



Anhydrobiosis in yeast: role of cortical endoplasmic reticulum protein Ist2 in *Saccharomyces cerevisiae* cells during dehydration and subsequent rehydration

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Abstract Two *Saccharomyces cerevisiae* strains, BY4741 and BY4741-derived strain lacking the *IST2* gene (*ist2Δ*), were used to characterise the possible role of cortical endoplasmic reticulum (ER) protein Ist2 upon cell dehydration and subsequent rehydration. For the first time, we show that not only protein components of the plasma membrane (PM), but also at least one ER membrane protein (Ist2) play an important role in the maintenance of the viability of yeast cells during dehydration and subsequent rehydration. The low viability of the mutant strain *ist2Δ* upon dehydration-rehydration stress was related to the lack of Ist2 protein in the ER. We revealed that the PM of *ist2Δ* strain is not able to completely restore its molecular organisation during reactivation from the state of anhydrobiosis. As the result, the permeability of the PM remains high regardless of the type of reactivation (rapid or gradual rehydration). We conclude that ER protein Ist2 plays an important role in ensuring the stability of molecular organisation and functionality of the PM during dehydration-

rehydration stress. These results indicate an important role of ER-PM interactions during cells transition into the state of anhydrobiosis and the subsequent restoration of their physiological activities.

Keywords Anhydrobiosis · Dehydration-rehydration · Yeast · Endoplasmic reticulum · Endoplasmic reticulum protein Ist2

Introduction

Anhydrobiosis is a unique state of living organisms which is caused by significant losses of water, resulting in temporary and reversible suspension of intracellular metabolism discovered by great Dutch naturalist Antonie van Leeuwenhoek in 1701 (van Leeuwenhoek 1702, 1705). Transition into the state of anhydrobiosis ensures the survival of yeast cells in adverse environmental conditions when there is no available water in the surrounding environment (Rapoport et al. 2019). Yeasts are described as one of the most resistant microorganisms against dehydration stress, and *Saccharomyces cerevisiae* is one of the model organism for the studies of anhydrobiosis because of its wide application in biotechnology and the food industry (Beker and Rapoport 1987; Rapoport et al. 1997, 2016, 2019; Clegg 2001; Rapoport 2017; Camara, Sant’Ana 2021). Detailed studies about yeast

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cell transfer into the state of anhydrobiosis have revealed main structural and functional changes as well as intracellular protective reactions that occur in cells under these conditions. Such information has been obtained in the studies of cell walls, plasma membranes, nucleus, mitochondria, vacuoles and lipid droplets (Beker et al. 1984; Beker and Rapoport 1987; Guzhova et al. 2008; Rapoport et al. 2009, 2019; Calahan et al. 2011; Borovikova et al. 2014, 2016; Grube et al. 2014; Rozenfelde and Rapoport 2014; Rapoport 2017; Kulikova-Borovikova et al. 2018; Camara, Sant’Ana 2021).

Plasma membranes and other intracellular membranes are one of the most important yeast cell structures affected by dehydration and subsequent rehydration, as the functionality of the lipid bilayer directly depends on the amount of available water in the surrounding environment. It is supposed that the maintenance of the molecular integrity of the cell plasma membrane and other intracellular membranes is one of the most important factors determining cell viability during dehydration-rehydration. Most respective studies have focused on the changes in lipid components of membranes, especially of the cell plasma membrane, whereas information about the role of membrane proteins in yeast survival at dehydration is scarce (Beker et al. 1984; Beker and Rapoport 1987; Dupont et al. 2010, 2014; Rapoport 2017, 2019; Rapoport et al. 2019). Recently, studies on the understanding of the possible role of plasma membrane protein components for yeast cell viability during dehydration and subsequent rehydration have been launched. The results show that the activities of potassium uptake transporter Trk2, glycerol transporters Stl1 (importer) and Fps1 (exporter) and maltose transporter Agt1 at the plasma membrane are important for yeast cell ability to survive during the transfer into the state of anhydrobiosis (Borovikova et al. 2014; Duskova et al. 2015; Kulikova-Borovikova et al. 2018). Yet almost nothing is known about the stress response of the endoplasmic reticulum (ER) in yeast cells during their transition into the state of anhydrobiosis (Rapoport et al. 2019). The first information about influence of dehydration treatment upon ER appeared recently in electron microscopy studies. It was shown that during the desiccation process approximately in 13% of dehydrated yeast cells may be revealed the appearance of ER whorls which are a multi-layered, ring-shaped structures (Ren

et al. 2020). Authors of this research suppose that these whorls are induced during drying by unfolded protein response (UPR). Besides that they found that desiccation causes expansion and disorganization of ER tangled around the nuclear membrane in yeast cells (Ren et al. 2020). These expanded membranes were different from the ER whorls, which were more organized and usually not associated with nuclear membrane. The ER close to plasma membrane was often observed broken into small pieces, forming circular structures (Ren et al. 2020).

Yeast ER consists of three distinct subdomains: cortical (cell peripheral) ER, cytoplasmic ER and perinuclear ER (Voeltz et al. 2002; Nakatogawa 2020). In accordance with modern ideas, the endoplasmic reticulum in the eukaryotic cells is considered a major membrane source. The main activities of the ER include folding, modification and assembly of newly synthesised proteins that may be used by the cells themselves or excreted from the cells (Hijazi et al. 2020). Ongoing work has elucidated a complex and interdependent relationship between mitochondria and the ER. Physical contact sites connect mitochondria and ER for lipid and calcium exchange (Phillips and Voeltz 2016), and adequate targeting of mitochondrial proteins is assisted by the ER (Hansen et al. 2018). The ER is a main reservoir of Ca^{2+} and participates in the maintenance of Ca^{2+} homeostasis in the cells (Knupp et al. 2019); it provides a platform for autophagosome biogenesis. Currently, it is assumed that the formation of autophagosomes is the result of interactions of many intracellular membranes and ER. The resulting autophagosomal membrane initiates from the ER and is tethered on the ER (Ktistakis 2020). In recent years, considerable attention has been paid to lipid droplets, which are cellular compartments linked to the storage of metabolic energy in the form of neutral lipids; their biogenesis takes place in the ER (Choudhary and Schneiter 2020; Choudhary et al. 2020). Under stress, increased protein misfolding may occur. When the demand for protein folding or repair of protein misfolding at the ER is too high, cells can experience ER stress. Under these conditions, cells activate a transcriptional program, the so-called “unfolded protein response” (UPR), which helps to maintain ER homeostasis by enhancing the capacity for protein folding and promoting transport of misfolded proteins for their destruction in lysosomes/phagolysosomes or vacuoles (Walter and Ron 2011;

Nakatsukasa 2021). Various stresses and unfavorable conditions (zinc deficiency, cadmium exposure, acetic acid stress, ethanol stress) evoke UPR which is triggered in yeast *Saccharomyces cerevisiae* by the ER-located transmembrane protein Ire1 (Nguyen et al. 2013; Miyagawa et al. 2014; Le et al. 2016; Kawazoe et al. 2017). It was revealed that the activation of UPR involved transcriptional changes of about 400 different genes (Tyo et al. 2012; Bernauer et al. 2021).

During ER stress, Ca^{2+} is released from the ER and enters the mitochondria; Ca^{2+} movement to the mitochondria is suggested to regulate oxidative phosphorylation and initially represents an adaptive response to acute ER stress (Kaufman and Malhotra 2014; Knupp et al. 2019). Recently, it has been shown that enhanced mitochondrial biogenesis, linked to improved efficiency of the electron transport chain, is a powerful strategy to block ROS accumulation and promote cell survival during ER stress in eukaryotic cells (Knupp et al. 2019). Yeast responds to ER stress with increased O_2 consumption, increased mitochondrial membrane potential and up-regulation of selected mitochondrial proteins (Hijazi et al. 2020). There are also interesting results that show the importance of mitochondrial ER junctions for cell decisions to live or die in the conditions of various environmental stresses. Besides that, they may influence mitochondrial morphology, inheritance and mitophagy (Smethurst et al. 2017).

The cortical ER in yeast cells consists of ER tubules and highly fenestrated cisternae (West et al. 2011; Chang et al. 2017). The ER-PM junctions in yeasts may cover about 40% of the plasma membrane, with a gap distance of about 30 nm (West et al. 2011; Chang et al. 2017). This distance allows direct protein–protein/lipid interactions. Most likely, the cortical ER participates in ER functions that suppress UPR in wild-type yeast cells (Chang et al. 2017). This information evidences that the ER state may have an important role at yeast cells response to dehydration stress and may be essential for their transition into anhydrobiosis. However, the underlying mechanisms are still unclear.

Although ER–PM junctions have been known for several decades, our understanding of the mechanism that tethers the cortical ER to the PM and why such connections are established remains limited. Also, relatively little is known about the architecture and structural components of ER–PM junctions. Several

proteins are involved in the regulation and functions of ER–PM junctions (Chang et al. 2017). The polytopic transmembrane protein Ist2 is a candidate for a factor involved in this process because it is specifically localised in the cortical ER and contains a lipid-binding domain that recognises phosphoinositide and other lipids at the cytosolic face of the PM (Fischer et al. 2009; West et al. 2011; Wolf et al. 2012, 2014; Manford et al. 2012). The Ist2 is involved, together with other tethering proteins (Scs2 and Scs22, Tcb1, Tcb2, Tcb3), in the formation of contacts between the plasma membrane and ER membranes. For now, it is assumed that Ist2 and Scs2 proteins are key ER-PM tethers (Fischer et al. 2009; Wolf et al. 2012, 2014; Manford et al. 2012).

In this study, we continue the investigations of the potential role of membrane proteins in yeast cell survival during dehydration-rehydration stress, with the aim to elucidate the importance of the endoplasmic reticulum and one of its proteins (Ist2) regarding yeast cell resistance during dehydration and subsequent rehydration.

Material and methods

Yeast strains, media and growth conditions

The *Saccharomyces cerevisiae* strains used in this study were BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and BY4741-derived *ist2Δ* strain (BY4741 *ist2Δ::loxP*; Papoušková et al. 2017).

The yeast strains were grown in 750 ml flasks containing 200 ml YPD nutrient medium (10 g yeast extract l^{-1} , 20 g peptone l^{-1} , 20 g glucose l^{-1}) on an orbital shaker at 180 rpm at 30 °C for 48 h (mid of the culture stationary growth phase). Cells were harvested by centrifugation (10 min) and subjected to dehydration stress.

For cell size measurements, cells were grown in minimal medium YNB-F (pH 5.8; Formedium; Navarette et al. 2010) supplemented with BSM mix of auxotrophic supplements (Formedium) and 200 mM KCl, harvested in the mid-exponential growth phase and transferred to the same medium supplemented with BSM, 50 mM KCl and 1 M NaCl.

Dehydration and rehydration procedures

The obtained yeast biomass was pressed through a sieve with a mesh diameter of 1 mm, spread in a 1-mm layer in the Petri dish and then subjected to drying. Yeast biomass dehydration was performed in a fan-free oven at 30 °C for 15–17 h, which is the minimum time needed for cells to reach a residual moisture content of 8–10%. The residual moisture of the cells was determined by drying yeast biomass to a constant weight at 105 °C.

Dehydrated yeast biomass was rehydrated using rapid or gradual rehydration. Rapid rehydration was performed in a test tube containing 5 ml distilled water for 10 min at room temperature, whereas gradual rehydration was performed in a glass water-vapour chamber at 37 °C for 2 h. A Petri dish with dehydrated yeast biomass was placed in the middle part of the glass chamber and water was poured in its lower part. After gradual dehydration in a water vapour, for further standard rehydration, partially rehydrated yeast cells were placed directly into the test tube containing 5 ml distilled water for 10 min at room temperature.

Determination of yeast cell viability after dehydration

The survival rates of dehydrated yeast cultures were determined using fluorescence microscopy with the fluorochrome primuline. The viability of dehydrated yeast samples was assayed after 10 min of rehydration in distilled water at room temperature. Samples were mixed with equal (1:1) volumes (10 µl) of fluorochrome primuline solution (1 mg/ml); relevant samples were stained for 3 min and then examined using fluorescence microscopy. This method for the determination of yeast cell viability is based on the principle that primuline does not diffuse through the plasma membrane of viable, more or less intact yeast cells, so that only the cell wall is fluorescent. In turn, in the dead cells, primuline penetrates into the cytoplasm, binds with its components and, as a result, the entire cell is fluorescent after its excitation with near-ultraviolet light (Rapoport and Meissel 1985).

Cell membrane permeability

Changes of plasma membrane permeability were quantified in percentages of cells dry matter losses during the rehydration, measured by differences of dry weight of the yeast samples before and after rehydration.

A certain amount of dehydrated or partially rehydrated biomass was placed in the test tubes. 5 ml of distilled water was added and rehydration was performed as described. The test tubes were then placed on a multi speed vortex at 3000 rpm for 10 min. The samples were then centrifuged for 10 min. The excess supernatant was decanted, but the biomass was quantitatively transferred into pre-weighed glass containers using distilled water. The yeast cell suspensions were placed in an oven at 105 °C and dried to constant weight.

Cell size measurement

To follow the changes in cell size upon osmotic shock, a cell counter (CASYTM model TT, Innovatis) equipped with a 60 µm capillary tube was used. The experiment was repeated twice with similar results. Each time, 2×10^4 cells were analysed for each strain and condition. Intervals containing the most typical 60% of the cell populations were visualised using a box plot diagram with the mean diameter from the observed interval (3–9 µm) inside the box.

Statistical analysis

All experiments (excluding those described in the section “Cell size measurement”) were performed in three or more repetitions. Results are presented as average value with standard deviation. To compare results between two groups, the Student’s t-test was performed. $P < 0.05$ was defined as the threshold of statistical significance.

Results and discussion

In this study two *S. cerevisiae* strains BY4741 and BY4741-derived strain lacking the *IST2* gene (*ist2Δ*) were used to characterize the possible role of cortical endoplasmic reticulum and its protein Ist2 upon maintenance of cell viability at their dehydration and

subsequent rehydration. Both strains were grown until the stationary phase and then subjected to dehydration until the cellular water content was reduced to the level of 8–10%. At such an intracellular water content, yeast cells may enter into the state of anhydrobiosis (Beker and Rapoport 1987; Rapoport 2017). The stationary phase of the culture growth was used because in this phase, yeast cells are more resistant to desiccation stress than at the exponential growth phase (Beker and Rapoport 1987; Calahan et al. 2011; Rapoport 2017). To reveal the resistance of these strains to drying, first of all, in our experiments, we determined their viability after dehydration. For this, dehydrated cells of the stains BY4741 and *ist2Δ* were subjected to rapid rehydration. The survival rates of these dehydrated-rehydrated cells are shown in Fig. 1.

The performed experiments showed that the viability of the parent strain BY4741 after dehydration was at the level of $36.6 \pm 5.2\%$; these results correspond with those previously published by our group (Duskova et al. 2015). In turn, the viability of the yeast strain with deletion of the *IST2* gene (*ist2Δ*) after dehydration and subsequent rehydration significantly decreased and was only $8.6 \pm 2.8\%$.

It is generally supposed that one of the main reasons why yeast cells lose their viability is a change in the molecular organisation of the plasma membrane, which, at the physiological level of the research, may be revealed using integral parameters such as the increase in membrane permeability and the significant

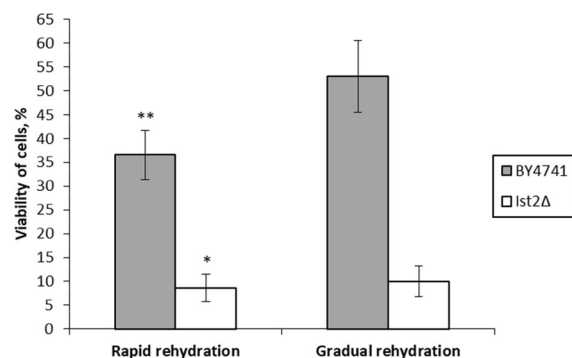


Fig. 1 Viability of *S. cerevisiae* BY4741 and BY4741-derived strain lacking the *IST2* gene after dehydration and subsequent rehydration treatment. * $P < 0.05$ indicates a statistically significant difference between dehydrated cell viability results of BY4741 and its mutant strain *ist2Δ* after rapid rehydration. ** $P < 0.05$ indicates a statistically significant difference between dehydrated cell viability results of BY4741 after rapid and gradual rehydration

leakage from the cell of various intracellular components (Beker and Rapoport 1987; Rapoport 2017; Rapoport et al. 2019). During the dehydration process, the membrane lipids may transfer from the liquid crystalline phase to the gel phase. Subsequently, during the rehydration of the cells, their membrane lipids are subjected to reverse phase transition. These phase transitions may cause changes in membrane permeability and a loss of cell viability. Based on previous studies, dehydration of cells increases the phase transition temperature of lipid bilayers, and for *S. cerevisiae*, this temperature may reach from 33 to 50 °C (Crowe et al. 1987, 1989, 2002; Leslie et al. 1994; Rapoport et al. 1997, 2009, 2019; Rapoport 2017). If rehydration of dehydrated cells occurs under adequate conditions, damages of the membrane state may be diminished, which in turn would lead to an increased viability of rehydrated cells. Up to the model of possible changes of plasma membranes during cells dehydration-rehydration, the decrease of membrane damages at cells rehydration may be reached if the reverse phase transition of membrane lipids would take place before dry cells placement in the water (Crowe et al. 1989). Such a result can be achieved by two approaches: by increasing the dry cell temperature above the membrane lipid phase transition temperature or by an initial gradual rehydration of dry cells in water vapour before their placement in the bulk of water (Crowe et al. 1989). Taking all this into account, the next step in our research was to use the second approach and to expose the dehydrated cells to gradual rehydration in the water vapor chamber, with the goal to reach the reverse phase transition of membrane lipids before their placement into the mass of water. Over a period of 2 h of gradual dry yeast rehydration, the amount of water in the cells reached the level of 29–31%. Earlier, it has been shown that the yeast cell plasma membrane restores its normal structure when the cell relative humidity reaches 20–30% (Beker and Rapoport 1987). The survival rates of dehydrated cells after gradual rehydration are shown in Fig. 1.

In strain *S. cerevisiae* BY4741, dehydrated cell viability after gradual rehydration was $53.0 \pm 7.5\%$. The viability of dehydrated cells after gradual rehydration significantly increased in comparison with the viability of cells after rapid rehydration ($36.6 \pm 5.2\%$), with a difference by 1.4 times. In turn, the viability of dehydrated *ist2Δ* strain cells after gradual rehydration did not significantly differ from

that of rapidly rehydrated cells, with $10.0 \pm 3.1\%$ in comparison to $8.6 \pm 2.8\%$. This leads us to infer that gradual rehydration increases the viability of the parent strain but not of the mutant strain cells. To explain whether this low viability of the mutant strain cells, irrespective of the type of rehydration, is associated with possible irreversible damages of the plasma membrane, a series of experiments were performed to compare the permeability of the plasma membrane depending on the type of rehydration (Table 1). This approach was successfully used in the previous studies of plasma membrane changes at yeast cells transition into anhydrobiosis (Rapoport et al. 2009, 2014; Kulikova-Borovikova et al. 2018 etc.).

Based on the results, the permeability of the plasma membrane in the cells of strain BY4741 during rapid rehydration was $22.5 \pm 1.6\%$, whereas after gradual rehydration, it was $16.5 \pm 3.7\%$. In turn, the permeability of the plasma membrane in the cells of *ist2Δ* strain did not differ with the type of rehydration and remained at a level of 23%. These results indicate that in the case of strain BY4741, one of the reasons why yeast cell viability rates decrease during dehydration–rehydration stress is linked to the changes in the molecular organisation of the plasma membrane, resulting in its permeability. In the case of *ist2Δ* strain, low rates of dry cell viability, at the same level of plasma membrane permeability as in the parent strain, cannot be explained by this phenomenon only, which means that the low viability rates of the mutant strain cells are not only due to the damage of the

plasma membrane during dehydration and subsequent rehydration stress. We therefore suggest that the significantly lower viability of *ist2Δ* strain cells after dehydration–rehydration, compared with the viability of parent strain cells (8.6% compared with 36.6%), can be somehow linked to the lack of the cortical endoplasmic reticulum protein Ist2 in the cells.

To further support our conclusion that absence of Ist2 plays an important role in survival of dehydration–rehydration stress and to see if Ist2 is involved in the water acquisition and thereby in cell volume control, we performed another type of experiment, comparing the changes in cell size upon osmotic shock. Osmotic shock, the first stage of dehydration, triggers the complex array of protective responses that can maintain the cells' viability under adverse conditions. Understanding the cellular biology of osmotic shock is therefore imperative to understanding the environmental tenacity of living systems. Osmotic shock is accompanied by a very rapid loss of water from cells, visible as a cell shrinkage. During the subsequent adaptation to osmotic shock cells transport back the lost water (usually in a passive mechanism driven by the accumulation of intracellular solutes, in *S. cerevisiae* mainly glycerol), restore their original volume and start to grow and divide. We exposed the cells of both strains to 1 M NaCl and followed the changes of cell size for 60 min. NaCl in 1 M concentration inhibits the growth of cells to the same level in both studied strains (not shown). Figure 2 summarizes obtained results and shows that indeed, the osmotic shock causes a significantly bigger loss of

Table 1 Plasma membrane permeability of dehydrated cells of *S. cerevisiae* BY4741 and BY4741-derived strain lacking the *IST2* gene after dehydration and subsequent rehydration treatment

Strain	Permeability of plasma membrane, %
<i>Rapid rehydration</i>	
BY4741	22.5 ± 1.6
<i>ist2Δ</i>	23.1 ± 1.4
<i>Gradual rehydration</i>	
BY4741	16.5 ± 3.7
<i>ist2Δ</i>	23.4 ± 3.1

Plasma membrane permeability was determined by losses of yeast dry weight during subsequent rehydration, %

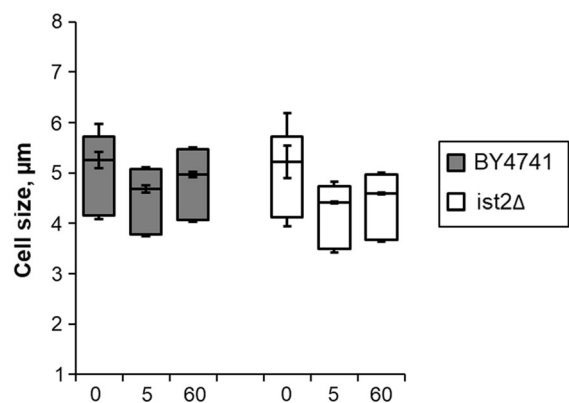


Fig. 2 Changes in cell size of *S. cerevisiae* BY4741 and BY4741-derived strain lacking the *IST2* gene before (0) and after 5 or 60 min of exposure to osmotic shock caused by 1 M NaCl

water from the *ist2*Δ cells than from the wild type, and also the recovery of cell volume is slower in cells lacking *Ist2*.

In this study we continue to investigate the role of the membrane proteins in the viability of yeast cells during dehydration-rehydration. Protein *Ist2* is a cortical ER resident protein involved in the formation of contacts between the plasma membrane and endoplasmic reticulum membranes (Fischer et al. 2009; West et al. 2011; Wolf et al. 2012, 2014; Manford et al. 2012; Kralt et al. 2014). Based on our results, loss of this ER-PM tether may influence the stability or organisation of the plasma membrane during dehydration-rehydration stress and during the response to osmotic stress.

The low viability rates of the mutant strain cells, which were revealed as the result of dehydration and subsequent rehydration stress, are most likely due to the loss of *Ist2* protein. Information about the physiological and functional role of *Ist2* in yeasts is rather limited (see Fischer et al. 2009; Manford et al. 2012; Wolf et al. 2012, 2014; Papoušková et al. 2017). It is hypothesised that the lack of this protein which determines the distance between the plasma membrane and cortical ER can influence an impairment of proper cortical ER and PM organisation. If *Ist2* is acting at the ER-PM contact sites, it is possible that it can be linked with metabolic processes at the PM and can affect protein internalisation, which can influence the stability and functionality of plasma membrane proteins, including leucine importers (such as *Bap2*), plasma membrane *Pma1* H⁺-ATPase and low-affinity alkaline metal cation transporters (Wolf et al. 2012, 2014; Manford et al. 2012; Papoušková et al. 2017).

Summarising the results of this study, we can make a number of interesting conclusions. One of them is that, for the first time, we show that not only protein components of the plasma membrane, but also at least one ER membrane protein (*Ist2*) can play an important role in maintaining the viability of yeast cells during dehydration and subsequent rehydration. Simultaneously, this means that the ER state and possible changes were underestimated, as they appear to have an essential influence upon the resistance/stability of yeast cells during their transition into the state of anhydrobiosis. Then, comparing permeabilities of dry parental and mutant strains at their rapid rehydration, which is an integral parameter that characterises the

level of plasma membrane damage, another conclusion can be drawn. Because these permeabilities are practically identical ($22.5 \pm 1.6\%$ and $23.1 \pm 1.4\%$), changes/damages of the plasma membrane at these processes are similar in the cells of both strains. This suggests that they cannot be responsible for such an essential decrease in the viability of the *ist2*Δ strain ($8.6 \pm 2.8\%$ compared with $36.6 \pm 5.2\%$ of the parental strain). As a consequence, we assume that such large ‘additional’ losses of viability may be attributed to the damages of cortical ER linked with the absence of *Ist2* protein. Finally, the absence of an increase in viability at the gradual rehydration of dry cells of *ist2*Δ strain as well as the absence of a decrease of plasma membrane permeability suggests that the deletion of this protein leads to losses of the ability to repair plasma membrane damage at the rehydration stage. This is confirmed by a larger cell shrinkage and a slower volume restoration of cells lacking *Ist2* upon an osmotic shock. Taking into account these results and that gradual rehydration led to reverse plasma membrane lipid phase transition before their placing into the mass of water, we can hypothesise that cortical ER connection with plasma membrane is extremely important for the maintenance (and in our case, for repair/restoration) of molecular organisation and the functioning of the plasma membrane. However, this hypothesis needs to be supported in detail in future studies.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The manuscript was written by all authors. All authors read and approved the final version of the manuscript.

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Data availability All data generated during this study are included in this paper.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent statement All authors agreed to participate in this paper. All authors agreed for publication of this paper in “Antonie van Leeuwenhoek Journal of Microbiology”, if accepted.

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