APPLIED MICROBIAL AND CELL PHYSIOLOGY

Air-drying kinetics affect yeast membrane organization and survival

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Abstract The plasma membrane (PM) is a key structure for the survival of cells during dehydration. In this study, we focused on the concomitant changes in survival and in the lateral organization of the PM in yeast strains during desiccation, a natural or technological environmental perturbation that involves transition from a liquid to a solid medium. To evaluate the role of the PM in survival during air-drying, a wild-type yeast strain and an osmotically fragile mutant ($erg6\Delta$) were used. The lateral organization of the PM (microdomain distribution) was observed using a fluorescent marker related to a specific green fluorescent protein-labeled membrane protein (Sur7-GFP) after progressive or rapid desiccation. We also evaluated yeast behavior during a model dehydration experiment performed in liquid medium (osmotic stress). For both strains, we observed similar behavior after osmotic and desiccation stresses. In particular, the same lethal magnitude of dehydration and the same lethal kinetic effect were found for both dehydration methods. Thus, yeast survival after progressive air-drying was related to PM reorganization, suggesting the positive contribution of passive lateral rearrangements of the membrane components. This study also showed that the use of glycerol solutions is an efficient means to simulate air-drying desiccation.

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Introduction

Microorganism dispersal allows yeasts to colonize new habitats and reach sites where resources favor growth, and enables their survival (Gorbushina and Broughton 2009; Gunde Cimerman et al. 2000; Quispel 1998). The yeast *Saccharomyces cerevisiae* occupies and flourishes in numerous habitats such as soil, plants, and rocks (Landry et al. 2006). Therefore, it can be found on surfaces constantly subjected to changes in atmospheric conditions with irregular fluctuations of temperature, humidity, and radiation (Gorbushina 2007). Among these stresses, the cell's capacity to resist dehydration and rehydration has been essential for the efficient colonization of terrestrial environments (Tunnacliffe and Lapinski 2003). Moreover, understanding of cellular mechanisms occurring during air-drying desiccation is essential to improve industrial preservation techniques (e.g., ferment, probiotics) (Beker and Rapoport 1987; Meng et al. 2008).

During moderate hydric perturbations (final a_w value >0.90), yeast cells actively maintain turgor pressure (Klipp et al. 2005). To adjust to such perturbations of the environment and to prevent the resulting loss of cytoplasmic water, microorganisms accumulate intracellular ions, such as K+, or organic solutes to reestablish the cell volume and, at the same time, preserve metabolic activities (Brown 1990; Santos and Da Costa 2002). For higher perturbations, no measurable metabolic activity can be detected anymore and only osmotic rules apply. The study of cell survival mechanisms that occur during desiccation, for instance on plants or soil, is scientifically complex to study and observe because of the change

from a liquid to a solid state. Thus, many data connecting the structural and functional changes in cells during dehydration stress are available in liquid media with high concentrations of osmolytes (Dupont et al. 2010; Mille et al. 2002; Simonin et al. 2007; Turk et al. 2007). The main results obtained from these approaches show that cell death caused by extreme dehydration and subsequent rehydration is the result of a sequence of plasma membrane (PM) events (Crowe et al. 1984; Ragoonanan et al. 2008; Scherber et al. 2009; Simonin et al. 2007).

The extensive loss of water causes PM condensation and restructuration, with a lipid phase transition (Crowe et al. 1992) and also an increase of the cell surface-to-volume ratio (s/v) (Ragoonanan et al. 2008; Wolfe et al. 1986). After rapid osmotic perturbations, invagination and structural instability promote internalization of the PM into the cell cytoplasm and result in a decrease of the cell's surface area. During rehydration and cell reexpansion, this lack of available surface area leads to PM rupture and further lysis of almost the whole cell population. By contrast, cell survival can be maintained after rehydration following more gentle and progressive osmotic perturbations. Recently, the mechanisms behind such cell survival have been identified as entailing lateral PM reorganization, which does not occur during abrupt perturbations (Dupont et al. 2010).

The occurrence of such mechanisms during yeast desiccation in air, which involves a transition from liquid to solid state, has not been demonstrated and experimental data are still lacking. During desiccation, the early stages correspond to the maintenance of cells in a concentrated liquid medium, as in a condition of osmotic stress. However, the subsequent events are more complex because of matrix solidification and the presence of air and particularly oxygen in direct contact with the yeast. Therefore, understanding how reductions in water availability influence the PM's behavior during air-drying desiccation is of great interest in the field of microbial ecology and exobiology.

This work was intended to determine whether the yeast survival mechanisms extensively studied in liquid medium are similar to those that occur during air-drying desiccation. For this, we examined how water limitation and high osmolarity influenced *S. cerevisiae* survival, respectively. Thus, three amplitudes of dehydration, all below the water activity value of 0.90, and two rates of change (kinetics), one rapid and one slow, were used to mimic the various disturbances of the yeast natural environments. To measure the PM damage induced by both dehydration methods, a wild-type and a mutant strain with an osmotically fragile PM (*erg6Δ*) were used. Instead of ergosterol, the mutant accumulates molecular precursors and it has already been shown that this strain exhibits lower PM plasticity with a low resistance to deformation and environmental stress (Abe and Hiraki 2009; Rodriguez-Vargas et al. 2007). Moreover, the yeast

PM contains two non-overlapping subcompartments called MCC (an ergosterol-rich membrane compartment of Can1, arginine/H$^+$-symporter) and MCP (a membrane compartment occupied by Pma1, H$^+$-ATPase protein) (Grossmann et al. 2007). Several proteins, such as Sur7 proteins, are integrated within the MCC microdomains. Under physiological conditions, when fused to green fluorescent protein (GFP), these proteins form microscopically visible patches in the plasma membrane (Malinska et al. 2004). A recent study showed that yeast survival to osmotic dehydration was accompanied by a lateral rearrangement of membrane components through modification of the distribution of specific proteins (Sur7-GFP) (Dupont et al. 2010). Therefore, to understand the role of the PM in the yeast's air-drying response, we focused on the behavior of these proteins, which constitute permanent fluorescent PM markers during desiccation.

Materials and methods

Yeast strains and growth conditions

The *S. cerevisiae* strain BY4742 Wild Type (WT) (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) and *erg6Δ* mutant (*MATα his3Δ1 leu2Δ0 lys2Δ0 YMR008c::kanMX4*) (Euroscarf, Frankfurt, Germany) were used in this study. Cells were grown aerobically at 25 °C in 250-mL conical flasks containing 100 mL of Malt Wickerham (MW) modified medium. The MW medium contained 10 g glucose, 3 g pancreatic peptone, 3 g yeast extract, and 1.5 g NaH$_2$PO$_4$ (Sigma-Aldrich, Saint Quentin Fallavier, France) in 1 L of water–glycerol, and had an osmotic pressure of 1.4 MPa. This osmotic pressure has been recommended for the optimal growth of *S. cerevisiae* (Anand and Brown 1968). For growth, a subculture (1 mL) was transferred into a conical flask containing MW medium. The cultures were placed on a rotary shaker (New Brunswick Scientific, Edison, NY, USA) at 250 rpm for 24 h and allowed to grow to the early stationary phase. The final population was nearly 10^8 cells mL^{-1}.

Yeast transformation

Plasmid amplification was carried out in the *Escherichia coli* DH5α host, grown at 37 °C in Luria–Bertani medium supplemented with 100 μg/mL ampicillin. The *S. cerevisiae* transformants were selected on Yeast Nitrogen Base casa plates (0.67 % Difco yeast nitrogen base without amino acids, 0.5 % NH$_4$Cl, 1 % glucose, 0.1 % Difco Bacto Casamino acids, and 1.8 % agar). The strain BY4742 was transformed with the plasmid YIp211SUR7GFP after linearization using *Eco*52I (*SUR7* gene was inserted as a *Hin*dIII–*Bam*HI fragment) (Grossmann et al. 2007; Dupont

et al. 2010). Strain transformation of *S. cerevisiae* with the linearized YIp211SUR7GFP plasmid was performed using the LiAc method (Ito et al. 1983).

Preparation of binary water–glycerol solutions of different osmotic pressures

Glycerol (Sigma-Aldrich) was the solute used for the hyperosmotic treatments in all experiments. The mass of solute to be added to 1,000 g of distilled water to obtain the desired water activity (a_w) was calculated using the Norrish equation (Norrish 1966):

$$a_w = (1 - X_s)e^{-KXs^2},$$

where X_s is the molar fraction of the solute and K is the Norrish coefficient of the solute used to increase the osmotic pressure. For glycerol, $K = 1.16$ (Chirife and Fontan 1980). Osmotic pressure π (Pa) and water activity are related by the following equation:

$$\pi = \frac{RT \ln(a_w)}{\overline{V_w}},$$

where R is the universal gas constant (J. mol^{-1}. K^{-1}), T the temperature (K), and $\overline{V_w}$ the partial molar volume of water (m^3. mol^{-1}). The quantities of glycerol were 51 g, 1,063 g, 3,986 g, and 6,654 g for 1,000 g of water to obtain final a_w values of 0.99, 0.80, 0.45, and 0.30. respectively. Cell suspensions and rehydration solutions were kept in an air-conditioned room at 25 °C. The a_w values of the solutions and the samples were checked using a dew-point osmometer (Decagon Devices Inc., Pullman, WA, USA).

Osmotic treatments

Three osmotic treatments were used: moderate ($a_w = 0.80$), which is slightly higher than that allowing osmoregulation, and two levels of severe ($a_w = 0.45$ and $a_w = 0.30$). Culture samples (20 mL) were centrifuged (5 min, 2,200×g), washed twice in the binary water–glycerol mixture ($a_w = 0.99$), and the pellets were resuspended in 10 mL of water–glycerol mixture. Growth of cells in the presence of glycerol leads to its uptake into the cytoplasm. Therefore, during osmotic treatments, rapid cell water outflow allows the equilibration of internal and external osmotic pressures and changes in cell volume (Dupont et al. 2011). The level of volume contraction reached after this one-step response remains constant as long as cells are not permeabilized (for at least 1 h after hyperosmotic shock). Thus, by preventing glycerol uptake during glycerol perturbations, the amount of water transfer and the volume contraction reached after dehydration in liquid and gaseous environments were similar. For rapid perturbations (osmotic shock,

166 MPa. s^{-1}), 1-mL aliquots of this suspension were placed in microsample tubes, which were then centrifuged (10 min, 5,100×g) and the supernatant was removed. Hyperosmotic shock was induced by quickly introducing 1 mL of a binary water–glycerol solution (final a_w values of 0.80, 0.45, and 0.30) to the pellets. For progressive perturbations (osmotic slope, 0.086 MPa. s^{-1}), the external osmotic pressure was increased linearly by slowly adding pure glycerol to an agitated conical flask containing the initial cell suspension at $a_w = 0.99$; the glycerol solution was injected using a syringe pump (KD Scientific, Boston, MA, USA). The cells were maintained under hyperosmotic conditions for 1 h before rehydration. For rapid rehydration, the hyperosmotic solution was removed from the microsample tube after centrifugation (10 min, 5,100×g) and 1 mL of the binary water–glycerol solution ($a_w = 0.99$) was added abruptly to the cell pellet. Progressive rehydration was performed using successive dilutions in solutions with increasing osmotic pressure to reach $a_w = 0.99$.

Desiccation

The desiccation chamber consisted of an airtight plastic box with controlled relative humidity (RH). Saturated salt solutions were used to control the average RH inside the box (Greenspan 1977). Samples were placed on a rack above the salt solutions and the area of exposure was kept as large as possible in order to allow free diffusion of water. The chambers were maintained at a temperature of 25 °C and ventilated to increase mass transfer. Three saturated salt solutions (CH_3COOK, K_2CO_3, or NaCl) were used to control the water activities to dry samples to obtain a_w values of 0.30, 0.45, and 0.75. Saturation was assured by mixing a large excess (50 %) of each salt with deionized water. Culture samples (40 mL) were centrifuged for 5 min at 2,200×g, washed twice with a NaCl solution (9 g/L), and the pellets were then resuspended in 10 mL of the binary NaCl solution ($a_w = 0.99$). For the fast treatment (shock), the cultures were harvested by filtration using a Buchner funnel. A thin layer was then deposited on a glass microscope slide and placed in the drying chamber for 1 h. For the progressive treatment (slope), the cultures were also harvested by filtration but they were extruded using a syringe (ø200 μm) forming small filaments, as in industrial processes, and placed in the drying chambers for 1 h. For rapid rehydration, yeast cells were plunged directly into water. Progressive rehydration was performed by placing the glass microscope slides in specific chambers (RH=100 %).

Measurement of yeast viability

Yeast viability was estimated in triplicate using the colony-forming unit (CFU) method. After osmotic dehydration or

air-drying, fully rehydrated cells were serially diluted and the appropriate dilutions were plated on MW medium with 15 g.L^{-1} of agar. Colonies were counted after incubation for 36 h at 25 °C and recorded as CFU/mL (liquid medium) or CFU/mL per gram of hydrated paste (gaseous environment).

Microscopy

WT yeast strains expressing Sur7-GFP were observed using a Nikon Eclipse TE 2000 U microscope with a multispectral confocal head (D Eclipse C1). Excitation was performed at 488 nm, and the emission signal was measured between 500 and 545 nm. Images were acquired with a 100× (NA=1.4) Plan Apo oil-immersion objective (Nikon) and collected using EZ-C1 software 3.50 (Nikon). Cells dehydrated in liquid medium were observed as described (Dupont et al. 2010). Dried yeast cells were harvested by filtration, and a thin layer was then deposited on a glass microscope slide and dried progressively (slope) or within a few milliseconds (shock). After dehydration, a drop of mineral oil was placed on the dried yeast, which was covered with a glass slide and subjected to microscopy.

Results

Impact of osmotic and desiccation stress on yeast viability

This study compared cell survival of two yeast strains dehydrated using two different techniques (osmotic dehydration and air-drying desiccation). The kinetics of the dehydration/rehydration phases and the dehydration amplitude were compared between the WT strain, which accumulates ergosterol in its PM, and the *erg6Δ* mutant, which is a membrane-sensitive strain.

Dehydration and rehydration kinetics

The kinetics of dehydration and rehydration in liquid and gaseous environments are presented in Fig. 1. Very similar values of cell water activity were observed between the two environments, during the dehydration and rehydration steps. The time delay during rehydration in a gaseous environment is due to the gain in moisture of the sample which depends on the transfer of water from the liquid to the air and from the air to the yeast. The results presented in Fig. 2a compare the viability of the WT strain after dehydration and rehydration cycles in liquid and gaseous environments. Progressive or rapid dehydration and progressive or rapid rehydration were tested, generating four possible combinations.

First, for osmotic dehydration and air-drying desiccation, the lowest survival rates were observed when the dehydration and rehydration steps were performed rapidly (0.35 % and 2.80 % survival, respectively, after osmotic and air-drying treatments), whereas the highest survival rates were observed with both techniques after progressive dehydration followed by progressive rehydration (78.7 % and 42.3 %, respectively). The two other treatments that combined a rapid and a progressive step showed intermediate cell survival rates: 19.2 % and 21.6 %, respectively, during osmotic and air-drying treatments after shock–slope cycles, and 42.6 % and 27.5 %, respectively, after slope–shock cycles.

The *erg6Δ* mutant yeast cells were subjected to the same four combinations (Fig. 2b). The viability measures of this strain after treatments in liquid and gaseous environments were, respectively, 3.65 % and 13.2 % after slope–slope,

Fig. 1 Dehydration and rehydration kinetics of *Saccharomyces cerevisiae* BY4742 from 1.4 to 166 MPa during air-drying and osmotic dehydration. Shock (*broken lines*) or slope (*gray squares*) air-dried cells and shock (*broken lines*) or slope (*gray diamonds*) osmotic dehydration in liquid medium. Temperature and relative humidity were checked with a thermocouple and a humidity data logger
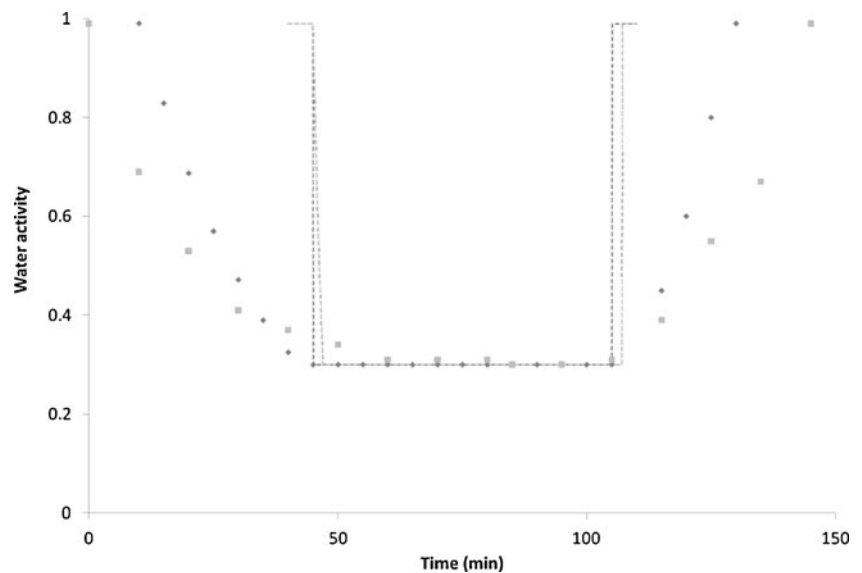
Fig. 2 Impact of different dehydration and rehydration kinetics on yeast viability. Survival of wild type strain (**a**) and *erg6Δ* mutant (**b**) were measured after osmotic treatments in water/glycerol solutions (*dark gray bars*) or air-drying (*light gray bars*). In both cases, viability was evaluated using the CFU method. *Error bars* correspond to the SD calculated from three repeated experiments. *Asterisks* denote statistical significance (*$P < 0.05$)
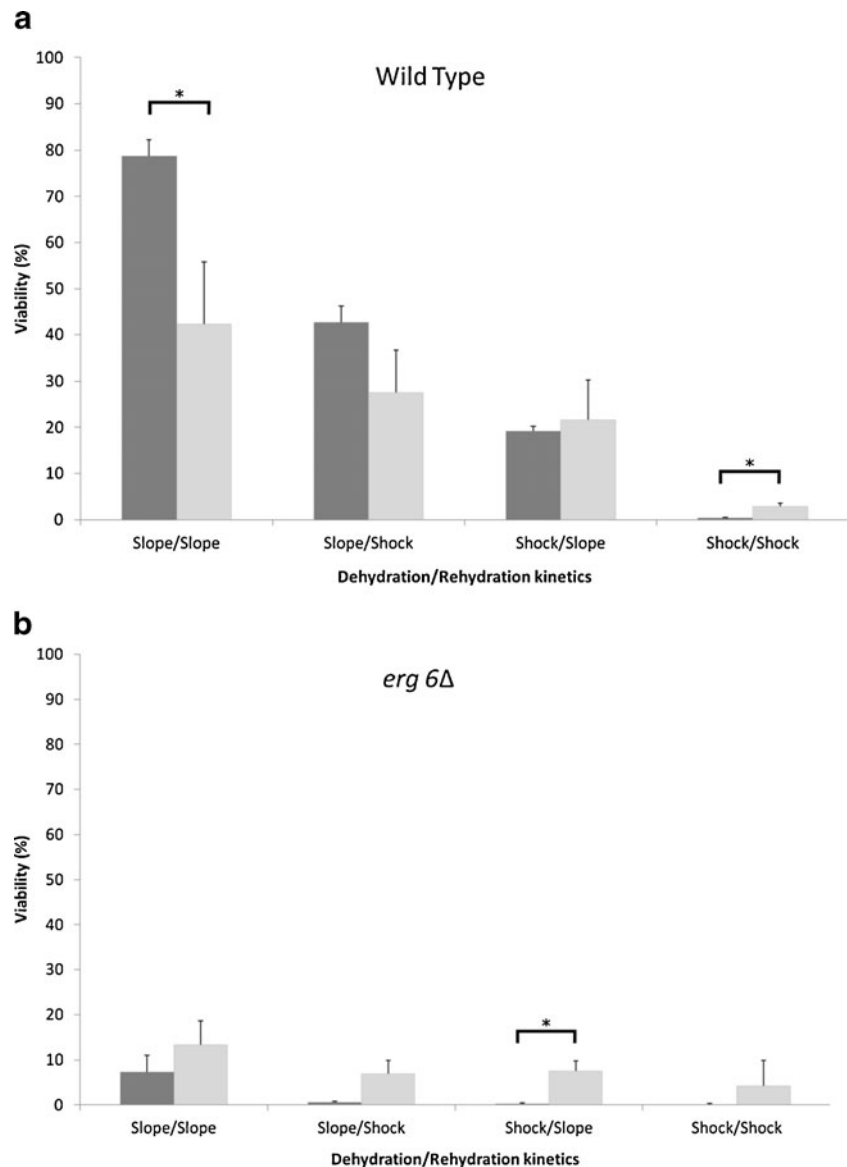
0.26 % and 6.92 % after slope–shock, 0.16 % and 7.58 % after shock–slope, and 0.30 % and 4.27 % after shock–shock cycles. These results confirm that this strain has a fragile PM that makes it particularly sensitive to osmotic dehydration and air-drying desiccation.

Magnitude of dehydration

For both strains, cells were treated with a shock–shock cycle (water activity of 0.80, 0.45, or 0.30) for 60 min and then rehydrated. Figure 3a presents cell viability of the WT strain after dehydration in liquid medium or in drying chambers. With these treatments, higher viability ratios were observed at a_w values of 0.80 with 82.5 % and 65.3 % in liquid and gaseous environments, respectively. The viability was, respectively, 12.5 % and 0.35 % after dehydration in liquid

medium and 30.1 % and 2.80 % after air-drying treatments at a_w values of 0.45 and 0.30. For the WT strain, dehydration in liquid medium or by air-drying led to similar behavior. Moreover, for both techniques, cell viability clearly decreased with decreasing water activity.

Figure 3b shows the cell viability of the *erg6Δ* mutant strain after the same dehydration–rehydration cycles. In this case, the threshold was lower, i.e., there was a stronger impact of the osmotic level on *erg6Δ* destruction than on the WT strain. For the three osmotic pressures tested, viability was between 3.29 % and 0.01 % after shock–shock cycles in liquid medium and between 3.50 % and 4.57 % in a gaseous environment. These results showed that dehydration, in liquid medium or by air-drying, led to low and very low survival rates, respectively. Thus, for *erg6Δ*, all cellular damage preventing cultivability and colonization occurred

Fig. 3 Impact of different dehydration and rehydration amplitudes (shock–shock cycle) on yeast viability. Survival of wild-type strain (**a**) and *erg6*Δ mutant (**b**) were measured after osmotic treatments in water/ glycerol (*dark gray bars*) or air-drying (*light gray bars*) using the CFU method. *Error bars* correspond to the SD calculated from three repeated experiments. *Asterisks* denote statistical significance (**P*<0.05)
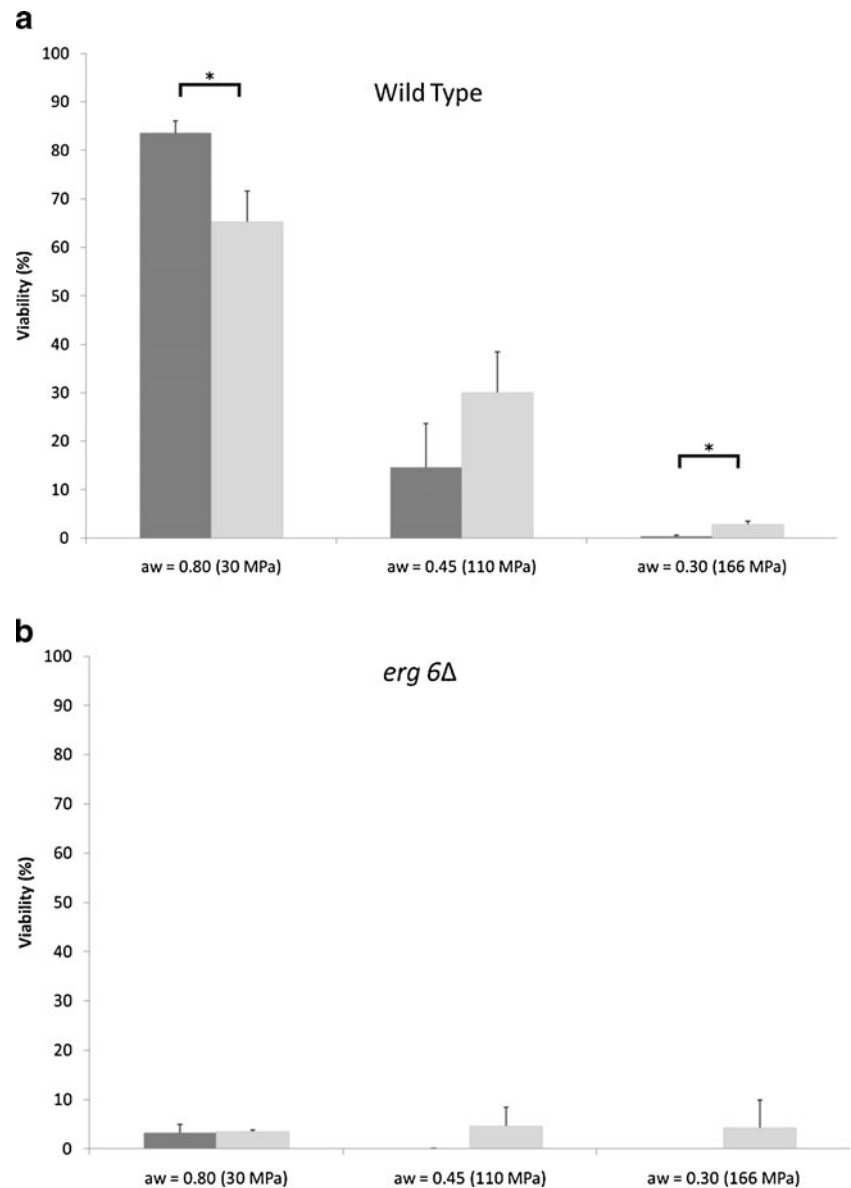
between a_w values of 0.99 and 0.80. As previously observed with the kinetic effect, the level of dehydration appeared to have no impact on survival rates of this strain.

Impact of osmotic and desiccation stress on PM organization

In addition to yeast survival, the PM organization of the WT strain was studied in order to observe whether the mechanisms of cell death/cell survival were the same in liquid and gaseous environments. Thus, the kinetic effect on the lateral organization of the PM through Sur7-GFP localization was studied for each technique, after dehydration and rehydration. The impacts of drying kinetics and the magnitude of dehydration in liquid and gaseous environments were also tested with the transformed WT strain and the results

obtained were similar to those obtained with the non-transformed strain (data not shown).

Lateral PM organization after dehydration

The results presented in Fig. 4a show the lateral organization of the PM after dehydration in liquid and gaseous environments. In the control cells, which were totally hydrated (a_w=0.99), Sur7-GFP proteins were concentrated in discrete patches (Fig. 4a, A). According to the literature, it is possible to observe around 15 GFP areas evenly distributed throughout the PM in yeasts (Dupont et al. 2010; Grossmann et al. 2007). After shock osmotic dehydration (Fig. 4a, C), the distribution of Sur7-GFP microdomains remained in distinct patches as in the control cells, whereas progressive dehydration in liquid medium (Fig. 4a, B) led to
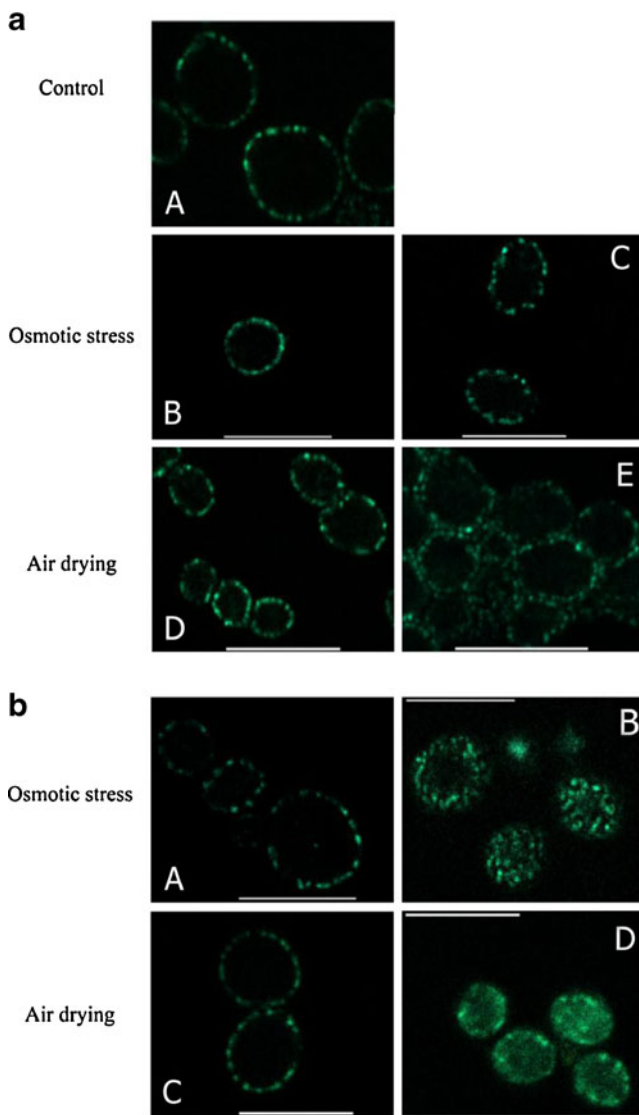
Fig. 4 Impact of dehydration (**a**) and rehydration (**b**) kinetics on Sur7-GFP microdomain distribution in the plasma membrane of transformed WT. Images (**a**) show control cells (*A*), cells in liquid medium after progressive (*B*) or rapid dehydration (*C*), and cells after progressive (*D*) or rapid air-drying (*E*). Images (**b**) show cells dehydrated in liquid medium after progressive (*A*) or rapid (*B*) rehydration and cells dehydrated in gaseous environment after progressive (*C*) or rapid rehydration (*D*). Median optical cross-sections of the cells are shown. *Scale bar*=5 μm

lateral redistribution of Sur7-GFP proteins. In the latter case, there were fewer but larger patches than in the control, suggesting PM reorganization.

After shock air-drying (Fig. 4a, E), the microdomains were still evenly distributed. These results were similar for the control and the yeast shock-dehydrated in liquid medium. After progressive dehydration in a gaseous environment (Fig. 4a, D), several distribution profiles were observed. Few cells showed evenly distributed microdomains, as seen in the controls. Nevertheless, most cells showed more

diffuse Sur7 areas, fewer but larger than in the controls. However, these domains were slightly different, being less extensive from those obtained in liquid medium (Dupont et al. 2010). This might have arisen from the observation technique under oil. Thus, microdomains stayed concentrated in discrete patches after rapid dehydration with both dehydration techniques, whereas the patches were larger and fewer after progressive dehydration suggesting kinetics-dependent PM reorganization.

Lateral PM organization after rehydration

Figure 4b shows lateral organization of the PM after rehydration visualized by Sur7-GFP localization. The effect of rehydration was studied by observation of the cells after dehydration (final a_w of 0.30) followed by rehydration (a_w of 0.99). While Sur7-GFP was still mainly confined in the PM after rapid dehydration in liquid or gaseous environments, after a shock–shock cycle, it was localized in the cell cytoplasm and was not observed in the PM either in liquid medium (Fig. 4b, B) or through air-drying (Fig. 4b, D). For both techniques, rapid rehydration resulted in the presence of the protein throughout the cell. This observation could be related to membrane rupture and disorganization of the cytoplasm during rehydration. After slope–slope cycles (Fig. 4b, C), numerous patches of Sur7-GFP were distributed in the PM. After rehydration, the reorganization of the PM in cells dehydrated in liquid medium or by air-drying was similar to the profiles of the control cells.

Discussion

This study was conducted to help understand the role of PM in the yeast survival mechanisms in response to hydric perturbations. For this, the resistance of two yeasts (WT and a membrane sensitive strain) to dehydration in a liquid medium (osmotic stress) or a gaseous environment (osmotic and matric stress) was measured. The effect of the dehydration technique on the lateral organization of the PM through Sur7-GFP localization was then studied. Our main conclusion is that there was similar behavior in liquid and gaseous environments for both yeast strains. Similarities were also found with PM modifications after observation using confocal microscopy. Finally, our results emphasize the major role of kinetics for optimal preservation of yeast cells during air-drying desiccation.

Similar yeast survival during osmotic dehydration and air-drying desiccation

For the WT strain, viability depended on the lowering rate of water activity with both methods of dehydration (Fig. 2a). A

comparison of yeast viability after shock–slope and slope–shock cycles showed the importance of controlling dehydration and rehydration kinetics to enhance yeast survival (Rodriguez-Porrata et al. 2008). As in liquid medium, the choice of optimal drying kinetics (progressive dehydration and rehydration) improved cell preservation. The largest difference between osmotic dehydration and air-drying desiccation (78.7 % and 42.3 %, respectively) was observed after the slope–slope cycle and can be explained by the more complex phenomena occurring during air-drying desiccation (Fig. 2a). Indeed, authors have reported in *S. cerevisiae* that drying leads to an increase in the intracellular level of malondialdehyde (MDA), a product of lipid peroxidation (Pereira et al. 2003). It has also recently been shown that cell oxidation levels increase significantly during air-drying desiccation (Garre et al. 2010). Therefore, the difference in yeast resistance between air-drying and osmotic dehydration might be associated with cell oxidation during the transition to an aerobic phase (França et al. 2007; Teixeira et al. 1996). Nevertheless, although the survival rates are slightly different, the general behavior of the strains is the same after osmotic dehydration and air-drying desiccation. Indeed, we can observe the same kinetic behavior (Fig. 2a) and the same decrease in viability with increasing osmotic magnitude (Fig. 3a), showing the importance of the mechanical component. Consequently, both effects (mechanical constraint and oxidative stress) exist but with different impacts on the yeast survival rate. Thus, oxidative stress has a minor effect compared to mechanical stress, certainly because the yeast *S. cerevisiae* can activate a broad response involving several defense mechanisms (enzymes, low molecular mass antioxidants) (Estruch 2000; Herdeiro et al. 2006; Landolfo et al. 2010).

After dehydration with moderate shock, cell survival remained relatively high in liquid and gaseous environments (82.5 % and 65.3 %, respectively), showing a limited kinetic effect at high water activity values (Fig. 3a). At an a_w of 0.80, cell growth and metabolism stopped but the viability was mainly preserved, whereas dehydration attaining water activities of 0.45 or 0.30 resulted in important cell destruction in both liquid and gaseous environments. This may be because of the membrane lipid phase transition from liquid crystalline to gel during dehydration and from gel to liquid during rehydration, which occurs between a_w values of 0.80 and 0.45 (Beker and Rapoport 1987). Authors showed in dry pollen that the transition from gel to liquid crystalline in membrane phospholipids that accompanies rehydration is the main cause of permeabilization and cell leakage (Crowe et al. 1989). A more recent study, conducted in liquid medium, showed that inappropriate kinetics or high levels of stress can cause cell death (Dupont et al. 2010). Indeed, rapid dehydration causes contraction of the yeast PM with progressive internalization leading to a reduction

of the PM surface area during dehydration and lysis of the cells because of a lack of surface area during shock rehydration. Conversely, if the PM has time to reorganize, the yeast's survival is better. This might be associated with the slowness of lyotropic chain-disordering phenomena (Milhaud 2004). Indeed, the redistribution of water molecules between the different parts of the sample could be a question of time. Moreover, the use of slow kinetics could allow the synthesis of protectants in the early stages of air-drying (Ratnakumar et al. 2011). However, the results obtained with the mutant strain suggest that the maintenance of PM integrity is the main parameter determining survival during air-drying desiccation. Indeed, the dehydration and rehydration kinetics had no impact on *erg6Δ* cell viability after osmotic and desiccation stress (Fig. 2b). The magnitude of dehydration also had no impact (Fig. 3b). It has already been shown that this strain, with a deleted ERG6 gene, is very sensitive to osmotic disturbances due to changes of PM mechanical properties (Dupont et al. 2011). Indeed, the nature of the membrane sterols governs the mechanical behavior of the PM during osmotic perturbation. These results also underline the importance of the mechanical constraint during air-drying desiccation. Thus, volume contraction resulting from the release of water—similar in liquid and gaseous environments—completely destabilizes the cells and determines their death at all the magnitudes and rates of dehydration tested (Simonin et al. 2007). Finally, the use of *erg6Δ* underlines how the yeast's PM composition, in particular the sterol composition, is essential for the survival of *S. cerevisiae* at the solid–liquid interface, where hydration is often subjected to instability.

Implication of the PM in yeast drying tolerance

To understand desiccation survival mechanisms at the atmosphere–lithosphere interface, the lateral organization of the PM through Sur7-GFP localization was studied for both dehydration methods on the WT strain. A microscopy technique under oil was used to maintain the effects of air-drying desiccation during observation. A redistribution of Sur7-GFP was observed after progressive disturbance in liquid and gaseous environments (Fig. 4a, B, D). These observations were accompanied by significant yeast survival (Fig. 2a) and might be linked with PM rearrangement during the lipid phase transition. The mechanisms responsible for cell resistance were perhaps not exactly identical but in both cases, they resulted in yeast survival (Figs. 2a and 4b, A, C). Observation of the Sur7-GFP distribution after shock–shock cycles revealed similar behavior after dehydration (Fig. 4a, C, E) and after rehydration (Fig. 4b, B, D) in both liquid and gaseous environments. Patches were distributed as in the controls on quickly dried cells, revealing that these proteins were not internalized during the first phase of

drying. After rehydration, cells showed a single cluster of Sur7-GFP, indicative of a cell bursting during rehydration, as obtained in liquid medium.

Whether in the case of cell death or survival, these results suggest similar mechanisms during desiccation to those already observed during dehydration in liquid medium, with similar PM behavior. During air-drying, the PM is a key structure in cell survival. Moreover, the cells had the same volume before dehydration and the yeast response was similar when the osmotic gradient was obtained in liquid or gaseous environments. Therefore, both dehydration strategies induced an accumulation of intracellular glycerol. Results from the WT and $erg6\Delta$ mutant strains showed that the mechanical component was the main stress experienced by the yeasts during hydric perturbations of their environment. For adapted microorganisms, such as the WT strain, slow kinetics seem to be essential in permitting partial disorganization of the PM, then survival and colonization.

In conclusion, for each of the two tested strains, survival rates were closed for air-drying desiccation and osmotic dehydration. There were similar patterns of PM disorganization and reorganization during progressive kinetics and preservation of the Sur7-GFP distribution and cell death after rapid kinetics. Therefore, the PM appears to be the main target of dehydration treatments and its slow lateral reorganization allows cell survival. Moreover, observation of dried cells under oil immersion optics is an interesting approach that could be used to stain other cellular structures and allow them to be studied in poorly hydrated conditions. The dehydration techniques used in this study, by addition of a solute (osmotic stress) and by elimination of water (osmotic and matric stress), were different in terms of molecular diffusion, matrix solidification, and cellular environment. Indeed, in the first case, cells were constantly surrounded by liquid and protected from the harmful effects of activated oxygen molecules. In the second case, as water flowed out, the different cellular structures were gradually exposed to air. Thus, if free radical production caused by hydric perturbation exists in liquid and gaseous environments (Petrovic 2006), yeasts are more exposed to oxidative stress associated with gaseous oxygen (singlet oxygen) during air-drying desiccation (Piette 1991). Although we observed a greater destructive effect in the case of air-drying desiccation, yeast survival, depending on the kinetics or amplitude, changed in parallel for both types of dehydration (glycerol and air-drying). Therefore, these results could be explained by the predominance of the mechanical dehydration component and modification of the PM surface area for both strains of yeast. This work allows proposing a hypothesis on the similar cellular behavior during dehydration in liquid medium and during air-drying desiccation. Thus, dehydration in liquid medium represents a convenient method for studying yeast survival mechanisms during air-drying

caused by natural fluctuations in the level of hydration. We plan to test other yeast mutants more sensitive to oxidative stress.

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