

# Tracing the ancient origins of plant innate immunity

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**Resistance to pathogens is one of the most ancient traits; mechanisms for discriminating self from non-self have evolved to accomplish this task. Animal and plant immune systems use a set of similar receptors to recognize pathogens. These receptors are located either at the cell surface or inside the cell. Kinases modulate further signalling and are either associated to the receptors or are part of the receptors themselves. In this review, we compare gene families and the nucleotide binding (NB) and the Toll-interleukin-1 receptor (TIR) domains of various kingdoms that are important for the immune systems. Possibilities to deconstruct and reconstruct evolutionary events contributing to the immune systems are explored together with functional aspects.**

## To live or let die – the ultimate sacrifice to ward off parasites

One of the most important aspects of the evolution of higher life is the ability to prevent parasitic symbiosis (see Glossary) by distinguishing pathogens from mutualistic symbionts and neighbouring cells of identical genetic composition (self recognition). The complex challenge posed by biotic stress throughout evolution is particularly important among multi-cellular eukaryotic organisms, where some cells undergo programmed cell death (PCD) for the greater good of the organism. One response to pathogens is a local cell suicide, denying the pathogen access to the nutrients in adjacent living cells. Immune responses are tightly associated with cell death processes and appear to have developed multiple times from components in the PCD machinery. This ‘altruism’ can also be seen among so-called social microbes – which primarily grow as unicellular organisms, but where genetically identical cells can organize into multicellular structures under certain conditions. For example, the slime mould *Dictyostelium discoideum*, a protist that diverged before the fungi and metazoan split, and *Volvox carteri*, a green algae, undergo PCD incited by stress and development [1]. This more ancient or primitive PCD mechanism shows common properties associated with multicellular organisms of the so-called crown group (plants, fungi and animals). The presence of common features in PCD among early

branching eukaryotes indicates a conserved core mechanism for PCD that pre-dates independent evolutionary events leading to multicellularity [1,2].

A common first line of defence found across phyla is the recognition of indispensable structures from pathogenic microorganisms, such as lipopolysaccharides (LPS), flagellin and chitin [3]. These essential components represent so-called pathogen-associated molecular patterns (PAMPs), which effectively identify alien cells. The term PAMPs can be misleading because this line of defence does not distinguish between mutualistic and parasitic symbiosis and accordingly should be denoted MAMPs (microbe-associated molecular patterns). MAMP recognition implies additional events downstream of the initial recognition that determine the level of host response and differentiation between pathogens and mutualistic or commensalistic microbes. In addition to intrinsic MAMPs, microbe-induced molecular patterns (MIMPs) derived from host molecules (either host cell degradation products or perturbed target proteins of a virulence factor) can be detected by a plant [4]. The MIMP concept is partly analogous to the wider definition of the ‘danger signal’ including damage-associated molecular patterns (DAMPs) used for animal innate immune triggers [5]. The two most well studied host systems for responses to pathogens and their pathogenicity components are those in plants and animals (Box 1). Despite a striking similarity in the general design of pathogen recognition receptors (PRRs) in the plant and animal phyla, many of the similarities are

## Glossary

**Biotroph:** an organism that can live and reproduce only on another living organism.

**Early branching eukaryotes:** eukaryotes not part of the crown group of plants, animals and fungi. Usually the term only refers to eukaryotes that diverged prior to the plant and animal split.

**Immunosuppression:** events leading to reduction of the efficacy of the immune system.

**Innate immunity:** a non-adaptive but dominant system of host defence.

**Necrotroph:** an organism that kills host tissue and lives on energy obtained from the dead cells.

**Rosetta stone principle:** the assumption that physically fused protein domains have functional links. This hypothesis can be used to predict conserved protein–protein interactions between proteins that swap domains (indicative of functional associations).

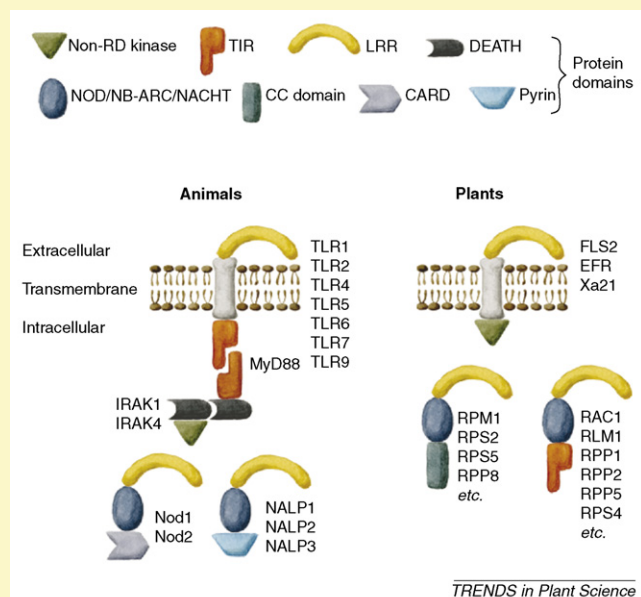
**Symbiosis:** two (or more) organisms in close association. Can be *commensalistic* (one member has benefits and the other is not affected), *mutualistic* (both members have benefits), or *parasitic* (one member has benefits and the other is harmed). Other relationships do also exist.

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### Box 1. Innate immunity in animals and plants

The two most studied immune systems, animals and plants, reveal striking similarities in domain usage and domain structures (Figure 1). In animals, recognition of pathogens at the cell surface is mainly carried out by a family of pathogen recognition receptors (PRRs) encoding Toll-like receptors (TLRs). TLRs are characterized by an extracellular leucine-rich-repeat (LRR) domain and an intracellular TIR protein–protein interaction domain. Activation of TLRs triggers a signalling pathway including activation of the nuclear factor-kappa B (NF- $\kappa$ B) transcription factor. TLRs also associate with the interleukin-1 receptor-associated kinase (IRAK) family and with receptor interacting-protein (RIP) kinases via adaptor proteins such as MyD88 and its own adaptor-like protein MAL [82]. MyD88 has an N-terminal death domain (DD) that recruits the IRAKs. A sub-class of IRAKs and RIPs lacks the RD motif (non-RD) kinases, and does not always take part in autophosphorylation, which often takes place upon pathogen recognition. Animals contain several classes of intracellular PRRs, of which nucleotide oligomerization domain (NOD) receptors are one important group known to detect bacterial pathogens. The NOD proteins are composed of variable domains, for example, LRRs, the caspase-recruitment domain (CARD) and NOD/NB-ARC/NACHT. The inflammasome, a multiprotein complex containing NALP proteins, activates pro-inflammatory caspases upon pathogen recognition. They belong to the NACHT-LRR family of cytoplasmic proteins, which also includes the NOD proteins. Many NALPs have a pyrin domain that is thought to interact with caspases crucial for proteolytic activation of downstream signals.

In plants, two classes of immune receptors have evolved. The membrane-resident receptor-like kinases (RLK), such as the flagellin receptor FLS2, recognize conserved microbe-associated molecular patterns (MAMPs) analogously to animal PRRs [83]. Non-RD kinases are intracellular domains of membrane-spanning receptors and, upon activation, a MAP kinase cascade triggers WRKY transcription factors, reactive oxygen species (ROS) also accumulate and a basal immune response occurs. However, a more specific system has evolved in plants, where NOD-nucleotide binding (NB) and LRR composed R proteins with N-termini consisting of either a coil-coil (CC) or TIR domain, recognize pathogen effector proteins intracellularly and trigger the immune responses. A link between PRR- and NB-LRR R protein triggered immunity in plants has recently been found involving repression and depression of MAMP- or PAMP-triggered basal defence via WRKY and MLA R proteins [51].



**Figure 1.** Schematic representation of the key proteins that are partners in the immune responses in both animals and plants.

likely to be the result of convergent solutions [6]. Different organisms have evolved ingenious solutions to overcome the problem of rapidly evolving pathogens. The jawed vertebrates have, in addition to innate immunity, evolved an adaptive immunity as a secondary defence to encounter any possible type of pathogen attack [7]. By contrast, plants only have innate immunity but have an impressive array of secondary (MIMP) detectors of pathogen suppression attempts of PAMP (MAMP) triggered immunity (PTI) [8,9]. This strategy relies on pluralism at the population level and the durability of a single resistance trait is dependent on the pathogen population structure and reproduction strategy [10]. The complex stress condition posed by pathogens on an organism that primarily relies on an innate immune system could be one of the main reasons for the evolution and maintenance of sexual reproduction and recombination, despite its fitness costs [11]. Many key regulatory components, such as the NF- $\kappa$ B transcription factors in animals and the WRKY transcription factors in plants, are not found outside their respective phyla, indicating that important aspects of immunity have developed in (at least) two independent events [6].

By contrast, there is also a range of components that show a striking similarity between the plant and animal innate immunities and PCD, which all derive from a pre-eukaryotic origin [12]. These components were supposedly part of a ‘murder programme’ of the early parasitic endosymbionts that later developed into chloroplasts and mitochondria. The cell death mechanism was then adopted by the eukaryotes to become essential in various processes. Today PCD is, for example, an integrated part of both plant development and defence. Some of these conserved protein domains also show conservation at higher levels of complexity in innate immunity – at the level of protein–protein interactions. Thus, the immunity of higher eukaryotes originates from a parasite – a beautiful irony of evolution. Based on our current knowledge, we have acquired some glimpses of the complex networks regulating the immune responses in plants and animals and their interactions with other cellular mechanisms (Box 1). The resemblance shows strong support for a common origin of several central components whereas others have independently evolved analogous solutions [13]. However, the functional context of the common ancestors of the components that are conserved has not been established. Because none of the ancestor organisms exist today, we need to infer the origin and function of the different conserved units by genome comparisons between present day early branching eukaryotes and the crown group [14]. Here the increasing amount of full genome sequences enables high-resolution reconstruction of the evolutionary events [15,16]. Cross-phylum comparisons in immunity have been suggested to be of mutual benefit for both plant and animal research, in particular in enhancing our understanding of the mechanisms of immune malfunction and PCD. For example in autoimmune diseases in animals and in lesion mimic mutants in plants [17].

Here we discuss how the emergence of genome sequences of the early branching organisms that diverged before the evolution of higher plants and animals offers a new possibility to trace back the recruitment of different

domain partners and the evolution of the mechanisms known today. We propose that establishment of the functional origin of the different components in the immune systems in an 'evo-devo' approach can give potential clues to its current function and its interactions with other cellular mechanisms.

### **NOD – a cell death domain twice recruited to NB-LRR proteins**

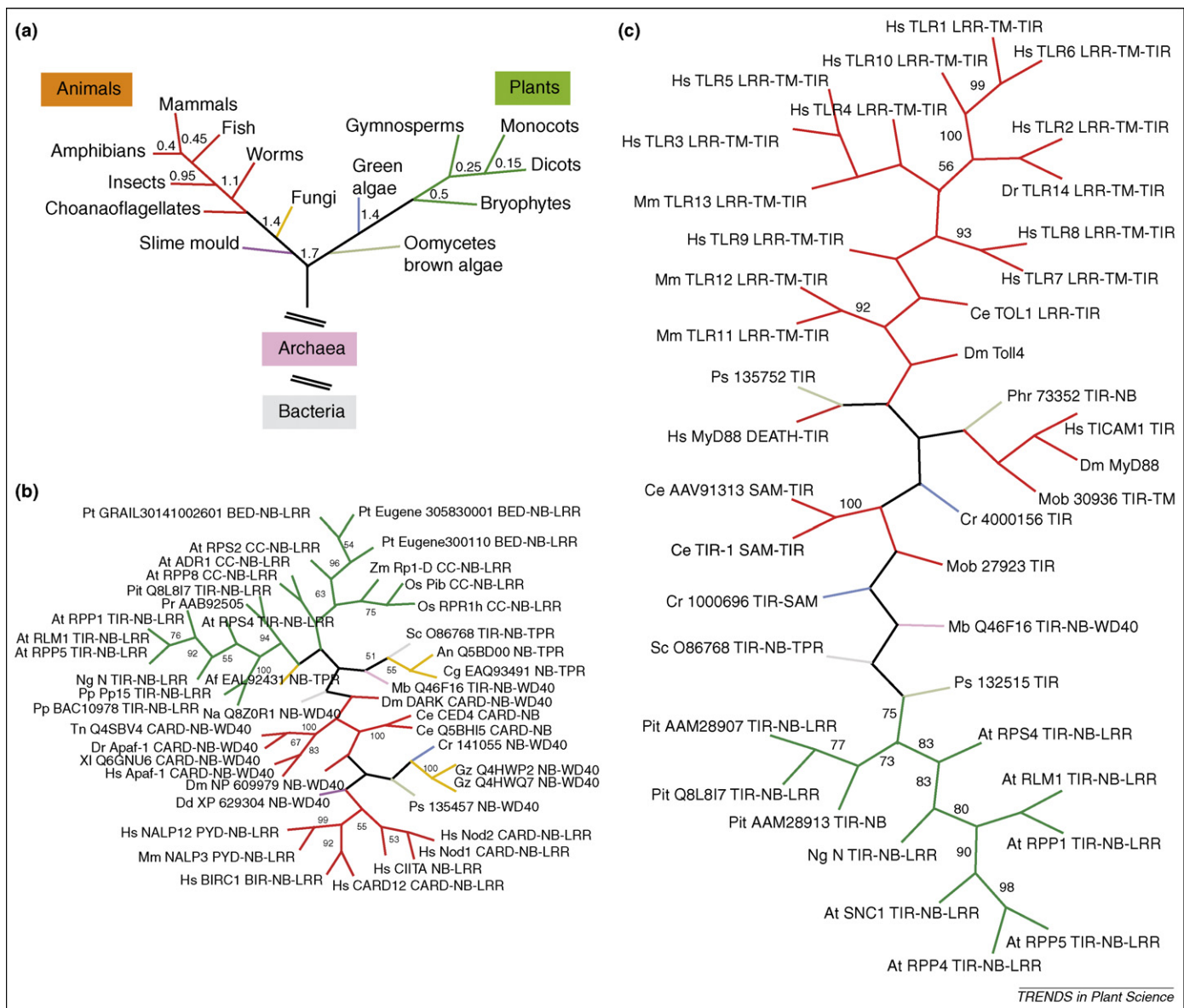
One of the largest gene families in plants encodes the nucleotide binding – leucine rich repeats (NB-LRR) class of disease resistance (R) proteins. NB-LRR R proteins have a tripartite domain structure, where the N-terminal can be composed of either a Toll/interleukin-1 receptor (TIR), a coiled coil (CC) or a *Drosophila melanogaster* BEAF and DREF (BED) zinc-finger domain [18]. Members of the NB-LRR family (CC-NB-LRR and TIR-NB-LRR so far) have been associated with disease resistance to a wide range of pathogens as diverse as viruses, bacteria, fungi, oomycetes, nematodes and insects. In *Arabidopsis thaliana* (*Arabidopsis*), the NB-LRR class comprises 149 genes, divided into 92 TIR-NB-LRR encoding genes, 51 genes that encode CC-NB-LRR proteins and six where the N-terminal domain is lost. In addition, there are also 58 genes encoding proteins lacking the LRR domain [19]. The plant NB-LRR resistance proteins can either be triggered via direct recognition of a pathogen component or via indirect detection of a pathogen immunosuppression attempt [20]. Upon pathogen recognition, a special form of rapid and highly localized PCD called hypersensitive response (HR) is induced. Several aspects of HR-PCD, such as cytological markers, sphingolipid elicitation and elicited cell death in plants expressing mammalian Bax, all occur commonly in animal apoptotic PCD (extensively reviewed in Ref. [21]). Nearly all NB-LRR proteins that have been associated to disease resistance confer resistance to biotrophic pathogens [22], but there are examples of NB-LRR R proteins in *Arabidopsis* that confer resistance to pathogens with a necrotrophic life style [23]. TIR-NB-LRR encoding genes have not been found in any of the monocots investigated and the entire gene family appears to be rapidly lost under certain conditions. To date, the dicot *Beta vulgaris* is unique because it lacks TIR-NB-LRR encoding sequences [24]. This discovery indicates that the conclusions about R protein motif distribution among monocots and dicots are over-simplified because of the limited genome information available. Despite the rapid loss of the entire gene family, TIR-NB-LRR encoding genes can be found as far back in plant evolution as gymnosperms (*Pinus monticola*, *Pinus taeda*) [25,26] and even bryophytes (*Physcomitrella patens*) [27]. The TIR domain has also been found associated to NB as far back as bacteria and archaea, indicating that this domain organization has an ancient origin (Figure 1). However, the NB domain of the TIR-NB-LRR family is more conserved than that of the CC-NB-LRR family, implying that the NB domain is under greater functional constraints when associated with TIR. This could be one reason why the age of the TIR-NB-LRR family was underestimated compared with that of the CC-NB-LRR family when only NB sequences from *Arabidopsis* were considered [28]. The *P. patens* full genome sequence, which

will be announced imminently, will hopefully reveal more information concerning the origin, domain distribution and evolution of the plant NB-LRR encoding R gene family.

The NB domain of plant disease resistance proteins is homologous to the NB domain of human Apaf-1/CED4 (apoptotic protease activating factor/Cell death 4, a central factor in apoptotic PCD among animals) and was denoted NB-ARC [29]. Both these proteins are members of the AP-ATPase sub-class of the STAND superfamily [30]. A general feature of the STAND NTPase class of proteins among eukaryotes is a rapid expansion and gene loss, leading to great diversity. The STAND sub-class NACHT GTPases is an important group of proteins in animal innate immunity [31] that was lost in the plant lineage at a relatively late stage of evolution given that green algae still contain proteins with this domain (Box 2). The AP-ATPase and NACHT GTPase domains cannot be clearly distinguished, leading to a proposed 'NOD' domain including both classes [31]. In animals, the NB-LRR (synonymous NLRs or CATEPILLER proteins) are also involved in innate immunity. For example, the human CARD-NB-LRR proteins NOD1 and NOD2 are involved in MAMP recognition and interact extensively with other parts of the innate immunity. Likewise, the human PYD-NB-LRR proteins NALP1 and NALP2 or NALP3 constitute the inflammasome, which activates pro-inflammatory caspases upon MAMP recognition [32]. A common feature in cell death induction between at least one plant NB-LRR protein (N) and human Apaf-1 and NOD proteins is an oligomerization upon elicitation [33]. However, there are far fewer NOD-type encoding genes in animals than in plants, only 26 in humans compared with 207 in *Arabidopsis* [31]. A striking exception is the purple sea urchin genome, which contains 203 genes of this class [34]. It is tempting to speculate that this animal has evolved this class of proteins as detectors of immunosuppression attempts analogously to plant R genes. The STAND NTPase superfamily of proteins among eukaryotes has only been found in multicellular organisms or social microbes. Analogously to the recognition roles of this class of proteins in plants and animals, NACHT-containing proteins have been implicated in self and non-self discrimination for parasexual fusion of vegetative mycelia among filamentous fungi to form heterokaryons [30,35].

### **TIR – a protein interaction domain used in immune responses**

The evolution of the TIR domain indicates a common pre-eukaryotic origin together with the NOD domain (Figure 1). This circumstance supports the early parasitic endosymbiont hypothesis of the origin of the two domains [12]. It also corroborates the notion that the TIR-NB-LRR domain structure would represent the ancestral R protein encoding gene in plants. In the animal lineage, the TIR domain has experienced considerable evolution from ancient duplications, which is in agreement with previous observations [36]. Contrary to previous assumptions based on observations from *Caenorhabditis elegans* [6], the TLR family and associated signalling components appear already in sponges. This indicates that the innate immune system found among modern animals arose early in



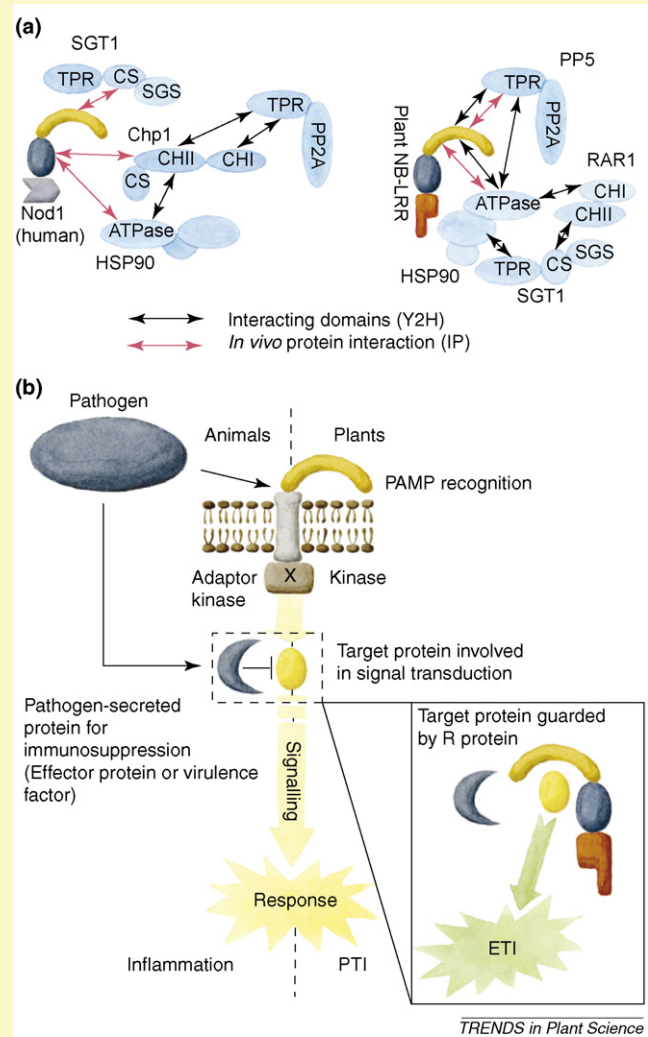
**Figure 1.** Comparative phylogenomics between nucleotide binding (NB) and Toll-interleukin-1 receptor (TIR) domains. The TIR and NB domains play a role in both plant (TIR-NB-LRR) *R* genes and in animal innate immunity (TLRs and NLRs). To investigate the relationships and origin of these two domains, sequences from multiple organisms in different phyla were used in the phylogenetic analysis. NB and TIR domain information from various organisms were retrieved from NCBI, JGI, BLAST and the PFAM databases (<http://www.sanger.ac.uk/Software/Pfam/>). The TIR and NB-ARC/NOD/NACHT domain amino acid sequences were retrieved from NCBI (<http://www.ncbi.nlm.nih.gov/entrez/>), AGI (<http://www.Arabidopsis.org>) and JGI (<http://www.jgi.doe.gov/>) based on protein domain annotations. The border of the domains for each entry was confirmed with multiple alignments in CLUSTALW (<http://www.ebi.ac.uk/clustalw/>). The phylogenetic analysis was performed using PAUP\* 4.0 b 10 [84]. The analysis reveals a clear split between NB-TPR and NB-WD40 ancestors. Both the prokaryotic TIR-NB-WD40 and TIR-NB-TPR sequences are found at this split, indicating an ancient divergence. Plant *R* genes evolved from an NB-TPR ancestor, whereas the animal sequences originate from the NB-WD40 lineage. This division does appear to be the result of a loss of sequences after the plant–animal split in both lineages given that fungi contain NB-TPR sequences and oomycetes and algae contain NB-WD40 sequences. The BED-NB-LRR class has recently been found in *Populus trichocarpa* and has not yet been associated with any disease resistance [18]. The BED-NB-LRR class appears to be relatively recent and evolved from a CC-NB-LRR origin. Analogously to the NB phylogeny, the prokaryotic TIR-NB proteins are located at the split between the plant and animal TIR sequences, indicating an ancient divergence. The presence of an oomycete TIR sequence indicates that the TIR domain found in animals diverged earlier than the plant–animal split. By contrast, the plant TIR-NB sequences show little variation, which could either be because of a late rapid expansion of this gene family or because of functional constraints that led to conservation of the TIR domain. (a) A schematic tree with estimated splits between phyla and organisms in billion years ago, supported by multiple independent sequences for each organism or complete genomes [15,85]. Part (a) is the colour template for cladograms in (b) and (c). (b) Distribution of the nucleotide binding domain (NB) between different phyla. (c) Distribution of the Toll-interleukin-1 receptor domain (TIR) between different phyla. The data shown in (b) derive from one of three most parsimonious trees (majority rule, 50%) inferred from NB domain sequences. Tree length = 5589 steps, consistency index (CI) = 0.610, retention index (RI) = 0.482. (c) One of the 13 most parsimonious trees (majority rule, 50%) inferred from TIR domain sequences. Tree length = 2415 steps, consistency index (CI) = 0.603, retention index (RI) = 0.499. The topologies obtained by the Neighbour-Joining method are the same in both cases. Bootstrap values 50 are indicated (1000 replicates). Abbreviations: Af, *Aspergillus fumigatus*; An, *Aspergillus nidulans*; At, *Arabidopsis thaliana*; Ce, *Caenorhabditis elegans*; Cg, *Chaetomium globosum*; Cr, *Chlamydomonas reinhardtii*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; Dr, *Danio rerio*; Gz, *Gibberella zeae*; Hs, *Homo sapiens*; Mb, *Methanosarcina barkeri*; Mm, *Mus musculus*; Mob, *Monosiga brevicollis*; Na, *Nostoc anabaena*; Ng, *Nicotiana glutinosa*; Os, *Oryza sativa*; Pr, *Pinus radiata*; Phr, *Phytophthora ramorum*; Pp, *Physcomitrella patens*; Ps, *Phytophthora sojae*; Pit, *Pinus taeda*; Pt, *Populus trichocarpa*; Sc, *Streptomyces coelicolor*; Tn, *Tetraodon nigroviridis*; XI, *Xenopus laevis*; Zm, *Zea mays*.

metazoan evolution [37]. This recent finding highlights the need for observation in multiple evolutionary lineages to draw conclusions about the evolutionary history. In the plant lineage, it appears as if a single ancestral TIR-NB

has remained relatively conserved and can be found as a free TIR domain in the oomycete *Phytophthora sojae*, but has been lost in the green algae *Chlamydomonas reinhardtii* (Figure 1). However, the green algae do contain several

### Box 2. Immune systems are conserved across phyla at higher levels of complexity

In plants, few host factors have been identified that directly interact with intercellular NB-LRR proteins and participate in receptor function. Among those identified, RAR1 (required for *Mla12* resistance), HSP90 (heat shock protein 90) and SGT1 (suppressor of the G2 allele of *skp1*) are bound to each other and are required for resistance to various plant pathogens. RAR1 contains two highly conserved zinc-binding domains, CHORDI and CHORDII (cysteine- and histidine-rich domain). SGT1 is required for accumulation of NB-LRR R proteins and probably controls steady-state levels of pre-activated R proteins [86]. Protein phosphatase 5 (PP5) contains a tetratricopeptide repeat (TPR) domain that is involved in protein-protein interactions with the NB-LRR complexes in both plants and animals (Figure 1) [46,47]. Despite being involved in protein-protein interactions, the TPR domain of SGT1 is not required for SGT1 to affect plant R genes. The TPR domain of PP5 has been found to modulate its protein phosphatase activity [46]. The mammalian NOD1 and NOD2 proteins share domain composition with many plant R proteins. Chp-1 is a mammalian homologue of RAR1 and interacts with the TPR domain of PP5 and the ATPase domain of HSP90 via CHORDI and CHORDII. NOD1 interacts with this HSP90 chaperone complex. The CS domain of Chp-1 is found in plant SGT1, indicating ancient conserved protein-protein interactions. To circumvent MAMP (PAMP)-triggered immunity (PTI), pathogens, particularly bacteria, have the ability to deliver intracellular effector proteins. In the situation when these proteins are not recognized, they suppress the MAMP-triggered immunity by interfering with the MAP kinase signalling, resulting in enhanced pathogen growth, or effector-triggered susceptibility (ETS). If appropriate R proteins are present, they recognize the effector proteins and trigger an immune response, effector-triggered immunity (ETI). One plant strategy is to use NB-LRR proteins to guard against effector proteins deployed to inhibit PTI. However, few cases of direct interaction between avirulence protein and NB-LRR protein exist [9]. Suppression of MAMP-induced responses is also required for infection of animals [76]. Pathogens that are able to infect both hosts are dependent on a set of common virulence factors, which could be involved in immunosuppression. Hence, it is likely that the target proteins of that virulence factor would be similar between plants and animals.



**Figure 1.** (a) Interactions between conserved NOD protein complexes in humans and plants based on different protein interaction analysis. (b) Schematic representation of the immunosuppression processes and secondary detection of immunosuppression in plants.

other TIR encoding sequences with sequence similarities to TIRs in protists and the animal lineage, resulting in multifaceted appearances. Taken together, the phylogeny of the TIR domain indicates that it separated from the NB partner and experienced an expansion before the plant and animal split. The TIR domain that remained associated to NB in the plant lineage represents a divergent branch of the domain, and other TIR encoding sequences were lost relatively late (after green algae) in plant evolution (Figure 1). It is possible that the TIR-NB group present in plants represents a later occurrence of horizontal gene transfer. A reciprocal conserving force on the TIR domain from the NB domain, analogous to what can be seen in the NB domain, is a more likely explanation. Despite the early split and obvious differences in domain composition for TIR-containing proteins in plants and animals, both are primarily used in proteins involved in immunity. Recent results also show that a role for the TIR domain in developmental processes exists for both plants and

animals [38]. The traditional view of the TIR domain is that it simply acts in protein-protein interactions, linking downstream signalling domains via a TIR::TIR dimer. A special feature of the TIR domain is that there seems to be high selectivity in the dimer formation [33]. One could speculate that signalling attenuation is particularly important in immune responses [39] and that the TIR domain dimerization would provide some specific regulatory possibilities that could explain its evolutionary conservation in immunity. Alternatively, the TIR domain could be implicated in as yet unknown functions. For example, the TIR domain from Toll-like receptors (TLRs) in animal innate immunity has been suggested, based on primary structure, to be a GTPase, but it has so far not been experimentally confirmed [40]. In plants, the TIR domain of RPP1A interacts with a small GTPase of the Rab family in a yeast-2-hybrid screening, which could suggest that TIR has other signalling roles than just the formation of homodimers [41].

### The 'rosetta stone principle' and conserved or convergent protein complexes

Plants and animals mediate early steps of the innate immune response via pathogen recognition receptors (PRRs), and several downstream components show striking similarities. There is no detectable homology or only a low level of similarity between plant PRRs and animal TLRs and TLR-recruited kinases, suggesting that they have evolved independently as part of a convergent evolution [3,6]. However, convergence does indicate that there are functional constraints or general conditions that must be met by an immune system. Some of the constraints leading to convergence might be other conserved components in the immune system or other cellular mechanisms. Domain swapping between interacting protein domains is, according to the 'rosetta stone principle', a common mechanism in evolution and can be used for predicting conserved interactions [42]. One could speculate that an ancient interaction between TIR-containing proteins and a trans-membrane LRR kinase could lead to the two different designs and explain the near-exclusive use of non-RD kinases lacking a conserved arginine in kinase sub-domain VI [43], and the presence of TIR domains in the immune responses of both systems. In humans, NOD1 influences TLR4-dependent responses to LPS [44] and also plant NB-LRR proteins have been found to act as signalling components downstream of trans-membrane LRR receptors [45]. Stronger proof for conserved interactions leading to domain swaps is found in the protein complex associated with NB-LRR proteins in plants and animals (Box 2). NB-LRR proteins in both systems interact with RAR1/Chp1, HSP90, PP5 and the CS domain present in SGT1 in plants and the animal Chp1 [46,47]. Interestingly, recent studies by two independent groups showed that the CS domain of human SGT1 interacts with NOD proteins and is required for immune responses [48,49]. The CS domain swap between SGT1 and Chp1 is evidence of conserved protein-protein interactions. Another possible similar evolutionary domain swap within the complex can be found among fungi and protists. Fungi and protists contain proteins with an NB-TPR domain structure (Figure 1), whereas the TPR domain in PP5 interacts with the LRR domain of NB-LRR proteins in plants and animals (Box 2). Another strong indication of evolution according to the 'rosetta stone principle' is the fusion of a WRKY domain to the LRR domain of RRS1-R [50], which indicates that WRKY transcription factors interact with plant NB-LRR R proteins. A concept recently shown to be valid in the barley-*Blumeria graminis* defence response [51].

### Caspases and caspase-like activities in cell death and immunity

Like the TIR and NOD domains, the caspase superfamily of proteases (caspases, paracaspases and metacaspases) shares a common pre-eukaryotic origin [12,52]. In animals, a family of Cys-dependent Asp-specific proteases called caspases initiate signalling cascades that can act either pro-apoptotic or pro-inflammatory (pyroptosis) depending on the protein target. Although the single paracaspase MALT1 in humans does not have the Asp proteolytic

activity associated with caspases and the Cys dependency for induction of immune responses is not definite, the MALT1 is a crucial component in several immune signalling pathways. The pro-inflammatory caspases represent a phylogenetic sub-group of the pro-apoptotic caspases indicating that immune responses are derived from cell death responses – a specialization that was initiated at least before the insect and vertebrate split [53]. Para- and metacaspases are ancestors of the caspases and are unequally distributed among phyla. *Dictyostelium discoideum* and animals contain paracaspase, whereas plants and fungi only contain metacaspases. Like the animal paracaspase, the plant metacaspases do not share the proteolytic activities that the true caspases possess. Plant metacaspases show an Arg/Lys-specific proteolytic activity rather than the Asp-specific activities that define the true caspases [54,55]. Caspase-1-like proteolytic activity via VPEg has been shown to be involved in plant immunity to both biotrophic and necrotrophic pathogens [56,57]. Some plant metacaspases are induced upon pathogen stress [58,59] and have been shown to induce downstream caspase-like proteolytic activity and PCD in yeast and in spruce embryonic development [59–61]. This could be a direct regulation via proteolytic activation of inactive proteases (zymogens), analogous to the caspase signalling cascades in animals, given that the metacaspases interact with proteins with caspase-like activities [62]. Interestingly, metacaspase activity in plants, like animal caspases, is regulated by S-nitrosylation via GSNOR [63], which is a conserved nitric oxide (NO) signalling immunity component [64,65].

Apoptosis is a relatively recent form of PCD and is not present among plants or fungi, which rely on a more ancient mechanism, autophagic PCD [1,66]. Conserved components for autophagic PCD in plants have been shown to be important for the restriction of runaway cell death from pathogen-induced HR, and similar roles for autophagy restriction of pathological cell death has also been seen during embryo development [62,67]. An appealing hypothesis would be that the true caspases represent a signalling shortcut, where the proteolytic activities induced by para- or metacaspases have been adopted by the caspases. A likely interpretation is that the ancestral organisms before the slime mould split and the fungi and animal split contained both meta- and paracaspases and that either one or the other was lost in the different lineages. It is not yet certain whether paracaspase evolved before or after the plant and animal split from a metacaspase ancestor, and no obvious roles in developmental or immunological PCD have been found [68].

### Signalling events, pathogen recognition and responses

Both plants and animals activate their immune responses via a MAP kinase signalling cascade and use a specific subclass of non-RD kinases for activation of the immune responses [43]. Small signalling proteins or peptides for the induction of defences are produced, but no detectable homology can be seen between animal cytokines and plant signalling peptides. Despite this, there are trans-membrane RING-finger ubiquitin ligases with a similarity to animal cytokine receptors that interact with NB-LRR

proteins and influence HR in plants [69]. Animal inflammatory responses as well as plant pathogen responses result in the induction of an array of antimicrobial proteins that show a remarkable similarity, such as the defensins [70], PR1-like proteins [71] and chitinases [72]. The similarities in the antimicrobial responses indicate that some antimicrobial responses were present before the split between plants and animals and, hence, also before the (independent) evolution of multicellularity in the two lineages.

### Pathogen-mediated immunosuppression – indirect evidence of similarities between plant and animal immunity?

Several pathogens, such as *Burkholderia cepacia*, *Cryptococcus neoformans*, *Enterococcus faecalis*, *Erwinia carotovora*, *Fusarium oxysporum*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are able to infect both plant and animal hosts. These pathogens harbour a common set of virulence factors that are required for infection [73–75] that is likely to suppress MAMP-triggered immunity [76,77]. For example, the plant pathogen *E. carotovora* has been shown to suppress LPS responses in cell cultures of *Drosophila* [78]. Conserved functions in immunosuppression can also be inferred based on comparisons between the effector protein YopT from the human pathogen *Yersinia pestis* and the avirulence protein AvrPphB from the plant pathogen *Pseudomonas syringae*, which belong to the same family of cysteine proteases [79] (Box 2). Recent analyses of oomycete plant pathogens have also revealed a striking similarity in the signal peptides of the effector proteins to the protist malaria parasite *Plasmodium falciparum* [80], suggesting that there are conserved invading pathways into the two hosts. The resemblance of the immunosuppression mechanisms is yet another intriguing sign of the similarities across phyla in the immune response. The constantly growing number of known virulence factors in both plant and animal pathogens and their targets in the pathogen–host interaction (PHI) database [81] might give us clues for future analyses of the relationships between the mechanisms of immunosuppression and the signal transduction pathways for plant and animal immune systems.

### Concluding remarks

The wide array of newly completed genome sequences that diverged relatively early from the characterized vascular plants, worms, insects and vertebrates provides unprecedented opportunities to deconstruct and reconstruct the evolutionary events leading to the immune systems known today via comparative genomics. Functional analysis of organisms more closely related to plants, such as the model algae *C. reinhardtii*, which contains homologues of plant immune components, such as EIN2, EDS5, LSD1 and RAR1, could shed some light on the origin and functional context of some of the known components in plant immunity. With the aid of various genomic and bioinformatics tools, it should be possible to assess the signalling pathways and conserved protein motifs. To understand immune systems fully, pathogens and their effector proteins must be fully integrated into the defence matrices,

as well as an understanding of the genetic variation and its potential for changes both in hosts and pathogens. Such knowledge will generate durable options to defeat diseases both in animals and plants.

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