

Review

Membrane transport of hydrogen peroxide

Gerd P. Bienert, Jan K. Schjoerring, Thomas P. Jahn*

Plant and Soil Sciences Laboratory, Department of Agricultural Sciences, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

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Abstract

Hydrogen peroxide (H_2O_2) belongs to the reactive oxygen species (ROS), known as oxidants that can react with various cellular targets thereby causing cell damage or even cell death. On the other hand, recent work has demonstrated that H_2O_2 also functions as a signalling molecule controlling different essential processes in plants and mammals. Because of these opposing functions the cellular level of H_2O_2 is likely to be subjected to tight regulation via processes involved in production, distribution and removal. Substantial progress has been made exploring the formation and scavenging of H_2O_2 , whereas little is known about how this signal molecule is transported from its site of origin to the place of action or detoxification. From work in yeast and bacteria it is clear that the diffusion of H_2O_2 across membranes is limited. We have now obtained direct evidence that selected aquaporin homologues from plants and mammals have the capacity to channel H_2O_2 across membranes. The main focus of this review is (i) to summarize the most recent evidence for a signalling role of H_2O_2 in various pathways in plants and mammals and (ii) to discuss the relevance of specific transport of H_2O_2 .

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Keywords: Aquaporin; Hydrogen Peroxide; Oxidative Stress; Reactive Oxygen Species; Signalling; Transport

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Abbreviations: ABA, Absciscic acid; AQP, Aquaporin; IMM, Inner mitochondrial membrane; MAPK, Mitogen activated protein kinase; NADH/NAD⁺, Nicotinamide adenine dinucleotide (reduced/oxidized); NADPH/NADP⁺, Nicotinamide adenine dinucleotide phosphate (reduced/oxidized); PTP, Protein-tyrosine phosphatase; ROS, Reactive oxygen species; TIP, Tonoplast intrinsic protein

* Corresponding author. Fax: +45 35 28 34 60.

E-mail address: tpj@kvl.dk (T.P. Jahn).

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1. Introduction

Hydrogen peroxide (H_2O_2) is a broad-range chemical catalyst with both reducing and oxidizing properties. H_2O_2 was discovered 1818 by Louis Jacques Thénard [1] and received rapidly increasing attention as an industrial product. Today the world production is over one million tons per year for use in a wide spectrum of applications ranging from cosmetic products to military technology.

In biological systems, H_2O_2 has long been known as a reactive oxygen species (ROS) with the potential to damage proteins, lipids and nucleic acids. Recently, the picture of H_2O_2 as a threat to cellular homeostasis has changed towards seeing H_2O_2 as having an indispensable role in a large variety of pathways. As Ca^{2+} , H_2O_2 appears to be both potentially toxic and a central signalling compound.

Irrespective of the fact that the concentration of a such multifunctional compound needs to be tightly controlled, transport of H_2O_2 is still widely believed to be due to free diffusion through the lipid bilayer of membranes. Various membrane systems, however, have been shown to be less permeable to H_2O_2 , leaving the possibility that diffusion might be regulated by changes in membrane composition. Alternatively, channel proteins may facilitate diffusion of H_2O_2 and the difference in permeability may reflect the relative presence and/or capacity of such channels in various membranes.

2. Structural and physical chemistry of hydrogen peroxide

The multifunctionality of H_2O_2 is due to its chemistry. The oxygens of H_2O_2 have an intermediate oxidation number of “−1”, explaining both reducing and oxidizing properties.

For thermodynamic reasons one would expect hydrogen peroxide to exist primarily in the linear or trans-planar conformation (Fig. 1A, B). In this way, the two hydrogen atoms would stay as far as possible from each other resulting in a non-polar arrangement due to a symmetrical distribution of charge.

The determination of the dipole moment by the Stark effect in a microwave spectrum, however, revealed a permanent dipole moment of 2.26×10^{-18} esu [2] which is even higher than that of water (1.85×10^{-18} esu) suggesting a skewed cis conformation (Fig. 1C) with the hydrogen atoms pointing in the same direction relative to the O–O bond and the lone electron pairs oriented in the opposite direction to the hydrogen atoms. The lone electron pairs do not allow free rotation around the O–O bond, resulting in a permanent dipole. So H_2O_2 indeed represents one of the smallest chiral molecules in nature and occurs as a racemic mixture containing equal amounts of both enantiomers [3]. With its two pK_a values of about 11 and 16 [4] it is uncharged and protonated at physiological pH.

Although chemically H_2O_2 is a ROS it shares surprisingly many physical features with water (Table 1). The permanent dipole is likely to be important for cellular function and transport of H_2O_2 .

3. The cellular chemistry of hydrogen peroxide

H_2O_2 is one of the most abundant ROS in aerobic organisms, including plants, being a constantly produced by-product of aerobic metabolism in mitochondria and of the photosynthesis

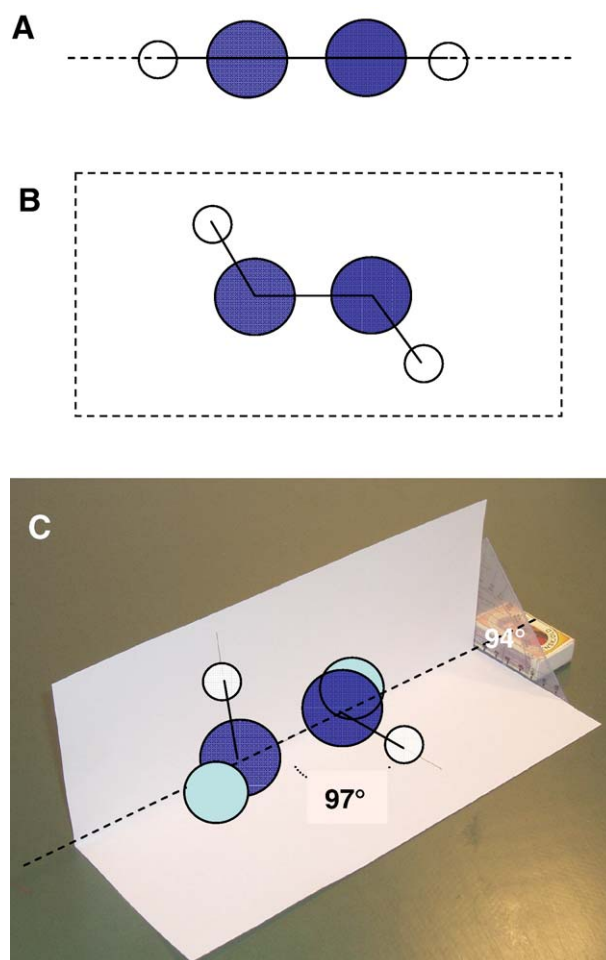


Fig. 1. Three possible conformations of hydrogen peroxide. Oxygen atoms are in blue and hydrogen atoms are in white. (A) Linear conformation of H_2O_2 resulting in a non-polar arrangement. (B) Trans-planar conformation of H_2O_2 resulting in a non-polar arrangement. (C) Skewed cis conformation of H_2O_2 resulting in a polar arrangement with both hydrogen atoms oriented to the same side relative to the oxygen–oxygen bond. Both oxygen–hydrogen bonds are 97° relative to the oxygen–oxygen bond. The two hydrogen atoms are in two different planes oriented to each other in an angle of 94° . The lone electron pairs are indicated as light blue balls.

Table 1
Physical properties of hydrogen peroxide and water (at 25 °C and 1 atm unless indicated otherwise)

Property	H ₂ O ₂	H ₂ O
Density _{liquid} 20°C, g cm ⁻³	1.450	0.998
Melting point, °C	-0.43	0
Boiling point, °C	150.2	100.0
Critical pressure, atm	214	218.2
Critical temperature, °C	457	374.2
Heat capacity _{liquid} , kJ kg ⁻¹ °C ⁻¹	2.625	4.172
Dielectric constant	73.1	80.4
Refractive index	1.4067	1.3325
Dipole moment, esu	2.26 × 10 ⁻¹⁸	1.85 × 10 ⁻¹⁸

Modified from Ardon [2].

in chloroplasts. H₂O₂ has effects on almost all organisms and can influence the life of every single cell. The physiological significance is based on two main features.

3.1. The intracellular redox state

Hydrogen peroxide belongs to the non-radical group of the ROS with an intermediate oxidation number and can be converted into other more reactive ROS by various means including enzymes (Fig. 2). H₂O₂ is relatively stable in vivo compared to other ROS molecules. The half-life of H₂O₂ in lymphocytes is 1 ms while that of superoxide (O₂⁻) is 1 μs [5]. By directly affecting other redox systems like NAD(P)H/NAD(P)⁺, ascorbate/dehydroascorbate, glutathione/oxidized glutathione, thioredoxin/oxidized thioredoxin and protein-SH/protein-SS-R, H₂O₂ is believed to regulate cell development, cell proliferation, cell death and signal transduction. Like the pH and ionic composition of the cytosol, the redox state of a cell influences the physiological environment and consequently the functionality of a wide range of proteins.

3.2. Oxidative modifications of cellular compounds

Under physiological conditions, H₂O₂ is relatively stable and less reactive compared to other ROS species but is able to perform a number of rather specific chemical reactions. A recent mass-spectrometry analysis revealed that protein modifications by ROS are not a random process but appear specific to certain target proteins [6]. Protein modifications in mammalian neuronal cell lines were dependent on the particular ROS used. Treatment with hydrogen peroxide either promoted or inhibited disulfide bonding of select proteins in a concentration-dependent manner. Interestingly, many of these targets are involved in translation and energy production [6]. This clearly demonstrates that oxidation by ROS is not simply destructive but rather a specific modification [7] used for signalling. Glyceraldehyde-3-phosphate dehydrogenase isoenzymes, aconitase, pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, fatty acid synthase, fructose biphosphatase and Cu–Zn superoxide dismutase are only a few examples of very important metabolic enzymes, which are directly or indirectly modified by H₂O₂ [8–10]. H₂O₂ can contribute to the carbonylation of proteins and oxidation of methionine residues or thiol groups of cysteines.

Oxidation of cysteines is the best-studied modification. Oxidation of the sulfhydryl group (–SH) of one cysteine molecule can lead to a sulfenic (–SOH), sulfinic (–SO₂H) or sulfonic (–SO₃H) derivate. This can change the enzyme activity of a protein or the binding capacity of a transcription factor. One investigated example is the regulation of the activity of the protein-tyrosine phosphatase protein family (PTP), which encompasses 103 members in human alone. It has been shown that the conserved catalytic cysteine residues sensitive to oxidation are essential for the catalysis and that the degree of oxidation determines PTP enzyme activity [11].

Protein structure and consequently protein function can be altered by the intra- and intermolecular oxidation of two cysteine residues causing formation of disulfide linkages. While intramolecular disulfides lead to alterations of enzyme activity by conformational changes, intermolecular disulfides cause di- or oligomerization. Such conformational changes can expose or shape the reaction centres of enzymes, shape DNA binding motifs or uncover localization signals like in the case of transcription factors. A well-studied example for a signalling pathway sensitive to oxidative stress is the transcription factor and redox sensor Yap1p in *Saccharomyces cerevisiae*. Yap1p regulates the expression of about 30 genes [12]. The redox state of the cell determines if two cysteines are oxidized to form a disulfide bridge. Upon intramolecular disulfide bonding, a nuclear export signal of Yap1p is masked to escape nuclear export and therefore activates transcription of the target genes.

4. Hydrogen peroxide—a signalling molecule

Cell death caused by oxidative stress has long been interpreted as an unavoidable consequence of damaging reactions evoked by ROS. However, the concentrations of ROS ultimately leading to cell death are often far below the concentration that could be considered dangerous. In contrast, a single ROS activated protein can be sufficient to switch on a

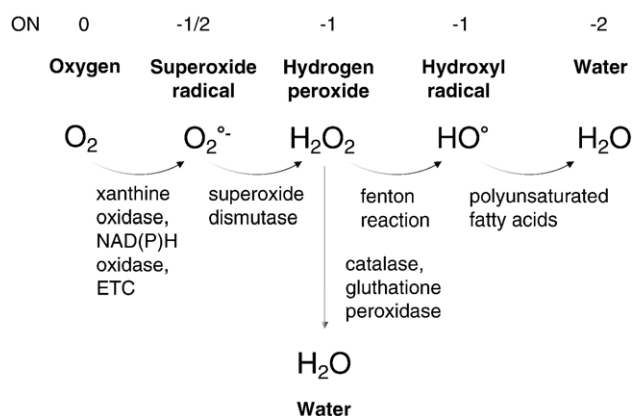


Fig. 2. Consecutive reduction of dioxygen yields reactive oxygen species. Step 1, the conversion of dioxygen to superoxide is endothermic. The following steps are exothermic. Enzymes for the conversion of dioxygen into superoxide radical and further conversion into hydrogen peroxide are known. The reduction via the hydroxyl radical to water occurs non-enzymatically. ON, oxidation number for the oxygen; ETC, electron transport chain.

genetic program resulting in cell death [13]. ROS, including H_2O_2 , are involved in developmental control by triggering apoptosis and cell proliferation [14].

The mechanism of mammalian cell growth involves specific reporters, growth signal transduction compounds such as protein kinases, protein phosphatases and transcription factors. Some of them are activated by oxidative stimuli, and H_2O_2 production has been observed after peptide growth factor addition [15,16] suggesting a signalling role in cell growth and development. Detailed reviews about the role of H_2O_2 in signalling in plants [17,18] and animals [19] have been published. Here we would like to focus on the most recent evidence.

4.1. Recent evidence for hydrogen peroxide signalling in plants

In plants, H_2O_2 mediates gravitropism and blue light-induced photomorphogenesis. In dark-grown wheat coleoptiles, the blue light-induced curvature can be simulated by unilateral exposure to H_2O_2 [20]. Pre-treatment with dithiothreitol (an antioxidant) abolishes the response to blue light. It was concluded that H_2O_2 acts as a translocating second messenger triggering Ca^{2+} fluxes, protein modifications and gene expression. Similar scavenging and unilateral application experiments revealed that H_2O_2 , or at least the generation of ROS, is an essential signal in maize root gravitropism [21]. Thus, H_2O_2 acts as a downstream component in auxin-mediated signal transduction.

In potassium (K^+) deprived *Arabidopsis* roots, the plasma membrane NADPH oxidase was shown to be important in the initiation of a response to K^+ deficiency [22]. A knockout mutant (*rhd2*) lacking NADPH oxidase, was not able to react adequately to K^+ deprivation. Exogenous H_2O_2 application restored the up-regulation of genes induced in wild-type plants under K^+ deficiency. Also nitrogen and phosphorus deficiency resulted in increased H_2O_2 levels. Similar genes were expressed under K^+ and nitrogen deficiency, while phosphorus deficiency induced another set of genes [23]. In sulphur-deprived tobacco plants, several genes related to oxidative stress response genes were isolated with the help of a suppression subtraction library [24].

Intracellular H_2O_2 mediates the salt stress signal transduction pathway by activating the expression of glutathione peroxidases [25], which is also induced by K^+ deprivation [22]. Increased levels of glutathione peroxidases and other antioxidant systems in response to elevated H_2O_2 concentrations could result in cross resistances of several biotic and abiotic stresses.

4.2. Intra- and intercellular signalling

Hydrogen peroxide acts as a signalling molecule in various cellular processes both as an autocrine (includes intracellular) as well as a paracrine (intercellular) signal. This is of particular interest here, since an intracellular signal needs to be kept within a cell, while an intercellular signal

implies that the signal molecule needs to be transported across at least one membrane. In neither case, unlimited diffusion of H_2O_2 across membranes would be compatible with its role as a signal molecule and both mechanisms again imply that there is a need to control transport of H_2O_2 across membranes.

4.2.1. Paracrine signalling

Examples of H_2O_2 -mediated intercellular signalling are rather recent and rare. However, the evidence is striking. H_2O_2 produced by human epidermal keratinocytes regulates melanogenesis of neighbouring melanocytes and contributes to the progression of vitiliginous lesions [26]. In another study, H_2O_2 secretion of myofibroblasts induced cell death in adjacent lung epithelial cells [27]. In both studies, coculture systems were used to demonstrate that H_2O_2 , produced from one cell type, was perceived as a signal in the other cell type, though both cell types were physically separated via a transwell system. Exogenous addition of catalase repressed the signal transfer. These experiments clearly demonstrate the occurrence of H_2O_2 transport across the plasma membrane of both donor and receiver cells and establish experimental model systems for paracrine H_2O_2 signalling.

In plants, H_2O_2 influences xylem differentiation and lignification in several ways. Primary H_2O_2 production maintains a high concentration in lignifying xylem tissues. Subsequently, this H_2O_2 is used for oxidative polymerisation of coumaryl, coniferyl and sinapyl alcohols to lignin, thereby serving a catalytic role. In addition, H_2O_2 also constitutes a signal, which activates transcription of lignin biosynthesis enzymes such as phenylalanine ammonia lyase [28] and peroxidases [29]. In this way, H_2O_2 initiates the secondary cell wall formation and ‘catalyses’ finally the programmed cell death in differentiating xylem cells [30]. Non-lignifying xylem parenchyma cells could be identified as the place of H_2O_2 production, necessary to activate transcription and to polymerise the alcohols in the neighbouring secondary cell walls of dying/dead xylem vessels [31], thus being a paracrine signal pathway.

4.2.2. Autocrine signalling

Stomatal closure, mediated by H_2O_2 in guard cells is an example of autocrine signalling [32,33]. Plants produce abscisic acid (ABA) in response to water deficit in their guard cells preceding stomatal closure. One downstream component of the signalling pathway is an NADPH oxidase. Elevated levels of H_2O_2 trigger further downstream signals finally resulting in stomatal closure. As the latter case elucidates, H_2O_2 can operate both as a direct effector molecule and as a second messenger in signal amplification.

The targets for H_2O_2 signalling in this pathway are still unclear. However, the regulation might take place at multiple levels in the signalling pathway from receptor to nucleus: (i) receptor kinases and phosphatases could be activated by oxidative oligomerization [34]. (ii) Oxidized second messenger like diacylglycerol could be shown to be a more effective signalling compound [35]. (iii) H_2O_2 can initiate changes in intracellular Ca^{2+} concentrations in a number of different cell

types [36], reviewed in [37]. (iv) Mitogen Activated Protein Kinases (MAPK) are serine/threonine kinases, highly conserved among eukaryotes, that transmit extracellular signals into cytoplasmic and nuclear responses. MAPKs seem to act in a variety of signal transductions pathways in response to stimuli such as osmotic stress [38], ozone [39] and plant–pathogen interactions [40]. H_2O_2 is linked to all of these responses via the redox network and it has been reported that MAPKs are activated in response to H_2O_2 [41–43].

5. Transport of hydrogen peroxide

H_2O_2 is a vital cellular component with various tasks in development, metabolism and homeostasis. In biological systems, H_2O_2 must be as old as the occurrence of aerobic metabolism. Since elevated concentrations are toxic to a broad range of macromolecules, scavenging or eliminating H_2O_2 must have developed early in evolution.

The concentration of H_2O_2 in a cell is defined by influx and intracellular formation as well as by scavenging and efflux.

$$[\text{H}_2\text{O}_2] = \text{Influx} + \text{Production} - \text{Efflux} - \text{Scavenging} \quad (1)$$

Eq. (1) Determination of the concentration of $[\text{H}_2\text{O}_2]$ inside a cell.

H_2O_2 has often been believed to freely cross membranes, an adequate explanation to explain various physiological facts. Contrary to this, recent studies [44–47] point out that some membranes are rather poorly permeable to H_2O_2 (Fig. 3A). This implies that transport of H_2O_2 may be regulated and that this regulation constitutes a major factor in the determination of cellular H_2O_2 concentration (Eq. 1). These differences in permeability could either be explained by changes in membrane lipid compositions or by diffusion-facilitating channel proteins or a combination of both.

Membrane composition indeed affects transmembrane H_2O_2 diffusion [47]. Also, mechanical changes imposed by osmotic stretching of lipid bilayers change H_2O_2 diffusion [48]. Consequently, cells are potentially able to control transmembrane H_2O_2 diffusion by changing their osmotic pressure.

5.1. Diffusion of hydrogen peroxide across membranes is limited

One of the first approaches to estimate H_2O_2 gradients across biomembranes was done by Antunes et al. [45]. By measuring enzyme latency, the authors could show that H_2O_2 gradients form when membranes separate the site of H_2O_2 production and H_2O_2 consumption. Enzyme latency is caused by trapping an enzyme in a membrane-surrounded compartment. The membrane constitutes a permeability barrier and the diffusion of the substrate to the enzyme can be measured as the delay of the reaction. In their calculations, Antunes and Cadenas [45] assumed that membranes separated three compartments, the extracellular compartment, the cytosol and the peroxisomes and their scavenging enzymes glutathione peroxidase (cytosol) and catalase (peroxisomes). By using this approach, significant

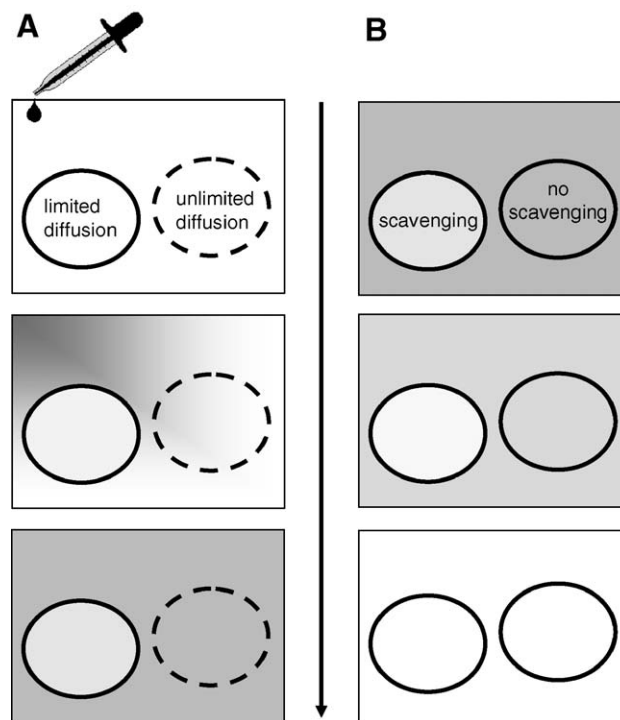


Fig. 3. Diffusion and scavenging together determine the cytoplasmic concentration of hydrogen peroxide applied to the extracellular medium. (A) Limited versus free diffusion of hydrogen peroxide across membranes. In the situation of limited diffusion, a concentration gradient will develop across the membrane. (B) The gradient across the membrane, however, will only be maintained with time when scavenging enzymes keep degrading hydrogen peroxide within the cell. Therefore, limited diffusion and scavenging are important to maintain gradients.

H_2O_2 gradients were measured in mammalian Jurak T-cells. The observation was explained firstly by the different capacities of the compartments to consume H_2O_2 and secondly by differential permeability characteristics of the different membranes. The permeability coefficient of H_2O_2 for the plasma membrane was $2 \times 10^{-4} \text{ cm s}^{-1}$ and the delay to establish a stable gradient was calculated to about 0.9 s. This clearly shows that H_2O_2 permeates rapidly across biomembranes but that the permeation is limited.

Seaver et al. [44] investigated H_2O_2 fluxes and compartmentation in growing *E. coli* cells. According to their results, bacterial membranes exhibit substantial, but limited, permeability to H_2O_2 . In their simple experiment, a catalase-proficient strain was co-cultured with a catalase-deficient strain in an H_2O_2 -containing media. While the catalase-proficient strain grew without delay, growth of the catalase-deficient cells was arrested in early growth. However, after the catalase-proficient strain had detoxified the growth medium, the catalase-deficient strain recovered and reached a similar growth rate as the catalase-proficient strain. If H_2O_2 diffusion had been unlimited, both strains would have had the same H_2O_2 concentration in the cytoplasm as in the medium and both strains would have been arrested in growth, until the catalase-proficient strain had lowered the H_2O_2 concentration below the toxic level. The growth advantage can thus only be explained by a relatively low H_2O_2 concentration in the catalase-proficient strain due to

detoxification and limited H_2O_2 diffusion across the plasma membrane (Fig. 3B).

Some bacteria use this limited membrane permeability for ROS to compete with other pathogens. These bacteria excrete substances which produce ROS, such as H_2O_2 , in the host cells while simultaneously expressing high amounts of scavenging enzymes in their own cytoplasm. This ensures a high oxidizing antibacterial environment inside the target cell. If H_2O_2 could cross membranes freely, the bacteria would poison themselves. Therefore, limited diffusion, as demonstrated in *E. coli* [44] must be important in this strategy.

Yeast (*Saccharomyces cerevisiae*) cells possess a mechanism to adapt to oxidative stress and to survive in media containing otherwise toxic concentrations of H_2O_2 if they are pre-cultured in a non-lethal H_2O_2 -containing medium. Although an up to 3-fold increase of antioxidant enzymes was measured in the adapted cultures, the rate of H_2O_2 disappearance from the growth medium was rather similar before and after conditioning [49]. The authors therefore suggested that one part of the adaptation to H_2O_2 must be a decrease in permeability and uptake at the plasma membrane. Sousa-Lopes et al. [47] could show that the permeability constant for H_2O_2 is 5 times lower in cells grown to stationary phase ($0.017 \pm 0.004 \text{ min}^{-1} \text{ OD}_{600}^{-1}$) than in exponentially growing cells ($0.083 \pm 0.028 \text{ min}^{-1} \text{ OD}_{600}^{-1}$). Conversely, H_2O_2 consumption was higher in exponentially growing cells, even though the amount of scavenging enzymes was lower.

These results have two immediate implications: (i) The scavenging of extracellular H_2O_2 is largely controlled by diffusion (see also Eq. (1)), and (ii) the diffusion coefficient can change in a membrane depending on the developmental stage of the cell.

5.2. The lipid composition influences transmembrane diffusion of hydrogen peroxide

Changing the biophysical membrane properties leads to changes in the permeability of lipophilic compounds. The length of fatty acids, the amount of unsaturated versus saturated fatty acids, the protein composition and the degree of phosphorylation and glycosylation of lipids all influence membrane permeability [50–52]. Water is commonly believed to cross biological membranes but only to limited extent. In artificial membranes, diffusion of water through lipid bilayers was dependent on the lipid composition [53]. In the yeast *Saccharomyces cerevisiae* the ergosterol content of the plasma membrane correlates with the membrane permeability of glycerol [52].

In another study, the permeability constant for H_2O_2 was 5 times higher in exponentially growing yeast cells compared to cells in stationary phase. Mutants in the ergosterol biosynthesis pathway (*erg3Δ*, *erg6Δ*), grown to stationary phase showed decreased plasma membrane permeability to H_2O_2 compared to wildtype while exponentially growing cells showed increased H_2O_2 permeability compared to wild type [47]. Thus, the differences in membrane permeability may be the result of changes in lipid composition during development. An alternative explanation could be differential expression of transport proteins.

The composition of lipid bilayers could be one way to regulate the flux of H_2O_2 across membranes. However, changes in membrane composition as a means to regulate diffusion is a rather limited tool. Firstly, this is a slow process and therefore limited to long-term adaptation as shown during subsequent growth phases in yeast. Secondly, changes in membrane composition is less specific and will affect many other processes including transport and activity of other membrane proteins. In the case of chloroplasts and mitochondria, membrane integrity is essential for their function. The membrane composition will for example directly affect the mobility of ubiquinone (mitochondria) and plastoquinone (chloroplasts) with possible negative effects on electron transfer. This could result in increased oxidative stress.

In conclusion, membrane composition can be useful as a long-term mechanism to set a general threshold permeability coefficient and may be important during development. However, fine adjustment and rapid regulation of diffusion of specific compounds is likely to involve specific channel proteins.

5.3. Transport of hydrogen peroxide by aquaporins

Aquaporins are known as diffusion facilitators for a growing number of non-charged and partially polar solutes such as glycerol, urea, CO_2 , polyols, purines, pyrimidines, NH_3 and trivalent inorganic forms of arsenic and antimony [54–60]. Water, for example, can cross membranes. Nevertheless, water also passes through aquaporins with much higher capacity, a diffusion facilitation that is believed to be physiologically highly relevant. Therefore, aquaporins have recently attracted interest as potential targets in therapies for disorders involving aberrant water movement known from clinical research [61].

5.3.1. Striking physico-chemical similarities between hydrogen peroxide and water

The size and the electro-chemical properties of solutes are the main factors determining the diffusion through aquaporins. Exactly these features qualify H_2O_2 as a possible aquaporin substrate. H_2O_2 possesses almost the same dipole moment, dielectric properties and capacity to form hydrogen bonds as does water (Table 1). The slightly larger dipole moment of H_2O_2 makes simple diffusion through the hydrophobic lipid bilayer even less likely than for water.

The mean diameter of about 0.25–0.28 nm [62] of H_2O_2 compared to the pore size of human AQP1, a typical aquaporin, of 0.30 nm [63] is also compatible with passage through aquaporins. The GlpF from *E. coli* as a typical aquaglyceroporin has an even wider pore with a diameter of 0.40 nm [64]. Taken together, the physico-chemical properties easily explain transport of H_2O_2 by aquaporins.

5.3.2. First experimental evidence

Henzler and Steudle [62] described a mathematical model including parameters for transport and metabolic degradation of H_2O_2 , to explain measurements on internodal cells of the algae *Chara corallina*, a well-studied model system for

plants. The model was used to simulate transport across membranes and to predict transport parameters. Testing the model, *Chara* internodal cells had an H_2O_2 permeability coefficient of $3.6 \times 10^{-6} \text{ m s}^{-1}$, which is very close to that of water ($7.7 \times 10^{-6} \text{ m s}^{-1}$). Mercury abolished H_2O_2 accumulation indicating transport through aquaporins. The reflection coefficient of H_2O_2 was calculated to be 0.33 ± 0.12 . The reflection coefficient (a value between 0 and 1) is a measure of membrane permeability. A value of “1” is equal to impermeable while a value of “0” means that the substance passes the membrane just as easily as water. To explain the relatively high reflection coefficient, the authors [62] suggested that some, but not all, “water channels in *Chara* serve as peroxoporins rather than as aquaporins”. The diffusion of H_2O_2 across erythrocyte membranes was insensitive to organic mercurials [65], suggesting that at least AQP1 should not transport H_2O_2 . H_2O_2 -transporting aquaporins have not been identified on the molecular level.

5.3.3. New molecular evidence

In our laboratory, we have now undertaken an approach to investigate the permeability of various single aquaporins for H_2O_2 (unpublished results). Using various yeast mutants ($\Delta\text{Tsa1,2}$; ΔYap1 ; ΔSkn7) compared to wild type with different sensitivities to exposure to H_2O_2 , we have identified aquaporins that, when expressed in yeast, increase the sensitivity to externally applied H_2O_2 . A screening was performed using a total of 24 aquaporins homologues from plants and mammals. Two aquaporins, hAQP8 and AtTIP1, increased the sensitivity to H_2O_2 by a factor of up to 10-fold suggesting a function in H_2O_2 transport (Fig. 4). The fact that only a limited number of aquaporins increased sensitivity of yeast cells to H_2O_2 is well in accordance with the interpretation that only some aquaporins are specific for H_2O_2 [62].

5.3.4. Physiological evidence from mammals

AQP8, an aquaporin highly permeable to water, is expressed in salivary glands, liver, pancreas, small intestine, colon, testis,

heart, kidney and respiratory tract. AQP8 displays a distinct gene organisation and seems evolutionarily separated from other mammalian AQPs. Besides its localization in the plasma membrane, AQP8 was the first aquaporin found in the inner mitochondrial membrane (IMM) of these various organs [66]. More than 15 different diseases (e.g., cancer, atherosclerosis, diabetes mellitus, Alzheimer’s disease and Parkinson disease) are associated with oxidative stress and mitochondrial dysfunction. It is known that mitochondria are very plastic and that their volume is dependent on respiratory activity and apoptosis. The existence of AQP8 in the IMM could help to understand the so far unexplained molecular pathway of water into mitochondria [66].

AQP8 in the IMM could also have a role in transporting H_2O_2 . In animal cells, mitochondria are the main source for H_2O_2 , regulating respiratory activity and apoptosis [67]. Scavenging H_2O_2 by antioxidant peroxidase systems in the matrix would consume electron equivalents in form of NAD(P)H [68], and thereby reduce respiration efficiency. One function of AQP8 may be to release H_2O_2 for detoxification elsewhere in the cell without consuming mitochondrial reduction equivalents. Such a reaction can only be performed by catalases, which are present in peroxisomes while there is only biochemical indication for catalase activity in mitochondria from rat heart and maize (reviewed in [68]). Release of H_2O_2 from mitochondria via AQP8 could be important under re-oxygenation after hypoxia, when oxygen supply leads to severe generation of H_2O_2 in the reduced environment (e.g., in heart and muscle).

5.3.5. Physiological evidence from plants

AtTIP1;1 was the first plant aquaporin described [69]. Subcellular location of TIP1 was detected in the vacuolar membrane in various plants. Based on expression profiles, it is one of the most highly expressed aquaporins in *Arabidopsis* [70,71].

While other aquaporin homologues are more variable in sequence, TIP1 homologues are highly conserved across species, even between monocotyledons and dicotyledons. This sequence similarity may suggest an important and unique physiological function. Interestingly, approaches to down-regulate TIP1;1 in *Arabidopsis* resulted in a dramatic and physiologically complex phenotype [72]. This severe effect through down-regulation of a single aquaporin homologue in *Arabidopsis* is surprising in view of the mild phenotypes found in other plant aquaporin knockout or antisense studies [70,73–75]. This suggests that the severe phenotype is the result of another and rather unique function of TIP1;1.

TIP1 could have a role in the scavenging of H_2O_2 in the vacuole. It has been known for a long time that flavonoids (anthocyanins, flavones and flavonols) in the human diet act as antioxidants. In vitro, flavonoids can directly scavenge ROS, like H_2O_2 or hydroxyl radicals [76]. Flavonoids are the most common secondary metabolites in plants and are primarily localized in vacuoles. The in vivo scavenging capacity has been questioned because the largest part of ROS is not formed in the vacuole but in the chloroplast, mitochondria, cytosol, peroxisomes and the cell wall, where it would be scavenged in situ

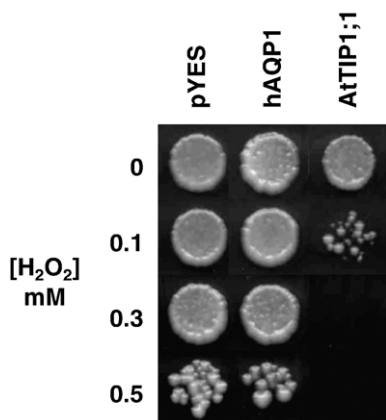


Fig. 4. Specific aquaporins increase the sensitivity of yeast towards externally applied hydrogen peroxide in a growth assay. AtTIP1;1 and hAQP1 were expressed in the *Saccharomyces cerevisiae* mutant Δyap1 . The empty vector pYES was used as a control. The different transformants were exposed to increasing concentrations of hydrogen peroxide in the media.

immediately. All these subcellular compartments already possess effective scavenging mechanisms. Therefore, the scavenging system in the vacuole was not believed to be important for primary detoxification.

A vacuolar scavenging system could, however, be important under conditions of elevated stress to increase detoxification capacity. Takahama and Egashira [77] could demonstrate that the vacuoles of mesophyll cells of *Vicia faba* have the capacity to detoxify H_2O_2 . Inhibitor studies indicated that peroxidases are involved in the scavenging. Several peroxidases are localized in vacuoles of different plants [78,79] and might be localized at the inner surface of the tonoplast [80]. Thus, the permeating H_2O_2 entering the vacuoles could be converted by peroxidases using flavonoids as electron donor. The concentration of the flavonoids in vacuoles is up to 100 times higher than the K_m values for vacuolar peroxidases [81]. Interestingly, flavonoid synthesis is additionally induced in a wide range of stresses (UV-light, wounding, pathogen infection, chilling, ozone, nutrient deprivation etc.) where also H_2O_2 levels increase significantly (reviewed in [82]). Red anthocyanin-rich parts of *Pseudowintera colorata* leaves have a high H_2O_2 scavenging capacity after mechanical injury. In contrast, green leaves only slowly decrease H_2O_2 levels [83]. And finally, Cd^{2+} -stress-induced H_2O_2 was localized to vacuolar membranes in pea leaves [84]. Thus, it is an attractive hypothesis that TIP1 may facilitate H_2O_2 diffusion and that a membrane-localized peroxidase in concert with TIP1 represents a highly sophisticated, high-capacity system to control H_2O_2 detoxification in plants.

6. Perspectives

Various new substrates have recently been identified for selected members of the aquaporin superfamily in plants and mammals. While water seems to be a general substrate to many or even all members, other substrates, such as urea, CO_2 , NH_3 , and now H_2O_2 , are restricted to a rather limited number of isoforms. Strikingly, the concentrations of these substrates in biological systems are usually at least 6 orders of magnitude below that of water. From this perspective, it is tempting to speculate that the diversity of aquaporins has developed to transport other substrates than water and that water transport is needed to balance osmotic gradients that naturally develop in the course of solute transport. In any case, the increasing number of substrates clearly shows that there is more than water to the large family of aquaporins.

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