THE EMBO JOURNAL

Rebuilding core abscisic acid signaling pathways of Arabidopsis in yeast

Moritz Ruschhaupt, Julia Mergner, Stefanie Mucha, Michael Papacek, Isabel Doch, Stefanie V. Tischer, Daniel Hemmler, David Chiasson, Kai H. Edel, Jörg Kudla, Philippe Schmitt-Kopplin, Bernhard Küster, and Erwin Grill

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Submission date:	21st Feb 2019
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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 17th Apr 2019

Thank you for submitting your manuscript for consideration by the EMBO Journal. I sincerely apologise for the unusual delay in the assessment of your work due to belated submission of referee reports. We have now received two reports on your manuscript, which are included below for your information.

As you will see from the comments, both reviewers appreciate the work and the quality of the data and recommend publication of the manuscript. Given these positive evaluations from two experts of the field, I would like to invite you to submit a revised version of your manuscript, in which you address the comments of reviewer #2.

REFEREE REPORTS:

Referee #1:

In general, a nice piece of work showing that yeast can be used to study the plant ABA signaling machinery. Grill's earlier discovery of the ABA receptor is perhaps one of the most important findings in plant basic research in the past two decades, up there with Estelle's discovery of the auxin receptor. The discovery that phosphatases are the direct target of the ABA receptor and that a kinase is also intimately involved are paradigms not seen in animal systems (yet), in terms of a protein phosphatase that is DIRECTLY regulated by a hormone receptor. Despite my great enthusiasm for Grill's earlier work and for the careful study described in this manuscript I am still left wondering whether the yeast system has really added a great deal of additional information not available with the protoplasts. However given the fact that yeast has not been used to reconstruct this machinery before and that without question it offers a great many new approaches to using synthetic biology for studying key aspects of the ABA dependent phosphatase and kinase, I highly recommend acceptance of this paper. This is not only excellent basic research but also, since ABA controls the movement of water out of a plant via stomata and also, so many other aspects of plant

life (embryo desiccation and then germination), that this will undoubtedly also prove to be a very important aspect of translational efforts for improving plant water use efficiency.

Referee #2:

This is a very interesting manuscript by Ruschhhaupt and colleagues. Here, the authors analyzed the core abscisic acid (ABA) signaling factors of Arabidopsis thaliana in yeast to dissect ligand-receptor specificities in a functional and multiplexed approach.

This study is very compelling and elegant. The authors took advantage of the yeast system to rapidly test many different combinations of pathway components and extract quantitative information. As mentioned in the manuscript, yeast was previously used successfully as a system to test many components of the auxin signaling pathways (Pierre-Jerome et al., PNAS 2014). In the present manuscript, Ruschhhaupt and colleagues go even deeper in the analysis of the ABA signaling core. They even identify SnRK2.1, 2.4, 2.5 and 2.10 (part of the subgroup I of the Arabidopsis SnRK2 protein kinase family) as putative candidates for being part of the ABA signaling core in Arabidopsis thaliana. The present manuscript should trigger new in planta studies, for example on roles of other SnRK2s in direct ABA signal transduction.

Overall, I believe the presented approach and results will be widely used and of broad interest to the research community.

Minor comments:

1) Line 135: the authors mention Fig. 1C but there is no such figure in the present manuscript.

2) Lines 148-149: RCAR1 does not activate signaling in yeast without exogenous ABA (Fig. 2A). However, this observation is true in Arabidopsis protoplasts (Fig. 2B).

3) Fig. 4C: is there any particular reason why OST1 was not used in this LUC-activity assay? OST1 is used throughout the manuscript for obvious reasons but not here. It should be included here as well.

4) Line 189: "subgroup II" is misspelled.

1st Revision - authors' response 1st Jul 2019

Referee #1:
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Overall, I believe the presented approach and results will be widely used and of broad interest to the research community.
We very much appreciate the input of the editor and both reviewers, and their positive feedback. We addressed the points raised one-by-one as stated below.
Minor comments:
1) Line 135: the authors mention Fig. 1C but there is no such figure in the present manuscript.
Response: Sorry, meant is Fig. 1B.
2) Lines 148-149: RCAR1 does not activate signaling in yeast without exogenous ABA (Fig. 2A). However, this observation is true in Arabidopsis protoplasts (Fig. 2B).
Response: There is an approximately twofold induction of ABA signaling by RCAR1 in yeast ($p < 0.001$; one-tailed t-test; see also now Table 2 of the appendix).
3) Fig. 4C: is there any particular reason why OST1 was not used in this LUC-activity assay? OST1 is used throughout the manuscript for obvious reasons but not here. It should be included here as well.
Response: The data for OST1 have been presented in Fig. 1A and was therefore omitted. We include now the data for better comparison in Fig. 4C.
4) Line 189: "subgroup II" is misspelled.
Response: corrected.

2nd Editorial Decision 9th Jul 2019

Thank you for submitting the revised version of your manuscript. The main issues have now been addressed and I am happy to inform you that your manuscript has been accepted for publication. Congratulations on a nice study!

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Erwin Grill
Journal Submitted to: EMBO J
Manuscript Number: 101859

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Analysis of large sample size experiments in yeast revealed that normal distribution occurred with an average s.d. of about 30%. For ABF transactivation studies in yeast we choose a sample size of 6 in total to analyze an effect of 3xsigma with a 0.05 α-probability and a power (1-β) of 90%. For comparisions of single core-cascades we increase n to 12 in general, to reduce the detectabel effect size to 1.5 x sigma. These parameters were evaluated using the G*Power 3.1.9.4 software for an unpaired, two-sided t-test.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Yeast samples showing no growth on selective induction media were excluded from the analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Analysis of large sample size experiments revealed normal distribution. Normal distribution was tested using OriginPro 2018b.
Is there an estimate of variation within each group of data?	No

USEFUL LINKS FOR COMPLETING THIS FORM

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http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

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http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	No

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	monoclonal ANTI-FLAG® M2 antibody; Sigma-Aldrich, #F1804
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Proteomic data can be downloaded from the ProteomeXchange consortium (ID: PXD013669) and the Panorama web repository server (https://panoramaweb.org/x8nkdT.url; ID: PXD013849).
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	Source Data of main figures are avalible online.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA