M sorbitol in 20 mM potassium phosphate, pH 7.4) and treated with lyticase (1,000–2,500 units/g cell wet weight) at 30°C for about 3 hrs to form spheroplasts. The spheroplasts harvested by centrifugation (3,000 \times g) were resuspended in lysis buffer (0.6 M sorbitol, 1 mM KCl and 0.5 mM EDTA in 5 mM MES-KOH buffer, pH 6.0) and homogenized by about 15 smooth strokes in a Dounce glass homogenizer. The cell debris were removed by low speed centrifugation (1,500 \times g), then the cellular organelles were separated from the soluble components by high speed centrifugation (27,000 \times g). The organelles were resuspended in 0.24 M sucrose solution containing 5 mM MES-KOH buffer (pH 6.0) and 1 mM EDTA, and then fractionated by ultracentrifugation on a step gradient prepared with 17, 25, and 35% (w/v) of Nycodenz solutions in the same buffer using a Kontron swing-out rotor, TST 55.5, at 100,000 \times g for 90 min at 4°C. Each fraction from the gradient was diluted 5-fold with 0.24 M sucrose solution containing 5 mM MES-KOH buffer (pH 6.0) and 1 mM EDTA, and the Nycodenz was removed by centrifugation at 15,000 rpm for 15 min (23).

The efficiency of the organelle fractionation was determined by assessing the compartmentation of the cytochrome c oxidase (16) and catalase (1). The distribution of CS1 and CS2 was analyzed by measuring the activity of citrate synthase in each fraction.

N-Terminal sequencing of citrate synthases

The N-terminal sequences of CS1 and CS2 were determined by the microsequencing method described by Matsudaira (17). The purified citrate synthases were firstly loaded onto a SDS-polyacrylamide gel and electrophoresed. Then the proteins were transferred to a PVDF membrane by electroblotting using a semi-dry transfer set. After the PVDF membrane was stained with freshly prepared 0.2% Ponceau S solution in 1% acetic acid, the protein band was cut into small rectangular pieces (5 \times 5 mm). The membrane pieces were rinsed in deionized water, air dried, and stored at -20°C. The proteins were sequenced on an Applied Biosystems model 477A sequanator equipped with on-line PTH analysis.

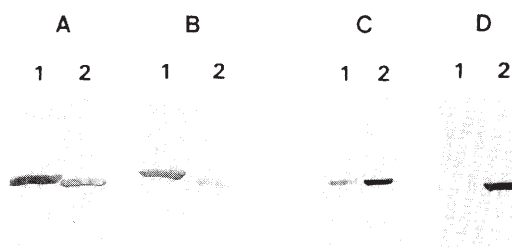


Fig. 1. Western blot analysis of anti-CS1 and anti-CS2 antisera.

A, anti-CS1 antiserum from rabbit; B, anti-CS1 antiserum from rabbit preabsorbed with a suspension of whole-cell acetone powder of *S. cerevisiae* pUCS2/PSY38; C, anti-CS2 antiserum from mouse; D, anti-CS2 antiserum from mouse preabsorbed with a suspension of whole-cell acetone powder of *S. cerevisiae* pLCS1/PSY40. Lane 1, CS1 purified from *S. cerevisiae* pLCS1/PSY40; Lane 2, CS2 purified from *S. cerevisiae* pUCS2/PSY38.

RESULTS AND DISCUSSION

Antisera

The specificities of the rabbit anti-CS1 antiserum and the mouse anti-CS2 antiserum were tested by Western blot analysis. As shown in Fig. 1A and 1B, it is apparent that both the anti-CS1 and anti-CS2 antisera have strong affinity toward their own antigens, CS1 and CS2, respectively. In addition, they showed considerable cross-reactivity, anti-CS1 toward CS2 and anti-CS2 toward CS1, respectively, which suggests that the two antigens share relatively large number of identical antigenic determinants and/or that the antigenic determinants of the two isozymes, although not identical, are stereochemically quite similar to each other. This result is consistent with the fact that they share approximately 75% of homology in their amino acid sequences (18).

To improve the specificities, the anti-CS1 antiserum was absorbed with the suspension of whole-cell acetone powder of *S. cerevisiae* pUCS2/PSY38 and the anti-CS2 antiserum with that of pLCS1/PSY40, respectively. As shown in Fig. 1C and 1D, the cross-reactivities of the antisera were markedly reduced while the affinities toward their own antigens were affected very little.

Intracellular localization of CS1 and CS2

For the immunoelectron microscopic observation of the subcellular localization of CS1 and CS2, Lowicryl-embedded ultrathin sections of the cells of *S. cerevisiae* PSY40 (*CIT1*, *cit2*) that lacks CS2 and PSY38 (*cit1*, *CIT2*) that lacks CS1 were incubated with preabsorbed anti-CS1 and anti-

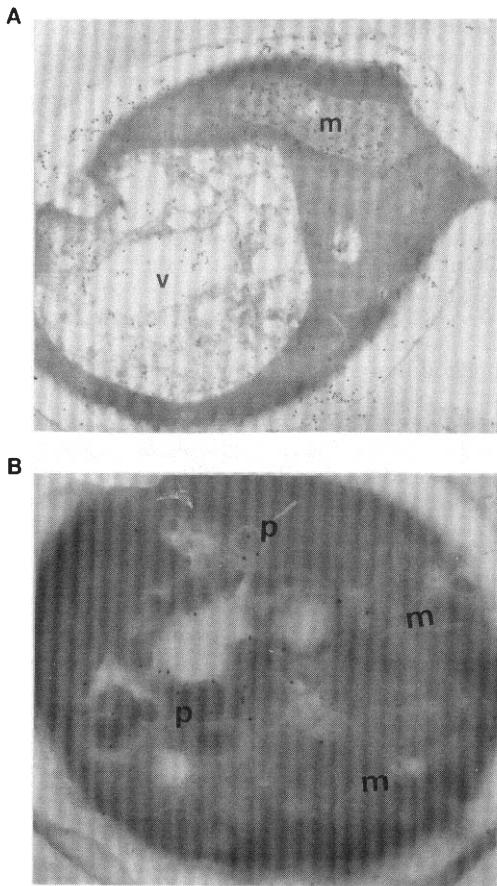


Fig. 2. Immunoelectron microscopic observation of intracellular organelle targeting of citrate synthases in *S. cerevisiae* by protein A-gold staining.

The localization of CS1 was observed in *S. cerevisiae* PSY40 that lacks CS2 using preabsorbed anti-CS1 antiserum from rabbit (A), and that of CS2 in *S. cerevisiae* PSY40 that lacks CS1 using preabsorbed anti-CS2 antiserum from mouse (B). Magnification: A, $\times 40,000$; B, $\times 50,000$. m, mitochondria; v, vesicle; p, peroxisomes.

CS2 antisera, respectively, followed by protein A-gold labeling. In the case of PSY40, despite of limitation of resolution and background gold particles probably due to somewhat improper sample preparation, many gold particles were found to be concentrated within a mitochondrion (Fig. 2A), which indicates that CS1 is localized into the matrix of this organelle. On the other hand, in the case of PSY38, gold particles were mainly detected in peroxisomes that were quite distinguishable from mitochondria which show

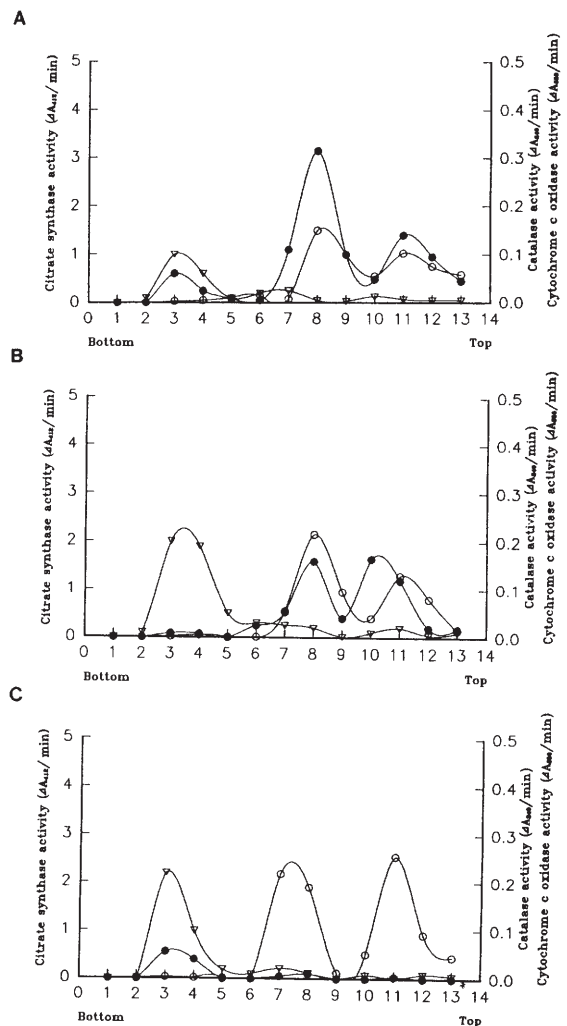


Fig. 3. Distribution of enzyme activities on discontinuous Nycodenz gradients from the cell lysates of *S. cerevisiae*.

A, *S. cerevisiae* PSY37 (*CIT1*, *CIT2*); B, *S. cerevisiae* PSY40 (*CIT1*, *cit2*); C, *S. cerevisiae* PSY38 (*cit1*, *CIT2*). ●, citrate synthase activity; ▽, catalase activity; ○, cytochrome c oxidase activity.

relatively clear intra-organelle crista structures, but no gold particle was seen in mitochondria (Fig. 2B). This result suggests that CS2 is not targeted to mitochondria but to peroxisomes.

To support the result from the immunogold localization experiments, the subcellular localization of CS1 and CS2 was also examined by cell fractionation on a Nycodenz stepped gradient. The distribution of CS1 and CS2 was analyzed by measuring the activity of citrate synthase in

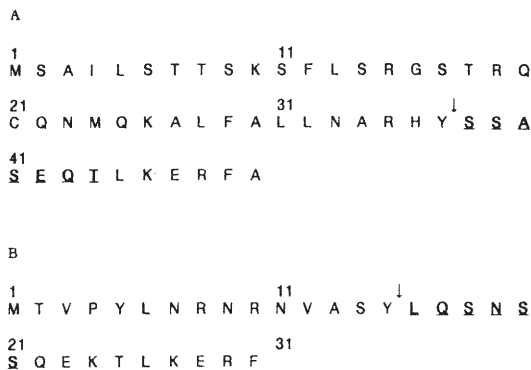


Fig. 4. Analysis of *N*-terminal cleavage motifs of citrate synthases of *S. cerevisiae*.

The *N*-terminal amino acid sequence of the CS1 precursor is derived from the sequence of *CIT1* gene (A) and that of the CS2 precursor from the sequence of *CIT2* gene (B). The boldfaced underlined parts designate the amino acid sequences determined by *N*-terminal sequencing of purified CS1 (A) and CS2 (B). The cleavage sites are indicated by ↓.

each fraction. Fractions were also analyzed for catalase as a peroxisomal marker enzyme and for cytochrome *c* oxidase as a mitochondrial marker enzyme. The activity profiles for PSY37 strain, which has wild-type alleles for *CIT1* and *CIT2*, are shown in Fig. 3A. The highest catalase activity was detected in fraction 3, bottom part of 25% Nycodenz layer, well separated from the activity of the mitochondrial enzyme, cytochrome *c* oxidase, that appeared in fraction 8, bottom part of 17% Nycodenz layer. The highest citrate synthase activity was measured in fraction 8 and a small amount of citrate synthase activity in fraction 3, which indicates that the two isofunctional citrate synthases, CS1 and CS2, are separately targeted to intracellular organelles: one to mitochondria the other to peroxisomes. The activity profiles for PSY40 strain (*CIT1*, *cit2*) which produces no CS2 (Fig. 3B) shows that CS1 is exclusively cosedimented with mitochondria. On the other hand, CS2, the sole citrate synthase produced by PSY38 strain (*cit1*, *CIT2*), was found in the peroxisomal fraction (Fig. 3C). Although Lewin *et al.* (15) have fractionated the cell lysate of *S. cerevisiae* by ultracentrifugation and shown that the CS2 activity cosedimented with peroxisomes, no immunoelectron microscopic observation has been attempted. It is thus in this experiment that the localization of CS2 was analyzed by both immunoelectron microscopy and by cell fractionation.

Identification of *N*-terminal cleavage sites of CS1 and CS2

The *N*-terminal sequence of purified CS1 was determined to be S-S-A-S-E-Q-T (Fig. 4A), which indicates that 37 *N*-terminal amino acids of the CS1 precursor are cleaved in the process of mitochondrial targeting (21, 18). As described in the previous paper (3), the molecular weight of mature CS1 subunit determined by SDS-PAGE is about 48.3 kDa while that of the CS1 precursor predicted from the open reading frame of *CIT1* is 53.5 kDa, which is in good accordance with the present result. In general, *N*-terminal mitochondrial signal peptides (mTPs) do not share any distinct consensus sequences, but they do exhibit some common features. They are usually rich in basic, hydrophobic, and hydroxylated residues, but lack acidic amino acids. In addition, there are at least four types of conserved motif for proteolytic cleavage of the mTPs during mitochondrial targeting, *i.e.*, R-none (X↓X-S), R-2 (R-X↓X-S), R-3 (R-X-Y↓S/A), and R-10 (R-X<↓>F/I/L-S-X-X-X-X-X-X↓X). Especially, the R-3 motif shows the highest confidence, 75% (7). There exists an R₍₃₅₎-H-Y-S₍₃₈₎ sequence in the predicted amino acid sequences of the CS1 precursor (Fig. 5A), and it was confirmed in this experiment that the R-3 motif in this precursor protein is cleaved correctly.

The *N*-terminal amino acid sequence of mature CS2 was revealed to be L-Q-S-N-S-S (Fig. 4B), which suggests that the 15 *N*-terminal amino acids are cut off from the CS2 precursor during its import into peroxisomes (18). There are at least two distinct types of peroxisomal targeting signals (PTSs) that are involved in the import of peroxisomal matrix proteins. The first PTS (PTS1) is *C*-terminal SKL sequence that is known to be ubiquitous among eukaryotes (8, 9, 10, 2, 13). In contrast, some peroxisomal proteins such as the 3-oxoacyl-CoA thiolases from *S. cerevisiae* (6) and from rat (22) do not contain the conserved PTS1, but they possess *N*-terminal PTS (PTS2) which consists of 11-16 amino acids cleaved proteolytically upon peroxisomal import instead. Catalases are also devoid of the conserved PTS1, although some contain fairly similar sequences in this position like SKF (4). Instead of PTS1, catalase A of *S. cerevisiae* contains an internal peroxisomal targeting signal in its *N*-terminal third (13). It has been shown that CS2 of *S. cerevisiae* has *C*-terminal SKL sequence, PTS1 (18). It is thus quite interesting that the *N*-terminal 15 amino acids are cut off notwithstanding the presence of PTS1, because none of the proteins containing PTS1 has been reported to have *N*-terminal sequences that are cleaved upon translocation of the protein into peroxisomes yet. However, more detailed analysis is required to clarify whether the *N*-terminal sequence of CS2

precursor truly functions as a PTS2 and thus CS2 precursor has both the PTSS, PTS1 and PTS2.

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REFERENCES

1. Baudhuin, P., H. Beaufay, Y. Rahman-Li, O.Z. Sellinger, R. Wattiaux, P. Jacques, and C. de Duve, 1964. Tissue fractionation studies: 17. Intracellular distribution of monoamine oxidase, aspartate amino transferase, alanine aminotransferase, D-amino acid oxidase and catalase in rat liver tissue. *Biochem. J.* **92**, 179-184.
2. Blattner, J., S. Swinkels, H. Dörsam, T. Prospero, S. Subramani, and C. Clayton, 1992. Glycosome assembly in trypanosomes: Variations in the acceptable degeneracy of a COOH-terminal microbody targeting signal. *J. Cell. Biol.* **119**, 1129-1136.
3. Cho, N.-S., K.S. Kim, and P.-J. Maeng, 1991. Purification and characterization of nonmitochondrial citrate synthase from *Saccharomyces cerevisiae*. *Kor. J. Microbiol.* **29**, 230-237.
4. Cohen, G., W. Rapatz, and H. Ruis, 1988. Sequence of *CTA1* gene and amino acid sequence of catalase A derived from it. *Eur. J. Biochem.* **176**, 159-163.
5. Duntze, W., D. Neumann, J.M. Gancedo, W. Atzpodien, and H. Holzer, 1969. Studies on the regulation and localization of the glyoxylate cycle enzymes in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **10**, 83-89.
6. Erdmann, R., 1994. The peroxisomal targeting signal of 3-oxoacyl-CoA thiolase from *Saccharomyces cerevisiae*. *Yeast* **10**, 935-944.
7. Gavel, Y. and G. von Heijne, 1990. Cleavage-site motifs in mitochondrial targeting peptides. *Protein Eng.* **4**, 33-37.
8. Gould, S.J., G.-A. Keller, and S. Subramani, 1988. Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins. *J. Cell. Biol.* **107**, 897-905.
9. Gould, S.J., G.-A. Keller, M. Schneider, S.H. Howell, L.J. Garrard, J.M. Goodman, B. Distel, H. Tabak, and S. Subramani, 1990. Peroxisomal protein import is conserved between yeast, plants, insects and mammals. *EMBO J.* **9**, 85-90.
10. Gould, S.J., G.-A. Keller, N. Hosken, J. Wilkinson, and S. Subramani, 1989. A conserved tripeptide sorts proteins to peroxisomes. *J. Cell. Biol.* **108**, 1657-1664.
11. Harlow, E. and D. Lane, 1988. Antibodies: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor.
12. Kim, K.S., M.S. Rosenkrantz, and L.P. Guarente, 1986. *Saccharomyces cerevisiae* contains two functional citrate synthase genes. *Mol. Cell. Biol.* **6**, 1936-1942.
13. Kragler, F., A. Rangeder, J. Raupachova, M. Binder, and A. Hartig, 1993. Two independent peroxisomal targeting signals in catalase of *Saccharomyces cerevisiae*. *J. Cell. Biol.* **120**, 665-673.
14. Krebs, H.A. and J.M. Lowenstein, 1960. The tricarboxylic acid cycle, p. 129-203. In D.M. Greenberg (ed.), *Metabolic pathways*. Academic Press, Inc., New York.
15. Lewin, A.S., V. Hines, and G.M. Small, 1990. Citrate synthase encoded by the *CIT2* gene of *Saccharomyces cerevisiae* is peroxisomal. *Mol. Cell. Biol.* **10**, 1399-1405.
16. Mason, T.L., R.O. Poyton, D.C. Wharton, and G. Schatz, 1973. Cytochrome c oxidase from bakers' yeast. *J. Biol. Chem.* **248**, 1346-1354.
17. Matsudaira, P., 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. *J. Biol. Chem.* **262**, 10035-10038.
18. Rosenkrantz, M., T. Alam, K.S. Kim, B.J. Clark, P.A. Srere, and L.P. Guarente, 1986. Mitochondrial and nonmitochondrial citrate synthase in *Saccharomyces cerevisiae* are encoded by distinct homologous genes. *Mol. Cell. Biol.* **6**, 4509-4515.
19. Sherman, F., G.R. Fink, and J.B. Hicks, 1983. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor.
20. Srere, P.A., 1969. Citrate synthase. *Methods Enzymol.* **13**, 3-26.
21. Suissa, M., K. Suda, and G. Schatz, 1984. Isolation of the nuclear genes for citrate synthase and fifteen other mitochondrial proteins by a new screening method. *EMBO J.* **3**, 1773-1781.
22. Swinkeis, B.W., S.J. Gould, A.G. Bodnar, R.A. Rachubinski, and S. Subramani, 1991. A novel, cleavable peroxisomal targeting signal at the amino-terminus of the rat 3-ketoacyl-CoA thiolase. *EMBO J.* **10**, 3255-3262.
23. Thieringer, R., H. Shio, Y. Han, G. Cohen, and P.B. Lazarow, 1991. Peroxisomes in *Saccharomyces cerevisiae*: Immunofluorescence analysis and import of catalase A into isolated peroxisomes. *Mol. Cell. Biol.* **11**, 510-522.
24. Towbin, H., T. Staehelin, and J. Gordon, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedures and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350-4354.

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초 록: *Saccharomyces cerevisiae*에서 Citrate Synthase의 세포내 소기관 Targeting

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*Saccharomyces cerevisiae*가 생성하는 두 가지 citrate synthase, 즉, CS1과 CS2의 세포내 소기관 targeting 양상을 분석하였다. 이를 위하여 CS1와 CS2에 대한 각각의 항혈청을 토끼와 mouse로부터 제조하였으며, 각 항혈청을 이용한 면역전자현미경법을 통하여 분석한 결과, CS1은 mitochondria로, CS2는 peroxisomes로 각각 targeting됨을 확인하였다. 또한, Nycodenz 농도 구배 초원심분리법을 이용한 세포분획을 통하여 분석했을 때에도 CS1은 mitochondria와 CS2는 peroxisomes와 각각 cosedimentation 하는 것으로 밝혀졌다. CS1의 N-말단 아미노산서열은 S-S-A-S-E-Q-T로 확인되었는데, 이는 이 효소의 전구체가 mitochondria로 targeting되는 과정에서 R(35)-H-Y↓S(38)와 같은 절단에 의하여 37개의 N-말단 아미노산들이 잘려 나간다는 것을 나타내며, 이로써 CS1 전구체는 mitochondrial targeting signal 내에 R-3 형의 cleavage motif를 갖고 있는 것으로 확인되었다. 한편, CS2의 N-말단 아미노산서열은 L-Q-S-N-S-S로 밝혀졌는데, 이는 CS2 전구체가 peroxisomes로 targeting되는 과정에서 A(13)-S-Y↓L(16)와 같은 절단에 의하여 15개의 N-말단 아미노산이 잘려 나간다는 것을 의미한다. 아직까지 C-말단 peroxisomal targeting signal (PTS1, 즉, S-K-L)을 갖는 단백질 중에서 peroxisomes로 targeting될 때 잘리는 N-말단 아미노산 서열을 갖는 것이 보고되어 있지 않은 상태이므로, CS2 전구체가 PTS1과 함께 peroxisomes로의 수송과정에서 잘리는 15개의 N-말단 아미노산을 갖고 있다는 것은 주목할 만한 일로 사료된다.