# Essentiality of Sis1, a J-domain protein Hsp70 cochaperone, can be overcome by Tti1, a specialized PIKK chaperone

Brenda Schilke and Elizabeth Craig

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## Transaction Report:

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RE: Manuscript #E21-10-0493
TITLE: Essentiality of Hsp70 J-domain protein cochaperone, Sis1, can be overcome by Tti1 subunit of specialized PIKK chaperone complex, TTT

Dear Dr. Craig,

thank you very much for submitting your work to MBoC. Your manuscript has now been reviewed by two experts, their comments are attached below. In light of their overall positive evaluation, we would be interested to receive a revised version that addresses the points raised by the two experts. As you can see, both ask for textual changes and some additional experiments. Particularly, experiments should be directed to clarify whether a potential recessive or dominant nature of your suppressor mutations underlies the observed phenotypes.

Thanks again for submitting your work to MBoC and we are looking forward to receiving your revised manuscript.

Sincerely,

Martin Ott
Monitoring Editor
Molecular Biology of the Cell

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Dear Prof. Craig,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

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To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

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Reviewer #1 (Remarks to the Author):

Hsp70 protein molecular chaperones are targeted to diverse substrates and pathways largely via the action of a large family of J domain-containing cofactors. In budding yeast, Sis1 is the only essential J-protein in the cytoplasm. Why it is required for survival when the abundant Ydj1 and other J proteins are available is not understood. In this brief report, Shilke and Craig identify mutations in the TTT complex protein Tti1 as loss of function suppressors of SIS1 deletion. Sis1 is shown to be required for stability of the TTT substrate PIKK kinases Mec1, Tra1 and both Tor kinases. Surprisingly, overexpression of Tti1 also suppresses sis1Δ lethality despite the stoichiometric imbalance with the other two TTT complex subunits. Finally, Tti1-suppressed cells exhibit differential activation of Hsf1 as demonstrated using reporter constructs with distinct HSE promoter architecture. Overall, this work establishes a previously unknown connection between the Hsp40/Hsp70 and TTT chaperone systems and highlights unique but still incompletely understood roles for Sis1 in Hsf1 regulation and beyond.

As a brief report, this manuscript generates some unique insights into chaperone biology, notably the unexpected connection between the TTT and Hsp40/70 systems. The experiments are impeccably performed but lean far more to the genetic than the cell biological in approach and outcomes. As a result, insight is gained but maybe not answers - what is Tti1 doing with Hsp70? With Hsp90? And what is going on with Sis1 and Hsf1? The experiments only provide a peek into what could be a larger and perhaps more impactful story.

I have a few minor comments:

1) In the abstract the second to last sentence describes effects on "genes activated by Hsf1." The experiments examine effects on fluorescent reporter constructs with promoter fragments containing the respective HSEs, not endogenous genes. While a full RNA-seq experiment would be very informative, it is probably beyond the scope of this work so the abstract wording should be modified accordingly.

2) The loss of function suppressors formally allow growth but the results text fails to accurately mention the exceedingly limited amount of growth observed. The spores in Fig.1 are just barely alive, consistent with a link between the two pathways but possibly a very tenuous one. In contrast, the overexpression phenotypes are far more robust and perhaps a better place to look for mechanistic insight.

3) It is hard to conclude too much from the Hsf1 experiments in Fig. 4, except that HSEs with different architecture respond differently to Hsf1 derepression. Given the recent advances in understanding the role of Ssa1/2/Hsp70 in direct binding to multiple Hsf1 domains to regulate transcriptional activity, it would be good to correlate Hsp70-Hsf1 binding in the suppressor strain to provide some level of mechanistic insight.

Reviewer #2 (Remarks to the Author):

This manuscript describes a genetic suppressor screen that links the function of the Hsp70 cochaperone and J-domain protein Sis1 to the expression levels and function of phosphatidylinositol 3-kinase related kinases (PIKKs). The study provides insight that is important for the further study of PIKKs and specifically how conformations/folding/stability of these kinases are regulated by the TTT complex and Hsp70.

1. This study is well executed and convincingly links Sis1 function to PIKKs on the phenotypic level. However phenotypic relationships, especially in the core proteostasis system, can be indirect and hence the study would benefit from providing evidence that Sis1 (or Hsp70) physically interacts with PIKKs, for example a coIP experiment.

2. The two suppressor mutations in TTI1 that enables growth of sis1D strains may represent gain of function or alternatively, loss of function. In light of that overexpression of wild-type TTI1 also results in suppression of the growth phenotype the interpretation provided is that Tti1 functions independent of its heterotrimeric TTT complex. This raises the question if the TTI1 suppressors are dominant or recessive. If for example the suppressor mutations liberates/discharges Tti1 from the TTT complex, dominant suppression is predicted. A recessive phenotype would instead imply that loss of TTT complex function underlies the suppression. Please provide clarification on the recessive or dominant nature of the suppression.

3. The section Hsf1-dependent expression is variable in sis1D cells (p. 5 and Fig. 4) does not describe how tti1S#1 suppression impacts of Hsf1 activity. This is important information for connecting the data to Fig. 1-3 so that the mechanism of suppression can be interpreted. Overall it looks like tti1S#1 does not suppress the sis1D induced Hsf1 activity. The data suggest that the decreased levels of PIKKs are not the cause of Hsf1 activation in Sis1 depleted cells. I suggest editing the text in this section to bring the focus to tti1S#1.

4. Please provide information on the actual TTI1 mutations on DNA level, only the resulting amino acid substitutions appear to

be presented in the manuscript.

5. The title of the manuscript is somewhat heavy, I suggest an edit.

RESPONSE TO REVIEWERS' COMMENTS

Editor Remarks:
Your manuscript has now been reviewed by two experts, their comments are attached below. In light of their overall positive evaluation, we would be interested to receive a revised version that addresses the points raised by the two experts. As you can see, both ask for textual changes and some additional experiments. Particularly, experiments should be directed to clarify whether a potential recessive or dominant nature of your suppressor mutations underlies the observed phenotypes.
RESPONSE: We have made changes in the text to address the reviewers' comments and have added data to address the dominant/recessive question (new Supplementary Figure 2D) and the results of coIP experiments using Sis1-specific antibodies (new Supplementary Figure 1F).

Reviewer #1 (Remarks to the Author):

Hsp70 protein molecular chaperones are targeted to diverse substrates and pathways largely via the action of a large family of J domain-containing cofactors. In budding yeast, Sis1 is the only essential J-protein in the cytoplasm. Why it is required for survival when the abundant Ydj1 and other J proteins are available is not understood. In this brief report, Schilke and Craig identify mutations in the TTT complex protein Tti1 as loss of function suppressors of SIS1 deletion. Sis1 is shown to be required for stability of the TTT substrate PIKK kinases Mec1, Tra1 and both Tor kinases. Surprisingly, overexpression of Tti1 also suppresses sis1Δ lethality despite the stoichiometric imbalance with the other two TTT complex subunits. Finally, Tti1-suppressed cells exhibit differential activation of Hsf1 as demonstrated using reporter constructs with distinct HSE promoter architecture. Overall, this work establishes a previously unknown connection between the Hsp40/Hsp70 and TTT chaperone systems and highlights unique but still incompletely understood roles for Sis1 in Hsf1 regulation and beyond.

As a brief report, this manuscript generates some unique insights into chaperone biology, notably the unexpected connection between the TTT and Hsp40/70 systems. The experiments are impeccably performed but lean far more to the genetic than the cell biological in approach and outcomes. As a result, insight is gained but maybe not answers - what is Tti1 doing with Hsp70? With Hsp90? And what is going on with Sis1 and Hsf1? The experiments only provide a peek into what could be a larger and perhaps more impactful story.
**RESPONSE**: We agree with the reviewer that this study raises as many, if not more, questions rather than answers. We think the importance of our findings is that it raises questions that no one had even thought about before, because there was no reason to think in the direction of a relationship between TTT/ PIKKS and Sis1. Our results provide a reason to think in this direction. Hopefully long-term experiments will lead to an understanding of the mechanistic connection - after some of the challenges in working with PIKKS biochemically are overcome.

I have a few minor comments:

1) In the abstract the second to last sentence describes effects on "genes activated by Hsf1." The experiments examine effects on fluorescent reporter constructs with promoter fragments containing the respective HSEs, not endogenous genes. While a full RNA-seq experiment would be very informative, it is probably beyond the scope of this work so the abstract wording should be modified accordingly.
**RESPONSE**: We have rephrased this comment. We were thinking about the HSP genes themselves, but as we point out later in the manuscript these have other regulatory elements in addition to HSEs. We agree that a genome-wide RNA-seq analysis is beyond the scope of this Brief Report.

2) The loss of function suppressors formally allow growth but the results text fails to accurately mention the

exceedingly limited amount of growth observed. The spores in Fig.1 are just barely alive, consistent with a link between the two pathways but possibly a very tenuous one. In contrast, the overexpression phenotypes are far more robust and perhaps a better place to look for mechanistic insight.
**RESPONSE**: We agree that the overexpression (both WT and suppressor variants) has more potential for providing mechanistic insight than the original suppressors. We have now pointed out more directly in the text that the growth supported by the suppressor under control of the native promoter is quite poor. We also agree that overexpression is more likely to provide insight in the long run, and is why the experiments in much of Fig 3 and 4 use the overexpression construct.

3) It is hard to conclude too much from the Hsf1 experiments in Fig. 4, except that HSEs with different architecture respond differently to Hsf1 derepression. Given the recent advances in understanding the role of Ssa1/2/Hsp70 in direct binding to multiple Hsf1 domains to regulate transcriptional activity, it would be good to correlate Hsp70-Hsf1 binding in the suppressor strain to provide some level of mechanistic insight.
**RESPONSE**: Knowing how some level of Hsf1 repression is maintained in the absence of Sis1 would indeed provide some insight into Hsf1 regulation, but we believe is beyond the scope of this report.

Reviewer #2 (Remarks to the Author):

This manuscript describes a genetic suppressor screen that links the function of the Hsp70 cochaperone and J-domain protein Sis1 to the expression levels and function of phosphatidylinositol 3-kinase related kinases (PIKKs). The study provides insight that is important for the further study of PIKKs and specifically how conformations/folding/stability of these kinases are regulated by the TTT complex and Hsp70.

1. This study is well executed and convincingly links Sis1 function to PIKKs on the phenotypic level. However phenotypic relationships, especially in the core proteostasis system, can be indirect and hence the study would benefit from providing evidence that Sis1 (or Hsp70) physically interacts with PIKKs, for example a coIP experiment.
**RESPONSE**: We have now included a coIP experiment using Sis1-specific antibody (new Supplementary Fig 1F) that shows pulldown of FLAG-tagged Mec1 and Tra1 from cell lysates. Although this coIP does not demonstrate direct interaction of Sis1 with PIKKs, because of the possibility of indirect association in larger protein complexes, it certainly is consistent with that possibility.

2. The two suppressor mutations in TTI1 that enables growth of sis1D strains may represent gain of function or alternatively, loss of function. In light of that overexpression of wild-type TTI1 also results in suppression of the growth phenotype the interpretation provided is that Tti1 functions independent of its heterotrimeric TTT complex. This raises the question if the TTI1 suppressors are dominant or recessive. If for example the suppressor mutations liberates/discharges Tti1 from the TTT complex, dominant suppression is predicted. A recessive phenotype would instead imply that loss of TTT complex function underlies the suppression. Please provide clarification on the recessive or dominant nature of the suppression.
**RESPONSE**: We have carried out and reported the results of a dominant/recessive experiment (Supplementary Figure 2D). Sup#1 is substantially dominant – the heterozygous diploid growing only slightly more poorly than the homozygous suppressor diploid.

3. The section Hsf1-dependent expression is variable in sis1D cells (p. 5 and Fig. 4) does not describe how tti1S#1 suppression impacts of Hsf1 activity. This is important information for connecting the data to Fig. 1-3 so that the mechanism of suppression can be interpreted. Overall it looks like tti1S#1 does not suppress the sis1D induced Hsf1 activity. The data suggest that the decreased levels of PIKKs are not the cause of Hsf1 activation in Sis1 depleted cells. I suggest editing the text in this section to bring the focus to tti1S#1.

**RESPONSE**: We have edited the text to try to better express the complexity of parsing the effects of Sis1 on PIKK function on overall protein homeostasis and direct effects on Hsf1 activity.

4. Please provide information on the actual TTI1 mutations on DNA level, only the resulting amino acid substitutions appear to be presented in the manuscript.
**RESPONSE**: These DNA changes are now provided in the methods section describing suppressor isolation.

5. The title of the manuscript is somewhat heavy, I suggest an edit.
**RESPONSE:** We have changed the title to: “Essentiality of Sis1, a J-domain protein cochaperone of Hsp70, can be overcome by Tti1, a specialized PIKK chaperone”.

RE: Manuscript #E21-10-0493R
TITLE: "Essentiality of Sis1, a J-domain protein Hsp70 cochaperone, can be overcome by Tti1, a specialized PIKK chaperone"

Dear Dr. Craig,

thanks a lot for sending the revised manuscript. You seem to have addressed all the points well. However, you indicated in your rebuttal letter that you performed a new Co-IP experiment, which shows a complex containing Sis1,Mec1 and Tra1. However, in the submitted material I cannot find this experiment (you indicated that it should be in the supplement 1F), can you upload new files containg this result? Plus it would be good to mention this result in the main text.

Sincerely,
Martin Ott
Monitoring Editor
Molecular Biology of the Cell

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In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL):
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Eric Baker
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Rebuttal letter 2:

thanks a lot for sending the revised manuscript. You seem to have addressed all the points well. However, you indicated in your rebuttal letter that you performed a new Co-IP experiment, which shows a complex containing Sis1,Mec1 and Tra1. However, in the submitted material I cannot find this experiment (you indicated that it should be in the supplement 1F), can you upload new files containg this result? Plus it would be good to mention this result in the main text.

**RESPONSE:** My mistake – the added co-IP experiment is Supplementary Figure 1**E,** not 1**F**. The co-IP result is mentioned on page 4 at the end of the third paragraph in the files uploaded on December 8. I apologize for the mistake and wasting your time.

RE: Manuscript #E21-10-0493RR
TITLE: "Essentiality of Sis1, a J-domain protein Hsp70 cochaperone, can be overcome by Tti1, a specialized PIKK chaperone"

Dear Prof. Craig:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Sincerely,
Martin Ott
Monitoring Editor
Molecular Biology of the Cell

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Dear Prof. Craig:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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Journal Production Manager
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