Nuclear V-type ATPase. Focus on “Vacuolar H\(^+\)-ATPase in the nuclear membranes regulates nucleo-cytosolic proton gradients”

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The nuclear envelope (NE) consists of two continuous membranes, with pores where the inner nuclear membrane (INM) meets the outer nuclear membrane (ONM) (7). V-type ATPases in the plasma membrane and intracellular membranes alter pH (\(\Delta pK\)) and generate a transmembrane electrical potential (\(\Delta \Psi\)) across plasma membranes and intracellular compartments (6).

In the accompanying article by Santos et al. (8) published in this issue of the American Journal of Physiology-Cell Physiology, the authors tested the hypothesis that the V-type ATPase was present and functional in the NE. The authors established that the nuclear pH is acidic, pH gradients across the nuclear membrane were bafilomycin and concanamycin sensitive and that the V-type ATPase was present in the NE by immunolocalization, and in both the ONM and INM by Western blots and ATPase assays.

The flow of ions across the nuclear membranes is restricted (3). Prior studies to determine whether there was an electrochemical gradient of protons across the nuclear membrane were contradictory and no previous studies examined whether V-type ATPases might be involved. The authors used the ratiometric dye SNARF-1 to determine nuclear-cyttoplasmic pH gradients. They examined RWPE-1 cells, a prostate epithelial cell line, LNCaP cells, a prostate cancer cell line, and highly tumorigenic and invasive CL-2 cells derived from LNCaP cells. They found that the steady-state pH of the nucleus was more acidic than the cytosol in RWPE-1 cells and LNCaP cells, but there was no difference in the steady-state pH between the nucleus and cytosol in CL-2 cells. The authors attribute the differences in their findings from those of others who also used ratiometric approaches to the failure of others to carry out in situ calibration for each cell type and each cell. In the present study, the authors used individual in situ calibration procedures for nucleus and cytosol, respectively, in contrast to other studies, which used either a single calibration curve obtained in vitro or in situ or the average of in situ calibration curves for nucleus and cytosol. The authors could have made a stronger case for their attribution of their differences from the studies of others by documenting how the different approach would change the measurements and by documenting that an average calibration could recapitulate the contradictory finding of others using SNARF-1.

They found that absolute pH values varied in the various compartments among cells, so all values were also reported as \(\Delta pK\). No differences were seen in the outward gradients between RWPE-1, LNCaP, and CL-2 cells in aggregate. However, there were larger inward H\(^+\) gradients in CL-2 cells than RWPE-1 or LNCaP cells, in aggregate. When \(\Delta pK\) was given as a percentage for each cell type, the majority of each cell type showed no \(\Delta pK\), but a larger percentage of CL-2 cells had inward H\(^+\) gradients. The differences in \(\Delta pK\) between the cell types and between individual cells suggest that there are unknown mechanisms for regulation of the pH of the nucleus. The heterogeneity of nuclear pH among cells in the same cultures suggests that \(\Delta pK\) and \(\Delta \Psi\) regulation of the nucleus is subject to molecular and biochemical signaling.

The authors showed that the inward and outward pH gradients were sensitive to bafilomycin in all cell types in aggregate and on a percentage basis, suggesting that V-type ATPase is responsible for gradient formation. Similar findings were observed using concanamycin in cancer cells.

The authors used antibodies to the \(a_2\) and \(c\) subunit of V-type ATPase and showed that it was found at the nucleus by immunocytochemistry. Immunoblots of the \(V_0\) subunit \(c\) in ONM with nesprin 3 as a marker and INM with lamin B as a marker demonstrated the presence of the V-type ATPase in both the INM and ONM membranes. Bafilomycin-sensitive ATPase activities were higher in the ONM, consistent with acidification of the nucleus, and the ATPase activity was highest in both INM and ONM of CL-2.

It has been shown by subtractive proteomics that numerous transporters, pumps, and channels exist in the nuclear envelope (2, 9). Although only a few functional studies have appeared on nuclear envelope transporters including patch clamp of K\(^+\) channels (5), it is known that K\(^+\) and Na\(^+\) gradients across the nucleus are regulated by the \(Na^+-K^+\)-ATPase (4), and there is evidence for regulation of nuclear Ca\(^{2+}\) (1).

\(\Delta pK\) and \(\Delta \Psi\) generated by the V-type ATPase will provide the driving force for many of the newly identified NE transporters and channels identified in proteomic studies (2, 9). \(\Delta pK\) and \(\Delta \Psi\) will also affect proteins and nucleic acids in the nucleus. In addition, it is likely that the V-type ATPase will be found to be involved in numerous other processes (6).

This is the first study to demonstrate that the V-type ATPase subunits are in the NE, an important finding on its own. The authors have been the first to show that the nucleus of a subset of cells in any given cell line studied generated a bafilomycin-sensitive inward H\(^+\) gradient. In all cell lines an outward H\(^+\) gradient was also present in a subset of cells. The majority of the cells in each cell line generated no H\(^+\) gradient. In aggregate, all of the cell lines studied generated an inwardly directed \(\Delta pK\). The basis for the heterogeneity of pH gradients has not been explained. It would be important to know if and how the cells transit between inwardly directed, outwardly directed, and no \(\Delta pK\) across the NE. Antibodies to \(V_0\) \(c\) subunit and \(a_2\) subunits were employed for immunocytochemistry, and antibodies to \(V_0\) \(c\) subunit were used for immunoblots. The literature did not provide a clear choice of markers for a nuclear V-type.
ATPase subunit in any cell type, and future studies will be required to characterize the isoforms in membranes of prostate epithelial and cancer cells.

Considering the key role of the highly regulated (10) V-type ATPase in generation of $\Delta pH$ and $\Delta \psi$, and its many other roles, these findings will lead to greater understanding of the regulation of nuclear physiology.

**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**

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**REFERENCES**
