THE EMBO JOURNAL

Manuscript EMBO-2010-76603

The biophysical and molecular basis of TRPV1 proton gating

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Review timeline:

Submission date:	25 November 2010
Editorial Decision:	15 December 2010
Additional correspondence (author)	05 January 2011
Additional correspondence (editor)	05 January 2011
Revision received:	07 January 2011
Accepted:	07 January 2011

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 15 December 2010

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has now been seen by two referees whose comments are shown below. As you will be pleased to see both referees are positive about the paper and support its publication here. Still, I would like to ask you to follow the suggestion by referee 1 in a revised manuscript to further broaden the paper.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Peer Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1 (Remarks to the Author):

In this manuscript, the authors demonstrate for the first time that low pH activates TRPV1 by shifting the voltage dependence of activation to lower voltages, similar to the effect of heat and capsaicin. Even more interesting, they identify a residue (660) that, when mutated, abolishes pH-induced shifts in voltage dependence, and even causes some inhibition of capsaicin-induced activation. The work is expertly performed, of high quality and of broad interest to the TRP field. Moreover, given the proposed correlation between effects on pH sensitivity and hyperthermia induced by TRPV1 antagonists, it may be of pharmacological/clinical importance.

Suggestion:
Previous work has identified other residues involved in pH sensing (e.g. Jordt et al.), but a detailed analysis of the voltage dependence of pH effects on these mutant channels is lacking. For comparison, it would be nice to see some of these mutants analysed in a similar robust manner as the 660 mutations.

Referee #2 (Remarks to the Author):

The TRPV1 channelsis implicated in nociception and can be activated/sensitized by capsaicin, temperature, and protons. Mechanisms
underlying capsaicin and heat activation/potentiation have been identified, yet how protons gate the channel is unknown. The manuscript by Aneiros et al. now addresses this issue. Using patch clamp and calcium flux experiments on HEK cells that express human TRPV1, the authors show that protons activate TRPV1 by shifting its voltage dependence, similar as was previously described for temperature activation. The authors further show that the proton activation of TRPV1 is selectively affected by mutations in position F660, which is implicated in heat activation in TRPV3. Amino acid replacements show that a non-basic aromatic amino acid at position 660 is essential for this proton activation, and data is presented that suggests that protons facilitate the voltage-dependent gating of TRPV1, thus potentiating the activation of TRPV1 by capsaicin.

The manuscript adds important new information to an issue of TRPV1 that has been discussed controversially in the past. The experiments all seem to be conducted carefully and the conclusions seem justified throughout. Also the manuscript is well written, and it will be interesting to see whether F660 has the same importance for TRPV1 proton activation and potentiation under in situ conditions. Given the novelty and clarity of the results, I positively inclined towards publication.

Additional correspondence (author) 05 January 2011

Thank you very much for your recent email regarding manuscript submission EMBOJ-2010-76603.We are very glad to see that both Referees are very positive about our work and highly supportive regarding publication in the EMBO journal.

While Referee 2 did not ask for additional experiments, Referee 1 suggested that an analysis of the voltage dependence of pH effects of residues previously shown to be involved in pH sensing of TRPV1 (e.g. Jordt et al., PNAS, 2000) may be performed for comparison. In the referred paper, the authors reported several putative proton sensing sites using combined mutagenesis and patch clamp techniques.

The main focus of our study was to investigate the mechanism of proton
activation of TRPV1 and to identify the critical residue(s) involved in voltage-dependent proton gating. From our point of view, to distinguish between pH sensing (proton binding) and voltage dependent gating of a certain residue, it is necessary to combine both the titration (with full series mutagenesis) and voltage dependence study, which was performed in our work. According to the classical ligand binding-gating model (Colquhoun et al., BJP, 1998), mutations affecting pH sensing or gating will both result in a shift of the voltage-dependent activation curve. The main difference between our mutants and the mutants described by Jordt et al. is that we did not observe a titration pattern with our mutants while Jordt et al. showed a clear titration phenotype (exemplified by E600) which suggested proton sensing is affected. Hence, analysis of the Jordt et al. mutants using our

voltage protocol would not provide novel information regarding the gating mechanism. Therefore, we strongly believe that the suggested study will not further strengthen our conclusions and hypotheses.

Both Referees agreed that we have provided convincing data to support all our hypotheses and conclusions in the current paper. The suggested additional experiment would require a significant amount of time but not further strengthen the data already presented or broaden the paper. In the interest of publishing these novel findings as soon as possible, we would like to kindly ask for publication without the suggested additional study.

Additional correspondence (editor) 05 January 2011

Thank you for your message asking us whether we would be able to publish the manuscript without additional experimental data. I have now had a chance to look into this issue. We will not insist on the additional experiments, but I would like to ask you to discuss the differences between your mutants and the ones used by Jordt et al. 2000 and the implications in the discussion section of the manuscript as well as in your point-by-point response.

I look forward to your amended manuscript.

Yours sincerely,

Editor
The EMBO Journal

1st Revision - authors' response 07 January 2011

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Authors: Thanks for the very positive comments.

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protocol would not provide novel information regarding the gating mechanism. Therefore, we strongly believe that the suggested study will not further strengthen our conclusions and hypotheses. Nevertheless, we added a paragraph in the manuscript to emphasize this point (see paragraph in manuscript Discussion highlighted in red).

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