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The TPR- and J-domain-containing proteins DJC31 and DJC62 are involved in abiotic stress responses in *Arabidopsis thaliana*

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Original submission

First decision letter

MS ID#: JOCES/2021/259032

MS TITLE: The tetratricopeptide repeat and J-domain containing proteins DJC31 and DJC62 are involved in abiotic stress response in Arabidopsis thaliana

AUTHORS: Sophie Dittmer, Tatjana Kleine, and Serena Schwenkert
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 raise a number of criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

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

Herein the authors characterized DJC31 and DJC62, two J-proteins that carry clamp type tetratricopeptide repeat domains. They showed that both proteins are attached to the cytosolic side of the endoplasmic reticulum membrane and interacted with cytosolic HSP70 and HSP90. The double mutant exhibited severe defects in growth and development, affecting almost all organs, are sensitive to osmotic stress and treatment with abscisic acid, but surprisingly exhibited enhanced tolerance to drought.

Comments for the author

The ms is well written, and executed. The results are clear and to the point.
The authors could detail a number of genes (down- or upregulated) found by transcriptome analysis by using RT-PCR. However, since this RNA analysis, at present, shows the stastus quo of the transcripts without any identification of gene regulatory scheme by these genes and because these genes/proteins are engaged in interactions with HSP90/70 that could influence the outcome of the transciptime, it may be not appropriate to pursuit it. However, I urge the authors to investigate, in the future, the interactome with the molecular chaperones and how this is influencing transcriptional control.

Reviewer 2

Advance summary and potential significance to field

Review of “The tetratricopeptide repeat and J-domain containing proteins DJC31 and DJC62 are involved in abiotic stress response in Arabidopsis thaliana”

This is an interesting Ms where the authors describe the function of two Arabidopsis genes encodes two chaperones that have important physiological roles. The study is nicely performed because there is no functional information on the role of these genes in plants.
They also showed a nice biochemical study and nice results indicating that these proteins are in the cytosolic side of the membrane...

Comments for the author

Review of “The tetratricopeptide repeat and J-domain containing proteins DJC31 and DJC62 are involved in abiotic stress response in Arabidopsis thaliana”
This is an interesting Ms where the authors describe the function of two Arabidopsis genes encodes two chaperones that have important physiological roles. The study is nicely performed because there is no functional information on the role of these genes in plants.

They also showed a nice biochemical study and nice results indicating that these proteins are in the cytosolic side of the membrane...
However, I have a few comments that could help to improve the Ms.
There is now a consensus that the use of BiFC Not enough at this stage to claim the interaction of two proteins, however I believe that with the previous information about the interaction with Hsp70 and 90 could be enough. The good thing about BiFC is that can provide subcellular information of the where the interaction takes place, but they did not comment on this.
Lines 34-35 and 36. There is something missing in this sentence. I really don´t get it.
Line 81. In addition to the homology, these proteins contain a similar structure. The next homolog is TTL4 so clearly makes sense to study these proteins together. I think they should comment something about this, homology seems a little informative.

These proteins have a clear IDR at the N-terminus using predictive programs. This is mentioned in the discussion, but I think is worth indicating it in the result section. IDRs are now a hot topic and, although its not a domain by itself can be considered a protein-protein interaction region that changes the structure depending of the interacting partner.

Line 104-106. What is the rationale for selecting this region of the protein? I guess that is because is up to the first TPR. Probably should be included in the text.

I found it interesting that the authors could not detect the protein after overexpression in benthamiana or Arabidopsis protoplasts, but they are about to detect the proteins in a western. The result is very convincing because in the western they use the Abs in the mutant analysis. To be honest, this is the first time I have seem this, enough endogenous proteins to be detected by Western but no expressed enough in protoplast or benthamiana… what could be the reason? Are they sure the constructs is correct? Is the same they used in the stable transgenics?

I am missing more information (or maybe I missed it) of the number of lines that they analyzed for the complementation studies. This is particularly important when the found that the mutated version of DJC31 and DJC31 did not complemented the phenotypes.
djc31xdjc62 is a strange way of indicating a double mutant. It is not the standard way to do it and it looks like a cross. Maybe is the way recommended by the journal.
I found strange that the authors first describe the phenotypes of single and double mutants and then show the molecular analysis of the T-DNA. It does not make sense to show that these lines, have the insertion, talk about phenotypes and then the complementation?

First revision

Author response to reviewers' comments

Reviewer 1:

The authors could detail a number of genes (down- or upregulated) found by transcriptome analysis by using RT-PCR. However, since this RNA analysis, at present, shows the status quo of the transcripts without any identification of gene regulatory scheme by these genes and because these genes/proteins are engaged in interactions with HSP90/70 that could influence the outcome of the transciptime, it may be not appropriate to pursuit it. However, I urge the authors to investigate, in the future, the interactome with the molecular chaperones and how this is influencing transcriptional control.

Answer: We would like to thank the reviewer for this suggestion, but agree that this is not in the scope of the current manuscript. We are certainly aiming to analyze the transcriptomic changes in more detail in the future.

Reviewer 2:

There is now a consensus that the use of BiFC is not enough at this stage to claim the interaction of two proteins, however I believe that with the previous information about the interaction with Hsp70 and 90 could be enough. The good thing about BiFC is that can provide subcellular information of the where the interaction takes place, but they did not comment on this.

Answer: We agree with the reviewer that alternative methods to verify the interaction would be beneficial. Unfortunately, the DJC proteins proved to be hard to overexpress and purify from E. coli in a soluble state, making the usage of assays requiring the recombinant protein impossible at this stage. Nevertheless, we will pursue these questions and hope to gain more information on the mode of interaction in the future.

We also have added a comment on the interaction site in lines 244-246, which reads: ‘Notably, the interaction was observed in the cytosol, thus strengthening the prior observation that DJC31 and DJC62 are facing the cytosolic side of the ER membrane.’

Lines 34-35 and 36. There is something missing in this sentence. I really don´t get it.

Answer: We have rephrased the sentence it now reads: ‘Among the HSP family, we find HSP70 (DnaK), HSP90 and the essential co-chaperone HSP40. The latter is a J-domain containing protein that regulates ATP hydrolysis activity as well as substrate release of HSP70.’

Line 81. In addition to the homology, these proteins contain a similar structure. The next homolog is TTL4 so clearly makes sense to study these proteins together. I think they should comment something about this, homology seems a little informative. These proteins have a clear IDR at the N-terminus using predictive programs. This is mentioned in the discussion, but I think is worth indicating it in the result section. IDRs are now a hot topic and, although it’s not a domain by itself can be considered a protein-protein interaction region that changes the structure depending of the interacting partner.

Answer: In the revised manuscript we highlighted the fact that DJC31 and DJC62 have a similar structure (line 82-84: Moreover, a highly similar structure is predicted for DJC31 and DJC62, indicating that the proteins might be redundant in function.) and added more information on the IDR region in the results section, lines 89-87: ‘Interestingly, both proteins also contain a J-domain at their extreme C-terminal ends. No known structural features are predicted for the large N-terminus, instead they show a tendency towards forming intrinsically disordered protein regions (Fig. S1A).’

Line 104-106. What is the rationale for selecting this region of the protein? I guess that is because is up to the first TPR. Probably should be included in the text.

Answer: Yes, the reviewer is right, we chose the region up to the TPR domain. We have now included this information in the text.

I found it interesting that the authors could not detect the protein after overexpression in benthamiana or Arabidopsis protoplasts, but they are about to detect the proteins in a western. The result is very convincing because in the western they use the Abs in the mutant analysis. To be honest, this is the first time I have seem this, enough endogenous proteins to be detected by Western but no expressed enough in protoplast or benthamiana… what could be the reason? Are they sure the constructs is correct? Is the same they used in the stable transgenics?

Answer: For the transient expression in N. benthamiana or A. thaliana a construct with a C-terminal GFP-tag was used to clarify the localization. For generation of the stable lines, however, the protein was expressed without the GFP tag (in the same vector, but with a stop codon before the GFP). We assume that the C-terminal GFP-tag caused problems in the transient experiments, possibly by interfering with the protein folding and by making it more prone to aggregation and/or degradation.

I am missing more information (or maybe I missed it) of the number of lines that they analyzed for the complementation studies. This is particularly important when the found that the mutated version of DJC31 and DJC31 did not complemented the phenotypes.

Answer: The in formation was indeed missing, thank you for pointing it out. We have now added a sentence in the figure legends stating that one representative of three independent lines is shown.

djc31xdjc62 is a strange way of indicating a double mutant. It is not the standard way to do it and it looks like a cross. Maybe is the way recommended by the journal.

Answer: We agree with the reviewer and have change it to djc31djc62.

I found strange that the authors first describe the phenotypes of single and double mutants and then show the molecular analysis of the T-DNA. It does not make sense to show that these lines, have the insertion, talk about phenotypes and then the complementation?

Answer: We agree with the reviewer and have changed the order of the experiments.

Second decision letter

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MS TITLE: The tetratricopeptide repeat and J-domain containing proteins DJC31 and DJC62 are involved in abiotic stress response in Arabidopsis thaliana

AUTHORS: Sophie Dittmer, Tatjana Kleine, and Serena Schwenkert
ARTICLE TYPE: Research Article

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

Reviewer 1

Advance summary and potential significance to field

DJC31 and DJC62 proteins with TPR domains could act as regulators of HSP70/HSP90 dependent signaling pathways involved in plant development and stress response.

Comments for the author

The paper is satisfactory as it is.