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Human trehalase is a stress responsive protein in *Saccharomyces cerevisiae*

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ABSTRACT

Three trehalases *ATH1*, *NTH1*, and *NTH2* have been identified in *Saccharomyces cerevisiae*. *ATH1*, and *NTH1* hydrolyze trehalose to glucose to provide energy and assist in recovery from stress. Human trehalase (*TREH*) is expressed in the intestine and kidney and probably hydrolyzes ingested trehalose in the intestine and acts as marker of renal tubular damage in kidney. Since trehalose is not present in circulation or kidney tubules, its renal effect suggests it has other yet unidentified actions. Here we examined the function of human trehalase in budding yeast. We constructed three yeast trehalase mutants (*NTH1Δ*, *NTH2Δ*, and *ATH1Δ*) and then transformed *TREH* into these mutants. *NTH1Δ* did not grow on media containing trehalose as the carbon source, and *TREH* did not rectify *NTH1Δ* dysfunction and also did not grow on trehalose medium, suggesting that *TREH* is not responsible for utilization of exogenous trehalose in yeast. In experiments involving exposure to heat, osmotic and oxidative stresses, *NTH1Δ* showed no recovery. Interestingly, *ATH1Δ-TREH* showed high sensitivity to all three stressors. *ATH1Δ* and *NTH2Δ* showed very low neutral trehalase activity and *NTH1Δ* did not show any neutral trehalase activity, and trehalose concentrations were higher. Increased neutral trehalase activity (equivalent to the wild type), reduction of trehalose content and brisk sensitivity to stressors were noted in *TREH-ATH1Δ* strain, but not in *TREH-NTH1Δ* or *-NTH2Δ*. Our results suggest that *TREH* acts as a stress–response protein in the kidney rather than involved in utilization of exogenous trehalose.

Trehalase (EC 3.2.1.28) is an intrinsic glycoprotein of the small intestine and renal brush-border membranes that hydrolyzes α,α-trehalose (1-α-D-glucopyranosyl α-D-glucopyranoside) to two glucose molecules in animals. In mammals, trehalase cDNA has been cloned from rabbit [1], rat [2], human [3], and mouse [4]. Studies on the properties of renal and urinary human trehalase suggest that this enzyme might be involved in glucose transport across the brush-border membranes in both the kidney and intestine [5]. Trehalase in the intestine is probably involved in the hydrolysis of ingested trehalose; isolated trehalose intolerance due to deficiency of intestinal trehalase has been reported to manifest clinically with diarrhea upon ingestion of trehalose-containing mushrooms [6]. On the other hand, urinary trehalase has been proposed as a specific marker of renal tubular damage [3]. Since trehalose is not present in the circulation and kidney tubules do not seem to be exposed to trehalose, the effect of trehalase on the kidney suggests that it has other yet unidentified actions in the kidney.

Three trehalases have been identified so far in *Saccharomyces cerevisiae*; *NTH1*, *NTH2*, and *ATH1*. The neutral trehalase 1 (*NTH1*) is a cytosolic protein with maximal activity at pH 7.0 involved in the hydrolysis of cytosolic trehalose, and is activated by cyclic AMP-dependent phosphorylation; Nth1p is responsible for intracellular mobilization and/or recycling of trehalose [7–9]. *NTH2* is a homolog of *NTH1*, but lacks both neutral and acid trehalase activity and has no detectable influence on trehalose concentration in intact cells [7,8]. *ATH1* is a vacuolar protein with maximal activity at pH 4.5, mainly localized in the periplasmic space and is an extracellular enzyme involved in splitting disaccharides into glucose [10]. Extensive investigation on the biological functions of the yeast trehalases has shown low trehalose concentrations through glucose formation by trehalose hydrolysis. In the budding yeast *S. cerevisiae*, the *NTH1* as well as its homolog *NTH2* protect yeast cells against stress damage, e.g., heat and osmotic shocks, nutrient starvation, dehydration, and exposure to toxic chemicals or oxidative agents [11–13]. Furthermore, the rapid hydrolysis of stored trehalose by Nth1p provides indispensable energy for accurate re-folding of partially denatured proteins after heat shock [14]. However, the exact functions of these enzymes are still not clear.

In the present study, we employed genetic and biochemical approaches using *S. cerevisiae* (introduction of human trehalase (*TREH*) into a series of yeast trehalase-deletion mutants; *NTH1Δ*, *NTH2Δ* and *ATH1Δ*) to investigate the function of human trehalase. The results showed that the transformed *TREH* into the yeast

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NTH1Δ did not rectify the defective growth on media containing trehalose as the carbon source, suggesting that human trehalase does not utilize exogenous trehalose. Yeast with *ATH1Δ-TREH* were sensitive to various cellular stresses. The results suggest that human trehalase is a stress–response protein in the kidney rather than being involved in utilization of exogenous trehalose.

Materials and methods

Yeast strain and medium. Saccharomyces cerevisiae strain W303-1*a* (*MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3 leu2-112 can1-100*) was used in this study. Cells were cultured either in YPAD medium (1% yeast extract, 2% polypeptone, 40 mg/ml adenine sulfate, and 2% glucose) or a synthetic medium (SD medium) containing 2% glucose, 6.7 g/L yeast nitrogen base (Difco Laboratory, Detroit, MI) without amino acids and supplemented with essential amino acids. When necessary, these media were supplemented with 500 mg/L G-418, a kanamycin derivative. For growth on solid trehalose medium, the respective strains were streaked out on YPAT plates and incubated at 30 °C for 2–3 days.

Construction of ATH1Δ, NTH1Δ, and NTH2Δ mutants. The three yeast trehalases (*NTH1*, *NTH2*, and *ATH1*) were deleted in the W303-1*a* strain using a deletion cassette carrying a kanamycin-resistant (*kan*^{r}) gene in *Escherichia coli*, *loxP-kanMX-loxP*, which was amplified by polymerase chain reaction (PCR) directed on the template plasmid pUG6 (a gift from Dr. J.H. Hegemann, Washington University, St. Louis, MO) [15] with synthetic oligonucleotides forward (knockF) and reverse (knockR) primers listed in Table 1. The W303-1*a* strain was transformed with the PCR product and trehalase-deleted transformants (*NTH1Δ*, *NTH2Δ*, and *ATH1Δ*) were selected by culturing cells on an YPAD plate containing the kanamycin derivative G-418 at 500 mg/L [15]. Deletion of the yeast trehalases was confirmed by detecting an 865-bp PCR product from the kanr gene using synthetic oligonucleotides KnockF (Table 1) and the kan^r-specific primer Kan-R378 [16] as the forward and reverse primers, respectively.

Construction of yeast expression plasmid of human TREH. The wild-type cDNA of human trehalase (*TREH)* was purchased from Open Biosystem (Huntsville, AL). To prepare the yeast expression plasmid of human *TREH*, EcoRI–HindIII fragment containing the full-length *TREH* was obtained by PCR directed against human trehalase cDNA, and inserted into the multicloning site of the centromere-based vector pXY122. The construct was confirmed by sequencing and then transformed into the yeast strains lacking the *NTH1* or *NTH2* or *ATH1*; the transformants were selected on SD medium plates containing histidine.

Stress treatment assay. The heat shock survival assay on solid media was performed as described previously [8]. Various trehalase mutant cells and *TREH* complementing cells were streaked out on medium YPAD plate and incubated at 30 °C for 3 days. The cells were replica plated onto fresh plates and shifted to 52 °C for 4 h. Then, cells were shifted back to 30 °C, and recovery was analyzed after 2 days. For heat stress treatment in the liquid media, the trehalase mutant cells and *TREH* complementing cells were cultured in SD medium supplemented with essential amino acids, incubated on 40 °C water bath for 40 min. For chemical stress, the mutants and *TREH* complementing cells were streaked out on YPAD plate containing 5 mM H_2O_2 (Sigma) or 1.5 M NaCl, then cultured at 30 °C for 3 days.

Neutral trehalase activity and trehalose concentrations. Crude enzyme extracts from heat stress-treated cells were prepared using glass beads. Neutral trehalase activity was measured using the method as described previously [17] by incubating 20 μl of equal amounts of protein with 180 μl of 0.5 M trehalose in 50 mM imidazole–HCl, pH 7.0, for 10 min at 37 °C. The reaction was stopped by boiling for 5 min at 95 °C. Intracellular levels of trehalose were determined by the method described previously [9,18]. Glucose concentrations were determined enzymatically using a commercially available kit (F-kit, JK International, Tokyo, Japan) using the instructions provided by the manufacturer. A unit of the enzyme was defined as the amount of enzyme that catalyzed the formation of 1 μmol of glucose per min. The specific activity was expressed as units per mg of protein. Protein was measured with a protein assay kit (Bio-Rad, Hercules, CA).

Results

TREH does not grow on trehalose as a carbon source

The cytosolic neutral trehalase hydrolyzes intracellular trehalose in intact cells. In contrast to neutral trehalase, the vacuolar acid trehalase is necessary for phenotype growth on trehalose and is involved in utilization of external trehalose [10]. These results prompted us to investigate the biological phenotype of human trehalase. We constructed serial deletion mutants including three yeast trehalases by PCR and complemented these mutants with *TREH*. Growth of the various mutants on solid trehalose medium was studied. As shown in Fig. 1B, deletion of the gene *NTH1* resulted in failure of growth of yeast cells on trehalose as a carbon source, suggesting that the neutral trehalase plays a key role in trehalose utilization and is responsible for intracellular trehalose hydrolysis. However, *ATH1Δ* and *NTH2Δ* were able to grow on YPA agar plates containing trehalose, the behavior of these two deletion mutants were similar to that of the wild type. Their growth was slower than in cultures on glucose medium (compare Fig. 1A and B). All three trehalase-deletion mutants transformed with *TREH* showed that the *TREH* did not complement *NTH1* dysfunction and failed to grow on YPA agar plate containing trehalose (Fig. 1B). These results suggest that the human trehalase does not utilize external trehalose.

TREH induces yeast cells sensitivity to heat shock, oxidative and osmotic stresses

In *S. cerevisiae*, trehalose concentration increases during heat stress, and trehalose levels correlated closely with tolerance to heat stress [19]. Other studies showed that Nth1 and Nth2 are multiple stress responsive proteins involved in cell recovery from severe heat shock. To investigate whether *TREH* is involved in the stress–response, we treated the *ATH1Δ*, *NTH1Δ*, *NTH2Δ* mutants

Table 1
Oligonucleotides used in this study for construction of yeast trehalase mutants.

Name	Sequence
ATH1-Knock F	5′-GTCCTTTATGAGAGCCTGCTAGTATCACCTAATATTGCATCTGCAGCTGAAGCTTCGTACGC-3′
ATH1-Knock R	5′-CGCTGGTGCAAAAGACCAATTTCAAGGTCACTACTATATAAGGTGCGCATAGGCCACTAGTGGATCTG-3′
NTH1-Knock F	5′-CAACTGCATAGATATAAGGAGATTACTAGATACAAGAACGCCTGCAGCTGAAGCTTCGTACGC-3′
NTH1-Knock R	5′-CATATAAAGAGGATTTAGGTACCTAGAGGACTGTACCTGGAGGCATAGGCCACTAGTGGATCTG-3′
NTH2-Knock F	5′-GGTAATTTGAAGTCCAATTTTTCCGAGCGGCGTGCCAGTTCCACCAGCTGAAGCTTCGTACGC-3′
NTH2-Knock R	5′-GCACTAGACCACGCAAAGGTGAACCGGCTTGGTACGAGAATTCGCATAGGCCACTAGTGGATCTG-3′