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Plant immunity: thinking outside and inside the box

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Keywords

Plant immunity, extracellular immunogenic pattern, intracellular immunogenic pattern, danger signal, pattern-triggered immunity, effector-triggered immunity, spatial immunity model

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Abstract

Extensive models describe the co-evolution between plants and microbial attackers. Such models distinguish between different classes of plant immune responses, based on the type of danger signal that is recognized or on the strength of the defence response that the danger signal provokes. However, recent molecular and biochemical advances have shown that these dichotomies are blurry.

With molecular proof in hand, we here propose to abandon the current classification of plant immune responses, and to define the different forms of plant immunity solely based on the site of microbe recognition; either extracellular or intracellular. Using this spatial partition, our 'spatial immunity model' facilitates a broadly including, but clearly distinguishing, nomenclature to describe immune signalling in plant-microbe interactions.

Models to describe plant-microbe interactions

Plants are able to sense attacking micro-organisms using a broad repertoire of receptors present at the cell surface, as well as inside the cell. At the plasma membrane (PM), cell surface receptors that are either receptor-like kinases (RLKs) or receptor-like proteins (RLPs), sense extracellular danger signals [1], to activate defence responses [2]. Extracellular danger signals comprise pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) [3], microbial effectors, and patterns originating from the host, namely damage-associated molecular patterns (DAMPs) and phytocytokines [4]. Recognition of intracellular danger signals, which can be of a similar nature as the extracellular danger signals described above, and subsequent defence activation, are facilitated by cytoplasmic receptors, mostly nucleotide-binding leucine-rich repeat receptors (NLRs) [5, 6] .

In the past 15 years, several models have been introduced to provide a conceptual framework describing plant-microbe interactions. Here we discuss some of the models that have been proposed to explain the molecular background of the constant evolutionary battle that is taking place between plants and pathogens. Many reports describe the outcome of plant-microbe interactions as a result of the recognition of two types of danger signals that become exposed during pathogen attack, namely structural patterns and effectors, by pattern recognition receptors (PRRs) and resistance (R) proteins, respectively. With new knowledge currently arising, it appears

that this dichotomy of danger signals is in fact blurry [7], as well as the nature of their receptors, and therefore these criteria cannot form a basis for a distinction between different signalling pathways leading to different types of plant immunity. As recognition of danger signals takes place either extracellularly via cell surface receptors present at the PM, or intracellularly via cytoplasmic receptors, we argue that if one aims to classify the immune responses triggered in plants, this should be based on the location of recognition.

The spatial partition: extracellular and intracellular immunogenic patterns (ExIPs and InIPs)

In the history of plant breeding and pathology, scientists have used various ways of describing resistance in plant-microbe interactions (Box 1). With novel molecular and biochemical tools becoming available, the processes determining host susceptibility and resistance in plant-microbe interactions have been more and more unravelled. Based on this knowledge, several models have been built to aid in describing the events driving the outcome of such interactions (Box 2). Among these models, the 'zigzag model' is still most commonly used, and it is continuously being refined [3].

Based on increased molecular insight, the distinction made in the zigzag model between patterns (PAMPs, MAMPs, or DAMPs) and effectors, has become blurry [7]. A few years after the introduction of the zigzag model, the term 'danger signal' was introduced to provide a broad term to describe exogenous immunogenic patterns derived from 'non-self' and endogenous ones originating from the host 'self', with the aim to link the fields of plant and mammalian immune signalling [1, 4]. Later, to accommodate all possible patterns and effectors, the broadly including term 'invasion pattern (IP)' was proposed for these host-recognised compounds in the so-called 'invasion model' [8]. This very general model states that recognition of IPs by IP receptors (IPRs) leads to IP-triggered responses (IPTRs). The broad term IP even includes manipulated plant virulence targets (VTs), double-stranded (ds)RNA from viruses, and molecular signals from arbuscular mycorrhizal fungi (myc-factors) and nitrogen-fixing rhizobia (Nod-factors) that initiate symbiosis [8, 9]. IPTR may eventually lead to successful defence (the end of symbiosis) or to a continued symbiosis with the

invading microbe, which can be either beneficial for both plant and microbe or only for the microbe (disease). Therefore, this model includes both beneficial and pathogenic plant-microbe interactions. Successful suppression of IPTR by IPs that function as effectors, allows continued symbiosis for biotrophic pathogens, and may cause additional IPs to be recognised by newly evolved IPRs. By contrast, necrotrophic pathogens exploit IPTRs, especially when host cell death is involved, and thereby are able to continue their symbiosis with the plant [8]. Although we support the broad concept of this model, invasion is not strictly necessary for recognition by the host, as mechanical wounding for instance can already lead to production and recognition of DAMPs. Therefore, we propose to move away from the invasion model and base ourselves on the danger model, which is widely accepted amongst biologists studying immunity in plans an metazoans.

The commonly used zigzag model (Box 2) provides an appropriate conceptual framework to describe the molecular arms-race between plants and pathogens, but distinctions made are too narrow. By contrast, the term danger signal is very broad, as any molecule that can potentially be recognized by the plant qualifies as such, and therefore this term does not allow to make any distinction between different types of plant immune responses.

To address both above shortcomings, we propose as a simple addition to the danger model, to include the location where the danger signal is recognized. This can be either extracellularly, therefore introducing the term extracellular immunogenic pattern (ExIP), or intracellularly, and therefore introducing the term intracellular immunogenic pattern (InIP) (Figure 1). Introducing this spatial bipartition, allows to facilitate a better differentiation of the immune signalling events taking place in plants, based on the location of immunogenic pattern recognition. In this ‘spatial immunity model’, recognition of ExIPs by cell surface receptors leads to extracellularly-triggered responses (ExTRs), which can result in extended symbiosis with the invading microbe or successful plant defence (the end of symbiosis), leading to extracellularly-triggered immunity (ExTI). Recognition of InIPs by cytoplasmic receptors, mainly NLRs, leads to intracellularly-triggered responses (InTR) and subsequent intracellularly-triggered immunity (InTI).

Extracellularly-triggered immunity (ExTI) provoked by various ExIPs depends on common mechanisms

Recognition of InIPs involves cytoplasmic receptors, which are mainly NLRs [10], whereas recognition of ExIPs involves cell surface receptors [2, 11, 12]. The ectodomain of cell surface receptors can carry different motifs, which determine the recognition specificity of the receptor. Different ectodomains facilitate the recognition of various types of ExIPs [2, 11, 12]. Cell surface receptors with an LRR-based ectodomain mediate the recognition of various extracellular hormones, proteins, and peptides [2, 11, 13, 14], and can be divided into receptors with and without an intrinsic kinase domain, referred to as RLKs and RLPs, respectively. In the following section, we mainly focus on LRR-type RLKs and RLPs, further referred to as RLKs and RLPs. Being devoid of a kinase domain, RLPs constitutively interact with the RLK SUPPRESSOR OF BIR1-1/EVERSHED (SOBIR1/EVR, further referred to as SOBIR1), to form bimolecular RLKs [15, 16]. Interestingly, the identification of several common downstream defence signalling components and mechanisms, i.e. RLKs from the SERK family and RLCKs playing a role downstream of cell surface receptors (see glossary), indicates that the signalling output of all cell surface receptors upon ExIP recognition can be classified into one category, namely ExTI.

Co-receptor recruitment by cell surface receptors is a common theme in extracellularly-triggered immunity (ExTI)

A common step after ExIP recognition by cell surface receptors is the formation of higher order complexes via recruitment of co-receptors. RLKs recruit the co-receptor BAK1, or other members of the SERK family, upon ExIP recognition [12, 17-20]. For example, for the well-studied RLK FLAGELLIN-SENSING 2 (FLS2), BAK1 recruitment was shown upon treatment with the bacterial flagellin-derived immunogenic peptide flg22 [17, 18]. Overall, BAK1 recruitment leads to transphosphorylation between the kinase domains of the ExIP-activated RLK and BAK1 that have now formed a stable complex with their two cytoplasmic kinase domains in close vicinity, and subsequent initiation of downstream signalling [21-30]. Interestingly, dependency on BAK1 has been shown for a plethora of RLKs in several plant species (Table 1).

Strikingly, also RLP/SOBIR1 bimolecular receptor complexes have recently been shown to recruit BAK1 and other SERK family members upon ExIP perception by the RLP that is associated with SOBIR1, suggesting that RLP/SOBIR1 complexes function as true bimolecular RLKs [31-34]. Additionally, many other RLPs have been described to depend on BAK1 for their function, although actual BAK1 recruitment has not yet been demonstrated for all of them (Table 1).

Likewise, cell surface receptors with ectodomains other than LRRs, also form higher order complexes as a result of co-receptor recruitment, suggesting a common mechanism of defence activation [2, 11]. For instance, the lysin motif (LysM)-containing CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) functions as a co-receptor for several cell surface receptors with LysM ectodomains upon perception of ExIPs like fungal chitin and bacterial peptidoglycan (PGN) [2]. However, in this review we restrict our focus to LRR-type cell surface receptors.

Remarkably, some RLPs historically classify as typical PRRs (triggering PTI), whereas others are referred to as resistance (R) proteins (triggering ETI) (Table 1). For example Cf-4, which is the tomato (*Solanum lycopersicum*) RLP that recognizes the apoplastic effector Avr4 of the extracellular fungal pathogen *Cladosporium fulvum*, triggers a strong defence response upon Avr4 recognition, including a hypersensitive response (HR) (Figure 2, right panel) [35, 36]. The *R* gene *Cf-4* and its matching *C. fulvum* effector gene *Avr4* form a classic example of a gene-for-gene couple [37]. By contrast, RLP23 from arabidopsis (*Arabidopsis thaliana*) triggers only a moderate defence response, including callose deposition and a swift burst of reactive oxygen species (ROS), but no HR (Figure 2, middle panel) [3, 31, 38]. RLP23 recognizes an epitope of NECROSIS and ETHYLENE-INDUCING PROTEIN 1 (NEP1)-LIKE PROTEINS (NLPs) [31]. NLPs are wide-spread, occurring in bacteria, fungi, and oomycetes, which classifies them as typical conserved microbial molecular patterns i.e. MAMPs [38]. Yet, both the activation of the Cf-4/SOBIR1 complex and the RLP23/SOBIR1 complex upon ExIP perception requires the recruitment of BAK1 [31, 32]. Consequently it can be assumed that, upon ExIP recognition and subsequent BAK1 recruitment, transphosphorylation events between the kinase domains of SOBIR1 and BAK1 occur in both complexes to activate downstream cytoplasmic signalling [39]. Such an event is reminiscent of the transphosphorylation events that take place between the kinase domains of FLS2 and BAK1 upon flg22 recognition [22,

27]. So, BAK1 recruitment is probably a general activation step for all RLK- and RLP/SOBIR1-containing complexes, regardless of the type of ExIP that is recognised and whether either strong or moderate ExTI is the outcome (Table 1) [16, 33, 34, 40].

Interestingly, while ExIP-recognizing RLKs provide their own kinase domain to facilitate transphosphorylation with BAK1, ExIP-recognizing RLPs in all cases provide the same SOBIR1 kinase domain. This suggests that the cytoplasmic signalling events that are induced by different RLP/SOBIR1 complexes in principle are identical, as in all cases the same SOBIR1/BAK1 kinase domain combination will transphosphorylate and initiate downstream signalling. The observed differences in overall immune signalling output, ranging from a strong defence response associated with an HR, versus a moderate response, might therefore be a consequence of several other variables, instead of fundamental differences between defence signalling pathways, as will be discussed below.

The striking finding that BAK1 recruitment is required for defence signalling by all LRR-type cell surface receptors tested thus far, prompts us to argue that the recognition of any ExIP by any cell surface receptor leads to the activation of similar immune signalling events. This is in contrast with the previous classification of these receptors into PRRs and R proteins, and their output according to the zigzag model into PTI and ETI, respectively.

Other common themes in extracellularly-triggered immunity

When we look further, recruitment of co-receptors like BAK1 is not the only common phenomenon occurring downstream of activated cell surface receptors. In this respect, receptor-like cytoplasmic kinases (RLCKs) represent an immediate downstream signalling component of the cell surface receptors reaching into the cytoplasm [41-43]. RLCKs have been shown to be involved in several signalling pathways downstream of RLKs, including links to ROS production, to the mitogen-activated protein kinase (MAPK) cascade [44, 45], and even to transcriptional reprogramming in the nucleus [46-49]. Recent findings also show roles of RLCKs downstream of RLP/SOBIR1 complexes [50]. Unexpectedly, BIK1, an RLCK from arabidopsis that plays a positive regulatory role in the defence response initiated by FLS2 and several other RLKs [28, 51], seems to negatively regulate RLP23-mediated responses as the ROS burst

triggered by the nlp20 epitope of NLPs is enhanced in a *bik1* knock-out of arabidopsis [50]. Further research is necessary to clarify whether BIK1 is a true negative regulator of defence responses initiated by RLPs in general, or that BIK1 plays varying roles downstream of different RLPs.

The production of ROS is a swift general output of activated cell surface receptors [46, 47, 52, 53], as are the typical Ca^{2+} spiking, the activation of MAPK cascades, and the activation of Ca^{2+}-dependent protein kinases (CDPKs) [50, 54-58]. These are all output responses that are common to cell surface receptors when activated, independent of the types of ectodomains that they contain, the co-receptors that they recruit, and the defence signalling ultimately leading to an HR or not [54-57, 59, 60]. Furthermore, activation of cell surface receptors in all cases leads to a substantial, overlapping, transcriptional reprogramming with the aim to support a solid defence response, including the production of phytohormones and defence-related proteins, for example through the activation of WRKY transcription factors [61-63].

All these commonalities support our proposed spatial immunity model, which differentiates plant immune responses based on the location where recognition of the immunogenic pattern, and thereby the attacking microbe, takes place (Figure 1).

ExTI and InTI are widely applicable terms

By introducing the spatial immunity model, we propose to move away from using the zigzag model to differentiate between PTI and ETI that is provoked by extracellular patterns and effectors, respectively [3]. The spatial partition will provide the framework to clearly describe recognition events in plant-microbe interactions. The distinction between extracellular and intracellular immunogenic patterns will remain true, as it is a division based on the biology of the interaction and not on an interpretation by scientists.

Although not discussed in detail here, cell surface receptors that do not depend on BAK1 recruitment for their functionality obviously also fit the spatial immunity model (Figure 1). Likewise, the different versions of recognition through guards and decoys are also not elaborated on here [64]. However, these different mechanisms of recognition are also included in the model, as they can all be regarded as different ways to recognize either ExIPs or InIPs (Figure 1). As part of the evolutionary arms-

race between plants and pathogens, the suppression of immune responses by effectors [65] is integrated into the spatial immunity model (Figure 1). ExIPs can suppress ExTR by defensive and offensive mechanisms in the apoplast [66], and the same holds for InIPs that exert their virulence function in the cytoplasm to suppress ExTR or InTR [67].

Possible causes of the existence of moderate and strong ExTI

Although all ExTI-related signalling is initiated by cell surface receptors and includes the recruitment of co-receptors like BAK1, the defence responses that are triggered have different intensities and characteristics (Figure 2). There are strong responses that follow the classic ETI principle, like Cf-4-triggered HR, in contrast to moderate responses that follow the PTI principle, like FLS2- and RLP23-induced defence responses [3, 36, 38, 68]. However, not only between cell surface receptors classically referred to as PRRs and R proteins there are differences concerning their output, but also among PRRs themselves there are significant variations in intensity and timing of the generated defence outputs [50]. A recent comparative study for example showed that the ROS burst that is triggered upon treatment with similar amounts of flg22, nlp20, or chitin differs in magnitude and timing [50]. These differences in intensities of the immune response might be explained by subtle differences that occur at one or more levels of the defence pathway employed by ExTI.

The effect of stability and affinity on the strength of the defence responses

The chemical nature of different immunogenic patterns is likely to influence the rate of diffusion into plant tissues and across the cell wall. For example, ExIPs present in the apoplast will not all be equally stable. Differences in the speed of ExIP diffusion and their stability will at least partially determine how many molecules of the compound are eventually being perceived by cell surface receptors, and thereby how fast and strong ExTI will be triggered. For instance, instable variants of Avr4, in most cases lacking one di-sulphide bond, have been shown to allow *C. fulvum* to evade Cf-4-mediated recognition and resistance, whereas these natural mutants retained their virulence function on tomato [69, 70]. Additionally, differences in the direct affinity of specific cell

surface receptors for particular ExIPs will be a factor also determining the differences in the intensity of signalling output [12, 71]. Furthermore, BAK1 has been described to specifically recognise the part of the flg22 peptide that is bound to the LRRs of FLS2 [72, 73]. This indicates that for different ligands, either directly or indirectly bound to their matching receptors, the probably varying affinity of BAK1 for these receptor-bound ExIPs contributes to the differences in signalling output.

Not only the stability of the ExIP, but also the stability and availability of the matching cell surface receptor will influence the intensity of ExTI. Cell surface receptor synthesis, recycling, and degradation have been shown to play an important role in regulating defence signalling [74-78]. Also the pace by which these processes take place will differ from one receptor to another [32, 79-82].

Do the short cytoplasmic tails of RLPs affect the intensity of ExTI?

As mentioned earlier, all RLPs that have been experimentally tested so far constitutively interact with SOBIR1, and BAK1 recruitment seems to be a common mechanism to initiate ExTI-related signalling (Table 1). Interestingly, for different primary ExIP-recognizing RLKs, BAK1 recruitment in each case provides a kinase domain forming a different couple of cytoplasmic kinases to trigger ExTI. By contrast, in the case of cell surface complexes consisting of RLPs interacting with SOBIR1, recruitment of BAK1 to the complex upon danger signal recognition by the RLP involved, in all cases leads to the formation of the same couple of cytoplasmic kinase domains; the one of SOBIR1 and the one of BAK1 (or another SERK member). So, in addition to the factors mentioned above, what could cause the observed differences in intensity of ExTI triggered by different RLPs?

One obvious difference between activated RLP/SOBIR1/BAK1-containing immune complexes is the short cytoplasmic tail of the particular RLP that is involved in the complex. These tails usually cover less than 30 amino acids, and apart from the presence of a conserved Trp and Phe residue, these tails do not seem to have an obvious common motif [16]. An ER-retention signal, consisting of the dilysine motif KKRY, is present at the cytoplasmic C-terminal end of both the Cf-4 and the Cf-9 protein [83]. However, this KKRY motif proved not to be essential for Cf-9 function, and it was suggested that this motif might be masked by Cf-9-interacting proteins, thereby

allowing the Cf protein to reach the cell surface [83]. Interestingly, one of these interactors could be SOBIR1, and in this way, only Cf-4/Cf-9 proteins constitutively interacting with SOBIR1, and thereby being functional, will not be retained in the ER and will properly localize at the PM [83]. Swapping of the cytoplasmic tails of RLPs either signalling for moderate ExTI or strong HR-associated ExTI, so for example between RLP23 and Cf-4, might provide results pointing to a role of these short cytoplasmic tails in determining the strength of the defence signalling output.

The influence of different SOBIR1 and BAK1/SERK proteins present in cell surface receptor complexes on ExTI intensity

SOBIR1 is only present as a single copy gene in arabidopsis. However, in Solanaceous plants, there is an additional homologue present, referred to as *SOBIR1-like*, which seems to have a redundant function next to *SOBIR1* itself [15]. *BAK1* on the other hand, which is also referred to as *SERK3*, is a member of the *SERK* family consisting of five homologues in arabidopsis. Also in Solanaceous plants and in for example rice, several *SERK* homologues have been annotated [40]. Possibly, a differential preference of various cell surface receptors for (combinations of) certain SERK proteins is a denominator to signal for either moderate or strong ExTI [84, 85]. In Cf-4-mediated signalling for example, BAK1/SERK3, as well as SERK1, have been shown to be involved in the activated complex [32, 86]. In RLP23-triggered signalling even four SERKs, namely SERK1, SERK2, BAK1/SERK3, and BKK1/SERK4, have been shown to play a role [31]. Likewise, the RLK ELONGATION FACTOR-TU RECEPTOR (EFR) functions together with BAK1 and other SERKs as co-receptors, while FLS2 makes preferential use of BAK1 [87]. However, the precise roles and preferences for the different SERKs of various RLK- and RLP-containing complexes, and their possible effect on the strength of the signalling output, needs to be further elucidated. This is challenging, as their redundancy makes it difficult to study the individual functions of the SERK family members in the activated cell surface complexes.

Additionally, not only the presence of different homologues of the SERKs, but also the presence of different amounts of SOBIR1 proteins, in addition to the various SERKs in the activated complexes might play a role. For instance, on western blots the co-immunopurifying band of SOBIR1 upon immunoprecipitation of Cf-4 is much

more intense than the band of SOBIR1 co-purifying with the RLP Ve1, providing resistance to *Verticillium dahliae* [15]. Furthermore, SOBIR1 has been shown to form homodimers [39]. These results lead to argue that multiple SOBIR1 (and/or SOBIR1-like) proteins might form a complex with a single Cf-4 protein in tomato. Possibly, different amounts of SOBIR1(/SOBIR1-like) and SERKs associating with an RLP direct the intensity of the defence responses that are triggered.

Regulating the activity of cell surface receptors

Several mechanisms have been shown to regulate the availability and activity of cell surface receptors. For instance pseudo-kinases, like BAK1-INTERACTING RLK 2 (BIR2), have been shown to negatively regulate the availability of BAK1 for its recruitment to activated cell surface receptors [88]. Different homologues of the BIR family, which contains four members in arabidopsis, might differentially regulate the availability of different pools of BAK1 and additional SERKs, which are present in various nanodomains [89, 90]. This highly complex regulation, taking place at multiple levels, could in its turn also contribute to the variety in the intensities of ExTI mediated by different cell surface receptors.

Differential phosphorylation of the kinase domains of cell surface receptors and their co-receptors is yet another mechanism to accomplish differential ExTI. For example, recently BAK1 was found to be differentially phosphorylated upon signalling for either immunity or development [91]. Differential phosphorylation of the cytoplasmic kinase domains of cell surface complexes upon recognition of various ExIPs possibly affects ExTI intensity. Although, in contrast to ExTI triggered by RLKs, RLP-triggered responses are always mediated by the kinase domains of SOBIR1 and BAK1, possibly minor differences in the overall structure of the activated complex, caused by small structural variations among the RLPs that are involved, might cause differences in the transphosphorylation events that take place.

One step further downstream, cytoplasmic RLCKs form a signalling hub that converges signals from cell surface receptors to signalling partners further downstream [20, 42, 46, 47, 92]. Possible differential phosphorylation of the same RLCK playing a role downstream of various cell surface receptors, adds to explaining the varying levels of ExTI that are generated. The RLCK family is extremely large, highly diverse, and

redundant, and plays very diverse roles in defence as well as in development [42, 92]. Therefore, downstream of different cell surface receptors, different RLCKs, their differential phosphorylation, and their intricate homeostasis, might contribute to further differentiation in the actual shape of the immune responses that are triggered [93].

Not only the amounts of the available cell surface receptors to be activated are regulated, but also the activity of these receptors themselves is strictly controlled [20]. For example, the phosphorylation status of the kinase domain of cell surface receptors, their co-receptors, and downstream components, like RLCKs, CDPKs, and MAPKs, is kept in check by various phosphatases [20]. For example, BAK1 and BIK1 are kept inactive in the resting state by PROTEIN PHOSPHATASE 2A (PP2A) and PP2C38, respectively [94, 95]. Also for the rice RLK Xa21, which confers resistance to the bacterial pathogen *Xanthomonas oryzae* pv *oryzae* secreting the matching effector Ax21, a control mechanism consisting of de-phosphorylation by a PP2C member has been shown [96]. The affinity of different phosphatases for distinct immuno-complexes, in combination with their efficiency to de-phosphorylate the various associated signalling partners, might also play a role in regulating the intensity of ExTI mediated by different cell surface receptors. If not kept in check, this could even lead to constitutive immunity, as was proposed for the HR observed when arabidopsis SOBIR1 is transferred to tobacco or *N. benthamiana* [97, 98].

Concluding remarks

The publication of the zigzag model in 2006 was a revolution in the field of molecular phytopathology, merging the field of responses triggered by ‘general elicitors’ and host resistance that is evoked upon recognition of avirulence proteins (effectors) [3]. Recent advances in molecular research on plant-microbe interactions have challenged the zigzag model. Therefore the danger model and invasion model were proposed, of which the danger model better covers the holistic concept of host immunity [4, 8]. With the current knowledge, we here propose a refinement of the danger model, which differentiates between extracellularly- and intracellularly-triggered immunity (ExTI versus InTI), both leading to resistance of plants to pathogens. This spatial immunity model will allow scientists, working in the field of molecular phytopathology, categorizing their findings concerning resistance and susceptibility in a clear way. Still,

411 future research is essential to explore the cause(s) of differences in the strength of the
412 immune responses triggered by ExIPs when activating their matching cell surface
413 receptor (see outstanding questions).

Highlights Box

Any distinction between the types of immune responses triggered in plants should be solely based on the location where the immunogenic pattern is perceived.

The dichotomy between patterns and effectors is blurry, which renders a classification of plant defence responses based on this dichotomy inappropriate.

All LRR-type cell surface receptors (both RLPs and RLKs) recruit the regulatory LRR-RLK BAK1 upon their activation by extracellular immunogenic patterns (ExIPs).

All LRR-RLPs studied appear to constitutively interact with SOBIR1 and to recruit BAK1 upon ExIP perception, thereby all providing a set of identical cytoplasmic kinase domains for downstream signalling.

LRR-RLPs trigger a plethora of defence responses, with intensities ranging from moderate immunity to a strong HR.

Outstanding questions Box

What causes RLP/SOBIR1/BAK1 complexes, harbouring different RLPs but employing identical SOBIR1 and BAK1 kinase domains for cytoplasmic signalling, to initiate ExTI with different strengths upon activation by their matching ExIPs?

Are the cytoplasmic kinase domains of SOBIR1 and BAK1, associated with different RLPs, differentially phosphorylated upon signalling for ExTI triggered by various ExIPs?

Which RLCKs are involved in positively and negatively regulating ExTI that is activated upon ExIP recognition by different cell surface receptor complexes?

Are ExTR and InTR linked, and if so, where do the responses that are triggered converge?

Box 1. The plant breeding point-of-view on plant-pathogen interactions

Plant breeding has been a human practice for thousands of years [99, 100]. Breeding focusses on crop qualities like higher yield, better tasting fruits, and increased drought and disease resistance. Throughout this history, several terms have been coined and models developed describing plant susceptibility and resistance. Recent insights in the molecular background of plant-pathogen interactions have rendered some of the established breeding terminology confusing or even obsolete.

Most plants are resistant to most pathogens. If all members of a plant species are resistant to all variants of a given pathogen, this type of resistance is referred to as 'non-host resistance' (NHR) [101]. Several molecular mechanisms have been shown to underlie NHR [102, 103], therefore this umbrella-term should be used as a general phenomenon, rather than to explain one particular mechanism.

The gene-for-gene model describes the evolutionary battle between plants and pathogens from a plant breeding point-of-view [104, 105]. In this molecular battle, during a continuous co-evolution between initially susceptible plants and virulent pathogens, the plant starts to recognise compounds from the pathogen, leading to host resistance and pathogen avirulence. Therefore, the gene of the pathogen that codes for the recognized compound is referred to as an *Avirulence* (*Avr*) gene. Recognition of a secreted Avr protein by the plant is based on the presence of a *resistance* (*R*) gene, and for each functional *R* gene present in the plant, there is a matching *Avr* gene in the pathogen. Loss, or mutation of the Avr protein by the pathogen, again results in host susceptibility and pathogen virulence. A plant-pathogen interaction in which matching *R* and *Avr* genes are present is referred to as 'incompatible'. When either a particular strain of the pathogen does not carry the matching *Avr* gene, and/or a certain plant genotype does not carry the matching *R* gene, the pathogen can infect the plant. This situation is called a 'compatible' interaction [37]. Obviously, for the pathogen it has no benefit to be recognized by the host plant, so the intrinsic function of Avr proteins for pathogens cannot be the triggering of their recognition by plants. Indeed, many Avr proteins have a function in pathogen virulence, and they promote colonisation of susceptible plants, thereby benefiting the pathogen [67, 106]. For this reason, Avr proteins are also referred to as virulence (Vir) proteins. At the time the gene-for-gene model was introduced, this nomenclature was a logical part of the model. However, current advances in the research on plant-pathogen interactions

481 have shed light on the intrinsic virulence function of the various proteins secreted by
482 pathogens during host colonisation. As a consequence, the term ‘Avr’ has become
483 very confusing and the term ‘effector’ has been introduced [107] (see Box 2).
484

Box 2. A brief history of the mechanistic point-of-view on plant-pathogen interactions

In recent years, the molecular mechanisms that underlie pathogen recognition and disease resistance in plants have started to be unravelled. This has led to the development of the 'zigzag model', which describes the evolutionary battle between plants and pathogens from a molecular point-of-view, and proposes the presence of two layers in the plant's immune system [3].

The first layer of plant defence, according to the zigzag model, involves the recognition of conserved structural molecular patterns of the pathogen (PAMPs or MAMPs)[3]. As an addition to this first layer of immunity, recognition of patterns of the host itself, which are generated upon damage caused by a pathogen or resulting from modified 'self' (so-called DAMPs), was introduced[4]. These patterns are recognized in the apoplast by cell surface-localized pattern recognition receptors (PRRs). Recognition of a PAMP leads to PAMP-triggered immunity (PTI), also referred to as MAMP-triggered immunity (MTI), and recently redefined as pattern-triggered immunity (also abbreviated as PTI) [20, 108]. To combat PTI, successful specialised pathogens have evolved effector proteins to interfere with PTI, thereby providing effector-triggered susceptibility (ETS).

The second layer of recognition is provided by resistance (R) proteins that are able to recognise these defence-suppressing effectors, allowing the plant to mount effector-triggered immunity (ETI). These R proteins can be PM-localized receptors, similar to PRRs, or cytoplasmic nucleotide-binding leucine-rich repeat (NLR) proteins. R proteins can either recognize effectors directly, or indirectly by guarding the host virulence target (VT) of the effector [64]. ETI generally is a stronger response than PTI, and often culminates in the hypersensitive response (HR) [3].

New insights in the mode of action of plant receptors, and the structure and occurrence of microbial patterns and effectors, has blurred the dichotomy between PTI and ETI [1, 4, 7, 109]. The continuum that is present between MAMPs and effectors, and in fact also between PRRs and R proteins, prompted Thomma and co-authors to introduce the term MAMP-triggered susceptibility (MTS), to stress the fact that also MAMPs can be involved in provoking susceptibility in plant-microbe interactions [7]. In 2014, the term effector-triggered defence (ETD) was proposed as another addition to the zigzag model [110]. ETD describes the defence responses triggered upon

recognition of apoplastic effectors by RLPs, which constitutively associate with the RLK SOBIR1 [15]. However, with the discovery that not all RLP/SOBIR1-complexes trigger a similar response upon their activation, this subdivision does not hold [15, 31].

Also the term 'apoplastic immunity' (AI) does not provide a satisfactory improvement of our understanding of plant defence mechanisms [111]. The term AI implies that immunity is established in the apoplast. However, for a successful immune response, after pathogen recognition in the apoplast, downstream signalling over the PM, into the cytoplasm is essential [111]. Therefore, this type of immunity is not strictly apoplastic, and the term AI is not appropriate.

Glossary

BAK1-INTERACTING RLKs (BIRs): a family of RLKs, mostly with a cytoplasmic pseudo-kinase domain, that negatively regulates defence responses by interacting with various cell surface receptors, especially BAK1.

BOTRYTIS-INDUCED KINASE 1 (BIK1): a cytoplasmic kinase that is anchored to the PM, and released into the cytoplasm upon its phosphorylation. As a member of the RLCK family, it mediates responses downstream of cell surface receptors.

BRI1-ASSOCIATED KINASE 1/SOMATIC EMBRYOGENESIS RECEPTOR KINASE 3 (BAK1/SERK3): an LRR-RLK, from the SERK family, that acts as a co-receptor for LRR-RLKs and LRR-RLP/SOBIR1 bimolecular complexes.

Danger signal: either an exogenous immunogenic signal being 'non-self' or an endogenous signal derived from the host 'self' (modified 'self'), that can be sensed by the host to initiate immune responses.

Effector: usually a small, stable, cysteine-rich protein, secreted by a pathogen into the apoplast or the cytoplasm of the host, upon attack, with the aim to prevent or circumvent plant defence and thereby promote disease. Typically, the expression of genes encoding effector proteins is highly induced *in planta*.

Extracellular immunogenic pattern (ExIP): any extracellular danger signal either externally encoded or representing a modified-self ligand, which betrays plant attack by cell surface receptors.

Intracellular immunogenic pattern (InIP): any intracellular danger signal either externally encoded or representing a modified-self ligand, which betrays plant attack by being recognized by intracellular receptors.

Pattern: a structurally conserved molecule derived from a (pathogenic) microbe (pathogen- or microbe-associated molecular pattern (PAMP or MAMP)), or from the host (damage-associated molecular pattern (DAMP)), which is released by an attacking microbe or exposed upon plant damage.

SUPPRESSOR OF BIR1-1/EVERSHED (SOBIR1/EVR): a regulatory LRR-RLK that constitutively interacts with RLPs, which lack a kinase domain themselves, to provide them with a kinase domain enabling downstream signalling.

Cell surface receptors: receptors that are localized at the plasma membrane to survey the apoplast for the presence of ExIPs, and as a result of ExIP perception initiate ExTI. They include RLPs and RLKs with various ectodomains.

Figure legends and tables

Figure 1; Schematic overview of the 'spatial immunity model'. Extracellular immunogenic patterns (ExIPs), which accumulate upon attack of the plant by microbes or as a result of cellular damage, are sensed by cell surface receptors that are present on the PM. ExIPs are so-called danger signals, which can be pathogen-derived patterns and effectors, or host-derived DAMPs (all shown as grey structures outside the cell) and effector-modified host derived virulence targets (VTs) (brown structure). Intracellular IPs (InIPs) are danger signals that are sensed by cytoplasmic receptors, mostly NLRs. InIPs can be pathogen-derived molecules (shown as grey structures inside the cell), or modified VTs (light brown structure). Both ExIP and InIP recognition leads to the activation of host defence responses, referred to as extracellularly- and intracellularly-triggered immunity (ExTI and InTI), respectively. ExIPs can act as effectors, and by their action in the extracellular space, they can suppress or circumvent the activation of ExTI (dotted line from the apoplast to the cytoplasm). InIPs can also act as effectors, with the potential to suppress immunity triggered by cell surface, as well as cytoplasmic receptors (dotted lines in the cytoplasm). Picture inspired by Dodds & Rathjen (2010) [65]. PM, plasma membrane; TTSS, type three secretion system; NLR, nucleotide binding leucine-rich repeat.

Figure 2; Immune signalling upon recognition of extracellular immunogenic patterns (ExIPs) should be referred to as extracellularly-triggered immunity (ExTI). (A) Numerous cell surface receptors are present at the PM that monitor the apoplast for the presence of extracellular immunogenic patterns (ExIPs). (B) Upon perception of ExIPs by cell surface receptors with an ectodomain consisting of leucine-rich repeats (LRRs), the co-receptor BAK1 is recruited, leading to the activation of defence responses. These ExIPs have originally been divided into so-called patterns (MAMPs/PAMPs/DAMPs) and effectors, and the responses that are initiated upon detection of these patterns and effectors have been classified as PTI and ETI, respectively. (C) The recent molecular proof of BAK1 recruitment by both LRR-RLKs (left) and LRR-RLPs (right) upon ExIP elicitation as shown in (B) now prompts to abandon this classic distinction between PTI and ETI, and to adopt the 'spatial immunity model'. In this model, we propose to use the general term ExIP for all different extracellular danger signals being either patterns or effectors, and perception of which leads to extracellularly-triggered immunity (ExTI).

599 **Table 1. LRR-RLKs and LRR-RLPs involved in immunity, their matching ExIPs, and the involvement of SERKs and SOBIR1 in their signalling.**
600

Receptor		Extracellular immunogenic pattern (ExIP)		Complex formation				Response	Refs.
RLKs	Plant origin	Name	Origin	Role of SOBIR1		Role of BAK1/SERKs		Previously classified as	
				Interaction (biochemical)	Dependence (genetic)	Interaction (biochemical)	Dependence (genetic)		
FLS3	Tomato	flgII-28 (MAMP)	Bacteria	n.a.	n.a.	Recruitment	Yes	PTI	Hind *et al.*, 2016
CORE	Tomato and *N. benthamiana* (Solanaceae)	csp22 (MAMP)	Bacteria	n.a.	n.a.	Recruitment	-	PTI	Wang *et al.*, 2016
Xa21	Rice	RaxX/Ax21 (MAMP)	Bacteria	n.a.	n.a.	Constitutive (recruitment not tested)	Yes	PTI	Chen *et al.*, 2014; Holton *et al.*, 2015; Pruitt *et al.*, 2015;
FLS2	Arabidopsis (very widespread)	flg22 (MAMP)	Bacteria	n.a.	n.a.	Recruitment	Yes	PTI	Gómez-Gómez & Boller, 2000; Chinchilla *et al.*, 2007; Heese *et al.*, 2007; Schulze *et al.*, 2010
EFR	Arabidopsis (Brassicaceae)	elf18 (MAMP)	Bacteria	n.a.	n.a.	Recruitment	Yes	PTI	Zipfel *et al.*, 2006; Chinchilla *et al.*, 2007; Schulze *et al.*, 2010; Macho *et al.*, 2014
PEPR1/PEPR2	Arabidopsis	Pep1 (DAMP)	Plants	n.a.	n.a.	Recruitment	-	PTI	Huffaker *et al.*, 2006; Schulze *et al.*, 2010; Postel *et al.*, 2010; Liu 2013; Tang *et al.*, 2015
RLPs									
Cf-4	Tomato	Avr4 (effector)	Fungus, biotrophic	Constitutive	Yes	Recruitment	Yes	ETI	Liebrand *et al.*, 2013; Postma *et al.*, 2016
Cf-9	Tomato	Avr9 (effector)	Fungus, biotrophic	Constitutive	-	-	-	ETI	Liebrand *et al.*, 2013
Ve1	Tomato	Ave1 (effector)	Fungus, hemi-biotrophic	Constitutive	Yes	-	Yes	ETI	Fradin *et al.*, 2009; Liebrand *et al.*, 2013
LeEIX2/leEIX1	Tomato	EIX (MAMP)	Fungi	Constitutive	-	Constitutive (recruitment not tested)	Yes	Not classifiable	Ron *et al.*, 2004; Bar *et al.*,

									2010; Liebrand *et al*., 2013
Cure	Tomato	*Cuscuta* factor (effector)	Parasitic plant	Constitutive	-	-	-	Not classifiable	Hegenauer *et al*., 2016
I	Tomato	Avr1 (effector)	Fungi, hemibiotrophic	Constitutive	-	-	-	ETI	Catanzariti *et al*., 2017
ELR	*S. microdontum* (potato)	Elicitins (MAMP?)	Oomycetes	Constitutive	Yes	Recruitment	Yes	Not classifiable	Chaparro-Garcia *et al*., 2011; Du *et al*., 2015; Domazakis *et al*., 2018
NbCSPR1	*N. benthamiana*	csp22 (MAMP)	Bacteria	Constitutive	No	Recruitment	Yes	PTI	Saur *et al*., 2016; Wang *et al*., 2016
NbRXEG1	*N. benthamiana*	XEG1 (MAMP)	Oomycete	Constitutive	Yes	Recruitment	Yes	Not classifiable	Wang *et al*., 2018
BnLEPR3	*Brassica napus*	AvrLm1 (effector)	Fungus, hemi-biotrophic	Constitutive	Yes	-	Yes	ETI	Ma and Borhan, 2015
BnRLM2	*B. napus*	AvrLm2 (effector)	Fungus, hemi-biotrophic	Constitutive	-	-	-	ETI	Larkan *et al*., 2015
ReMAX/AtRLP1	Arabidopsis	eMAX (MAMP)	Bacteria	-	Yes	-	-	PTI	Jehle *et al*., 2013a and 2013b
RLP23	Arabidopsis	NLPs, nlp20 (MAMP)	Bacteria, fungi, oomycetes	Constitutive	Yes	Recruitment	Yes	PTI	Bi *et al*., 2014; Albert *et al*., 2015
RLP30	Arabidopsis	SCFE1 (MAMP)	Fungi, necrotrophic	-	Yes	-	Yes	PTI	Zhang *et al*., 2013
RLP42/RBPG1	Arabidopsis	Polygalacturonases (PGs) (MAMP)	Fungi	Constitutive	Yes	Does not interact or recruit	No	Not classifiable	Zhang *et al*., 2014

-, no data available; n.a., not applicable.

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