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Receptor-like proteins

Searching for functions

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Receptor-like proteins (RLPs) are cell surface receptors, which are composed of several distinct domains including a signal peptide, an extracellular leucine-rich repeat (LRR) region, a transmembrane domain and a short cytoplasmic tail. RLPs are implicated in plant growth and development as well as in disease resistance. Our previous genome-wide functional analysis of 57 Arabidopsis *RLP* genes (*AtRLPs*) revealed that mutant phenotypes were only observed for a few genes including the two reported genes *CLAVATA2* (*CLV2*) and *TOO MANY MOUTHS*, despite the wide range of growth and developmental stages and treatments that were tested. In a recent study, we reported on further insights into the biological role of a few AtRLPs closely-related to CLV2. Two AtRLPs (AtRLP2 and AtRLP12), which share high sequence similarity with CLV2, were found to be able to rescue the *clv2* mutant phenotype when expressed under the control of the *CLV2* promoter, suggesting that the specialization among *CLV2*, *AtRLP2* and *AtRLP12* is largely ascribed to differences in their expression patterns. Our data further indicated that the island domain of CLV2 is dispensable for its function and the C3-F domain of CLV2 could be replaced by that of AtRLP38. In this Addendum, we are elaborating on further strategies concerning the function of largely unknown AtRLPs.

The proper balance between maintenance of undifferentiated stem cells and cell division is critical for organ initiation and formation. Insight into in the maintenance of stem cells in the shoot apical meristem (SAM) has revealed three *CLV* genes, *CLV1*, *CLV2* and *CLV3*, which are implicated in this process.[1,2] Loss-of-function of any of the *CLV*s causes the progressive accumulation of undifferentiated stem cells, resulting in an enlarged meristem, increased floral organ numbers and altered phyllotaxy.[3-5] Although *CLV1*, *CLV3* and their closely related genes are studied extensively,[6-8] *CLV2* and its homologs, to a large extent, are ignored.

CLV2 encodes a LRR-RLP lacking a cytoplasmic kinase domain that is present in CLV1 (**Fig. 1**).[4,9] In addition to its role in meristem maintenance and various organs development, CLV2 also has a function in root development.[10] Overexpression of *CLV3*, *CLV3* embryo surrounding region 19 (*CLE19*) and *CLE40* causes the consumption of the root meristem,[11,12] which is prevented in a *clv2* mutant as has been shown for *CLE19*.[12] The root apical meristem of *clv2* mutants no longer responds to in vitro application of CLE peptides, corresponding to the conserved CLE motif, suggesting the involvement of the CLV2 receptor in the perception of CLE peptides in the root.[10] The newly identified *coryne/suppressor of overexpression of LLP1-2 (crn/sol2)* mutant,[13] like *clv2*, shows an enlarged SAM and similar resistance to various CLE peptides, and is defective in floral organ development.[14,15] CRN is a

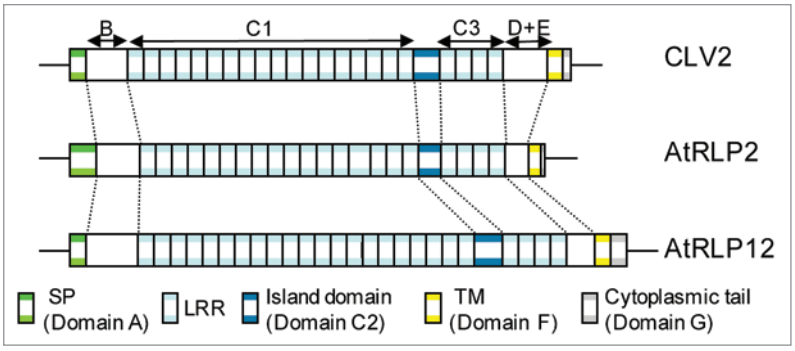

Figure 1. Domain composition of CLV2, AtRLP2 and AtRLP12. Domains B, C1, C3, D and E are indicated on top. The signal peptide (SP, domain A), LRR domains, Island domain (C2 domain), trans membrane domain (TM, domain F) and cytoplasmic tail are depicted by colored boxes.

RLK containing a short non-LRR extracellular domain and a cytoplasmic kinase domain.[14,15] CRN is responsible for meristem maintenance and is suggested to act synergistically with CLV2 in a receptor complex. Taken together, the CLV3 signal is probably relayed through two separate receptor complexes, one comprising CLV1, and the other one comprising CRN and CLV2.[15,16] Indeed, it has been shown that CLV2 can directly interact with CRN in the absence of CLV3, and CLV1 can weakly interact with CRN, but cannot interact with CLV2.[17]

In the Arabidopsis genome, fifty-seven RLPs were identified that share homology with the CLV2 protein.[18,19] Previously, a T-DNA insertion mutant collection was assembled to functionally characterize the function of *AtRLP* genes with respect to plant growth, development and sensitivity to various stress responses including pathogen susceptibility. However, only a few new phenotypes were discovered through this genome-wide functional analysis, suggesting a high level of functional redundancy among the *AtRLPs*.[19]

In our recent publication,[20] we described the functional analysis of the CLV2 subfamily members and identification of possible functional domains of CLV2 that contribute to specificity. To gain new insights into the biological roles of AtRLPs, we identified eight AtRLPs that share a high sequence similarity with CLV2 and have a similar domain organization. We found that out of four Arabidopsis *CLV2* homologues tested, *AtRLP2* and *AtRLP12* could functionally rescue the *clv2* mutant when expressed under the control of the *CLV2* promoter (**Fig. 1**). Together with previous results,[19] this reinforces our understanding of functional redundancy and diversity among *CLV2* homologues. However, the double mutants *atrlp2 atrlp12, atrlp2 clv2-7* and *atrlp12 clv2-7* exhibited no phenotype nor significant alterations when compared with wild type or *clv2-7*. We further carried out deletion analyses to dissect functional domains of CLV2 and made chimeric molecules between *CLV2* and a related but functionally distinct *RLP, AtRLP38*, to determine the domains of CLV2 that specify its function. Our results indicated that the island domain is not important for CLV2 function, whereas the C1 and C3 regions of the LRR domain are essential (**Fig. 1**). Analyses of the CLV2RLP38 chimeras have revealed that the C3-F region of CLV2 can be functionally replaced by the corresponding part of AtRLP38, while the C1 domain can not be replaced. This indicates that the C3-F region might be functionally conserved among AtRLPs and may be involved in dimerization, whereas the C1 fragment may determine the functional specificity of CLV2.

The function of most *AtRLPs*, including *AtRLP2* and *AtRLP12*, still remains unclear, despite the fact that *AtRLP2* and *AtRLP12* can replace the function of *CLV2* when expressed under the control of the *CLV2* promoter. The present challenge still will be to understand the biological function of almost all *AtRLP* genes. A major problem in determining the function of *AtRLPs* is the lack of obvious phenotypes in a single mutant background. Regarding this observation, one reason could be the lack of suitable physiologically screening conditions. For instance, we did not include insects/nematodes or some abiotic stress inducer such as UV-B light, toxic compounds or damage in our assays.[19,20] Secondly, the phenotype might only be visible at microscopic level or in a tissue/stage-specific manner. Therefore, it may be necessary to test a broad-range of physiological conditions in combination with high-resolution screening.

Another important factor is the high-degree of functional redundancy among *AtRLP* genes.[19,20] This phenomenon is very common in any large gene family, which obscures the understanding of the function of a single AtRLPs. Indeed, most of the closely-related *AtRLP* genes are located at one locus on the chromosomes as seen in the case of *AtRLP2*/*AtRLP3* and *AtRLP11*/*AtRLP12*,[19,20] which makes it impossible to create double or high-order mutant combination by crossing individual T-DNA insertion lines.[20] The RNA interference approach might be useful to silence multiple related AtRLP genes simultaneously and thus could simplify the study of functional redundancy among *AtRLP* genes. However, the RNAi targeted domains should be selected with great care to avoid targeting other close relatives.[20]

A more systematical approach to determine the function of *AtRLP* genes is the study of transcript expression profile. Therefore, public microarray databases could be examined for their expression profile,[21,22] and genes that are co-expressed with *AtRLPs*.[23,24] Searching two tissue-specific microarray databases revealed that only very few *AtRLPs* are expressed in root and SAM.[25,26] A survey of the SAM expression map revealed that *AtRLP4*, *AtRLP29*, *AtRLP32* and *AtRLP54* exhibit an expression in the shoot apex, whereas their roles in the regulation in the SAM remains to be elucidated.[20,26] It could be very useful to perform a systematic investigation of the transcriptional profile of *AtRLP* genes with respect to different conditions including biotic/abiotic stress and hormones. In this way, it could be possible to get an indication of the function of some of *AtRLPs* which display a change in expression upon external stimuli.

Another emerging approach is to study the AtRLPs by proteomics techniques.[27]

Even though transcriptional profiling is useful; it is not enough for understanding the role of AtRLPs. Proteomics can certainly complement the gene expression profile.[27] Proteomics study of the brassinosteroid response provides an excellent example of applying proteomics to gain a better understanding of receptor functions.[28,29] An important aspect of AtRLP signaling is heterodimerization with RLK(s),[7] forming a receptor complex. The identification of different possible receptor combinations under various conditions and stimuli by proteomics will provide an insight in the signaling pathways mediated by the AtRLP family.

In conclusion, as noted in our studies, a major problem in determining the function of *AtRLPs* is the lack of phenotypes in single loss-of-function mutants.[19,20] This is partially due to the lack of suitable physiologically screening conditions, and partially because of the high level of functional redundancy among AtRLPs. The availability of large amounts of microarray data now permits large-scale data mining of '-omics' for identification of candidates for further studies based on their (co)-expression profile. Combing transcriptional and proteomics studies will give new insights on the different components of the signal transduction pathways and downstream targets that are regulated by different AtRLP receptors.

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