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AGT1, Encoding an α-Glucoside Transporter Involved in Uptake and Intracellular Accumulation of Trehalose in *Saccharomyces cerevisiae*

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The trehalose content in *Saccharomyces cerevisiae* can be significantly manipulated by including trehalose at an appropriate level in the growth medium. Its uptake is largely dependent on the expression of *AGT1*, which encodes an α-glucoside transporter. The trehalose found in a *tps1* mutant of trehalose synthase may therefore largely reflect its uptake from the enriched medium that was employed.

Most work on trehalose metabolism in yeast concerns factors governing its endogenous level (6, 14, 15). Trehalose synthase is a multimeric protein composed of four subunits encoded by *TPS1*, *TPS2*, *TSL1*, and *TPS3* (2, 18), of which only Tps1p catalyzing the formation of trehalose-6-phosphate from UDP-Glc and glucose-6-phosphate is essential for growth on rapidly fermentable carbon sources like glucose and fructose (8, 22). The molecular mechanism underlying this defect is not yet understood (21). The deletion of *TPS1* in principle results in the loss of trehalose accumulation. However, the existence of another functional pathway for trehalose synthesis in yeast has been postulated, based on data showing that trehalose accumulation is somehow related to maltose metabolism or constitutive maltose gene expression (5, 15) and on one report of a putative ADP-Glc-dependent trehalose synthase activity (17). However, most demonstrations in wild-type and *tps1* mutant strains have employed an enriched medium such as yeast extract-peptone (YEP), and our analysis of yeast extract from various commercial sources (Difco and BIOKAR Diagnostic) by using ionic exchange chromatography with pulsed amperometric detection (4) shows it to contain ca. 1.5% trehalose by weight, which would result in a yield of 150 μg of free trehalose per ml of YEP medium. Since *Saccharomyces cerevisiae* is known to show both a high- and a low-affinity trehalose uptake (13, 19, 20), we speculated that the trehalose accumulation of *tps1* mutant cells might be related to uptake from the medium, perhaps involving *AGT1*, a maltose-controlled gene encoding a transporter with broad specificity for α-glucosides, including trehalose (10).

Yeast mutant strains used in this work were derived from the prototrophic CEN.PK113-7D strain (the kind gift of K.-D. Entian and P. Kötter, Frankfurt, Germany), which possesses a *MAL2-8*^{c} dominant mutation. Culturing was carried out at 30°C in YEP medium (10 g of yeast extract and 10 g of Bacto Peptone per liter) or in mineral medium (MIN) prepared according to the method described in reference 23 and buffered at pH 5.8 by the addition of 10 g of succinic acid and 6 g of NaOH per liter. The carbon source was added to the media at a final concentration of 10 g · liter^{-1}, and auxotrophic requirements, when required, were added at 100 mg · liter^{-1}. The deletion of *TPS1* and *AGT1* was performed according to the PCR and short homolog fragment procedure of Wach et al. (24) by using the pUG6 plasmid bearing the *loxP-kanMX4-loxP* module (9) for *TPS1* and pUG6lacZ bearing *lacZ-kanMX4* (3) for *AGT1*. The oligonucleotides used to construct the deletion cassette were as follows: d-TPS1 (5′-ATGACTACGGATAACGCTAAGGCGCAACTGACCTCGTCTTCAGCTGAAGCTTCGTACGC-3′), containing the sequence from nucleotide +1 (A of the start codon) to +40 of the *TPS1* open reading frame (ORF), and f-TPS1 (5′-TCAGTTTTTGGTGGCAGAGGAGCTTGTTGAGCTGATGATGCATAGGCCACTAGTGGATCTG-3′), containing the complementary sequence from nucleotide +1445 to +1488 of the *TPS1* ORF, and S1-LAGT1 (5′-ATGAAAAATATCATTTCATTGGTAAGCAAGAAGAAGGCTGCCTCAAAATTCGTACGCTGCAGGTCGAC-3′), containing the sequence from nucleotide +1 (A of the start codon) to +48 of the *AGT1* ORF, and S2-AGT1 (5′-TAATTCTCGCTGTTTTATGCTTGAGGACTGACTGATACTCTCATCAGCGCATAGGCCACTAGTGGATCTG-3′), containing the complementary sequence from nucleotide +1783 to +1830 of the *AGT1* ORF.

Amplifications were carried out with Expand high-fidelity polymerase (Boehringer, Mannheim, Germany) according to standard procedures, and the amplified fragments (5 μg) were used for gene disruption as described in reference 9. For the deletion of *ATH1*, a 4.4-kb fragment bearing the full gene was amplified by using a pair of primers (ATH1-250 [5′-CGTATCACGACAAACCAACAGCC-3] and ATH1-500 [5′-CAAACCCTACTGACGAGAGAAG]) and genomic DNA from CEN.PK113-7D as a template. The PCR fragment cloned into the pGEM-T vector (Promega) was digested with *Eco*RV-*Hpa*I, which was replaced by a 1.5-kb *Eco*RV-*Sma*I *kanMX4* frag-

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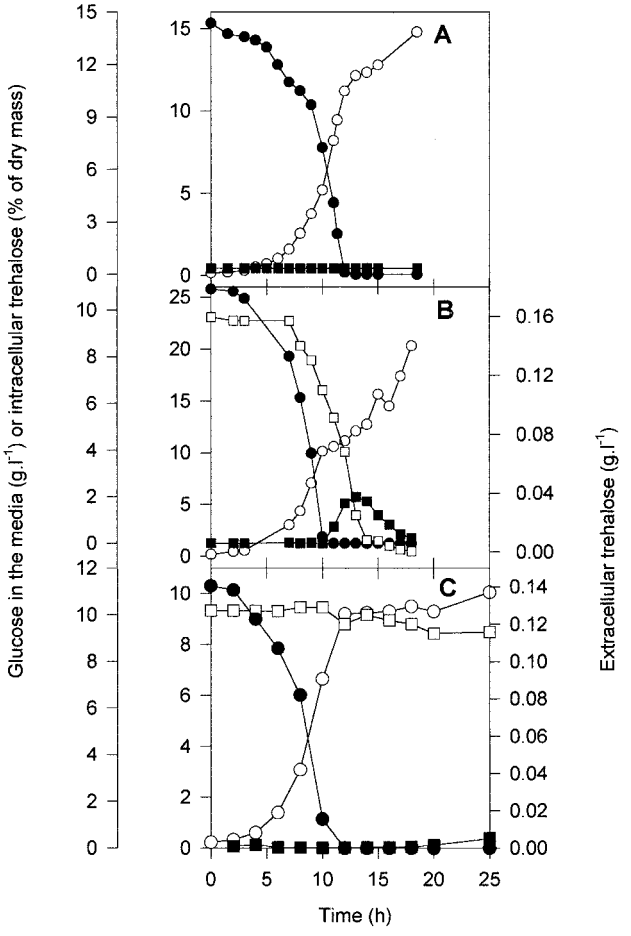

FIG. 1. Patterns of extracellular and intracellular trehalose levels during growth of wild-type CEN.PK113-7D (A and B) and RWY13 (Δ*agt1* [C]) on galactose-MIN without (A) or with 0.15 g of trehalose · liter^{-1} (B and C). Shown are the optical densities at 660 nm (○), the level of glucose in the medium (□), the level of extracellular trehalose (●), and the level of intracellular trehalose (■).

ment from pFAkanMX4 (24). This construct was cut with *Sca*II and *Spe*I, and the 3.45-kb fragment was gel purified and used for transformation (7). For the deletion of *NTH1*, a 1.2-kb *Stu*I-*Sna*BI of plasmid pTZ18RNTH1 (12) was replaced by a 1.4-kb *Eco*RV-*Sma*I *kanMX* module from pFAKanMX4 to yield pΔATH1. This plasmid was cut with *Pvu*II-*Alf*II, and the 3.6-kb fragment was used for transformation. Gene disruption was verified either by PCR (24) or by Southern blotting. The Δ*agt1* Δ*tps1* and Δ*nth1* Δ*ath1* double mutants were obtained by the crossing of haploid mutant strains. The correct Δ*agt1* Δ*tps1* mutant was characterized by its inability to grow on YEP-dextrose and its ability to turn dark blue on maltose due to the expression of *AGT1-lacZ*. The correct Δ*nth1* Δ*ath1* mutant was obtained from a typical tetrad as the only one lacking both acid and neutral trehalase activity. The level of intracellular trehalose was determined by the procedure described previously (16).

In agreement with the idea that the trehalose content can be manipulated by including trehalose in the growth medium, Fig. 1A shows that although there was a barely detectable level of trehalose in a wild-type strain (with a *MAL*^{c} mutation) cultivated on galactose-MIN, this level was significantly increased when the medium also contained 0.15 g of trehalose · liter^{-1} (Fig. 1B), but this accumulation was prevented by the deletion of *AGT1* (Fig. 1C). Table 1 shows that the maximal content of trehalose during the growth of a wild-type strain on YEP-galactose was about 10% of the dry mass and that this level dropped to 3.2% in the *tps1* isogenic strain. By contrast, in galactose-MIN, the content of the disaccharide in the wild-type strain reached ca. 1% and was not detectable in the *tps1* mutant. Both strains, however, were able to accumulate about 2% trehalose when the medium was initially supplemented with 0.15 g of trehalose · liter^{-1}.

The use of varying exogenous trehalose concentrations shows that with 1 g · liter^{-1} (ca. 3 mM), a 9% intracellular concentration of trehalose can be attained even in galactose-MIN. For a cell sap of 2.4 ml per g of cell dry mass (1, 11), this content corresponds to 110 mM, suggesting an active uptake of trehalose. The accumulation of trehalose was largely abolished by the deletion of *AGT1*, with a residual internal trehalose concentration probably contributed by trehalose synthase activity, as indicated by its loss in the *agt1 tps1* double mutant. However, at a much higher exogenous trehalose concentration (10 g · liter^{-1} or 30 mM), its endogenous level was on the order of 1% in both the single (*agt1*) and the double (*agt1 tps1*) mutants. This latter result is in agreement with previous work showing the existence of a nonconcentrative low-level K_m uptake system for trehalose (13, 19). Since acid and neutral tre-

TABLE 1. Levels of trehalose in wild-type and isogenic mutant strains with *AGT1*, *TPS1*, *NTH1*, or *ATH1* deleted during growth on different media

Growth medium	% Trehalose in dry mass of yeast with genotype[a]				
	Wild type	*AGT1 tps1*	*agt1 TPS1*	*agt1 tps1*	*nth1 ath1 AGT1*
YEP + galactose	9.50	3.2	ND	0.0	ND
MIN + galactose	1.20	BD	ND	0.0	ND
+ trehalose (0.15 g · liter^{-1})[b]	2.0	1.80	0.40	0.0	ND
+ trehalose (0.1 g · liter^{-1})	1.28	ND	0.38	0.0	ND
+ trehalose (1.0 g · liter^{-1})	8.80	ND	0.41	0.01	ND
+ trehalose (10 g · liter^{-1})	12.9	ND	1.34	0.91	ND
MIN + glucose	0.39	ND	0.0	ND	2.0
+ trehalose (0.1 g · liter^{-1})	1.28	ND	0.0	ND	3.80
+ trehalose (1.0 g · liter^{-1})	8.80	ND	ND	ND	9.50
+ trehalose (4.0 g · liter^{-1})	9.70	ND	ND	ND	11.10

[a] Although the intracellular trehalose level was determined during growth, the only values reported were the maximal ones attained for each growth condition. BD, below detection; ND, not done.

[b] Value of trehalose contained in YEP medium.

halases counteract the endogenous formation of trehalose (14), their influence on intracellular trehalose content was investigated by deleting the corresponding *NTH1* and *ATH1* genes. It is shown in Table 1 that the content of trehalose determined at the end of growth on glucose-MIN increased from 0.39% in a wild-type strain to 2% in cells lacking both trehalases. However, their absence had much less influence on the level of intracellular trehalose as its external concentration was increased, suggesting that yeast cells have a limiting capacity to store a maximum of 12 to 13% trehalose. Taken together, these results conclusively demonstrate that the accumulation of trehalose in yeast is mediated by at least two pathways: the first is via the endogenous UDP-Glc-linked trehalose synthase complex and the second is the uptake of exogenous trehalose via the high-affinity α-glucoside transporter encoded by *AGT1*.

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REFERENCES

1. **Alexandre, H., L. Plourde, C. Charpentier, and J. François.** 1998. Lack of correlation between trehalose accumulation, cell viability and intracellular acidification as induced by various stresses in *Saccharomyces cerevisiae*. Microbiology **144:**1103–1111.
2. **Bell, W., W. Sun, S. Hohmann, S. Wera, A. Reinders, C. De Virgilio, A. Wiemken, and J. M. Thevelein.** 1998. Composition and functional analysis of the *Saccharomyces cerevisiae* trehalose synthase complex. J. Biol. Chem. **273:** 3311–3319.
3. **Boles, E., P. de Jong-Gubbels, and J. T. Pronk.** 1998. Identification and characterization of *MAE1*, the *Saccharomyces cerevisiae* structural gene encoding mitochondrial malic enzyme. J. Bacteriol. **180:**2875–2882.
4. **Dallies, N., J. François, and V. Paquet.** 1997. A new method for quantitative determination of polysaccharides in the yeast cell wall. Application to the cell wall defective mutants of *Saccharomyces cerevisiae*. Yeast **14:**1297–1306.
5. **Ferreira, J. C., J. M. Thevelein, S. Hohmann, V. M. F. Paschoalin, L. C. Tugo, and A. D. Panek.** 1997. Trehalose accumulation in mutants of *Saccharomyces cerevisiae* deleted in the UDP-dependent trehalose synthase-phosphatase complex. Biochim. Biophys. Acta **1335:**40–50.
6. **François, J., M. A. Blazquez, J. Arino, and C. Gancedo.** 1997. Storage carbohydrates in the yeast *Saccharomyces cerevisiae*, p. 285–311. *In* F. K. Zimmermann and K.-D. Entian (ed.), Yeast sugar metabolism. Technomics Publishing Co., Inc., Lancaster, Pa.
7. **Gietz, R. D., R. H. Schiestl, A. R. Willems, and R. A. Woods.** 1995. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast **11:**355–360.
8. **Gonzales, M. I., R. Stucka, M. A. Blazquez, H. Feldmann, and C. Gancedo.** 1992. Molecular cloning of *CIF1*, a yeast gene necessary for growth on glucose. Yeast **8:**183–192.
9. **Güldener, U., S. Heck, T. Fiedler, J. Beinhauer, and J. H. Hegemann.** 1996. A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res. **13:**2519–2524.
10. **Han, E.-Y., F. Cotty, C. Sottas, H. Jiang, and C. A. Michels.** 1995. Characterization of *AGT1* encoding a general α-glucoside transporter from *Saccharomyces cerevisiae*. Mol. Microbiol. **17:**1093–1107.
11. **Hottiger, T., C. De Virgilio, M. N. Hall, T. Boller, and A. Wiemken.** 1994. The role of trehalose synthesis for the acquisition of thermotolerance in yeast. II. Physiological concentrations of trehalose increase the thermal stability of proteins *in vivo*. Eur. J. Biochem. **219:**187–193.
12. **Kopp, M., H. Müller, and H. Holzer.** 1993. Molecular analysis of the neutral trehalase gene from *Saccharomyces cerevisiae*. J. Biol. Chem. **268:**4766–4774.
13. **Kotyk, A., and D. Michaljanivova.** 1979. Uptake of trehalose by *Saccharomyces cerevisiae*. J. Gen. Microbiol. **110:**323–332.
14. **Nwaka, S., and H. Holzer.** 1998. Molecular biology of trehalose and the trehalases in the yeast *Saccharomyces cerevisiae*. Prog. Nucleic Acid Res. Mol. Biol. **58:**197–237.
15. **Panek, A. D., and A. C. Panek.** 1990. Metabolism and thermotolerance function of trehalose in *Saccharomyces cerevisiae*: a current perspective. J. Biotechnol. **14:**229–238.
16. **Parrou, J. L., and J. François.** 1997. A simplified procedure for a rapid and reliable assay of both glycogen and trehalose in whole yeast cells. Anal. Biochem. **248:**186–188.
17. **Paschoalin, V. M. F., J. T. Silva, and A. D. Panek.** 1989. Identification of an ADPG-dependent trehalose synthase in *Saccharomyces cerevisiae*. Curr. Genet. **16:**81–87.
18. **Reinders, A., N. Bürckert, S. Hohmann, J. M. Thevelein, T. Boller, A. Wiemken, and C. DeVirgilio.** 1997. Structural analysis of the subunits of the trehalose-6-phosphate synthase/phosphatase complex in *Saccharomyces cerevisiae* and their function during heat shock. Mol. Microbiol. **24:**687–695.
19. **Stambuk, B. U., P. S. de Araujo, A. D. Panek, and R. Serrano.** 1996. Kinetics and energetics of trehalose transport in *Saccharomyces cerevisiae*. Eur. J. Biochem. **237:**876–881.
20. **Stambuk, B. U., A. D. Panek, J. H. Crowe, L. M. Crowe, and P. S. de Araujo.** 1998. Expression of high-affinity trehalose-H^+ symport in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **1374:**118–128.
21. **Thevelein, J. M., and S. Hohmann.** 1995. Trehalose synthase: guard to the gate of glycolysis in yeast? Trends Biochem. Sci. **20:**3–10.
22. **Van Aelst, L., S. Hohmann, B. Bulaya, W. de Koning, L. Sierkstra, et al.** 1993. Molecular cloning of a gene involved in glucose sensing in the yeast *Saccharomyces cerevisiae*. Mol. Microbiol. **8:**927–943.
23. **Verduyn, C., E. Postma, A. Scheffers, and J. P. Van Dijken.** 1991. Effect of benzoic acid on metabolic fluxes in yeasts: a continuous-culture study on the regulation of respiration and alcoholic fermentation. Yeast **8:**501–516.
24. **Wach, A., A. Brachat, R. Pöhlmann, and P. Philippsen.** 1994. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast **10:**1793–1808.