Review

Leucine-Rich Repeat (LRR) Domains Containing Intervening Motifs in Plants

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Abstract: LRRs (leucine rich repeats) are present in over 14,000 proteins. Non-LRR, island regions (IRs) interrupting LRRs are widely distributed. The present article reviews 19 families of LRR proteins having non-LRR IRs (LRR@IR proteins) from various plant species. The LRR@IR proteins are LRR-containing receptor-like kinases (LRR-RLKs), LRR-containing receptor-like proteins (LRR-RLPs), TONSOKU/BRUSHY1, and MJK13.7; the LRR-RLKs are homologs of TMK1/Rhg4, BRI1, PSKR, PSYR1, Arabidopsis At1g74360, and RPK2, while the LRR-RLPs are those of Cf-9/Cf-4, Cf-2/Cf-5, Ve, HcrVf, RPP27, EIX1, clavata 2, fascinated ear2, RLP2, rice Os10g0479700, and putative soybean disease resistance protein. The LRRs are intersected by single, non-LRR IRs; only the RPK2 homologs have two IRs. In most of the LRR-RLKs and LRR-RLPs, the number of repeat units in the preceding LRR block (N_1) is greater than the number of the following block (N_2); $N_1 \gg N_2$ in which N_1 is variable in the homologs of individual families, while N_2 is highly conserved. The five families of the LRR-RLKs except for the RPK2 family show $N_1 = 8 - 18$ and $N_2 = 3 - 5$. The nine families of the LRR-RLPs show $N_1 = 12 - 33$ and $N_2 = 4$; while $N_1 = 6$ and $N_2 = 4$ for the rice Os10g0479700 family and the $N_1 = 4 - 28$ and $N_2 = 4$ for the soybean protein family. The rule of $N_1 \gg N_2$ might play a common, significant role in ligand interaction, dimerization, and/or signal transduction of the LRR-RLKs and the LRR-RLPs. The structure and evolution of the LRR domains with non-LRR IRs and their proteins are also discussed.

1. Introduction

LRR (leucine rich repeat) regions are present in over 14,000 proteins in the data bases-PFAM, SMART, PROSITE, and InterPro [1–4]. LRR-containing proteins have been identified in viruses, bacteria, archaea, and eukaryotes. *Arabidopsis thaliana* and *Oryza sativa* subsp. japonica (rice) contain over 700 and 1,400 LRR proteins, respectively [5]. Most LRR proteins are involved in protein-ligand and in protein-protein interactions; these LRR proteins include plant immune response and mammalian innate immune response [6–10]. Most LRR repeating units are 20–30 residues in length. All LRR units can be divided into a HCS (highly conserved segment) and a VS (variable segment). The HCS part consists of an 11 residue stretch, LxxLxLxxNxL, or a 12 residue stretch, LxxLxLxxCxxL, in which "L" is Leu, Ile, Val, or Phe, "N" is Asn, Thr, Ser, or Cys, and "C" is Cys, Ser or Asn [7,11–14]. Eight classes of LRRs have been characterized by different lengths and consensus sequences of the VS part of the repeats. They are "RI-like", "CC", "Bacterial", "SDS22-like", "plant specific (PS)", "Typical", "TpLRR", and "IRREKO". Plant specific LRRs (class: PS-LRR) are 23 to 25 residues long and contain a conserved consensus sequence of the VS part, SGxIPxxLxxLxx, in which "S" is Ser or Thr, "G" is Gly or Ser, "I" is Ile or Leu, and "L" is Leu, Ile, Val, Phe, or Met, and "x" is any amino acid [14]. The structures of polygalacturonase inhibiting protein (PGIP) and brassinosteroid insensitive 1 (BRI1), which have PS-LRRs, are available [15–17].

LRR-containing proteins from plants have diverse overall structures and functions. Several classes contain LRR-containing receptor-like kinases (LRR-RLKs) [18,19], LRR-containing receptor-like proteins (LRR-RLPs) [20], nucleotide binding site LRR (NBS-LRR) proteins [21,22] and PGIPs [23–25]. They provide an early warning system for the presence of potential pathogens and activate protective immune signaling in plants [26–28]. In addition, they act as a signal amplifier in the case of tissue damage, establishing symbiotic relationships and effecting developmental processes.

Evolution of plant, disease resistance (*R*) genes that encode an LRR region has been studied by many researchers [18,22,29–45]. The generations of *R* genes are proposed to be mainly due to gene duplication, genetic recombination, diversifying selection, sequence divergence in the intergenetic region, composition of the transposable elements, gene conversion, and unequal crossover [41–43].

Non-LRR, island regions (IRs) interrupting LRRs are widely distributed; they are referred to as "islands" or "loop outs" [46,47]. A large number of plant LRR proteins have non-LRR IRs which are called LRR@IR proteins; they include LRR-RLKs and LRR-RLPs [46–61]. Some experimental studies on the function of non-LRR IRs within LRR@IR proteins have been performed [62–64]. TLRs 7, 8, and 9 out of Toll-like receptors (TLRs) are also LRR@IR proteins [65–67]; TLRs initiate an innate immune response [68–71].

A method—LRRpred—identify the repeat number of LRRs and phasing (that is, what segment or residue corresponds to the beginning of a repeating unit) was developed, which incorporates protein secondary structure prediction [65,72]. LRRpred predicts the repeat number and phasing of LRRs to

be completely consistent with, or almost so, with those revealed by structure analyses [72]. Furthermore, to identify non-LRR IRs, a method (called LRR@IRpred) utilizing LRRpred was developed and used to find LRR@IR proteins from organisms other than plants [47]. The present article reviews 19 families of plant LRR@IR proteins identified by LRR@IRpred and describes some features of their LRR domains. The structure, function and evolution of the LRR domains as well as the LRR@IR proteins are discussed.

2. Structures of Plant LRR Proteins

All of the LRR domains in one protein form a single continuous structure and adopt an arc or horseshoe shape [73]. Three residues at positions 3 to 5 in the HCS, LxxLxLxxNxL or LxxLxLxxCxxL, form a short β-strand. On the inner, concave face there is a stack of the parallel β-strands and on the outer, convex face there are a variety of secondary structures such as α-helix, 3_{10}-helix, polyproline II helix, or a tandem arrangement of β-turns, which are connected by two loops. Most of the known LRR structures have caps, which shield the hydrophobic core of the first LRR unit at the *N*-terminus and/or the last unit at the *C*-terminus. In extracellular proteins or extracellular regions, the *N*-terminal and C-terminal caps frequently consist of Cys clusters including two or four Cys residues; the Cys clusters on the *N*- and *C*-terminal sides of the LRR arcs are called LRRNT and LRRCT, respectively [8–10].

The crystal structures of PS-LRR domains of *Phaseolus vulgaris* PGIP and *A. thaliana* BRI1 (an LRR@IR protein) have been determined [15–17]. The structure of the BRI1 LRR domain forms a right-handed superhelix composed of 25 PS-LRRs (Figure 1A) [16,17]; most of these 25 PS-LRRs are 24 residues long. The helix completes one full turn, with a rise of ~70 Å. The concave surface is formed by α- and 3_{10} helices that produce inner and outer diameters of ~30 and ~60 Å, respectively. The consensus sequence LxGx(I/L)P at positions 11 to 16 likely forms a second β-strand, which characterizes the fold of the PS-LRRs. Thus, the structural LRR units may be represented by β-β-3_{10}. BRI1 has both an LRRNT with Cx_6C and an LRRCT with Cx_6C; both the LRRNT and LRRCT form two disulfide bonds. The disulfide bonds contribute to the stability of the N-terminal cap structure (*N*-Cap) consisting of one β-strand and two α-helices and the *C*-terminal cap structure (*C*-Cap) consisting of two short helices.

The crystal structures of LRR domains of *A. thaliana* transport inhibitor response 1 (TIR1) and coronatine-insensitive protein 1 (COI1) (that are F-box proteins) are also available [74–76]. TIR1 has 18 LRRs of various lengths (from 22 to 35 residues) of which 13 are noncanonical, imperfect LRRs and have long β-strands of 4–6 residues. Most VS parts adopt α-helix. Thus, the structural LRR units may be represented by β-α. The TIR1 LRR domain form a right-handed superhelix of one full turn, which is represented by one closed ring, as well as the BRI1 LRR domain [74,75]. The top surface of the TIR1 superhelix has three long intra-repeat loops (loop-2 in ***LRR2***, loop-12 in ***LRR12*** and loop-14 in ***LRR14***). The loop-2 plays a pivotal role in constructing the auxin- and substrate-binding surface pocket by interacting with the nearby concave surface of the TIR1 LRR structure. The COI1 LRR domain adopts a very similar structure to that of TIR1 [76]. Similarly, three long intra-repeat loops are involved in the bindings of hormone (jasmine) and polypeptide substrates [76].

Figure 1. Three-dimensional structures of the PS-LRR domains of BRI1 and PGIP. (**A**) BRI1 [3RGZ]; (**B**) PGIP [1OGQ]. The LRRs are colored blue, the cap structures at the N-terminal and C-terminal side orange, the non-LRR IR in BRI1 pink, and the disulfide bonds yellow. All figures were prepared with PYMOL.

(A) BRI1

(B) PGIP
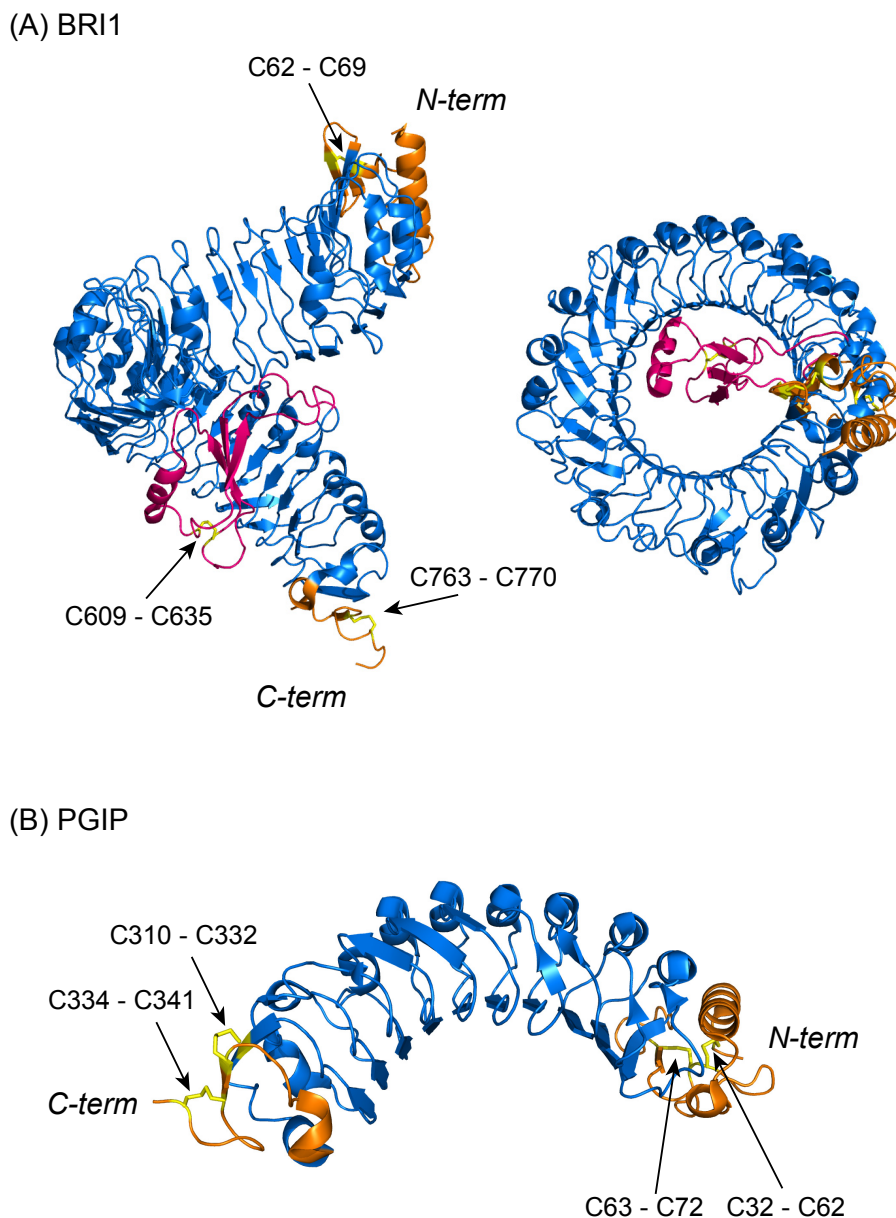
3. Plant LRR@IR Proteins

Plant LRR@IR proteins found through previous research by Matsushima *et al.* [47] and by use of keywords in the references are described. Homologs of an individual protein family from various plant species were collected by the following procedures. First, LRRs in a representative LRR@IR protein of each family were identified by LRR@IRpred; the number of repeat units in the preceding LRR block (N_1), its number in the following block (N_2), and the non-LRR IR sequence of the LRR region were determined. Second, database searches using the amino acid sequences of the non-LRR IR and one LRR unit at the N-terminal and C-terminal IR region were performed by FASTA at the Bioinformatic Center, Institute for Chemical Research, Kyoto University on February 15, 2012.

Third, PS-LRR proteins with highly significant similarity (E-value $< 10^{-10}$) were identified and then they were regarded as putative homologs in which the results of amino acid sequence alignments of full lengths and non-LRR IRs, and their domain architecture, were taken account of. Finally, LRRs in the homologs of each family were identified by LRR@IRpred. When a candidate region is not an LRR unit and its length is longer than average length of the repeating unit of LRRs, it was defined as a non-LRR IR.

The following sequence analyses were also carried out: signal sequence analysis by the program SignalP (http://www.cbs.dtu.dk/services/SignalP/) [77], transmembrane predictions by TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) [78], and the identification of other characteristic regions by SMART (http://smart.embl-heidelberg.de/smart/set_mode.cgi? GENOMIC = 1) [2].

Finally, the 19 families of 344 LRR@IR proteins are described (Supplementary Table S1). The 19 families are grouped into LRR-RLKs, LRR-RLPs, and intracellular proteins. At least one protein in each family has clear experimental evidence for its existence or expression data (such as existence of cDNA(s), RT-PCR or Northern hybridizations) of the existence of a transcript. TMHMM predicts that *A. thaliana* RSYR1 and RPP27 contain a transmembrane region at the N-terminal side (Supplementary Table S1). However, orthology or domain structure was taken account of, and then these two proteins were regarded as LRR-RLKs. SignalP predicts no signal peptide in *A. thaliana* At1g74360 and soybean putative disease resistance protein. Similarly, these proteins were regarded as an LRR-RLK and an LRR-RLP, respectively.

LRR-RLKs count 165/233/239 proteins from *A. thaliana*, 292/357 proteins from *O. sativa* subst. Japonica (rice) and 440 from *Popula trichocarpa* (poplar) [42,79,80]. LRR-RLPs count 90 LRR-RLPs from rice (*O. sativa*) and 48/56 from *A. thaliana* [42,46]. There are LRR-RLKs and LRR-RLPs having no non-LRR IRs, such as FLS2, Xa21, and TMM [81]. LRR- containing receptor-like cytoplasmic kinases (LRR-RLCKs) that lack an extracellular domain have no non-LRR IRs [79,82].

The present review could not describe all families of LRR@IR proteins in plants because of a limited survey of LRRs having non-LRR, IRs which comes from LRR@IRpred.

3.1. Six Families of LRR-RLKs

LRR-RLKs have an extracellular LRR region with an N-terminal signal peptide, a single transmembrane-spanning region, and an intracellular serine-threonine kinase region [18,19],. Transmembrane kinase 1 (TMK1), brassinosteroid insensitive 1 (BRI1), *A. thaliana* At1g74360 protein, phytosulfokine receptor (PSKR), tyrosine-sulfated glycopeptide receptor 1 (PSYR1), and LRR receptor-like serine/threonine-protein kinase RPK2 are members of the LRR-RLKs family. The LRR-RLKs are LRR@IR proteins in which the LRRs are intersected by a single non-LRR IR; only RPK2 has two IRs (Figure 2 and Table 1, and Supplementary Table S1 and Figure S1).

Figure 2. Schematic representation of six LRR-RLKs having LRR domains intersected by non-LRR island regions. *Arabidopsis thaliana* TMK1 [TMK1_ARATH]; *A. thaliana* BRI1 [BRI1_ARATH]; *Daucus carota* PSKR [PSKR_DAUCA]; *A. thaliana* PSYR1 [PSYR1_ARATH]; *A. thaliana* At1g74360 [Y1743_ARATH]; *A. thaliana* RPK2 [RPK2_ARATH].
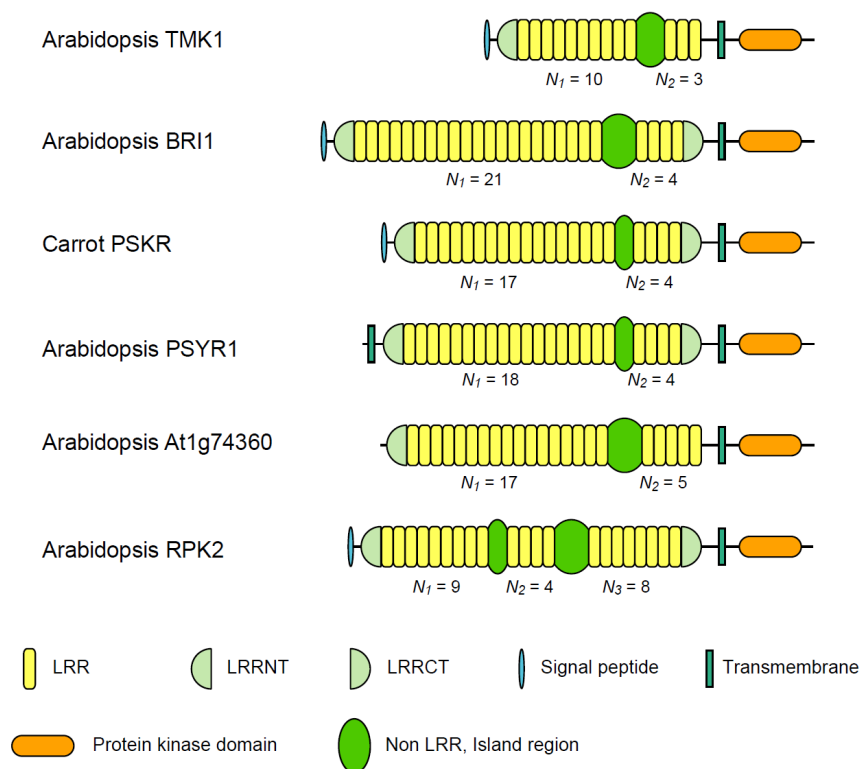

Table 1. Nineteen families of plant LRR proteins having LRR domains intersected by non-LRR island regions. [a] "N_1" is the repeat number of LRRs of the first LRR block in the homologs of each family. [b] "N_2" is the repeat number of LRRs of the second LRR block in the homologs of each family. [c] "N_1/N_2" is average values. [d] The LRR domain in *Arabidopsis* RPK2 contains two non-LRR IRs. The number "13" is the sum of repeat number of LRRs of the first and second LRR blocks. The number "8" is the repeat number of the third LRR block.

	Nineteen Families of Plant LRR Proteins	Species	Repeat number of LRRs			Lengths of non-LRR Island
			N_1^a	N_2^b	N_1/N_2^c	
(A)	*Six families of LRR-RLKs*					
	ArabidopsisTMK1/Soybean Rhg4	14	8~10	3	3.33	57~61
	Arabidopsis BRI1	24	10~22	4	4.94	67~70
	Carrot PSKR	11	17~18	4	4.36	36~38
	Arabidopsis PSYR1	9	17~18	4	4.47	37~38
	Arabidopsis At1g74360	10	16~17	5	3.40	75~77
	Arabidopsis RPK2	4	13[d]	8[d]	1.63	71~75

Table 1. *Cont.*

Nineteen Families of Plant LRR Proteins	Species	Repeat number of LRRs			Lengths of non-LRR Island
		N_1^a	N_2^b	N_1/N_2^c	
(B) *Eleven families of LRR-RLPs*					
Tomato Cf-9/Cf-4	8	17~23	4	5.31	41~46
Tomato Cf-2/Cf-5	3	18~33	4	6.30	37~41
Tomato Ve	12	28~30	4	7.27	41~49
Appl HcrVf	1	22~28	4	6.52	39~46
Arabidopsis RPP27	2	12~26	4	5.78	65~71
Tomato EIXi	1	27	4	6.75	47~49
Arabidopsis CLV2	11	18	4	4.50	41~44
Maize fascinated ear2	4	10~14	4	3.04	41~42
Arabidopsis AtRLP2	2	18	4	4.50	35~38
Rice Os10g0469700	6	6	4	1.50	39~40
Soybean disease resistance protein	5	4~28	4	4.73	41~46
(C) *Two families of plant intracellular proteins*					
Arabidopsis TONSOKU	6	10~13	1	12.00	78~131
Arabidopsis MJK13.7	11	12	8	1.50	59~62

The transcript concentration of *O. sativa TMK1* increase in the rice internode in response to gibberellins [83]. *Nicotiana tabacum TMK1* mRNA accumulation in leaves was stimulated by $CaCl_2$, methyl jasmonate, wounding, fungal elicitors, chitins, and chitosan [84]. TMK1 orthologs were identified from 14 plant species and its paralogs are present in 10 species, including *A. thaliana*, *Glycine max,* and *O. sativa* (Figure 2 and Table 1, and Supplementary Table S1 and Figure S1). Also *G.max Rhg4*, which is a soybean cyst nematode resistance gene [85], was identified as a *TMK1* homolog; while *G.max* Rhg1 [C9VZY3] contains 13 PS-LRRs of 24 residues in which only **LRR6** is 29 residues long. The TMK1 homologs contain 13 LRRs intercepted by a 56 to 76-residue, non-LRR IR. The number of repeat units in the preceding LRR block (N_1) is greater than the number of the following block (N_2), which means $N_1 \gg N_2$ with $N_1 = 10$ and $N_2 = 3$. The non-LRR IRs have a cluster of four Cys residues with the pattern of $Cx_{6-7}Cx_{29-30}Cx_{6-11}C$ and a conserved motif of $Lx_8Yx_{7-8}WxG$ where "Y" is Tyr or Phe, "W" is Trp, and "G" is Gly; this motif is similar to Yx_8KG found in many LRR-RLPs [46]. An LRRNT (with Cx_6C) is observed, but not an LRRCT. Putative C-Cap regions are rich in Gly, Ser, and Pro residues.

BRI1/SR160 is a receptor complex for brassinosteroids that are necessary for plant development, including expression of light- and stress-regulated genes, promotion of cell elongation, normal leaf and chloroplast senescence, and flowering [86–92]. BRI1 orthologs were identified from 24 species and its paralogs are also present in 10 species. The BRI1/SR160 homologs contain 21–26 LRRs with a single non-LRR IR. The N_1 value is relatively variable among species and is 10–22, while $N_2 = 4$; $N_1 \gg N_2$. (Figure 2 and Table 1, and Supplementary Table S1 and Figure S1). *A. thaliana* BRI1 contains 25 LRRs interrupted by a 70-residue IR between **LRR21** and **LRR22.** The non-LRR IR, together with **LRR22**, binds brassinosteroids [62]. The non-LRR IRs of the BRI1 homologs are 68–70 residues long and have a cysteine cluster of $Cx_{25-26}C$ and have a conserved motif of R(I/V/M/L)Y. An LRRNT (with

Cx_6C) and an LRRCT (with Cx_6C) were observed; only soybean BR [C6ZRS8] and *Ricinus communis* LRR-RLK [B9T4K2] have LRRNTs with $Cx_{26-27}C$. The LRRCT regions are rich in His, Arg, and Lys residues, and thus are basic.

PSKR is a PSK receptor that regulates, in response to PSK binding, a signaling cascade involved in plant cell differentiation, organogenesis, and somatic embryogenesis [55,63,93,94]. PSKR orthologs and paralogs were identified (Figure 2 and Table 1, and Supplementary Table S1 and Figure S1). The PSKR homologs contain LRRs with a 36 to 38-residue, non-LRR IR. $N_1 = 17 - 18$ and $N_2 = 4$ (Figure 2 and Table 1, and Supplementary Table S1 and Figure S1). The non-LRR IRs have a conserved motif of (Y/F)x_{5-12}Yx_5F. Most LRRCT regions are basic. *Daucus carota* PSKR contains 22 LRRs intersected by a 36-residue IR between **LRR17** and **LRR18**. An LRRNT (with $Cx_{33}CCx_6C$) that is similar to that in PGIP [15] and LRRCT (with Cx_6C) are observed. A 15-residue region within the non-LRR IR is a binding site of PSK [63]. The corresponding regions in the homologs are relatively variable.

A. thaliana RSYR1 regulates, in response to tyrosine-sulfated glycopeptide binding, a signaling cascade involved in cellular proliferation and plant growth [95]. The RSYR1 homologs from seven species contain 21–22 LRRs with a 37-residue, non-LRR IR ($N_1 = 17 - 18$ and $N_2 = 4$) (Figure 2 and Table 1, and Supplementary Table S1 and Figure S1). The non-LRR IRs have a conserved motif of Yx_2LPVFx_4Nx_4Qx_{2-3}QLSxL. The LRRNT (with four, five, or seven Cys residues) and the LRRCT (with Cx_7C) are observed. The LRRCT regions are basic.

A. thaliana At1g74360 is a BRI1-related protein (Figure 2 and Table 1, and Supplementary Table S1 and Figures S1). Putative orthologs and paralogs were identified from 10 species. The At1g74360 family contains 21–22 LRRs with a single IR. The N_1 value is relatively conserved among species; $N_1 = 16 - 17$, while not $N_2 = 4$ but $N_2 = 5$. The non-LRR IRs of 76-residue are longer than those in BRI1 and have a cysteine cluster with the pattern of $Cx_{25}Cx_{16}C$. The IRs are highly conserved among the homologs.

A. thaliana RPK2 is a key regulator of anther development (e.g., lignifications pattern), including tapetum degradation during pollen maturation (e.g., germination capacity) [96–98] and contributes to shoot aptical meristerm homeostasis [99,100]. The RPK2 homologs from *Arabidopsis lyrata subsp. Lyrata*, *Populus trichocarpa*, and *R. communis* contain 21–22 LRRs with two non-LRR IRs. The first IR is between **LRR9** and **LRR10**. The second IR is between **LRR13** and **LRR14** (Figure 2 and Table 1, and Supplementary Table S1 and Figure S1). The second IRs are highly conserved among homologs. There are an LRRNT (with $Cx_6Cx_{25}Cx_{16}C$) and an LRRCT (with $Cx_{11}C$). The LRRCT region is rich in Ser and Pro residues. Sawa and Tabata [101] have reported the RPK2 homologs from other plant species-*Musa acuminate*, *O.sativa* Japonica Group, *Vitis vinifera*, *Sorghum bicolor*, *Physcomitrella patens*, and *Marchantia polymorpha*.

3.2. Eleven Families of LRR-RLPs

LRR-RLPs have a short cytoplasmic tail instead of the kinase region in LRR-RLKs (Figure 3) [20]. LRR-RLPs are involved both in resistance of plant–pathogen interactions and development [34,102]. Tomato *Cf* genes confer resistance to the fungal pathogen *Cladosporium fulvum* [43,56,103,104]. Tomato Verticillium wilt disease resistance gens (*Ve1*) and *Ve2*, apple *HcrVf2*, Arabidopsis *RPP27* are

involved in resistance to *Verticillium*, *Venturia*, and *Peronospora*, respectively [105–107]. Furthermore, the tomato LeEIX initiates defense responses upon elicitation with a fungal ethylene-inducing xylanase (EIX) of non-pathogenic *Trichoderma* from tomato that confer resistance against the fungal pathogen *Cladosporium fulvum* [108,109]. The *clavata2* (*CLV2*) functions in both shoot and root meristems of *Arabidopsis* [58,110–112] and also affects autoregulation of nodulation of pea and *Lotus japonicus* [113,114]. *Zea mays fascinated ear2* is involved in meristem development [59]. *A. thaliana* RLP2 is involved in the perception of CLV3 and CLV3-like peptides, that act as extracellular signals regulating meristems maintenance [64]. The LRR-RLPs are all LRR@IR proteins in which the LRRs are intersected by a single non-LRR IR (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1).

Figure 3. Schematic representation of 11 LRR-RLPs having LRR domains intersected by non-LRR island regions. Currant tomato Cf-9 [Q40235I]; Currant tomato Cf-2.1 [Q41397]; Tomato Ve1 [Q94G61]; Apple HcrVf1 [Q949G9]; *A. thaliana* RPP27 [Q70CT4]; Tomato EIX1 [Q6JN47]; *A. thaliana* CLV2 [Q9SPE9]; Maize fascinated ear2 [Q940E8]; *A. thaliana* RLP2 [RLP2_ARATH]; *Oryza sativa* Os10g0469700 [Q337L7]; Soybean disease resistance protein [C6ZS07].
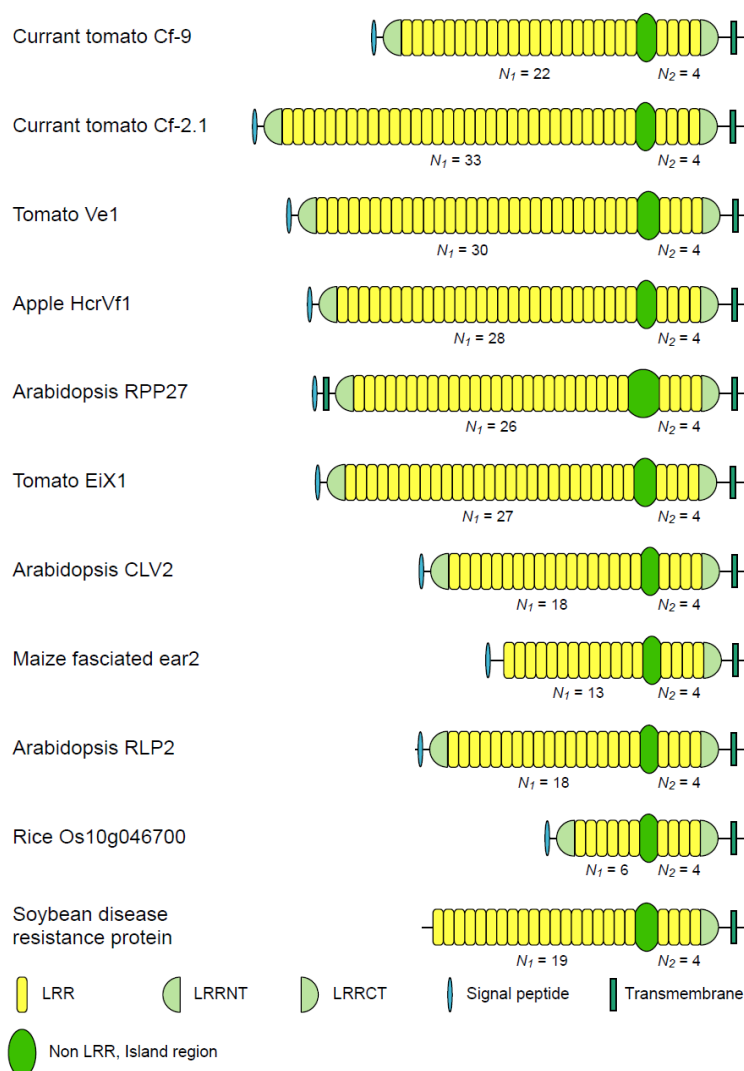
Tomato Cf-9/Cf-4 homologs were identified from six species. Elicitor-inducible LRR receptor-like protein (EILP) from *N. tabacum* [115] was identified as ortholog of tomato Cf-9/Cf-4. The number of N_1 is 18 to 22, while N_2 keeps 4, and the non-LRR IRs are 40–44 residues long (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1) and have a conserved motif of $MKx_3Ex_6Yx_{5-8}Yx_7TKG$ in which hydrophilic residues are conserved. The EILP protein also contains 27 LRRs with $N_1 = 23$ and $N_2 = 4$. Most of the homologs have LRRNT consisting of six Cys residues with the pattern of $Cx_{24-29}Cx_{13-23}CCx_6Cx_{12-13}C$. However, peru 1 and peru 2 have an LRRNT of four Cys's with the pattern of $Cx_{47}CCx_6C$ [116]. The C-terminal side of the LRRCT is rich in Glu and Asp residues and thus is acidic.

Tomato Cf-2/Cf-5 homologs were identified from two species (*Lycopersicon esculentum,* and *L. pimpinellifolium*). The number of N_l is highly variable; $N_1 = 20 - 33$, while N_2 keeps 4, and the non-LRR IRs are 37–41 residues long. The IRs are hydrophilic. The variability of N_1 has been reported by other researches in between the paralogs and orthologs [43,46,103,104] (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1). Interestingly, the N-terminal LRRs include tandem repeats of the super-motif of two highly conserved LRRs; for example, LxxLxLxxNxLSGxIPxxIGYLRS and LxxLxLSxNxLNGxIPxxFGxLxN in currant tomato Cf-2.1 [103].

Tomato Ve orthologs and paralogs were identified from twelve species including *Solanum neorickii*, *S. aethiopicum*, *Mentha longifolia*, and *M. spicata* [105,117,118]. The Ve homologs contain 32–34 LRRs intercepted by a 44 to 49-residue, non-LRR IR with $N_1 = 28 - 30$ and $N_2 = 4$ (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1). The non-LRR IRs have a conserved motif of $YYx_8K(G/R)$ and are relatively hydrophilic.

Apple *HcrVfs* (**H**omologs of **C**ladosporium fulvum **r**esistance genes of **Vf** region) are scab resistance genes [119,120]. *Mentha longifolia HcrVfs* are orthologs of tomato *Ve* genes [105,117,118]. The HcrVfs paralogs contain 32–34 LRRs intercepted by a 41 to 46-residue, non-LRR IR with $N_1 = 22 - 28$ and $N_2 = 4$ (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1). The non-LRR IRs have a conserved motif of VTKGxExEYx(K/E)ILxFxKxxDLSCNF in which hydrophilic residues are conserved. The C-terminal side of the LRRCT is rich in Gly and Pro residues.

A. thaliana RPP27 homologs were also identified from *A. lyrata.* The LRR@IR proteins contain 16–30 LRRs intercepted by a 61 to 71-residue, non-LRR IR with $N_1 = 11 - 26$ and $N_2 = 4$ (Figure 2 and Table 1, and Supplementary Table S1 and Figure S1). The IRs have a conserved motif of FxxKxRYD. The C-terminal side of most LRRCT regions is acidic.

Tomato LeEIX1 and LeEIX2 contain 31 LRRs intercepted by a 47 to 49-residue, non-LRR IR with $N_1 = 27$ and $N_2 = 4$ (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1). The C-terminal side of the LRRCT is acidic.

A. thaliana CLV2 homologs were identified from 11 species. The CLV2 homologous proteins contain 22 LRRs intercepted by a 41 to 43-residue, non-LRR IR with $N_1 = 18$ and $N_2 = 4$ (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1). The IRs have a conserved motif of LxFxYxL. The C-terminal side of most LRRCT regions is acidic. *A. thaliana* CLV1 is an LRR-RLP but not LRR@IR protein.

Z. mays fascinated ear2 is an ortholog of Arabidopsis CLV2. The homologs were also identified from *O. sativa subsp. Japonica*, and *indica*, and *S. bicolor*. The fascinated ear2 homologous proteins contain 17–18 LRRs intercepted by a 41 to 42-residue, IR with $N_1 = 10 - 14$ and $N_2 = 4$ (Figure 3 and

Table 1, and Supplementary Table S1 and Figure S1). The IRs and the LRRCT regions are rich in Gly. Both regions may be flexible.

A. thaliana RLP2 contains 23 LRRs that are intercepted by a 44-residue, IR with N_1 = 18 and N_2 = 4 (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1). There are an LRRNT and an LRRCT. The extracellular region including the 23 LRRs is homologous to that in *A. thaliana* PSYR1 [121].

O. sativa Os10g0469700 is an LRR@IR protein; the function is unknown (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1). The homologs from four species contain 10 LRRs with a single IR with N_1 = 6 and N_2 = 4. The non-LRR IRs with 39–40 residues is represented by MKxP(K/E)IxSSx$_{2-3}$LDGSxYQDRIDIxWKGx$_3$FQx$_4$L.

A putative disease resistance protein from soybean [C6ZS07] is an LRR@IR protein (Figure 3 and Table 1, and Supplementary Table S1 and Figure S1). The homologs were identified from four species and contain 8–32 LRRs with a single IR with N_1 = 4 − 28 and N_2 = 4. The N_1 number is highly variable in both the paralogs and orthologs. The IRs have a conserved motif of Yx$_2$Sx$_5$Kx$_7$(R/K)I.

3.3. Two Families of Plant Intracellular Proteins

A. thaliana TONSOKU(TSK)/MGOUN3(MGO3)/BRUSHY1(BRU1), which is localized in the nucleus and is preferentially expressed in the shoot apex than in the leaves and stems, is required for cell arrangement in root and shoot apical meristems and involved in structural and functional stabilization of chromatin [122–124]. The TONSOKU protein may represent a link between response to DNA damage and epigenetic gene silencing [125].

Potential homologs of *A. thaliana* TONSOKU have been identified in eight species. The UniProKB database describes that *A. thaliana* TONSOKU contains three LRRs and eight TPRs, while the data bases - InterPro, Gene3D, SMART and PROSITE-identify only TPR. LRR@IRpred identifies 14 LRRs with a single IR; N_1 = 13, N_2 = 1 (Figure 4 and Table 1, and Supplementary Table S1 and Figure S1) [47]. The LRRs are not "plant-specific" motifs but presumably "RI-like" motifs. Thus, the structural LRR units may be represented by β-α instead of β-β-3$_{10}$. The LRR domain is predicted to adopt a typical horseshoe shape seen in ribonuclease inhibitor [126]. The non-LRR IRs are 70–131 residues long and are rich in Ser and Gly. The IRs may be unstructured or flexible.

A. thaliana MJK13.7 is considered to be intracellular protein. The function is unknown. *A. thaliana* MJK13.7 homologs were identified from 11 species. The homologs contain 20 LRRs intersected by a single IR; N_1 = 12, N_2 = 8 (Figure 4 and Table 1, and Supplementary Table S1 and Figure S1). All of the non-LRR IRs are 60–62 residues long and have conserved Lys residues at five positions. The consensus of the LRRs is LxxLxLxxNxLxxLPxxLxxLxx of 23 residues that are present in many proteins from bacteria to human (data not shown). The LRR motif does not belong to PS-LRR and the structure of the LRR domain is not available. However, the LRR motifs are contained in part of the LRR domains in toll-like receptor 1 (TLR1) and glycoprotein Ibα (GpIbα) of which the crystal structures are available [127–130]. Four LRRs are <u>IKVLDLHSNKI</u> K**SIP**KQ<u>V</u>VK<u>L</u>EA and <u>LQELNVASNQL</u> K**SVP**DGIFDR<u>L</u>TS in TLR1, and <u>LGTLDLSHNQL</u> QS**LPL**LGQT<u>L</u>PA and <u>LDTLLL</u>QENSL YT**IPK**G<u>F</u>FGSHL in GpIbαe. The structures revealed that the LRRs may be characterized by extended conformations at the bold sequences [127–130].

Figure 4. Schematic representation of two plant intracellular LRR@IR proteins having LRR domains intersected by non-LRR island regions. *A. thaliana* MJK13.7 [Q9M7W9]; *A. thaliana* TONSOKU [Q6Q4D0].
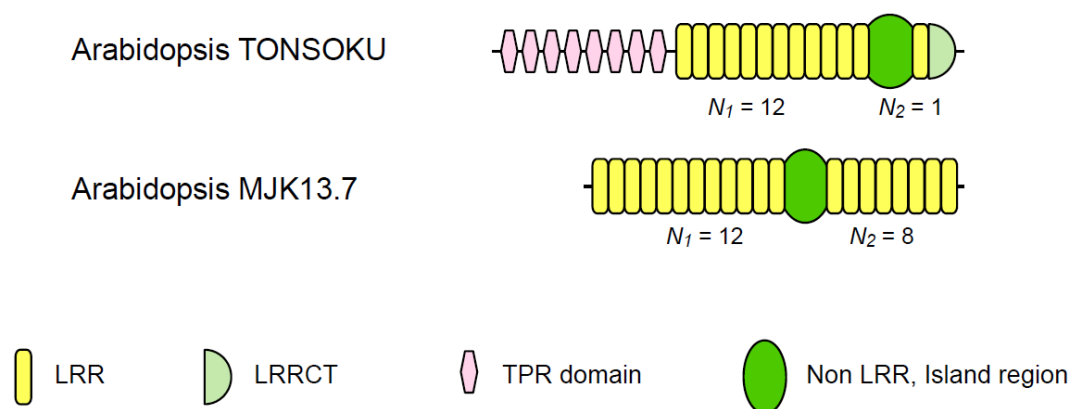
Moreover, *A. thaliana* MJK13.7 forms a family with its homologs from insect species, *Strongylocentrotus purpuratus, Nematostella vectensis*, and *Paramecium tetraurelia* and LRRC40 from vertebrates species [47]. The *S. purpuratus* protein has 163 residues containing two repeats of 64 residues each [47].

3.4. An NBS-LRR Protein

Rice blast resistance gene *Pi-ta* encodes an NBS-LRR protein with 928 residues [44,45]. The Pi-ta protein [Q9AY26] lacks a canonical LRR [44]. The C-terminal region contains highly imperfect LRRs with 10 repeats of various lengths (from 16 to 75 residues) based on the consensus LxxLxxL. The Pi-ta protein appears to be an LRR@IR protein. LRR@IRpred predicts 13 LRRs of 20–54 residues with one non-LRR, IR between ***LRR6*** **and** ***LRR7*** (Supplementary Figure S1). The secondary structure prediction prefers α-helix in the VS's. The Pi-ta LRR domain might adopt a similar structure to those of TIR1 and COI1 [74–76].

4. Features, Structure, Function, and Evolution of the LRR Domains in Plant LRR@IR Proteins

4.1. Fundamental Features

Most plant LRR@IR proteins that are LRR-RLKs or LRR-RLPs keep the rule of $N_1 \gg N_2$; $N_1 = 10 - 30$ and $N_2 = 3 - 5$ (Table 1). The same rule of $N_1 \gg N_2$ is observed in other LRR@IR proteins of toll receptors and toll-related proteins from insect species, that have one single transmembrane-spanning region and an intracellular Toll IL-receptor (TIR) domain as well as TLRs instead of the kinase region in LRR-RLKs [131]. Most toll receptors and toll-related proteins contain 21–30 LRRs interrupted by a single non-LRR IRs of 81–120 residues with $N_1 \gg N_2$; $N_1 = 17 - 24$ and $N_2 = 4 - 6$ (data not shown). Fritz-Laylin *et al.* [46] have performed sequence analysis of 90 LRR-RLPs of rice (*O. sativa*) and 56 Arabidopsis (*A. thaliana*). Many LRR-RLPs contain 18–28 LRRs intercepted by a 30 to 80-residue, single IR with $N_1 \gg N_2$; $N_1 = 14 - 24$ and $N_2 = 4$ [46].

The non-LRR IRs in plant LRR@IR proteins may be classified into two groups; one group is non-LRR IRs having cysteine clusters, while the other has no cysteine clusters. The IR cysteine

clusters are characterized by $Cx_{6-7}Cx_{29-30}Cx_{7-11}C$ in *A. thaliana* TMK1 homologs, $Cx_{25}C$ in BRI1 homologs, and $Cx_{25}Cx_{16}C$ in At1g74360 homologs. The other non-LRR IRs frequently have a conserved motif of Yx_8KG which are observed in the homologs of *A. thaliana* TMK1, tomato Cf-9/Cf-4, tomato Cf-2/Cf-5, tomato Ve, *M. longifolia* HCrVf, *A. thaliana* CLV2, and *Z. mays* fascinated ear2, and *O. sativa* Os10g0469700. Non-LRR IRs in many LRR-RLPs from Arbidopsis and rice contain a conserved motif of Yx_8KG [46].

Most of the LRRNTs consist of two, four, or six Cys residues of which the patterns are $Cx_{6-7}C$, $Cx_{23-34}CCx_6C$, and $Cx_{24-29}Cx_{13-23}CCx_6Cx_{12-13}C$. They probably form one, two, and three disulfide bonds, respectively. The LRRCTs consist of two Cys's with the pattern of $Cx_{4-29}C$ which probably form one disulfide bond (Supplementary Table S1 and Figure S1). The disulfide bonds should contribute to the structural stabilization of the N-terminal and C-terminal caps.

4.2. Possible Structures

The structure of a non-LRR IR is available in *A. thaliana* BRI1 (Figure 1A). The BRI1 LRR domain forms a superhelix with 25 LRRs. The 70-residue, non-LRR, IR in BRI1 between **LRR21** and **LRR22** forms a small domain that folds back into the interior of the superhelix, where it makes extensive polar and hydrophobic interactions with LRRs 13–25 [16,17]. The LRR domain fold is characterized by an anti-parallel β-sheet, which is sandwiched between the LRR core and a 3_{10} helix and stabilized by a disulphide bridge of the Cys cluster with $Cx_{25}C$ in the non-LRR, IR. Cys clusters are also present in non-LRR, IRs in the homologs of TMK, At1g74360 and TONSOKU. Thus, the non-LRR IRs may adopt similar structures with disulfide bridges. All of the non-LRR IRs would fold back into the interior or exterior of a superhelix of the LRR domains.

4.3. Possible Function(s)

The non-LRR IRs of BRI1 and PSKR participate in ligand/protein-protein interactions. The BRI1 non-LRR IR binds brassinosteroids [62]. The insertion of a folded domain into the LRR repeat is probably an adaptation to the challenge of sensing a small steroid ligand [16]. The PSKR non-LRR IR also binds PSK [63]. The non-LRR IRs in TLRs 7, 8, and 9 was also predicted to contribute to nucleic acid-protein interaction [66,132].

The non-LRR IRs in plant LRR@IR proteins have frequently conserved motifs that are characterized by hydrophilic residues such as Lys, Arg, Glu and Asp, as noted. Some non-LRR IRs are presumably flexible. The conservation of hydrophilic residues in the IRs is also observed in the respective families of LRRC40, LRRC9, and *C. elegans* LRK-1 which are LRR@IR proteins from organisms including vertebrate other than plants [47]. The IRs might contribute to ligand/protein-protein interactions [47]. Moreover, Afzals *et al.* [133] suggested, based on circular dichroism data, that non-LRR IRs are intrinsically unstructured, providing binding diversity to the domains.

The first LRR block in tomato Cf-9, Cf-4, and Cf-2 recognize fungal avirulence proteins [134–138]. The recognitional specificity of Cf-2 with 37 LRRs lies between leucine-rich repeat **LRR3** and **LRR27**, a region that differs from Cf-5 with 31 LRRs by six extra LRR and 78 amino acid substitutions [134]. Although crudely defined, this region of specificity corresponds to those in Cf-4, Cf-9, and Cf-9B

responsible for recognition of their cognate ligands [135–138]. Biochemical studies show that CLV2 is essential for the stability of CLV1, in which CLV1 and CLV2 may form a disulfide-linked heterodimer of 185 kD [58]; CLV1 is an LRR-RLP having no non-LRR IR.

Drosophila Toll and vertebrate TLRs 7, 8, and 9 are LRR@IR proteins [65–67] which contain one single transmembrane-spanning region as well as LRR-RLKs and LRK-RLPs from plant. Homo- or heterodimerization are involved in ligand-interactions of vertebrate TLRs [68–71]. A model for Drosophila Toll activation by ligand Spatzle has been proposed; the first LRR block interacts with Spatzle and the second LRR block forms strong dimer contacts that are prevented by the first block, which in the absence of ligand provides a steric constraint [67,131]. The BRI1 receptor activation involves homodimerization [139]; although Hothorn *et al*., [16] suggested that the superhelical BRI1 LRR domain alone has no tendency to oligomerize, indicating that BRI1 receptor activation may not be mediated by ligand-induced homodimerization of the ectodomain.

Taken together, non-LRR IRs in plant LRR@IR proteins might participate in ligand/protein-interactions, dimerization or both, although an LRR-RLP, *A. thaliana* CLV2, remains functional without non-LRR IR, while the first and the second LRR blocks are essential for functionality [64]. $N_1 \gg N_2$ brings close proximity of the non-LRR IRs to interact with ligand/protein and a transmembrane region. $N_1 \gg N_2$ might facilitate signaling in the cytoplasm through the ligand/protein- interactions.

There is a possibility that Cys residues in LRRs are involved in dimerization of LRR@IR proteins. The conserved hydrophobic residues of the PS-LRR consensus sequence of LxxLxLxxNxLSGxIPxxLxxLxx at positions 1, 4, 6, 11, 15, 19, and 22 contribute to the hydrophobic cores in the LRR arcs [8,9]. The conserved hydrophobic residues at positions 1, 19 and 22, and "N" at position 9, are frequently occupied by Cys in the PS-LRRs. Moreover, Cys residues are frequently observed in noncanonical PS-LRRs which, as examples, are longer LRR motifs of 25–30 residues with the consensus of LxxLxLxxNxLSGxIPxxLCxxxxx(x/-)(x/-)(x/-)(x/-)(x/-), in which "-" indicates a possible deletion site. At the present stage it remains unknown whether the Cys residues contribute to the hydrophobic core of the LRR arcs or are exposed to solvent. However, some LRR@IR proteins contain PS-LRRs having Cys at positions 2, 3, or 5 in the HCS part (Supplementary Table S1). The Cys residues are likely to be exposed to solvent in the LRR arc and thus might induce dimerization.

4.4. Implications for Evolution

What is the evolutionary origin of non-LRR IRs interrupting LRRs? Previous research provided evidence that a direct duplication of the super motifs containing non-LRR regions naturally leads to the occurrence of non-LRR IRs in LRR@IR proteins, including LRR-containing 17 protein (LRRC17), LRRC32, LRR33, chondroadherin-like protein, trophoblast glycoprotein precursor, and *Leishmania* proteophosphoglycans, not from plants but from other eukaryotes [47]. The non-LRR IRs in plant LRR@IR proteins might originate from such similar events.

The tomato Cf-2/Cf-5 homologs have PS-LRRs that include tandem repeats of the super-motif of two highly conserved LRRs, as noted [103]. The duplications of the super-motif were suggested to have occurred in the Cf-2/Cf-5 homologs [43]. Super-motifs of LRRs are observed in many LRR proteins. The SLRP subfamily (biglycan, decorin, asporin, lumican, fibromodulin, PRELP, keratocan, osteoadherin, epiphycan, osteoglycin, opticin, and podocan), the TLR7 family (TLR7, TLR8 and

TLR9), the FLRT family (FLRT1, FLRT2, and FLRT3), and OMGP [65,140,141] contain tandem repeats of a super-domain of ***STT***, where "***T***" is "typical" LRR and "***S***" is "Bacterial" LRR. Ribonuclease inhibitor also has RI- LRRs consisting of a super-motif of 57 residues that encode two LRRs [142]. The super-repeats as well as Cf-2/Cf-5 have been contributed to the duplication of their super-motifs.

5. Evolution of Plant LRR@IR Proteins

A large number of LRR-RLPs resembling the extracellular domains of LRR-RLKs are found in the Arabidopsis genome; although not all RLK subfamilies have corresponding RLPs [121]. Indeed, the present analysis indicates that the extracellular domain in PSYR1 is highly similar to that in RLP2. The same distributions also occur in LRR@IR proteins from other plants, such as *S. bicolor* and *O. sativa* (Supplementary Figure S2). Here four examples are described: Sb10g028170/Sb10g028210 (LRR-RLK/LRR-RLP), and Os06g0691800/Os06g0692700; all the four proteins contain 22 LRRs intersected by a single non-LRR IR of 33 residues with $N_1 = 18$ and $N_2 = 4$. The others are Os07g0597200/Os03g0400850, and OsI_26735/OsI_11946; the LRR-RLKs-Os07g0597200 and OsI_26735 are homologs of Arabidopsis At1g74360. The pair-wise comparisons of the amino acid sequences exceed 50% of the identity in respective pairs. The above observations indicate that the LRR-RLKs and LRR-RLPs evolved from gene duplications and recombination [39].

Two putative uncharacterized proteins from *Z. mays* with 717 residues [B8A2X8] and with 623 residues [B8A383_MAIZE] are paralogs of *Z. mays* TMK1 with 958 residues (Supplementary Figure S2). The 717-residue protein contains 6 LRRs; $N_1 = 3$ and $N_2 = 3$. There are other examples; a hypothetical protein from *Z. mays* with 247 residues [C0PL86] and fasciated ear2 with 613 residues, *O. sativa* Os02g0782800 with 441 residues [Q6K7E5] and BRUSHY1 with 1,332 residues [Q6K7D3]. The occurrence of these proteins is attributed to gene duplication and deletions.

6. Conclusions

Most plant LRR@IR proteins have LRRs intersected a single IR with $N_1 \gg N_2$ in which N_1 is variable in their individual homologs, while N_2 is highly conserved. For all known LRR-RLPs, $N_1 = 4$. The rule of $N_1 \gg N_2$ plays a common, significant role in ligand-interaction, dimerization, and/or signal transduction of the LRR-RLKs and the LRR-RLPs. All of the LRR domains consisting of PS- LRRs are predicted to form a superhelix and non-LRR IRs in plant LRR@IR proteins fold back into the interior or exterior of the superhelix. The present analyses suggest that some LRR-RLKs and LRR-RLPs evolved from gene duplications and recombination. The present review will stimulate various experimental studies to understand the structure and evolution of the LRR domains with non-LRR IRs and their proteins.

Acknowledgments

References

1. Finn, R.D.; Tate, J.; Mistry, J.; Coggill, P.C.; Sammut, S.J.; Hotz, H.R.; Ceric, G.; Forslund, K.; Eddy, S.R.; Sonnhammer, E.L.; *et al*. The Pfam protein families database. *Nucleic Acids Res.* **2008**, *36*, D281–D288.

2. Letunic, I.; Doerks, T.; Bork, P. SMART 7: Recent updates to the protein domain annotation resource. *Nucleic Acids Res.* **2011**, *40*, D302–D305.

3. Sigrist, C.J.; Cerutti, L.; de Castro, E.; Langendijk-Genevaux, P.S.; Bulliard, V.; Bairoch, A.; Hulo, N. PROSITE, a protein domain database for functional characterization and annotation. *Nucleic Acids Res*. **2010**, *38*, D161–D166.

4. Burge, S.; Kelly, E.; Lonsdale, D.; Mutowo-Muellenet, P.; McAnulla, C.; Mitchell, A.; Sangrador-Vegas, A.; Yong, S.Y.; Mulder, N.; Hunter, S. Manual GO annotation of predictive protein signatures: The InterPro approach to GO curation. *Database (Oxford)* **2012**, *2012*, bar068.

5. IPR001611 Leucine-rich repeat. Available online: http://www.ebi.ac.uk/interpro/ IEntry?ac=IPR001611 (accessed on 5 May 2012).

6. Kobe, B.; Deisenhofer, J. The leucine-rich repeat: A versatile binding motif. *Trends Biochem. Sci.* **1994**, *19*, 415–421.

7. Kobe, B.; Kajava, A.V. The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct Biol.* **2001**, *11*, 725–732.

8. Matsushima, N.; Enkhbayar, P.; Kamiya, M.; Osaki, M.; Kretsinger, R. Leucine-Rich Repeats (LRRs): Structure, Function, Evolution and Interaction with Ligands. *Drug. Design Rev.* **2005**, *2*, 305–322.

9. Matsushima, N.; Tachi, N.; Kuroki, Y.; Enkhbayar, P.; Osaki, M.; Kamiya, M.; Kretsinger, R.H. Structural analysis of leucine-rich-repeat variants in proteins associated with human diseases. *Cell Mol. Life Sci.* **2005**, *62*, 2771–2791.

10. Bella, J.; Hindle, K.L.; McEwan, P.A.; Lovell, S.C. The leucine-rich repeat structure. *Cell Mol. Life Sci.* **2008**, *65*, 2307–2333.

11. Kajava, A.V. Structural diversity of leucine-rich repeat proteins. *J. Mol. Biol.* **1998**, *277*, 519–527.

12. Ohyanagi, T.; Matsushima, N. Classification of tandem leucine-rich repeats within a great variety of proteins. *FASEB J.* **1997**, *11*, A949.

13. Matsushima, N.; Miyashita, H.; Mikami, T.; Kuroki, Y. A nested leucine rich repeat (LRR) domain: The precursor of LRRs is a ten or eleven residue motif. *BMC Microbiol.* **2010**, *10*, 235.

14. Kajava, A.V.; Anisimova, M.; Peeters, N. Origin and evolution of GALA-LRR, a new member of the CC-LRR subfamily: From plants to bacteria? *PLoS One* **2008**, *3*, e1694.

15. Di Matteo, A.; Federici, L.; Mattei, B.; Salvi, G.; Johnson, K.A.; Savino, C.; De Lorenzo, G.; Tsernoglou, D.; Cervone, F. The crystal structure of polygalacturonase-inhibiting protein (PGIP), a leucine-rich repeat protein involved in plant defense. *Proc. Natl. Acad Sci. USA* **2003**, *100*, 10124–10128.

16. Hothorn, M.; Belkhadir, Y.; Dreux, M.; Dabi, T.; Noel, J.P.; Wilson, I.A.; Chory, J. Structural basis of steroid hormone perception by the receptor kinase BRI1. *Nature* **2011**, *474*, 467–471.

17. Chai, J.J.; She, J.; Han, Z.F.; Kim, T.W.; Wang, J.J.; Cheng, W.; Chang, J.B.; Shi, S.A.; Wang, J.W.; Yang, M.J.; *et al*. Structural insight into brassinosteroid perception by BRI1. *Nature* **2011**, *474*, 472–496.

18. Afzal, A.J.; Wood, A.J.; Lightfoot, D.A. Plant receptor-like serine threonine kinases: Roles in signaling and plant defense. *Mol. Plant Microbe Interact* **2008**, *21*, 507–517.

19. Gish, L.A.; Clark, S.E. The RLK/Pelle family of kinases. *Plant J.* **2011**, *66*, 117–127.

20. Dievart, A.; Clark, S.E. LRR-containing receptors regulating plant development and defense. *Development* **2004**, *131*, 251–261.

21. DeYoung, B.J.; Innes, R.W. Plant NBS-LRR proteins in pathogen sensing and host defense. *Nat. Immunol.* **2006**, *7*, 1243–1249.

22. McHale, L.; Tan, X.; Koehl, P.; Michelmore, R.W. Plant NBS-LRR proteins: Adaptable guards. *Genome Biol.* **2006**, *7*, 212.

23. Di Matteo, A.; Bonivento, D.; Tsernoglou, D.; Federici, L.; Cervone, F. Polygalacturonase-inhibiting protein (PGIP) in plant defence: A structural view. *Phytochemistry* **2006**, *67*,528–533.

24. Di, C.; Zhang, M.; Xu, S.; Cheng, T.; An, L. Role of poly-galacturonase inhibiting protein in plant defense. *Crit. Rev. Microbiol.* **2006**, *32*, 91–100.

25. Wang, G.; Fiers, M. Receptor-like proteins: Searching for functions. *Plant Signal. Behav.* **2010**, *5*, 540-542.

26. Jones, D.; Jones, J. The role of leucine-rich repeat proteins in plant defenses. *Adv. Bot. Res.* **1997**, *24*, 89–167.

27. Jaillais, Y.; Belkhadir, Y.; Balsemao-Pires, E.; Dangl, J.L.; Chory, J. Extracellular leucine-rich repeats as a platform for receptor/coreceptor complex formation. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 8503–8507.

28. Tor, M.; Lotze, M.T.; Holton, N. Receptor-mediated signalling in plants: Molecular patterns and programmes. *J. Exp. Bot.* **2009**, *60*, 3645–3654.

29. Michelmore, R.W.; Meyers, B.C. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. *Genome Res.* **1998**, *8*, 1113–1130.

30. Couch, B.C.; Spangler, R.; Ramos, C.; May, G. Pervasive purifying selection characterizes the evolution of I2 homologs. *Mol. Plant Microbe Interact.* **2006**, *19*, 288–303.

31. Liu, J.; Liu, X.; Dai, L.; Wang, G. Recent progress in elucidating the structure, function and evolution of disease resistance genes in plants. *J. Genet. Genomics* **2007**, *34*, 765–776.

32. Friedman, A.R.; Baker, B.J. The evolution of resistance genes in multi-protein plant resistance systems. *Curr. Opin. Genet. Dev.* **2007**, *17*, 493–499.

33. McDowell, J.M.; Simon, S.A. Molecular diversity at the plant-pathogen interface. *Dev. Comp. Immunol.* **2008**, *32*, 736–744.

34. Wulff, B.B.; Chakrabarti, A.; Jones, D.A. Recognitional specificity and evolution in the tomato-Cladosporium fulvum pathosystem. *Mol. Plant Microbe Interact* **2009**, *22*, 1191–1202.

35. Hulbert, S.H.; Webb, C.A.; Smith, S.M.; Sun, Q. Resistance gene complexes: Evolution and utilization. *Annu. Rev. Phytopathol.* **2001**, *39*, 285–312.

36. Dodds, P.N.; Lawrence, G.J.; Catanzariti, A.M.; Teh, T.; Wang, C.-I.A.; Ayliffe, M.A.; Kobe, B. Ellis, J.G. Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 8888–8893.

37. Parniske, M.; Hammond-Kosack, K.E.; Golstein, C.; Thomas, C.M.; Jones, D.A.; Harrison, K.; Wulff, B.B.; Jones, J.D. Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the Cf-4/9 locus of tomato. *Cell* **1997**, *91*, 821–832.

38. Wei, F.; Wing, R.A.; Wise, R.P. Genome dynamics and evolution of the Mla (powdery mildew) resistance locus in barley. *Plant Cell* **2002**, *14*, 1903–1917.

39. Leister, D. Tandem and segmental gene duplication and recombination in the evolution of plant disease resistance gene. *Trends Genet.* **2004**, *20*, 116–122.

40. Baumgarten, A; Cannon, S.; Spangler, R.; May, G. Genome-level evolution of resistance genes in Arabidopsis thaliana. *Genetics* **2003**, *165*, 309–319.

41. Zhou, B; Dolan, M.; Sakai, H.; Wang, G.L. The genomic dynamics and evolutionary mechanism of the Pi2/9 locus in rice. *Mol. Plant Microbe Interact* **2007**, *20*, 63–71.

42. Mondragon-Palomino, M.; Gaut, B.S. Gene conversion and the evolution of three leucine-rich repeat gene families in Arabidopsis thaliana. *Mol. Biol. Evol.* **2005**, *22*, 2444–2456.

43. Dixon, M.S.; Hatzixanthis, K.; Jones, D.A.; Harrison, K.; Jones, J.D. The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. *Plant Cell* **1998**, *10*, 1915–1925.

44. Bryan, G.T.; Wu, K.S.; Farrall, L.; Jia, Y.; Hershey, H.P.; McAdams, S.A.; Faulk, K.N.; Donaldson, G.K.; Tarchini, R.; Valent, B. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene Pi-ta. *Plant Cell* **2000**, *12*, 2033–2046.

45. Jia, Y.; McAdams, S.A.; Bryan, G.T.; Hershey, H.P.; Valent, B. Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *Embo J.* **2000**, *19*, 4004–4014.

46. Fritz-Laylin, L.K.; Krishnamurthy, N.; Tor, M.; Sjolander, K.V.; Jones, J.D. Phylogenomic analysis of the receptor-like proteins of rice and Arabidopsis. *Plant Physiol.* **2005**, *138*, 611–623.

47. Matsushima, N.; Mikami, T.; Tanaka, T.; Miyashita, H.; Yamada, K.; Kuroki, Y. Analyses of non-leucine-rich repeat (non-LRR) regions intervening between LRRs in proteins. *Biochim. Biophys. Acta* **2009**, *1790*, 1217–1237.

48. Torii, K.U. Leucine-rich repeat receptor kinases in plants: Structure, function, and signal transduction pathways. *Int. Rev. Cytol.* **2004**, *234*, 1–46.

49. van der Hoorn, R.A.; Wulff, B.B.; Rivas, S.; Durrant, M.C.; van der Ploeg, A.; de Wit, P.J.; Jones, J.D. Structure-function analysis of cf-9, a receptor-like protein with extracytoplasmic leucine-rich repeats. *Plant Cell* **2005**, *17*, 1000–1015.

50. Wang, G.; Ellendorff, U.; Kemp, B.; Mansfield, J.W.; Forsyth, A.; Mitchell, K.; Bastas, K.; Liu, C.M.; Woods-Tor, A.; Zipfel, C.; *et al*. A genome-wide functional investigation into the roles of receptor-like proteins in Arabidopsis. *Plant Physiol.* **2008**, *147*, 503–517.

51. Li, J.; Chory, J. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell* **1997**, *90*, 929–938.

52. Nomura, T.; Bishop, G.J.; Kaneta, T.; Reid, J.B.; Chory, J.; Yokota, T. The LKA gene is a BRASSINOSTEROID INSENSITIVE 1 homolog of pea. *Plant J.* **2003**, *36*, 291–300.

53. Scheer, J.M.; Ryan, C.A., Jr. The systemin receptor SR160 from Lycopersicon peruvianum is a member of the LRR receptor kinase family. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9585–9590.

54. Montoya, T.; Nomura, T.; Farrar, K.; Kaneta, T.; Yokota, T.; Bishop, G.J. Cloning the tomato curl3 gene highlights the putative dual role of the leucine-rich repeat receptor kinase tBRI1/SR160 in plant steroid hormone and peptide hormone signaling. *Plant Cell* **2002**, *14*, 3163–3176.

55. Matsubayashi, Y.; Ogawa, M.; Morita, A.; Sakagami, Y. An LRR receptor kinase involved in perception of a peptide plant hormone, phytosulfokine. *Science* **2002**, *296*, 1470–1472.

56. Jones, D.A.; Thomas, C.M.; Hammond-Kosack, K.E.; Balint-Kurti, P.J.; Jones, J.D. Isolation of the tomato Cf-9 gene for resistance to Cladosporium fulvum by transposon tagging. *Science* **1994**, *266*, 789–793.

57. Kruijt, M.; Kip, D.J.; Joosten, M.H.; Brandwagt, B.F.; de Wit, P.J. The Cf-4 and Cf-9 resistance genes against Cladosporium fulvum are conserved in wild tomato species. *Mol. Plant Microbe Interact* **2005**, *18*, 1011–1021.

58. Jeong, S.; Trotochaud, A.E.; Clark, S.E. The Arabidopsis CLAVATA2 gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. *Plant Cell* **1999**, *11*, 1925–1934.

59. Taguchi-Shiobara, F.; Yuan, Z.; Hake, S.; Jackson, D. The fasciated ear2 gene encodes a leucine-rich repeat receptor-like protein that regulates shoot meristem proliferation in maize. *Genes Dev.* **2001**, *15*, 2755–2766.

60. de Kock, J.D.M.; Brandwagt, F.B.; Bonnema, G.; de Wit, P.J.G.M.; Lindhout, P. The tomato Orion locus comprises a unique class of Hcr9 genes. *Mol. Breed.* **2005**, *15*, 409–422.

61. Chow, B.; McCourt, P. Plant hormone receptors: Perception is everything. *Genes Dev.* **2006**, *20*, 1998–2008.

62. Kinoshita, T.; Cano-Delgado, A.; Seto, H.; Hiranuma, S.; Fujioka, S.; Yoshida, S.; Chory, J. Binding of brassinosteroids to the extracellular domain of plant receptor kinase BRI1. *Nature* **2005**, *433*, 167–171.

63. Shinohara, H.; Ogawa, M.; Sakagami, Y.; Matsubayashi, Y. Identification of ligand binding site of phytosulfokine receptor by on-column photoaffinity labeling. *J. Biol. Chem.* **2007**, *282*, 124–131.

64. Wang, G.; Long, Y.; Thomma, B.; de Wit, P.; Angenent, G.; Fiers, M. Functional analyses of the CLAVATA2-like proteins and their domains that contribute to CLAVATA2 specificity. *Plant Physiol.* **2010**, *152*, 320–331.

65. Matsushima, N.; Tanaka, T.; Enkhbayar, P.; Mikami, T.; Taga, M.; Yamada, K.; Kuroki, Y. Comparative sequence analysis of leucine-rich repeats (LRRs) within vertebrate toll-like receptors. *BMC Genomics* **2007**, *8*, 124.

66. Mikami, T.; Miyashita, H.; Takatsuka, S.; Kuroki, Y.; Matsushima, N. Molecular evolution of vertebrate Toll-like receptors: Evolutionary rate difference between their leucine-rich repeats and their TIR domains. *Gene* **2012**, *503*, 235–243.

67. Gay, N.J.; Gangloff, M.; Weber, A.N. Toll-like receptors as molecular switches. *Nat. Rev. Immunol.* **2006**, *6*, 693–698.

68. Kumar, H.; Kawai, T.; Akira, S. Toll-like receptors and innate immunity. *Biochem. Biophys. Res. Commun.* **2009**, *388*, 621–625.

69. Kumar, H.; Kawai, T.; Akira, S. Pathogen recognition in the innate immune response. *Biochem. J.* **2009**, *420*, 1–16.

70. Kawai, T.; Akira, S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* **2011**, *34*, 637–650.

71. Botos, I.; Segal, D.M.; Davies, D.R. The Structural Biology of Toll-like Receptors. *Structure* **2011**, *19*, 447–459.

72. Matsushima, N.; Miyashita, H.; Mikami, T.; Yamada, K. A New Method for the Identification of Leucine-Rich Repeats by Incorpolating Protein Secondar Structure Prediction. *Bioinformatics: Genome Bioinformatics and Computational Biology*; NOVA Sience Pulishers: Hauppauge, NY, USA, 2011.

73. Enkhbayar, P.; Kamiya, M.; Osaki, M.; Matsumoto, T.; Matsushima, N. Structural principles of leucine-rich repeat (LRR) proteins. *Proteins* **2004**, *54*, 394–403.

74. Tan, X.; Calderon-Villalobos, L.I.; Sharon, M.; Zheng. C.; Robinson, C.V.; Estelle, M.; Zheng, N. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* **2007**, *446*, 640–645.

75. Hayashi, K.; Tan, X.; Zheng. N.; Hatate, T.; Kimura, Y.; Kepinski, S.; Nozaki, H. Small-molecule agonists and antagonists of F-box protein-substrate interactions in auxin perception and signaling. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5632–5637.

76. Sheard, L.B.; Tan, X.; Mao, H.; Withers, J.; Ben-Nissan, G.; Hinds, T.R.; Kobayashi, Y.; Hsu, F.F.; Sharon, M.; Browse, J.; *et al*. Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. *Nature* **2010**, *468*, 400–405.

77. Bendtsen, J.D.; Nielsen, H.; von Heijne, G.; Brunak, S. Improved prediction of signal peptides: SignalP 3.0. *J. Mol. Biol.* **2004**, *340*, 783–795.

78. Sonnhammer, E.L.; von Heijne, G.; Krogh, A. A hidden Markov model for predicting transmembrane helices in protein sequences. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **1998**, *6*, 175–182.

79. Lehti-Shiu, M.D.; Zou, C.; Hanada, K.; Shiu, S.H. Evolutionary history and stress regulation of plant receptor-like kinase/pelle genes. *Plant Physiol.* **2009**, *150*, 12–26.

80. Hwang, S.G.; Kim, D.S.; Jang, C.S. Comparative analysis of evolutionary dynamics of genes encoding leucine-rich repeat receptor-like kinase between rice and Arabidopsis. *Genetica* **2011**, *139*, 1023–1032.

81. Nadeau, J.A.; Sack, F.D. Control of stomatal distribution on the Arabidopsis leaf surface. *Science* **2002**, *296*, 1697–1700.

82. Shiu, S.H.; Bleecker, A.B. Plant receptor-like kinase gene family: Diversity, function, and signaling. *Sci. STKE* **2001**, *2001*, re22.

83. van der Knaap, E.; Song, W.Y.; Ruan, D.L.; Sauter, M.; Ronald, P.C.; Kende, H. Expression of a gibberellin-induced leucine-rich repeat receptor-like protein kinase in deepwater rice and its interaction with kinase-associated protein phosphatase. *Plant Physiol.* **1999**, *120*, 559–570.

84. Cho, H.S.; Pai, H.S. Cloning and characterization of ntTMK1 gene encoding a TMK1-homologous receptor-like kinase in tobacco. *Mol. Cells* **2000**, *10*, 317–324.

85. Meksem, K.; Ruben, E.; Hyten, D.L.; Schmidt, M.E.; Lightfoot, D.A. High-throughput genotyping for a polymorphism linked to soybean cyst nematode resistance gene Rhg4 by using Taqman (TM) probes. *Mol. Breed.* **2001**, *7*, 63–71.

86. Friedrichsen, D.; Chory, J. Steroid signaling in plants: From the cell surface to the nucleus. *Bioessays* **2001**, *23*, 1028–1036.

87. Bishop, G.J. Brassinosteroid Mutants of Crops. *J. Plant Growth Regul.* **2003**, *22*, 325–335.

88. Napier, R. Plant hormone binding sites. *Ann. Bot. (Lond)* **2004**, *93*, 227–233.

89. Belkhadir, Y.; Wang, X.; Chory, J. Brassinosteroid signaling pathway. *Sci. STKE* **2006**, *2006*, doi:10.1126/stke.3642006cm4.

90. Belkhadir, Y.; Chory, J. Brassinosteroid signaling: A paradigm for steroid hormone signaling from the cell surface. *Science* **2006**, *314*, 1410–1411.

91. Bajguz, A.; Hayat, S. Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiol. Biochem.* **2009**, *47*, 1–8.

92. Tang, W.; Deng, Z.; Wang, Z.Y. Proteomics shed light on the brassinosteroid signaling mechanisms. *Curr. Opin. Plant Biol.* **2010**, *13*, 27–33.

93. Matsubayashi, Y.; Ogawa, M.; Kihara, H.; Niwa, M.; Sakagami, Y. Disruption and overexpression of Arabidopsis phytosulfokine receptor gene affects cellular longevity and potential for growth. *Plant Physiol.* **2006**, *142*, 45–53.

94. Irving, H.R.; Kwezi, L.; Ruzvidzo, O.; Wheeler, J.I.; Govender, K.; Iacuone, S.; Thompson, P.E.; Gehring, C. The phytosulfokine (PSK) receptor is capable of guanylate cyclase activity and enabling cyclic GMP-dependent signaling in plants. *J. Biol. Chem.* **2011**, *286*, 22580–22588.

95. Amano, Y.; Tsubouchi, H.; Shinohara, H.; Ogawa, M.; Matsubayashi, Y. Tyrosine-sulfated glycopeptide involved in cellular proliferation and expansion in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 18333–18338.

96. Nodine, M.D.; Yadegari, R.; Tax, F.E. RPK1 and TOAD2 are two receptor-like kinases redundantly required for arabidopsis embryonic pattern formation. *Dev. Cell* **2007**, *12*, 943–956.

97. Nodine, M.D.; Tax, F.E. Two receptor-like kinases required together for the establishment of Arabidopsis cotyledon primordia. *Dev. Biol.* **2008**, *314*, 161–170.

98. Mizuno, S.; Osakabe, Y.; Maruyama, K.; Ito, T.; Osakabe, K.; Sato, T.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Receptor-like protein kinase 2 (RPK 2) is a novel factor controlling anther development in Arabidopsis thaliana. *Plant J.* **2007**, *50*, 751–766.

99. Kinoshita, A.; Betsuyaku, S.; Osakabe, Y.; Mizuno, S.; Nagawa, S.; Stahl, Y.; Simon, R.; Yamaguchi-Shinozaki, K.; Fukuda, H.; Sawa, S. RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in Arabidopsis. *Development* **2010**, *137*, 3911–3920.

100. Betsuyaku, S.; Takahashi, F.; Kinoshita, A.; Miwa, H.; Shinozaki, K.; Fukuda, H.; Sawa, S. Mitogen-activated protein kinase regulated by the CLAVATA receptors contributes to shoot apical meristem homeostasis. *Plant Cell Physiol.* **2010**, *52*, 14–29.

101. Sawa, S.; Tabata, R. RPK2 functions in diverged CLE signaling. *Plant Signal. Behav.* **2011**, *6*, 86–88.

102. Kruijt, M.; de Kock, M.J.D.; de Wit, P.J.G.M. Receptor-like proteins involved in plant disease resistance. *Mol. Plant Pathol.* **2005**, *6*, 85–97.

103. Dixon, M.S.; Jones, D.A.; Keddie, J.S.; Thomas, C.M.; Harrison, K.; Jones, J.D. The tomato Cf-2 disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. *Cell* **1996**, *84*, 451–459.

104. Thomas, C.M.; Jones, D.A.; Parniske, M.; Harrison, K.; Balint-Kurti, P.J.; Hatzixanthis, K.; Jones, J.D. Characterization of the tomato Cf-4 gene for resistance to Cladosporium fulvum identifies sequences that determine recognitional specificity in Cf-4 and Cf-9. *Plant Cell* **1997**, *9*, 2209–2224.

105. Kawchuk, L.M.; Hachey, J.; Lynch, D.R.; Kulcsar, F.; van Rooijen, G.; Waterer, D.R.; Robertson, A.; Kokko, E.; Byers, R.; Howard, R.J.; *et al*. Tomato Ve disease resistance genes encode cell surface-like receptors. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 6511–6515.

106. Vinatzer, B.A.; Patocchi, A.; Gianfranceschi, L.; Tartarini, S.; Zhang, H.B.; Gessler, C.; Sansavini, S. Apple contains receptor-like genes homologous to the Cladosporium fulvum resistance gene family of tomato with a cluster of genes cosegregating with Vf apple scab resistance. *Mol. Plant Microbe Interact* **2001**, *14*, 508–515.

107. Tor, M.; Brown, D.; Cooper, A.; Woods-Tor, A.; Sjolander, K.; Jones, J.D.; Holub, E.B. Arabidopsis downy mildew resistance gene RPP27 encodes a receptor-like protein similar to CLAVATA2 and tomato Cf-9. *Plant Physiol.* **2004**, *135*, 1100–1112.

108. Ron, M.; Avni, A. The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. *Plant Cell* **2004**, *16*, 1604–1615.

109. Sharfman, M.; Bar, M.; Ehrlich, M.; Schuster, S.; Melech-Bonfil, S.; Ezer, R.; Sessa, G.; Avni, A. Endosomal signaling of the tomato leucine-rich repeat receptor-like protein LeEix2. *Plant J.* **2011**, *68*, 413–423.

110. Fiers, M.; Golemiec, E.; Xu, J.; van der Geest, L.; Heidstra, R.; Stiekema, W.; Liu, C.M. The 14-amino acid CLV3, CLE19, and CLE40 peptides trigger consumption of the root meristem in Arabidopsis through a CLAVATA2-dependent pathway. *Plant Cell* **2005**, *17*, 2542–2553.

111. Fiers, M.; Golemiec, E.; van der Schors, R.; van der Geest, L.; Li, K.W.; Stiekema, W.J.; Liu, C.M. The CLAVATA3/ESR motif of CLAVATA3 is functionally independent from the nonconserved flanking sequences. *Plant Physiol.* **2006**, *141*, 1284–1292.

112. Song, X.; Guo, P.; Li, C.; Liu, C.M. The cysteine pairs in CLV2 are not necessary for sensing the CLV3 peptide in shoot and root meristems. *J. Integr. Plant Biol.* **2010**, *52*, 774–781.

113. Krusell, L.; Sato, N.; Fukuhara, I.; Koch, B.E.; Grossmann, C.; Okamoto, S.; Oka-Kira, E.; Otsubo, Y.; Aubert, G.; Nakagawa, T.; *et al*. The Clavata2 genes of pea and Lotus japonicus affect autoregulation of nodulation. *Plant J.* **2011**, *65*, 861–871.

114. Reid, D.E.; Ferguson, B.J.; Hayashi, S.; Lin, Y.H.; Gresshoff, P.M. Molecular mechanisms controlling legume autoregulation of nodulation. *Ann. Bot.* **2011**, *108*, 789–795.

115. Takemoto, D.; Hayashi, M.; Doke, N.; Mishimura, M.; Kawakita, K. Isolation of the gene for EILP, an elicitor-inducible LRR receptor-like protein, from tobacco by differential display. *Plant Cell Physiol.* **2000**, *41*, 458–464.

116. Wulff, B.B.; Kruijt, M.; Collins, P.L.; Thomas, C.M.; Ludwig, A.A.; De Wit, P.J.; Jones, J.D. Gene shuffling-generated and natural variants of the tomato resistance gene Cf-9 exhibit different auto-necrosis-inducing activities in Nicotiana species. *Plant J.* **2004**, *40*, 942–956.

117. Chai, Y.; Zhao, L.; Liao, Z.; Sun, X.; Zuo, K.; Zhang, L.; Wang, S.; Tang, K. Molecular cloning of a potential Verticillium dahliae resistance gene SlVe1 with multi-site polyadenylation from Solanum licopersicoides. *DNA Seq.* **2003**, *14*, 375–384.

118. Fei, J.; Chai, Y.; Wang, J.; Lin, J.; Sun, X.; Sun, C.; Zuo, K.; Tang, K. CDNA cloning and characterization of the Ve homologue gene StVe from Solanum torvum Swartz. *DNA Seq.* **2004**, *15*, 88–95.

119. Patocchi, A.; Vinatzer, B.A.; Gianfranceschi, L.; Tartarini, S.; Zhang, H.B.; Sansavini, S.; Gessler, C. Construction of a 550 kb BAC contig spanning the genomic region containing the apple scab resistance gene Vf. *Mol. Gen. Genet* **1999**, *262*, 884–891.

120. Joshi, S.G.; Schaart, J.G.; Groenwold, R.; Jacobsen, E.; Schouten, H.J.; Krens, F.A. Functional analysis and expression profiling of HcrVf1 and HcrVf2 for development of scab resistant cisgenic and intragenic apples. *Plant Mol. Biol.* **2011**, *75*, 579–591.

121. Shiu, S.H.; Bleecker, A.B. Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis. *Plant Physiol.* **2003**, *132*, 530–543.

122. Guyomarc'h, S.; Vernoux, T.; Traas, J.; Zhou, D.X.; Delarue, M. MGOUN3, an Arabidopsis gene with TetratricoPeptide-Repeat-related motifs, regulates meristem cellular organization. *J. Exp. Bot.* **2004**, *55*, 673–684.

123. Suzuki, T.; Inagaki, S.; Nakajima, S.; Akashi, T.; Ohto, M.A.; Kobayashi, M.; Seki, M.; Shinozaki, K.; Kato, T.; Tabata, S.; *et al*. A novel Arabidopsis gene TONSOKU is required for proper cell arrangement in root and shoot apical meristems. *Plant J.* **2004**, *38*, 673–684.

124. Guyomarc'h, S.; Benhamed, M.; Lemonnier, G.; Renou, J.P.; Zhou, D.X.; Delarue, M. MGOUN3: Evidence for chromatin-mediated regulation of FLC expression. *J. Exp. Bot.* **2006**, *57*, 2111–2119.

125. Takeda, S.; Tadele, Z.; Hofmann, I.; Probst, A.V.; Angelis, K.J.; Kaya, H.; Araki, T.; Mengiste, T.; Mittelsten Scheid, O.; Shibahara, K.; *et al*. BRU1, a novel link between responses to DNA damage and epigenetic gene silencing in Arabidopsis. *Genes Dev.* **2004**, *18*, 782–793.

126. Kobe, B.; Deisenhofer, J. Crystal structure of porcine ribonuclease inhibitor, a protein with leucine-rich repeats. *Nature* **1993**, *366*, 751–756.

127. Jin, M.S.; Kim, S.E.; Heo, J.Y.; Lee, M.E.; Kim, H.M.; Paik, S.G.; Lee, H.; Lee, J.O. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. *Cell* **2007**, *130*, 1071–1082.

128. Dumas, J.J.; Kumar, R.; Seehra, J.; Somers, W.S.; Mosyak, L. Crystal structure of the GpIbalpha-thrombin complex essential for platelet aggregation. *Science* **2003**, *301*, 222–226.

129. Uff, S.; Clemetson, J.M.; Harrison, T.; Clemetson, K.J.; Emsley, J. Crystal structure of the platelet glycoprotein Ib(alpha) N-terminal domain reveals an unmasking mechanism for receptor activation. *J. Biol. Chem.* **2002**, *277*, 35657–35663.

130. McEwan, P.A.; Andrews, R.K.; Emsley, J. Glycoprotein Ibalpha inhibitor complex structure reveals a combined steric and allosteric mechanism of von Willebrand factor antagonism. *Blood* **2009**, *114*, 4883–4885.

131. Valanne, S.; Wang, J.H.; Ramet, M. The Drosophila Toll signaling pathway. *J. Immunol.* **2011**, *186*, 649–656.

132. Wei, T.; Gong, J.; Jamitzky, F.; Heckl, W.M.; Stark, R.W.; Rossle, S.C. Homology modeling of human Toll-like receptors TLR7, 8, and 9 ligand-binding domains. *Protein Sci.* **2009**, *18*, 1684–1691.

133. Afzal, A.J.; Lightfoot, D.A. Soybean disease resistance protein RHG1-LRR domain expressed, purified and refolded from Escherichia coli inclusion bodies: Preparation for a functional analysis. *Protein Expr. Purif.* **2007**, *53*, 346–355.

134. Seear, P.J.; Dixon, M.S. Variable leucine-rich repeats of tomato disease resistance genes Cf-2 and Cf-5 determine specificity. *Mol. Plant Pathol.* **2003**, *4*, 199–202.

135. van der Hoorn, R.A.; Roth, R.; de Wit, P.J. Identification of distinct specificity determinants in resistance protein Cf-4 allows construction of a Cf-9 mutant that confers recognition of avirulence protein Avr4. *Plant Cell* **2001**, *13*, 273–285.

136. Wulff, B.B.; Thomas, C.M.; Smoker, M.; Grant, M.; Jones, J.D. Domain swapping and gene shuffling identify sequences required for induction of an Avr-dependent hypersensitive response by the tomato Cf-4 and Cf-9 proteins. *Plant Cell* **2001**, *13*, 255–272.

137. Chakrabarti, A.; Panter, S.N.; Harrison, K.; Jones, J.D.; Jones, D.A. Regions of the Cf-9B disease resistance protein able to cause spontaneous necrosis in Nicotiana benthamiana lie within the region controlling pathogen recognition in tomato. *Mol. Plant Microbe Interact* **2009**, *22*, 1214–1226.

138. Wulff, B.B.; Heese, A.; Tomlinson-Buhot, L.; Jones, D.A.; de la Pena, M.; Jones, J.D. The major specificity-determining amino acids of the tomato Cf-9 disease resistance protein are at hypervariable solvent-exposed positions in the central leucine-rich repeats. *Mol. Plant Microbe Interact* **2009**, *22*, 1203–1213.

139. Wang, X.; Li, X.; Meisenhelder, J.; Hunter, T.; Yoshida, S.; Asami, T.; Chory, J. Autoregulation and homodimerization are involved in the activation of the plant steroid receptor BRI1. *Dev. Cell* **2005**, *8*, 855–865.

140. Matsushima, N.; Ohyanagi, T.; Tanaka, T.; Kretsinger, R.H. Super-motifs and evolution of tandem leucine-rich repeats within the small proteoglycans—biglycan, decorin, lumican, fibromodulin, PRELP, keratocan, osteoadherin, epiphycan, and osteoglycin. *Proteins* **2000**, *38*, 210–225.

141. Matsushima, N.; Kamiya, M.; Suzuki, N.; Tanaka, T. Super-motifs of leucine-rich repeats (LRRs) proteins. *Genome Inform.* **2000**, *11*, 343–345.

142. Haigis, M.C.; Haag, E.S.; Raines, R.T. Evolution of ribonuclease inhibitor by exon duplication. *Mol. Biol. Evol.* **2002**, *19*, 959–963.