LETTER TO THE EDITOR

# Mitochondrial Aquaporin-8: a Functional Peroxiporin?

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We have read with great interest the News & Views article: "Tyrosine kinase signal modulation: a matter of $H_2O_2$ membrane permeability?" by Bertolotti and colleagues (1).

We would like to raise some concerns regarding the interpretation of data in mitochondria studies. The authors conducted aquaporin-8 (AQP8) silencing experiments in HeLa cells without assessing AQP8 protein expression and mitochondrial localization in these cells. One of the authors' conclusions is that AQP8 is dispensable for the mitochondrial import of $H_2O_2$. Although it may be true, our concern is that they may have mistakenly assumed mitochondrial AQP8 expression in HeLa cells, since these cells do not endogenously express significant levels of AQP8 (2). What is more, in a recent study (3), we actually demonstrated that AQP8 present in inner mitochondrial membranes of human hepatic HepG2 cells facilitates the release of $H_2O_2$ from mitochondria.

The authors state that the lack of an AQP8-dependent $H_2O_2$ mitochondrial entry reinforces the view that no functional AQP8 resides in mitochondria, citing Yang et al (7). AQP8 has been demonstrated to work as a multifunctional channel that conducts either $H_2O_2$ or ammonia in addition to water. Although the water channel activity of mitochondrial AQP8 seems not to be relevant (5, 7), there is convincing evidence that mitochondrial AQP8 is involved in ammonia uptake and ureagenesis in hepatocytes (5, 6) and ammonia exit and response to acidosis in renal proximal tubule cells (4). Thus, at least in hepatic and kidney cells, mitochondrial AQP8 seems to play important functional roles as an ammoniaporin.

As shown in Bertolotti et al. (1), plasma membrane AQP8 mediates $H_2O_2$ uptake to modulate signaling pathways. Although mitochondrial AQP8 is also able to function as a peroxiporin (3), its significance in cellular redox signaling pathways is a matter of further studies.

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**Response to the Letter:**

We are glad that our colleagues Marinelli and Marchissio found our recent paper "Tyrosine kinase signal modulation: a matter of H2O2 membrane permeability?" (1) of interest.

Owing to the strict space limitations of News and Views articles, we could not present RT-PCR data showing the presence of AQP8 in our HeLa cells and its strong reduction upon silencing. In our model, entry of H2O2 into mitochondria was efficient also in cells in which AQP8 was knocked down and –as a consequence- transport across the plasma membrane was severely impaired (1). Thus, the conclusion that AQP8 is dispensable for mitochondrial H2O2 import remains valid, even though this aquaporin may facilitate the release of H2O2 from these organelles, when present (3).

Clearly, more has to be learned on the role of intracellular aquaporins in transporting substances other than water. In our opinion, a matter of particular interest is how AQP8 can be targeted to the exocytic pathway and/or to mitochondria in cells in which a mitochondrial function has been described. As an example of alternative intracellular routing, the cotranslational translocation of PrP and other proteins normally destined to the secretory pathway is inhibited in cells undergoing ER stress (2,4).

Finally, we entirely agree that the mechanisms controlling H2O2 transport across membranes deserve further studies, in view of their paramount pathophysiological relevance.

*Milena Bertolotti, Stefano Bestetti, Iria Medrano Fernandez and Roberto Sitia*

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