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The grapevine tonoplast aquaporin TIP2;1 is a pressure gated water channel

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ABSTRACT

In plants, the vacuole is a multifunctional organelle with an important role in the maintenance of the intracellular space. Tonoplast membranes are highly permeable to water due to their content in aquaporins TIPs (Tonoplast Intrinsic Proteins) that allow the rapid water influx creating an internal turgor pressure responsible for cell expansion, elongation and shape.

The aim of the present study was to evaluate if the grapevine *Vitis vinifera* TIP2;1 would operate as a possible volume regulator gated by membrane surface tension.

For that, the wild type *Vv*TIP2;1 and a non-functional mutated form were heterologous expressed in yeast. Using an experimental strategy in which cells are incubated in external media that induce an increase in internal hydrostatic pressure and consequently membrane surface tension, we were able to compare the osmotic permeability (P_f) and the activation energy for water transport (E_a) of yeast strains expressing the functional and a non-functional TIP2;1. We found P_f and E_a dependence on internal turgor pressure only for the strain harboring the functional aquaporin indicating that TIP2;1 activity is regulated by membrane tension changing from an open to a closed state in an internal pressure dependent manner. This turgor dependent gating of TIP2;1 might be a mechanism to regulate vacuolar size and shape in plants withstanding hostile drought conditions such as grapevine.

1. Introduction

Aquaporins (AQPs) are membrane protein channels widespread in cell membranes of animals, plants and microorganisms that facilitate the transport of water and/or small neutral solutes such as glycerol. Additionally they were proposed to be key players in the detection of osmotic and turgor pressure gradients [1].

Plant performance is highly dependent on the tight regulation of water permeation across cellular membranes and tissues. An important role for plant aquaporins, more than to promote paths for water movements, is to provide regulation of water flow triggered by environmental modifications, and essential under stress conditions [2]. Phosphorylation and regulation by intracellular calcium or pH [3,4] and the organization of membrane heterotetramers [5–7] are well-documented examples of these control mechanisms. In addition, the disclosure of aquaporin regulation by membrane tension in different cell systems has been reported [8–12]; in all cases, an increase in membrane tension induced a decrease in aquaporin activity.

In plants, the vacuole is a multifunctional organelle with an important role in the maintenance of the intracellular space. The vacuole biogenesis and its increase in volume relies on the transport of osmotically active species through the tonoplast followed by a rapid water influx into the vacuolar interior that creates an internal turgor pressure responsible for cell expansion, elongation and shape. Tonoplast membranes are highly permeable to water due to their content in aquaporin TIPs (Tonoplast Intrinsic Proteins) allowing the rapid water influx with low activation energy. In this context, pressure dependent gating of TIPs could be a possible mechanism of vacuole size and shape regulation [13].

Grapevine (*Vitis vinifera* L.) genome contains 28 genes coding for aquaporins, which acting in a concerted and regulated manner appear relevant for plant withstanding extremely unfavorable drought conditions essential for the quality of berries and wine

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[14]. In a previous study, *V. vinifera* cv. Touriga nacional *Vv*TnTIP2;1 was expressed in a yeast strain devoid of its native aquaporins and water transport activity was characterized [4]. *Vv*TnTIP2;1 expression enhanced the water permeability with a concomitant decrease of the activation energy of water transport. Acidification of yeast cytosol resulted in loss of *Vv*TnTIP2;1 activity [4].

The aim of the present study was to evaluate if the water channel TIP2;1 from *V. vinifera* would operate as a possible volume regulator, gated by membrane surface tension, as previously suggested for other aquaporins, namely rabbit and human AQP1 [9,12] and *Saccharomyces cerevisiae* Aqy1 [11]. For that purpose, the wild type *Vv*TnTIP2;1 and a non-functional mutated form were heterologous expressed in yeast. The osmotic permeability coefficient (P_f) and the activation energy for water transport (E_a) of the yeast strains expressing the functional and the non-functional TIP2;1 were evaluated and compared under different membrane surface tension levels. Results clearly demonstrate that *Vv*TnTIP2;1 activity is regulated by membrane tension changing from an open to a closed state in an internal pressure dependent manner.

2. Materials and methods

2.1. Yeast strains, maintenance and growth conditions

Yeast strains and plasmids used in this work are listed in Table 1. All *S. cerevisiae* strains derive from *S. cerevisiae* 10560-6B *MATα leu2::hisG trp1::hisG his3::hisG ura3-52 aqy1::KanMX4 aqy2::HIS3*. The double mutant *S. cerevisiae aqy1aqy2* was used as recipient in complementation experiments with the plasmids listed in Table 1. Transformant strains were maintained and grown in YNB medium without amino acids (DIFCO) with 2% (w/v) glucose (plus 2% (w/v) agar for solid medium) supplemented with the adequate requirements [15].

For stopped-flow assays, cells were grown in the same medium with orbital shaking, at 28 °C up to $OD_{640} \approx 1$, centrifuged (10,000 × *g*; 3 min; 25 °C), re-suspended in YPD medium (6 g l^{-1} wet weight) and incubated 1 h at 28 °C. Cells were then harvested by centrifugation (10,000 × *g*; 3 min; 4 °C), washed, and re-suspended in ice cold 1.4 M sorbitol (3 g ml^{-1} wet weight).

2.2. Cell volumes and stopped-flow fluorescence assays

Cells equilibrated in sorbitol buffer with different osmolarities ($(osm_{out})_o$ from 0.1 to 1.4 M) were loaded with a concentration-dependent self-quenching fluorophore 5-and-6-carboxyfluorescein diacetate (CFDA) previous to the osmotic challenges with sorbitol, an impermeant solute, at different tonicities Λ (defined as the ratio between final and initial media osmolarities, $\Lambda = (osm_{out})_\infty / (osm_{out})_o$) [11].

Cell initial and final equilibrium volumes were obtained under a fluorescent microscope equipped with a digital camera [11]. An average of 6 pictures with 4–6 cells each were taken before (V_o) and within 10–40 s after the osmotic challenge (V_∞).

The stopped-flow technique was used to monitor cell volume changes [16]. Experiments were performed on a HI-TECH Scientific PQ/SF-53 stopped-flow apparatus (with 2 ms dead time), at temperatures ranging from 7 to 38 °C, as described in detail [11]. For each experimental condition, at least 5 runs were usually performed and analyzed.

The changes in fluorescence due to carboxyfluorescein (CF) fluorescence quenching were recorded. The calibration of the fluorescence stopped-flow traces (F) into relative cell volume ($v_{rel} = V/V_o$) was accomplished using a linear relation between V/V_o and F previously demonstrated for the same yeast cell system [11].

2.3. Evaluation of osmotic permeability and activation energy

Permeability coefficients P_f were evaluated from the calibrated time dependent stopped flow traces, as described in detail [11]. Briefly, considering that in the time span of these studies no solute movement occurs, the total intracellular solute quantity remains constant and equal to $Qt = osm_{in}\, V_{osm}$, where osm_{in} is the intracellular media osmolarity and V_{osm} is the cellular osmotic volume that equals the total cell volume minus the constant non-osmotic volume, $V_{osm} = V - V_{Nosm}$. Defining the relative non-osmotic volume β ($=V_{Nosm}/V_o$) and v_{osm} as the ratio of the osmotic volume over the initial osmotic volume, we reach $v_{osm} = (v_{rel} - \beta)/(1 - \beta)$. When cells are in osmotic equilibrium the hydrostatic and osmotic pressure differences are equal ($\Delta P = P_{in} - P_{out} = \Delta\Pi = RT[(osm_{in}) - (osm_{out})]$, where R is the gas constant and T the absolute temperature). When applying an osmotic shock by changing the external media osmolarity (from $(osm_{out})_o$ to $(osm_{out})_\infty$), the initial osmotic pressure changes abruptly and the osmotic equilibrium is disturbed; β remains constant while v_{osm} (and v_{rel}) starts changing driving the changes of osm_{in} and of ΔP. When cells reach the new osmotic equilibrium the new condition $\Delta\Pi_\infty = \Delta P_\infty$ is attained, and the changes in v_{rel} induced by the water flux in or out of cells, across the membrane area A, are proportional to P_f, according to the following equation where $p = \Delta P/[RT(osm_{out})_o]$.

$$\frac{dv_{rel}}{dt} = \frac{A}{V_o} P_f V_w (osm_{out})_o \left[\left(\frac{1 + p_o}{v_{osm}} - p \right) - \Lambda \right] \quad (1)$$

In this equation, the only time dependent variables are the v_{rel}, v_{osm} and p. When the final osmotic equilibrium is reached all these variables reach their final equilibrium value, $(v_{rel})_\infty$, $(v_{osm})_\infty$ and p_∞ and the term in brackets in this equation vanishes; consequently, $\Lambda = [(1 + p_o)/(v_{osm})_\infty] - p_\infty$.

This term was evaluated in our previous work for this same yeast strain pre-equilibrated in different osmolarities [11] and used to calculate P_f by numerically integrating and curve fitting the calibrated data v_{rel}, using the Berkeley Madonna software (http://www.berkeleymadonna.com/). From p_o the initial

Table 1
Plasmids and strains used in this work.

Name in this work	Relevant characteristics	Source
Plasmids		
Empty plasmid	pUG35	[23]
Functional TIP2;1	pUG35-*Vv*Tn*TIP2*;1	[4]
Non-functional TIP2;1	pUG35-*Vv*Tn*TIP2*;1 E146G	[4]
	Genotype	
Yeast strains		
S. cerevisae YSH1172	10560-6B *MATa leu2::hisG trp1::hisG his3::hisG ura3-52 aqy1::KanMX4 aqy2::HIS3*	P. Van Dijck
LT21	YSH1172 + pUG35-*Vv*Tn*TIP*2;1	[4]
LT21n	YSH1172 + pUG35-*Vv*Tn*TIP*2;1 E146G	[4]

membrane tension can be calculated considering LaPlace's law for a sphere $\sigma_o = \Delta P_o r_o/2$, where $\Delta P_o = p_o(osm_{out})_o RT$ and r_o is the initial radius for each population.

The activation energy (E_a) of water transport was evaluated from the slope of the Arrhenius plot (ln P_f as a function of $1/T$).

3. Results

3.1. Cloning and expression of V. vinifera TIP2;1

V. vinifera TIP2;1 aquaporin (from now on referred as functional TIP2;1) was cloned in a single-copy plasmid harboring the corresponding ORFs, driven by the MET25 promoter, tagged with the GFP sequence at their 3′ end (Table 1) and expressed in a *S. cerevisiae aqy-null* strain (LT21) [4]. For negative control, we used the strain LT21n harboring a non-functional mutated TIP2;1 (from now on referred as non-functional TIP2;1) in which glutamic acid E146 is replaced by a glycine residue (Table 1) [4].

GFP-tagging confirmed the plasma membrane localization of both functional and non-functional TIP2;1 (Fig. 1A and B), with equivalent amounts of the tagged proteins confirmed by similar fluorescence intensities.

3.2. Functional assessment of water transport

Fig. 1C shows the shrinking rate of the two yeast strains expressing the functional and non-functional TIP2;1, equilibrated in 1.4 M sorbitol (when initial hydrostatic pressure is low) and subjected to an osmotic shock of $\Lambda = 1.25$ at 23 °C. When compared to the functional TIP2;1, a reduction on the shrinking rate for the non-functional was detected. The P_f values were $(9.5 \pm 0.8) \times 10^{-4}$ and $(2.0 \pm 0.7) \times 10^{-4}$ cm s^{-1} for functional and non-functional TIP2;1 respectively.

Temperature dependent P_f measurements (1.4 M and $\Lambda = 1.25$) enabled the evaluation of the activation energy (E_a) for water transport. Fig. 1D shows the Arrhenius plot (ln P_f vs $1/T$) obtained where the calculated E_a values were 4.5 ± 0.5 for the functional and 14.6 ± 1.0 kcal mol^{-1} for non-functional TIP2;1. Altogether these results confirm that the E146G mutation rendered the aquaporin inactive as we reported before [4].

3.3. Volume changes depend on media osmolarity

Yeast strains harboring functional and non-functional TIP2;1 loaded with CFDA and equilibrated in buffer osmolarities ranging from 0.1 to 1.4 M (osm_{out}) were exposed to a 1.25 tonicity shock. The initial and final equilibrium volumes were measured by fluorescence microscopy and the volume ratio (V_∞/V_o) was plotted against its initial buffer osmolarity (Fig. 2A). Equivalent results were obtained for both strains, highlighting a singular performance: a 1.25 tonicity shock induces larger volume changes in cells pre-equilibrated in higher osmolarity media.

In our previous work [11] it was unequivocally demonstrated that incubation in low osmolarity media gives rise to an internal hydrostatic pressure inducing a membrane surface tension and sustaining the yeast cells in a pre-swollen state. This internal hydrostatic pressure and the resulting surface tension increase as the media osmolarity decreases. Upon a hyperosmotic shock, the pressure starts dissipating, but a volume change can only be detected if the magnitude of the shock is sufficient to reduce the pressure below a certain level. Since the same yeast strains were used in both studies, identical levels of tension were expected. The results of Fig. 2A reinforce these assumptions, showing that a comparable level of pressure was obtained for the same initial $(osm_{out})_o$, independently of aquaporin expression (yeast *aqy-null* strain, yeast overexpressing aqy1 in the previous study or expressing TIP2;1 in this study). Thus, for P_f evaluation, we used the estimated value of $V_{Nosm} = 3.96 \pm 0.34$ μm^3 and the experimental curves of Λ vs $1/(v_{osm})_\infty$ [11].

3.4. Membrane tension modulates TIP2;1 permeability

To evaluate the effect of membrane surface tension on aquaporin activity, cells were equilibrated in $(osm_{out})_o$ from 0.1 to 1.4 M, thus creating different levels of pressure and membrane surface tension. Subsequently, cells were confronted with osmotic shocks able to induce measurable volume changes, but unable to totally dissipate the initial pressure ($\Lambda = 1.25$ for $(osm_{out})_o \geqslant 0.5$ M and $\Lambda = 1.5$ for $(osm_{out})_o < 0.5$ M). The calculated P_f are plotted in Fig. 2B. For the non-functional TIP2;1 the P_f obtained was low and independent of the internal pressure, whereas for cells expressing the active aquaporin, a strong dependence was clearly depicted. Cells pre-incubated in higher osmolarity media, above 1 M, showed approximately a 5-fold P_f compared to the non-functional TIP2;1. However, when incubated in external media

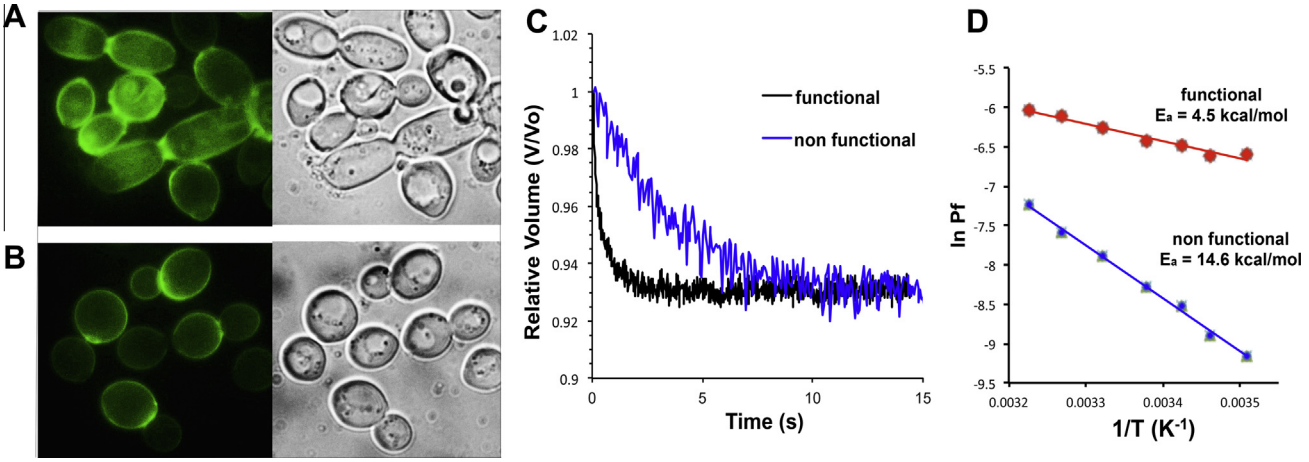

Fig. 1. Localization and function of GFP-tagged *V. vinifera* TIP2;1 in *S. cerevisiae*. Epifluorescence (left panels) and phase contrast (right panels) images of *S. cerevisiae aqy-null* strains YSH1172 transformed with centromeric plasmids harboring (A) functional TIP2;1 (LT21) and (B) non-functional TIP2;1 (LT21n). Cells show aquaporin localization at the yeast plasma membrane. (C) Time course of volume change for *S. cerevisiae* expressing the functional and the non-functional TIP2;1. Cells were equilibrated in osm_{out} 1.4 M and exposed $\Lambda = 1.25$ at 23 °C. (D) Arrhenius plots of water transport.

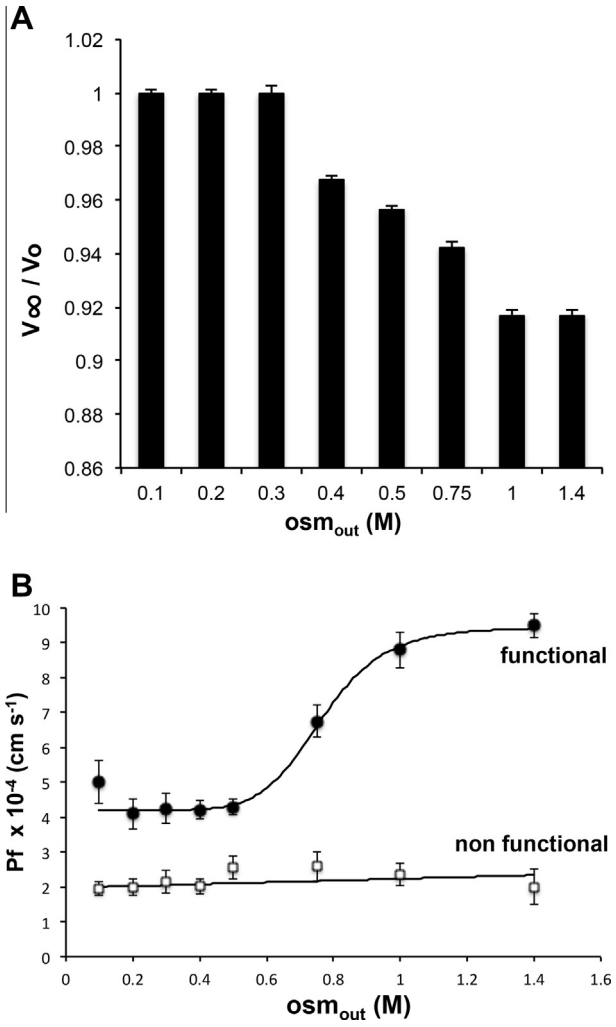

Fig. 2. Cell volumes and permeabilities of *S. cerevisiae* expressing functional and non-functional TIP2;1. (A) Equilibrium cell volumes of *S. cerevisiae* expressing functional TIP2;1 equilibrated in different initial osmolarities (V_o) and exposed to a hypertonic shock of tonicity 1.25 (V_∞). (B) P_f for *S. cerevisiae* expressing functional and non-functional TIP2;1. Cells were equilibrated in osm_{out} from 0.1 to 1.4 M, and subjected to hypertonic shocks. Data shown are mean ± SD of triplicates of three independent experiments.

below 0.5 M, P_f decreased approaching the non-active aquaporin, reducing the difference to 1.5-fold. This pattern reveals a closure of TIP2;1 by increased internal pressure and consequent membrane tension, a process similar to the described for yeast endogenous aquaporin Aqy1 [11].

Table 2
Water permeability (P_f) and activation energy (E_a) for *S. cerevisiae* expressing TIP2;1 under increasing levels of membrane tension.

$(osm_{out})_o$ (M)	Tonicity (Λ)	Functional TIP2;1 (LT21)		Non-functional TIP2;1 (LT21n)	
		P_f[a] (cm s^{-1} × 10^4)	E_a (kcal mol^{-1})	P_f[a] (cm s^{-1} × 10^4)	E_a (kcal mol^{-1})
1.4	1.25	9.50 ± 0.8	4.5 ± 0.5	2.00 ± 0.7	14.6 ± 1.0
0.5	1.25	4.68 ± 0.3	9.7 ± 0.4	2.56 ± 0.5	
	3.50	10.0 ± 0.7	4.1 ± 0.6	2.25 ± 0.6	13.6 ± 0.5
0.3	1.50	5.15 ± 0.6	10.7 ± 0.5	2.15 ± 0.7	
	1.70	7.62 ± 0.4	6.1 ± 0.3	2.05 ± 0.8	
	3.50	9.87 ± 0.9	5.4 ± 0.4	2.21 ± 0.6	13.3 ± 0.9

[a] Values obtained at 23 °C.

Table 2 shows the P_f and E_a for the two strains departing from non-tense (equilibrated in 1.4 M) or pre-swollen (equilibrated in 0.5 and 0.3 M) states. Cells were subjected to hyperosmotic gradients of different magnitudes to discriminate stages of pressure dissipation. As referred above, the functional TIP2;1 pre-equilibrated in 1.4 M showed a much higher P_f and lower E_a characteristic of an active aquaporin, while its non-functional mutant showed lower P_f and higher E_a. In addition, for the functional TIP2;1 strain, as the buffer osmolarity decreased (0.5 M) for the same tonicity shock (Λ = 1.25), the E_a increased in line with the observed decrease in P_f, indicating that the dissipation of the initial pressure was not sufficient. As the hyperosmotic gradient increased (Λ = 3.5), the E_a decreased and P_f increased approaching the values obtained for cells departing from a non-tense state, showing the dissipation of the initial pressure and activation of the channel. Identical behavior was observed for cells incubated in 0.3 M, where tonicity shocks of 1.5 and 1.7 could not dissipate the initial pressure, and only a Λ = 3.5 gave E_a and P_f compatible with active aquaporins. However, the non-functional TIP2;1 showed stable high E_a and low P_f independently of the experimental conditions applied.

4. Discussion

There is increasing evidence that aquaporin activity is regulated by pressure induced membrane tension [9–12]. According to LaPlacés law for a sphere, the tension is directly related to the product of pressure and radius, meaning that for cells of different sizes to reach the same membrane tension, larger cells need much lower levels of pressure (sometimes hardly discernible), making the disclosure of aquaporin modulation by tension difficult to assess. For large plant cells, the heterologous expression of plant aquaporin in yeast might be a good strategy to surpass this difficulty.

Yeast strains incubated in different low osmolarity buffers can develop similar levels of membrane surface tension independently of protein expression (native [11] or heterologous, this study).

In this work, a P_f dependence on initial osm_{out} was found for *V. vinifera* TIP2;1, while no such dependence was detected for the non-functional aquaporin, showing that TIP2;1 becomes inactive when membrane tension is high. Furthermore, temperature dependence studies also revealed that if cells equilibrated in low osm_{out} are subjected to increasing osmotic gradients, the E_a values decrease approaching the values obtained for the non-tense state. These results reinforce the idea that, for cells equilibrated in low osm_{out}, as the tonicity of the osmotic shock increases, the initial tension starts dissipating leading to the opening of the channel.

When departing from a tense state and applying increasing osmotic gradients, similar patterns of P_f as a function of tonicity were found for rabbit AQP1 [10], yeast Aqy1 [11] and *V. vinifera* TIP2,1 (this work). Equivalent results were also obtained for human AQP1 expressed in emptied-out oocytes [12]. Interestingly, the ratios of highest and lowest permeability values (P_{fmax}/P_{fmin}) are very similar and independent of aquaporin isoform or system where they are expressed: this ratio equals 2.25 for rabbit AQP1 in kidney vesicles, for human AQP1 expressed in emptied-out oocytes and for TIP2;1 expressed in yeast, and equals 3.1 for Aqy1 in yeast.

Fig. 3A shows the channel activity ($(P_f - P_{fmin})/P_{fmax}) \times 100$) for Aqy1 (data from [11]) and TIP2;1 (this study) as a function of osm_{out} with 50% channel opening at 0.51 M for Aqy1 and 0.77 M for TIP2;1 respectively. The plot also shows values of the initial hydrostatic pressure differences ΔP_o (N cm^{-2}) for the different osm_{out} estimated from the slope of the experimental curves Λ vs $1/(v_{osm})_\infty$ [11]. Pairing the values for the same osm_{out} of these activity curves with the ΔP_o curve and plotting their respective data points, the sigmoidal curves of Fig. 3B show that 50% inhibition

was achieved for $\Delta P_o = 2.91 \times 10^2$ N cm^{-2} for Aqy1 and 2.41×10^2 N cm^{-2} for TIP2;1.

If the estimated ΔP_o was fully sensed by the yeast plasma membrane, the resulting surface tension could be calculated using LaPlacés law for a sphere, with the correspondent initial radius r_o for each osm_{out}. Thus, 50% aquaporin inhibition was estimated at $\sigma_{0.5} = 2460$ mN m^{-1} for Aqy1 and 1980 mN m^{-1} for TIP2;1. These levels of membrane tension are much higher (≈500 times) than the reported for mammalian aquaporins, being less than 6 mN m^{-1} for rabbit AQP1 in kidney vesicles [9] and for human AQP1 expressed in emptied-out oocytes [12].

Reasoning about the great discrepancies of the $\sigma_{0.5}$ values, we should keep in mind the values for pre-lysis tension σ_{lysis} reported for these systems. The σ_{lysis} was estimated for empty out [12] and whole oocytes [17] as 555 and as 178 mN m^{-1}, respectively, while much lower values were found for rabbit kidney vesicles (18.7 mN m^{-1}) [9], for erythrocytes (10–12 mN m^{-1}) [18] and for liposomes (40 mN m^{-1}) [19]. The $\sigma_{0.5}$ for the mammalian aquaporins expressed in oocytes or in their natural membranes are lower than the measured σ_{lysis} of these membrane systems. For intact yeast, lysis does not occur, but yeast protoplasts were found to lyse when exposed to media osmolarity below 0.8 M sorbitol solution and were only fully stable at 1.2 M solutions [20]. Considering that the estimated ΔP_o at 1.2 M medium osmolarity is 1.40×10^2 N cm^{-2} (Fig. 3A), the corresponding σ_{lysis} for yeast protoplasts would be around 1000 mN m^{-1}. This value is lower than the $\sigma_{0.5}$ for Aqy1 or TIP2;1 calculated above. However, these calculations were made with the assumption that ΔP_o (for each osm_{out}) was acting solely upon the plasma membrane.

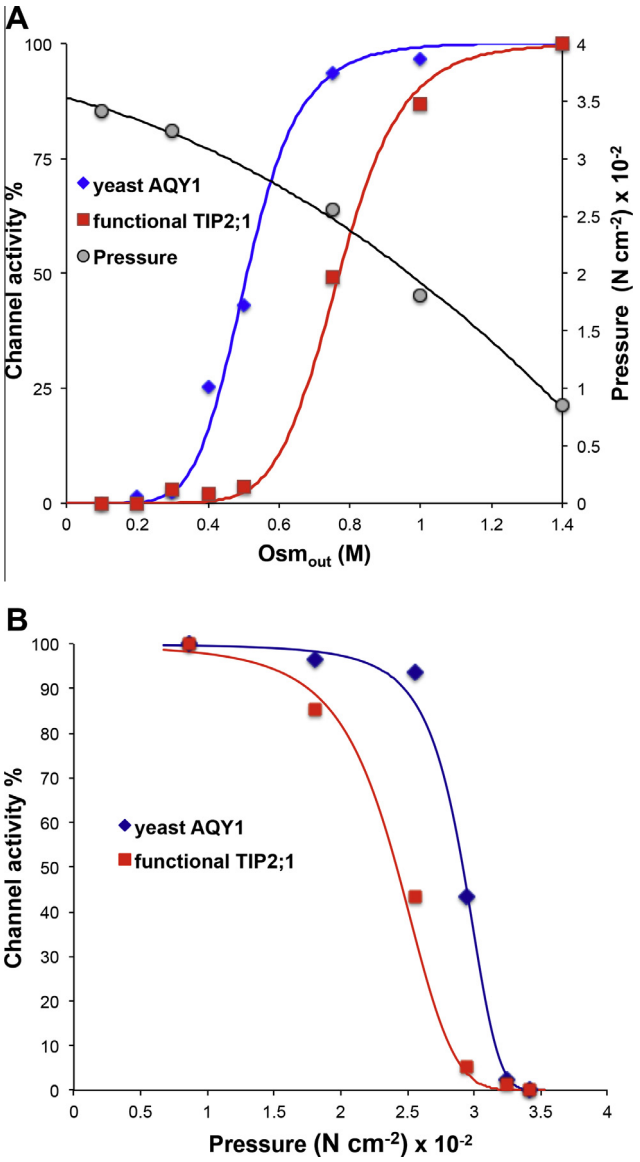

Fig. 3. Channel activity ($(P_f - P_{fmin})/P_{fmax}) \times 100$) for yeast Aqy1 and TIP2;1. (A) Channel activity as function of osm_{out} with 50% channel opening at 0.51 M for Aqy1 and 0.77 M for TIP2;1, respectively. The plot also shows the initial hydrostatic pressure differences ΔP_o (N cm^{-2}) for the different osm_{out} estimated from the slope of the experimental curves Λ vs $1/(v_{osm})_\infty$. (B) Channel activity as function of ΔP_o, obtained by pairing the values for the same osm_{out} of channel activity and ΔP_o. 50% inhibition was obtained for $\Delta P_o = 2.91 \times 10^2$ N cm^{-2} for Aqy1 and 2.41×10^2 N cm^{-2} for TIP2;1.

As discussed by Ozu et al. [12] the maximal tension obtained in emptied-out oocytes is around 5 times larger than the measured for whole oocytes. The authors explained their results based on the work in astrocytes by Spagnoli et al. [21] that suggested that the protein lattice connected to the plasma membrane and the cytoskeleton acting as a cross linking gel in the cytoplasm are factors that, upon cell swelling, buffer the total hydrostatic pressure, thus reducing its effect on the membrane. Furthermore, in yeast cells, besides these components there are also transmembrane protein sensors anchored to the cell wall, which under low osmolarity stress conditions increase in number and in clustering thus contributing to cell wall integrity [22]. This indicates that the cell wall is also an important part of the pressure buffer system. Taking all this into account, it is reasonable to admit that the cytoplasm, the membrane cytoskeleton lattice and the cell wall can greatly reduce the calculated ΔP_o values felt by the membrane and the correspondent membrane tension.

Although the levels for $\sigma_{0.5}$ inhibition for Aqy1 and TIP2;1 expressed in yeast are overestimated, this system of expression is very useful to disclose the regulation of channel activity by tension, in particular in plants where their native system is unsuitable for these studies.

This work deals with *Vv*TnTIP2;1, a water channel located in the tonoplast of *V. vinifera*, a plant usually withstanding hostile drought conditions essential for the quality of berries and wine. One of the main roles of the vacuole is to maintain turgor pressure against the cell wall. In the present study, we disclosed the gating of TIP2;1 by turgor induced membrane tension, changing from an open to a closed state in an internal pressure dependent manner. This turgor dependent gating might be a mechanism to buffer osmotic cytosol fluctuations and regulate vacuolar size and shape in plants withstanding hostile drought conditions such as grapevine. Thus, conditions of maximal turgor and membrane tension may induce water channels closure, preventing fast water loss. The rapid modification of water permeability not dependent on intracellular signaling is crucial for regulating vacuolar/cytosol water contents; as if aquaporins behave as valves favoring water traffic when the vacuole is flaccid. In turgid state, necessary for a best plant performance, the valves remain closed keeping water inside the vacuole.

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