Biochemical Journal (2021) **478** 1511–1513
https://doi.org/10.1042/BCJ20210109

PORTLAND PRESS

Commentary

Protecting P-type plasma membrane H^+-ATPases from ROS

Sarah C. Stainbrook and Joseph M. Jez

Department of Biology, Washington University in St. Louis, St. Louis, MO 63130, U.S.A.

Correspondence: Joseph M. Jez (jjez@wustl.edu)

P-type ATPase are ubiquitous transport proteins across all kingdoms of life. These proteins share a common mechanism involving phosphorylation of an invariant aspartate to facilitate movement of substrates from protons to phospholipids across cellular membranes. In this issue of the *Biochemical Journal*, Welle et al. identify a conserved cysteine near the functionally critical aspartate of P-type plasma membrane H+-ATPases that protects the protein from reactive oxygen species.

Movement of molecules across biological membranes is a common feature in metabolism and physiology and a shared challenge for organisms across all the kingdoms of life. The structural organization of proteins for the myriad transport processes are incredibly diverse, as are their biochemical/biophysical mechanisms and regulatory controls [1,2]. P-type ATPases are ubiquitous in nature and move ions across membranes for a wide range of biological processes including generation of membrane potential, muscle contraction, removal of toxic ions from cells, and regulation of cytosolic pH. Their operation and regulation have been extensively studied at the molecular and cellular levels [3–5]. Recent work by Welle et al. [6] suggests a new mechanism for protecting P-type plasma membrane H^+-ATPases from reactive oxygen species and heavy metals, which would enable these critical proteins to function under stress conditions.

P-type ATPases use ATP to drive the transport of molecules ranging from protons to phospholipids across cellular membranes. Their name, i.e. 'P-type', derives from the use of a phosphorylated protein intermediate to move substrates across the membrane [7,8]. A substrate molecule binds to the ATPase triggering phosphorylation of the transporter to mediate the conformational changes that cycle the enzyme between two states with different affinities for the substrate and nucleotide. Thus, P-type ATPases use ATP to maintain a substrate gradient across the membrane. The structural features of the P-type ATPases, which include three cytosolic domains (A, actuator; P, phosphorylation; and N, nucleotide binding) and core transmembrane transport/ion binding domain, are conserved across the five subfamilies of these proteins with members of each subfamily sharing substrate preference [3,4,9]. Biochemical studies have intensively examined the P2 and P3 ATPases from animals and plants, as these proteins generate and maintain membrane potential for central metabolism [4–6]. The P2 subfamily includes the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and Na^+/K^+-ATPase and the P3 subfamily includes the plasma membrane H^+-ATPases [9]. Within the P-domain of the P2 and P3 ATPases, an invariant aspartate serves as the phosphorylation site that drives the translocation cycle; however, two residues upstream in the protein sequence is another invariant residue — a cysteine whose function was previously unknown.

The versatility of cysteine's nucleophilic thiol is a mainstay of biological chemistry from enzyme reaction mechanisms to scavenging of reactive oxygen species (ROS) to redox-linked signaling [10–12]. Depending on cellular environment, cysteine modification can alter protein activity for redox-dependent regulation or provide protection against oxidative damage to ensure protein function and/or stability [13].

Received: 9 March 2021
Revised: 29 March 2021
Accepted: 30 March 2021

Version of Record published:
21 April 2021

PORTLAND PRESS

Biochemical Journal (2021) **478** 1511–1513
https://doi.org/10.1042/BCJ20210109

As noted by Welle et al. [6], oxidative stresses, including heavy metals, can alter the activity of P2 and P3 ATPases. Previously, the residue in SERCA which corresponds to the conserved cysteine was identified as sensitive to nitrosylation and highly reactive to peroxides, but the role of these modifications was unclear as protein activity was largely unaffected. Likewise, mutations of the conserved cysteine in other P-type ATPases yielded only minor changes in nucleotide hydrolysis, despite its evolutionary conservation and high reactivity. Using the plasma membrane H^+-ATPase (AHA2) from *Arabidopsis thaliana* (thale cress), Welle and co-workers examined the possible role of the invariant cysteine (Cys327) near the phosphorylation site [6]. The authors expressed the Arabidopsis protein recombinantly in yeast. Because the yeast paralog is essential for growth, they used a clever system in which the native protein is only expressed in the presence of galactose — without induction, only the Arabidopsis protein is detectable. Using cysteine-labeling agents, initial experiments identified tryptic-digest peptide fragments of AHA2 which contained modified Cys327, reaffirming that the thiol group was highly reactive and was accessible to external compounds even though the residue is not located on the protein surface. Next, a series of point mutations were introduced into AHA2 and examined for ATP hydrolytic activity and proton pump activity, which were generally unaffected by mutations which did not disrupt the protein's fold. As in other P2 and P3 ATPases, the invariant cysteine of the plant plasma membrane H^+-ATPase is not essential for protein function.

Given the proximity of Cys327 in AHA2 to the active site, the authors speculated that residue may interact with heavy metals, like Cu^{2+}, that promote ROS formation. Interestingly, mutations of the invariant cysteine led to increased sensitivity of the plasma membrane H^+-ATPase to Cu^{2+}, but not to Zn^{2+} or Cd^{2+}, compared with wild-type protein. Similarly, mutant proteins treated with hydrogen peroxide (H_2O_2) or peroxynitrite ($ONOO^-$) displayed increased ROS sensitivity based on ATPase activity assays. Overall, the experiments of Welle et al. [6] suggested a new role for the conserved cysteine in the plasma membrane H^+-ATPases: the residue may bind Cu^{2+} ions to protect the active site from oxidation under stress conditions that induce ROS formation. Intriguingly, it appears that evolution selected for robustness in the essential role of the plasma membrane H^+-ATPases that enables their adaption to changing cellular conditions.

Robustness describes the ability of a system to perform its function despite perturbations or suboptimal conditions. Organisms evolve robustness in response to complex and changeable environments. They face an unpredictable repertoire of biotic and abiotic challenges such as changes in temperature, water and nutrient availability, competition, pathogens and predators. Successful organisms are those which have evolved strategies to cope with these selective pressures and build robustness into biological functions at every scale. Because organisms are typically studied in a carefully controlled laboratory environment, the extent of this robustness is just beginning to be appreciated. Recently, mechanisms for robustness to mutation, protein expression, initiation of translation, and cell size have been described [14–17]. In these systems, various proteins, such as heat shock proteins or DNA repair enzymes, serve specific roles to act as buffer against these environmental changes.

Now, Welle et al. [6] reveal another mechanism for functional robustness in which proteins with other primary roles in the cell themselves harbor protective residues to maintain activity during stress conditions. In the case of the plasma membrane H^+-ATPases, the extensive conservation of the key cysteine residue across homologs from various kingdoms suggests a long evolutionary selection for robustness to changes in copper concentration and ROS generation. Interestingly, there are other examples of unanticipated metal chelating activity in proteins with other primary functions. Copper chelating activities have been recently identified in granulins, chaperonins, and lens crystallins [18–20]. It is possible that evolution of metal chelation serves a protective measure to maintain robust molecular function under ROS generating conditions.

For the first time, the mystery of the invariant cysteine in the P3 subfamily of plasma membrane H^+-ATPases appears to be solved. A logical next step is to examine whether members of the P2 subfamily, like SERCA and the Na^+/K^+-ATPases, also evolved a similar protection mechanism to maintain robust cellular function during other ROS-generating stress conditions.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Acknowledgements

The authors acknowledge funding from the National Science Foundation (IOS-1907288 to S.C.S. and MCB-1614539 and MCB-1818040 to J.M.J.)

Abbreviations

ROS, reactive oxygen species; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase.

References

1 Reis, R. and Moraes, I. (2019) Structural biology and structure-function relationships of membrane proteins. *Biochem. Soc. Trans.* **47**, 47–61 https://doi.org/10.1042/BST20180269
2 Drew, D. and Boudker, O. (2016) Shared molecular mechanisms of membrane transporters. *Annu. Rev. Biochem.* **85**, 543–672 https://doi.org/10.1146/annurev-biochem-060815-014520
3 Kühlbrandt, W. (2004) Biology, structure and mechanism of P-type ATPases. *Nat. Rev. Mol. Cell. Biol.* **5**, 282–295 https://doi.org/10.1038/nrm1354
4 Dyla, M., Kjaergaard, M., Poulsen, H. and Nissen, P. (2020) Structure and mechanism of P-type ATPase ion pumps. *Annu. Rev. Biochem.* **89**, 583–603 https://doi.org/10.1146/annurev-biochem-010611-112801
5 Falhof, J., Pederson, J.T., Fuglsang, A.T. and Palmgren, M. (2016) Plasma membrane H^+-ATPase regulation in the center of plant physiology. *Mol. Plant* **9**, 323–337 https://doi.org/10.1016/j.molp.2015.11.002
6 Welle, M., Pedersen, J.T., Ravnsborg, T., Hayashi, M., Maaß, S., Becher, D. et al. (2021) A conserved, buried cysteine near the P-site is accessible to cysteine modifications and increases ROS stability in the P-type plasma membrane H+-ATPase. *Biochem. J.* **478**, 619–632 https://doi.org/10.1042/BCJ20200559
7 Albers, R. (1967) Biochemical aspects of active transport. *Annu. Rev. Biochem.* **36**, 727–756 https://doi.org/10.1146/annurev.bi.36.070167.003455
8 Post, R.L., Hegyvary, C. and Kume, S. (1972) Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. *J. Biol. Chem.* **247**, 6530–6540 https://doi.org/10.1016/S0021-9258(19)44725-X
9 Palmgren, M.G. and Axelsen, K.B. (1998) Evolution of P-type ATPases. *Biochim. Biophys. Acta* **1365**, 37–45 https://doi.org/10.1016/S0005-2728(98)00041-3
10 Reczek, C.R. and Chandel, N.S. (2015) ROS-dependent signal transduction. *Curr. Opin. Cell Biol.* **33**, 8–13 https://doi.org/10.1016/j.ceb.2014.09.010
11 Yang, J., Carroll, K.S. and Liebler, D.C. (2016) The expanding landscape of the thiol redox proteome. *Mol. Cell. Proteomics* **15**, 1–11 https://doi.org/10.1074/mcp.O115.056051
12 Reichmann, D., Voth, W. and Jakob, U. (2018) Maintaining a healthy proteome during oxidative stress. *Mol. Cell* **69**, 203–213 https://doi.org/10.1016/j.molcel.2017.12.021
13 Winterbourn, C.C. (2008) Reconciling the chemistry and biology of reactive oxygen species. *Nat. Chem. Biol.* **4**, 278–286 https://doi.org/10.1038/nchembio.85
14 Zheng, J., Guo, N. and Wagner, A. (2020) Selection enhances protein evolvability by increasing mutational robustness and foldability. *Science* **370**, eabb5962 https://doi.org/10.1126/science.abb5962
15 Osterwalder, M., Barozzi, I., Tissières, V., Fukuda-Yuzawa, Y., Mannion, B.J., Afzal, S.Y. et al. (2018) Enhancer redundancy provides phenotypic robustness in mammalian development. *Nature* **554**, 239–243 https://doi.org/10.1038/nature25461
16 Tsai, A., Alves, M.R. and Crocker, J. (2019) Multi-enhancer transcriptional hubs confer phenotypic robustness. *Elife* **8**, e45325 https://doi.org/10.7554/eLife.45325
17 Shi, H., Colavin, A., Bigos, M., Tropini, C., Monds, R.D. and Huang, K.C. (2017) Deep phenotypic mapping of bacterial cytoskeletal mutants reveals physiological robustness to cell size. *Curr. Biol.* **27**, 3419–3429 https://doi.org/10.1016/j.cub.2017.09.065
18 Bhopatkar, A.A. and Rangachari, V. (2021) Are granulins copper sequestering proteins? *Proteins* **89**, 450–461 https://doi.org/10.1002/prot.26031
19 Ahmad, M.F., Singh, D., Taiyab, A., Ramakrishna, T., Raman, B. and Rao, C.M. (2008) Selective Cu^{2+} binding, redox silencing, and cytoprotective effects of the small heat shock proteins alphaA- and alphaB-crystallin. *J. Mol. Biol.* **382**, 812–824 https://doi.org/10.1016/j.jmb.2008.07.068
20 Ansari, M.Y., Batra, S.D., Ojha, H., Dhiman, K., Ganguly, A., Tyagi, J.S. et al. (2020) A novel function of *Mycobacterium tuberculosis* chaperonin paralog GroEL1 in copper homeostasis. *FEBS Lett.* **594**, 3305–3323 https://doi.org/10.1002/1873-3468.13906