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# Hydrogen peroxide in the ER: A tale of triage

Nabil A. Rashdan, Christopher B. Pattillo*

*Molecular and Cellular Physiology, LSU Health Science Center, 1501 Kings Highway, Shreveport, 71130, LA, USA*

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ABSTRACT

Oxidative protein folding in the endoplasmic reticulum (ER) is a significant source of hydrogen peroxide ($H_2O_2$). For correct protein folding the redox state of the ER must be efficiently regulated. As such, several mechanisms with varying degrees of overlap manage the redox state of the ER. $H_2O_2$ also functions as a second messenger playing a role in most aspects of cellular physiology and pathology, requiring tight control of the concentration and flux of $H_2O_2$. Bestetti et al. have demonstrated a role for Aquaporin 11 in transport of $H_2O_2$ out of the ER.

The role of Hydrogen peroxide ($H_2O_2$) as a messenger molecule has progressively become more studied over the past two decades. The ability of $H_2O_2$ to regulate the spatial and temporal organization of cellular events, such as differentiation and development, as well as responses to environmental stimuli, necessitates a detailed understanding of its regulation and trafficking. The various roles of $H_2O_2$ are possible through tight spatial and temporal regulation of its subcellular concentration. One well studied mechanism by which cells are able to create localized high concentrations of $H_2O_2$ is through Nadph oxidase isoforms that have been shown to colocalize to specific cellular machinery [1,2]. Another important source of $H_2O_2$ occurs during the folding of proteins in the endoplasmic reticulum (ER). This process involves protein disulfide isomerase (PDI) working in concert with ER oxidoreductin 1 (Ero 1) to catalyze the formation of disulfide bonds. Molecular oxygen is the ultimate electron acceptor in this process and yields one $H_2O_2$ molecule for every disulfide bond formed [3]. In highly active cells there are rapid increases in $H_2O_2$ within the ER; if uncontrolled this increased ROS load would lead to ER stress activating the unfolded protein response (UPR), leading to apoptosis. To prevent ROS overload the ER needs a robust antioxidant mechanism. The most abundant reducing agent in the cell is reduced glutathione (GSH), however, while the ratio of reduced to oxidized (GSSG) glutathione is in excess of 100:1 in the cytosol [4], such a highly reducing environment would be unfavorable for disulfide bond formation. Indeed, the ER maintains a GSH:GSSG ratio closer to 3:1 [5].

Bestetti et al.. in this current issue of Redox Biology demonstrate that aquaporins play a major role in transporting $H_2O_2$ out of the ER [6]. Aquaporins (AQP) are transmembrane channels that were originally described as being either predominantly water permeable or glycerol and water permeable [7]. Since those studies several small uncharged solutes have been described as substrates for AQPs including nitric oxide [8], carbon dioxide [9], ammonia [10,11], and $H_2O_2$. In mammals AQP1 [12], AQP3 [13,14], AQP8 [15,16], and AQP9 [17] have been demonstrated to facilitate $H_2O_2$ transport across membranes. These aquaporins modulate cell signaling cascades by creating localized increases in $H_2O_2$. $H_2O_2$ in turn inhibits phosphatases thereby amplifying kinase mediated signaling [15]. Indeed, silencing of AQP8 blunts the epidermal growth factor induced influx of $H_2O_2$ as well as the accumulation of tyrosine-phosphorylated proteins. AQP8 silencing decreases $H_2O_2$ transit into the ER in digitonin permeabilized cells [15]. This transit was measured using the florescent $H_2O_2$ sensor HyPer with an ER targeting domain; however, the localization of AQP8 was not interrogated in this system. AQP8 null mice develop normally and are phenotypically normal except for enlarged testicles [18]. In contrast to this, AQP11 mice die before weaning due to advance polycystic kidney disease, characterized by increased ER stress inducing apoptosis and vacuolization [19–21]. Bestetti et al. interrogate the role of AQP11 in ER $H_2O_2$ transit [6]. They demonstrate that AQP11 colocalizes to the ER, interestingly, they also report some degree of colocalization to the mitochondrial-associated membrane (MAM) which are signaling hubs with essential functions in regulating signal transduction. By adding tags on either terminus of recombinant AQP11 they were able to elegantly demonstrate the conformation of APQ11 within the ER membrane; by utilizing HyPer localized to cytosol, mitochondria, or the ER (graphical abstract) they demonstrate the specificity of AQP11 to ER $H_2O_2$ transport. They further demonstrate AQP8 primarily localizes to the plasma membrane suggesting that although it may contribute to ER $H_2O_2$ transport it is likely this effect occurs while in transit to the plasma membrane.

Unfortunately, redox sensitive dyes are plagued with various caveats and pH sensitivity is the one caveat to consider when using HyPer. Weller et al., in 2014 went so far as to suggest that any use of HyPer

* Corresponding author.
*E-mail address:* cpatt7@lsuhsc.edu (C.B. Pattillo).

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should be performed in the presence of a pH indicator such as SypHer [22]. However, Bestetti et al. have performed extra experiments that suggest the movement of $H_2O_2$ through AQP11 is a real observation. The ER is a pH neutral location, although accumulation of $H_2O_2$ could decrease the pH. In this manuscript, however, exogenous $H_2O_2$ was shown to increase HyPer signal, DTT (reducing conditions) influenced HyPer signal, and cells with low basal HyPer signal were used, allowing a more robust ability to measure smaller swings in $H_2O_2$ accumulation [6].

Taken together these data demonstrate a central role of AQP11 mediated $H_2O_2$ transit into and out of the ER and suggest a mechanism to prevent $H_2O_2$ toxicity during protein folding. The potential that AQP11 associates with the MAM and can allow movement of $H_2O_2$ is another exciting aspect of this manuscript suggesting a role for this channel in several pathologies. Dysfunctional MAMs have been implicated in diseases ranging from neurodegenerative diseases [23], aging [24], neoplasia [25], to heart failure [26].

## Declaration of competing interest

The authors have no conflicts of interest to declare.

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