The FEBS Journal

FEBS PRESS science publishing by scientists

COMMENTARY

New 'kids' on the voltage-gated proton channel block

Éva Korpos[1,2] and Ferenc Papp[1]

1 Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Hungary
2 MTA-DE Cell Biology and Signalling Research Group, Faculty of Medicine, University of Debrecen, Hungary

Keywords
Aplysia californica; Hv1; patch-clamp; voltage-gated proton channel

Correspondence
F. Papp, Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen H-4032, Hungary
Tel: +36 52 258 603
E-mail: papp.ferenc@med.unideb.hu

(Received 7 October 2022, accepted 25 October 2022)

doi:10.1111/febs.16670

So far one gene for Hv1 has been detected in studied species. The work presented by Chaves et al. in *The FEBS Journal* reported an 'Unexpected expansion of the voltage-gated proton channel family'. They searched for proton channel candidates and found three sequences in the genome of *Aplysia californica* (Ac), which were named AcHv1, AcHv2 and AcHv3. Based on electrophysiological experiments, AcHv1 and AcHv2 are voltage-gated channels. While AcHv1 behaves like Hv1 in other species, that is, it is voltage and pH-dependent, it can be inhibited by zinc and conducts protons outwardly, AcHv2 conducts protons inwards at symmetrical pH. AcHv3 constantly leaks protons, and its C-terminal part contains several cytoplasmic retention motifs. Through carefully designed and carried out electrophysiological experiments, Chaves et al. determined the biophysical parameters of all three proton channels, such as the voltage and the pH dependence, the threshold-voltage, the gating charge and the time constants of activation and inactivation.

Comment on: https://doi.org/10.1111/febs.16617.

Introduction

After completing the sequencing of the human genome, researchers in the voltage-gated ion channel field were looking for new voltage sensor proteins using bioinformatic tools. The discovery of voltage-sensing phosphatase (VSP) revealed that voltage-sensing domains (VSD) can exist in proteins independent of ion-conducting pore [1]. The first member of voltage-gated proton channels (Hv1) was subsequently discovered and shown to consist of only a VSD that conducts protons in response to membrane depolarization [2,3]. The next identified candidate protein was encoded by the c15orf27 gene (called TMEM266 now), which contained the most critical residues found in VSD of other voltage sensors. Based on sequence alignment and structural prediction, Hv1 showed the highest similarity to TMEM266 [4], suggesting the existence of a new member in the voltage-gated proton channel family. However, experiments showed that TMEM266 is not a voltage-gated proton channel. In Decoursey's laboratory, TMEM266 served as a non-conducting negative template to identify the selectivity filter of Hv1 [5]. In Swartz's laboratory, we were able to make functional chimera ion channels in which the S4 domain of TMEM266 was transferred to Hv1 or Shaker channels, indicating that the S4 domain of TMEM266 could function as part of voltage sensor units [6]. Furthermore, our voltage clamp fluorometry (VCF) experiments have demonstrated that TMEM266 can sense voltage, but it is not a new Hv channel [6].

The Hv1 proton channel has been described in many different species from unicellular organisms to

Abbreviations
AcHv, *Aplysia californica* voltage-gated proton channel; Hv, voltage-gated proton channels; NAPDH, nicotinamide adenine dinucleotide phosphate; VCF, voltage clamp fluorometry; VSD, voltage-sensing domain; VSP, voltage-sensing phosphatase.

mammals and in many different cell types [7]. In the last years, the number of publications on Hv1 has increased and it was identified in species and cell types that were not known to express the Hv1 [8]. The main characteristics of the proton channel are that it has high proton selectivity, it opens upon membrane depolarization and it is sensitive to pH gradient [7]. Hv1 regulates the intracellular pH by extruding protons from the inside to the outside of the cell [9]. One exception has been described in dinoflagellates, in *Karlodinium veneficum*, where the proton channel conducts proton current inwardly [10].

Hv1 has many different functions, such as contributing to the production of bioluminescent light in dinoflagellates or calcifying the skeleton of coccolithophores [10]. Mouse and human Hv1 channels have been identified in many different immune cell types, and the best-known functions of Hv1 are related to the regulation of reactive oxygen species production via NAPDH oxidase, but they also play a role in B-cell receptor signalling [11], T-cell activation [12] and modulation of sperm motility (in humans) [13]. Also, Hv1 has been associated with tumour progression, via promotion of tumour cell proliferation and migration [14].

So far one gene for Hv1 has been detected in studied species. For human Hv1 gene, three transcript variants were described, two of them encoded the same protein; however, the third variant encodes a shorter protein that was linked to malignant B cells [15].

From 2006 until recently, we had been waiting for new members to join the Hv family: the Musset laboratory has published two new members: Hv2 and Hv3 [12].

The new members of Hv

The work presented by Chaves et al. in *The FEBS Journal* reported an 'Unexpected expansion of the voltage-gated proton channel family' [12]. They searched for proton channel candidates and found two sequences with the criteria for Hv channels in the genome of *Aplysia californica* (California sea hare, marine mollusc; Ac; Fig. 1A), which were named AcHv1 and AcHv2. Furthermore, they recognized another channel candidate gene (AcHv3); however, that one had two sequence anomalies compared to Hv1. Based on electrophysiological experiments, AcHv1 and AcHv2 are voltage-gated channels. Whereas AcHv1 behaves like Hv1 in other species, that is, it is voltage and pH-dependent, it can be inhibited by zinc and conducts protons outwardly, AcHv2 conducts protons inwards at symmetrical pH (Fig. 1B). So, while the primary function of mammalian proton channels is to release protons from the cell, AcHv2 conducts inward proton currents that acidify the cell and maybe more importantly, depolarize the cell membrane. Because of this latter, it is possible that AcHv2 could generate proton-mediated action potential in *Aplysia californica* supposed by Chaves et al. AcHv3 is the longest protein among the three proton channels with 980 amino acids (AcHv1 has 281, while AcHv2 has 339). AcHv3 is not likely to be present in the plasma membrane but in intracellular membranes *in vivo* for two reasons: it is constantly leaking protons, and its C-terminal part contains several cytoplasmic retention motifs. Two possible explanations for the proton selective leakage

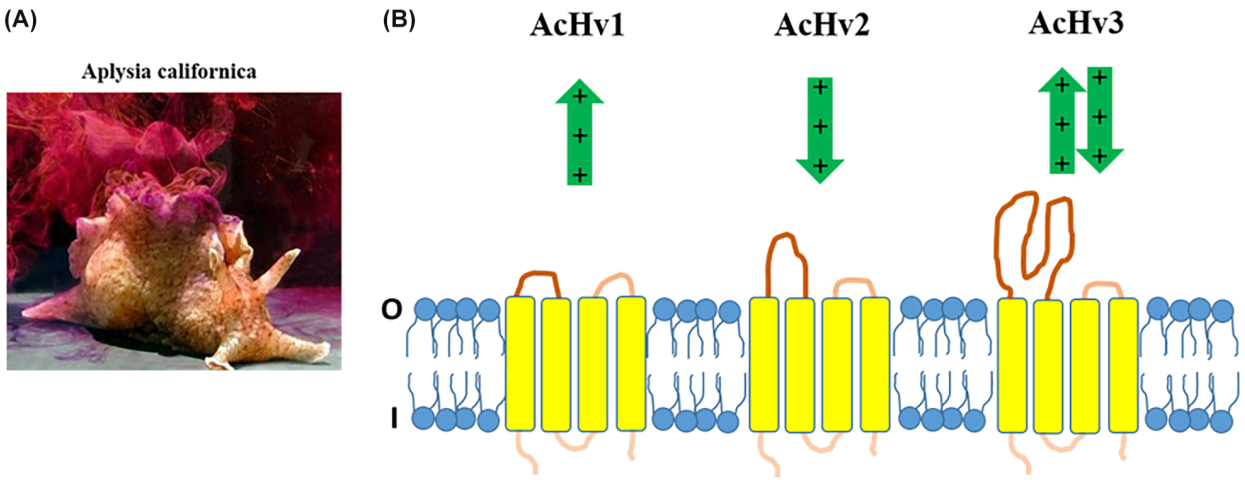

Fig. 1. Schematic representation of the *Aplysia californica* (A, internet source: Aplysia Genome Project¦Broad Institute), the model organism used in the present study and of the three AcHv proton channels (B). The AcHv1 conducts proton current outwardly, the AcHv2 inwardly and AcHv3 leaks current. I, inner layer of the cell membrane; O, outer layer of the cell membrane.

current are mentioned by Chaves et al.: (a) weak voltage dependence of gating. (b) closed-channel proton leak. Both could come from the missing of the first arginine residue in the VSD (called R1 in the classical Hvs) and the existence of a proline right before the second arginine (R2). Through carefully designed and carried out electrophysiological experiments, Chaves et al. determined the biophysical parameters of all three proton channels, such as the voltage and the pH dependence; the threshold-voltage; gating charge; the time constants of activation and inactivation.

Conclusion

The discovery and detailed electrophysiological characterization of the three proton channels of *Aplysia californica* by Musset and his colleagues is a major contribution to the field of proton channel research. Beside the characterization of the AcHv channels, Chaves et al.'s preliminary data suggest that the presence of three proton channels is characteristic not only to *Aplysia californica* species but also to other members of Mollusca phylum. Will be interesting to learn more about the function of these novel proton channel proteins, to understand why it is necessary to conduct the current inwardly, and what is the need for the AcHv3 proton channel. It should be mentioned here that inward proton currents have been reported before in the case of a symmetric pH, namely through the KvHv1 channel discovered in '*Karlodinium veneficum*'. It may then be worth investigating further and wondering whether the proton channel named KvHv1 should perhaps be called KvHv2. A precise identification of cell types expressing the different proton channels will also be meaningful. The authors keep the readers tuned up, since it is mentioned that the 'story' of the novel proton channels in Mollusca phylum will be continued in an upcoming paper. We are looking forward to that!

Acknowledgements

This work was supported by OTKA Bridging Fund 1G3DBKB0BFPF247 (FP); by János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00355/21/8) (FP); by the ÚNKP-21-5-DE-420 New National Excellence Program of the Ministry for Innovation and Technology (FP), by MTA-DE Cell Biology and Signalling Research Group (ÉK).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

ÉK and FP wrote and revised the manuscript.

References

1 Murata Y, Iwasaki H, Sasaki M, Inaba K, Okamura Y. Phosphoinositide phosphatase activity coupled to an intrinsic voltage sensor. *Nature*. 2005;**435** (7046):1239–43.
2 Ramsey IS, Moran MM, Chong JA, Clapham DE. A voltage-gated proton-selective channel lacking the pore domain. *Nature*. 2006;**440**(7088):1213–6.
3 Sasaki M, Takagi M, Okamura Y. A voltage sensor-domain protein is a voltage-gated proton channel. *Science*. 2006;**312**(5773):589–92.
4 Kulleperuma K, Smith SME, Morgan D, Musset B, Holyoake J, Chakrabarti N, et al. Construction and validation of a homology model of the human voltage-gated proton channel hHV1. *J Gen Physiol*. 2013;**141** (4):445–65.
5 Musset B, Smith SME, Rajan S, Morgan D, Cherny VV, DeCoursey TE. Aspartate 112 is the selectivity filter of the human voltage-gated proton channel. *Nature*. 2011;**480**(7376):273–7.
6 Papp F, Lomash S, Szilagyi O, Babikow E, Smith J, Chang T-H, et al. TMEM266 is a functional voltage sensor regulated by extracellular Zn^{2+}. *Elife*. 2019;**8**:e42372.
7 DeCoursey TE, Morgan D, Musset B, Cherny VV. Insights into the structure and function of HV1 from a meta-analysis of mutation studies. *J Gen Physiol*. 2016;**148**(2):97–118.
8 Rangel-Yescas G, Cervantes C, Cervantes-Rocha MA, Suárez-Delgado E, Banaszak AT, Maldonado E, et al. Discovery and characterization of Hv1-type proton channels in reef-building corals. *Elife*. 2021;**10**: e69248.
9 Thomas RC, Meech RW. Hydrogen ion currents and intracellular pH in depolarized voltage-clamped snail neurones. *Nature*. 1982;**299**(5886):826–8.
10 Smith SME, Morgan D, Musset B, Cherny VV, Place AR, Hastings JW, et al. Voltage-gated proton channel in a dinoflagellate. *Proc Natl Acad Sci USA*. 2011;**108** (44):18162–7.
11 Capasso M, Bhamrah MK, Henley T, Boyd RS, Langlais C, Cain K, et al. HVCN1 modulates BCR signal strength via regulation of BCR-dependent generation of reactive oxygen species. *Nat Immunol*. 2010;**11**(3):265–72.
12 Chaves G, Ayuyan AG, Cherny VV, Morgan D, Franzen A, Fieber L, et al. Unexpected expansion of the voltage-gated proton channel family. *FEBS J*. 2022. https://doi.org/10.1111/febs.16617
13 Lishko PV, Botchkina IL, Fedorenko A, Kirichok Y. Acid extrusion from human spermatozoa is mediated

by flagellar voltage-gated proton channel. *Cell*. 2010;**140**(3):327–37.

14 Fernandez A, Pupo A, Mena-Ulecia K, Gonzalez C, et al. Pharmacological modulation of proton channel Hv1 in cancer therapy: future perspectives. *Mol Pharmacol*. 2016;**90**(3):385–402.

15 Hondares E, Brown MA, Musset B, Morgan D, Cherny VV, Taubert C, et al. Enhanced activation of an amino-terminally truncated isoform of the voltage-gated proton channel HVCN1 enriched in malignant B cells. *Proc Natl Acad Sci USA*. 2014;**111**(50): 18078–83.