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STIM1 interacts with termini of Orai channels in a sequential manner

Liling Niu, Fuyun Wu, Kaili Li, Jing Li, Shenyuan L. Zhang, Junjie Hu and Qian Wang
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Original submission

First decision letter

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MS TITLE: STIM1 interacts with termini of Orai channels in a sequential manner

AUTHORS: Liling Niu, Fuyun Wu, Jing Li, Shenyuan L Zhang, Junjie Hu, and Qian Wang
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but raised some critical points that will require amendments to your manuscript. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

They have demonstrated direct interaction between the Orai-NT and STIM1. They have also defined roles for residues not previously known to be important in this interaction. This represents new insight into how STIM and Orai interact.

Comments for the author

The manuscript entitled, “STIM1 interacts with termini of Orai channels in a sequential manner” is an interesting study that makes several new findings regarding the nature of the STIM-Orai interaction. This area has been very heavily investigated over the last 10+ years. However, using a biochemical strategy, the authors provide new evidence in support of direct interactions between the Orai-NT and STIM1. This is a significant observation as the possibility that STIM1 interacts with the Orai-CT only has been proposed. However, data presented here indicates that STIM1 has a higher affinity for the Orai-NT vs. the Orai-CT. This data is strongly supported by the authors finding that these interactions can be disrupted by point mutations, also revealing several key residues in the STIM and Orai interactions not previously established. That they have made new observations in such a crowded field leaves me with considerable enthusiasm for this study, however, I do have some concerns, as noted below. Addressing these concerns would improve the quality and impact of this study.

Specific comments:

1. I found the introduction to be a little outdated and off-topic. The references are all around 10 years old and, in some cases do not reflect current thinking. For example, it is stated that R91 regulates pore opening, a concept not well supported by recent studies. I also question the validity of the claim that the second half of the cytosolic portion of STIM1 lacks secondary structure. I do not personally believe that; without a formally solved structure, no such statement can be supported. That said, this information is not entirely helpful to the paper; I think that the introduction should be rethought.

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This manuscript by Niu et al. shows that N-terminal and C-terminal peptides of Orai1 and Orai3 engage the functional STIM1 fragment, residues 342-531. The majority of the binding analysis is carried out with Orai3 peptides and peptide mutants. There is a workmanlike investigation of likely STIM-interacting residues in the Orai peptides, some previously reported, some new, and the in vitro analysis is backed by measurements of Orai channel currents in HEK293 cells.

The major new conclusion is that binding of Orai3 N-terminal peptide to STIM1(342-531) alters the binding of Orai3 C-terminal peptide in a way suggesting that STIM1 interaction with the C-terminal region and the N-terminal region are sequential steps in physiologcial STIM1 binding to the full Orai1 channel. Further confirmation of this hypothesis is needed, but this is an important observation, in part because it may stimulate new definitive work in an area that has remain unsettled in the literature.

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(1) The manuscript is not accurate in citing previous work in this area. The only recognition of earlier studies on STIM-ORAI peptide binding comes on p. 14, “As previously suggested, the NT and CT peptides of Orai both bind to purified STIM1”, without citations. The binding was not merely suggested. Park et al. 2009, Zhou et al. 2010, and Gudlur et al. 2014 all published direct biochemical demonstrations of STIM1 binding to the N-terminal and C-terminal peptides. Although two of these papers are cited in other contexts, neither is cited for demonstrating binding.

(2) Equally baffling, the manuscript does not mention a controversy in the literature that is inextricably connected to the results reported. In contrast with Park et al. 2009, Zhou et al. 2010, and Gudlur et al. 2014 which have been cited as consistent with involvement of a STIM-Orai N-terminal peptide interaction in gating Zhou et al. 2016 (Nat. Commun. 7:13725) have argued that STIM does not bind to the N terminus of Orai1 in activating the channel. (The crux of their argument is that mutations in the Orai1 N terminus that impair STIM binding also impair ion flux in an Orai1 channel mutant that conducts constitutively, independent of STIM1.
Zhou et al. conclude that previous work had mistakenly attributed the effects of the mutations to impaired STIM binding, whereas they actually impair intramolecular interactions within Orai1 that support Orai channel gating.) There are considerations on both sides, and the issue needs to be aired, particularly in a report that depends on the interpretation that STIM1 gates the channel by interacting with the N-terminal region.

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Given these issues, it is unclear how the authors can be confident interpreting the data of Figure 2B and 2C in terms of simple changes in an affinity or dissociation rate.

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First revision

Author response to reviewers' comments

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We have rewritten the intro accordingly and updated references.

2.Figures are presented out of order. Please re-organize the manuscript so that this is not the case.

We have made changes accordingly.

3.The manuscript is written referencing Orai-NT and Orai-CT, however, the vast majority of the data is generated using peptides from Orai3. I have no problem with the use of Orai3 peptides, however, the authors should take more care in describing the data. My suggestion is that the text related to the first 3 figures should be limited to discussion of Orai3. Relevance to Orai1 is established in the full length construct in figure 5 and can be discussed further in the discussion.

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The two peaks are of statistical difference. Nevertheless, we have tune down the conclusion here as suggested.

Reviewer 2 Advance summary and potential significance to field
This manuscript by Niu et al. shows that N-terminal and C-terminal peptides of Orai1 and Orai3 engage the functional STIM1 fragment, residues 342-531. The majority of the binding analysis is carried out with Orai3 peptides and peptide mutants. There is a workmanlike investigation of likely STIM-interacting residues in the Orai peptides, some previously reported, some new, and the in vitro analysis is backed by measurements of Orai channel currents in HEK293 cells. The major new conclusion is that binding of Orai3 N-terminal peptide to STIM1(342-531) alters the binding of Orai3 C-terminal peptide in a way suggesting that STIM1 interaction with the C-terminal region and the N-terminal region are sequential steps in physiological STIM1 binding to the full Orai1 channel. Further confirmation of this hypothesis is needed, but this is an important observation, in part because it may stimulate new definitive work in an area that has remain unsettled in the literature.

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We have made changes accordingly.

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We have made changes accordingly. We confirmed that R77A K78A blocks the ANSGA channel (see below) and acknowledged the possibility in discussion.

(3) The pull-down assay of Figure 2A is acceptable evidence for NT-CT competition. The limited trypsin digests of Figure 2D are further evidence that binding of the Orai3 N-terminal peptide elicits a conformational change in STIM1(342-531), and thereby support the conclusion about competition.

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biolayer interferometry assay, it could lead to many binding modes, with added complexity due to possible multiple STIM1 conformations. Given these issues, it is unclear how the authors can be confident interpreting the data of Figure 2B and 2C in terms of simple changes in an affinity or dissociation rate.

As shown in Fig. S1E, no homotypic or heterotypic interaction was seen with 3NT and 3CT. We have removed the speculative sentences in the discussion to avoid confusion. We agree that the curve fitting in Fig. 1D,E is not perfect, however, it still fits best with global 1:1 association-then-dissociation model.

(5) Gudlur et al. 2014 found that the substitutions L81A/S82A/K85E compromise the binding of Orai1 N-terminal peptide to STIM1 C terminus, STIM1(233-685). Thus, at a minimum, there is a STIM1 conformation for which Orai1 peptide binding is sensitive to mutations in the part of the Orai1 N terminus spanning residues 81-85. In the current manuscript, either an L56A substitution in Orai3 (corresponding to L81 in Orai1) or a K60A substitution (corresponding to K85 in Orai1) has little effect on binding. Is S57 (S82 in Orai1) necessary for STIM1 binding? Or are the STIM1 fragment STIM1(342-531) and the full STIM1 C terminus in very different conformations?

We have performed the binding assays with additional mutants as suggested (Fig. 3C). The defects in the triple mutants are mainly caused by L56A. These new results are consistent with previous findings.

(6) The effect of the R77A/K78A replacements in Figure 5 may be open to the same objection that Zhou et al. have made to other mutations in the N-terminal region of Orai1. (Impairment of STIM1 binding is inferred here from impairment of binding by the corresponding replacements, R52A/R53A, in the Orai3 peptide, shown in Figure 3A.) Do these replacements block channel function of the constitutively conducting “ANSGA” channel, in which case the Zhou et al. critique is applicable, or not?

We have tested R77A K78A in the context of the “ANSGA” channel as suggested (Fig. S5C,D). We found that the mutant indeed blocked the channel. We have added this point in the text.

(7) Figure S1D does not in fact display “marginal” binding to the uncoated sensor as stated on p. 7. On the other hand, measurements with the uncoated sensor are not a good control. A properly coated reference sensor is what matters.

We have changed the wording here accordingly. The control experiments performed in S1D are indeed with biocytin-coated sensor.

(8) The experiments in Figure 5A would have given cleaner data if Orai1-/- or Orai1 knockdown cells were used. The reductions with R77A/K78A, V271A, and L282A are clear, but it is incorrect to say that Ca2+ entry was “reduced to endogenous levels” with R77A/K78A, because there may still be function— a truly nonfunctional subunit might well assemble into heteromultimers and drive influx below endogenous levels. Likewise, it is not clear that S269A “caused no significant reduction” because the high bakground with Vector+STIM1 collapses the dynamic range.

As suggested, we have tested these mutants now with Orai triple knockout cells (Fig. 6A,B). Similar results were obtained.

(9) “2 M Ca2+“ and “2 M EDTA” on p. 6, “2 M Ca2+“ on p. 11, and “2 M Ca2+“ and “2 M EGTA” in Figure panel S1B are typographical errors.

Corrected.

Second decision letter

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Comments for the author

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Summary previously provided.

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Comments (2) and (6): The revised manuscript still does not address the controversy over whether STIM binding to the ORAI N-terminal region is involved in channel activation. The R77A/K78A replacements partially block the constitutive activity of the “ANSGA” channel, according to Figures S5D and S5E. This is arguably comparable to the partial block of STIM-dependent activation in Figure 6A. The authors need to explain why they reject the interpretation of Zhou et al. that the

ORAI N-terminal peptide supports channel activation purely by intra-ORAI interactions, and conclude instead that “these conserved basic residues in Orai-NT likely play dual roles ... interacting with STIM and maintaining channel integrity”. The latter conclusion may prove to be correct, in the end, but what are the counterarguments to the Zhou et al. interpretation?
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Comment (9): The typographical errors (on p. 4 and p. 7 in the revised manuscript, and on the figure panel and in the legend to Figure S1B) have not been corrected.

Second revision

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We have changed the wording to “demonstrated” (line 296) as suggested.

Comments (2) and (6): The revised manuscript still does not address the controversy over whether STIM binding to the ORAI N-terminal region is involved in channel activation. The R77A/K78A replacements partially block the constitutive activity of the “ANSGA” channel, according to Figures S5D and S5E. This is arguably comparable to the partial block of STIM-dependent activation in Figure 6A. The authors need to explain why they reject the interpretation of Zhou et al. that the ORAI N-terminal peptide supports channel activation purely by intra-ORAI interactions, and conclude instead that “these conserved basic residues in Orai-NT likely play dual roles ... interacting with STIM and maintaining channel integrity”. The latter conclusion may prove to be correct, in the end, but what are the counterarguments to the Zhou et al. interpretation?

We have changed the text accordingly (lines 240-241 and 271-273). We acknowledged that current evidence argues a direct gating role of R77/K78. Whether the NT also interacts with STIM1 for gating remains to be tested.

Comment (4): The criticism still stands. It is not clear how the authors can draw kinetic conclusions from Figures 2B and 2C. The overall conclusions that binding of the N-terminal peptide elicits a conformational change in the STIM1 fragment, and that it reduces binding of the C-terminal ORAI peptide, are supported by Figures 2A and 2D. It would be preferable to make the argument on those grounds alone.

We would favor to keep the additional evidence brought by the BLI analysis (Figures 2B and 2C). We have modified the text to avoid implication in kinetics (line 139).

Comment (9): The typographical errors (on p. 4 and p. 7 in the revised manuscript, and on the figure panel and in the legend to Figure S1B) have not been corrected.

We have corrected the typo on p. 7 (line 222). We added 2 mM Ca^{2+} in the culture media for SOCE. The experiments shown in S1B (described on p. 4) is indeed with 2 μM Ca^{2+} or 2 μM EDTA. The chosen concentration is close to cytosolic concentrations of Ca^{2+}, where STIM1 meets Orai. We have also tried 2 mM Ca^{2+} for the assays in S1B and obtained same results.

Third decision letter

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I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.