Research Signpost
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

Recent Res. Devel. Plant Biol., 2(2002): 201-214 ISBN: 81-7736-149-X

Plant leucine-rich repeat proteins: Diverse roles in signal transduction and development

Daniel M. Vernon and Nancy Forsthoefel
Department of Biology, Whitman College, Walla Walla, WA 99362 USA

Abstract

Leucine-rich repeat proteins (LRRPs) constitute a large and diverse superfamily with members found in all eukaryotes. LRRPs are defined by the presence of a characteristic leucine-rich motif usually present in multiple copies, arranged in tandem. Leucine-rich repeat domains serve as platforms for protein-protein interactions. Many LRRPs from animal and fungal systems have roles in cellular signal transduction. In plants, several types of LRRPs with different biological functions have been identified through molecular and biochemical studies, and genetic studies using the model system Arabidopsis thaliana have identified plant LRR proteins with important roles in developmental and hormonal signal transduction, and pathogen resistance. The recent completion of the Arabidopsis genome sequence has led to the rapid identification of hundreds of additional genes encoding plant LRR proteins. Many, such as LRR-receptor

Correspondence/Reprint request: Dr. Daniel M. Vernon, Department of Biology, Whitman College, Walla Walla, WA 99362 USA
E-mail: vernondm@whitman.edu

kinases, appear to be members of large, previously identified LRRP families. Others appear to represent novel classes unique to plants. One example of the latter is the SLATs, a class of Arabidopsis LRR proteins related to animal and fungal LRRPs that function in RAS signal transduction.

While the SLAT LRR consensus sequence is related to that of animal LRRPs, other structural features define the SLATs as a new, plant-specific class of LRRPs. Functional genomic analyses such as reverse genetics, as well as traditional developmental, physiological, molecular, and biochemical studies, will be needed to define the roles of the SLATs and the many other recently identified plant LRRPs at the organismal, cellular, and biochemical levels. Such efforts will also provide insights into plant signal transduction, cell biology, and development.

Introduction

Leucine-rich repeat proteins (LRRPs) constitute a large and widespread protein superfamily, with members found in all eukaryotes and in bacteria [1]. Many have important roles in signal transduction and in regulating crucial aspects of development. In this review, we introduce the LRR superfamily, and provide examples of their structural and functional diversity. We then describe the classes of LRRPs found in plants. We put particular emphasis on LRRPs identified through recent genetic and molecular analysis of *Arabidopsis thaliana*, because this small weed has become the premier system for identifying genes and determining their functions. The picture that emerges is one of diversity and biological significance: plants contain numerous LRRPs, many belonging to families not found in other eukaryotes. Plant LRRPs, like those in animals and fungi, appear to carry out important roles in signal transduction and development, but many function in biological contexts unique to plants. With the completion of the *Arabidopsis* genome sequence, a major challenge will be to determine the functions of the many recently identified plant LRRPs. Research on these LRRPs will also provide insights into diverse aspects of plant signal transduction, cell biology, and development.

The leucine-rich repeat superfamily defined

Members of the LRR superfamily are characterized by the presence of a repeated 23-25 amino acid leu-rich motif, found in from 1 to >30 copies, usually arranged in tandem into a distinct LRR domain. The fundamental consensus sequence for this motif, shared by most LRR proteins, is xLxxLxLxNxaxxa xxxxaxxax (where "x" represents any amino acid, and "a" stands for hydrophobic amino acids). This motif represents only a generalized consensus: the numbers of amino acids (x) between conserved residues can vary at some positions, resulting in slight variation in motif length. Also, protein families within the LRR superfamily may have additional conserved amino acids that elaborate on this basic motif [1,2]. LRR domains form a platform for highly specific protein:protein interactions [3]. Variation in length and amino acid composition in the unit motifs are likely to impart binding specificity to LRR domains of different proteins [2]

Beyond the defining feature of the consensus motif, LRRPs are a diverse superfamily. Diversity can be found on many levels. At the most basic structural level, the amino acid sequence of the repeating unit motif, more than 6 major structural classes

of LRRPs have been defined [2,4]. Such protein classification is possible because all of the unit motifs within a given LRR protein share the same class of consensus structure; repeat motifs from different LRRP classes are not found together in a single protein. This may reflect a requirement for consistent motif structure for proper folding of the LRR into a functional protein interaction domain [2].

In terms of overall protein structure, members of the LRR superfamily can differ in the size of their LRR domains (i.e., the number of unit repeats), and in the location of those domains within the proteins. Table 1 provides a few representative examples of LRR protein diversity with respect to LRR motif sequence and protein location and function. Figure 1 illustrates the structural diversity of these proteins.

Table 1. Structural and functional diversity of LRR proteins in animals and yeast

LRR protein (function)	Leu-rich unit motif consensus	Location: Protein / LRRs	# LRR motif
basic LRR superfamily motif	a...a..a..L..L.L..N.a...	-	-
Human GP1bα (cell adhesion)	LP.GL.....L..L.L..N.L..	plasma membrane/ extracellular	7
FLI1 (fly embryogenesis)	aP..a..L..L..L.LS.N.L..	cytoplasm/cyto-plasm	16
SUR8 (Ras signaling)	LP..IG.L..L..L^{N}_{D}L..N.L..	cytoplasm/cyto-plasm	18
Trk (transmembrane receptor kinase)	S.........LR.aNLSQN.L...	Plasma membrane/ extracellular	2-3
GRR1 (ubiquitin targeting complex)	a..L..L...L..a.L..C.NaTD ..	nucleus/nucleus	9

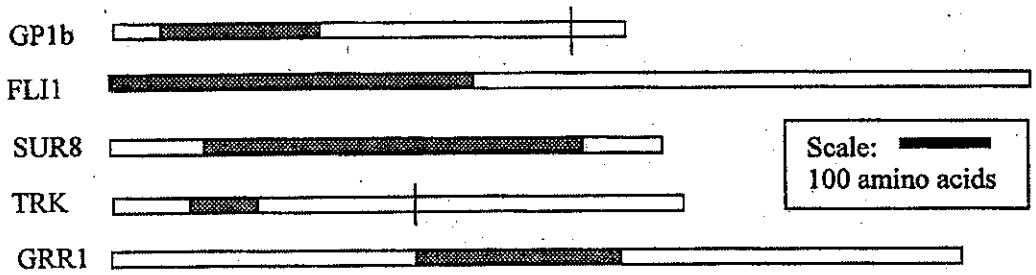

Figure 1. Diagrams of representative LRRPs from animals and yeast, illustrating the variety of LRR domain size and location within proteins in the LRR superfamily. Shaded regions: LRR domains. Vertical bars: transmembrane sections. Further information on these proteins is provided in Table 1.

LRRP functions in animals and yeast

LRRPs were first identified in animals and yeast, and LRRPs from these sources were the first for which functions were determined. LRRPs as a group are diverse in

function, as they are in structure. They are found throughout the cell (and extracellularly), where they take part in such varied processes as (for example) cell adhesion, DNA repair, enzyme inhibition, cytoskeletal organization, and cell cycle control [1, 2, 4, 5, 6].

Despite the structural and functional diversity found within the LRR protein superfamily, two themes are evident: 1) LRRPs take part in protein:protein interactions to fulfill their biological functions, and 2) most LRRPs are involved in signal transduction. Indeed, many of the animal and fungal LRRPs for which functions have been defined act in signal transduction and development. Examples include the mammalian TRK receptor kinase, which has an extracellular ligand-binding LRR domain, Drosophila FLI1, an intracellular LRRP that regulates cytoskeletal organization during development, and human ERBIN, which facilitates localization and signaling from the transmembrane ERB receptor [1,4,7]

One particular class of animal and fungal LRRPs illustrates well the importance of LRRPs in signal transduction: "Ra*s* group" LRRPs, which all share a characteristic LR motif (xxaxxLxxLxxLD/NLSxNxaxxaP) [4]. Most Ras group LRRPs appear to be cytoplasmic, unlike the majority of LRRPs, which are extracellular or associated with the plasma membrane. One member of the Ras group with a prominent role in signal transduction is yeast/fungal adenylate cyclase, which interacts directly with RAS via a carboxy-terminal LRR domain [8]. Other Ras group proteins include SUR8, originally identified in *C. elegans* by extragenic suppressor mutations that suppress dominant *ras* mutations. SUR-8 interacts directly with RAS through its LRR domain [9]. Another RAS-group LRRP is RSU-1/RSP-1, a mammalian protein that interacts with the small GTPase RAF, and which can suppress *ras* transformation in mammalian cell cultures [10,11]. Both SUR8 and RSU1 may be members of large multi-component signaling complexes mediated by Ras and Raf GTPases [12]. Drosophila FLI1 is also member of this Ras group, and has been proposed to serve as a link between signal transduction pathways and control of cytoskeleton [4].

LRR proteins in plants

Molecular and biochemical studies in diverse plant species have identified LRRPs that carry out a wide range of functions, and in-depth genetic studies of processes such as pathogen resistance and hormonal signaling have greatly accelerated the discovery of new plant LRRPs. Indeed, most of the LRRPs for which functions have been best defined have been identified through the analysis of developmental mutants, in which genes responsible for intriguing phenotypes were cloned by positional methods or gene tagging. The recent completion of *Arabidopsis thaliana* genome has further increased the number of known (or predicted) LRRPs, and defined the size of LRRP-encoding gene families in plants. Overall, the diversity of LRRPs in plants is on a scale similar to that found in animal systems: many classes of LRRPs have been identified, with diverse cellular locations and functions, and each class with characteristic protein architecture. Some of these belong to LRRP classes widely represented among the eukaryotes, although the plant representatives of such classes often carry out functions specific to plant physiology or development. Other plant LRRPs define novel classes unique to plants, with plant-specific roles in the biology of the cell wall, pathogen response, and cell communication. Interestingly, although the biological contexts in which plant

LRRPs function often differ from those in animals, some major generalizations about LRRP function in animals hold true plants: 1) a large number of plant LRRPs are involved in signal transduction; 2) many are crucial for coordination of multicellular development; 3) many (but not all) are localized extracellularly, or in association with the plasma membrane; and 4) as with LRRPs from animal and fungal systems, plant LRRP function often involves protein:protein interactions mediated through the LRR domain.

Below, we provide examples of classes of plant LRRPs identified by a variety of approaches. Some representative proteins are listed in Table 2. Our intent is not to exhaustively catalog plant LRRPs; rather, we describe major, functionally-defined LRRP classes, illustrate the diversity of plant LRRP proteins, and underscore the importance of these proteins to plant signal transduction and development. Because *Arabidopsis* has become such an important system for gene identification and functional characterization, many of our examples are drawn from that species.

Table 2. Representative proteins from major classes of plant LRRPs

LRR class (example)	Leu-rich unit motif consensus[a]	LRR-domain location	# LRR unit motifs	Reference #
General LRR superfamily	a...a..a..L..L.L..N.a...	-	-	1
PGIPs	IP..a..L..L..L.LS.N.L.G.	extracellular	10	17
R-Gene (RPS2)	....a..L..L..$^{I}/_{L}$.L..	internal	13	30
R-gene (Cf-9)	IPS.L..l..l..LDLSSNNL.G.	extracellular	28	26
Plant F-box/LRR (COI1)	L..a...C..L..L.a..	internal	16	38
Plant LRR-RPK (BRI1)	IP..F..F..L..L.LS.N.FSG.	extracellular	25	49
Plant F-box/LRR (COI1)	L..a...C..L..L.a..	internal	16	38
SLATs	LP.SIG.L..L..L$^{N}_{D}$aS.N.a..	internal	9-11	56

[a] Capital letters indicate residues conserved in 50-100% of unit motifs. Lower-case "a" stands for any hydrophobic amino acid.

Structural and functional diversity of plant LRRPs

LRR proteins from a range of plant species have been found through molecular investigations into different aspects of plant biology. Such studies identified structurally diverse plant LRRPs, located in different parts of the cell and involved in a wide range of processes. For example, the *Arabidopsis* DRT100 protein was identified through its ability to partially complement recA deficiency in *E. coli*. DRT100 , which contains a relatively short internal LRR domain near its N-terminus, is predicted to be localized to chloraplasts, where it may be involved in DNA repair or replication [13]. Another intriguing *Arabidopsis* LRRP, LRX1, was recently identified by its similarity to tomato cell wall extensins [14]. LRX1 is an extracellular protein with an N-terminal signal peptide, an internal LRR domain, and a carboxy-terminal domain that resembles cell wall extensin proteins. Reverse-genetic analysis of *lrx1* knockout mutants showed that the protein is required for root hair morphogenesis. Over-expression of the LRR domain resulted in a dominant abnormal root-hair phenotype, suggesting this domain interacts

with other factors to affect cell morphology. Other plant LRRPs identified through molecular methods have less well-defined functions, but further reflect the diversity of plant LRRPs. FIL2 and SF17 are two examples. FIL2 was identified as a flower-specific gene product, regulated by the DEFICIENS transcription factor in *Antirrhinum* [15]. FIL2 contains a large, central LRR domain, is glycosylated, and is localized extracellularly. The presence of this domain, as well as its cellular location and developmentally-regulated expression, has led to speculation that FIL2 is important in cell communication during floral organ formation. Another example of an LRRP identified on the basis of its expression is SF17 from sunflower. Screening a cDNA library identified the SF17 mRNA as a pollen-specific transcript [16]. The predicted protein contains an internal LRR domain consisting of 9 LRRs in the carboxy-terminal half of the protein. SF17 is predicted to be cytoplasmic; no biological function has been determined.

Polygalacturonase inhibitor proteins

In contrast to the examples above, some classes of plant LRRPs have very-well defined biochemical functions. One example is the polygalacturonase inhibitor proteins (PGIPs). These secreted proteins have been isolated and characterized from several species, including tomato and apple [17, 18]. They feature a large internal LRR domain flanked by short N- and C-terminal regions, are expressed in developing fruit, and their expression can be induced by wounding or infection. PGIPs selectively bind and inhibit fungal polygalacturonases. The PGIP LRR domain is crucial for function: recognition and binding to PGs from specific fungal species is mediated by residues of the LRR domain, as demonstrated by site-directed mutagenesis and in vitro binding analysis [19]. An intriguing extracellular plant LRRP related to PGIPs has been identified in carrot: it inhibits ice recrystallization and has antifreeze activity [20].

LRR proteins involved in plant disease resistance

Two of the largest classes of functionally-defined plant LRRPs are involved in pathogen or disease resistance. Disease resistance (R) genes trigger resistance responses in the host plant in response to "avirulence" (avr) proteins expressed by microbial pathogens. Recognition and response are often highly specific to microbial strains and their avr proteins, reflecting specificity of protein:protein interaction between avr genes and host factors. The products of some plant R genes may be directly involved in this recognition. In addition, they also must interact with host factors to elicit a response. Recent genetic and biochemical evidence suggests that LRR domains on plant R genes play a key role in such interactions. Plant R genes have received much attention in recent years and the topic has been the focus of several recent reviews [21,22,23,24,25]. Thus we just briefly summarize current knowledge and provide some representative examples of these LRR proteins here.

R genes from numerous species, including tomato, tobacco, flax, barley, and *Arabidopsis* have been cloned and characterized, and they encode LRRPs that fall into two major classes (Table 2). One class, exemplified by the tomato Cf-9 and Cf-2 proteins is characterized by large transmembrane proteins with large extracellular leu-rich domain consisting of 25 to 38 tandemly repeated leu-rich motifs [26,27]. Based on the presence of a predicted transmembrane region and extracellular LRRs this class of R-

protein has been proposed to serve as initial receptors for microbial avirulence factors that trigger resistance responses. The LRR domains in tomato Cf proteins display pronounced variation in LRR copy number that correspond to differences in response specificity [28].

The second major R-protein class consists of NBS-LRR proteins, exemplified by the tobacco N protein and the *Arabidopsis* RPS and RPM proteins [29,30,31]. These proteins contain a prominent C-terminal LRR domain consisting of 14 leu-rich repeats, with centrally-located nucleotide binding sites (NBS). The NBS-LRRs constitute a large class of R proteins, constituting approximately 1% of the *Arabidopsis* genome, and this class actually contains two sub-classes: 1) the " TIR" NBS-LRRPS, which contain an N-terminal region resembling a signal-transducing domain found in the Drosophila TOLL protein and interleukin receptor; and 2) the leucine zipper-LRR class, which feature an N-terminal coiled coil structure (often a leucine-zipper), as well as NBS and LRR domains [21,32]. The structural differences between the TIR and non-TIR NBS-LRR proteins may reflect differences in biochemical function; it has been proposed that these different subclasses of NBS-LRR R proteins trigger responses through different pathways (reviewed in ref. 32).

The precise mechanisms by which NBS-LRR proteins trigger responses have not been determined and are the focus of much current research. While it was proposed that these proteins might serve receptors that contact pathogen factors extracellularly, it is now believed that NBS-LRR proteins are intracellular factors that are likely peripherally associated with the cytoplasmic face of the plasma membrane, where they respond to infecting avr factors [31,33]. Detailed genetic analyses of R-protein alleles with variation in LRR domains, and with chimeric R-genes with different LRR domains, have pinpointed the LRR domain as important for NBS-LRR function and specificity [22,34,35]. The precise role of the LRR domain in this context has been debated. It could be important for recognition and binding of pathogen avr proteins; however, genetic evidence and domain swap experiments with different RPS2 alleles suggest that the LRR domain interacts with plant host factors [34,36]. Whatever the case, it appears that the LRR domain of NBS-LRR R proteins, like LRR domains of many animal and fungal proteins, functions in signal transduction by mediating key protein:protein interactions.

Hormonal signaling

Several plant LRRPs for which functions have been defined have important roles in growth regulator responses. Characterization of *Arabidopsis* mutants defective in hormone signaling has revealed that two very different types of plant hormones- auxins and jasmonates- apparently act through LRRPs resembling yeast and human GRR and SKP2 proteins. GRR1 and SKP2 are intracellular LRRPs that contain a conserved F-box motif and a C-terminal LRR domain consisting of 16 leu-rich repeats. They act as members of large, multi-protein complexes that target other cellular factors (such as cell cycle regulatory proteins) for ubiquitination and subsequent degradation. By degrading negative regulators, these complexes can trigger a cellular process or response. The TIR1 and COI1 proteins, which are required for auxin and jasmonate signaling, respectively, share the F-box and LRR domain structure of GRR1 and SKP2 [37,38]. They apparently both mediate their respective hormone responses by a mechanism

similar to GRR1 and SKP2, although they act in different pathways that affect different aspects of plant development: TIR1 affects auxin regulated growth such as hypocotyl elongation, while COI1 is required for jasmonate-mediated defense responses, and pollen fertility [37,38]. The LRR domain of TIR1 and COI1 might allow assembly of the LRRP into the mult-subunit active ubiquitin-targeting complex, or they might play a role in substrate recognition and binding by the ubiquitin targeting complex.

LRR-receptor like kinases

Another class of plant LRRPs with established roles in cell signaling is the LRR-receptor-like kinase (LRR-RPK) family. The general architecture of these proteins resembles that of receptor kinases from animal systems: they contain a cytoplasmic kinase domain (ser/thr kinase in plants), a transmembrane segment, and an extracellular domain composed largely of LR repeats [39,40]. The size of the LRR domain can vary, ranging from 6 to more than 20 motif units. The role of the LRR domain on LRR-RLKs has not been established, although genetic analyses has indicated that LRR domains are important for function (see below). An obvious hypothesis is that it serves as an extracellular ligand-recognition domain. Ligands could include peptides as well as smaller organic molecules [41,42].

LRR-RPKs constitute one of the largest protein families in plants. More than 170 are encoded by the *Arabidopsis* genome, making them the largest category of plant receptor-like kinases [43]. An additional 60+ genes encode LRR-receptor-like molecules without cytoplasmic kinase domains [44]. Thus, LRR-RLKs and related proteins are thought to be of central importance to plant signal transduction in myriad biological processes, ranging from environmental responses to developmental cell-cell communication [40,41,45].

The functions of a number of LRR-RPKs from a variety of species have been defined in recent years, and the family has been reviewed extensively [e.g.; 40,45]. As might be expected, many have important roles in development, and were identified through analysis of developmental mutants. The best characterized of examples include the *Arabidopsis* CLV1 and BRI1 proteins. CLV1 was identified by analysis of *Arabidopsis clv1* mutants, which exhibit enlarged shoot meristems with an overabundance of undifferentiated cells. The CLV1 LRR-RLK has a large extracellular LRR domain consisting of 21 LR repeats. It is expressed in meristems and is proposed to control the fate of meristem cells, maintaining the balance between undifferentiated stem cells and cells specified to contribute to organ formation [46]. CLV1 interacts with another LRR protein, CLV2, which is a receptor-like transmembrane LRR that lacks a kinase domain [47]. The extracellular LRR domain likely serves as a ligand-binding domain for a peptide factor, CLV3, which is required for proper meristem activity and which is present in an active multi-component complex with CLV1 [48]. Thus, CLV1 appears to function as a classic receptor kinase that functions in cell signaling necessary for normal meristem function. BRI 1 has a different sort of role in development. It is required for perception of brassinosteroids (BRs), a class of growth regulator required for growth and cell elongation. Mutations in BRI1 result in a pleiotropic phenotype, the most obvious aspect of which is severely dwarfed growth [49,50]. BRI1 has a large extracellular LRR domain of 25 leu-rich repeats, which is interrupted by a "non-LRR island" [49]. The LRR domain is important for function, as mutations in this region

compromise protein function [50]. Indeed, the LRR domain is capable of perceiving external BRs and triggering a cellular response in conjunction with the cytoplasmic kinase domain: a chimeric RLK consisting of the BRI1 LRR domain fused to the cytoplasmic domain of a pathogen-sensing kinase triggered pathogenesis-specific responses in cells exposed to brassinolides [51]. Thus, while it has not been directly shown that the LRR domain directly interacts with brassinosteroids, it is clear that this region takes part in interactions central to BRI1's function in signal transduction.

CLV1 and BRI1 are but two of the best characterized examples of functionally-defined plant LRR-RLKs. Others include the *Arabidopsis* ERECTA protein, which regulates the morphology of shoot meristem-derived organs [52], and HAESA, which controls floral organ abscission [53]. It should be noted that other LRR-RLK proteins are involved in signal transduction in non-developmental contexts. One example is FLS2, which recognizes the microbial flagellin protein and triggers defense responses [54]. Mutations in the LRR of FLS2 disrupt flagellin binding, again implicating the LRR domain of this class of receptors in ligand interaction.

SLATs: A novel class of plant LRRP related to Ras-group LRRPs

Characterization of LRR-RLKs and R proteins has provided insight into initial steps of various signal transduction pathways in plants. However, little is known about downstream components of signaling pathways. Our laboratory has identified a group of genes encoding a previously unidentified class of plant LRRPs with potential roles in intracellular signal transduction. The first of these genes was fortuitously identified and cloned during characterization of the *Arabidopsis emb88* mutant [55]. We then identified 9 additional related genes in *Arabidopsis* genome sequence, using BLASTP and tBLASTN searches. These proteins and their features are listed on Table 3. LRR motif structure and protein relationships were investigated using Clustal alignments and visual inspection of amino acid sequences and corresponding gene structures. SLATs all contain an internal LRR domain of 8 to 11 copies of a signature leu-rich repeat motif,

Table 3. *SLAT* genes and SLAT protein characteristics.

	Gene location			Protein	
SLAT	Chromosome	Genomic BAC accession	EST availability	Size (aa)	# LRRs
1	V	[AB005237]	Yes	506	9
2	III	[AB028611]	Yes	470	9
3	I	[AC012187]	No EST	463	9
4	IV	[AL117188]	Yes	549	10.5
5	II	[AC002329]	Yes	526	10
6	II	[AC003058]	No EST	380	9
7	IV	[AL161575]	No EST	374	8.5
8	IV	[AL049483]	Yes	382	9
9	III	[AC024081]	Yes	584	17
10	III	[AC073395]	Yes	537	9

flanked by highly hydrophilic N- and C-terminal domains (Figure 2) [56]. Their consensus leu-rich motif structure resembles that of the RAS-group LRRPs from animals and fungi, including *C. elegans* SUR-8. We have therefore named this new class of plant LRRPs "SLATs", for SUR-8-like LRRs of *Arabidopsis thaliana*. While BLAST alignments and the leu-rich motif structure indicate that SLATs are more closely related to animal Ras-group LRRPS than to other plant LRRPs, it should be noted that they are not structural orthologs of any Ras-group proteins. The SLATs' shorter LRR domains, and hydrophilic N- and C-terminal regions, distinguish them as a novel and plant-specific class of LRRPs. Genes encoding similar proteins are found in other plants, as shown by EST sequences from rice, castor bean, and alfalfa, as well as genomic Southern blot hybridization to maize and pea [D.M. Vernon, unpublished].

Although they share common structural features, the SLATs can be grouped into 4 families (each consisting of 1 to 4 proteins), based on protein and gene structures (Figure 2) [57]. Thus, it is likely that various SLATs interact with different cellular factors and act in several different pathways and biological contexts. As a cytoplasmic class of plant LRRPs related to Ras-group proteins, some SLATs may function as internal, downstream components of plant signaling pathways. It is tempting to speculate that the SLATs, like the related Ras-group LRRPs of animals (which interact with Ras and Raf) , may play a role in plant GTPase-mediated signaling pathways. Although no direct Ras orthologs appear to be present in plants, plants do contain small GTPases, called Rops, belonging to the Ras superfamily, which are important in numerous developmental processes [58]. We are currently investigating SLAT function by reverse genetics, and prospective knock-out alleles have been identified for 9 of the 10 *SLAT* genes [57]; analysis of mutant phenotypes, and creation of double or multiple mutants homozygous for mutations in the most closely related SLATs, should provide insights into SLAT function at the biological level. Other approaches, such as two-hybrid screens and ectopic expression of LRR domains, should help identify the role of the LRR domain and cellular components with which SLATs interact. In addition to elucidating the role of the SLATs themselves, such studies may provide insight into intracellular aspects of plant signal transduction pathways.

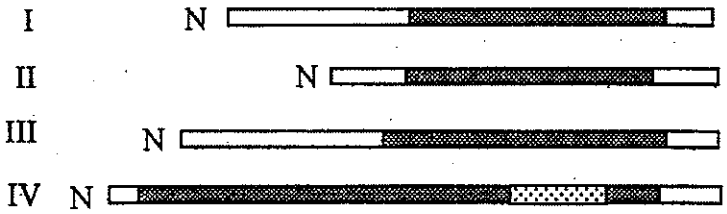

Figure 2. Four families of SLAT proteins. Diagrams illustrate the characteristic protein architecture of 4 classes of SLATs (I-IV). More details of protein and LRR structure are provided in Table 3. "N" designates N-terminus. The shaded regions represent LRR domains. Family I: SLATs 1, 2,3, and 10. Family II: SLATs 6, 7, and 8. Family III: SLATs 4 and 5. Family IV: SLAT 9. Light shading represents degenerate LRR repeats present in SLAT 9.

Future research on plant lRRPs

The recent completion of the *Arabidopsis* genome sequence has revealed the numbers and diversity of LRRPs in plants. It has also helped frame major questions to

guide further research. These include: 1) In what biological processes do the many recently-identified LRRP genes take part?; 2) What are the specific functions of these proteins at the cellular and biochemical levels?; and 3) how has the superfamily evolved, both in plants and in eukaryotes in general?

Without doubt, these questions will be addressed through research on a variety of plant systems, including maize, rice, tomato, and others in which LRRPs have already been investigated, and for which genomic -level characterizations are underway. To address questions on LRRP function on a larger scale, however, work will likely focus on the genes already identified in *Arabidopsis*, because this weed provides a powerful genetic system that allows gene identification as well as sophisticated genetic analysis of gene function. And *Arabidopsis*' genome is already complete and available to the public. Different approaches will contribute ot our understanding of plant LRRP function. Classical genetic analysis of mutants defective in specific aspects of physiology and development has provided the most in-depth knowledge of LRRP functions to date in both plants and animals; it is likely to continue to do so for processes that receive the attention of genetic investigation, and for LRRPs that yield observable phenotypes when mutated. As more is known about the genetic control of various aspects of development, genetics studies will go beyond straightforward mutant analysis, and will include such in-depth such genetic approaches as suppressor and enhancer screens, which will identify genes that modify previously identified mutant phenotypes. This sort of approach allows the identification of genes/proteins that do not yield an obvious phenotype when mutated by themselves. Indeed, suppressor screens are how SUR8, the RAS-interacting LRRP from *C. elegans*, was identified [9]. Such 'indirect" genetic approaches may identify LRR protein functions in plants in much the way they have in animal systems.

Other approaches already being employed to reveal the biological functions of large numbers of previously uncharacterized LRRP genes include reverse genetics and activation tagging. For reverse genetics, thousands of insertion-mutagenized *Arabidopsis* lines are available to identify knock-out mutants defective in specific proteins [59]. These lines can be screened by PCR using primers designed from publicly-available genomic sequence. Phenotypic analysis of such knockout mutants can then provide information on function of the targeted gene, and double or multiple mutants defective in >1 gene can be generated by crosses between knockout lines defective in related genes. Thus, through the identification of knockout mutants, LRRP fucntions can be systematically investigated. Our own laboratory is pursuing this approach to investigate the SLAT LRRPs [Forsthoefel, 2001], and another, larger scale knock-out screen is underway to investigate the functions of the hundreds of LRR-RLK (and receptor-like) proteins encoded by the *Arabidopsis* genome [F.E. Tax, University of Arizona, personal communication]. Activation-tagged mutagenized lines are available to identify genes that yield a phenotype if over- or ectopically-expressed [60]. This approach may prove valuable for LRRPs that are functionally-redundant with related LRRPs, but which may yield dominant negative phenotypes if they are over-expressed. LRRPs are good candidates for creation of dominant negative phenotypes through over-expression, as their LRR domains may interact with other cellular components and disrupt process or pathways.

At the biochemical level, the most productive routes for investigation of LRRP function will likely focus on identifying protein:protein interactions. By identifying

binding partners of LRRP proteins, it should be possible to associate LRRPs with proteins that act in previously identified processes or signaling pathways. Yeast two-hybrid screens, as well as new large-scale proteomic approaches that identify protein interactions on a genomic scale, represent two means toward identifying cellular components that interact with LRRPs. Gene expression can also provide clues to gene function. Identifying genes that are co-regulated with various LRRPs could identify networks of genes that act in the same processes. Thus, as with the methods that identify protein:protein interactions, gene expression analyses should allow association of specific LRRPs with other genes/proteins of known function. Microchips allow such investigations to be carried out on a genomic scale. Microchip analyses can also be used to obtain more general clues to LRRP expression, by surveying LRRP expression under differing developmental or environmental conditions. Together, these different approaches, which address the biological context as well as the biochemical interactions of LRRPs, should reveal the roles of LRRPs at biological, cellular, and biochemical levels.

Acknowledgements

We thank Mardi Brink and Tori Yamamoto (Whitman College) for help with structural and reverse-genetic analysis of the SLAT LRR proteins, and Dr. Frans Tax (University of Arizona) for critical reading of this review. Work on LRR proteins in our laboratory has been supported by the National Science Foundation (9604344 to DMV) and the Murdock Charitable Trust.

References

1. Kobe, B., and Deisenhofer, J. 1994, TIBS, 415.
2. Kajava, A.V. 1998, J Mol Biol, 277, 519.
3. Kobe, B.,and Deisenhofer, J. 1995, Nature, 374, 183.
4. Claudianos, C., and Campbell, H.D. 1995, Mol. Biol. Evol., 12, 405.
5. Liu, Y.T., and Yin, H.L., 1998, J Biol Chem, 273(14), 7920.
6. Li, F.N., and Johnston, M., 1997, The EMBO, 16(18), 5629.
7. Borg, J.P., Marchetto, S., Le Bivic, A., Ollendorff, V., Jaulin-Bastard, F., Saito, H., Fournier, E., Adelaide, J., Margolis, B., and Birnbaum, D., 2000, Nat Cell Biol, 7, 407.
8. Suzuki, N., Choe H.R., Nishida Y., Yamawaki-Kataoka, Y., Ohnishi S., Tamaoki, T., Kataoka, T. , 1990, Proc Natl Acad Sci USA, 87, 8711.
9. Sieburth, D.S., Sun, Q., and Han, M., 1998, Cell, 94, 119.
10. Cutler, M.L., Bassin, R.H., Zanoni, L., and Talbot, N., 1992, Molecular and Cellular Biology, 12(9), 3750.
11. Masuelli, L., and Cutler, M.L., 1996, Molecular and Cellular Biology, 16(10), 5466.
12. Sternberg, P.W., and Alberola-Ila, J., 1998, Cell, 95, 447.
13. Pang, Q., Hays, J.B., and Rajagopal, I., 1992, Proc. Natl. Acad. Sci. USA, 89(17), 8073.
14. Baumberger, N., Ringli, C., and Keller, B., 2001, Genes & Development 15,1128.
15. Steinmayr, M., Motte, P., Sommer, H., Saedler, H., and Schwarz-Sommer, Z., 1994, The Plant Journal, 5(4), 459.
16. Reddy, J.T., Dudareva, N., Evrard, J.L., Kräuter, R., Steinmetz, A., and Pillay, D.T.N., 1995, Plant Science, 111, 81.
17. Stotz, H.U., Contos, J.J., Powell, A.L., Bennett, A.B., and Labavitch, J.M., 1994, Plant Mol. Biol., 25(4), 607.
18. Yao, C., Conway, W.S., Ren, R., Smith, D., Ross, G.S., and Sams, C.E., 1999, Plant Mol Biol, 39(6), 1231.

19. Leckie, F., Mattei, B., Capodicasa, C., Hemmings, A., Nuss, L., Aracri, B., De Lorenzo, G., Cervone, F., 1999, EMBO J, 18(9):2352.
20. Worrall, D., Elias, L., Ashford, D., Smallwood, M., Sidebottom, C., Lillford, P., Telford, J., Holt, C., and Bowles, D., 1998, Science, 282(5386),115.
21. Dangle, J.L., 1995, Cell, 80, 363.
22. Ellis, J., Dodds, P., Pryor, T., 2000, Trends Plant Sci, 5(9), 373.
23. Staskawicz, B.J., 2000, Plant Physiol, 125, 73.
24. Nimchuk, Z., Hohmer L., Chang J.H., Dangle J.L., 2001, Curr. Opin. Plant Biol., 4, 288.
25. Jones, J.D.G., 2001, Curr. Opin. Plant Biol., 4, 281.
26. Jones, D.A., Thomas, C.M., Hammond-Kosack, K.E., Balint-Kurti, P.J., and Jones, J.D.G., 1994, Science, 266, 789.
27. Dixon, M.S., Jones, D.A., Keddie, J.S., Thomas, C.M., Harrison, K., and Jones, J.D., 1996, Cell, 84(3):451.
28. Dixon, M.S., Hatzixanthis, K., Jones, D.A., Harrison, K., and Jones, J.D., 1998, Plant Cell, 10(11), 1915.
29. Whitham, S., Dinesh-Kumar, S.P., Choi, D., Hehl, R., Corr, C., and Baker, B., 1994, Cell, 78(6), 1105.
30. Bent, A.F., Kunkel, B.N., Dahlbeck, D., Brown, K.L., Schmidt, R., Giraudat, J., Leung, J., and Staskawicz, B.J., 1994, Science, 263, 1856.
31. Boyes, D.C., Nam, J., & Dangl, J.L.,1998, Proc. Natl. Acad. Sci. USA, 95, 15849.
32. Young, N.D., 2000, Current Opinion in Plant Biology, 3, 285.
33. Leister, R.T., Ausubel, F.M., and Katagiri, F., 1996, Proc. Natl. Acad. Sci. USA, 93(26), 15497.
34. Warren, R.F., Henk, A., Mowery, P., Holub, E., Innes, R.W., 1998, Plant Cell, 10(9), 1439.
35. Dodds, P.N., Lawrence, G.J. and Ellis, J.G., 2001, Plant Cell, 13, 163.
36. Banerjee, D., Zhang, X., and Bent, A.F., 001, Genetics, 158, 439.
37. Ruegger, M., Dewey, E., Gray, W.M., Hobbie, L., Turner, J., and Estelle, M., 1998, Genes & Development, 12, 198.
38. Xie, D., Feyes, B.F. James, S., Nieto-Rostro, M., and Turner, J.G., 1998, Science, 280, 1091.
39. Walker, J.C., 1994, Plant Mol Biol, 26(5), 1599.
40. Becraft, P.W., 1998, TIPS, 3(10), 384.
41. McCarty, D.R. and Chory, J., 2000, Cell, 103, 201.
42. Cock, J.M., and McCormick, S., 2001, Plant Physiology, 126, 939.
43. The Arabidopsis Genome Iniative, 2000, Nature, 408, 796.
44. Chory, J., Wu, D., 2001, Plant Physiology, 125, 77.
45. Lease, K., Ingham, E., and Walker, J.C., 1998, Current Opinion in Plant Biology, 1, 388.
46. Clark, S.E., Williams, R.W., Meyerowitz, E.M., 1997, Cell, 89(4), 575.
47. Jeong, S., Trotochaud, A.E., and Clark, S.E., 1999, Plant Cell, 11(10), 1925.
48. Trotochaud, A.E., Hao, T., Wu, G., Yang, Z., and Clark, S.E., 1999, The Plant Cell, 11, 393.
49. Li, J., Chory, J., 1997, Cell, 90(5), 929.
50. Noguchi, T., Fujioka, S., Choe, S., Takatsuto, S., Yoshida, S., Yuan, H., Feldmann, K.A., and Tax, F.E., 1999, Plant Physiology, 121, 743.
51. He, Z., Wang, Z.Y., Li, J., Zhu, Q., Lamb, C., Ronald, P., and Chory, J., 2000, Science, 288(5475), 2360.
52. Torii, K.U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whittier, R.F., and Komeda, Y., 1996, Plant Cell, 8(4), 735.
53. Jinn, T.L., Stone, J.M., and Walker, J.C., 2000, Genes Dev., 14(1), 108.
54. Gómez-Gómez, L., Bauer, Z., and Boller, T., 2001, Plant Cell, 13, 1155.
55. Tax, F.E., and Vernon, D.M., 2001, Plant Physiology, 126, 1527.
56. Vernon, D.M., Brinck, M.D., Brady, M.A., Eastberg, J.H., and Forsthoefel, N., 2000, Meeting of the American Society of Plant Physiologists, San Diego, CA, Abs. #51.
57. Forsthoefel, N., Yamamoto, T. and Vernon, D.M., 2001, 12th Annual Conference on Arabidopsis Research, Madison, WI, Abstract #177.

58. Li, H., Shen, J., Zheng, Z, Lin, Y, and Yang, Z., 2001, Plant Physiology, 126, 670.
59. Krysan, P.J., Young, J.C., and Sussman, M.R., 1999, The Plant Cell, 11, 2283.
60. Weigel, D. and 19 other authors, 2000, Plant Physiology, 122, 1003.