Receptor Kinases in Plant Pathogen Interactions: More than Pattern Recognition

Dingzhong Tang, Guoxun Wang, and Jian-Min Zhou

Plant Cell. Advance Publication March 16, 2017; doi: 10.1105/tpc.16.00891

Corresponding author: Jian-Min Zhou (jmzhou@genetics.ac.cn)

Review timeline:

TPC2016-00891-REV	Submission received:	Nov. 27, 2016
	1st Decision:	Jan. 26, 2017 *accept with minor revisions*
TPC2016-00891-REV1	1st Revision received:	Feb. 17, 2017
	2nd Decision:	Mar. 1, 2017 *acceptance pending, sent to science editor*
	Final acceptance:	Mar. 16, 2017
	Advance publication:	Mar. 16, 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.)

TPC2016-00891-REV 1st Editorial decision – *revision requested* Jan. 26, 2017

We have received reviews of your manuscript entitled "Receptor kinases in plant pathogen interactions: more than pattern recognition." Thanks for your submission of this well-written review article. On the basis of the advice received, the board of reviewing editors would like to accept your manuscript for publication in The Plant Cell. This acceptance is contingent on revision based on the comments of our reviewers. In particular, please consider the following:

All three reviewers make important comments that should be relatively easy to address.

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

[Reviewer comments shown below along with author responses]

TPC2016-00891-REV1 1st Revision received Feb. 17, 2017

Reviewer comments and **author responses**:

Reviewer #1:

This review manuscript presents a very nice summary of recent updates in plant RK studies, with a particular focus on plant-pathogen interactions. It is timely and will be appreciated across a broad readership well beyond the plant immunity field. I really enjoyed reading this manuscript, but have some concerns outlined below.

Point 1. These studies support the view that SERKs are co-receptors for the three PRRs, but did not undertake similar (structural) approaches.

RESPONSE: We now clearly state which structures of receptor complexes have been done and have added Figure 1 to illustrate mechanistic similarity among different complexes.

Point 2. Is there solid (structural) evidence that SERKs also bind their ligands and act as co-receptors for all these RKs?

RESPONSE: See response above.

Point 3. BAK1 and SOBIR1 both act as a co-receptor or adapter for receptors. Additional explanation is needed to "in the absence of ligands".

RESPONSE: We now state "BAK1 and SOBIR1 constitutively associate with each other only in seedlings lacking BIR1, suggesting that BIR1 prevents the interactions between BAK1 and SOBIR1 in the resting state".

Point 4. Overall, this paragraph is quite superficial. It seems to me over-ambitious to introduce an enormous volume of studies on pathogen effectors in such a limited space. It is unclear how the authors put a particular focus on MPK-targeting effectors or AvrPiz-t and APIP6, in addition to those directly targeting PRR complex components. An effector can indirectly influence an early PTI output, even when acting far from PRR complexes. In my view, it is better to simply present Fig. 3 to have a summary and refer to other recent reviews on the topic, and then discuss, e.g., how these effector studies help advance our understanding of RK/PTI signaling, as new effective approaches to RK/PTI regulatory mechanisms are central to this review article.

RESPONSE: Excellent suggestion. We have deleted most of this part and added review papers for readers (lines568–569). We then discussed how these studies advanced our understanding of PRR signaling (lines 575–585).

Reviewer #2:

The manuscript "Receptor kinases in plant-pathogen interactions: more than pattern-recognition" is a thorough, timely and nicely written review reporting on the many roles played by receptor kinase in plants. The work nicely reflects the maturity of this research field. Thus, I recommend publication wholeheartedly and anticipate that the review will be highly cited by researchers working on plant immunity and by researchers working on plant development as well.

Point 1. When the authors present the csp22/NbCSPR receptor ligand pair reported by Saur et al., 2016 PNAS, it would be nice to include and discuss the recent work performed in the Felix laboratory: "The pattern-recognition receptor CORE of Solanaceae detects bacterial cold-shock protein" by Wang et al., 2016 Nature Plants.

RESPONSE: We have added this important work: "A recent success story is the identification of CORE as a receptor for csp22 (Wang et al., 2016b). csp22 sensitivity was observed in many, but not all, Solanaceae species. Exploitation of natural variation among cultivated and wild tomato species allowed the isolation of CORE required for csp22 perception. Transformation of CORE into Arabidopsis, which lacks CORE, confers csp22 sensitivity and disease resistance to *P. syringae.*"

Point 2. Recent work reports on the role of FERONIA on PRR receptor complex formation. I would therefore encourage the authors to include and discuss the new data in the section where the *Fusarium oxysporum* RALF peptide (F-RALF) is discussed.

RESPONSE: We have added this important work: "A previous study suggested that FLS2 and BAK1 exist in a preformed complex prior to flg22 treatment (Sun et al., 2013). A very recent study identified FER as a protein modulating flg22, elf18, and chitin signaling, as a loss-of-function *fer* mutant displays reduced PAMP responses and increased susceptibility to *P. syringae* (Stegmann et al., 2017). FER is also enriched in microdomain after flg22-treatment (Keinath et al., 2010) and weakly interacts with both FLS2 and BAK1 (Stegmann et al., 2017). The flg22-induced FLS2-BAK1 and elf18-induced EFR-BAK1 interactions are compromised in *fer* seedlings, indicating that FER promotes ligand-induced dimerization of the receptors and co-receptor (Stegmann et al., 2017). Furthermore, co-treatment of seedlings with RALF23 peptide or overexpression of *RALF23* decreases PAMP-induced responses and FLS2-BAK1 and EFR-BAK1 interactions (Stegmann et al., 2017). Together, the study supports that FER can act as a scaffold protein mediating PAMP-induced PRR complex formation and that RALFs actively regulate this process. Similar to the FLS2-FER interaction, the Arabidopsis Malectin-like/LRR-RLK IMPAIRED OOMYCETE SUSCEPTIBILITY1 (IOS1) was recently shown to interact with FLS2, EFR and CERK1, and contributes to pattern-triggered immunity (Yeh et al., 2016). An intimate interaction between different receptor complexes may facilitate cross-talk between different pathways. It will be extremely interesting to test whether this is a common feature for RKs and RPs."

Point 3. The stereotypic signaling system that perceives the CLE peptides are not mentioned anywhere. I think that the work would greatly benefit from a mention. In this respect, the two major receptor complexes CORYNE (CRN)

and CLV2 act together, and in parallel with CLV1, to perceive the CLV3 signal. It would be nice to speculate whether effector CLE peptides from other organisms, e.g., nematodes have evolved specifically to target one system in particular or rather the two systems together. Of note, the paradigms for CLV3 perception by two receptor complexes reminds me of the perception of csp22 by either an RLK or an RLP signaling system (see point #1). This could be discussed as well. Along this line, chitin perception also seems to involve RLK and RLP receptor complexes. I see here very nice and overlapping discussion points.

RESPONSE: We have incorporated this excellent suggestion: "The perception of chitin by the Arabidopsis LysM-RK LYK5 and rice LysM-RP CEBiP echoes the recent findings that bacterial cold shock protein epitope csp22 is perceived by the tomato LRR-RK CORE and tobacco LRR-RP NbCSPR (Saur et al., 2016; Wang et al., 2016b). Interestingly, the peptide hormone CLAVATA3 (CLV3p) is perceived by not only the LRR-RK CLV1, but also by a likely LRR-RP CLV2, although direct ligand binding has been shown only for CLV1 (Soyars et al., 2016). Thus, an RK and an RP can perceive the same ligand with a shared ECD."

Reviewer #3:

Overall, this review is well written and fills in a gap in the literature, making it worthy of publication. Most recent reviews in the field focus on single classes of receptors or on receptors that play roles in either immunity or growth/development. A larger scale allows for a discussion of the issues and commonalities that allow for cross-talk and coordination of these pathways. A few changes to the text I think could accentuate these unique features and in addition to adding recent advances in the field would make this review a good addition for the community.

Point 1. I think that the flow of the article could be greatly improved if the long lists of items are removed from the text. For example, the lists of the known PRRs and their ligands are not necessary. Instead the text should make reference to the tables, which are never referred to in the text.

RESPONSE: We have removed many of the descriptions of PRRs and ligands and kept them in the table. We have also removed much of the effectors targeting PRR complexes.

Point 2. If these items are removed there is more room to instead discuss examples that highlight interesting research findings and techniques. For example, an extended discussion of the structural insights available in the FLS2-BAK1-flg22 complex could be added and used as a starting point for a more complete discussion of the general binding features of these signaling complexes.

RESPONSE: We have expanded our discussion on structural work: "Crystal structures have been solved for ECDs of FLS2, PEPR1, RGF receptor RGFR1, PSK receptor PSKR1, IDA receptor HAESA, and TDIF receptor PXY (phloem intercalated with xylem) in complex with their respective ligands and/or a SERK ECD (Sun et al, 2013; Tang et al., 2015; Song et al., 2016; Santiago et al., 2016; Wang et al., 2016a; Zhang et al., 2016). The FLS2-flg22-BAK1 complex showed that flg22 adopts a linear conformation and binds in an N-terminal to C-terminal orientation to FLS2 LRR3-16 (Figure 1; Sun et al., 2013). Strikingly, Gly18 of flg22 fits in an "inner-curved loop" between Thr52 and Val54 at the N-terminus of BAK1 ECD, and this interaction is further stabilized by hydrogen bonds between flg22 Leu19 and BAK1 Thr52 and Val54. This interaction effectively bridges the dimerization between FLS2 and BAK1, demonstrating that BAK1 is a co-receptor for flg22. FLS2 LRR23-26 additionally interacts with a cluster of bulky BAK1 amino acids, forming a stable complex. The binding of flg22 does not lead to a conformational change in the FLS2. The PEPR1-Pep1, HAESA-IDA-SERK1, PXY-TDIF-SERK2, and RGFR1-RGF1-BAK1 complexes also adopt a conformation strikingly similar to the FLS2-flg22-BAK1 complex (Figure 1; Tang et al., 2015; Santiago et al., 2016; Song et al., 2016; Zhang et al., 2016). The PSKR1-PSK-SERK1 structure showed a different mechanism for ligand-binding and receptor complex formation. PSK presents as a β-strand and forms an anti-parallel β-sheet conformation with the island domain of PSKR1 ECD (Wang et al., 2016a). This induces an allosteric change in PXY, allowing for the recruitment of SERKs. Taken together, SERKs are likely common co-receptors for many LRR-RKs perceiving proteinaceous ligands."

Point 3. The other area in which an expanded discussion may be warranted is around the size of the ECD of receptors and co-receptors. The authors already allude to this observation in noting that BAK1 has only five LRR repeats, while FLS2 and EFR, for example, have much larger ECDs. Perhaps a more direct discussion of this apparent dichotomy and its implications for signaling complex formation and predictive methods of PRR discovery is warranted.

RESPONSE: See above. We have added a new Figure 1 on alignment of different receptor complexes to highlight similarities in LRR-RK complexes.

TPC2016-00891-REV1 2nd Editorial decision – *acceptance pending* **Mar. 1, 2017**

We are pleased to inform you that your Review Article entitled "Receptor kinases in plant pathogen interactions: more than pattern recognition" 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. 16, 2017**