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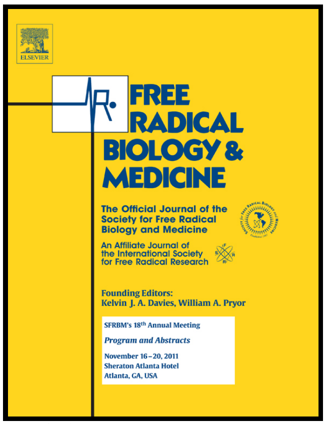

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Transit of H_2O_2 across the endoplasmic reticulum membrane is not sluggish

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Abstract

Cellular metabolism provides various sources of hydrogen peroxide (H_2O_2) in different organelles and compartments. The suitability of H_2O_2 as an intracellular signaling molecule therefore also depends on its ability to pass cellular membranes. The propensity of the membranous boundary of the endoplasmic reticulum (ER) to let pass H_2O_2 has been discussed controversially. In this essay, we challenge the recent proposal that the ER membrane constitutes a simple barrier for H_2O_2 diffusion and support earlier data showing that (i) ample H_2O_2 permeability of the ER membrane is a prerequisite for signal transduction, (ii) aquaporin channels are crucially involved in the facilitation of H_2O_2 permeation, and (iii) a proper experimental framework not prone to artifacts is necessary to further unravel the role of H_2O_2 permeation in signal transduction and organelle biology.

Principles of reduction-oxidation signaling

The life of a multicellular organism is organized in a complex network of intercellular communication. In this vein, individual cells react to external cues such as hormones or other receptor-based agonists by the activation of signal transduction cascades, which faithfully transfer the extracellular signals to the intracellular addressees. Similar processes are activated also in single cell organisms in response to pheromones or nutrient signals. The single steps of these signaling cascades are designed to proceed by optimized spatial and temporal dynamics [1].

An important element in intracellular signal transduction is the transient formation of diffusible second messengers, which allow amplification of the signal due to their multiple places of action. Amongst many other second messengers, hydrogen peroxide (H_2O_2) is now widely being recognized to serve as such a mobile signaling molecule [2, 3]. H_2O_2 is one of the reactive oxygen species that are produced upon reduction of molecular oxygen and is itself an oxidant. It primarily acts by specifically oxidizing target proteins on specialized, sensitive cysteine residues to modulate their function [4]. Therefore, H_2O_2-mediated signaling is referred to as reduction-oxidation (redox) signaling. Of note, H_2O_2 is a relatively poorly reactive oxidant, which allows it to travel further from its site of generation than can superoxide ($O_2^{\cdot -}$) or hydroxyl radical, before it encounters a peroxidase, catalase or signaling target [4, 5].

A prime example of redox signaling is the role of H_2O_2 during growth factor-stimulated signal transduction [6, 7]. Here, the binding of extracellular growth factor ligands to receptor tyrosine kinases (RTKs) on the cell surface frequently co-activates members of the NADPH oxidase (Nox) family [8-10]. Nox family members locally produce $O_2^{\cdot -}$, which rapidly dismutates to H_2O_2. This increase in $O_2^{\cdot -}$ and H_2O_2 generation is required for sustained receptor tyrosine phosphorylation and downstream signaling events, because H_2O_2 inactivates protein tyrosine phosphatases (PTPs) on a reactive cysteine in their active site [11, 12]. Membrane topology is a critical aspect in this process. The $O_2^{\cdot -}$-producing active sites of Nox complexes are located on the exoplasmic side of the membrane, whereas PTPs localize to the cytosol. This topological problem is solved by membrane-embedded aquaporin channels (AQPs). By serving as H_2O_2 pores they facilitate the formation of local areas with an elevated H_2O_2 concentration on both sides of the plasma membrane [13-16].

H_2O_2 can readily permeate through the endoplasmic reticulum membrane

RTK signaling is not restricted to the plasma membrane. For instance, epidermal growth factor (EGF) receptor can be internalized upon stimulation by endocytosis and brought into proximity with the endoplasmic reticulum (ER) membrane [17]. As a notable consequence, ER-associated proteins such as Nox4 [18, 19] and the phosphatase PTP1b [20-22] play important roles during EGF receptor signaling by acting in analogy to their cognate signaling components at the plasma membrane [17]. Nox4, which can directly, i.e. irrespective of a dismutase, generate H_2O_2 in the ER lumen [23-27], is coupled to the transient oxidative inactivation of PTP1b on the cytosolic side of the ER membrane [17]. This sequence of events premises that H_2O_2 must be able to pass the ER membrane at a time scale that copes with EGF receptor signaling.

While observations of redox signaling at the ER are relatively scarce at this stage, it is clear that H_2O_2 is widely utilized as a signaling molecule *in vivo* [28] and it is quite predictable that further mechanisms specific to the ER will be uncovered in the future [18]. Other examples, which are connected to H_2O_2 transit across the ER membrane, are granulocyte colony-stimulating factor receptor signaling [29], oxidative DNA damage in response to cellular stresses [30-32], activation of survival pathways upon H_2O_2 generation in the ER [33, 34], and the regulatory roles of ER-luminal peroxidases in various settings of cytosolic signal transduction [29, 35-38]. These findings clearly indicate the permeability of the ER membrane for H_2O_2.

Ample H_2O_2 permeability at the ER membrane has additionally been demonstrated by studying over-expressed ER oxidoreductin 1α (Ero1α). This ER-luminal oxidase produces H_2O_2, which is immediately detoxified by the Ero1α-associated peroxidase GPx8 [18]. Depletion of GPx8, however, leads to the overflow of H_2O_2 to the cytosol [39]. By contrast, depletion of the ER-luminal high-abundance-high-affinity-high-turnover-peroxidase peroxiredoxin 4 [40, 41] does not cause similar leakage of Ero1α-derived H_2O_2 into the cytosol [39]. Thus, the shielding of the cytosol against Ero1α-derived H_2O_2 takes place at the Ero1α-GPx8 interface through catalytic elimination [42]. If hindered diffusion of H_2O_2 at the ER membrane was to provide an additional shielding mechanism, Ero1α-derived H_2O_2 would certainly be eliminated by peroxiredoxin 4 already within the ER and not found in the cytosol upon depletion of GPx8.

Aquaporins regulate the permeability of the ER membrane to H_2O_2

Is the transport of H_2O_2 at the ER facilitated by AQPs in analogy to the situation at the plasma membrane? AQP8 fulfills a major function in the transport of H_2O_2 at the plasma membrane [13]. In addition, knockdown of AQP8 strongly diminishes the entry of exogenous H_2O_2 into the ER of plasma membrane-permeabilized cells [13]. This indicates that AQP8 can accelerate the transit of H_2O_2 also across the ER membrane when expressed at physiological levels. Since cell surface AQP8 is synthesized at the ER before trafficking to the plasma membrane, a physiological function in the ER is conceivable. This is also supported by its steady-state localization both at the plasma membrane and in “intracellular vesicles” [43]. In addition, AQP8 appears to be involved in the transit of H_2O_2 from mitochondria in certain cell types [44].

AQP8 and other AQPs show specific tissue distributions. The rich collection of human AQPs enables a versatile regulation of transmembrane permeation of water throughout the body by harboring specific differences in transcriptional regulation, post-translational modification, protein stability, water permeability, and subcellular distribution [43]. Accordingly, it is likely that AQPs other than AQP8 play complementary, tissue- and context-specific roles with regard to H_2O_2 transport at the ER. One obvious candidate is AQP11, the subcellular localization of which is strongly shifted to the ER [45, 46]. AQP11 loss-of-function causes destructive symptoms of ER stress, which mainly manifest in the proximal tubular epithelial cells of the kidney [45-47] but also in other organs such as the liver [48]. The failure of AQP11-deficient cells is accompanied by elevated levels of intracellular H_2O_2 [45]. Whether or not ER stress and H_2O_2 dysregulation are linked to a change in H_2O_2 permeability of the ER membrane remains to be shown.

In addition to the tissue-specific expression level of ER AQPs, the H_2O_2 permeability of the ER membrane is likely regulated by post-translational modifications. For instance, the permeability of AQP8 is reversibly inhibited in response to diverse stress conditions through the targeting of cysteine 53 (Iria Medraño-Fernandez, Stefano Bestetti, and R.S.; unpublished observations) and the overproduction of ER-luminal H_2O_2 appears to stimulate its own passage through the ER membrane in liver cells of living mice [49].

Based on biophysical and structural data, it has been deduced that all AQPs that are able to transport water can also transport H_2O_2 [50]. Thus, not only the highly conducting aquaammoniaporin AQP8 but also the water-permeable AQP11 is predicted to serve as a *bona fide* H_2O_2 channel.

The ER membrane is not refractory to rapid H_2O_2 diffusion

In a recent publication, the ER membrane was postulated to comprise a significant barrier to H_2O_2 diffusion [51]. This postulate was based on an experiment, in which oxidation of intracellular H_2O_2 probes in response to increasing concentrations of extracellular H_2O_2 were recorded. As already worked out elsewhere [39], the H_2O_2-dependent oxidation of the genetically encoded probe HyPer [52] was recorded upon concomitant addition of the disulfide reductant dithiothreitol (DTT). In this setup, ER-targeted HyPer was less readily oxidized than cytosolic HyPer [51]. This appears to be a trivial observation though, as exogenous H_2O_2 on its way to the ER must cross the cytosol, which is equipped with a plethora of powerful peroxidases. In a comparable experimental setup, most H_2O_2 was consumed before it could reach the depth of the cell [53]. Konno et al. addressed this issue by using a cell line that expresses relatively low levels of some cellular antioxidant enzymes [51], a measure that can modulate but not eliminate the problem of cytosolic dissipation of H_2O_2. The less efficient oxidation of ER-targeted HyPer compared to cytosolic HyPer therefore cannot only be interpreted to reflect hampered permeability of the ER membrane to H_2O_2.

In addition to cytosolic and ER-targeted HyPer, Konno et al. used mitochondrial HyPer, which showed similar H_2O_2-induced fluorescence changes as cytosolic HyPer [51]. This is surprising, because, as for the ER, mitochondria can only be reached *via* the cytosol, which would be expected to decrease the H_2O_2-sensitivity of mitochondrial HyPer below the sensitivity of cytosolic HyPer (see above). How can this be explained? HyPer is not only sensitive to oxidation but also to alkalinisation [52], which is typically controlled for by also analyzing the response of cysteine-mutant HyPer [54]. Of potential relevance, treatment of cells with H_2O_2 induces the transient alkalinisation of the mitochondrial matrix [55]. Furthermore, we note that the responses to extracellular H_2O_2 of chemical, pH-independent H_2O_2 sensors are similarly slow in mitochondria and ER and slightly faster in cytosol and nucleus [56]. Apart from pH, other organelle-specific differences in the handling of HyPer could also be relevant. It is possible, for example, that the rich collection of thiol-disulfide isomerases in the ER (for review see [23, 57]) catalyzes the reduction of ER-targeted HyPer by DTT particularly well. This in turn would decrease the net steady-state oxidation of ER-targeted HyPer as compared to mitochondrial HyPer at the lower doses of H_2O_2, as has been observed [51]. Although these explanations are yet hypothetical, we suggest that some mitochondrion-specific feature rather than the relative impermeability of the ER membrane causes the more pronounced response to H_2O_2 of mitochondrial HyPer compared to ER-targeted HyPer.

In summary, all published data strongly support the notion that facilitated permeability to H_2O_2 is a designated and likely regulated feature of the ER membrane, which is in line with the central signaling role of this fascinating organelle.

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Highlights

- Ample H_2O_2 permeability of the ER membrane is critical for signal transduction
- Aquaporins facilitate the transmembrane permeation of H_2O_2
- The ER H_2O_2 pool appears not to be isolated from other cell compartments