Ligand-receptor pairs in plant peptide signaling

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Summary

Extensive studies on plant signaling molecules over the past decade indicate that plant cell-to-cell communication, as is the case with animal systems, makes use of small peptide signals and specific receptors. To date, four peptide-ligand–receptor pairs have been identified and shown to be involved in a variety of processes. Systemin and phytosulfokine (PSK), the first and second signaling peptides identified in plants, were isolated by biochemical purification based on their biological activities. Furthermore, their receptors have been biochemically purified from plasma membranes on the basis of specific ligand-receptor interactions. By contrast, the two other peptide signals, CLAVATA3 (CLV3) and the pollen S determinant SCR/SP11, were genetically identified during searches for specific ligands for receptors that had already been cloned. Systemin functions in the plant wound response, whereas PSK appears to cooperate with auxin and cytokinin to regulate cellular dedifferentiation and redifferentiation. CLV3 is important for meristem organization, binding to a heterodimeric receptor comprising the CLV1 and CLV2 proteins. SCR/SP11 instead plays a role in self-incompatibility, where it activates a signalling cascade that leads to rejection of pollen with the same *S* haplotype. These ligands all seem to bind to receptors that possess intrinsic kinase activity, and al least two of them are generated by proteolytic processing of larger precursor proteins.

Key words: Peptide hormone, Receptor-like kinase, Plant, Leucine-rich repeat

Introduction

In animal systems, peptide hormones and specific receptors play a major role in cell-to-cell communication, coordinating cell growth and differentiation in various organs. In contrast, most intercellular communication involved in plant growth and development has been explained on the basis of signaling by the six non-peptide plant hormones: auxin, cytokinin, ethylene, gibberellin, abscisic acid and brassinolides. There is no doubt about the significance of these hormones in plant growth, but discoveries over the past decade indicate that plant cell communication also makes use of small peptide signals and specific receptors.

To date, researchers have identified four peptide-ligand–receptor pairs in plants (reviewed by Ryan et al., 2002), which are involved in a variety of processes, such as wound responses, cellular dedifferentiation, meristem organization and self-incompatibility (Fig. 1, Table 1). However, these must be only part of the story, because plant genome sequencing has revealed many genes predicted to encode small peptide ligands and receptor-like kinases, whose functions remain to be uncovered (The Arabidopsis Genome Initiative, 2000; Shiu and Bleecker, 2001). Furthermore, mutations in possible prohormone processing proteases have been shown to disrupt plant growth and development. For example, the *Arabidopsis sdd1* mutant, which has a defect in a subtilisin-like serine protease, shows stomatal clustering and an increase in stomatal density (Berger and Altmann, 2000). *Arabidopsis amp1* mutants show pleiotropic phenotypes, including altered shoot apical meristems, increased cell proliferation, and increased cyclin expression. The *AMP1* gene encodes a protein with significant similarity to glutamate carboxypeptidases (Helliwell et al., 2001). In addition, the *ALE1* gene, which is required for proper differentiation of epidermis, encodes a subtilisin-like serine protease (Tanaka et al., 2001). All this evidence strongly suggests that a number of undiscovered peptide ligands that are produced by proteolytic processing from larger proteins are involved in plant growth and development.

Here, I outline the roles of the four known ligand-receptor pairs in plant peptide signaling from a biochemical point of view, and discuss the current limitations of the methodology used in identifying new ligand-receptor pairs and possibilities for future studies.

Systemins: systemic inducers of the plant wound response

Higher plants respond to wounding by herbivores and pathogens by expressing a set of defense proteins in leaves and stems. In tomato and potato plants, wounding triggers the expression of serine protease inhibitors such as protease inhibitor I and II (Green and Ryan, 1972). These proteins accumulate not only in wounded leaves but also in undamaged leaves distal from the damage sites, indicating the presence of a mobile factor that induces a systemic defense response. This factor is present in crude extracts of wounded tomato leaves and activates proteinase inhibitor genes when applied to young excised tomato plants through their cut stems. Biochemical purification of this factor on the basis of its proteinase inhibitor-inducing activity led to the identification of an 18-residue peptide named systemin (Pearce et al., 1991). Chemically synthesized systemin induces the expression of

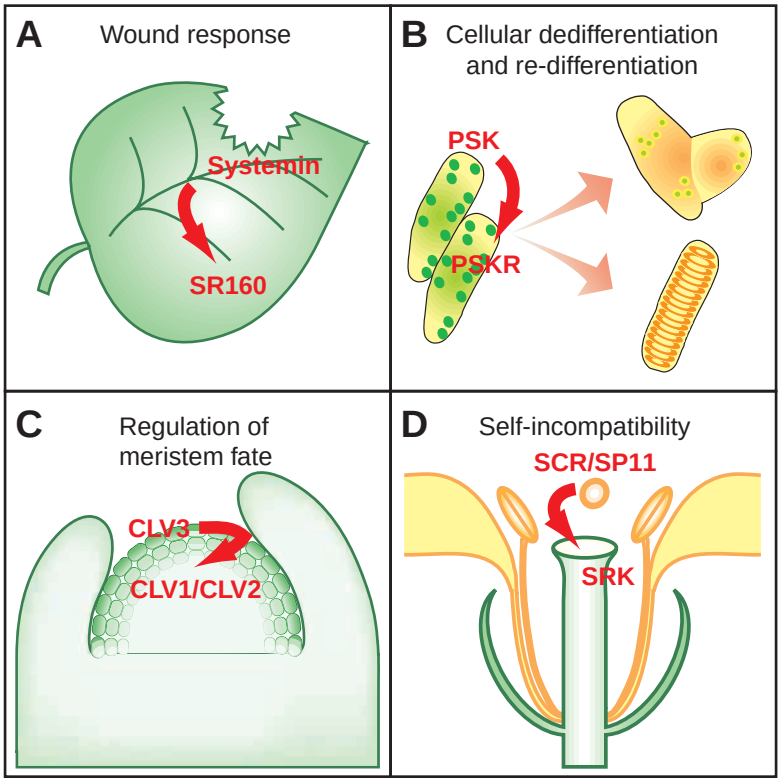

Fig. 1. Ligand-receptor pairs in plant peptide signaling. Systemin is produced by post-translational processing of a precursor, prosystemin, and recognized by SR160 receptor-like kinase, which induces defense gene activation. PSK is produced from the precursor proPSK by sulfation and post-translational processing. Secreted PSK interacts with receptor-like kinase PSKR and activates a set of genes responsible for cellular dedifferentiation and re-differentiation. CLV3 is translated, secreted and binds a CLV1/CLV2 receptor-like kinase complex, which regulates the balance between meristem cell proliferation and differentiation. SCR/SP11 is translated, secreted and interacts with the *S* locus receptor-like kinase SRK, which triggers self-incompatibility responses.

proteinase inhibitors in the leaves of young tomato plants when supplied at nanomolar levels through their cut petioles. In addition, when [^{14}C]systemin is applied to wound sites, radioactivity can subsequently be detected in the upper leaves, as well as in wounded leaves, indicating that systemin is mobile in plants.

Cloning of systemin cDNA revealed that the peptide is proteolytically processed from the C-terminus of a 200-residue precursor called prosystemin (McGurl et al., 1992). This precursor, however, does not exhibit a signal sequence at its N terminus, which suggests that it does not enter the secretory pathway but is probably systhesized on free ribosomes in the cytosol. Prosystemin orthologs have also been identified in other solanaceous species, such as potato, black nightshade and bell pepper (Constabel et al., 1998), but not in tobacco. Two prosystemin isoforms produced by alternative splicing of prosystemin pre-mRNA have been found, and both isoforms are active as signals in the wound response pathway (Li and Howe, 2001). Promoter analysis of the prosystemin gene indicates a low, constitutive level of expression in unwounded leaves and the presence of wound-inducible elements that can be activated in cells associated with the vascular bundles of petioles (Jacinto et al., 1997). Although systemin peptide has been detected in the phloem, how the systemin peptide is incorporated into this transport system and how it is transported from the phloem to the outside of distal leaf cells to activate defense genes have not been established.

The significance of systemin in the defense response was revealed by experiments in which tomato plants were transformed with sense or antisense prosystemin cDNAs under the control of the constitutive 35S promoter (McGurl et al., 1994). Overexpression of prosystemin resulted in constitutive expression of defense response genes, as if the plant were in a permanently wounded state. In addition, grafting of wild-type tomato plants onto root stocks overexpressing the prosystemin gene caused the wild-type scions to express defense genes in the absence of wounding. By contrast, transgenic plants

Table 1. Peptide ligands and their specific receptors in higher plants

Peptide ligand	Sequence	Function	Receptor(s)
Systemin	AVQSKPPSKRDPPKMQTD (Tomato systemin) RGANLPOOSOASSOOSKE (TobHypSys I*) NRKPLSOOSOKPADGQRP (TobHypSys II*) RTOYKTOOOOTSSSOTHQ (TomHypSys I*) GRHDYVASOOOOKPQXXXXX (TomHypSys II*) GRHDSVLPOOSOKTD (TomHypSys III*)	Defense signaling	SR160 (LRR-receptor-like kinase)
PSK	Y(SO_3H)IY(SO_3H)TQ	Cellular dedifferentiation and re-differentiation	PSKR (LRR-receptor-like kinase)
CLV3	MDSKSFVLLLLLFCFLFLHDASDLTQAHAHVQGLSNRKMMMM KMESEWVGANGEAEKAKTKGLGLHEELRTVPSGPDPLHHHVN PPRQPRNNFQLP (Deduced sequence from cDNA)	Growth regulation of meristem	CLV1/CLV2 (LRR-receptor-like kinase dimer)
SCR/SP11	NLMKRCTRGFRKLGKCTTLEEEKCKTLYPRGQCTCSDSKMNTH SCDCKSC (S_8-SP11)	Self incompatibility	SRK (receptor-like kinase)

Predicted signal sequence is underlined.

*These systemin peptides are glycosylated and contain hydroxyproline (one-letter abbreviation: O) residues. The amino acid sequence of TomHypSys II was estimated to be 20 amino acids in length by MALDI-MS analysis, but the amino acid analysis by Edman degradation was incomplete and only 15 residues were unambiguously assigned.

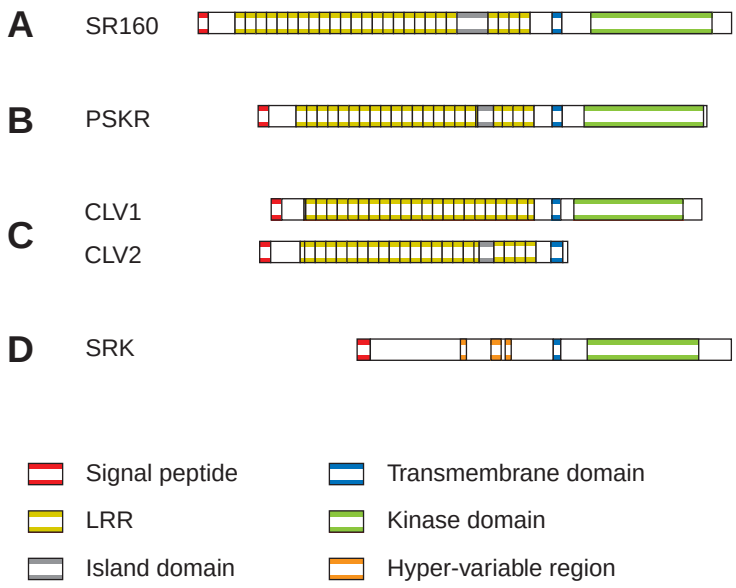

Fig. 2. Protein domain configurations of SR160, PSKR, CLV1/CLV2 and SRK.

expressing anti-sense systemin transcripts showed a severe depression of systemic proteinase inhibitor induction as well as decreased resistance towards herbivorous larvae (Orozco-Cardenas et al., 1993).

Although several solanaceous species contain homologs of prosystemin genes, no orthologous tobacco gene had been identified until recently. Pearce et al., however, have now isolated systemic hydroxyproline-rich glycopeptides (TobHypSys I and II) from tobacco leaves by biochemical purification (Pearce et al., 2001). Both peptides contain 18 residues, although they share no sequence similarity with tomato systemin, and have tobacco-trypsin inhibitor-inducing activity similar to that of tomato systemin. District peptides might therefore serve the same functions in different plant species. Interestingly, the tobacco peptides arise from a single 165-residue precursor protein that has a signal sequence at its N-terminus; such a scenario is common in peptide-ligand precursors in animals (Fisher et al., 1988). Recently, Pearce and Ryan (Pearce and Ryan, 2003) reported the isolation of three hydroxyproline-rich glycopeptides from tomato leaves, of 20, 18 and 15 amino acids in length, that act as signals for activation of defense genes, and function similarly to the systemin peptide. These three glycopeptides (TomHypSys I, TomHypSys II, and TomHypSys III) are also encoded by a single precursor gene.

A systemin receptor was detected in plasma membranes from tomato cells (Scheer and Ryan, 1999). A mono-iodinated systemin analog rapidly, reversibly and saturably binds to the receptor with nanomolar binding affinity. Scheer and Ryan have purified the 160 kDa receptor, SR160, from tomato plasma membranes (Scheer and Ryan, 2002). It has a typical leucine-rich repeat receptor-like kinase (LRR-RLK) sequence, including a putative signal sequence, a leucine zipper motif, 25 LRRs interrupted by an island domain, a single transmembrane domain, and a protein kinase domain (Fig. 2A). Interestingly, SR160 has also been isolated as tBRI1 (tomato brassinosteroid insensitive 1), a membrane receptor for the plant steroid hormone brassinolide, which is essential for normal plant development (Montoya et al., 2002). Brassinolide, however, does not compete with systemin for binding to SR160. To confirm that SR160/tBRI1 is a bona fide systemin receptor, it should be determined whether tBRI1 mutants lack systemin-binding activity and exhibit defective systemin signaling.

Phytosulfokine: a key factor regulating cellular dedifferentiation and re-differentiation in plants

In contrast to animal cells, a high proportion of plant cells, even when fully differentiated, can dedifferentiate and proliferate in vitro as totipotent stem cells, called calli, following treatment with plant hormones such as auxin and cytokinin. Callus cells differentiate into various organs, which eventually form a new plant, indicating that plant cells from a given tissue can differentiate into cells of all tissue types (Skoog and Miller, 1957). The relative rate of dedifferentiation and callus growth in vitro, however, strictly depends on the initial cell density, even if sufficient amounts of auxins and cytokinins are supplied. Cellular dedifferentiation and callus formation efficiently progress at high cell density but are significantly suppressed at low density. Interestingly, this suppression is alleviated by addition of conditioned medium in which cells have previously been grown at high density, which indicates that individual cells secrete a growth factor responsible for dedifferentiation and callus growth (Somers et al., 1985; Bellincampi and Morpurgo, 1987; Jorgensen et al., 1992; Folling et al., 1995).

This growth factor has been purified from conditioned medium derived from suspension culture of dispersed asparagus mesophyll cells (Matsubayashi and Sakagami, 1996). It is a five-residue peptide that has the sequence Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln, and because of the presence of a sulfate ester, is named phytosulfokine (PSK). Sulfated tyrosine residues are often found in secreted peptides in animals (Huttner, 1982) but, to date, PSK is the only example of post-translational sulfation of tyrosine residues in plants. PSK with an identical structure is present in conditioned medium derived from many plant cell lines, including dicotyledons and monocotyledons, which indicates the peptide is widely distributed in higher plants. PSK induces dedifferentiation and callus growth of dispersed plant cells at low nanomolar concentrations, even at initial cell densities as low as ~300 cells/ml. Interestingly, it also stimulates tracheary element differentiation of *Zinnia* mesophyll cells without intervening cell division (Matsubayashi et al., 1999) and stimulates somatic embryogenesis in carrot (Kobayashi et al., 1999). Such cellular dedifferentiation and re-differentiation, however, cannot be induced by PSK alone, but require in addition certain ratios and concentrations of auxin and cytokinin.

Organogenesis in vitro generally has three distinct phases, which were revealed by the temporal requirements of explants for a specific balance of phytohormones in the control of organogenesis (Christianson and Warnick, 1985). In the first phase, explants acquire competence, which is defined as the ability to respond to induction signals such as auxin and cytokinin. These competent explants can then be canalized and analysed for specific organ development under the influence of

the auxin/cytokinin balance through the second phase. During the third phase, morphogenesis proceeds independently of exogenously supplied hormones. One possibility is that PSK confers competence on individual cell plants and that auxin and cytokinin then determine cell fate.

Five paralogous genes encoding ~80-residue precursors of PSK have been identified in *Arabidopsis*. Each predicted protein has a probable secretion signal at the N-terminus and a single PSK sequence close to the C-terminus (Yang et al., 2001). In addition, there are dibasic amino acid residues immediately upstream from the PSK domain. It is generally accepted that, in animal prohormone precursor proteins, the primary processing recognition sequence for endoproteolysis is a pair of basic amino acid residues that bracket the peptide hormone (Harris, 1989). Gene families encoding putative PSK precursors also exist in many other plant species, including rice, carrot and asparagus, but these genes are extremely diverse, with only a few residues being conserved throughout the family. *PSK* mRNAs are found not only in callus cells but also in the leaves and roots of intact plants, indicating that *PSK* expression is not limited to the region in which individual cells actively divide. Overexpression of *PSK* genes slightly promotes callus formation in the presence of auxin and cytokinin (Yang et al., 2001) but does not affect growth of plants (Y.M., unpublished).

Acidic amino acid residues flanking the mature PSK sequence in PSK precursors are suggested to be involved in tyrosine sulfation, which is catalyzed by a tyrosylprotein sulfotransferase in the Golgi apparatus (Hanai et al., 2000). Since elimination of sulfate esters of tyrosine residues within the mature PSK sequence abolishes its biological activities, tyrosylprotein sulfotransferase must be a key enzyme in PSK biosynthesis.

Studies using radiolabelled PSK have provided evidence for the existence of high-affinity binding sites for PSK in plant plasma membranes (Matsubayashi et al., 1997; Matsubayashi and Sakagami, 1999). Photoaffinity labeling experiments have shown that 120 kDa and 150 kDa glycosylated proteins are putative PSK receptors (Matsubayashi and Sakagami, 2000), and a PSK receptor has been purified from membrane fractions of carrot cells (Matsubayashi et al., 2002). The cDNA encodes a typical LRR-RLK that has 21 LRRs and a 36-residue island between the 17th and 18th LRRs (Fig. 2B). Overexpression of this receptor-like kinase in carrot cells enhances callus growth in response to PSK and substantially increases the number of PSK-binding sites, indicating that PSK and this receptor-like kinase act as a ligand-receptor pair.

Now that the in vitro function of PSK and the molecular basis of ligand-receptor interaction in PSK signaling have been established, the next phase of research is characterization of the in vivo role of PSK and its downstream signaling pathway in plants. The carrot PSK receptor shares significant sequence identity with At2g02220, an LRR receptor-like kinase found in *Arabidopsis*. The sequencing of the *Arabidopsis* genome is now complete, and large collections of gene-disruption lines are available. Once the PSK-binding activity of At2g02220 is confirmed, direct clues to in vivo function of PSK will be provided by the phenotypes of knockout mutants.

Three *CLAVATA* loci: genes encoding ligand-receptor pairs regulating meristem fate

In plants, the shoot meristem can be thought of as having two zones: a central zone containing meristematic cells in an undifferentiated state; and a surrounding peripheral zone where cells enter a specific developmental pathway toward a differentiated state. The *Arabidopsis* genes *CLAVATA1* (*CLV1*) and *CLAVATA3* (*CLV3*) appear to play important roles in the regulation of shoot meristem development. The *CLV* loci promote the transition towards differentiation of cells in the shoot and floral meristems or restrict the proliferation of cells at the center of these meristems (Clark et al., 1993; Clark et al., 1995). Loss-of-function mutations in either *CLV1* or *CLV3* cause identical phenotypes, in which stem cells accumulate and there is a progressive enlargement of shoot and floral meristems. Double *clv1-clv3* mutants have the same phenotype, which suggests that *CLV1* and *CLV3* function in a common signaling pathway.

CLV1 encodes a predicted 105 kDa RLK that has 21 extracellular LRRs, a single transmembrane domain and an intracellular Ser/Thr kinase domain (Clark et al., 1997) (Fig. 2C). *CLV3* encodes a 96-residue peptide containing an N-terminal secretion signal (Fletcher et al., 1999). The protein does not contain a potential dibasic processing site that could be recognized by a processing enzyme, and anti-CLV3 antibodies detect an unprocessed polypeptide in *Arabidopsis* extracts, suggesting a lack of further processing in CLV3 biosynthesis. Both *CLV3* and *CLV1* are expressed in shoot apical meristems, and there is a strong possibility that CLV3 is a ligand for the CLV1 receptor-like kinase. An alternative *clv* mutant, *clv2*, displays a phenotype that is weak but similar to those of *clv1* and *clv3*. CLV2 is structurally similar to CLV1 but lacks a cytoplasmic kinase domain (Jeong et al., 1999) (Fig. 2C).

Biochemical studies show that CLV1 exists in two distinct complexes of 185 kDa and 450 kDa (Trotochaud et al., 1999). The 185 kDa molecule is proposed to be a disulfide-linked heterodimer of CLV1 and CLV2. The larger 450 kDa complex contains, in addition to the CLV1-CLV2 dimer, a Rho-GTPase-related protein (Rop) (Trotochaud et al., 1999) and the kinase-associated protein phosphatase (KAPP) (Stone et al., 1998). Rop represents a plant-specific subfamily of the RHO family of small GTPases, but its function in CLV signaling is not known. KAPP is a type 2C protein phosphatase, first isolated by screening an *Arabidopsis* cDNA expression library for interactions with the cytoplasmic domain of serine/threonine receptor-like kinase RLK5 (Stone et al., 1994). In *clv3* mutants, CLV1 occurs only as the 185 kDa protein, which suggests that formation of the 450 kDa complex requires CLV3. CLV3 and CLV1 coimmunoprecipitate in vivo, and yeast cells expressing CLV1 and CLV2 bind to native CLV3 from meristem extracts (Trotochaud et al., 2000). CLV3 associates with the active CLV1 protein complex but does not interact with a mutated CLV1 lacking kinase activity. Kinase activity must therefore be required for ligand binding. The CLV1 cytoplasmic domain has kinase activity and phosphorylates both itself and KAPP. In contrast, KAPP binds and dephosphorylates CLV1 (Williams et al., 1997). CLV3 peptide thus appears to bind to and activate the 185 kDa CLV1-CLV2 heterodimer, inducing its autophosphorylation and subsequent formation of a 450 kDa complex including Rop and KAPP. KAPP functions as a negative regulator of the CLV1 signal transduction pathway by dephosphorylating the CLV1 cytoplasmic domain.

CLV3 is expressed specifically in the central zone of the outermost meristem, whereas *CLV1* mRNA accumulates in

deeper cell layers. *CLV2* mRNA is detected in all tissues. It has been proposed that the CLV3 protein is secreted from stem cells at the apex of the meristem, travels through the extracellular space and interacts with the CLV1-CLV2 receptor complex at the plasma membrane of the underlying cells to restrict the size of the stem cell population (Fletcher et al., 1999). This speculation is supported by the observation that a tagged CLV3 fusion protein, which has the same biological activity as native CLV3, is localized to the extracellular space (Rojo et al., 2002). Interestingly, database searches have revealed a large family of genes that share homology with *CLV3* in several plants (Cock and McCormick, 2001). The majority of the predicted polypeptides have signal sequences in the N-terminal and are actually exported to the extracellular space (Sharma et al., 2003). Because CLV1 is a member of the LRR receptor-like kinase family, some of the CLV3 homologs are expected to be ligands for orphan LRR receptors.

In *clv1* mutants, missense mutations within the LRR domain often produce mutants that exhibit stronger phenotypes than null mutants, which suggests they have dominant negative effects in these mutants. Recently, it was confirmed that a chimeric CLV1 receptor kinase whose kinase domain is replaced with that of another receptor kinase acts in a dominant negative manner in the regulation of meristem development (Diévart et al., 2003). One possibility is that multiple receptor kinases that functionally overlap act within the meristem.

SCR/SP11 and SRK: determinants of *Brassica* self-incompatibility

Many flowering plants possess self-incompatibility (SI) systems in which pollen from closely related individuals is recognized and rejected by the pistil to prevent inbreeding and maintain genetic diversity within a species. Classical genetic analysis revealed that SI is controlled by a single multiallelic locus named the sterility locus (*S*-locus) (Bateman, 1955). When pollen and pistil share the same allele, a molecular interaction between male and female determinants triggers an SI response in which metabolic activation of the pollen grain and subsequent growth of the pollen tube are completely inhibited (Kanno and Hinata, 1969). The fact that these SI responses occur immediately after the primary contact between the pollen grain and the stigma surface strongly suggests the involvement of specific cell surface molecules in SI systems. During the past two decades, SI determinants in *Brassica* species have been identified through molecular cloning of *S*-locus genes whose products are expressed specifically in the stigma, pollen or anther.

Molecular and biochemical studies have identified two *S*-locus-derived proteins, *S*-locus glycoprotein (SLG) and *S*-locus receptor-like kinase (SRK), specifically expressed on the stigma surface. SLG is a soluble extracellular glycoprotein containing several *N*-linked sugar moieties, polymorphic regions and 12 conserved cysteine residues (Takayama et al., 1987; Nasrallah et al., 1987). SRK is a typical receptor-like kinase, consisting of an SLG-like extracellular domain, a single transmembrane domain and a cytoplasmic Ser/Thr kinase domain (Stein et al., 1991) (Fig. 2D). Mutations within the SRK sequence block the SI response, suggesting that SRK has a key role in SI signaling (Goring et al., 1993; Nasrallah et al., 1994). To determine directly the functions of SLG and SRK, each gene has been independently introduced into *Brassica* plants. Transformation with an *SRK* transgene results in acquisition of the corresponding SI specificity, that is, rejection of pollen that has an *S* haplotype the same as that of the transgene (Takasaki et al., 2000). In contrast, transgenic plants expressing *SLG* alone showed no SI specificity. The *SLG* transgene, however, enhances the SI response in the presence of an *SRK* transgene derived from a plant with an *S* haplotype the same as that of the *SLG*. These results demonstrate that *SRK* alone regulates the female (stigmatic) SI specificity and that *SLG* has an accessory role in SI. In some *Brassica* species, there appears to be no SLG requirement for SI (Suzuki et al., 2000; Suzuki et al., 2003).

An alternative target in SI research is the identification of a male (pollen) determinant. Extensive work by two groups has identified a highly polymorphic small gene located between *SRK* and *SLG* at the *S*-locus (Schopfer et al., 1999; Takayama et al., 2000). This gene, designated *S locus cysteine-rich protein* (*SCR*) or *S locus protein 11* (*SP11*), encodes a highly polymorphic peptide containing a putative signal peptide cleavage site and is expressed predominantly in the anther. Transformation of *Brassica* plants homozygous for one *S* haplotype with an *SCR/SP11* gene derived from another haplotype results in acquisition by transgenic pollen of the SI specificity encoded by the transgene. Furthermore, addition of bacterially expressed SP11 protein to the stigma induced *S*-haplotype-specific inhibition of pollen (Takayama et al., 2000). These experiments have confirmed that the *SCR/SP11* gene product is necessary and sufficient to determine pollen SI. Immunohistochemical experiments suggested that SP11 is secreted from the tapetal cell into the anther locule as a cluster and translocated to the pollen surface at the early developmental stage of the anther. During the pollination process, SP11 is translocated from the pollen surface to the papilla cell and then penetrates the cuticle layer of the papilla cell to diffuse across the pectin cellulose layer (Iwano et al., 2003).

S-haplotype-specific ligand-receptor interactions between SCR/SP11 and SRK were directly demonstrated by experiments that used synthetic radiolabeled SP11 (Takayama et al., 2001). Ligand-binding assays indicated the presence of high- and low-affinity binding sites in the stigmatic membranes of the cognate *S*-haplotype. The labeled SP11 could be specifically crosslinked to the 120 kDa SRK and a 65 kDa protein that might correspond to SLG or a truncated SRK produced by alternative splicing. In addition, synthetic SP11 induced autophosphorylation of SRK in an *S*-haplotype-specific manner. Specific ligand-receptor interactions have also been detected by experiments using bacterial recombinant SCR and native stigma SRK (Kachroo et al., 2001).

The next phase of SI research will be to focus on identifying components of the SRK-mediated signaling cascade. The low efficiency of transformation and lack of a genome database for *Brassica*, however, are making further studies of SI responses difficult. To overcome these problems, a self-incompatible *Arabidopsis* plant in which the SRK and SCR genes are incorporated has been established (Nasrallah et al., 2002). *A. thaliana* is normally a self-fertilizing plant, but successful complementation studies demonstrate that the signaling cascade leading to rejection of self-related pollen is nevertheless present. Analysis of SI responses will be

facilitated by the availability of the complete genome sequence of this species.

Perspectives

Arabidopsis genome sequencing has revealed the presence of at least 610 putative RLK genes, 222 of which belong to the large LRR-RLK subfamily (The Arabidopsis Genome Initiative, 2000; Shiu and Bleecker, 2001). Proteins containing LRR motifs are thought to be involved in protein-protein interactions, and the specificity of these interactions might be determined by the composition of the variable amino acids in the consensus core of the LRRs (Kobe and Deisenhofer, 1995). Receptors for systemin, PSK and CLV3 are members of the LRR-RLK subfamily, and the plant steroid hormones (brassinolides) and a bacterial peptide elicitor flagellin also bind to LRR-RLKs (Li and Chory, 1997; Gomez-Gomez and Boller, 2000). Although brassinolides are not peptides, recent evidence suggests the involvement of putative secreted brassinosteroid-binding proteins in the binding of brassinosteroid to the BRI1 (Li et al., 2001). However, most members of this family remain orphan receptors.

There are several ways to identify ligand-receptor pairs in plants. The most general approach is extensive screening of mutants on the basis of their phenotype. Once a large collection of mutant lines showing a unique phenotype is assembled, there is a chance that one can identify the ligand-receptor pairs in a particular peptide signaling pathway. A successful precedent for this approach, however, is found only in the CLV signaling pathway, suggesting limitations in the genetic approach. The main difficulty in finding mutant lines that have informative phenotypes may be due to functional redundancy arising from the genome structure of *Arabidopsis*. Indeed, although *Arabidopsis*, *Drosophila* and *C. elegans* share a similar number of gene types, multigene families, present as clustered and dispersed copies, are particularly frequent in *Arabidopsis* (The Arabidopsis Genome Initiative, 2000). In fact, five paralogous genes encoding precursors of PSK have been identified in *Arabidopsis*, and loss-of function approaches have not given rise to visible, directly informative phenotypes; this suggests functional redundancy among these five PSK precursor genes. The same may be true of the members of the LRR-RLK family. There are three BRI1-like receptor proteins in *Arabidopsis*, two of which actually show specific binding to brassinolide (Yin et al., 2002). The existence of an additional receptor kinase(s) that has a functional overlap with CLV1 is also suggested by the analysis of dominant negative mutants (Diévart et al., 2003). In this context, definitive phenotypes may only emerge when combinations of knockouts for all homologous redundant genes are available.

The classical methodologies for receptor cloning are those based on direct ligand-receptor binding. A key factor in the use of ligand-based affinity chromatography is the ability to derivatize peptide ligands without loss of functional binding activity. In PSK receptor studies, the finding that [Lys^5]PSK retains significant activity after derivatization of the side chain of Lys^5 provided the breakthrough in a series of experiments aimed at visualization and purification of PSK receptors. Similarly, receptor-based affinity chromatography has also been used for ligand-fishing experiments. Bartley et al. isolated a protein ligand for the ECK receptor protein-tyrosine kinase by using the extracellular domain of the receptor as an affinity reagent in a single-step purification (Bartley et al., 1994). In addition, progress in biosensor technology, based on surface plasmon resonance, has had a great impact on the ability to detect and measure biospecific interactions in real time. Davis et al., for example, constructed a probe consisting of the extracellular domain of the receptor-like tyrosine kinase, TIE2, fused to the Fc portion of human IgG, and coupled this to the surface of a BIAcore sensor chip, which they then used to screen conditioned media from a variety of cell lines for specific binding to TIE2 (Davis et al., 1996). Once tagged versions of the plant receptor-like Ser/Thr kinases are functionally expressed and immobilized on such a biosensor chip, this biochemical system should offer the most direct approach for ligand fishing in plants.

Although ligand-receptor binding depends on a large interacting surface between two essentially correctly folded and disulfide-paired proteins, which usually occurs efficiently only in the secretory pathway, the yeast two-hybrid system, which detects protein-protein interactions that can occur within the reducing environment of the yeast cell cytoplasm, may, in some cases, be a sensitive tool for studying ligand-receptor interactions. To find the ligands for pollen-specific RLKs, LePRKs, Tang et al. conducted a yeast two-hybrid screen using the extracellular domains of LePRKs as bait to search for interacting proteins encoded by a pollen cDNA library (Tang et al., 2002). They identified numerous secreted and plasma-membrane-bound candidate ligands. One of these, the Cys-rich protein LAT52, is known to be essential during pollen hydration and pollen tube growth (Twell et al., 1989; Muschietti et al., 1994). In vivo coimmunoprecipitation demonstrates that LAT52 is capable of forming a complex with LePRK2 in pollen and that the extracellular domain of LePRK2 is sufficient for the interaction. Although there is much to be done, interactions between LePRK2 and LAT52 might represent an autocrine pollen signaling system that plays a vital role in regulating the initiation and maintenance of pollen tube growth.

Despite the large numbers of putative RLKs encoded in the genomes of plants, a general model for how these receptors carry out signal transduction has yet to be determined. To overcome this problem, the chimeric receptor approach has been used for the characterization of a brassinosteroid receptor, BRI1, one of the LRR-RLKs in plants (He et al., 2000). A rice LRR-RLK named XA21 (Song et al., 1995) confers resistance to *Xanthomonas oryzae* pv. *oryzae*, and activation of XA21 signaling leads to rapid and strong induction of transcription of the rice defense genes chitinase *RCH10* and phenylalanine ammonia lyase. Interestingly, a chimeric receptor, consisting of the extracellular and transmembrane domains of BRI1 and 65 amino acids of a juxtamembrane domain fused to the kinase domain of XA21 is able to elicit cell death, an oxidative burst, and the defense pathway, suggesting that a mechanism of signaling conserved between BRI1 and XA21 may be extrapolated to the large number of LRR-RLKs found in plant genomes. This chimeric receptor approach, using the XA21 signaling outputs, should provide an alternative: an assay system that is applicable to the discovery of ligands for the LRR-RLKs.

For many years, peptide signaling, despite its overwhelming importance in animals, has been largely neglected because six

lipophilic non-peptide plant hormones play various roles in plant growth and development. Now, we are beginning to be aware of the possibility that some of the cell-to-cell interactions in plants are mediated by small hydrophilic ligands such as peptides. Our continued efforts to identify novel peptide ligands and their receptors should eventually yield a paradigm for local intercellular communication in plants and will clarify both distinct and similar aspects of peptide signal transduction in plants and animals.

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Note added in proof

Recently, Clark and co-workers retracted their previous report regarding CLV3 protein [Trotochaud, A. E., Jeong, S. and Clark, S. E. (2000). CLAVATA3, a multimeric ligand for the CLAVATA1 receptor-like kinase. *Science* **289**, 613-617]. In this paper they concluded that CLV3 acted as a ligand for the CLV1 receptor kinase based on immunoprecipitation and western blots using polyclonal antibodies to CLV3. However, subsequent examination by Clark et al. revealed that these polyclonal antibodies can detect neither native CLV3 nor bacterially expressed CLV3. Thus, there is currently no evidence for the ligand-receptor interaction between CLV1 and CLV3 [Nishihama, R., Jeong, S., DeYoung, B. and Clark, S. E. (2003). Retraction. *Science* **300**, 1370].

Recently, it has been confirmed that systemin binds SR160 expressed in tobacco suspension-cultured cells. In addition, it has been reported that *cu-3*, a *SR160/tBRI1* null mutant in tomato, exhibits a severely reduced response to systemin. These results indicate that SR160/tBRI1 is a component of the functional systemin receptor in tomato [Scheer, J. M., Pearce, G. and Ryan C. A. (2003). Generation of systemin signaling in tobacco by transformation with the tomato systemin receptor kinase gene. *Proc. Natl. Acad. Sci. USA* **100**, 10114-10117].

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