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PERSPECTIVES

SARS-CoV-2: pushing the E(nvelope)

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Linked articles: This Perspectives article highlights an article by Cabrera-Garcia *et al.* To read this paper, visit https://doi.org/10.1113/JP281037.

Halting SARS-CoV-2's relentless spread now appears within reach – in the form of multiple, approved vaccines directing the immune system to recognize SARS-CoV-2's spike (S) protein. Nonetheless, the spectre of limited efficacy against rapidly evolving viral variants remains of concern. Coronaviral proteins – especially S, nucleocapsid (N), membrane (M) and envelope (E) – drive the cycle of infection, replication, assembly and release. Understanding processes downstream of S-receptor binding affords strategies to mitigate disease. Consider the final steps of assembly and release: without either, infection and replication are arrested. Identifying and exploiting vulnerabilities in both processes requires detailed insight into E protein's many facets. So, what is known about E proteins in general, and specifically about SARS-CoV-2's E protein?

Envelope proteins of other viruses, notably the influenza A virus M_2 protein, function as ion channels – viroporins – modulated by extracellular pH (Pinto *et al.* 1992). M_2 viroporins render host cell intracellular compartments more alkaline, protecting nascent virions throughout assembly and release (Sugrue *et al.* 1990). Like M_2, E proteins of coronaviruses assume a similar compact transmembrane topology, chararacterized by a single membrane-spanning helix.

Evidence from bilayer experimentation (reviewed in McClenaghan *et al.* 2020) suggests that coronavirus E proteins also organize to function as ion channels. Although consistent with the notion that SARS-CoV-1 E viroporins are permeable to cations, the reliance on non-cellular systems precludes direct determination of how it promotes a virion-friendly environment within the endoplasmic reticulum–Golgi intermediate compartment (ERGIC).

Westerbeck and Machamer (2019) previously demonstrated that E protein of infectious bronchitis virus (IBV), also a coronavirus, renders intraluminal pH of the ERGIC alkaline, thereby functionally shielding nascent virion from an inhospitable environment. They favour a mechanism, however, that the observed pH change arises from interaction of *monomeric* IBV E with host proteins, or alternatively, by otherwise disrupting normal Golgi function.

The study of Cabrera-García et al. (2021) in this issue of *The Journal of Physiology* offers the first steps toward connecting these parts. Using the SARS-CoV-2 E protein, the work represents an exceptionally timely and significant effort. The authors developed a strategy for robust E protein expression, as well as identify a promising platform for evaluating novel antiviral therapeutics.

The straightforward strategy of incorporating a carboxyl-terminal fluorescent tag, mKate2, allowed localization of tagged wild-type E to perinuclear components overlapping with the ERGIC in NIH3T3 cells. Fluorescence quenching of a pH-sensitive dye indicated compartmental pH to be relatively more alkaline over a duration of 48 h in E protein-expressing cells *versus* mock-transfectants. Despite the limitations of a relative intensity readout rather than one permitting pH calibration and ratiometric measurements, the data qualitatively align with previous findings for IBV (ΔpH ~0.3 pH units; see Westerbeck & Machamer, 2019). Significantly, they provide the first indication that SARS-CoV-2 E also raises ERGIC luminal pH.

But is it SARS-CoV-2 E viroporin that causes this pH rise, and if so, what ions move through it? The intracellular localization and apparent lack of plasma membrane trafficking in HEK293 cells confounded conventional electrophysiological strategies.

To this end, Cabrera-García *et al.* devised a series of informed molecular modifications, ultimately achieving successful plasma membrane targeting by disabling protein interaction and retention motifs, and incorporating a Golgi export signal. Plasma membrane trafficking was sufficient to produce small currents using the whole-cell recording configuration. Membrane-trafficked E protein produced currents due to permeation of small cations ($P_K > P_{Cs} \approx P_{Na}$), but excluded *N*-methyl-D-glucamine as well as Cl^-. Of special note is the inward current rectification that appears subsequent to acidifying extracellular pH (pH_e); increased pH reduced current, but did so without producing rectification.

Ideally, the functional signature emerging from established cellular expression systems would translate directly to a screening platform. Realizing the limited signal-to-noise ratio in HEK293 cells, Cabrera-García *et al.* turned to *Xenopus* oocytes, a model system offering the allure of robust expression and rapid screening by two-microelectrode voltage clamp. Although currents were broadly similar – increasing with acidic pH_e and decreasing with alkaline pH_e – two points bear mention.

First, at pH 6.0, E protein currents were linear in oocytes, rather than inwardly rectifying. Such discrepancies between currents measured in mammalian cell and amphibian oocyte heterologous expression systems are not unprecedented. There are many explanations for the differing rectification properties. For example, SARS-CoV-2 E viroporin assemblies may associate with different host plasma membrane-resident proteins that act effectively as β-subunits. An additional modifier might be the membrane lipid composition.

Second, raising pH_e revealed voltage-dependent current decay in the oocyte currents. The authors provide two possible explanations: voltage-dependent block consequent to deprotonation at high pH_e, or alternatively, electrochemical gradient collapse from high expression levels and correspondingly large currents. Current expression levels correlate with quantity of cRNA injected, so the latter is feasible by testing if the decay persists with lower-level expression.

The findings collectively suggest SARS-CoV-2 E functions as a viroporin that produces currents both modulated by

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and consequential to extracellular/luminal pH, making it feasible to probe further functional details. If resolvable, do isolated channels in cellular expression systems behave similarly to those in reconstitution systems? The ability to express large currents having clearly defined responses offers exciting possibilities for screening E protein-targeted, anti-viral drugs, as well as blockers of other known viroporins. If E indeed acts as a viroporin, is it a proton channel, can pH sensitivity be altered, and how does it ultimately impact ERGIC pH? The present findings (Cabrera-García *et al.* 2021) highlight many future paths of inquiry, and firmly chart approaches for ensuring a diverse armamentarium to manage viral disease.

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Additional information

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