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Review

*Research review*

# Self/nonself perception and recognition mechanisms in plants: a comparison of self-incompatibility and innate immunity

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## Summary

Analyses of emerging concepts indicate that parallels exist between self-incompatibility and pathogen recognition. In the case of surveillance of 'nonself', plant immune responses are triggered either by pattern recognition receptors (PRRs) that detect conserved pathogen-associated molecular patterns (PAMPs) or by resistance (R) proteins recognizing isolate-specific pathogen effectors. PAMP detection is an important component of innate immunity in plants and serves as an early warning system for the presence of potential pathogens and activation of plant defense mechanisms. In the Brassicaceae, the recognition of 'self' and self-incompatibility are components of a receptor-ligand based mechanism that utilizes an S receptor kinase (SRK) to perceive and reject 'self'-pollen. SRK is an S-domain receptor-like kinase (RLK), which in turn is part of the RLK family, some members of which represent PRRs involved in the detection of PAMPs. S-domain RLKs also occur in species that do not exhibit self-incompatibility and are up-regulated in response to wounding, PAMPs and pathogen recognition. Although evolution may have driven expansion of certain RLK families to serve roles in particular physiological processes, this may not exclude these receptor types from functioning in different programs. Recent findings on self/nonself recognition are reviewed and conceptual and mechanistic links between microbial recognition and self-incompatibility are discussed.

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## Introduction

The two separate mechanisms of innate immunity and self-incompatibility (SI) are remarkably similar. The similarities and differences between the two mechanisms in terms of functions, functional outcomes, selective processes, responses, recognition molecules, recognition receptors, and signal transduction and perception are summarized herein. In

order to elucidate how innate immunity fits into the global picture of overlapping and complex plant defense mechanisms, a short overview is presented first. In addition, an overview of SI is given to elucidate the molecular and biochemical mode of SI in the Brassicaceae. This is followed by a discussion of the role of receptor-like kinases (RLKs) in defense mechanisms and SI. While the role of S-domain RLKs in SI within the Brassicaceae is well described, the role of these receptors in pathogen perception and defense is not widely recognized.

Plant innate immunity, with its associated defense mechanisms, exhibits similar characteristics to the mammalian and insect mechanisms (Nürnberger *et al.*, 2004; Zipfel & Felix, 2005). Although they express an apparent passivity associated with their sedentary lifestyle, and are simultaneously exposed to evolving pathogens as well as environmental stresses, plants have evolved a unique metabolic plasticity that allows them to perceive pathogens and unleash effective defense strategies. The innate immune system in plants is unable to acquire or specifically adapt like the animal adaptive immune system (Goldsby *et al.*, 2000) and relies on a spectrum of predetermined receptors expressed in nonmobile cells. The question therefore arises as to how such a system could perceive so many diverse pathogen-derived signals, whilst being limited to an ancient disposition (i.e. a mechanism that originated before the evolving variables in potential invaders).

Plants therefore appear to utilize evolutionary genetic events, such as changes in gene sequence and/or genetic architecture and alterations in gene regulation, in self-defense against simultaneously evolving pathogens. Evolutionary events are also reliant on exon swapping, as well as domain recruitment through the incorporation of exons into new loci (Shapiro, 2002). Subsequently, plants must be able to re-use sections of translatable codons to produce proteins with similar morphologies or proteins that are able to multitask between different functions.

## Surveillance of 'nonself': innate immunity

The ability to distinguish 'self' from 'nonself' is the most fundamental aspect of an immune system. Basal or general resistance against disease in plants used to be described by the term 'nonhost immunity', referring to an evolutionarily ancient, multilayered resistance mechanism consisting of constitutive and inducible components (Thordal-Christensen, 2003). Nonhost immunity remains operative even in susceptible plants to limit pathogen growth and is associated with the release of molecules (ligands or elicitors) derived from the pathogen and/or molecules such as oligogalacturonides and peptides released by the host plant as endogenous elicitors, analogous to the 'danger signals' of the vertebrate immune system (Matzinger, 2002). By contrast, host immunity is more recently evolved, acts within the species level and is controlled by polymorphic host genes, such as *R* (resistance) genes, the products of which interact, directly or indirectly, with secreted 'avirulence' proteins or effectors of the pathogen (Jones & Takemoto, 2004).

The surface receptors are known to detect both pathogen-derived elicitors (or pathogen-associated molecular patterns (PAMPs) if the molecule contains a conserved 'pattern') and avirulence effectors. They include receptor-like kinases (RLKs), receptor-like proteins (RLPs) and extracellular binding proteins that may form part of multicomponent recognition complexes. Intracellular receptors are the nucleotide-binding (NB) leucine-rich repeat (LRR) class of receptors for the detection of pathogen effectors (reviewed by Nürnberger & Kemmerling, 2006; Altenbach & Robatzek, 2007; Tameling & Takken, in press, and summarized in Fig. 1).

Two branches of the plant immune system are now recognized: PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) (Chisholm *et al.*, 2006; Jones & Dangl, 2006). PTI refers to the inducible responses activated upon recognition of conserved PAMPs, such as the lipopolysaccharides (LPSs), peptidoglycan and flagellin of bacteria, and the chitin and glucan of fungi. Recent evidence indicates that some identified pattern recognition receptors (PRRs) are members of the RLK family (e.g. flagellin sensing 2 (FLS2) and the Ef-Tu receptor (EFR); Gomez-Gomez & Boller, 2000; Zipfel *et al.*, 2006). Work on flagellin and EF-Tu by the Boller group indicates that there must be a requirement for numerous such signal perception and transduction systems in plants able to recognize all potential invaders (Gomez-Gomez & Boller, 2002; Zipfel *et al.*, 2006). Sequencing of the *Arabidopsis thaliana* genome has revealed the presence of > 400 RLK sequences with various receptor configurations, of which those containing an LRR in the extracellular domain constitute the largest group, with 216 members (Shiu *et al.*, 2004; Ingle *et al.*, 2006). The diversity and large number of plant RLKs suggest that they may be involved in the perception of a wide range of stimuli (discussed in the section 'RLKs in plant innate immunity and self-incompatibility'). Other PRRs are also found amongst non-RLK proteins such as *Glycine max* beta-glucan elicitor binding proteins (GmGBP), *Lycopersicon esculentum* ethylene-inducing xylanase (LeEIX2) and chitin elicitor-binding protein (CeBIP), for perception of beta-glucans (soybean (*Glycine max*)), xylanase (tomato (*Lycopersicon esculentum*)) and chitin fragments (rice (*Oryza sativa*)), respectively (Umemoto *et al.*, 1997; Ron & Avni, 2004; Kaku *et al.*, 2006).

By contrast, ETI, the second branch, acts mostly inside the cell, using polymorphic NB-LRR proteins encoded by *R* genes. Some R proteins structurally resemble RLK and RLP receptors and probably evolved from PAMP receptors (reviewed by Liu *et al.*, 2007; Tameling & Takken, in press). *R* gene-mediated resistance is a form of host immunity activated upon recognition of an avirulence factor, a pathogen effector that elicits resistance, via recognition of the effector by the plant. Few *R* genes confer broad-spectrum resistance as

 

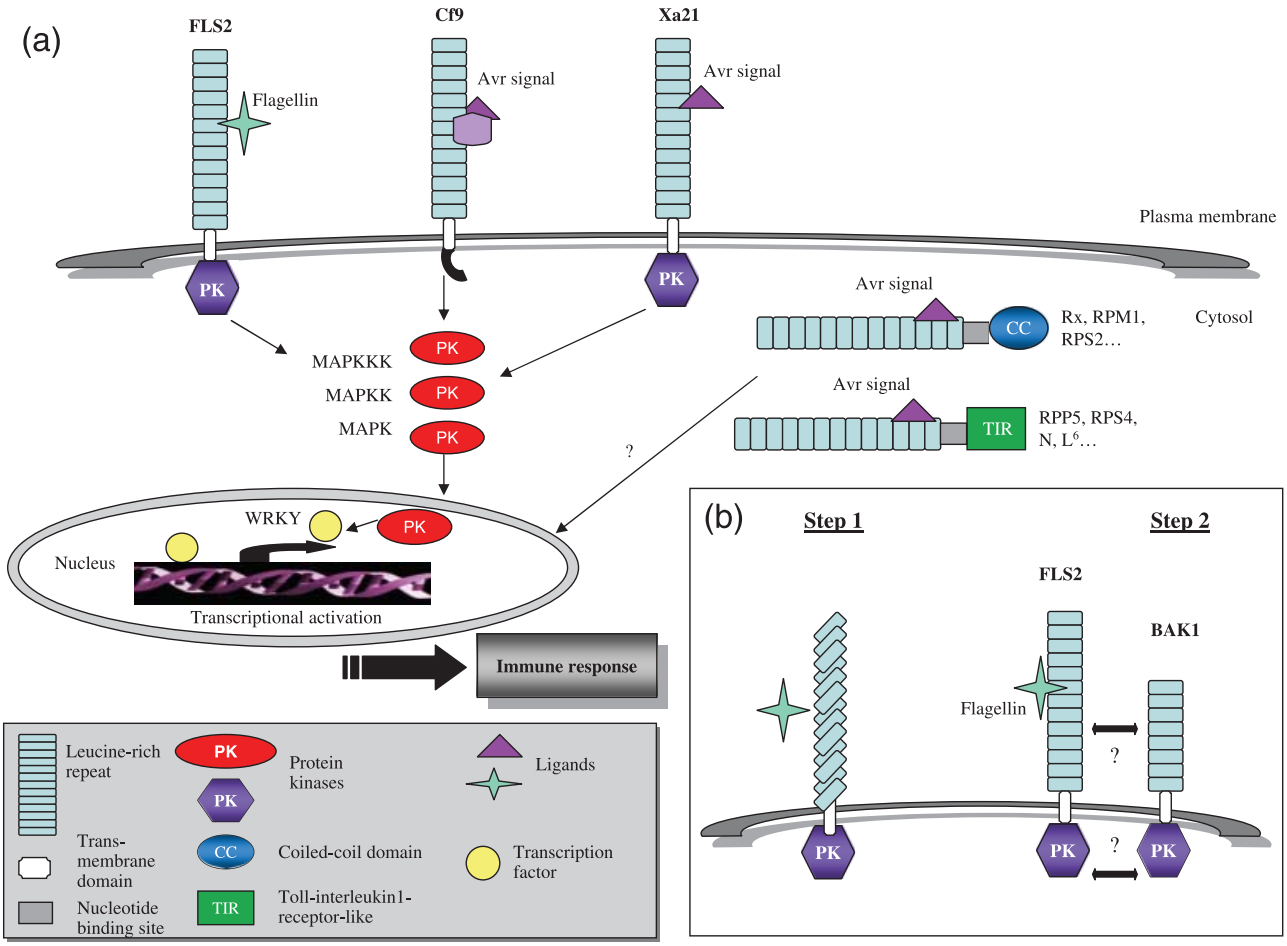


**Fig. 1** Signaling cascades in the innate immune response of plants (adapted from Nürnberger *et al*., 2004). Note that the non-receptor-like kinase (RLK)/receptor-like protein (RLP) pattern recognition receptors (PRRs) were not included and pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) signaling pathways do not necessarily converge. (a) Pathogen recognition by the innate immune system relies on interactions between pathogen-derived molecules and corresponding host receptors. PAMPs on microbial surfaces, as well as other molecules produced by infecting pathogens, trigger innate immunity. In plants, various leucine-rich repeat (LRR)-type proteins appear to be involved in pathogen defense activation. Flagellin sensing 2 (FLS2) and the R proteins Cf9 and Xa21 are transmembrane receptors that are able to recognize PAMPs, such as flagellin, or avirulence (Avr) effector signals. Avr9 is recognized by a high-affinity binding site in tomato. This complex interacts with Cf9 and activates at least two mitogen activated protein kinase (MAPKs). *Arabidopsis thaliana* FLS2 and rice Xa21 are likely to transduce the pathogen signal through their cytoplasmic protein kinase domains. The amino-terminal fragment of flagellin (flg22) directly binds to FLS2 and activates MAPKs, AtMPK3 and AtMPK6. Translocation of PAMP-activated plant MAPKs into the nucleus has been demonstrated, where these enzymes are likely to contribute to the activation of transcription factors of the WRKY type. In turn, intracellular plant R proteins recognizing Avr signals confer pathogen race/plant cultivar-specific immunity to viral (tobacco resistance gene to tobacco mosaic virus (N) and potato resistance gene to potato virus X (Rx)), bacterial (Arabidopsis resistance gene to *Pseudomonas syringae* pv tomato strain DC3000 (RPS4), Arabidopsis resistance gene to *Pseudomonas syringae* pv tomato strains producing AvrRpm1 or AvrB (RPM1) and Arabidopsis resistance gene to *Pseudomonas syringae* pv tomato strains that produce AvrRpt2 (RPS2)), oomycete (Arabidopsis resistance gene to *Peronospora parasitica* (RPP5)), or fungal (flax rust resistance gene ($L^6$)) pathogens (Gomez-Gomez & Boller, 2002). Intracellular nucleotide binding site (NBS)-LRR proteins are linked to coiled-coil (CC) or Toll-interleukin 1 receptor (TIR) domains. PGN, peptidoglycans; Pto, a tomato protein kinase. (b) Ligand-induced dimerization. During the two-step address-message mechanism, binding of flagellin to the N-terminal part is the first step and activation of responses with the C-terminus is the second step (Meindl *et al*., 2000). flg22-dependent heterodimerization of FLS2 and brassinosteroid receptor-associated receptor kinase (BAK)1 occurs, where FLS2 binds flg22 independently of its association with BAK1. BAK1 probably does not determine the specificity of the signal output, but is likely to have a role as an adaptor or coreceptor for regulation of various receptors, as suggested by Chinchilla *et al*. (2007).

they act in a race-specific manner. *R* gene-mediated immunity is often associated with the hypersensitive response (HR). It results in local induced resistance (LIR), acting at the site of infection to contain the invader, and systemic acquired resistance (SAR), which induces defenses in distal, noninfected parts of plants after activation of local resistance. It should be noted that SAR has also been demonstrated to be induced by PAMP recognition (Mishina & Zeier, 2007).

 

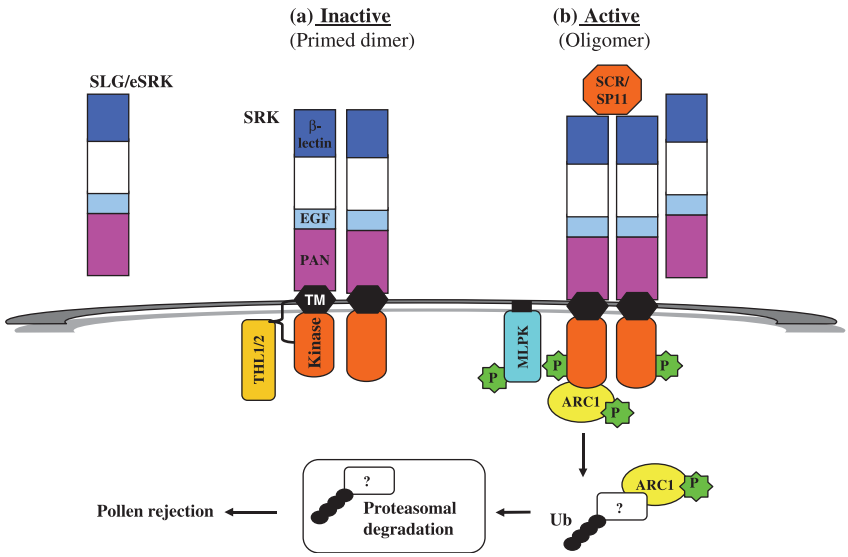


**Fig. 2** S receptor kinase (SRK) signaling in the *Brassica* self-incompatibility response (adapted from Haffani *et al*., 2004; Takayama & Isogai, 2005). (a) Inactive, but primed. Ligand-independent dimerization might provide a 'primed' state that allows rapid recruitment and activation of the receptor on ligand binding (Naithani *et al*., 2007). In the absence of incompatible pollen, SRK is inhibited by thioredoxin *h* proteins (THL1/2). This inhibition is released upon the haplotype-specific S cysteine-rich (SCR)/*S* locus protein 11 (SP11) pollen ligand binding (Bower *et al*., 1996; Cabrillac *et al*., 2001). *S* locus glycoprotein (SLG) and a soluble extracellular domain produced from SRK (eSRK) represent low-affinity binding sites for SCR/SP11. (b) Active. During incompatible pollination, the SCR/SP11 ligand binds to and activates SRK (Giranton *et al*., 2000). Whether SLG participates in the SRK complex is unclear, as the haplotype-specific SLG is not always required. Activated SRK is proposed to autophosphorylate serine and threonines, and some of these phosphorylation sites represent docking sites for downstream signaling proteins such as armadillo-repeat-containing 1 (ARC1) and the M-locus protein kinase (MLPK).

Dangl and Jones have questioned the likelihood of the known *R* genes, which are linked to defense, being able to recognize all the possible effector signals (Dangl & Jones, 2001). The mechanisms of *R* gene-mediated immunity may thus be explained by the 'gene-for-gene' genetic model or the 'guard hypothesis' molecular model. A 'guard' can refer to a typical R protein, whereas the 'guardee' represents a target of pathogen effectors (Dangl & Jones, 2001). Many plant R proteins might be activated indirectly by pathogen-encoded effectors, and not by direct recognition (Dangl & Jones, 2001). This form of 'guard hypothesis' implies that R proteins are able to indirectly recognize pathogen effectors by monitoring the structural integrity of the host cell targets, which is altered by effector action. The R proteins in question are thus activated as sensors of 'pathogen-induced self' or 'altered self' molecular patterns and can potentially perceive the presence of more than one effector protein. This can explain how plants can potentially recognize a diverse set of pathogens and pathogen-specific molecules, using a relatively limited number of pathogen receptors.

A 'zigzag' model to illustrate the quantitative output of the plant immune system, as well as to illustrate the evolutionary relationship between PTI and ETI, was recently proposed (Jones & Dangl, 2006). In phase 1, PAMPs or microbe-associated molecular patterns (MAMPs) (i.e. pathogens or microbes) are recognized by PRRs, resulting in PTI, which can stop further colonization. In phase 2, successful pathogens deploy effectors that contribute to pathogen virulence. When effectors interfere with PTI, this results in effector-triggered susceptibility (ETS). In phase 3, an effector is specifically recognized by an R protein, which results in ETI. Therefore, recognition is either indirect, or through direct NB-LRR recognition of an effector. ETI is an accelerated and amplified PTI response, which results in disease resistance and may lead to the HR at the infection site. In phase 4, natural selection drives pathogens to avoid ETI. This is achieved either by shedding or diversifying the recognized effector gene, or by acquiring effectors that suppress ETI. Thereafter, natural selection results in the evolution of new *R* specificities leading to ETI being triggered again.

Transcriptional analysis of genes expressed in *A. thaliana* in response to elicitation by flagellin (flg22) (Navarro *et al*., 2004) indicates that a considerable number of the up-regulated genes can be classified as being involved in signal perception (*RLK* and *R* genes) and signal transduction. This is indicative of positive feedback regulation operating in innate immunity with transcriptional activation of the components involved in the perception and signaling (Zipfel *et al*., 2004). The up-regulated expression of *RLK* and *R* genes presumably leads to an enhanced sensitivity of the plant to further stimuli, which allows sensing of the presence of invading microorganisms with other PAMPs or effector signals; that is, a primed or sensitized state.

 

## Surveillance of 'self': self-incompatibility

The established system of 'self'/'nonself' recognition in SI systems utilizes receptor–ligand type interactions to perceive, recognize and reject incompatible pollen. Thus, SI prevents 'self'-pollination (Fig. 2). Although SI responses are generally comprised of a 'self' and 'nonself' recognition process, SI systems have evolved independently and do not utilize one molecular mechanism exclusively. Rather, SI encompasses a collection of divergent cellular responses leading to pollen rejection (Takayama & Isogai, 2005; Wheeler & Franklin-Tong, 2007).

The molecular signatures of 'self' and 'nonself' in plant SI are unambiguous. The recognition and rejection of 'self'-pollen is generally regulated by two or more multiallelic and tightly linked *S* genes (comprising *S* haplotypes). In sporophytic systems such as in the Brassicaceae, 'self' is derived from the expression of matched products from the same *S* haplotype in the interacting pistil and pollen parent, whereas 'nonself' is derived from the expression of the unmatched products from different *S* haplotypes (Takayama & Isogai, 2005). This system uses a receptor–ligand based mechanism, with the S-domain receptor kinase (SRK), to perceive a ligand, the S cysteine-rich (SCR)/*S* locus protein 11 (SP11), present on the pollen coat. The multiallelic *SRK* gene is the 'female' determinant of specificity in the SI response of the Brassicaceae (Takasaki *et al.*, 2000; Silva *et al.*, 2001). SRK spans the plasma membrane of stigmatic epidermal cells, and it is activated in an *S* haplotype-specific manner upon binding of the pollen ligand to a hypervariable subdomain in its extracellular region (Fig. 2; Kachroo *et al.*, 2001; Takayama *et al.*, 2001; Kemp & Doughty, 2007; Shimosato *et al.*, 2007). The multiallelic *SCR*/*SP11* gene is the 'male' determinant in this system (Schopfer *et al.*, 1999; Takayama *et al.*, 2000). Activation of SRK leads to cellular signaling pathways in the stigmatic papillae causing a block in pollen hydration, germination and pollen tube growth.

Another protein implicated in the *Brassica* SI system is the multiallelic *S* locus glycoprotein (SLG), a secreted glycoprotein encoded in the *S* locus region and expressed in the stigma. SLG shows a sequence similarity to the ectodomain of SRK, but does not contribute to the *S* haplotype specificity of this system (Takasaki *et al.*, 2000; Silva *et al.*, 2001). The expression of SLG has been proposed to facilitate the processing or accumulation of SRK (Dixit *et al.*, 2000), or to enhance the SI response (Takasaki *et al.*, 2000), although it is not always required (Silva *et al.*, 2001). SLG was co-immunoprecipitated as part of a chemically cross-linked SRK complex and may function as a coreceptor with SRK to form a heteromeric receptor complex that perceives the signal carried by the pollen (Giranton *et al.*, 2000). However, SLG and a soluble extracellular domain produced from SRK (eSRK) primarily exist as monomers. Recently, the soluble SLG and eSRK were found only to present low-affinity binding sites for SCR/SP11, while the membrane-bound SRK and a truncated membrane-bound form of SRK (tSRK) presented high-affinity binding sites for SCR/SP11 (Shimosato *et al.*, 2007). While tSRK could participate in complexes with SRK for high-affinity SCR/SP11 binding, it is less likely that SLG and eSRK participate in these complexes.

The classical view of ligand-activated animal receptors involves receptor homodimerization or oligomerization, which is induced by ligand binding and serves to bring the receptor intracellular domains into close proximity for transphosphorylation and recruitment of effector cytoplasmic proteins (Heldin, 1995). SRK has been found to exist as a dimer in unpollinated pistils (i.e. in the absence of ligand), suggesting that the classical animal-based receptor model does not apply (Fig. 2; Giranton *et al.*, 2000). In this state, the SRK dimer provided a high-affinity binding site for the *S* haplotype-specific SCR/SP11 ligand, and, interestingly, high-affinity binding of SCR/SP11 appears to be a consequence of the presence of preformed dimers (Shimosato *et al.*, 2007). The ligand-independent dimerization of SRK might provide a 'primed' condition that allows the rapid recruitment and activation of the receptor on ligand binding. SCR/SP11 ligand binding may then cause the rearrangement of existing SRK dimers or stabilize the ligand–receptor complex, leading to the phosphorylation and activation of SRK (Shimosato *et al.*, 2007). The recruitment of this additional step for full receptor activation has also been reported for animal receptors (Heldin, 1995; Giranton *et al.*, 2000).

## Self-incompatibility vs innate immunity

Plant SI and plant immunity evolved in response to different pressures, namely, avoidance of inbreeding in the former case and avoidance of parasitism in the latter. The recognition and rejection of 'self' in plant SI can be compared to recognition and defense activation in plants. The *Brassica* SI SRK complex serves to recognize, and mounts a response to, 'self' ligands. By contrast, in plant defense, pathogen-derived 'nonself' ligands are recognized by RLKs and pathogen-induced changes to 'guardee' molecules are recognized as 'altered self' by *R* gene products.

Hogenboom (1983) noted the close parallels between the genetics of SI and plant–pathogen interactions. Hodgkin *et al.* (1988) compared SI responses with pathogen recognition and pointed out that parallels between SI and host–pathogen interactions include the penetration of the 'host' by a tubular cell emanating from a spore-like structure. Support for this idea came later when the wheat (*Triticum aestivum*) wheat leaf rust kinase (*WLRK*) defense genes were found to be structurally related to *SRK* genes, and this led to speculation that genes involved in SI and defense might have had a common ancestor (Feuillet *et al.*, 1998). Nasrallah (2005) has discussed the evolutionary origins of plant SI, focusing on the hypothesis that SI evolved from a defense pathway (Hiscock *et al.*, 1996).

 

**Table 1** Summarized comparison between plant innate immunity and self-incompatibility

| | Innate immunity | Self-incompatibility (specific reference to Brassicaceae) |
|---|---|---|
| **Physiological function** | Basal defense | Reproduction |
| **Selective process** | Recognition of:<br>nonself: (PAMP), PTI<br>altered self: (Avr-R), ETI,<br>endogenous elicitors | Recognition of:<br>self |
| **Response** | Mounts response to nonself or altered self | Mounts response to self |
| **Recognition molecule** | PAMPs<br>Avr effectors<br>Host-derived oligouronides and peptides | SCR protein ligand |
| **Recognition receptor** | RLK PRRs (e.g. for PAMPs)<br>R proteins (directly or indirectly, e.g. for pathogen effectors)<br>non-RLK PRRs (e.g. for endogenous oligogalacturonides, glucans, or chitin fragments) | S-domain RLKs for pollen epitopes |
| **Receptor activation** | PRRs bind to PAMPs with or without coreceptor | S-domain RLKs form oligomers and bind to pollen |
| **Subsequent reactions during response** | Downstream intracellular phosphorylation cascades are triggered<br>Deposition of callose at site of plant cell in host–pathogen interaction<br>Cross protection; an incompatible host–pathogen interaction can affect the outcome of a compatible interaction<br>Induction of general inhibitory compounds, e.g. phytoalexins<br>$Ca^{2+}$-dependent signaling network<br>Programmed cell death | Downstream intracellular signaling cascades are triggered<br>Deposition of callose at surface of stigma cells<br>Individual interaction; an individual pollen grain interacts with an individual papillar cell<br>No induction of general inhibition that would interfere with compatible pollen reactions<br>$Ca^{2+}$-dependent signaling network[1]<br>DNA fragmentation and morphological changes in mitochondria, Golgi and endoplasmic reticulum[1] |
| **Functional outcome** | Plant rejects pathogen by block in pathogen penetration and proliferation | Plant rejects pollen by block in pollen hydration, tube penetration and growth |

[1]As seen in the poppy system, but not in *Brassica*.
Avr, avirulence; Avr-R, avirulence-resistance; ETI, effector-triggered immunity; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; PTI, PAMP-triggered immunity; RLK, receptor-like kinase; SCR, S cysteine-rich.

Parallels exist where plant SI and plant immunity have similar outcomes, such as the elimination of undesirable cells or organisms. Also, both immunity and at least some SI systems (the crucifer and possibly the poppy systems) use highly variable receptors to recognize highly variable ligands. In addition, SI systems that have bio-destructive activity towards pollen tubes use components that are also used in defense; for example, programmed cell death and other reactions triggered in the incompatible pollen tubes of poppy are also induced during the plant immune response (Jordan *et al.*, 2000; Dangl & Jones, 2001; Geitmann *et al.*, 2004; Thomas & Franklin-Tong, 2004).

Another example relates to the nature of SCR/SP11, the pollen determinant of SI specificity in crucifers. SCR/SP11 is similar in structure, although not in primary sequence, to defensins, the molecules of innate immunity that present a first line of defense to microbial challenge in plants and animals (Mishima *et al.*, 2003; Chookajorn *et al.*, 2004). The similarity between the two classes of molecules suggests an evolutionary link, albeit a distant one, between crucifer SI and innate immunity.

The most notable parallels, however, emerge from comparisons of the self-recognition loci and genes of plant SI with those that control self/nonself recognition in a variety of recognition systems, the vertebrate major histocompatibility complex (MHC) in particular (Janeway & Medzhitov, 2002). In both plants and animals, and from immunity to reproduction, self/nonself discrimination systems have been molded by similar selective pressures for diversification and coevolution of recognition functions, and by a shared requirement to maintain the genetic linkage of coadapted gene complexes (Nasrallah, 2005).

 

**Table 2** Summary of putative associations of S-domain receptor-like kinases (RLKs) with defense mechanisms

| Gene | Accession number | Organism | Association | Reference |
|---|---|---|---|---|
| *ARK1* | gi/18408364 | *Arabidopsis thaliana* | Bacteria and wounding inducible | Pastuglia *et al*. (2002) |
| *ARK3* | gi/30685418 | *Arabidopsis thaliana* | Bacteria and wounding inducible | Pastuglia *et al*. (2002) |
| HAP3–15 | gi/67568666/gb/DR109311.1 | *Nicotiana tabacum* | Putative S-domain receptor-like kinase with protein–protein or protein–carbohydrate interactions | Sanabria & Dubery (2006) |
| *Pi-d2* | | *Oryza sativa* | *R/RLK* gene with extracellular S domain | Chen *et al*. (2006) |
| *RKS1* | gi/4008007/gb/AF084035.1 | *Arabidopsis thaliana* | Salicylic acid inducible | Takahashi *et al*. (1998) |
| *RKS2* | gi/4008009/gb/AF084036.1 | *Arabidopsis thaliana* | Salicylic acid inducible | Takahashi *et al*. (1998) |
| *RLK1* | gi/18424408/NM_125483 | *Arabidopsis thaliana* | Salicylic acid inducible | Walker (1993) |
| *SFR1* | gi/2598268/emb/Y14285.1 | *Brassica oleracea* | Bacteria and salicylic acid inducible | Pastuglia *et al*. (2002) |
| *SFR2* | gi/1783311/emb/X98520.1 | *Brassica oleracea* | Bacteria and salicylic acid inducible | Pastuglia *et al*. (2002) |
| *SI-RLK1* | gi/146739162/EF560751 | *Oryza sativa* | Salt/stress inducible | Unpublished, direct submission to NCBI |
| | At5g60900 | *Arabidopsis thaliana* | S-receptor kinase homolog 2 precursor | T. Nürnberger (unpublished)* |
| | At5g18470 | *Arabidopsis thaliana* | Putative protein S-receptor kinase PK3 precursor | T. Nürnberger (unpublished)* |
| | At1g70530 | *Arabidopsis thaliana* | Putative protein kinase similar to C-terminal region of S-receptor kinase precursor | T. Nürnberger (unpublished)* |

*TAIR accession expression set 100808727. *ARK1*, *Arabidopsis* receptor kinase 1; *ARK3*, *Arabidopsis* receptor kinase 3; *HAP3–15*, *Hind* III arbitrary primer; *Pi-d2*, resistance gene *Pi-d(t)2*, renamed as *Pi-d2*, confers resistance to the *M. grisea* strain ZB15; *RKS1*, receptor-like protein kinase 1; *RKS2*, receptor-like protein kinase 2; *RLK1*, receptor-like protein kinase; *SFR1*, S family receptor 1; *SFR2*, S family receptor 2; *SI-RLK1*, stress-induced receptor-like kinase 1.

In this context, the similarities between plant SI and plant innate immunity have, however, received scant attention. Table 1 summarizes the major similarities and differences between innate immunity and SI in plants with regard to functions, functional outcomes, selective processes, responses, recognition molecules, recognition receptors, and signal transduction and perception.

## RLKs in plant innate immunity and self-incompatibility

The proposed evolutionary relationships among receptor kinase family members arose from an ancient duplication event leading to the divergence of RLK/Pelle from the receptor tyrosine kinase (RTK)/Raf group, which consists of serine/threonine kinases. Thereafter, a more recent gene duplication led to the divergence of RTK from Raf, followed by the divergence of plant and animal lineages, resulting in the ancestral sequences that gave rise to the extant receptors and related kinases (Shiu & Bleecker, 2001). The evolutionary history of plant RLKs indicates that the kinase domains were recruited numerous times by fusion with different extracellular domains to form the subfamilies found in *A. thaliana*. Based on the presence or absence of extracellular domains, members of this gene family are categorized as RLKs or receptor-like cytoplasmic kinases (RLCKs). Subfamilies are assigned based on kinase phylogeny and are grouped according to the domain organization of the majority of members in a given subfamily (comparative summary reported in Shiu & Bleecker, 2001).

It has been suggested that a drastic expansion of the *RLK* gene family occurred in the land plant lineage and that this abundance of plant RLKs represents a plant-specific utilization for extracellular signal sensing. Diverse sequence motifs are present in the extracellular domains of RLKs (Shiu & Bleecker, 2001) and these motifs are potentially responsible for interactions with other proteins, carbohydrates or lipids. The data indicate that RLKs involved in resistance or defense responses may have been duplicated or retained at higher rates in a lineage-specific fashion (Shiu *et al*., 2004). The preferential expansion of defense/resistance-related RLKs could be the consequence of strong selection pressure for recognizing pathogens (Shiu *et al*., 2004). The large family of plant RLK proteins therefore contains distinct protein kinases, each of which might play a unique role in cellular signaling (Walker, 1994; Haffani *et al*., 2004), and probably comprise receptors for further PAMP recognition (Zipfel *et al*., 2006).

An example of independent recruitment of biochemical components for different functions is the LRR motif. LRR domains are found in transmembrane proteins, transmembrane kinases and intracellular R proteins. Collectively, LRRs appear to be involved in a range of processes from development to intercellular communication and disease resistance (Zhang, 1998; Torii, 2004; Chisholm *et al*., 2006). A number of LRR transmembrane and intracellular proteins act as integral components of ligand perception complexes during ETI (Dangl & Jones, 2001). In addition, the LRR motif also plays an important role in PRRs in the evolutionarily older PTI (Nürnberger & Kemmerling, 2006). Although leucine-rich repeat RLKs (LRR-RLKs) (particularly the members of

'clade XII') have been implicated in plant immunity, and the S-domain RLKs (particularly SRK) have been associated with SI, this does not exclude the possibility that other receptor types are involved in either program. The role of LRR-RLKs in defense mechanisms is now recognized (e.g. FLS2, EFR and the endogenous peptide ligand of the AtPEP 1 receptor (PEPR1): Gomez-Gomez & Boller, 2000; Yamaguchi *et al.*, 2006; Zipfel *et al.*, 2006), but intermittent reports on the potential involvement of S-domain RLKs in defense mechanisms (Table 2) have received little attention (Pastuglia *et al.*, 1997, 2002; Bassett *et al.*, 2005).

S-domain *RLK* genes belong to large subfamilies with 40 and 147 members in *A. thaliana* and rice, respectively (Morillo & Tax, 2006). *RLK* genes containing the S domain are regarded as unique intermediates between RLKs mediating developmental and resistance functions (Shiu & Bleecker, 2001; Shiu *et al.*, 2004). Except in the case of the SRK involved in *Brassica* SI, very little is known about their functions. It has been suggested that the development of the SRK-mediated SI response is an evolutionarily relatively recent event in the Brassicaceae and may have occurred through the recruitment of pre-existing genes that performed other related functions (Shiu & Bleecker, 2001). The widespread occurrence of S-domain RLKs in *A. thaliana* and other plants, expressed in nonreproductive tissues, and in species that do not exhibit SI (Pastuglia *et al.*, 2002; Bassett *et al.*, 2005), would also argue for additional functions for S-domain RLKs. This leads to the questions of whether the conserved S domain has a function other than in SI and whether the SI-linked SRKs have an earlier S-domain ancestor, linked to defense.

S-domain RLKs, like other RLKs, contain all the elements required for PAMP perception and signal transduction: the proteins are single-pass transmembrane serine/threonine kinases displayed on the plasma membrane. The extracellular domains of S-domain RLKs include a mannose-binding agglutinin/B-lectin domain, a cysteine-rich S domain and an epidermal growth factor (EGF)-like or plasminogen/apple/nematode (PAN) motif (Tordai *et al.*, 1999; Shiu & Bleecker, 2001). The PAN motif represents a conserved module, also found in the ectodomain of several animal receptors, that functions in protein–protein interactions, similarly to LRR domains, but also in protein–carbohydrate (e.g. mannose binding) interactions (Tordai *et al.*, 1999). The region containing the predicted PAN motif plays a primary role in dimerization of the receptor, and the S-domain motif plays a secondary role. The extracellular domain of SRK showed a preference, mediated by a small, highly variable region within the PAN motif, for homodimers over heterodimers with the products of other SRK alleles. Thus, the polymorphic extracellular domain of SRK is not only responsible for *S* haplotype-specific binding of the SCR/SP11 ligand (Kemp & Doughty, 2007), but also appears to play a role in the allele-specific homodimerization of SRK (Naithani *et al.*, 2007).

Another feature possibly exhibited by S-domain RLKs (Giranton *et al.*, 2000), and LRR-RLKs involved in PAMP perception, is the involvement of a coreceptor that may modulate specificity (Chinchilla *et al.*, 2007; Heese *et al.*, 2007; see legend to Fig. 1b). As discussed in the section 'Surveillance of "self": self-incompatibility', oligomeric complexes of receptors with bound ligands lead to transphosphorylation of the receptor and triggering of a signaling cascade (Figs 1, 2). Signal transduction downstream of RLKs involves proteins that interact with and are phosphorylated by the cytoplasmic domain of the RLK. In the case of the *Brassica* SI system, the plant-specific signaling protein armadillo-repeat-containing 1 (ARC1) binds to SRK (Gu *et al.*, 1998; Stone *et al.*, 1999). The ARC1 protein belongs to the U-box family of E3 ligases, and, interestingly, other related members have been implicated in plant cell death and defence responses in *A. thaliana*, tobacco (*Nicotiana tabacum*) and rice (Zeng *et al.*, 2004; Gonzalez-Lamothe *et al.*, 2006; Yang *et al.*, 2006). A reduced expression of *ARC1* led to a partial breakdown of SI, providing evidence that ARC1 acts immediately downstream of SRK as a positive modulator of SI (Stone *et al.*, 1999). A U-box in ARC1 suggests a role for ubiquitination in the SI response (Newbigin & Vierstra, 2003). Ubiquitin is well known in defense mechanisms as the tag that directs targeted proteins to the machinery that eliminates them (Devoto *et al.*, 2003). Phosphorylated ARC1 relocates from the cytosol to the proteasome present on the cytosolic face of endoplasmic reticulum membranes and promotes the ubiquitination and proteasomal degradation of unknown substrate proteins, thereby leading to pollen rejection (Stone *et al.*, 2003).

SRK may also interact with other signal transduction-associated molecules such as calmodulin and the kinase-associated protein phosphatase (KAPP; Vanoosthyse *et al.*, 2003). It should be noted that FLS2 also interacts with KAPP (Gómez-Gómez *et al.*, 2001; Ding *et al.*, 2007). In addition, SRK interacts with the M-locus protein kinase, a member of the RLCK family, that is required downstream of SRK to promote the SI response and is efficiently phosphorylated by SRK *in vitro* (Murase *et al.*, 2004; Kakita *et al.*, 2007).

A number of S-domain *RLK* genes have been found to have up-regulated expression in response to pathogen infections, wounding, or treatment with defence-related compounds such as salicylic acid (a metabolite that plays an important role in potentiating local and systemic induced resistance) and LPS (Table 2). Interestingly, the first report of salicylic acid-induced expression of *RLK* genes was found in a member of the *Brassica* S-domain RLK family, S family receptor 2 (*SFR2*). *SFR2* expression was found to be induced by bacterial pathogens, wounding, and treatment with salicylic acid. The transient induction of the *SFR2* gene exhibited a kinetic and induction pattern typical of defense genes (Pastuglia *et al.*, 1997). A second closely related S-domain *RLK* gene, *SFR1*, was also found to be induced by bacterial infection and salicylic acid treatment (but not wounding) in *Brassica oleracea* (Pastuglia *et al.*, 2002).

 

In addition, two closely related *A. thaliana* S-domain *RLK* genes, *Arabidopsis* receptor kinase (*ARK*)*1* and *ARK3*, were found to have increased mRNA accumulation following bacterial infections and wounding (Pastuglia *et al.*, 2002). Two other *A. thaliana* S-domain *RLK* genes, receptor-like protein kinase (*RKS*)*1* and *RKS2*, are also induced by salicylic acid treatment (Ohtake *et al.*, 2000). Finally, it has been reported that LPS elicitation of *N. tabacum* cell suspensions resulted in the differential expression of a putative S-domain *RLK* (Sanabria & Dubery, 2006). In addition, three S-domain *RLK* genes (At5g60900, At5g18470 and At1g70530) were found to be up-regulated in a transcriptional microarray analysis of genes expressed in *A. thaliana* in response to elicitation by LPS (T. Nürnberger, unpublished: TAIR accession expression set 100808727), suggesting a putative function in LPS perception.

What is probably the first direct genetic evidence for the role of S-domain receptor kinases in plant disease resistance comes from Chen *et al.* (2006), who characterized an *R* gene (Pi-d2) that confers resistance to blast disease in rice. The gene encodes a bulp-type mannose-specific binding (B)-lectin receptor kinase and belongs to the S-domain-2b RLK subfamily of lectin receptor kinases (LecRKs). Although several *R* genes that encode RLKs have been cloned and characterized, this is the first reported to have an extracellular lectin domain and to belong to the S-domain RLKs. Despite the presence of this domain, an indirect role for Pi-d2 in pathogen recognition was proposed without considering the variable features of the extracellular domain of S-domain RLKs that allow them to multitask in developmental and defense responses.

A summary of S-domain RLKs and their association with defense mechanisms is given in Table 2.

## Conclusions

In addition to PAMP perception as a 'nonself' surveillance mechanism in innate immunity, the evolutionary solution in plants to the problems of perceiving and responding to pathogens involves 'self' surveillance, which is conceptually similar to SI. The two separate mechanisms of innate immunity and SI are remarkably similar, leading to speculation about a common ancestor for genes involved in SI and defense and the hypothesis that SI evolved from a defense pathway.

Similar to the animal innate immune system (Medzhitov & Janeway, 2002), different and possibly overlapping receptor types may be implicated in different physiological programs in plants, such as immunity and SI. There is currently no conclusive evidence for evolutionary conservation of an ancient PAMP detection system (Dangl & Jones, 2001) and independent recruitment of components during evolution is equally plausible (Ingle *et al.*, 2006). Moreover, there are also various examples where a specific type of biochemical module or protein appears to be used to fulfil a requirement in more than one process, that is, to show dual functioning, and the re-use of highly evolved processes for diverse functions was recently pointed out by Ausubel (2005) in his perspective on immune signaling pathways. There are thus some indications that certain defense genes are structurally related to the S-domain *RLK* genes which can be regarded as intermediates between *RLK* genes mediating developmental and resistance functions. Specific domains in RLK proteins can be utilized to fulfil a number of biochemical functions and receptor modules are not necessarily reserved for one physiological purpose only. Although evolution may have driven expansion of particular RLK families (LRR-RLKs and S-domain RLKs) to serve roles in particular physiological processes (defense/development and SI, respectively), this may not exclude these receptor types from functioning in different programs, lending support to the hypothesis that subsets of molecules involved in innate immunity were co-opted to perform 'self' recognition functions in reproduction. It is thus plausible that S-domain *RLK* genes could be utilized to function as *R* genes or as PRRs in perception of PAMPs of a nonprotein nature.

Unfortunately, the role of S-domain RLKs in defense mechanisms has previously not been widely recognized, or thoroughly explored. Further research is therefore warranted in order to broaden our understanding of the involvement or dual-functioning of S-domain RLKs in PAMP surveillance and perception. This includes aspects of receptor function and ligand–receptor interaction, the sharing of receptors between ligands with common molecular signatures and the modulating role of potential co-receptors in interaction, specificity and priming.

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