Bound by Fate: Reactive Oxygen Species in Receptor-Like Kinase Signaling

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Review timeline:

TPC2016-00947-REV	Submission received:	Dec. 20, 2016
	1st Decision:	Feb. 6, 2017 *revision requested*
TPC2016-00947-REVR1	1st Revision received:	Mar. 8, 2017
	2nd Decision:	Mar. 16, 2017 *acceptance pending, sent to science editor*
	Final acceptance:	Mar. 29, 2017
	Advance publication:	Apr. 3, 2017

REPORT: (The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2016-00947-REV 1st Editorial decision – *revision requested* Feb. 6, 2017

We have received reviews of your manuscript entitled "Bound by fate - reactive oxygen species in receptor-like kinase signaling." Thank you for submitting your best work to The Plant Cell. The editorial board agrees that the work you describe is substantive, falls within the scope of the journal, and may become acceptable for publication pending revision, and potential re-review.

We ask you to pay attention to the following points in preparing your revision.

The editors agree with Reviewer 3 that the article could be re-organized, and this reviewer gives good suggestions for the focus of sections. Currently it is not entirely well-organized: for example the first major section is one short paragraph, followed by a lengthy second section that is split into four subsections, and so on. The structure suggested by the reviewer would also help to provide logical sequence to the focus and main points/conclusions. For example, the short first section could be moved to a later section focused on ROS in the apolplast. Likewise, the first comment of Reviewer 2 could be addressed by rewriting and moving the section on systemic signaling to a major section as outlined by Reviewer 3. All other comments (which are not extensive) of all three reviewers should be addressed to the best of your ability. We encourage the use of a professional editing service to ensure that the work is presented in the best manner and reaches the broadest possible audience.

---------- Reviewer comments:

[Reviewer comments shown below along with author responses]

TPC2016-00947-REVR1 1st Revision received Mar. 8, 2017

Reviewer comments and **author responses**:

We reorganized the manuscript according to the suggestions of Reviewer 3. Specifically, we moved the short first section to the section dealing with the apoplast and named the section "The apoplast: an arena for ROS signaling". The sections on oxidative modifications and apoplastic pH now appear as subsections under this header. We follow with the section "RLKs: mediators of apoplastic ROS perception", which is in turn divided into subsections on localized ROS perception, intracellular perception of apoplastic ROS, and the interplay of RLKs and ROS during long-distance signaling. Finally, we separated the section on "Guard cells: a model for ROS and MAMP/DAMP perception research" into two more subsections to balance the manuscript.

We also used a professional editing service to further improve the manuscript.

Reviewer #1:

This is timely, informative and though-provoking review that gives a useful overview of current knowledge and concepts regarding the relationships between reactive oxygen species (ROS) and receptor-like kinase (RLK) signalling in plants. Current knowledge concerning interactions between the different signalling systems in the apoplast/cell wall compartment is particularly well explained giving a current overview of the plant chemosensory system, which facilitates sensing and discrimination between different signals.

RESPONSE: We thank the reviewer for the positive comments about our manuscript.

Relationships between RLK-mediated and ROS-mediated and RAPID ALKALINIZATION FACTOR (RALF) are discussed. If I understand correctly, the authors suggest that these are largely independent pathways that may act either in concert or independently. F-RALF co-opts the receptor-like kinase FERONIA (FER) FER to induce extracellular alkalinisation in roots, thereby enhancing fungal invasiveness and suppressing the plant immune response. Similarly, ROS-generating enzymes from the plant and the interacting micro-organism also act in concert.

RESPONSE: Interestingly, shortly after submitting our manuscript, an article describing the intricate roles of FERONIA as a scaffold that regulates immune responses to different RALF peptides was published (Stegmann et al., 2017, Science 355(6322):287-9). We integrated information from this article into the section on the control of ROS production by RLKs.

Reviewer #2:

This is a very nice review that addresses the role of RLKs in ROS dependent signaling in response to different signals and stimuli. The corresponding author was involved in at least one other review that presented RLKs as mediators of ROS signaling (Wrzaczek, M., Brosché, M., and Kangasjärvi, J. (2013). ROS signaling loops - production, perception, regulation. Curr. Opin. Plant Biol. 16: 575-582). However, the current review is an improvement on the previous ones and includes new data published by other labs since. The review is very nicely written and the discussion is very good and in depth. The figures are also very helpful. I also liked the discussion of the apoplast as a site for signaling/ROS and the relationship between pH changes and signaling at that site.

RESPONSE: We thank the reviewer for the positive comments about our manuscript.

Points that I feel should be addressed:

Point 1. The paper mainly summarizes responses to pathogens and not abiotic stress (see Table 1), and the part about systemic signaling is a little pre-mature and reminds me of an opinion paper in TiPS (not a review in TPC). There simply isn't enough evidence currently to support this hypothesis. May be this part should be downplayed a bit or incorporated within a different section of the paper.

RESPONSE: We agree with the reviewer that, while there are striking parallels and common mechanisms between systemic RLK and ROS signaling, there are currently no data that convincingly link the roles of RLKs and ROS in long-distance signal transduction. To comply with the reviewer's comment, we shortened this section and emphasized the hypothetical nature of the text. Furthermore, the section was moved to a subsection within the larger section "RLKs: mediators of apoplastic ROS perception".

Point 2. In fig 2 it would be nice to add an arrow showing what reactions are enhanced by low pH (as in the apoplast), also, what is the role of GSH and NO in these processes?

RESPONSE: We modified Figure 2 in agreement with the reviewer's suggestion and have also included a brief discussion on the roles of GSH and NO.

Point 3. In Table 1 it would be helpful to know what results came from studying mutants (and the mutant names) and what were based on pharmacological evidence or other types of experiments.

RESPONSE: We reformatted the table and included the requested information.

Reviewer #3:

The authors provide a comprehensive, timely and up-to-date review which ties together two in the past separately addressed topics of ROS and Receptor-Like Kinase signalling. ROS, originally linked (and restricted) to "oxidative stress", has gained increasing attention in the context of abiotic and biotic stress signalling and in particular recently in the signal distribution via long distances in a plant. Likewise, ligand/RLK receptor-mediated ROS generation had almost exclusively been discussed in the field of plant pathology, whereas "developmental ROS" such as in root growth or pollen development had been considered an entirely different process.

This review accommodates recent literature in which ROS is manifested as overall signal component associated to RLK signalling in controlling plant life within a plant as well as with its environment.

RESPONSE: We thank the reviewer for recognizing the relevance of the topic of our manuscript and for the very helpful suggestions discussed below.

In principle, I am tempted to subdivide this review in several chapters (more and less along the text):

1) RLK mediate ROS activation (ROS downstream of RLK)

2) ROS in the apoplast (- role of pH)

3) Potential molecular mechanisms which allow the sensing of ROS

4) RLKs as ROS sensors (RLK downstream of ROS)

5) Examples

RESPONSE: We agree with the structure proposed by Reviewer 3. This aligns with comments from the editors and Reviewer 2 (please see the responses above). In brief, we moved the short first section of the original manuscript to the section dealing with the apoplast in the revised version, which is now named "The apoplast: an arena for ROS signaling". The sections on oxidative modifications and apoplastic pH are now presented as subsections under this header. We follow with the section "RLKs: mediators of apoplastic ROS perception", which is in turn divided into subsections on localized ROS perception, intracellular perception of apoplastic ROS, and the interplay between RLKs and ROS during long-distance signaling. Finally, we separated the section on "Guard cells: a model for ROS and MAMP/DAMP perception research" into two more subsections to balance the manuscript.

Points in favour:

The authors provide an impressive comprehensive amount of literature, encompassing mechanistic details as well as biological data to support the emerging and exciting topic of RLK - ROS interrelation. Points of discussions are - in principle - well supported by citations and more than once are thought-provoking, exactly what one would expected from a high quality review.

In this context the chapter of ROS perception via oxidative protein modification appears particularly useful and informative. By integrating molecular and biochemical mechanisms with up-to-date developmental or stress-physiological results in a homogenous text of clear interpretations and even hypotheses, this review has potential becoming a citation classic.

RESPONSE: We very much appreciate this encouraging statement from the reviewer!

Points distracting:

The review does not yet seem entirely balanced in the way single topics are developed or presented as main chapters or as a side information. In part the review lacks coherence and focus, it is not clear what is meant, and language is not always used with sufficient precision.

It has to be clear when data are cited from original literature (ideally one may even like to know the experimental system - plant/approach, in vitro, in vivo, heterologous system), which is to be preferred, or when reviews are used to support (not to substitute for!) an interpretation. Also, it has to be clear, for what a citation is mentioned. (Otherwise, apart from being incorrect, funny things may happen, see: l. 418 "nearly a decade ago", which here is followed by a citation from 2014). A recent review about ROS in guard cell signalling (Sierla et al, 2016) is cited fairly often, understandably given the topic, but this must not replace independent development of argumentation!

RESPONSE: We agree with the reviewer and have improved the presentation by referring to original data instead of simply citing reviews to support our arguments throughout the manuscript.

Data should be clearly separated from their interpretation/sorting by the authors enabling an independent line of argumentation.

RESPONSE: We separated the data from interpretations more clearly throughout the manuscript.

Finally, the generation of well justified hypotheses / speculation (the "icing of the cake" for every review that is fun to read) should again kept distinct - at the end of a chapter (?).

RESPONSE: We moved the discussions of hypotheses towards the ends of sections wherever possible.

Overall concept:

The authors may want to consider the most prominent issues to sell their topic (see above): these should be sorted in a logic order, highlighted (already in the ABSTRACT), these should get main attention and focus, and their dilution by side aspects should be avoided. (To me one of the most novel, prominent aspects pointing into the future is RLK as potential ROS "sensor"?).

RESPONSE: We have rewritten the Abstract in accordance with the reviewer's suggestion. We also modified the order of the text in accordance with suggestions from the editors as well as Reviewers 2 and 3.

If it the hypothesis of a ROS "receptor" is discussed (nothing against it!), or the differentiation between "sensor" and "receptor" is to be addressed, the authors may want to consider that in biochemical terms "receptor-mediated" perception is defined by dose-response effects, saturation, reversibility, physiological concentrations of "ligand-binding". How does this relate to ROS "sensing" by oxidative modification of proteins and to the experiments cited out of the literature?

RESPONSE: This is a very important comment. Indeed, we do not propose that RLKs "bind" ROS in a traditional sense (as is the case for peptide receptors such as FLS2 and PEPR1 and for the perception of the plant hormone brassinosteroid by its receptor, BRI1). Thus, we now consistently use the term ROS sensor, as suggested by Reviewer 3.

Clarity in language/ topic:

It is not always clear what the authors mean when using the term "specificity", "ROS-specificity" or "crosstalk".

RESPONSE: We clarified our use of the terms "specificity", "ROS-specificity", and "crosstalk".

l. 264/265: The first two lines are contradictory and hint to a general point of discussion in the review in its present form: Are all RLCKs (and are RLKs) able to directly phosphorylate RBOHs? As it stands, the authors use the terms "to target", "to be a phosphorylation substrate", or "to mediate" synonymously. The same holds true to ROS "sensor" and ROS "receptor". In biochemical speaking this is incorrect. Language used to explain non-covalent associations to guide ligand-receptor interaction (l. 104 - 107) is as presented at least misleading (and not sufficient) if not wrong.

RESPONSE: We agree that lines 265–265 (in the original manuscript) were confusing, as we mixed up the findings that several RLCKs can in parallel or redundantly participate in the activation of NADPH oxidase-dependent ROS production, while other RLCKs participate in the negative regulation of ROS production. We therefore rephrased the statement to improve the clarity of the text.

We also clarified the terms "target", "phosphorylate", and "mediate". We use phosphorylate for cases where direct phosphorylation of a substrate by a kinase has been experimentally verified. In cases where this information is lacking but comparison of data suggests that direct phosphorylation of the substrate by the kinase could take place, we mention that this has not been experimentally shown. We now use "mediate" to describe more indirect effects, as in, e.g., "The FER-ROPGEF-ROP module also controls ROS-mediated pollen tube reception in the female gametophyte".

We agree with the reviewer's comment about the ROS receptor and have therefore changed "ROS receptor" to "ROS sensor" throughout the manuscript.

We rephrased the description of ligand–receptor interactions and refer to a recent review (Hohmann et al., 2017

Annual Reviews in Plant Biology) for further reading.

If a paragraph is outlined to discuss apoplastic ROS (l. 58/59), is should be about apoplastic ROS; if different subcellular locations or intracellular ROS is topic of interest, then it should be addressed accordingly.

RESPONSE: We appreciate the comment and have rephrased the introductory paragraph to which the reviewer is referring. We are reluctant to entirely omit a discussion of intracellular ROS production in plants. However, the paragraph now begins with the statement that RLK signaling frequently induces ROS production in the apoplast. We then describe different types of ROS and explain that ROS can generally be produced in many different subcellular compartments. We finish by emphasizing that it is currently unknown how plant cells differentiate between different types of ROS and the subcellular origin of ROS.

Author actions:

Focus on key topics (eventually re-sort order of presentation and omit sub-topic), re-design review but rather to cumulate in subject of most current novelty and thought/experiment-provoking hypotheses.

RESPONSE: As described above, we restructured the manuscript in accordance with the suggestions of the editors and reviewers. We opted not to omit subsections, but rather to balance the use of sections and subsections throughout the manuscript to improve the clarity of the text.

Separate data from their interpretation and from hypotheses.

RESPONSE: We separated data from interpretation and hypotheses throughout the manuscript wherever possible.

Clearly differentiate in context and in language between direct and indirect effects in RLK-ROS interconnections.

RESPONSE: We improved the precision of the language used when referring to direct and indirect effects.

TPC2016-00947-REVR1 2nd Editorial decision – *acceptance pending* Mar. 16, 2017

We are pleased to inform you that your review article entitled "Bound by Fate: Reactive Oxygen Species in Receptor-Like Kinase Signaling" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff. At this stage, your manuscript will be evaluated by a Science Editor with respect to scientific content presentation, compliance with journal policies, and presentation for a broad readership.

Final acceptance from Science Editor Mar. 29, 2017