Identification of a Gene for a Polyamine Transport Protein in Yeast*

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Properties of a membrane protein encoded by YLL028w were examined using yeast cells transformed with the gene. The transformed cells became resistant to polyamine toxicity, and the resistance was overcome by bafilomycin A1, an inhibitor of vacular H+ -ATPase. Although spermine uptake activity of the transformed cells was almost the same as that of wild type cells, the uptake activity of vacuolar membrane vesicles from the transformed cells was higher than that from wild type cells. The transformed cells became resistant to MGBG (methylglyoxal bis(guanylhydrazone)) and paraquat, which stimulate polyamine uptake have been reported (8–10). To identify a gene encoding a protein that catalyzes excretion of spermidine, we searched for amino acid homology between Blt and proteins encoded by yeast genome (17) and detected four candidate genes, we then examined polyamine toxicity and transport activity using yeast transformed with the genes. A membrane protein on vacuoles, which catalyzes proton gradient-dependent polyamine (putrescine, spermidine, and spermine) transport (18), was encoded by one of the genes (YLL028w). This is the first report of the isolation of a gene for a polyamine transporter (TPO1) from eukaryotic cells.

EXPERIMENTAL PROCEDURES

Yeast Strains and Culture Conditions—Yeast strain YW5-1B (MATa, trp1, ura3–52, leu2–3, 112) and its polyamine transport-deficient mutant YTH27-1 (Alexander et al. (21)) was introduced into YW5-1B by the lithium acetate method of Ro et al. (21). A YLL028w gene-disrupted mutant (YTH27-1) of yeast strain YPH499 (MATa, ade2–101, his3-D200, leu2–801, trp1-D63, ura3–52) was generated by one-step gene disruption (22) using the YLL028w::HIS3 PCr product. The YLL028w::HIS3 cassette was constructed by inserting the HIS3 gene (1.8 kilobase pair) into the Neo and Aaf restriction sites of YLL028w. Correct disruption was verified by Southern blot analysis. Yeast strain YPH499 was also transformed by pYLL028w as described above.

Spermine Transport Assay with Intact Cells and Vacular Membrane Vesicles—Yeast cells were harvested during the exponential phase (A600 = 0.5), washed twice with 5 ml of 20 mM Na-Hepes buffer (pH 7.2) containing 10 mM glucose and suspended at 2 mg dry weight/ml in the same buffer, and incubated at 30 °C. The reaction was started by the addition of [14C]spermine (37 MBq/mmol) at a final concentration of 100 μM, and 0.5-ml aliquots were filtered through cellulose acetate filters (pore size, 0.45 μm) at certain intervals (5, 10, and 20 min). The reaction was linear during incubation. The radioactivity trapped on the filters was counted in a liquid scintillation counter. Preparation of vacuolar membrane vesicles and the assay of spermine uptake were performed as described previously (18).

Measurement of Polyamine and Protein Contents—Polyamine contents were determined with high performance liquid chromatography as described previously (23) after extraction with hot trichloroacetic acid. Protein was determined by the method of Lowry et al. (24).

RESULTS AND DISCUSSION

Effect of the Membrane Protein Encoded by YLL028w on Polyamine Toxicity—To identify a gene encoding a protein that catalyzes excretion of polyamines, we searched for amino acid sequence homology between Blt, a protein involved in the excretion of spermidine (16), and membrane proteins encoded by yeast genome (17), and detected four candidate genes, YLL028w, YBR180w, YKR105c, and YCR023c. The activity of proteins encoded by these genes was first tested by polyamine toxicity in yeast.

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† The abbreviations used are: PCR, polymerase chain reaction; MGBG, methylglyoxal bis(guanylhydrazone).
We reported previously that cell growth of yeast was inhibited by polyamines in magnesium-limited (50 μM Mg²⁺) synthetic medium (19) and that the inhibition of cell growth did not occur in a polyamine transport-deficient mutant YTM22-8 (8). Thus, if a protein catalyzing excretion of polyamines is encoded by one of the genes, polyamine toxicity in wild type cells should be decreased when transformed by that gene. As shown in Fig. 1, A–C, inhibition of cell growth of wild type yeast was observed by addition of 75 mM putrescine, 3 mM spermidine, or 0.3 mM spermine (SPM) was followed by measuring A₅₄₀ ○, wild type yeast YW5-1B, YEp351; ▲, polyamine transport-deficient mutant YTM22-8; ●, TPO1-transformed yeast YW5-1B/pYLL028w. D–F, cells were grown in the presence and absence of polyamine. ○, wild type yeast YPH499 cultured without polyamine; ●, wild type yeast YPH499 cultured with 65 mM putrescine (PUT), 1.5 mM spermidine (SPD), or 0.2 mM spermine (SPM); ▲, TPO1-disrupted mutant YTH27-1 cultured without polyamine; ●, TPO1-disrupted mutant YTH27-1 cultured with polyamine indicated above.

FIG. 1. Effect of YLL028w (TPO1) on cell growth in the presence and absence of polyamine. A–C, cell growth in the presence of 75 mM putrescine (PUT), 3 mM spermidine (SPD), or 0.3 mM spermine (SPM) was followed by measuring A₅₄₀ ○, wild type yeast YW5-1B/YEp351; ▲, polyamine transport-deficient mutant YTM22-8; ●, TPO1-transformed yeast YW5-1B/pYLL028w. D–F, cells were grown in the presence and absence of polyamine. ○, wild type yeast YPH499 cultured without polyamine; ●, wild type yeast YPH499 cultured with 65 mM putrescine (PUT), 1.5 mM spermidine (SPD), or 0.2 mM spermine (SPM); ▲, TPO1-disrupted mutant YTH27-1 cultured without polyamine; ●, TPO1-disrupted mutant YTH27-1 cultured with polyamine indicated above.

The above results were confirmed by making the YLL028w gene-disrupted mutant. The toxicity of 65 mM putrescine, 1.5 mM spermidine, or 0.2 mM spermine was much stronger in the mutant than in wild type yeast (Fig. 1, D–F).

Properties of the Membrane Protein Encoded by YLL028w (TPO1)—Polyamine contents in wild type yeast cells and in YLL028w (TPO1), a gene for a transporter of polyamines)-transformed or -disrupted cells were compared. When TPO1 was transformed into YW5-1B cells, the accumulation of spermine in the cells cultured in the presence of 0.3 mM spermine increased significantly compared with that in wild type cells (Figs. 2A, 1 and 2). When TPO1 was disrupted in YPH499 cells, the final accumulation of spermine in the mutant YTH27-1 was much lower than that in wild type cells (Figs. 2A, 3 and 4).

Spermine uptake activities in wild type cells or TPO1-transformed or -disrupted cells were nearly equal (Fig. 2B). We then measured spermine uptake activity of vacuolar membrane vesicles, since the existence of proton potential-dependent poly-
A1, a specific inhibitor of vacuolar H+ ATPase (25), inhibits polyamine uptake activity of vacuolar membrane vesicles prepared from *TPO1*-disrupted mutant YTH27-1 was small, suggesting that on the vacuolar membrane by *TPO1*. When amino acid sequences of the yeast membrane protein and PotE were compared, the protein possessed a longer hydrophilic NH2-terminal region, in which many serine and threonine residues are included. Thus, the NH2-terminal region of the protein may be important for regulation by *PTK1*.

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**FIG. 4.** Effect of cations (1 mM MGBG (A), 0.5 mM paraquat (B), 0.05 mM Ni2+ (C), and 0.05 mM Co2+ (D)) on cell growth of wild type yeast and *YLL028w* (*TPO1*-transformed yeast). Wild type yeast Y51-1B/pSc535 cultured with cations; a, wild type yeast Y51-1B/pSc535 cultured with cations; ●, *TPO1*-transformed yeast Y51-1B/pSc535 cultured with cations; □, *TPO1*-transformed yeast Y51-1B/pYLL028w cultured with cations.

The results, taken together, indicate that a membrane protein encoded by *YLL028w* (*TPO1*) is a polyamine transport protein on the vacuolar membrane. However, the change of spermine uptake activity of vacuolar membrane vesicles in *TPO1*-transformed or -disrupted cells was small, suggesting that there is at least one more polyamine transport protein on the vacuolar membrane.

**Characteristics of the Membrane Protein Encoded by *YLL028w* (*TPO1*)—**The *YLL028w* (*TPO1*) is located on chromosome XII and encodes a membrane protein consisting of 586 amino acid residues (17). The protein has 12 putative transmembrane segments, and three glutamic acids, which may interact with polyamines, are located in similar positions to those of PotE, a putrescine excreting protein in *E. coli* (6). Polyamine transport in yeast is positively regulated by protein kinases (*PTK1* and *PTK2*) (8–10). The activity of our polyamine transport-deficient mutant YTM22-8 was recovered by *PTK2* in a single-copy vector (9) and by *PTK1* in a multicopy vector (8). Thus, we hypothesize that polyamine transport on the plasma membrane is regulated by *PTK2* and that on the vacuolar membrane by *PTK1*. When amino acid sequences of the yeast membrane protein and PotE were compared, the protein possessed a longer hydrophilic NH2-terminal region, in which many serine and threonine residues are included. Thus, the NH2-terminal region of the protein may be important for regulation by *PTK1*.