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Increasing intracellular trehalose is sufficient to confer desiccation tolerance to *Saccharomyces cerevisiae*

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Contributed by Douglas Koshland, April 3, 2015 (sent for review March 10, 2015)

Diverse organisms capable of surviving desiccation, termed anhydrobiotes, include species from bacteria, yeast, plants, and invertebrates. However, most organisms are sensitive to desiccation, likely due to an assortment of different stresses such as protein misfolding and aggregation, hyperosmotic stress, membrane fracturing, and changes in cell volume and shape leading to an overcrowded cytoplasm and metabolic arrest. The exact stress(es) that cause lethality in desiccation-sensitive organisms and how the lethal stresses are mitigated in desiccation-tolerant organisms remain poorly understood. The presence of trehalose in anhydrobiotes has been strongly correlated with desiccation tolerance. In the yeast *Saccharomyces cerevisiae*, trehalose is essential for survival after long-term desiccation. Here, we establish that the elevation of intracellular trehalose in dividing yeast by its import from the media converts yeast from extreme desiccation sensitivity to a high level of desiccation tolerance. This trehalose-induced tolerance is independent of utilization of trehalose as an energy source, de novo synthesis of other stress effectors, or the metabolic effects of trehalose biosynthetic intermediates, indicating that a chemical property of trehalose is directly responsible for desiccation tolerance. Finally, we demonstrate that elevated intracellular maltose can also make dividing yeast tolerant to short-term desiccation, indicating that other disaccharides have stress effector activity. However, trehalose is much more effective than maltose at conferring tolerance to long-term desiccation. The effectiveness and sufficiency of trehalose as an antagonizer of desiccation-induced damage in yeast emphasizes its potential to confer desiccation tolerance to otherwise sensitive organisms.

trehalose | desiccation | yeast | anhydrobiosis | stress

Water is an essential molecule whose absence can lead to a variety of detrimental and often lethal effects on cells and organisms (1–3). Severe water removal, termed desiccation, has been proposed to lead a variety of detrimental stresses (3). Which of these stresses leads to lethality in desiccation-sensitive organisms is unclear. Organisms capable of surviving desiccation, commonly termed anhydrobiotes, are found among bacteria, fungi, plants, and invertebrates (1, 3). Anhydrobiotes harbor stress effectors that are known or postulated to mitigate the different stresses associated with desiccation (2, 4). These stress effectors include osmolytes, heat shock proteins, redox balancing enzymes, nonreducing disaccharides (trehalose, sucrose), and hydrophilins (short unstructured hydrophilic proteins—also known as LEAs) (1). A reasonable assumption might be that many if not all of these stress effectors are necessary for desiccation tolerance given the multitude of stresses imposed by desiccation. However, this assumption is challenged by the uncertainty in the number and degree of lethal stresses generated by desiccation and the versatility and coordination/cooperation of multiple stress effectors in ameliorating such lethal stresses. Thus, a critical question in the anhydrobiosis field is whether a single stress effector is sufficient to promote desiccation tolerance.

One of the most studied desiccation-associated stress effectors is the simple nonreducing disaccharide, trehalose (α,α-1,1-glucoside) (5). It is found in extremely high concentrations in most anhydrobiotes, including in the model organism *Saccharomyces cerevisiae* (6, 7). In this yeast, exponentially dividing cells have very low levels of trehalose and are extremely desiccation sensitive (8). However, in saturated cultures, yeast cells accumulate high levels of a number of stress effectors, including extremely high levels of trehalose (up to 15% of dry cell mass) (6, 7). We recently showed that high levels of trehalose are necessary for yeast cells in saturated cultures to survive weeks to months of desiccation (long term), but not a few days (short term) (9). Trehalose dispensability during short-term desiccation is due in part to overlapping functions with the heat shock factor Hsp104. This overlap led us to discover that trehalose functions as a chemical chaperone capable of preventing the aggregation of both membrane and cytoplasmic proteins (9). Work in the nematode *Caenorhabditis elegans* demonstrated that worms unable to synthesize trehalose display hallmarks of membrane damage, consistent with trehalose playing a role in preserving membrane structure (10). Indeed, trehalose has been found to be lipidated in nematodes and these "maradolipids" are required for efficient desiccation tolerance (11). Due to the different and versatile mechanisms by which trehalose confers desiccation tolerance in anhydrobiotes, we hypothesize that trehalose, in the absence of other stress effectors, will be sufficient in conferring desiccation tolerance.

A simple way to address this hypothesis is to increase the intracellular levels of trehalose in desiccation/dehydration-sensitive cells or organisms then assess whether they acquire desiccation tolerance. Two strategies for increasing intracellular trehalose have been previously used. These were engineering high level expression of trehalose biosynthetic enzymes or importing extracellular trehalose via fusion with lipid vesicles (12–16). Both methods only generated small increases in trehalose levels and minor increases in

Significance

Diverse organisms across taxa are desiccation tolerant, capable of surviving extreme water loss. Remarkably, desiccation-tolerant organisms can survive years without water. The study of desiccation tolerance has the potential to provide novel insights into many aspects of stress and stress responses. Additionally, the study of desiccation tolerance may generate biomedical (blood storage) and agricultural (drought tolerance) applications. The disaccharide, trehalose, is one of several factors predicted to mitigate the adverse effects of desiccation tolerance. Here, we establish the sufficiency of trehalose in the desiccation tolerance of budding yeast. This study provides insights into the in vivo potential of small chemical chaperones to act as stress effectors and informs on the potential for in vivo metabolism under extreme desiccation conditions.

Author contributions: H.T. and D.K. designed research; H.T. and L.Y. performed research; D.F. and C.R.B. contributed new reagents/analytic tools; H.T. analyzed data; and H.T. and D.K. wrote the paper.

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dehydration but not desiccation tolerance. This weak effect could reflect the need for additional stress effectors. Alternatively, trehalose alone could indeed be sufficient for desiccation tolerance but was missed for two reasons. First, high physiological levels of trehalose observed in desiccation-tolerant organisms were not reached so a potential critical threshold level of trehalose was not met. Second, the biosynthetic strategy not only increased trehalose but also trehalose-6-phosphate, a potent regulator of glucose metabolism that has deleterious effects on cell and organism fitness. Thus, it remains untested whether trehalose alone is sufficient for generating desiccation tolerance.

The correlative evidence for trehalose being sufficient for desiccation tolerance was provided from our previous study comparing desiccation sensitivity of saturated and exponentially dividing cultures of yeast (8). Cells in a saturated culture rapidly lose desiccation tolerance when they divide upon dilution into fresh media. Shortly, after dilution, the levels of many stress factors, including trehalose, diminish. Trehalose levels drop as a consequence of activation of two intracellular trehalases, *NTH1* and *NTH2*, and the inhibition of the trehalose biosynthetic enzyme Tps1 (6, 7). The diluted cells retained their desiccation tolerance significantly longer when trehalose depletion was slowed by inactivation of the trehalases (9). This result is consistent with the notion that sustaining high trehalose levels, while reducing the levels of other stress effectors, is sufficient to promote desiccation tolerance. Encouraged by this result, we decided to investigate further the potential sufficiency of trehalose for desiccation tolerance, exploiting the ability of the *AGT1* sugar transporter to import extracellular trehalose (17). Here, we show that when *AGT1* overexpressing cells are grown in the presence of trehalose, they acquire high levels of intracellular trehalose and desiccation tolerance similar to that of saturated cultures. We characterize this novel acquisition of desiccation tolerance and provide important insights into the roles of trehalose concentration and trehalose structure in both short- and long-term desiccation tolerance.

Results

Import of Extracellular Trehalose Confers Robust Desiccation Tolerance to Dividing Yeast. We hypothesize that exponentially dividing yeast cells are sensitive to desiccation because of their inability to accumulate high levels of intracellular trehalose. To increase trehalose in exponentially dividing cells, we used yeast strains overexpressing Agt1, a specialized disaccharide transporter (17). Agt1 was first identified as a high-affinity maltose transporter (*MAL11*). However, it also has the ability to transport other disaccharides like trehalose, albeit less efficiently, hence the name alpha-glucoside transporter (*AGT1*) (17). *AGT1* is normally only expressed in the presence of maltose, so we replaced its promoter with the *TDH3* promoter (*TDH3pr-AGT1*), which allows constitutive *AGT1* expression. Wild-type cells with or without *TDH3pr-AGT1* were grown to early log phase in rich media (YPD). Cells were subsequently transferred to rich media alone or containing trehalose, incubated for 1 h, then resuspended in buffer lacking trehalose and subjected to desiccation. Viability upon rehydration provided a quantitative measure of their desiccation tolerance.

Wild-type cells exhibited extreme desiccation sensitivity regardless of the presence or absence of trehalose as expected from previous studies of exponentially dividing yeast (Fig. 1*A*) (8). Wild-type cells constitutively expressing *TDH3pr-AGT1* were also extremely sensitive to desiccation when trehalose was not present in the media. By comparison, wild-type cells expressing *TDH3pr-AGT1* when grown in the presence of trehalose became approximately four orders of magnitude more desiccation tolerant (Fig. 1*A*). Intracellular trehalose concentrations correlated with the presence of the transporter, the presence of trehalose in the media, and importantly, with desiccation tolerance (Fig. 1 *A* and *B*).

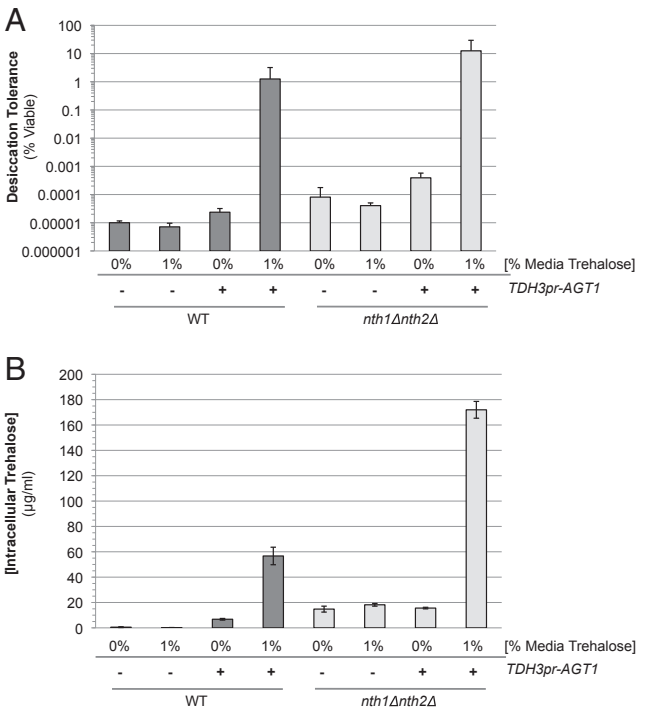

Fig. 1. Trehalose increases short-term desiccation tolerance in WT and trehalase delete cells. (*A*) Yeast cells were grown to midexponential phase (OD < 0.5) in rich media (YPD). Cells were then transferred to either rich media (YPD, 0% trehalose) or rich media with trehalose (YPD, 1% trehalose) for 1 h. Cells were collected, washed, and air dried for 2 d at 23 °C, 60% relative humidity (RH), then rehydrated and assessed for viability by counting colony forming units (cfus). Strains tested are as follows: WT (wild type), WT + *TDH3pr-AGT1* (wild type, expressing trehalose transporter, *AGT1*), *nth1/2Δ* (trehalase delete), and *nth1/2Δ* + *TDH3pr-AGT1* (trehalase delete, expressing trehalose transporter, *AGT1*). (*B*) Intracellular trehalose concentrations of strains examined in *A*, before desiccating. Trehalose concentrations are expressed as micrograms per milliliter of ~10^7 cells.

The fraction of cells in saturated cultures that survive desiccation varies between 20% and 40%; ~20-fold higher relative to logarithmically dividing *TDH3pr-AGT1* cells grown in the presence of trehalose. Interestingly, saturated cells have a 5-fold higher intracellular trehalose concentration (9). To test whether further elevation of intracellular trehalose would increase desiccation tolerance of dividing cells, we examined both desiccation tolerance and trehalose levels in a strain missing both cytoplasmic trehalases, NTH1 and NTH2 (*nth1Δ nth2Δ*), with the expectation that deletion of a cell's trehalases should allow greater accumulation of intracellular trehalose and in turn increased desiccation tolerance. The *nth1Δ nth2Δ* strain with *TDH3pr-AGT1* was able to accumulate trehalose to levels that are 3-fold higher than wild-type cells with *TDH3pr-AGT1* (Fig. 1*B*). These cells were also an order of magnitude more desiccation tolerant than wild-type cells expressing *TDH3pr-AGT1* (Fig. 1*A*). Thus, inactivating intracellular trehalases increased both a cell's ability to accumulate intracellular trehalose and conferred the highest induced desiccation tolerance recorded for logarithmically growing cells (8, 9, 18).

By growing *nth1Δnth2Δ* cells expressing *TDH3pr-AGT1* in media with different amounts of trehalose, we can modulate the intracellular concentration of trehalose and test its impact on desiccation tolerance. Increasing trehalose concentrations in the media causes a corresponding increase in both the intracellular trehalose concentration and desiccation tolerance of this strain in a concentration-dependent manner. Desiccation tolerance levels and intracellular trehalose concentrations increase four

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orders of magnitude with the addition of trehalose in concentrations ranging from 0.1% to 5% (Fig. 2). Three orders of magnitude of this increase occurred with only a fourfold increase in intracellular trehalose, demonstrating a nonlinear requirement for trehalose in desiccation tolerance. Higher concentrations of trehalose in the media (>5%) did not increase desiccation tolerance or intracellular trehalose concentrations (Fig. S1). Hence, it is unclear whether trehalose levels or some additional factor determine the upper limit of tolerance.

Trehalose likely induced desiccation tolerance in logarithmically dividing cells through its known roles as potent stress effector against protein aggregation and membrane damage. However, trehalose can also serve as an energy source (6, 7). This indirect mechanism was eliminated by the observation that trehalose-induced desiccation tolerance increased in trehalase-defective cells, where trehalose degradation to glucose was blocked (6, 7). A second potential indirect mechanism is that trehalose might promote tolerance indirectly by modulating the levels of trehalose-6-phosphate, a metabolic regulator and biosynthetic precursor to trehalose (19). To test this possibility we introduced the Agt1 transporter into *tps1Δ* cells that cannot make trehalose-6-phosphate. Trehalose import induced the same high level of desiccation tolerance in dividing *tps1Δ* cells expressing *TDH3pr-AGT1* as wild-type cells expressing *TDH3pr-AGT1* (Fig. 3*A*). Thus, the induction of tolerance by trehalose is independent of the metabolic regulatory functions of trehalose-6-phosphate or *TPS1*.

A third potential indirect mechanism for trehalose-induced desiccation tolerance came from the observation that wild-type cells grown in minimal medium expressing *TDH3pr-AGT1* exhibit slower growth rates after addition of trehalose to the media and induced the environmental stress response (ESR) after ~2 h (20). The ESR induces increased synthesis of additional stress effectors besides trehalose (21). Importantly, in our experiments, the growth rate of cells expressing *TDH3pr-AGT1* in rich media was not altered by the addition of trehalose for the first few hours of growth, indicating that the presence of trehalose was unlikely to induce ESR under these conditions (Fig. S2). Growth rate of cells capable of importing trehalose slowed when grown for extended periods of time (>4–6 h); however, all of our strains were allowed to grow/induce for only 1 h after trehalose addition.

To determine whether trehalose import induced the synthesis of any products that also contributed to desiccation, we tested the ability of trehalose to induce tolerance when the protein synthesis was blocked with cycloheximide. Wild-type or trehalase deletion strains expressing *AGT1* were grown to midexponential phase in rich media, then transferred for 1 h to rich media containing 1% trehalose with either cycloheximide or its carrier DMSO. After 1 h, cells were desiccated. Importantly, the presence of cycloheximide did not further reduce desiccation tolerance compared with DMSO (Fig. 3*B*). Trehalose-induced desiccation tolerance was impaired ~10-fold by the DMSO carrier alone. This result suggests that trehalose-induced desiccation tolerance is independent of the de novo synthesis of any protein including other potential stress effectors within the ESR. A small reduction in desiccation tolerance was observed in the wild-type cells expressing *TDH3pr-AGT1* treated with cycloheximide compared with the DMSO control, which corresponded with a lower accumulation of intracellular trehalose in this strain. This small reduction in intracellular trehalose and desiccation tolerance was likely due to the established up-regulation of intracellular trehalase activity by cycloheximide (22). Together, these results argue against models where trehalose indirectly induces desiccation tolerance through changes in metabolism or induction of other stress factors. Rather, these results suggest that induction of desiccation tolerance by trehalose import is caused directly by an inherent chemical property(s) of trehalose.

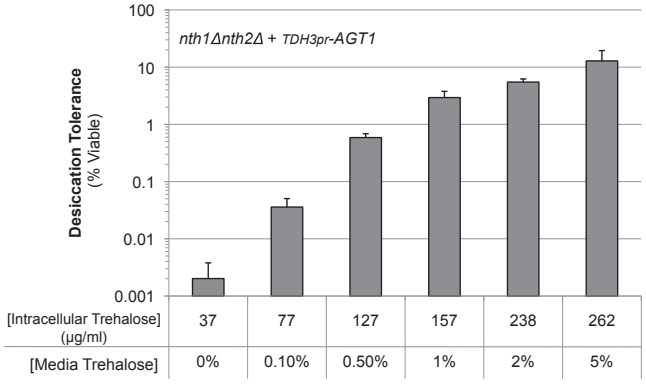

Fig. 2. Increasing trehalose concentrations increases desiccation tolerance in trehalase delete cells. Yeast cells were grown to midexponential phase (OD < 0.5) in rich media (YPD). Cells were then transferred to either rich media (YPD, 0% trehalose) or rich media with trehalose (YPD, 0.1%, 0.5%, 1%, 2%, or 5% trehalose) for 1 h. Cells were collected and treated as in Fig. 1*A*. Strains tested are as follows: *nth1/2Δ + TDH3pr-AGT1* (trehalase delete, expressing trehalose transporter, *AGT1*). Intracellular trehalose concentrations of *nth1/2Δ + TDH3pr-AGT1* were determined as in Fig. 1*B*, before desiccating. Trehalose concentrations are expressed as micrograms per milliliter of ~10^7 cells.

Maltose Confers Short-Term Desiccation Tolerance. Not all anhydrobiotes have elevated levels of trehalose (23). In these organisms, other disaccharides have been postulated to serve the same function, primarily sucrose (3). However, evidence that any of these disaccharides have a causal role in desiccation tolerance is lacking. With this in mind, we wondered whether elevated levels of maltose could confer desiccation tolerance to yeast. To this end, we examined desiccation tolerance of wild-type cells expressing *TDH3pr-AGT1* in media containing maltose rather than trehalose. Maltose should be readily imported into this strain because Agt1 is even more proficient at importing maltose than trehalose (17, 24).

Addition of 1% maltose increased desiccation tolerance by five orders of magnitude in wild-type yeast cells expressing *TDH3pr-AGT1* (Fig. 4*A*). This increase was about 10-fold more than the trehalose-induced increase in the same strain (Fig. 4*A*). We wondered whether the additional desiccation tolerance induced by maltose resulted from its hyperaccumulation in the cytoplasm because *AGT1* is a specialized, high-affinity maltose transporter. There are currently no simple enzymatic assays to measure maltose. Therefore, we measured the concentration of both trehalose and maltose in our different wild-type strains expressing or not expressing *TDH3pr-AGT1*, using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE/PAD). Intracellular trehalose and maltose concentrations increased dramatically in strains expressing *TDH3pr-AGT1* when grown in media with trehalose and maltose, as expected (Fig. 4*B*). The maltose levels were found to accumulate to double the concentration of trehalose despite being added exogenously at equal concentrations (1%). This doubling in disaccharide concentration was likely the explanation for the elevated desiccation tolerance because doubling the concentration of intracellular trehalose caused a similar order of magnitude increase in desiccation tolerance (Fig. 2). These results show that trehalose is not unique in its ability to confer desiccation tolerance, but rather may reflect chemical properties shared by other disaccharides.

Trehalose, Not Maltose, Confers Long-Term Desiccation Tolerance. Whereas maltose can confer desiccation tolerance, it has never been observed to accumulate in any desiccation-tolerant species. Furthermore, whereas our data suggest that the accumulation of

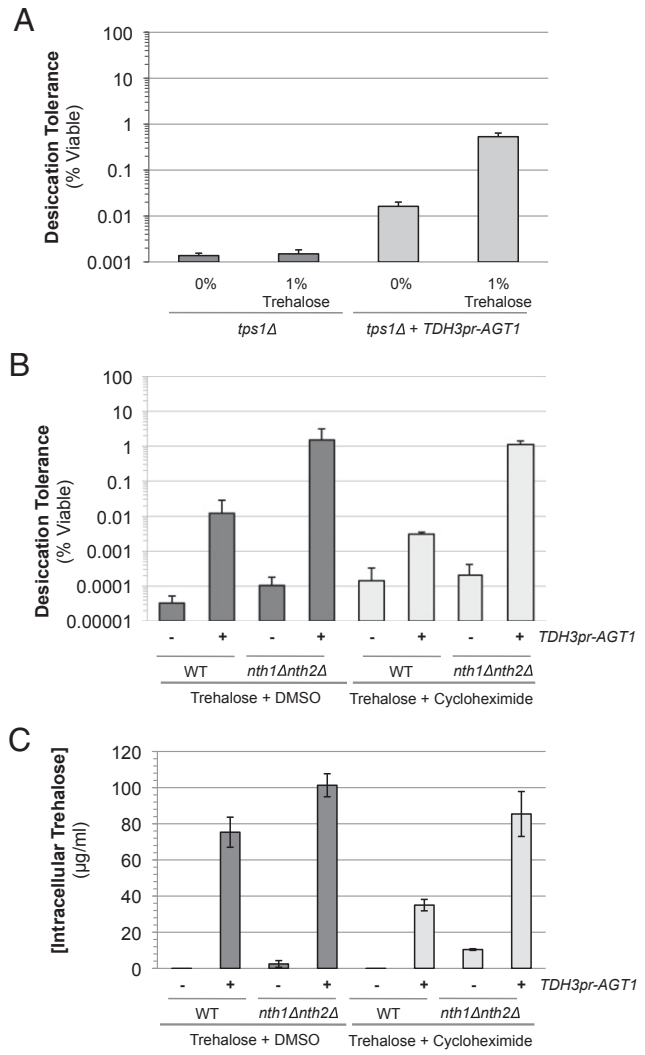

Fig. 3. Trehalose increases short-term desiccation tolerance independent of *TPS1* and the eukaryotic stress response. (*A*) Yeast cells (*tps1Δ*) were grown to midexponential phase (OD < 0.5) in rich media (YPGal). Cells were then transferred to rich media (YPGal, 0% trehalose) or rich media with trehalose (YPGal, 1% trehalose) for 1 h. Cells were collected, and treated as in Fig. 1*A*. Strains tested are as follows: *tps1Δ* (defective trehalose biosynthesis) and *tps1Δ* + *TDH3pr-AGT1* (defective trehalose biosynthesis, expressing trehalose transporter, *AGT1*). (*B*) Yeast cells were grown to midexponential phase (OD < 0.5) in rich media (YPD). Cells were then transferred to either rich media with trehalose and the carrier DMSO (YPD, 1% trehalose, 0.2% DMSO) or rich media with trehalose and cycloheximide (YPD, 1% trehalose, 10 μg/mL cycloheximide) for 1 h. Cells were collected and treated as in Fig. 1*A*. Strains tested are as follows: WT (wild type), WT + *TDH3pr-AGT1* (wild type, expressing trehalose transporter, *AGT1*), *nth1/2Δ* (trehalase delete), and *nth1/2Δ* + *TDH3pr-AGT1* (trehalase delete, expressing trehalose transporter, *AGT1*). (*C*) Intracellular concentrations of strains examined in *B*, before desiccating. Trehalose concentrations are expressed as micrograms per milliliter of ~10^7 cells.

other disaccharides may act as surrogates for trehalose, most desiccation-tolerant organisms accumulate trehalose but never maltose. A potential clue as to why organisms choose trehalose comes from our previous studies that showed that trehalose is particularly important for long-term desiccation because its stable stress-effector activities continue to protect yeast when the activity of other stress effectors like Hsp104 dissipates (9). We wondered whether this stability might also have been the evolutionary selection that made it the preferred disaccharide to protect against the insults of desiccation. For example, although just two enzymes degrade intracellular trehalose, many enzymes capable of maltose breakdown exist [(maltases: *MAL12* and *MAL32*), invertases (*SUCX*), glucosidases (*SGA1*)]. Moreover, maltose is a reducing sugar, whereas trehalose is not. This difference may be important due to the propensity of reducing sugars to participate in protein browning via Maillard reactions (25). Therefore, we decided to test the efficacy of trehalose and maltose in conferring long-term desiccation tolerance to dividing yeast. Dividing cultures of yeast with or without *TDH3pr-AGT1* were grown for 1 h in the presence of trehalose or maltose, desiccated, and then rehydrated after 2, 15, 30, 90, or 180 d and assayed for viability.

First, we asked whether trehalose imported into dividing cells was as key a determinant in long-term desiccation tolerance as we previously showed it was in saturated cultures. We observed that, whereas dividing wild-type cells expressing *TDH3pr-AGT1* were significantly more desiccation tolerant than wild-type cells not expressing *TDH3pr-AGT1*, this desiccation tolerance decreased much more rapidly with time than that seen previously in saturated cultures (9). However, like saturated cells, the loss of trehalose-induced tolerance in dividing cells was greatly attenuated in *nth1Δnth2Δ* cells overexpressing *AGT1*. This result suggests that trehalose is critical for long-term desiccation tolerance

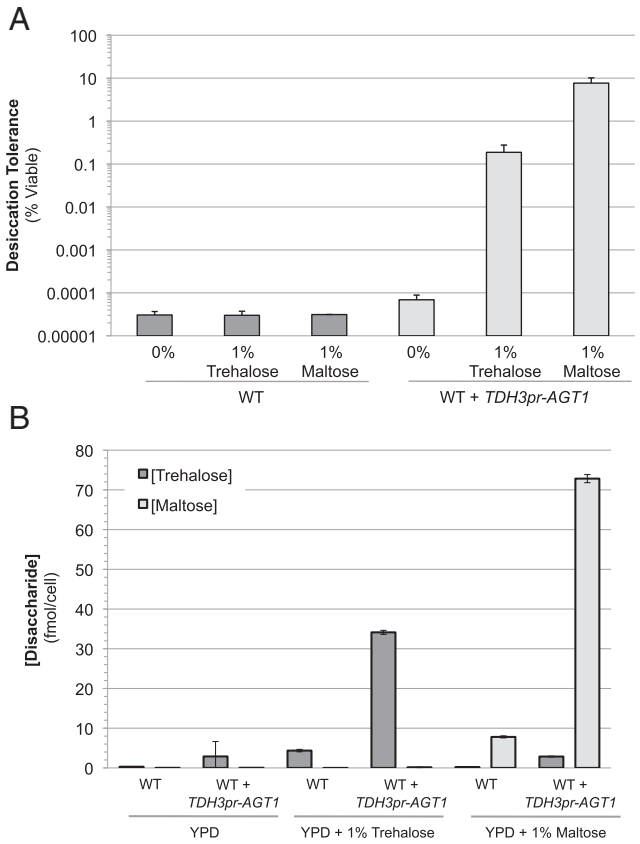

Fig. 4. Trehalose and maltose confer short-term desiccation tolerance. (*A*) Yeast cells were grown to midexponential phase (OD < 0.5) in rich media (YPD). Cells were then transferred to rich media (YPD, 0% trehalose), rich media with trehalose (YPD, 1% trehalose), or rich media with maltose (YPD, 1% maltose) for 1 h. Cells were collected and treated as in Fig. 1*A*. Strains tested are as follows: WT (wild type) and WT + *TDH3pr-AGT1* (wild type, expressing trehalose transporter, *AGT1*). (*B*) Intracellular carbohydrate concentrations of WT and WT + p*AGT1* treated according to Fig. 1*A*. Concentrations were determined by HPAE/PAD, before desiccating. Disaccharide concentrations are expressed as femtomoles per cell of ~10^7 cells.

and that its degradation in dividing cells by trehalases was likely more severe than in saturated cultures. We then examined long-term desiccation tolerance in the presence of maltose. Despite starting with 10-fold higher levels of desiccation tolerance, dividing cells with maltose lost tolerance more rapidly than those with trehalose, falling to an absolute level of survival that was 10-fold lower than dividing cells with trehalose (Fig. 5). Taken together, our results suggest that although trehalose and maltose can both promote high levels of survival to short-term desiccation, trehalose is significantly better at promoting tolerance to long-term desiccation.

Discussion

The conditional desiccation tolerance of budding yeast, exemplified by their extreme sensitivity when dividing and extreme tolerance when stationary, provides an ideal tool to dissect the molecular basis of desiccation tolerance. However, the induction of many stress effectors in stationary cells generates potential functional overlaps that can obscure the specific contribution of individual effectors like trehalose. In this study, we eliminate this complexity by importing trehalose into dividing cells constitutively expressing the Agt1 (*TDH3pr-AGT1*) transporter. We demonstrate that trehalose import from the media into dividing *TDH3pr-AGT1* cells is sufficient to rapidly and dramatically improve their desiccation tolerance by four orders of magnitude. Intracellular trehalases that are encoded by *NTH1* and *NTH2* degrade trehalose to glucose (6, 7). In a strain lacking these intracellular trehalases, trehalose-induced desiccation tolerance is enhanced another order of magnitude, reaching a level of survival (10%) close to that seen for desiccation-tolerant nondividing cells in saturated cultures (20–40%).

The remarkable sufficiency of trehalose to confer desiccation tolerance coupled with our previous studies informs both on the versatility of trehalose, as well as on the possible stresses that it might mitigate. We have previously shown that desiccation induces the aggregation of both cytoplasmic and membrane proteins, a detrimental effect that is counteracted by trehalose and Hsp104 during short-term desiccation and by trehalose during long-term desiccation (9). If the proteotoxicity mitigated by trehalose and Hsp104 were solely responsible for desiccation sensitivity, then stationary cells lacking these two stress effectors should be as sensitive to desiccation as dividing cells. However, stationary cells lacking Hsp104 and trehalose are still three orders of magnitude more tolerant to short-term desiccation than dividing cells (9). From this result, we infer that proteotoxicity is insufficient to explain all of the desiccation sensitivity of dividing cells; another stress(es) likely contributes to desiccation sensitivity. With this and the knowledge that trehalose alone can provide high level of tolerance to dividing cells (this study), we propose the following model. Desiccation induces proteotoxicity and another stress that both contribute to lethality. Trehalose and Hsp104 mitigate proteotoxicity, whereas trehalose and unknown effectors mitigate secondary stresses. Identifying the additional stress and the factors that partner with trehalose is an important future direction. One likely candidate for the additional stress is membrane damage as has been suggested from studies in nematodes (10, 11).

How does trehalose mitigate desiccation-induced stresses? An important insight into this question came from our system of trehalose import that allowed us to systematically change the intracellular concentrations of trehalose and observe its impact on desiccation tolerance. We show that high levels of intracellular trehalose are required for tolerance and dramatic nonlinear increases in tolerance occur with small increases in trehalose concentration. This conclusion explains observations in our previous studies of stationary cells. During long-term desiccation or upon rehydration of desiccated cells, we observed that even small reductions in the high level of intracellular trehalose caused dramatic decreases in desiccation tolerance. We suggest that under these conditions the additional stress effectors are suppressed or overwhelmed, revealing the same dramatic dependence of desiccation tolerance on trehalose concentration we observe in dividing cells where the other stress effectors are also suppressed. Furthermore, the tolerance in the dividing cells is not as high as that seen in stationary cells. This difference is explained by the fact that dividing cells never achieve the same high level of intracellular trehalose as stationary cells to generate the initial level of tolerance. The lower initial levels also mean that the degradation of trehalose during long-term desiccation will cause cells to fall more quickly below the critical threshold of trehalose needed for tolerance. Indeed, the trehalose-induced desiccation tolerance is lost more quickly in dividing cells than stationary cells. Taking all these observations together, we suggest that a threshold of trehalose is needed such that when water is removed, trehalose acquires a chemical activity essential for desiccation tolerance, perhaps vitrification (26).

Our vitrification model contradicts many studies suggesting a role for trehalose in protecting against heat, osmotic, or oxidative stress under aqueous conditions. The majority of studies that have previously implicated trehalose as a stress protectant rely on purely correlative data and are based on in vitro studies. Indeed, in a companion paper, Gibney et al. demonstrate that introduction of trehalose into naïve yeast cells does not confer tolerance to heat shock, nor does it aid in a variety of other roles to which trehalose has been previously assigned (20).

A number of observations in other organisms are consistent with this hypothesis. First, if this putative chemical property of trehalose is critical for desiccation tolerance, then other organisms should also require absolute high levels of trehalose to achieve desiccation tolerance, and should show dramatic changes in tolerance with small changes in trehalose concentrations. Interestingly, nematodes grown under normal conditions are desiccation sensitive and have ~75 μg/mg trehalose (10). Preconditioning the nematodes with very mild dehydration causes an increase in trehalose concentration to 300 μg/mg and the acquisition of desiccation tolerance (10). Thus, threefold changes in trehalose concentration cause

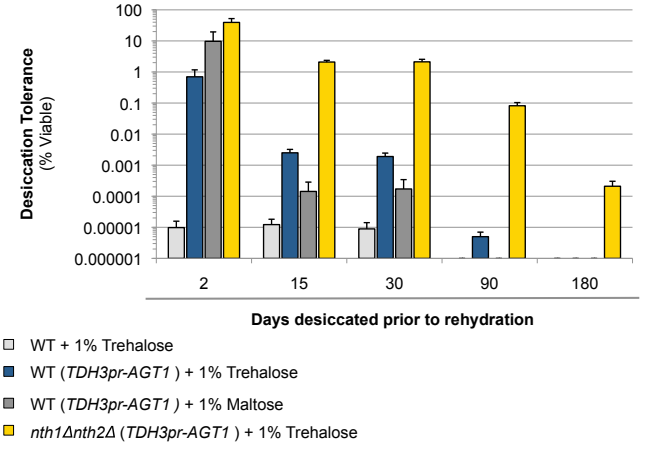

Fig. 5. Trehalose, not maltose, confers long-term desiccation tolerance. Yeast cells were grown to midexponential phase (OD < 0.5) in rich media (YPD). Cells were then transferred to rich media (YPD, 0% trehalose), rich media with trehalose (YPD, 1% trehalose) or rich media with maltose (YPD, 1% maltose) for 1 h. Cells were collected, washed, and air dried for 2, 15, 30, 90, or 180 d at 23 °C, 60% relative humidity (RH), and then rehydrated and assessed for viability by counting colony forming units (cfus). Strains tested are as follows: WT (wild type), WT + *TDH3pr-AGT1* (wild type, expressing trehalose transporter, *AGT1*), and *nth1/2Δ* + *TDH3pr-AGT1* (trehalase delete, expressing trehalose transporter, *AGT1*).

dramatic increases in desiccation tolerance as observed in yeast. Second, engineering plants to make trehalose have failed to improve their drought tolerance (13–16). In retrospect, this failure is not surprising because the engineering never achieved trehalose levels even close to this high threshold level necessary in yeast and worms.

Vitrification is a general property of sugars; we hence reasoned that other disaccharides should be capable of promoting desiccation tolerance (26). Indeed, trehalose is found in most but not all desiccation-tolerant organisms. When not present, sucrose has been speculated to act as a surrogate for trehalose, but no in vivo evidence exists for a causal role in desiccation tolerance for any disaccharide other than trehalose. Here we show that maltose can promote robust desiccation tolerance. This result strongly suggests that the stress effector activity of trehalose is a general chemical property of disaccharides and not a unique property of trehalose. Consistent with this conclusion, in vitro experiments analyzing disaccharide activity on protein stability found little differences between maltose and trehalose (27). Furthermore, trehalose and maltose were both equally effective in preventing damage to vesicles in vitro (28). If other disaccharides like maltose are sufficient to confer desiccation tolerance, why does nature preferentially use trehalose? We demonstrate that trehalose is more proficient at long-term desiccation, likely because as a nonreducing disaccharide trehalose is more stable.

The ability of trehalose to confer robust desiccation tolerance has significant practical implications. Developmental defects and poor drought tolerance of plants engineered to make trehalose apparently caused researchers to abandon the development of trehalose as a tool for desiccation tolerance. Our results suggest that this abandonment may have been premature. First, cells engineered to have high levels of trehalose can provide equally high levels of desiccation tolerance. Second, the different developmental defects that arise in trehalose-producing plants may be a consequence of intermediates of trehalose biosynthesis, as we do not observe any impairment of yeast growth when alternative carbon sources like glucose are available. Thus, engineering plants to make high trehalose levels through direct import, which avoids making these intermediates, may be an effective strategy for improving drought/desiccation tolerance.

Materials and Methods

Detailed descriptions of growth conditions, desiccation tolerance assay, and disaccharide analysis are provided in *SI Materials and Methods*.

Desiccation Tolerance Assay. Cells were grown to midexponential phase (OD < 0.5) in rich media, YPD, or YPGal, depending on strain necessities. Cells were then transferred to inducible media: YPD/YPGal ± 1% trehalose or maltose, for 1 h. Following induction, ~10^7 cells were withdrawn from liquid cultures and washed twice in water and then brought to a final volume of 1 mL. Undesiccated controls were plated for colony counting. 200-μL aliquots were then transferred to a 96-well tissue culture plate centrifugated, and water was removed without disturbing the cell pellet. Cells were allowed to desiccate in a 23 °C incubator with constant 60% relative humidity (RH), with the lid raised, for at least 48 h. (Trehalose % and time of desiccation varies by experiment.)

Enzymatic Trehalose Determination. Cells were treated according to desiccation tolerance assays. Approximately 10^7 cells were withdrawn from liquid cultures. Samples were washed with ice-cold H_2O and resuspend in 250-μL 0.25 M Na_2CO_3. Samples were boiled at 95–98 °C for 4 h followed by addition of 150-μL 1 M acetic acid and 600-μL 0.2 M sodium acetate. Half of the sample was transferred to a new tube (Control), and the remaining half was incubated with 0.025 units/mL trehalase at 37 °C overnight. Samples were centrifugated at 14,000 rpm in a microcentrifuge for 3 min and assayed for glucose using a Glucose Assay Kit (Sigma-Aldrich).

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