Distinct evolution rate between receptor-like kinases and receptor-like proteins

Introduction

Plants are exposed constantly to the attacks from potential virulent microbes. As the sessile organisms, plants have developed innate immunity to detect and fight against the invading pathogens in order to survive.

Three components are involved in plant resistance and susceptibility to disease. Firstly, microbes have pathogen-associated molecular patterns (PAMPs) – the conserved components from pathogens, such as bacterial flagellin and fungal chitin, which plants have evolved the capacity to recognize and respond to, and this results in PAMP-triggered immunity (PTI) (Silke et al. 2002). Secondly, to achieve successful invasion, pathogens make and deliver effector proteins into plant cells to suppress PTI (Thomas et al. 2009). Thirdly, plants have employed a repertoire of resistance (R) genes that encode guarding proteins that sense and recognize specific pathogen effectors, either directly or indirectly via effector action on host cellular components. R gene-dependent recognition results in effector-triggered immunity (ETI) (Thomas et al. 2009), resulting in restored resistance. To understand resistance, we need to better understand PTI, not only because of its intrinsic interest and important role in crop protection, but also because host components involved in PTI are targets of pathogen effectors.

To trigger PTI, PAMPs from microbial pathogens need to be perceived by cell surface receptors (Silke et al. 2002), known as pattern recognition receptors (PRRs). PRRs include receptor-like kinases (RLKs) and receptor like proteins (RLPs). RLKs and RLPs both contain a single transmembrane domain, an extracellular ligand binding domain. Detection or binding of PAMPs will trigger intracellular responses transduced by the RLKs cytoplasmic Ser/Thr kinase domain (Christiaan et al. 2012) which is absent in RLPs. Instead, RLPs are likely to transduce signals by recruiting RLKs partners upon ligand binding. Being the most frontiers to detect the existence of potential invaders by binding PAMPs, RLKs and RLPs are supposed to be facing similar selective pressure. On the other hand, the intracellular domain of RLKs is involved in signal transduction and interactions with different partners upon different stimuli, as well as becoming the targets of effectors of pathogens, all of could render RLKs to a higher selective pressure (Mariana et al. 2005).

In this study, all the available validated RLKs and RLPs protein sequences in different plant species are collected from Plant Resistance Gene database (PRGdb). The sequences are then analyzed to determine the evolution rate of RLKs and RLPs with regards to the similarities and dissimilarities in their roles in recognizing PAMPs and downstream signaling initiation.

Methods

Sequence Alignment

All the protein sequences downloaded from PRGdb were aligned using Mafft installed in HPC-class (Iowa State University) with "auto" strategy and default setting of BLOSUM62 model and gap penalty -1.53, +0.00, +0.00.

Construction of Phylogenetic Tree

Neighbor-joining tree was constructed using online FastME program available at the ATGC bioinformatics platform website. RLKs and RLPs amino acid sequence alignment file was uploaded for FastME with substitution model MtREV, Gamma distribution rate across the sites and tree refinement with SPR were chosen to run the program. Random sampling using bootstrap was used to evaluate the support of clade in inferred neighbor-joining tree.

Result

Phylogenetic analyses reveal different evolution rates between RLKs and RLPs

A total of 18 protein sequences of the receptor-like kinases (RLKs) and receptor-like proteins (RLPs) are downloaded from PRGda. Genes encoding the 18 RLKs and RLPs have been investigated with published data supporting their roles in recognizing PAMPs and therefore the participation in PTI response. Detailed information about the corresponding interactive pathogens and the related diseases is provided from PRGda website. The inferred neighbor-joining (NJ) tree by FastMe suggests distinct evolution rates between RLKs and RLPs (Fig. 1). With 3 exceptions, the overall amino acid substitution rate indicated by branch length shows that RLKs have a significantly higher substitution rate than that of the RLPs, ranging from 1.28 of ER RLK in *Arabidopsis thaliana* to 3.33 of RPG1 RLK in *Hordeum vulgare*. In contrast, the longest branch length among RLPs is 0.19 (LeEIX2 in *Solanum lycopersicum*).

RLKs intracellular domain is not responsible for the higher substitution rate

The extracellular domain of RLKs and RLPs contain multiple leucine rich repeat (LRRs) units for ligand recognition and perception (Shin-Han et al. 2001). Besides the extracellular domain, both RLKs and RLPs contain a similar single transmembrane domain. However, the cytoplasmic kinase domain for initiating downstream signal transduction in RLKs is absent in RLPs. RLPs contain a short cytoplasmic tail without any identified functional domain. To investigate whether the intracellular kinase domain of RLKs, which might be under positive selection, contribute to the higher substitution rate in RLKs, the kinase domain and the adjacent N terminal amino acids are removed from all RLKs protein sequences. The trimmed RLKs amino acid sequences containing extracellular domain, transmembrane domain and a short intracellular tail are re-aligned with RLPs sequences to infer the NJ tree using exactly the same parameters as the one used for full sequence alignment and NJ clustering. Surprisingly, the branch length in new NJ tree (1.33 to 4.06 for RLKs and 0.00 to 0.21 for RLPs) remain comparable to the branch length in the NJ tree using full sequences (Fig. 2), indicating that kinase domain is not responsible for the higher substitution rate among RLKs receptors.

Discussion

The neighbor-joining tree using protein sequences of RLKs and RLPs involved in plant PTI response reveals distinct evolution rate between RLKs and RLPs. More than half RLKs sequences tested in this analysis have longer branch lengths. In contrast, the branch lengths of all the RLP protein sequences are significantly shorter, indicating a much lower substitution rate among RLP members. 3 sequences in RLKs show extremely short branch length are BAK1 from *Arabidopsis thaliana* and Serk3A andSerk3B from *Nicotiana benthamiana*. The branch lengths of BAK1, SERK3A and SERK3B are similar to those of the RLPs. Compared to other RLKs, BAK1 has less LRR repeats and may not be involved in direct ligand perception. Growing evidence shows that BAK1 can be instantaneously recruited by other RLKs, FLS2 for example. It acts as a co-receptor of FLS2 and is required for the full activation of FLS2 triggered immunity (Antje et al. 2007, Yadong et al. 2013). Notably, the closely related SERK proteins have gain increasing support to associate with RLKs and regulate their function, suggesting their role as co-receptors upon ligand binding. RLPs are capable of binding PAMPs directly, however, they also act as co-receptors in a way that additional RLKs are required to transduce the signals upon ligand perception. The role of RLPs, RLK BAK1 and SERKs acting as co-receptors may somehow distinguish them from other RLKs facing distinct selective pressure. NJ tree constructed using RLKs partial amino acid sequences after removing the intracellular kinase domain sequences indicate the kinase domain alone doesn't contribute to the relatively fast evolution rate of RLKs. The distinct substitution rate may be a result of dominant and ancillary roles in signal transduction and PTI initiation between different receptor-like kinases and receptor-like proteins. Further investigations need to be done, and meanwhile, more RLKs and RLPs to be identified and included for further analysis will help address this question.

Reference

1 Antje Heese, Dagmar R. Hann, Selena Gimenez-lbanez, Alexandra M.E. Jones, Kai He, Jia Li, Julian I. Schroeder, Scott C. Peck and John P. rathjen. The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proc.Natl.Acad.Sci*. 104:12217-12222 (2007)

2 Christiaan Greeff, Milena Roux. John Mundy and Morten Petersen. Receptor-like kinase complexes in plant innate immunity. *Frontiers in Plant Science* 3: 209-216 (2012)

3 Mariana Mondragon-Palomino, Brandon S. Gaut. Gene conversion and the evolution of three leucine-rich repeat gene families in Arabidopsis thaliana. *Molecular Biology and Evolution* 22: 2444-2456 (2005)

4 Shin-Han Shiu and Anthony B. Bleecker. Plant receptor-like kinase gene family: diversity, function and signaling. *Science's STKE* 113: 22-28 (2001)

5 Silke Stracke, Catherine Kistner, Satoko Yoshida, Lonneke Mulder, Shusei Sato, Takakazu Kaneko, Satoshi Tabata, Niels Sandal, Jens Stougaard, Krzysztof Szczyglowski and Martin Parniske. A plant receptor-like kinase required for both bacterial and fungal symbiosis. *Nature* 417: 959-962 (2002)

6 Thomas Boller and Sheng Yang He. Innate immunity in plants: an arm race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science* 324: 742-744 (2009)

7 Yadong Sun, Lei Li, Alberto P. Macho, Zhifu Han, Zehan Hu, Cyril Zipfel, Jian-Min Zhou, Jijie Chai. Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex.