Expression of Drosophila Trehalose-Phosphate Synthase in HEK-293 Cells Increases Hypoxia Tolerance*

Qiaofang Chen‡, Kevin L. Behar‡, Tian Xu‡, Chenhao Fan‡, and Gabriel G. Haddad‡*‡

From the Departments of Pediatrics and Neurosciences, Albert Einstein College of Medicine, Bronx, New York 10461 and the Department of Psychiatry and Howard Hughes Medical Institute, Department of Genetics and Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut 06520

Increasing hypoxia tolerance in mammalian cells is potentially of major importance, but it has not been feasible thus far. The disaccharide trehalose, which accumulates dramatically during heat shock, enhances thermotolerance and reduces aggregation of denatured proteins. Previous studies from our laboratory showed that over-expression of Drosophila trehalose-phosphate synthase (dtps1) increases the trehalose level and anoxia tolerance in flies. To determine whether trehalose can protect against anoxic injury in mammalian cells, we transfected the dtps1 gene into human HEK-293 cells using the recombinant plasmid pcDNA3.1(-)-dtps1 and obtained more than 20 stable cell strains. Glucose starvation in culture showed that HEK-293 cells transfected with pcDNA3.1(-)-dtps1 (HEK-dtps1) do not metabolize intracellular trehalose, and, interestingly, these cells accumulate intracellular trehalose during hypoxic exposure. In contrast to HEK-293 cells transfected with pcDNA3.1(-) (HEK-v), cells with trehalose were more resistant to low oxygen stress (1% O2). To elucidate how trehalose protects cells from anoxic injury, we assayed protein solubility and the amount of ubiquitinated proteins. There was three times more insoluble protein in HEK-v than in HEK-dtps1 after 3 days of exposure to low O2. The amount of Na+-K+ ATPase present in the insoluble proteins dramatically increased in HEK-v cells after 2 and 3 days of exposure, whereas there was no significant change in HEK-dtps1 cells. Ubiquitinated proteins increased dramatically in HEK-v cells after 2 and 3 days of exposure but not in HEK-dtps1 cells over the same period. Our results indicate that increased trehalose in mammalian cells following transfection by the Drosophila tsp1 gene protects cells from hypoxic injury. The mechanism of this protection is likely related to a decrease in protein denaturation, through protein-trehalose interactions, resulting in enhanced cellular recovery from hypoxic stress.

The sensitivity of organisms to hypoxia varies, with most mammals being extremely vulnerable to O2 deprivation. Irreversible injury may occur in mammalian tissues within 5–10 min of severe hypoxia or ischemia, whereas animals such as the turtle, Pseudemys scripta elegans, and invertebrates such as Drosophila melanogaster can recover from hours of experimental O2 deprivation without a trace of injury. Some of the reasons for tolerance to low O2 are as follows. (a) Hypoxia induces a decrease in membrane permeability (i.e., ion "channel arrest") that dramatically reduces the energy costs of ion flux (1). (b) These organisms, especially the turtle, have an unusually well developed capacity for rapid entry into, and return from, metabolically depressed steady states using anaerobic metabolism to sustain reduced rates of energy turnover during hypoxia. Clearly, the net effect of this regulated metabolic depression is that it conserves fermentable fuel, reduces deleterious end-product formation, and extends survival time. (c) The ability to re-allocate cellular energy to essential (and away from non-essential) demands such as energy supplies become limited. For example, energy spared by the reduction in protein synthesis is re-allocated to more critical cell functions involved in osmotic and ionic homeostasis. Therefore, compared with anoxia-tolerant animals, anoxia-sensitive organisms show little or no reduction in the absolute ATP demand of the ion-motive ATPases in response to O2 lack. Other ways of increasing tolerance to hypoxia have also been discovered. Up-regulation of heat shock proteins takes place during O2 limitation and has been shown to render a variety of cell types more resistant to hypoxia in vitro (2, 3).

It has been demonstrated that the disaccharide trehalose can protect proteins from unfolding and from degradation by the ubiquitin-proteasome system during stress conditions such as dehydration and freezing (4, 5). Furthermore, we have recently shown that over-expression of tsp1, a fly gene responsible for catalyzing trehalose synthesis, renders Drosophila even more tolerant than they are naturally (6). In this work, we constructed a mammalian cell expression vector, pcDNA3.1(-)-dtps1, and transfected it into human cell line HEK-293 to determine whether the fly gene tsp1 can induce tolerance to hypoxia in human cells. We have demonstrated that expression of Drosophila tsp1 in HEK-293 cells leads to varied concentrations of trehalose and that these cells show much less injury and higher viability during O2 deprivation as compared with control cells with the vector alone. This protection is probably because of a reduction of denatured proteins during hypoxia.

** Experimental Procedures

Construction of Mammalian Cell Expression Vector—The vector for mammalian cell expression was bought from Invitrogen (pcDNA3.1(-), V795-20). Drosophila tsp1 cDNA was cut from the plasmid pUAST-tsp1 that we constructed previously (6) and inserted into pcDNA3.1(-) between EcoRV and Kpnl.

** Transfection of HEK-293 Cells and Screening of Stable Cell Strains—The CalPhos mammalian transfection kit (K2051-1, Clontech) was used as described in the manual. We seeded 4 × 10⁴ cells in a 35-mm diameter tissue culture dish the day before transfection, replaced the medium with 2 ml of fresh culture medium 0.5–3 h before transfection, and prepared solutions as follows. We mixed 3 μg of plasmid DNA,
Trehalose and Hypoxia Tolerance in Mammalian Cells

Lactate Dehydrogenase (LDH) Assay—To further support the notion of cell injury, LDH activity in the culture medium as well as in the cells was measured using an LDH kit (Promega, CytoTox 96 nonradioactive cytotoxicity assay, catalog no. G1780) and a Bio-Rad microplate imaging system. The culture medium was sampled and centrifuged to remove cellular debris from the supernatant. Subsequently, 50 μl of the sample was added. Cells were washed twice with PBS and suspended in 1 ml of PBS, and then the culture plates were kept at −80 °C for 30 min, followed by 37 °C 15 min to lyse cells. Cell lysate was collected and centrifuged, and 10 μl of supernatant was added to a 96-well plate for assay.

Protein Preparation—This is modified from the method of Soldatenkov and Dritschilo (7). Cells were washed twice with PBS and lysed at 4°C for 15 min in buffer with Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 5 μM N-ethylmaleimide). The debris was removed by centrifuging at 700 × g at 4°C for 10 min. The supernatant was centrifuged for 30 min at 4°C at 16,000 × g to further separate insoluble from soluble proteins. The pellet (insoluble proteins) was washed with 1 ml of buffer and centrifuged at 30 min at 4°C at 16,000 × g again to eliminate contamination of soluble proteins.

RESULTS

Drosophila TPS1 Synthesizes Trehalose in HEK Cells

More than 20 stable cell strains were obtained after transfection of HEK-293 cells with pCDNA3.1(−)dtps1. Cells (1 × 10^6) were collected, and total trehalose was measured. The concentration of trehalose varied with various cell strains from just detectable to as much as 600 μg/ml cells.

Intracellular Trehalose Increases during Hypoxia in HEK-dtps1

To investigate whether intracellular trehalose concentration changed with hypoxic exposure, we measured the trehalose concentration in cells cultured under both normoxic as well as hypoxic conditions for 1, 2, and 3 days. As shown in Fig. 1, trehalose in HEK-dtps1 cells cultured under normoxic conditions amounted to 211 ± 14 μg/mg protein, and this increased to about 371 ± 25 μg/mg protein at 2 days (means ± S.D. (n = 6); *, p < 0.05, ***, p < 0.0001). The plasmid vector pCDNA3.1(−) used in the experiment contains a cytomegalovirus promoter, and the promoter itself is not hypoxia-inducible.

HEK-dtps1 Cells Are More Resistant to Hypoxia Than HEK-v

Morphology—After 48 h in culture at 1% O_2, HEK-v cells (without dtps1) started to change their morphology, as shown in Fig. 2. A significant change was seen at 96 h in almost all cells; most cells had lost their three-dimensional structure and appeared injured or dead. They also appeared detached from the culture plate after 4 days. In contrast to HEK-v, HEK-dtps1 cells had roughly the same morphology after 48 h of exposure to 1% O_2, were still attached, and the overwhelming majority appeared healthy after 96 h.

Cell Viability Assay—After 96 h of exposure to 1% O_2, cells were stained with calcein AM and ethidium homodimer-1 as shown in Fig. 3. There were four times more dead cells in HEK-v (9.4 ± 1.0%) than in HEK-dtps1 (2.4 ± 1.2%) (p < 0.01, means ± S.E.).

Cell Apoptosis Assay—Annexin V-FITC and propidium io-
Hypoxia induces an accumulation of trehalose in cells. HEK-dtps1 and HEK-v cells were equally seeded in 25-cm² tissue culture flasks after being cultured under normoxic conditions for 2 days (cell confluence was reached at about 50–60%) and subjected to hypoxia for 3 days. The concentration of trehalose in HEK-dtps1 cells increased during hypoxic exposure. No trehalose was detected in HEK-v cells. The values are mean ± S.D. (n = 6). *, p < 0.05, ***, p < 0.0001 compared to day 0.

FIG. 1. Hypoxia induces an accumulation of trehalose in cells. HEK-dtps1 and HEK-v cells were equally seeded in 25-cm² tissue culture flasks after being cultured under normoxic conditions for 2 days (cell confluence was reached at about 50–60%) and subjected to hypoxia for 3 days. The concentration of trehalose in HEK-dtps1 cells increased during hypoxic exposure. No trehalose was detected in HEK-v cells. The values are mean ± S.D. (n = 6). * p < 0.05, ***, p < 0.0001 compared to day 0.

FIG. 2. Morphology of the cells after being exposed to 1% O₂. HEK-v and HEK-dtps1 cells were cultured in the incubator at 1% O₂ and were cultured in the incubator at 1% O₂. HEK-v cells (without dtps1) started to change their morphology, and a significant change was seen at 96 h in almost all cells. In contrast to HEK-v, HEK-dtps1 cells had roughly the same morphology after 48 h of exposure to 1% O₂ and were still attached; the overwhelming majority appeared healthy after 96 h. Scale bars = 100 μm.

LDH Assay—Cells were cultured under normoxic conditions for 2 days and then exposed to 1% O₂ after reaching 50–60% confluence. LDH activity of both the supernatant and attached cells was measured before hypoxic exposure and at 1, 2, 3 days after the start of the exposure. Medium LDH activity/total LDH activity did not change significantly after 1 or 2 days of exposure, but it rose dramatically after 3 days in HEK-v cells (35 ± 6%, p < 0.01), and this increase was much smaller in HEK-dtps1 cells (16 ± 1%) (Fig. 5).

Intracellular Trehalose Reduces Insoluble and Ubiquitinated Proteins during Hypoxia

The above data showed that expression of Drosophila tps1 dramatically increased the resistance of HEK-dtps1 cells to 1% O₂. To understand the mechanism of how trehalose induces the change in this phenotype, we asked whether trehalose reduces the amount of insoluble proteins and protein ubiquitination during hypoxic stress. Therefore, cells were exposed to 1% O₂ for 1, 2, and 3 days, and the total and insoluble proteins were assayed. The percentage of insoluble proteins in HEK-v cells rose from 1.5 ± 0.2% at day 0 to 11.9 ± 1.8% at day 3, whereas in HEK-dtps1 cells, insoluble proteins increased from 1.9 ± 0.2% at day 0 to 3.6 ± 0.6% at day 3 (Fig. 6). Western blot with anti-ubiquitin antibody showed that there was almost no difference in ubiquitinated proteins per total protein between HEK-v and HEK-dtps1 cells at days 0 and 1 of hypoxia. However, as exposure time to hypoxia increased, there was a significant difference on days 2 and 3. The amount of ubiquitinated proteins in HEK-dtps1 cells was half that of HEK-v after hypoxia exposure for 3 days (Fig. 7).
Intracellular Trehalose Prevents Na\textsuperscript+-K\textsuperscript+-ATPase Aggregation during Hypoxia

To investigate the fate of specific proteins, we examined the solubility of Na\textsuperscript+-K\textsuperscript+-ATPase, which is a protein known to unfold and aggregate in low O\textsubscript{2} conditions (8). Western blot of insoluble proteins revealed that Na\textsuperscript+-K\textsuperscript+-ATPase aggregation was reduced markedly in HEK-\textit{dtps1} cells as compared with HEK-\textit{v} cells after exposure to hypoxia on days 2 and 3 (Fig. 8).

HEK-\textit{dtps1} Cells and Intracellular Trehalose during Glucose Depletion

To determine whether intracellular trehalose is used as an energy source during hypoxia, glucose was depleted in HEK-\textit{dtps1} cultures, and total trehalose was measured. Trehalose changed little after 1, 2, and 3 days of depletion, as shown in Fig. 9.

** P<0.01, compared to HEK-\textit{dtps1} cells

** P<0.01 compared to HEK-\textit{dtps1} cultures

Fig. 6. Intracellular trehalose helps maintain protein solubility. Cells were exposed to 1% O\textsubscript{2} for 0, 1, 2, and 3 days, and total and insoluble proteins were prepared (see "Experimental Procedures"). The percentage of insoluble proteins/total proteins in HEK-\textit{v} cells rose dramatically at 2 and 3 days, whereas no or little change was observed in HEK-\textit{dtps1} cells during the same period in culture. Values are means ± S.D. (n = 31), *, p < 0.05; **, p < 0.001.

Intracellular Trehalose Prevents Na\textsuperscript+-K\textsuperscript+-ATPase Aggregation during Hypoxia

To investigate the fate of specific proteins, we examined the solubility of Na\textsuperscript+-K\textsuperscript+-ATPase, which is a protein known to unfold and aggregate in low O\textsubscript{2} conditions (8). Western blot of insoluble proteins revealed that Na\textsuperscript+-K\textsuperscript+-ATPase aggregation was reduced markedly in HEK-\textit{dtps1} cells as compared with HEK-\textit{v} cells after exposure to hypoxia on days 2 and 3 (Fig. 8).

HEK-\textit{dtps1} Cells and Intracellular Trehalose during Glucose Depletion

To determine whether intracellular trehalose is used as an energy source during hypoxia, glucose was depleted in HEK-\textit{dtps1} cultures, and total trehalose was measured. Trehalose changed little after 1, 2, and 3 days of depletion, as shown in Fig. 9.

DISCUSSION

On the basis of our previous study using \textit{Drosophila} as a genetic model and our current work (6), we have made several important observations regarding the role of \textit{Drosophila} \textit{tps1} gene in human embryonic kidney (HEK-293) cells. First, the structure of \textit{Drosophila} \textit{TPS1} has homologies to both \textit{Saccharomyces cerevisiae} trehalose-phosphate synthase (TPS1) and trehalose-phosphate phosphatase (TPS2); TPS2 is not present in \textit{Drosophila} (6). The \textit{tps1} gene in \textit{Drosophila}, the only gene in flies (as compared with \textit{Escherichia coli} and yeast (two genes)) that catalyzes the synthesis of trehalose, could also synthesize trehalose in mammalian cells transfected with this gene. In many organisms, the trehalose biosynthetic machinery exists in a complex in which the two biosynthetic enzymes (\textit{tps1} and \textit{tps2}) are associated with other subunits that play regulatory roles in trehalose biosynthesis (9). It is interesting to note that the \textit{tps1} gene in the fly has domains with high similarity to both genes in \textit{E. coli}. Our study demonstrates that expression of \textit{Drosophila} \textit{tps1} is sufficient to result in trehalose biosynthe-
sis. Therefore we believe that dtps1, the gene we transfected into mammalian HEK-293 cells, functions as both tps1 and tps2, which are usually present in yeast and E. coli.

Second, we found that 1% O2 induces an accumulation of trehalose in transfected HEK-dtps1 cells but not in cells with the vector alone. The question that can be raised is how trehalose accumulates in HEK-dtps1. It is well known that certain stress-tolerant organisms accumulate large amounts of trehalose during extreme conditions such as high temperature, dehydration, osmotic stress, and oxidant injury (10). For example, S. cerevisiae acquires tolerance to different types of environmental stresses and is associated with enhanced expression of a number of genes of the trehalose synthase system (tps1, tps2, and their regulatory units) that contains stress-responsive elements in their promoters (11). Our expression system contains a human cytomegalovirus (hCMV) promoter with no stress-responsive elements, and hence the accumulation of trehalose during hypoxia cannot be the result of an up-regulation of the gene by stress-responsive elements. The increase in trehalose during hypoxia is interesting. The possible reasons for this are a decreased breakdown or an increased synthesis in trehalose. The first possibility is unlikely because in our work we have shown that trehalose breakdown does not change under stressful conditions such as when glucose is absent under normoxic conditions or when it is present under hypoxia. Can it be then that trehalose synthesis is increased? It can if for example the substrates are increased. During hypoxia, it is likely that this would not be the case because the level of free intracellular glucose drops. Trehalose 6-phosphate can inhibit hexokinase, which, in turn, can decrease the availability of substrates that are needed for trehalose. One other possibility is that trehalose can itself control the level of trehalose formation, i.e. can act as a feedback inhibitor of tps1. One possibility is an enhanced interaction and binding of trehalose to proteins during hypoxia, which can, in turn, result in a decrease in free cytosolic trehalose. We speculate that this decrease in trehalose could constitute a signal for an increase in trehalose synthesis.

Third, we have clearly demonstrated in this work that the HEK-dtps1 cells that express trehalose are much more tolerant of very low O2 concentrations in their microenvironment than those transfected with the vector alone. Those cells transfected with vector alone die within a short period of time when exposed to severe hypoxia, as expected for mammalian cells such as in neuronal cultures (12). Indeed this is the first demonstration of the role of the Drosophila tps1 gene in inducing increased resistance to hypoxia in mammalian cells.

Although it is known that LDH coincides well with the injury of cell membranes and cell death, an increase in LDH lagged behind the increase in apoptotic cells. One reason for that difference between the annexin V and LDH results could be that annexin V is an earlier marker in the process and therefore precedes membrane disruption and LDH leakage.
Trehalose and Hypoxia Tolerance in Mammalian Cells

The function of trehalose has been studied in plants, yeast, and invertebrate animals (10, 13). Previous investigators have argued about a number of possibilities that could explain the mode of action of trehalose. In flies, trehalose is a major sugar in the hemolymph and is consumed as an energy source during flight. Fungal spores hydrolyze trehalose during dormancy and early germination, and they can serve as a carbon source for ATP generation (10, 14, 15). Is it possible therefore that trehalose is used as another carbon source to enhance the metabolic machinery in the transfected cells during hypoxic stress? We doubt that the sole action of trehalose is to provide an alternative energy source, for at least two reasons. (i) Intracellular trehalose in HEK-dtps1 cells was not consumed even during glucose depletion (trehalose was about 122 μg/flask before glucose depletion and about 125 μg/flask at 1, 2, and 3 days after glucose depletion). The lack of trehalose hydrolysis is consistent with the lack of trehalase. (ii) Rather than being used during periods of hypoxia and increased anaerobic glycolysis, trehalose increased in cells during hypoxia.

Of major interest for the interpretation of our data is that trehalose has been demonstrated to be a stabilizer and protector of proteins and membranes. For example, trehalose has been studied during dehydration and heat, two stresses that can destabilize lipid bilayers. Studies by laser light scattering and other techniques demonstrate that trehalose inhibits both fusion and phase transitions between vesicles during drying (16). Evidence also indicates that the stabilizing effect of trehalose is due to its structure. X-ray diffraction studies have shown that trehalose fits well between the polar head groups of membrane proteins, trehalose probably interacts directly with dry proteins through the formation of hydrogen bonds between its hydroxyl groups and polar residues in the protein (18). Although it is not clear why this interaction results in stabilization, trehalose is a nonreducing sugar, preventing the browning reaction that normally occurs between the aldehyde group on reducing sugars and the amino groups on proteins, which may lead to denaturation. Thus, the interaction of trehalose with proteins may prevent their denaturation and aggregation, which ultimately can lead to protein degradation.

Trehalose also reduces heat-induced protein aggregation. Trehalose is known to stabilize proteins exposed to high temperatures (19, 20); using two different temperature-sensitive reporter proteins, investigators have shown that enzymes are better able to retain activity during heat shock in cells that are producing trehalose. These studies showed an additional and important role of trehalose, that of suppressing aggregation of proteins that have already been denatured.

In our experiments, cells were treated with 1% O2 for several days. As proteins are very sensitive to changes that occur during hypoxia, such as ATP reduction, acidosis, and enzymatic activation (e.g. kinases, phosphatases), they can undergo conformational changes and denaturation. Denatured proteins are targeted by the ubiquitin-proteasome system and degraded. Because ubiquitination and degradation is ATP-dependent, and if little ATP is available, as encountered during severe hypoxia, then an accumulation of denatured proteins takes place, and such an accumulation, in turn, can lead to cell death (21, 22). To understand the mechanisms underlying the protective role of trehalose during hypoxia, we studied 1) the insoluble protein fraction, 2) the ubiquitinated protein fraction, and 3) the amount of Na+-K+ATPase (as a specific protein example) that is present in the insoluble fraction in both HEK-v and HEK-dtps1 cells. Our results, showing that there was much less insoluble and ubiquitinated proteins in the HEK-dtps1 cells than in cells with the vector alone, indicate that one potential explanation for the resistance to hypoxia is that trehalose can maintain cellular proteins in a soluble form and hence can avoid being targeted by the ubiquitin-proteasome system (23, 24). The observation that Na+-K+ATPase was maintained to a greater degree as the soluble protein in HEK-dtps1 cells, but not in HEK-v cells, during the hypoxic period, support this notion.

In summary, the results presented here demonstrate that over-expression of Drosophila trehalose-phosphate synthase (dtps1) in mammalian cells (HEK-293) leads to trehalose synthesis in these cells. Furthermore, trehalose increases upon exposure to 1% O2. Finally, intracellular trehalose renders cells more resistant to hypoxia due, in part if not wholly, to a reduction in protein denaturation and aggregation.

REFERENCES

1. Boutilier, R. G. (2001) J. Exp. Biol. 204, 3171–3178
2. Zhong, N., Zhang, Y., Fang, Q. Z., and Zhou, Z. N. (2000) Acta Pharmacol. Sin. 5, 467–472
3. Suzuki, K., Smolenksi, R. T., Jayakumar, J., Murtuza, B., Brand, N. J., and Yacoub, M. H. (2000) Circulation 102, Suppl. 3, III216–III221
4. Puhler, I., Guo, N., Brown, D. R., and Levine, F. (2001) Cryobiology 42, 207–217
5. Beatie, G. M., Crowe, J. H., Lopez, A. D., Cirulli, V., Ricordi, C., and Hayek, A. (1997) Diabetes 46, 519–523
6. Chen, Q., Ma, E., Behar, K. L., Xu, T., and Haddad, G. G. (2002) J. Biol. Chem. 277, 3274–3279
7. Soldatenkov, V. A., and Dritschilo, A. (1997) Cancer Res. 57, 3881–3885
8. Amfrich, C., Ardito, T., Thulin, G., Kashgarian, M., Siegel, N. J., and Van Why, S. K. (1996) Am. J. Physiol. 274, F215–F222
9. Bell, W., Sun, W., Hohmann, S., Wera, S., Reinders, A., De Virgilio, C., Wiemken, A., and Thevelein, J. M. (1998) J. Biol. Chem. 273, 33311–33319
10. Fyfe, A. D., Pan, Y. T., Fursttussuk, I., and Carroll, D. (2003) Glycobiology 13, 17–27
11. Winderickx, V., and Jentsch, S. (2002) Eur. J. Biochem. 269, 470–482
12. Banaszk, K. J., Xia, Y., and Haddad, G. G. (2000) Prog. Neurobiol 62, 215–249
13. Arguelles, J. C. (2000) Arch. Microbiol. 174, 217–224
14. Barton, J. K., Den Hollander, J. A., Hopfield, J. J., and Shulman, R. G. (1982) 131, 177–185
15. Thevelein, J. M., den Hollander, J. A., and Shulman, R. G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3503–3507
16. Crowe, J. H., and Crowe, L. M. (1999) in Water Science Reviews (Franks, F., ed) pp. 1–23, Cambridge University Press, Cambridge, UK
17. Rudolph, A. S., Chandrasekhar, E., Gaber, B. P., and Nagumo, M. (1990) Chem. Phys. Lipid 53, 243–261
18. Carpenter, J. F., and Crowe, J. F. (1989) Biochemistry 28, 3916–3922
19. De Virgilio, C., Hottinger, T., Dominguez, J., Boller, T., and Wiemken, A. (1994) Eur. J. Biochem. 219, 179–186
20. Singer, M. A., and Lindquist, S. (1998) Trends Biotechnol. 16, 460–468
21. Passamani, L. A., Beso Moreno, M., Lopez Salon, M., and Soto, E. F. (2002) Neurochem. Res. 27, 1401–1410
22. Jesenberger, V., and Jentsch, S. (2002) Nat. Rev. Mol. Cell. Biol. 3, 112–121
23. Illing, M. E., Rajan, R. S., Bene, N. F., and Kropf, R. (2002) J. Biol. Chem. 277, 34150–34160
24. Klimaschewski, L. (2003) Neur Physiol. Sci. 18, 29–33
Expression of *Drosophila* Trehalose-Phosphate Synthase in HEK-293 Cells Increases Hypoxia Tolerance

Qiaofang Chen, Kevin L. Behar, Tian Xu, Chenhao Fan and Gabriel G. Haddad

*J. Biol. Chem. 2003, 278:49113-49118.*

doi: 10.1074/jbc.M308652200 originally published online September 16, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M308652200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 19 references, 6 of which can be accessed free at http://www.jbc.org/content/278/49/49113.full.html#ref-list-1