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NOTES

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 CEN.PK113-7D strain (the kind gift of K.-D. Entian and P. Kötter, Frankfurt, Germany), which possesses a MAL2-8’ 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 sucinic 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’-ATGACTACCGGATAACGTAAGGCGCACTCTGCTTCAGTAGCACAAGCTTCGTAGCCGCGTAGTGAGCTGATGATGCATAGGCCACTAGTGATCTG-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’-TCAGTTTGTGCGGCAGAGAGGACCTGTTAGTGATGATCAGGCGCCACTAGTGCGAGCCCGTTG-3’), containing the complementary sequence from nucleotide +1445 to +1488 of the TPS1 ORF, and S1-LAGT1 (5’-ATGAAAAATATCATTTCTATAGTTGTAAGCAGAAAGGAAGAGCTGCTTCATAAAATTCGTACGCTGCAGGTGACG-3’), containing the sequence from nucleotide +1 (A of the start codon) to +48 of the AGT1 ORF, and S2-AGT1 (5’-TAATTCCTCCTGCTTATTATGCGACGACTAGCTACTCTCATAAGCGCATAAGCCACTAGTGAGCTTG-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’-CGTATCAGCAGAAAAACCAACAGCC-3] and ATH1-500 [5’-CCTACTGACGAGAAGGAGG-3]) and genomic DNA from CEN.PK113-7D as a template. The PCR fragment cloned into the pGEM-T vector (Promega) was digested with EcoRV-HpaI, which was replaced by a 1.5-kb EcoRV-SmaI kanMX4 frag-
1.4-kb EcoRV-SmaI kanMX module from pFAkanMX4 to yield pΔATH1. This plasmid was cut with PvuII-Al/I, and the 3.6-kb fragment was used for transformation. Gene disruption was verified either by PCR (24) or by Southern blotting. The Δagt1 Δtps1 and Δnth1 Δath1 double mutants were obtained by the crossing of haploid mutant strains. The correct Δagt1 Δtps1 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 Δnth1 Δath1 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 MAL6 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 when the medium also contained 0.15 g of trehalose · liter−1. This latter result is in agreement with previous work showing the existence of a nonconcentrative low-level Km uptake system for trehalose (13, 19).

Since acid and neutral tre-
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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