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The APEX approaches: a unified LRR-RK network revealed

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Abstract

Leucine-rich repeat receptor kinases (LRR-RKs) represent a large and functionally diverse family of transmembrane proteins critical for signal recognition and transduction at the plant cell plasma membrane. Here, we discuss a recent report which used a systems-level approach to validate key paradigms by constructing an LRR-RK interaction network model.

LRR-RKs form a perception network at the plasma membrane

The plant cell plasma membrane is a dynamic and functionally decorated surface in constant interface with the environment and proximal cells. Leucine-rich repeat receptor kinases (LRR-RKs) are an important family of transmembrane proteins which act as critical regulators of plant development and immunity [1]. LRR-RKs contain a highly variable extracellular LRR domain, a single transmembrane domain and an integral intracellular kinase domain [1]. The LRR domain contributes to both ligand recognition and co-receptor association. Notably, molecular phylogenies imply that this gene family underwent significant expansion and neofunctionalization in land plants [2]. Structural studies of LRR-RK extracellular domains (ECDs) have indicated that the formation of a heterodimeric LRR-RK complex is often required for signal transduction, and that the formation of this complex is often induced by the presence of a corresponding extracellular ligand [3]. A thorough elucidation of the LRR-RK interaction network likely holds critical insights into the subtleties of plant cell function, but this network's complexity has remained challenging to study due to its size and intricacy.

A systems-level approach to LRR-RK interaction dynamics

To assay the interaction potential of LRR-RKs in arabidopsis (*Arabidopsis thaliana*), Smakowska-Luzan *et al.* implemented a high-throughput technique previously used to reveal the extracellular interactions of immunoglobulin and LRR proteins in *Drosophila* [4, 5].

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Each ECD of 200 arabidopsis LRR-RKs was heterologously expressed in, and subsequently secreted from a *Drosophila*-based expression platform. The supernatant of the resulting cell suspension containing the LRR domain of interest was directly used in an *in vitro* alkaline phosphatase activity-based bidirectional interaction screen. The interaction confidence of each pair was quantified, and the subsequent “WalkTrap” computational analysis revealed four subnetworks of LRR-RKs [5]. After comparing the contributions of small (< 12 LRR repeats) and large (> 12 LRR repeats) LRR-RKs to network connectivity, small LRR-RKs such as BRI1-ASSOCIATED KINASE 1 (BAK1) appeared to have the strongest influence [5].

The proposed network analysis was indicated by the previous findings which identified interacting pairs such as FLAGELLIN-SENSING 2 (FLS2) and BAK1. In addition, the network prediction-guided genetic elimination analysis predicted two novel functionally relevant LRR-RKs, designated FLS2-INTERACTING RECEPTOR (FIR) and APEX [5]. FIR was identified as an interactor of FLS2, BRI1, and BAK1; and was demonstrated to positively regulate FLS2 and BRI1 signaling. Further, the presented evidence suggests that FIR might be required for the ligand-induced interaction of FLS2 and BAK1. By contrast, APEX was shown to negatively regulate the formation of the FLS2-BAK1 complex despite that it is several steps away in the predicted interaction network. Additionally, APEX was shown to interact with PLANT ELICITOR PEPTIDE (Pep) RECEPTOR 1 (PEPR1) and PEPR2 in a ligand-independent manner [5]. The appropriate dosage of APEX appears to be required for Pep-induced responses. Together, this work provides insights into the system-wide regulation of the plant cell surface receptor interaction network.

APEXes of LRR-RK interaction networks

In ecology, trophic networks often converge on keystone species called “apex predators”, without which the ecosystem collapses. Similarly, the aptly named APEX, and short LRR-RKs like it, were more frequently identified as critical nodes that support the integrity of the predicted cell-surface interaction network [5]. BAK1, a well-characterized short LRR-RK, was identified by the PageRank algorithm as the most interconnected and important node in the network. This validates the consensus that BAK1 contributes to various developmental and immune processes by serving as a communal co-receptor for several LRR-RKs [6]. In addition, APEX was proposed as a network articulation point which connects at least two discrete subnetworks. Despite that both the immune-signaling deficient mutant *bak1-5* and the *apex* single mutant are morphologically wild-type, the *apex bak1-5* double mutant is developmentally impaired. This supports the assertion that APEX has an essential contribution to network integrity. The mechanism underlying the paradoxical universality and functional specificity of APEX and BAK1 in LRR-RK signal transduction remains unknown. However, this work provides an important scaffold and mineable resource which can facilitate future studies on LRR-RKs.

Beyond the ECD: unifying a regulatory network

While this high-throughput assay is self-evidently powerful and efficient, it still relies on a removal of the proteins of interest from their biochemical context on the plasma membrane.

Despite this, the results of this study indicate that this technique is a valuable and instructive predictor of LRR-RK interactions. To refine the findings of this work, this network might be used as a guide for future biochemical and genetic studies which consider the host of other variables that influence receptor complex formation on the plasma membrane. For example, it is clear that the presence of a ligand in the extracellular environment plays a critical role in the recruitment of co-receptors to their associated complexes. Conserved motifs in the transmembrane domain of certain LRR-RKs have also been demonstrated to encourage co-receptor complex formation [7]. In addition, plants possess an expanded number of LRR receptor-like proteins (LRR-RLPs). LRR-RLPs contain no cytosolic kinase domain, but are otherwise similar to LRR-RKs in structure. LRR-RKs are often observed to complex with LRR-RLPs, and are thought to be required for LRR-RLP-mediated signaling [8]. Certain LRR-RLPs can function as a switch to modify the ligand recognition specificity of some LRR-RKs [9]. It is tempting to speculate that LRR-RLPs add another dimension to the plasticity of the LRR-RK network.

As expected, almost every protein included in the network analysis has more than one predicted interactor [5]. However, the set of functionally relevant interactions might be reduced by considering that *LRR-RK* gene expression is often spatially and temporally regulated [10]. Additionally, the plant cell plasma membrane is a highly organized and dynamic structure with partitioned membrane domains [11]. Whether and how these membrane microdomains and their associated lipid compositions affect LRR-RK interaction networks remains unknown. Experiments which integrate the regulatory contexts of these LRR-RKs into the network have the potential to refine its predictive power. As the methods for studying transmembrane proteins in a more natural context are limited, supplementing high-throughput screens with additional biochemical and genetic evidence may prove to be useful in developing a more robust picture of the cell-surface interactome.

The PageRank algorithm, originally implemented to assess website importance as a function of the quantity and importance of sites to which it is linked, was used in this work to identify the most important actors in this biological system [12]. To hierarchically agglomerate the nodes to predict subnetwork structure, the WalkTrap algorithm was also implemented. Together, these analyses point to the primary advantage of graph databases in biology: the capacity to identify both important nodes and complicated patterns in an otherwise intractable interaction network. One potential drawback of graph theory in molecular biology is that it may usher in a new era of data-mining where information from separate experiments is layered onto existing networks to support hypotheses in an unscientific manner. By contrast, this work is an example of a prudent use of graph theory in protein-protein interactions because it was implemented here as a predictive tool to generate new hypotheses rather than the other way around. This implementation of both link analysis (PageRank) and community detection (WalkTrap) algorithms holds the potential to serve as a model for future interactome studies which seek to identify otherwise opaque connections in the cellular machinery of other model organisms. From social media to terrorist communication networks, graph theory has been broadly implemented to model and understand disordered systems. In a biological context, the careful implementation of these and similar algorithms with the recent explosion of post-genome era multidimensional

datasets—including protein-protein interaction and gene expression networks— will continuously reveal unprecedented insights into the subtler functions of biological systems.

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APEX as a connector of receptor subnetworks.

The short LRR-RK APEX (center; red), was demonstrated to act as an articulation point in the LRR-RK cell-surface interaction network. The interconnectedness of the various receptor subnetworks (represented top left/right) was predicted to largely rely on APEX and short LRR-RKs like it. APEX likely acts in a manner similar to BAK1 by complexing with several LRR-RKs (represented left/right; green/blue) or indirectly affecting the integrity of other subnetworks. Examples of features which govern the interaction specificity and affinity for the formation of these co-receptor complexes (ligand-induced heterodimerization, transmembrane-helix interactions, intracellular kinase trans-phosphorylation) are labeled throughout the figure. The illustrated LRR-RK models do not represent true molecular structures.