# How do plants achieve immunity? Defence without specialized immune cells

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Abstract | Vertebrates have evolved a sophisticated adaptive immune system that relies on an almost infinite diversity of antigen receptors that are clonally expressed by specialized immune cells that roam the circulatory system. These immune cells provide vertebrates with extraordinary antigen-specific immune capacity and memory, while minimizing self-reactivity. Plants, however, lack specialized mobile immune cells. Instead, every plant cell is thought to be capable of launching an effective immune response. So how do plants achieve specific, self-tolerant immunity and establish immune memory? Recent developments point towards a multilayered plant innate immune system comprised of self-surveillance, systemic signalling and chromosomal changes that together establish effective immunity.

Phytopathogens
Microbial organisms that are specialized in attacking plant hosts. They use a variety of infection strategies, ranging from feeding on live plant cells to destroying plants cells to feed on their contents.

Callose
Following pathogen infection, this polysaccharide is produced by plant cells and deposited near the site of attempted penetration to reinforce the cell wall.

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An optimal immune system for long-lived organisms requires high specificity, self-tolerance and immune memory. The immune system in jawed vertebrates is the best studied and most sophisticated. Here, the relatively nonspecific innate immune system is complemented by the highly refined adaptive immune system, which uses vast repertoires of structurally similar receptors — namely, B cell immunoglobulins and T cell receptors (TCRs) — that have an almost infinite number of antigen-binding specificities generated through somatic recombination and mutation. These receptors are clonally expressed by lymphocytes (B and T cells), which travel through the circulatory system to detect pathogens or mutated cells. Antigen recognition by a receptor leads not only to the clonal expansion of lymphocytes expressing that receptor, but also to the formation of long-lived memory cells that produce receptors with the same antigen-binding specificity, allowing secondary immune responses to the corresponding antigen to be faster and more effective.

By comparison, the immune system of plants seems to be far less complex. Because plants lack a circulatory system and mobile immune cells, they cannot use circulating immune receptors to detect non-self. Nonetheless, plants are capable of establishing immune responses that are highly specific, with restricted self-reactivity, and that often generate a lifelong 'memory' of the encountered pathogens. So, these features of vertebrate immunity can be achieved in plants using different immune strategies. The intriguing question is: how does the plant immune system do this?

The initial obstacle that phytopathogens encounter is the plant cell wall, which can be reinforced by the deposition of callose (glucan polymers) following the activation of host defence pathways. The first active line of defence occurs at the plant cell surface when microorganism-associated molecular patterns (MAMPs) — such as lipopolysaccharides, peptidoglycans and bacterial flagellin — are detected by pattern-recognition receptors (PRRs) (BOX 1). Although there are some overall structural similarities between PRRs from plants and animals, such as the use of the leucine-rich repeat (LRR) domain for ligand binding[1], they are thought to have arisen through convergent evolution rather than divergent evolution[1–3]. This is exemplified by the analogous transmembrane flagellin receptors FLAGELLIN-SENSITIVE 2 (FLS2) in *Arabidopsis thaliana* and Toll-like receptor 5 (TLR5) in humans, which are only similar in terms of the LRR domain. Although both receptors recognize conserved epitopes of bacterial flagellin, they bind to different epitopes within this MAMP[4,5]. Moreover, a recent finding indicates that FLS2 might have a different substrate range than TLR5, as it can also detect additional, structurally unrelated MAMPs[6]. These differences in the structures of the PRRs, as well as in their downstream signalling components, indicate that pattern-triggered immunity arose independently in plants and animals.

To circumvent pattern-triggered immunity, adapted pathogens can deliver effector molecules directly into the plant cell. For example, *Pseudomonas syringae* strains

Box 1 | **Plant immune responses induced by pattern-recognition receptors**

Pattern-recognition receptors (PRRs) are found in both plants and animals, and they enable the detection of microorganism-associated molecular patterns (MAMPs). In plants, PRRs are membrane-bound receptor-like kinases (RLKs) or receptor-like proteins (RLPs)[92]. The *Arabidopsis thaliana* genome encodes a large number of RLKs (~615); this property is reminiscent of the sea urchin genome, which unlike that of other animal species contains a recently expanded repertoire of transmembrane receptors (comprising some 222 Toll-like receptor genes)[93]. Some of the *A. thaliana* RLKs are involved in immunity, whereas others have key roles in plant development, symbiosis and self-incompatibility in pollination. XA21 in rice (*Oryza sativa*) was the first PRR to be identified, and it confers resistance against diverse strains of the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*. However, the ligand for XA21 was cloned only recently and found to be a sulphated peptide of the protein Ax21 (activator of XA21-mediated immunity), which is present in all sequenced *Xanthomonas* species and is predicted to function as an inducer of quorum sensing[94–96]. MAMP–PRR interaction in plants was first studied between the amino-terminal 22 amino acids of flagellin (flg22) of *Pseudomonas syringae* and the flg22 receptor, FLAGELLIN-SENSITIVE 2 (FLS2), in *A. thaliana*[97]. FLS2 contains an extracellular leucine-rich repeat (LRR) domain for ligand binding, a transmembrane domain and a serine/threonine kinase domain. Notably, some plant PRRs (such as the chitin receptor CEBIP in rice) contain a lysine motif rather than the LRR domain for ligand recognition. FLS2 is localized in the plasma membrane and is endocytosed following binding to flg22 (REF. 98). When activated, FLS2 interacts with the co-receptor BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) to initiate pattern-triggered immunity[99,100]. The signal-specific activation of plant PRRs by different MAMPs leads to seemingly generic responses. These responses include ion fluxes, the oxidative burst, activation of a downstream mitogen-activated protein kinase cascade, transcriptional changes and the production of antimicrobial compounds, such as pathogenesis-related (PR) proteins (BOX 2) and phytoalexins.

Convergent evolution
A process by which organisms from different lineages independently evolve similar traits that help them to adapt to their environment.

Pattern-triggered immunity
A basal type of immunity conferred by the recognition of conserved microorganism-associated molecular patterns by specific transmembrane receptors that protect hosts against non-specialized pathogens.

Effector molecules
Pathogen-produced proteins that are injected into the host cell, where they suppress the function of host immune regulators to promote pathogen virulence.

Effector-triggered immunity
A type of immunity triggered by resistance (R) proteins that sense perturbations of host signalling hubs caused by pathogen-produced effector molecules. Effector-triggered immunity frequently culminates in programmed cell death of the infected cell.

Hypersensitive response
A plant immune response that occurs locally to isolate and prevent the growth of pathogens or insects whose life cycles depend on live host cells. This response is triggered when the presence of a pathogen effector is detected by a host resistance (R) protein and is characterized by the rapid death of cells at the infection site.

Programmed cell death
Unlike cell senescence, this is an active form of cell death that occurs through a regulated process during normal development and has a physiological function.

Systemic acquired resistance
A long-lasting, broad-spectrum immune response that is induced throughout the entire plant following attempted local infection.

contain dozens of such effectors. Some of these effectors, such as AvrPto1 of *P. syringae* pv. *tomato*, have been shown to promote pathogen virulence by suppressing immune-related proteins[7,8]. Through co-evolution with pathogens, plants have developed intracellular immune receptors known as resistance (R) proteins that can recognize the presence of certain pathogen effector molecules. Thus, plants can use these immune receptors to detect pathogen 'avirulence' signals and activate effector-triggered immunity. The hallmark of effector-triggered immunity (in other words, R gene-mediated resistance) is a hypersensitive response. This response is typically associated with programmed cell death of the infected cells and the production of antimicrobial molecules — such as the hydrolytic enzymes chitinase and β-1,3-glucanase — in the surrounding tissue, leading to local resistance to the pathogen. Unlike pattern-triggered immunity, which is a general response to a limited number of MAMPs that are conserved between the major microbial groups (for example, fungi and Gram-positive and Gram-negative bacteria), effector-triggered immunity is specific for effectors that are highly polymorphic between different pathogen strains. With a given genome size, how do plants recognize the virtually unlimited number of pathogen effectors?

A local hypersensitive response can also 'immunize' plants against future infection. This phenomenon was named systemic acquired resistance (SAR) by A. Frank Ross, who discovered that the local inoculation of tobacco plants with tobacco mosaic virus (TMV) could protect them against infection with not only TMV but also other pathogens[9]. This broad-spectrum resistance could last for at least 20 days in tobacco plants. So what is the systemic signal for SAR? And how do plants 'remember' prior pathogen exposure?

In this Review, we focus on discussing the design principles of effector-triggered immunity that allow plants to respond to a large array of pathogen effectors while avoiding autoimmunity. We also discuss how SAR is established, leading to long-lasting, broad-spectrum immunity throughout the induced plant and possibly in its progeny. This is not intended to be a comprehensive review of all potential mechanisms of plant immunity but rather a discussion that highlights the similarities and differences with mammalian immune systems based on the most recent publications.

## Specificity and self-tolerance

Functional genomic surveys of pathogen effectors indicate that these proteins are highly diverse in sequence as well as in molecular function[10–13]. Surprisingly, the cognate R proteins in plants are structurally conserved. Numerous R proteins have been identified (150 in *A. thaliana*[14] and more than 600 in rice (*Oryza sativa*)[15]), and they typically consist of a variable amino terminus followed by a nucleotide-binding site (NBS) domain in the middle and an LRR domain at the carboxyl terminus. Interestingly, these NBS–LRR proteins have a similar domain structure to animal NLR proteins (nucleotide-binding oligomerization domain (NOD)- and LRR-containing proteins), which are intracellular immune receptors. Based on homology modelling to the well-studied potato R protein Rx, a general mechanistic model for R protein activation has been proposed[16]. In the absence of ligand, intramolecular interactions occur between the variable N terminus, the NBS or NOD and the LRR domain of an R protein or NLR protein. This limits nucleotide exchange and hydrolysis in the central NBS or NOD, thereby inhibiting the activity of the receptor[16–18]. Following ligand binding, this intramolecular inhibition is thought to be alleviated, resulting in receptor activation, which is associated with nucleotide exchange and hydrolysis. In addition, receptor activation leads to possible conformational changes — mediated by interaction with a conserved eukaryotic chaperone complex that contains heat shock protein 90 (HSP90) and suppressor of G2 allele of SKP1 (SGT1) — and to downstream signalling events[18,19].

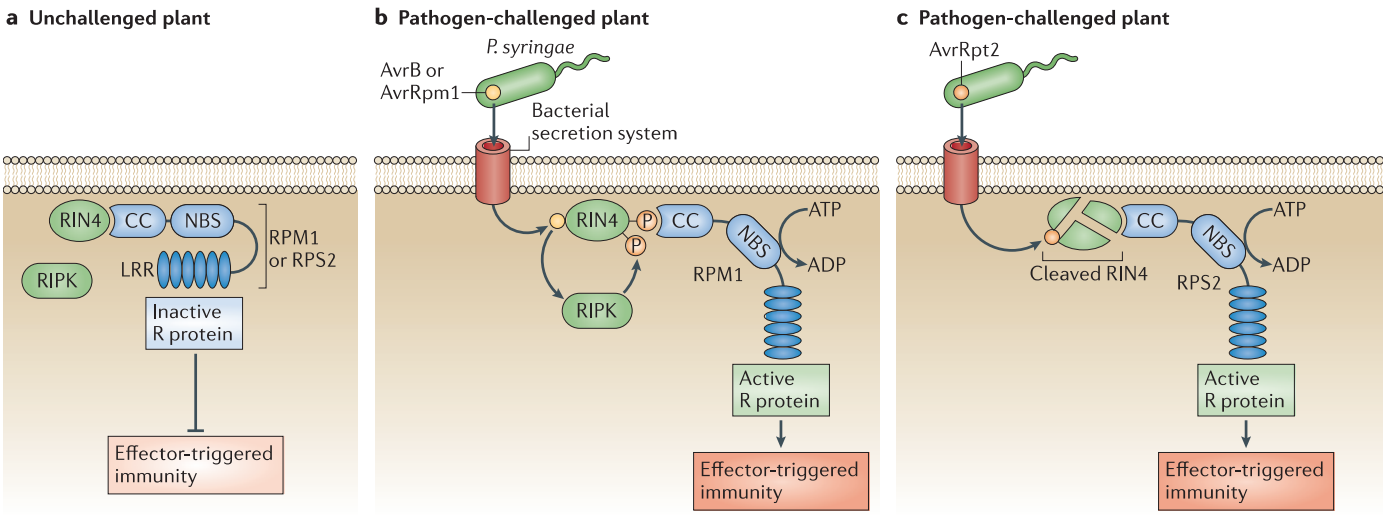


Figure 1 | **The guard model: surveillance of the host immune regulator RIN4 by the R proteins RPM1 and RPS2.** **a** | In unchallenged plants, resistance (R) proteins that have a CC–NBS–LRR (coiled coil–nucleotide-binding site–leucine-rich repeat) domain structure detect unmodified RPM1-INTERACTING PROTEIN 4 (RIN4). This interaction maintains these R proteins (which include RPM1 and RPS2) in an inactive state. **b** | Infection with pathogens such as *Pseudomonas syringae*, which injects the effector molecules AvrB and AvrRpm1 into the plant cell, results in RPM1-INDUCED PROTEIN KINASE (RIPK)-mediated phosphorylation of RIN4. Phosphorylated RIN4 is detected by RPM1, resulting in its activation, which possibly occurs through intramolecular conformational changes (as demonstrated for other R proteins). Active RPM1 induces downstream signalling pathways that lead to effector-triggered immunity. **c** | Infection with pathogens such as *P. syringae* that inject the effector molecule AvrRpt2 into the plant cell results in the cleavage of RIN4, which leads to the activation of RPS2 and effector-triggered immunity.

***Recognition of pathogen-induced host perturbations.*** The highly specific nature of R protein-mediated immunity was discovered more than 50 years ago by studies showing that single dominant Mendelian R loci in flax (*Linum usitatissimum*) varieties could confer resistance to specific strains of a rust fungus[20]. However, based on our current knowledge of the number of R genes in plant genomes, the gene-for-gene model that was proposed at the time[20] — which states that each plant R gene matches with an effector-coding gene in the pathogen — cannot explain the broad immune capacity of plants. A similar puzzle in mammalian immune diversity was reconciled by the discovery of somatic DNA rearrangements of immunoglobulin and TCR gene loci during lymphocyte development. In plants, however, although R genes are present in gene clusters that have higher rates of recombination than the genome average[21], no somatic rearrangement events similar to those in mammals have been observed. Moreover, with only a few exceptions[22–25], most of the R proteins studied so far do not interact with their cognate pathogen effectors directly.

To solve this major puzzle in plant immunity, the guard hypothesis was put forward, stating that unlike immunoglobulin and TCR molecules, which are receptors of non-self signals, plant R proteins bind to and 'guard' pathogen-targeted self proteins. R protein activation is triggered when self proteins are perturbed or modified by pathogen effectors. The best-studied R protein-guarded cellular target is the *A. thaliana* protein RPM1-INTERACTING PROTEIN 4 (RIN4). Consistent with RIN4 being a 'guardee' of R proteins, it not only interacts physically with the R proteins RPM1 and RPS2, but is also targeted and modified by three distinct pathogen effectors from *P. syringae* (namely, AvrRpm1, AvrB and AvrRpt2)[26,27]. Recently, purification of a RIN4-containing complex led to the identification of RPM1-INDUCED PROTEIN KINASE (RIPK), a member of the receptor-like cytoplasmic kinase family. RIPK was shown to phosphorylate RIN4 at several threonine residues in response to the pathogen effectors AvrB and AvrRpm1 (REF. 28). Phosphorylation of threonine 166 of RIN4 was particularly important for R protein activation, as RIN4 mutants with a phosphomimetic amino acid at this position could trigger RPM1-mediated immunity even in the absence of the pathogen effectors AvrRpm1 and AvrB[28,29]. By contrast, the activity of AvrRpt2 on RIN4 is more direct, as RIN4 is cleaved by this bacterial cysteine protease, resulting in RPS2 activation[27,30,31] (FIG. 1).

These findings lead to the next question: why is RIN4 a favourable target for pathogen effectors? To answer this question, the normal cellular function of RIN4 has to be investigated. Studies carried out in plants that do not express the RIN4-guarding R proteins RPM1 and RPS2 show that the *P. syringae* effectors AvrRpm1, AvrB and AvrRpt2 target RIN4 to suppress pattern-triggered immunity, indicating that RIN4 has an important role in plant host defence[26–29,31,32]. However, the molecular mechanism by which RIN4 regulates host defence is largely unknown. One possible mechanism was hinted at by the discovery that RIN4 interacts with plasma membrane-associated $H^+$-ATPases that regulate the apertures of plant stomata, which are a primary site of pathogen entry into the plant leaf and a major target of MAMP action[33].

NLR proteins
(Nucleotide-binding oligomerization domain (NOD)- and leucine-rich repeat (LRR)-containing proteins). A group of intracellular immune receptors that have a structure that closely resembles that of resistance (R) proteins in plants. In contrast to R proteins, NLRs in mammals detect microorganism-associated molecular patterns rather than pathogen effectors.

Taken together, these data strongly suggest that the plant immune system uses R proteins predominantly to monitor pathogen effector-triggered perturbations of self molecules, rather than to detect non-self molecules (FIG. 1). This strategy provides plants with the potential to specifically recognize groups of pathogens that use similar infection strategies (in other words, pathogens that use effectors converging on the same host targets, such as RIN4). Despite the numerous different effectors that pathogens inject into plant cells to promote virulence, they might target relatively few conserved 'hubs' in the plant signalling network that controls plant defence, metabolism and signalling[34]. These signalling hubs are probably essential host proteins and so might be difficult to identify through genetic approaches. A recent study used a genome-wide yeast two-hybrid screen to identify physical interactions between *A. thaliana* immune-related proteins (including 30 R proteins) and effectors from two evolutionarily separated pathogens. In this study, it was shown that pathogen effectors might converge on a limited set of host proteins that are highly interconnected hubs with important regulatory roles in plant immune signalling[35]. Moreover, rather than physically associating with pathogen effectors, plant R proteins were found to interact with effector-targeted host proteins. This work provided crucial information regarding how a few hundred R genes might be sufficient to protect plants from a much larger array of potential pathogen-encoded effectors.

***R protein-mediated programmed cell death.*** The (indirect) activation of R proteins by pathogen effectors often leads to programmed cell death at the site of attempted infection. This defence strategy is effective against viral, bacterial, fungal and oomycete pathogens, as well as nematodes that feed on live plant cells. However, unlike NLRs in animals, which are known to trigger cell apoptosis through the activity of caspases that activate pro-inflammatory cytokines[36], plants lack the homologous caspases, and several potential alternative mechanisms have been proposed for R protein-mediated programmed cell death. A recent publication shows that one type of β-subunit of the 26S proteasome (namely, PBA1) contributes to the caspase-3-like activity that is observed during the resistance response in *A. thaliana* to the bacterial pathogen *P. syringae* pv. *tomato*[37]. This activity is required for membrane fusion between the central vacuole and the plasma membrane of the plant cell, which leads to the release of antibacterial factors and programmed cell death-promoting signals from the vacuole, and consequently pathogen resistance. Another recent paper indicates that R protein-triggered programmed cell death can be mediated by metacaspases. Specifically, *A. thaliana* METACASPASE 1 (AtMC1) was shown to function as a positive regulator of programmed cell death. Elimination of its catalytic residues rendered AtMC1 unable to trigger cell death[38]. The notion that R protein-mediated programmed cell death might involve perturbation of multiple cellular processes came from a report showing that, in *A. thaliana*, the resistance conferred by the R protein RPP4 against the obligate biotroph *Hyaloperonospora arabidopsidis* is not mediated by a single gene but rather by multiple downstream genes[39]. Phenotypic analysis of plants with mutations in these genes showed that programmed cell death is the major defence mechanism against *H. arabidopsidis*. Interestingly, these programmed cell death-promoting genes encode proteins, mostly enzymes, with very diverse functions, including a receptor-like kinase, a calcineurin-like phosphoesterase, a protease, a UDP-glucosyl transferase, an ABC transporter and an ATPase. However, it is still debatable whether cell death is the cause or a consequence of resistance, because in some mutant plants as well as in transgenic plant cell lines that express cell death inhibitors, cell death is abolished but R protein-mediated pathogen resistance is not perturbed[38,40,41]. It is plausible that, on these genetic backgrounds, pathogen growth is blocked before the R protein-mediated induction of cell death. Epistasis experiments may be helpful for testing this hypothesis.

***Strategies to prevent autoimmunity.*** As R proteins are expressed by all plant cells, controlling their activity is crucial for plant survival, as well as for defence against pathogens. Plant R proteins have evolved to recognize modified self antigens, so there should be strong selective pressure to eliminate R proteins that can be activated by normal (unmodified) self antigens. Nonetheless, recent findings indicate that this design principle of the plant immune system can occasionally give rise to autoimmunity in genetically diverse populations. For example, in *A. thaliana* (a predominantly self-pollinating species), about two percent of manually performed intraspecific crosses result in offspring that are severely necrotic, sterile or nonviable[42]. This phenomenon, known as hybrid necrosis, is associated with the spontaneous, systematic activation of immune-related genes. Mapping of the loci responsible for hybrid necrosis has repeatedly identified R genes[42–44], indicating that the inherent self-tolerance of R protein-mediated immunity might be compromised by incompatible genetic interactions. Hybrid necrosis occurs when R genes from one parent plant are mixed with a corresponding incompatible target locus (potentially encoding an R protein guardee) from the other parent plant. An important clue to the nature of such a locus was recently revealed in a study of hybrid necrosis caused by interspecific crosses in lettuce (*Lactuca sativa*). One of the two interacting loci found in this study encoded a RIN4 orthologue. Interestingly, substitution of three polymorphic residues of RIN4 in one parent with the corresponding residues of the other parent averted necrosis in the hybrid offspring[45]. In another study, it was shown that autoimmunity arose from incompatible interactions between the *A. thaliana RPP1* cluster of R genes and allelic variations in the gene encoding the receptor-like kinase STRUBBELIG-RECEPTOR FAMILY 3 (SRF3), indicating that *RPP1*-cluster proteins might monitor SRF3 for perturbations induced by pathogen effectors[46]. Taken together, these findings imply that intraspecific or interspecific crosses can lead to a mismatch between R proteins and the targets of pathogen effectors that they

Metacaspases
Arginine- and lysine-specific proteases that are related to animal caspases. Metacaspases are found in plants, fungi and protists, where they have an essential role in programmed cell death responses.

Hybrid necrosis
A post-zygotic incompatibility resulting from intraspecific or interspecific crosses that is typified by severe tissue necrosis, stunting and auto-activation of immune responses.

guard. Consequently, in hybrid plants, R proteins from one parent recognize the effector targets from another parent as modified self, and this results in auto-activation of the R protein in the absence of pathogen attack (FIG. 2). As noted by others[44], it will be interesting to determine how autoimmunity is avoided in outcrossing plants, such as maize, in which hybrid necrosis has not been observed despite the intensive mixing of heterologous genetic backgrounds during domestication.

Another important research focus for understanding the mechanism that controls autoimmunity is how R protein signalling is normally turned off in plants. Gain-of-function mutations in the R genes *SNC1* and *SSI4* in *A. thaliana* indicate that dysregulation of R proteins poses an imminent autoimmune threat. Mutant *snc1* and *ssi4* plants show signs of autoimmunity — including immune-related transcriptional reprogramming, accumulation of hydrolytic enzymes with antimicrobial activities, and spontaneous cell death that resembles that induced by the hypersensitive response — and this culminates in stunted growth and altered morphology[47,48]. It is therefore likely that the activities of R proteins are normally under strict cellular control. Several reports have shown that the overexpression of R proteins results in autoimmunity[49,50], indicating that the activity levels of some R proteins are linked to their cellular levels. Indeed, it was recently shown that mutation of the gene encoding the tetratricopeptide repeat domain-containing protein SRFR1 resulted in autoimmune responses owing to transcriptional upregulation of several co-regulated R genes[51,52]. Accordingly, SRFR1 has substantial sequence similarity to various eukaryotic transcriptional repressors in which the tetratricopeptide repeat interacts with other transcriptional (co)regulators[53]. In another recent study, protein degradation mediated by a SKP1–CULLIN 1–F-box protein complex was shown to have a role in controlling R protein levels, as a loss-of-function mutation in the gene encoding the F-box protein CPR1 resulted in the accumulation of higher levels of the R proteins SNC1 and RPS2, as well as in autoimmunity[54]. The autoimmune phenotype of the *cpr1* mutant was largely suppressed by knocking out *SNC1*, indicating that it was the result of R protein over-accumulation. It is plausible that high levels of R proteins out-titrate regulatory factors, such as chaperone complexes, that normally control their activities. R protein stability is tightly controlled by a highly conserved eukaryotic chaperone complex that includes HSP90, SGT1 and the cysteine- and histidine-rich domain-containing protein RAR1. It is thought that this chaperone complex maintains R proteins in a recognition-competent state and, after they recognize pathogen effector-modified self proteins, facilitates the conformational change of R proteins to induce downstream immune signalling. Accordingly, mutation of genes encoding chaperone components markedly affects R protein stability[19].

R protein levels, and thus their activities, are not simply under constitutive or static cellular control. Rather, they can follow a dynamic pattern of expression and accumulation. The *A. thaliana* R gene *RPP4*, which confers immunity against the downy mildew disease agent *H. arabidopsidis*, was shown to adopt a rhythmic pattern of expression controlled by the circadian regulator CCA1. Intriguingly, peak expression of *RPP4* and RPP4-dependent genes occurred at dawn, coinciding with the time of *H. arabidopsidis* sporulation[39]. Accordingly, artificial infection with *H. arabidopsidis* at dusk increased the susceptibility of plants to this pathogen as compared with infection at dawn. Thus, plants seem to anticipate infection by *H. arabidopsidis* strains at dawn through the regulated expression of particular R genes. It is plausible that this mechanism is widely used by plants to minimize the risk of autoimmunity, as these R genes are only temporarily expressed when necessary.

In summary, signal-specific immunity in plants is provided by structurally similar R proteins that guard key cellular signalling hubs. Perturbation of these hubs by pathogen effectors activates R proteins, which trigger a programmed cell death response and establish immunity. The intrinsic autoimmune reactivity of R proteins requires plant cells to tightly regulate their expression and activity to minimize self-reactivity.

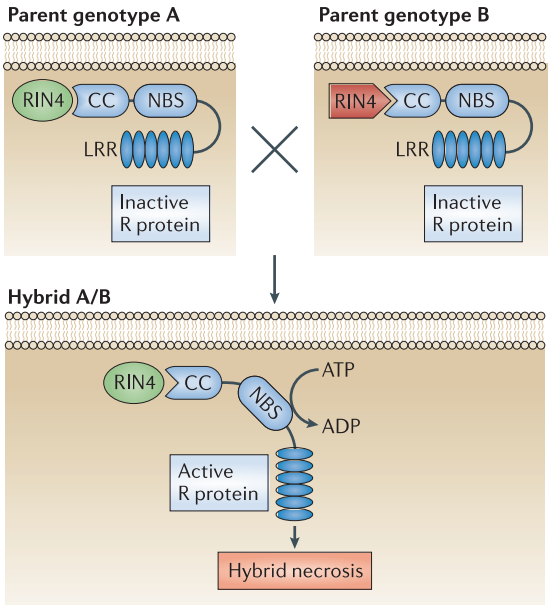


Figure 2 | **Autoimmunity in necrotic hybrids might be caused by a mismatching of R proteins and the targets of pathogen effectors that they guard.** The figure shows a proposed model for the involvement of resistance (R) proteins and the host targets of pathogen effectors in hybrid necrosis. Parental genotypes A and B have sequence variations in the effector target RPM1-INTERACTING PROTEIN 4 (RIN4) and the R proteins that guard RIN4. Hybrid progeny inherit RIN4 variants and the respective guarding R proteins from both parents. Consequently, the R protein from one parent might recognize RIN4 from the other parent as a modified protein, resulting in the activation of immune responses in the absence of pathogen challenge and causing autoimmune-induced hybrid necrosis. CC, coiled coil; LRR, leucine-rich repeat; NBS, nucleotide-binding site.

## Systemic activation of immunity

Mobile immune cells and secreted opsonins (antibodies) of the humoral immune system provide animals with specific immunity throughout their entire body. Plants do not have a circulatory system, but the experiments outlined below indicate that their non-circulatory vascular system nonetheless transports immune signals from the site of infection to systemic uninfected tissues to establish SAR. Unlike adaptive immunity in animals, which is antigen specific, SAR is typically induced following effector-triggered immunity (although induction by pattern-triggered immunity has also been reported[55]) and is effective against a wide range of biotrophic pathogens[56].

***Mobile immune signals.*** Since the discovery of SAR, much research has been devoted to identifying the mobile immune signal that is responsible for this phenomenon. Such a signal should be generated in the infected tissue and be rapidly transported to uninfected parts of the plant. It might also be able to encode detailed information about the primary pathogen infection. If the last criterion holds true, then more than one kind of mobile signal, functioning in a synergistic manner, might be needed to relay such complex information to systemic tissues. The currently available data seem to support this hypothesis.

The onset of SAR is accompanied by increased accumulation of the signalling hormone salicylic acid in the phloem[57]. Moreover, the removal of salicylic acid by constitutive expression of a salicylate hydroxylase abolishes SAR[58]. Although these findings strongly suggest that salicylic acid is a transported immune signal, grafting experiments showed that salicylic acid is dispensable for signal generation at the site of infection; rather, it is required for SAR development in systemic tissues[59]. More-recent grafting experiments using tobacco plants showed that salicylic acid methyltransferase activity, which converts salicylic acid into methylsalicylic acid (MeSA), is required in the tissue that generates the immune signal. Conversely, MeSA esterase activity, which converts MeSA back into salicylic acid, is required for signal perception in systemic tissues. Taken together with the observation that MeSA accumulates in the phloem following the activation of SAR, these results suggest that MeSA might be the transported immune signal[60]. Although exogenous application of MeSA induced systemic immunity in wild-type tobacco plants[60], it is not known whether MeSA can bypass the requirement for salicylic acid methyltransferase to induce SAR. Moreover, experiments using *A. thaliana* found that knockout mutant plants that lacked salicylic acid methyltransferase failed to accumulate MeSA, but still retained the ability to systemically accumulate salicylic acid and activate SAR. In fact, most of the MeSA produced following infection of *A. thaliana* escaped the plant by volatile emission[61]. These data indicate that, contrary to the findings made in tobacco plants, MeSA is dispensable for SAR in *A. thaliana*. Thus, it remains uncertain whether MeSA is a necessary and/or sufficient mobile immune signal for SAR in general. It seems that the composition of the mobile immune signal in SAR might differ depending on the plant species and the type of plant–pathogen interaction.

A labour-intensive genetic screen for mutant *A. thaliana* plants deficient in systemic immunity identified the *defective in induced resistance 1-1* (*dir1-1*) gene[62]. Importantly, mutant *dir1-1* plants can still launch local immune responses, indicating that *DIR1* is only required for systemic immunity. Vascular exudates from pathogen-inoculated wild-type plants induced immune-related genes, whereas those from mutant *dir1-1* plants did not. In accordance with this, and the fact that *DIR1* is predicted to encode an apoplastic lipid-transfer protein, it was concluded that DIR1 has a role in immune signal generation and/or transports a lipid-based immune signal to systemic tissues. The hormone jasmonic acid fits the profile for such an immune signal, as it is a lipid-derived molecule and its accumulation in the phloem is associated with the induction of SAR[63]. However, mutant plants with defects in jasmonic acid biosynthesis or signalling have varying degrees of SAR. This was again dependent on the type of plant–pathogen interaction[61,63], thereby casting doubts on the role of jasmonic acid as a mobile immune signal. Indeed, co-infiltration of jasmonic acid or methyl jasmonate with vascular exudates from SAR-deficient plants failed to induce pathogen resistance. Moreover, fractionation of SAR-induced vascular exudates revealed that jasmonic acid did not co-purify with the SAR-inducing activity[64].

A breakthrough was made with the discovery that *A. thaliana* mutants with an impairment in the biosynthesis of the organophosphate compound glycerol-3-phosphate (G3P) failed to activate SAR[65,66]. Importantly, the development of SAR in distal tissues was rescued in these mutant plants by the local application of exogenous G3P or SAR-induced vascular exudates from wild-type plants. Conversely, SAR-induced vascular exudates from mutant plants with defective G3P biosynthesis failed to induce SAR in wild-type plants unless supplemented by G3P[66]. These data imply that G3P is a signal that is generated following the infection of primary tissues and subsequently translocated to distal parts of the plant to induce systemic immunity. Intriguingly, the authors reported that exogenous G3P was most effective in inducing SAR when it was applied together with vascular exudates from mock-treated plants, which indicates that a cofactor might be required for the immune activity of G3P. Indeed, G3P-induced SAR was shown to be dependent on DIR1 and vice versa. Although a physical association between G3P and DIR1 was not found, these findings strongly suggest that cooperative movement of these mobile immune signals confers SAR.

In addition to G3P, azelaic acid has been identified as a mobile immune signal through the analysis of infection-induced plant vascular exudates[67]. Azelaic acid was shown to prime plants for salicylic acid accumulation and the activation of immune-related genes. Moreover, it induced the expression of *AZELAIC ACID INDUCED 1* (*AZI1*), which is predicted to encode a secreted lipid-transfer protein. Reciprocal application of vascular exudates from wild-type and *azi1* mutant plants indicated that AZI1 is involved in the production and/or translocation of a mobile immune signal.

Phloem
The plant vascular tissue, which transports organic nutrients (such as sugars) from photosynthetic 'source' tissues to nutrient-consuming 'sink' tissues throughout the entire plant.

Apoplastic
Localized to the free diffusional space outside the plasma membrane of plant cells.

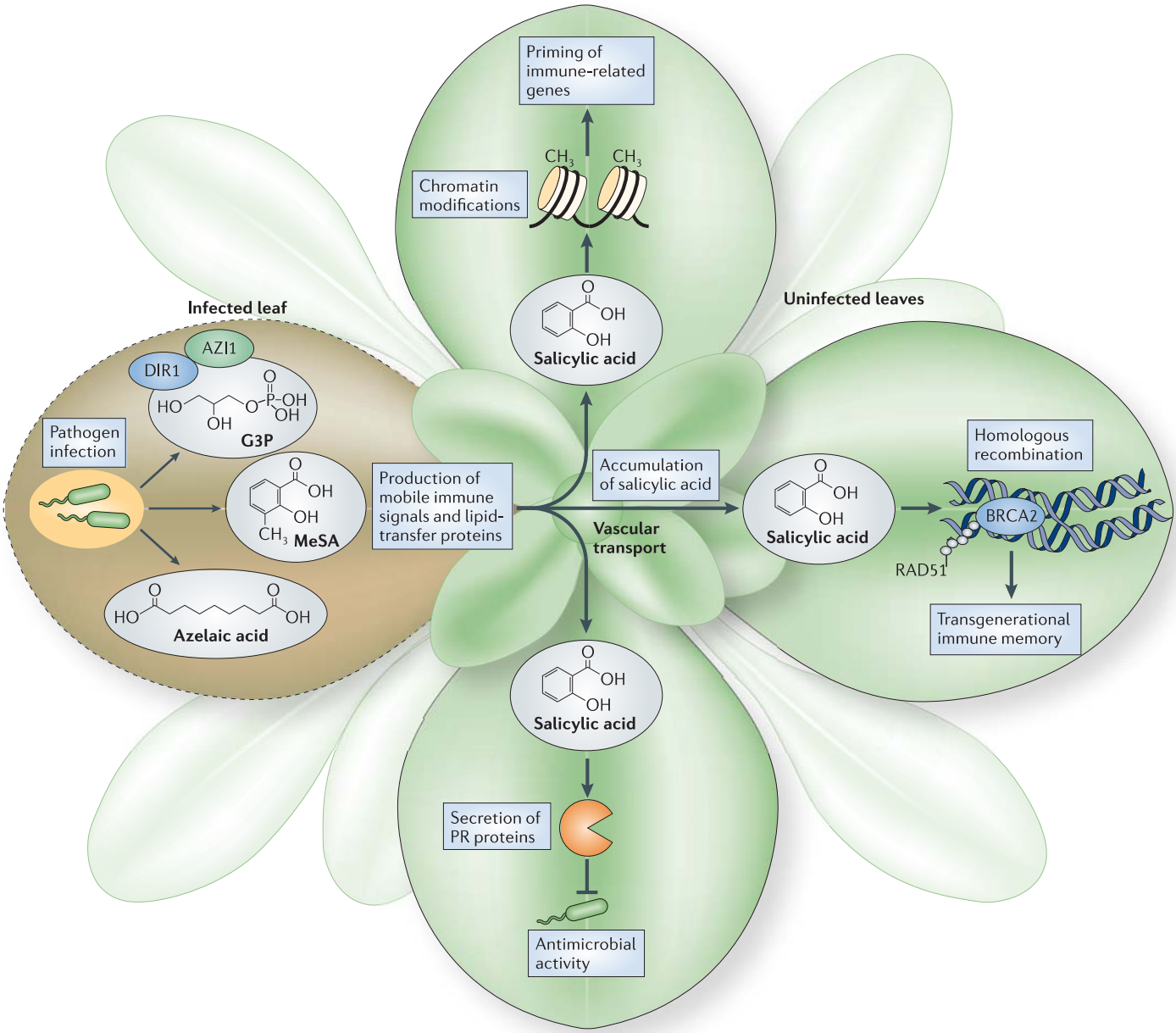


Figure 3 | **Translocation of mobile immune signals induces systemic immunity and immune memory.** Local pathogen infection results in the production of the mobile immune signals methylsalicylic acid (MeSA), azelaic acid and glycerol-3-phosphate (G3P), and the lipid-transfer proteins DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1) and AZELAIC ACID INDUCED 1 (AZI1). These mobile signals are transported through the vasculature to systemic, uninfected parts of the plant, where through an unknown mechanism they induce the accumulation of salicylic acid, which is a signal molecule for systemic acquired resistance. Accumulation of salicylic acid induces: the secretion of pathogenesis-related (PR) proteins with antimicrobial activities; histone methylation and other chromatin modifications that prime immune-related genes for increased expression and establish immune memory; and somatic homologous recombination through the actions of BREAST CANCER SUSCEPTIBILITY 2 (BRCA2) and RAD51 to potentially establish a transgenerational memory of immunity.

Taken together, the data indicate that the mobile immune signal in plants consists of multiple proteins as well as lipid-derived and hormone-like molecules (FIG. 3). There are several indications that these different components coordinate each other's activities to establish systemic immunity. For example, azelaic acid could not induce immunity in the *dir1-1* mutant plants, indicating that its activity requires DIR1 (REF. 67). Moreover, *dir1-1* mutant or *DIR1*-silenced plants have increased expression of salicylic acid methyltransferases, resulting in increased production of MeSA at the cost of salicylic acid accumulation and disease resistance[68]. It is plausible that the interplay between different mobile immune signals in plants might relay specific information about the type of primary pathogen encountered and consequently determine the level of immune response that is most appropriate for systemic tissues.

***Cellular reprogramming prioritizes immunity.*** Early studies showed that the arrival of mobile immune signals in systemic tissues is associated with an increased accumulation of salicylic acid[57–59]. However, the mechanism by which this is accomplished is largely unknown, except for the conversion of mobile MeSA to salicylic acid as described above. The regulation of salicylic acid metabolism in plants by the salicylic acid biosynthetic enzyme isochorismate synthase and the salicylic acid-inactivating enzyme salicylic acid glucosyltransferase might hold the key to this question and should be studied in more detail.

Signalling downstream of salicylic acid has been studied intensely because the exogenous application of salicylic acid to plants can mimic pathogen-induced SAR. This method, which was serendipitously discovered using aspirin (acetylsalicylic acid)[69], is not only convenient for conducting genetic screens, but also the basis for the development of synthetic salicylic acid analogues — such as 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) — for commercial use in controlling plant disease. Treating *A. thaliana* plants with these compounds results in marked transcriptional changes in more than 2,000 genes, including those that encode pathogenesis-related (PR) proteins with antimicrobial activity[70] (BOX 2). This transcriptional reprogramming is largely dependent on the transcription cofactor NONEXPRESSER OF PR GENES 1 (NPR1) (BOX 3). NPR1 not only activates a myriad of immune-related genes (such as PR genes) and genes encoding transcription factors that initiate further transcriptional cascades, it also downregulates genes involved in basic cellular processes, such as photosynthesis, thereby prioritizing immune responses at the cost of plant growth[70,71].

Box 2 | **Pathogenesis-related proteins are the executioners of plant immunity**

The production of pathogenesis-related (PR) proteins was first associated with tobacco mosaic virus (TMV) infection of tobacco plants[101]. Later studies showed that PR proteins include hydrolytic enzymes (such as β-1,3-glucanase and chitinase) and defensins, which have potent antimicrobial activities through the hydrolysis of pathogen cell walls and the disruption of the pathogen membrane, respectively. Their synthesis is induced not only by pathogens, but also by immune signals such as salicylic acid in the absence of pathogen challenge. Therefore, the term 'PR proteins' is really a misnomer, as these antimicrobial proteins are the executioners of plant immunity. Fourteen classes of PR protein (PR1–PR14) are currently recognized in plants[102]. Early efforts in engineering disease resistance in plants through the overexpression of PR proteins showed that they are not as effective when induced individually compared with when they are coordinately expressed[103]. It is known that distinct sets of PR proteins are induced in response to different pathogens. In *Arabidopsis thaliana*, PR1, PR2 (a β-1,3-glucanase) and PR5 (thaumatin) are induced by salicylic acid in response to biotrophic pathogens, whereas PR3 (a chitinase), PR4 (a chitinase) and PR12 (a defensin) are induced by jasmonic acid in defence against necrotrophic pathogens[104]. Moreover, the regulation of a large set of endoplasmic reticulum-resident proteins is required to ensure proper folding, transport and secretion of PR proteins[105]. Plant genomes have the capacity to produce a large array of PR proteins. For defensins alone, 317 defensin-like sequences were found through a search of the *A. thaliana* genome[106]. The defensin genes are present in clusters, probably as a result of gene duplication and divergent or purifying selection. Defensins are found not only in plants, but also in insects and mammals, and they have diverse immune functions against bacterial and fungal pathogens as well as herbivorous insects. Therefore, understanding the regulation and the activities of PR proteins is a crucial part of immunological research.

Taken together, the available data indicate that the establishment of systemic immunity involves the transport of multiple mobile signals from the site of initial infection to the entire plant. The perception of these signals in systemic tissues leads to the accumulation of salicylic acid, which mediates transcriptional reprogramming through activation of the co-activator NPR1. Ultimately, this results in the expression of antimicrobial proteins, the concerted action of which confers broad-spectrum pathogen resistance.

## Immune memory

Adaptive immunity in animals confers long-lasting resistance after primary antigen recognition owing to the formation of memory immune cells. Consequently, secondary exposure to the same antigen triggers an accelerated and more-effective immune response. Despite the absence of specific memory immune cells, the SAR response in plants also confers a long-lasting memory of primary pathogen attack but is far less specific than adaptive immune memory in animals. Consequently, SAR provides an enduring, heightened state of resistance against secondary attack by a broad range of pathogens. Moreover, some studies seem to indicate that this immune memory not only can provide life-long protection for the plant, but might also be transmitted to subsequent generations.

***Establishing long-lasting immunity.*** Similarly to investigations of SAR in plants, studies of immunity in invertebrate animals (which also do not have a typical adaptive immune response) show that primary pathogen exposure can induce life-long protection against secondary infection[72], indicating that immune memory might be a common phenomenon. Immune memory in plants and invertebrate animals has been associated with cell priming, which results in a sensitized state that allows cells to respond faster and with greater amplitude to secondary pathogen attack, thereby rapidly limiting pathogen proliferation and spread. However, the molecular mechanisms of cell priming are not well understood. Priming is widely speculated to result from the cellular accumulation of signalling components that are activated only following exposure to a secondary pathogen attack. Recent work in plants seems to confirm this hypothesis. In *A. thaliana*, chemical- or pathogen-induced priming correlates with the accumulation of the mitogen-activated protein kinases MPK3 and MPK6 (at both the mRNA transcript and protein levels). However, these kinases are maintained in an inactive state in primed cells and require pathogen or chemical challenge for activation[73]. Interestingly, priming of MPK3 and MPK6 expression was abolished in *npr1* mutant plants. This is consistent with the previously mentioned role of NPR1 in the transcriptional reprogramming of cells to prioritize immunity. Moreover, primary pathogen attack was previously shown to induce the accumulation of transcriptionally active NPR1 monomers in systemic tissues[74]. Thus, the accumulation of immune signalling components, such as MPK3, MPK6 and NPR1, could confer long-lasting resistance to secondary pathogen attack.

Box 3 | **Regulation and function of the systemic acquired resistance co-activator NPR1**

Several reports have shown that salicylic acid and its analogues trigger transient oxidative and reductive changes in plant cellular redox states[107]. These changes regulate the conformation of NONEXPRESSER OF PR GENES 1 (NPR1), allowing it to switch reversibly between a disulphide bond-mediated oligomeric complex and a monomeric state in the cytoplasm[74,108]. Monomeric NPR1 translocates to the nucleus, where it forms a complex with members of the TGA family of transcription factors, some of which may also undergo redox-regulated conformational changes[109,110]. The marked change in global transcription induced by NPR1 is reminiscent of that induced by the master immune regulator nuclear factor-κB (NF-κB) in mammals. In contrast to the nuclear translocation of NPR1, which is controlled by a redox-sensitive oligomer–monomer exchange, the nuclear translocation of NF-κB occurs when its inhibitor (IκB) is phosphorylated and degraded by the proteasome. In addition to the nucleocytoplasmic regulation of their activities, NPR1 and NF-κB also have in common a pulsatile accumulation in the nucleus (with a period of ~100 minutes for NF-κB; NPR1 pulses have not yet been measured at the single-cell level). For NF-κB, this is largely due to a delayed negative feedback loop created by NF-κB-dependent transcriptional activation of the gene encoding IκB[111–113]. Interestingly, the persistence, period and amplitude of NF-κB pulses seem to differentially activate immune-related genes[112,113]. In the case of NPR1, its transient accumulation in the nucleus as a transcriptionally active monomer is regulated by changes in the cellular redox state, coupled with its proteasome-mediated clearance from the nucleus[108,114,115]. Although the details of how NPR1 pulses control downstream transcriptional events require further investigation, blocking the proteasome-mediated degradation of NPR1 in the nucleus delays and decreases the transcription of certain target genes[114]. This implies that NPR1 proteins that have initiated a transcription event might need to be cleared from the gene promoter to efficiently release RNA polymerase II and/or to reset the promoter to allow the re-initiation of transcription.

It is not completely clear how NPR1 brings about the chromosomal changes that prime target genes for enhanced transcription. Surprisingly, salicylic acid induces the recruitment of RAD51, BREAST CANCER SUSCEPTIBILITY 2 (BRCA2) and SUPPRESSOR OF SNI1 2 (SSN2; a homologue of the yeast protein Sws1) to the promoters of NPR1 target genes[75,76]. These are highly conserved proteins in eukaryotes that are involved in DNA repair and homologous recombination. Moreover, salicylic acid and DNA damaging agents (such as bleomycin) have synergistic effects on immune gene induction (S. Yan and X.D., unpublished observations). It is plausible that chromatin remodelling by proteins involved in DNA repair and homologous recombination might underpin gene priming, but the specific mechanism of this priming effect needs to be further elucidated.

Changes in the methylation and acetylation status of DNA and histones have been associated with the activation of immune-related genes in plants[77]. The latest evidence now indicates that epigenetic modifications might also have an important role in providing plants with a long-lasting immune memory. Local pathogen infection was shown to modify the methylation and acetylation status of histones at gene promoters in systemic tissues[78]. In particular, trimethylation of histone H3 lysine 4 (H3K4me3) at certain gene promoters was strongly induced in distal tissues following local pathogen infection, and this modification correlated with the potentiated expression of immune-related genes following challenge. Intriguingly, both H3K4me3 modification and the associated potentiation of immune gene transcription required NPR1, indicating that this co-activator also orchestrates epigenetic transcriptional poising[78]. Hence, a combination of epigenetic control mechanisms and an abundance of signalling components seems to be responsible for the development of long-lasting immune memory in plants (FIG. 3).

Redox state
A term that can be used narrowly to describe the ratio of interconvertible oxidized and reduced forms of a specific redox couple (such as $NAD^+$–NADH), but that can also be used broadly to describe the cellular redox environment, which is determined by the states of all of the redox couples combined.

***Transgenerational memory of immunity.*** R genes have been found to reside in clusters within plant genomes. It is thought that such clusters are the result of successive rounds of duplication and unequal recombination, enabling diversification of the genes within these clusters and subsequent selection for greater specificity and effectiveness[14,79]. Curiously, epigenetic changes have been suggested to influence the stability of these gene clusters. Hypomethylation in the *A. thaliana bal* variant (which was generated in the *ddm1* (*decreased DNA methylation 1*) background) was shown to be associated with the tandem duplication of a 55-kb region containing six R genes[80]. Moreover, rearrangements in N gene-like loci, which contain R genes that may confer resistance against TMV, correlate well with local DNA hypomethylation in tobacco plants[81]. In addition, following on from pioneering work in maize[82], more-recent reports have indicated that biotic stress increases genome instability[77,81,83,84]. Taken together, these findings make it tempting to speculate that increased pathogen pressure promotes the formation of new R genes by locally changing the epigenetic chromatin landscape to destabilize R gene clusters and allow for gene rearrangements. The rare R gene recombination events that are beneficial under a particular pathogen pressure could then be inherited by the plant progeny (FIG. 3).

The repressor of plant immunity SNI1 might be involved in regulating the chromatin landscape of immune-related genes. A loss-of-function mutation in *SNI1* changes the acetylation and methylation status of the chromatin encompassing the immune marker gene *PR1*, and this change mimicks the pathogen-induced state of this gene[85]. Mutant *sni1* plants also have enhanced levels of somatic homologous recombination[86], suggesting that SNI1 might control recombination rates through chromatin remodelling. Notably, genetic screens for mutations that suppress the constitutive defence phenotype of *sni1* mutant plants have so far exclusively identified genes involved in DNA repair and homologous recombination, such as *BRCA2* and *RAD51*, implying that the mutant *sni1* phenotype is largely

due to an increase in the activity of DNA repair machinery[75,76,86]. A major future challenge remains to determine whether pathogen-induced DNA rearrangements occur at specific genomic sites, including R gene loci.

How could stress-induced somatic homologous recombination lead to a transgenerational memory of stress? Stress-induced somatic homologous recombination in a cell can lead to the formation of a sector within the plant that has an enhanced stress-resistance trait; such sectors have an advantage within the plant, as they are more successful than other sectors. Unlike animals, plants do not have a preset embryonic germ line; instead, plant gametes arise from somatic tissues. Thus, successful somatic sectors that give rise to gametes allow reproduction and preferential transmission of the stress-resistance trait[87]. Indeed, it was previously reported that an ultraviolet B radiation-induced somatic rearrangement of a reporter construct in *A. thaliana* was stably transmitted to subsequent generations, indicating that somatic homologous recombination events can introduce permanent genetic changes in plant populations[88].

Interestingly, it has been reported that the progeny of parental plants that were exposed to a MAMP or pathogen maintain increased levels of somatic homologous recombination in the absence of pathogen stress[81,83]. This indicates that successive plant generations might be heritably poised to cope with environments of high pathogen pressure. Many abiotic stresses also induce homologous recombination, but in only a select few cases does the increased level of somatic homologous recombination in the parent plant persist in unstressed progeny[83,89,90]. This suggests that transgenerational memory is not a general response to environmental stresses, but is instead specific to certain types of stress, such as pathogen attack. The molecular basis for this phenomenon is likely to be epigenetic and subject to dynamic changes. Such a hypothesis can now be tested, as genome-wide high-resolution mapping of DNA methylation has been carried out in wild-type *A. thaliana* as well as in the DNA methyltransferase-null mutant (*ddc*; for the *drm1*, *drm2* and *cmt3* triple mutant)[91]. Similar surveys can be repeated in response to immune induction in treated parental plants and in the untreated progeny.

In summary, long-lasting immune memory might be established by the enhanced accumulation of signalling components and by epigenetic changes that prime gene promoters. Moreover, it has been suggested that plants can establish transgenerational immune memory through epigenetic changes and by increasing the rate of DNA rearrangement to generate new R genes. But whether this is a widespread phenomenon requires further investigation.

## Perspectives

We have described how the plant immune system adopts unique strategies that render it highly pathogen specific with intrinsic autoimmune tolerance owing to R protein-mediated cellular surveillance, which enables plants to induce immunity in distal tissues through the long-distance transport of hormones and lipid-derived molecules. These strategies also provide potentially life-long or transgenerational memory of immunity through cellular priming and somatic homologous recombination. However, it should be noted that we have only discussed defence mechanisms against biotrophic pathogens, which rely on live host cells either completely or partly in their life cycle. Immune responses against necrotrophic pathogens (which feed on dead host cells) and herbivorous insects are mechanistically distinct from or even antagonistic to those used against biotrophs. Nevertheless, these responses are also highly specific and in some cases can generate long-lasting memory. We also know very little about the immune mechanisms used by roots and anticipate that they may be quite different from those used in leaves. Similarly to the digestive tracts of animals, roots are constantly associated with microorganisms, most of which are beneficial to plant health. Therefore, immune responses in roots have to be well controlled to distinguish friend from foe. Major challenges remain in understanding the dynamic and spatial regulation of these various immune responses and their interplay with other cellular functions. There is also a need for molecular studies on how plant immune function and memory operate in large populations and in long-lived plants, such as trees. Hence, plant immunity still has many mysteries that remain to be solved.


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### Competing interests statement
The authors declare no competing financial interests.

### FURTHER INFORMATION
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