Plant and Animal Innate Immunity Complexes: Fighting Different Enemies with Similar Weapons

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Review

Plant and Animal Innate Immunity Complexes: Fighting Different Enemies with Similar Weapons

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Both animals and plants express intracellular innate immunity receptors known as NLR (NOD-like receptors or nucleotide-binding domain and leucine-rich repeat receptors, respectively). For various mammalian systems, the specific formation of macromolecular structures, such as inflammasomes by activated NLR receptors, has been extensively reported. However, for plant organisms, the formation of such structures was an open scientific question for many years. This year, the first plant 'resistosome' structure was reported, revealing significant structural similarities to mammalian apoptosome and inflammasome structures. In this review, we summarize the key components comprising the mammalian apoptosome/inflammasome structures and the newly discovered plant resistosome, highlighting their commonalities and differences.

Highlights

The recent characterization of macromolecular structures involved in plant innate immunity, the resistosomes, highlights the commonalities observed between plant and animal innate systems.

Plants and animals have a repertoire of intracellular immune receptors with similar domain structure, which perceive pathogens and initiate a cascade, leading, in both systems, to cell death. Their mode of action is similar, although in plants more sophisticated pathways of recognition have been reported, such as the 'integrated sensor/decoy' model.

dATP exchange in the nucleotide-binding domain of the core proteins of apoptosome/resistosome is crucial for their activation.

In animals, caspases seem to be key players in immunity responses, with a similar activity not yet confirmed for plant proteases.

Plant and Animal Defense Mechanism: An Overview

To understand plant and animal defense mechanisms, the most important challenge is to characterize the macromolecular structures that underlie host immunity machinery. In both plant and animal innate immunity systems, specific intracellular immune receptors, called **NLRs** (see Glossary), protect the organisms against pathogen invasion by activating immunity upon detection of pathogen invasion-associated molecules [1]. In both systems, pathogen perception by NLRs could lead to activation of a localized **programmed cell death (PCD)** [2]. PCD is a central mechanism for the development and homeostasis of all metazoans [3], while in plants, the NLR-related PCD is known as the **hypersensitive response (HR)** and its main role is to restrict pathogens from spreading to host tissues [2,4].

The two main macromolecular complexes that have been identified to play a central role in two animal PCD pathways, **apoptosis** and **inflammation**, are the **apoptosomes** and the **inflammasomes**, respectively [3]. In plants, however, whether there is a formation of macromolecular structures similar to apoptosomes/inflammasomes has been an open question for many years. Recently, such a structure has been reported. In the light of this exciting finding, the focus of this review is to highlight the key components comprising mammalian apoptosome/inflammasome in parallel with the newly emerging structure of the plant '**resistosome**'.

NLR Receptors in Plants and Animals

Animal and plant NLRs reveal significant structural similarities [5] and have been proposed to follow similar activation/inactivation molecular rules [2]. They form part of a structural class termed signal transduction ATPases with numerous domains (STAND) (Figure 1) [6]. The core domain of plant NLRs is a nucleotide-binding domain, termed the nucleotide binding sequence with an ARC domain (NB-ARC domain), shared between the human apoptotic protease-activating factor-1 (APAF-1), R proteins, and *Caenorhabditis elegans* death-4 protein (CED4) [7]. The animal analog is termed NACHT domain [NLR family apoptosis inhibitory protein (NAIP)], MHC class 2 transcription activator (CIITA), HET-E (incompatibility locus protein from *Podospora anserina*), and telomerase-associated protein (TP1) [8]. A striking difference between the nucleotide-binding domains of animal and plant NLRs is related to the absence of the third helical domain (HD) subdomain (Figure 1), which is substituted by a short linker [9]. In plants, the NB-ARC domain is followed by a leucine-rich repeat (LRR) domain, while their amino terminal domain, which has been proposed to play a role in signaling, varies. Plant NLRs are divided into two major groups, depending on the presence at their amino

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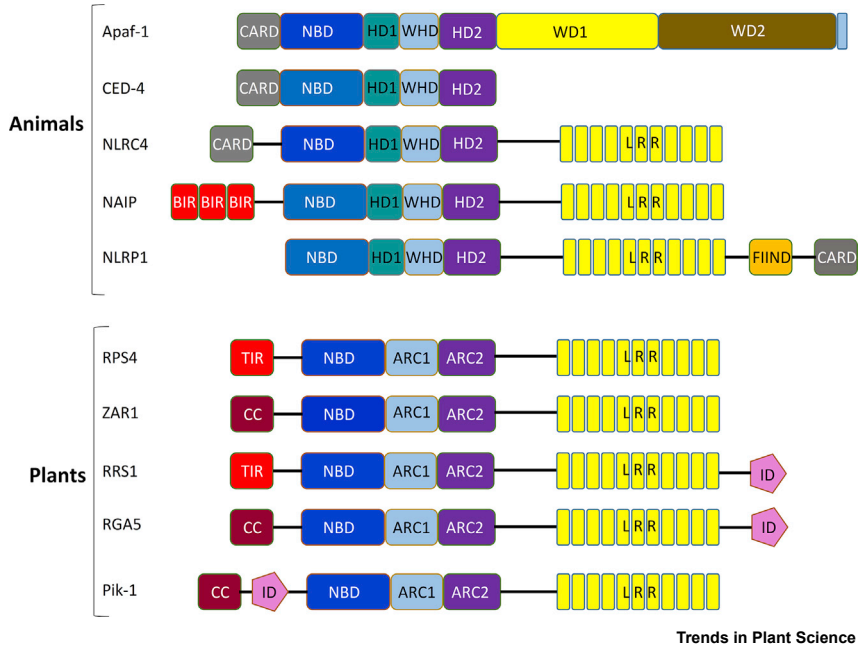

Figure 1. Schematic Representation of the Key Domains Comprising the Mammalian and Plant NLRs.
The domains are depicted as colored boxes. Abbreviations: BIR, Baculovirus inhibitor of apoptosis protein repeat; CARD, caspase recruitment domain; CC, coiled coil domain; HD, helical domain; ID, integrated domain; LRR, leucine-rich repeat; NBD, nucleotide-binding domain; NLR, nucleotide-binding domain and leucine-rich repeat receptors; TIR, Toll/Il-1 receptor; WHD, winged helix domain.

terminal of a Toll/interleukin1 receptor (TIR), the TIR-NLRs (TNLs), and the non-TIR-NLR (nTNL); the second group contain mostly a coiled-coil (CC) domain and thus are often called CNL [10–12]. The mammalian NLRs contain a **caspase** recruitment domain (CARD), a pyrin domain (PYD), or a baculovirus inhibitor of apoptosis protein repeat (BIR) at the N terminus. Phylogenetic analysis of the plant and animal NLR ATPase domains suggests that the acquisition of these domains probably occurred independently in each kingdom [13].

Apart from these three domains, an interesting feature of the plant NLRs has recently been identified. Certain plant NLR receptors carry fusions of additional integrated domains (IDs) to the canonical TIR/CC-NB-ARC-LRR structure [14] (Figure 1). According to studies in *Arabidopsis thaliana* [15,16] and rice [17,18], IDs seem to serve as decoys for **effectors** by mimicking their real subcellular target in the host cell. *In silico* analyses have shown that a large number of NLRs contain IDs and that some of these IDs overlap with previously identified pathogen targets [14]. To accommodate the mode of function of these plant NLRs with IDs (NLR-ID), the so called 'integrated decoy/sensor' model has been proposed [15,19,20] (discussed below). However, animal NLRs with IDs have only been identified in two cases, mouse NLRP1b [21,22] and the human NLRX1 [23] (discussed below). Whether animal NLRs with IDs are as widespread as in plants remains an open question.

A major difference between animal and plant NLR receptors relates to the type of the stimuli that leads to their activation. The animal NLR receptors typically detect conserved **pathogen-associated molecular patterns (PAMPs)** or **damage-associated molecular patterns (DAMPs)** [24–27]. NOD1 and NOD2, for example, recognize specific peptidoglycan components of bacterial origin (γ-D-glutamyl-meso-diaminopimelic acid and muramyl dipeptide, respectively) [28,29], while NLRP3 can be activated by various PAMPs and DAMPs (e.g., pore-forming toxins, adenosine triphosphate, and others) [30].

Glossary

Apoptosis: a process essential for the maintenance of cellular homeostasis in mammals, which involves a series of events leading to a programmed cell death.
Apoptosome: a multimeric protein complex formed during the process of apoptosis. Apoptosome formation is triggered by the release of cytochrome-c from the mitochondria in response to an internal ('intrinsic' pathway) or an external ('extrinsic' pathway) cell death stimulus.
Caspases: a family of endoproteases important in apoptosis and inflammation. They are initially produced as inactive monomeric procaspases that require dimerization and often cleavage for activation. This process is performed mainly in apoptosomes and inflammasomes.
Damage-associated molecular patterns (DAMPs): host molecules released after cellular stress or damage that can initiate a noninfectious inflammatory response in mammals.
Effector: molecules secreted by plant pathogens into plant cells; they facilitate infection by modulating plant immunity.
Hypersensitive response (HR): a type of rapid cell death used by plants to restrict the spread of invading pathogens in the infected area.
Inflammasome: multimeric protein complexes that assemble intracellularly in response to pathogens and other damage associated signals. Inflammasome assembly leads to the activation of inflammatory responses resulting to a type of cell death, distinct from apoptosis, termed pyroptosis.
Inflammation: a biological response of the animal's immune system triggered by infectious (pathogens) or noninfectious agents (e.g., irradiation).
NLRs: nucleotide-binding domain and leucine-rich repeat receptors that protect the organisms against pathogen invasion by activating immunity upon detection of pathogen invasion-associated molecules.
Pathogen-associated molecular patterns (PAMPs): microbial components that are not found in multicellular hosts (e.g., flagellin,

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Plant NLRs, however, typically recognize intracellular pathogen-translocated virulence components, known as effector proteins. The phytopathogen effectors were initially characterized as 'avirulence' (Avr) proteins, based on the quick defense activation they trigger, which, upon their recognition by an NLR receptor, regularly leads to HR activation. This recognition typically leads to the prevention of the disease development and inhibition of pathogen growth and spread to other plant tissues [31]. For some plant NLRs, pathogen recognition occurs via the direct interaction between NLR and specific pathogen effectors. For example, the LRR domains of the plant RPP1 and Pi-ta NLR receptors interact with their cognate ligands [32,33]. However, in some cases, it has been reported that the TIR- or the CC-domains can interact with the Avr ligands [34,35]. For a number of plant NLRs, pathogen recognition occurs indirectly [2]. To explain this indirect recognition, 'guard/decoy' models have been proposed. According to these models, some plant NLRs guard specific host components involved in plant immunity (guardee), or proteins with no obvious role in plant immunity (decoy) that have been proposed to function as pathogen 'effector baits', and detect modifications triggered by pathogen effectors during host colonization [2]. Both the guard and decoy recognition models propose effiient mechanisms by which a host plant is able to use a limited number of NLR receptors to recognize different pathogens through the specifi guarding of a limited number of host proteins. To date, the only animal NLR reported to function in a similar way is the human NOD1 receptor. NOD1 detects modifications imposed by a virulence factor of the enteric pathogen *Salmonella* (SoPE) on distinct Rho GTPases and initiates the NOD1 signaling pathway [36]. Activation of Rho GTPases is also sensed by other non-NLRs (e.g., pyrin), leading to inflammatory responses [37,38].

A modification of this guard/decoy model is the integrated decoy/sensor model, in which key players are NLR receptors with integrated domains (NLR-IDs) that usually are members of NLR receptors pairs [15,19,20]. According to studies in *A. thaliana* [15,16] and rice [17,18], IDs serve as decoys for pathogen effectors, by mimicking their real subcellular target in the host cell. The interaction of the ID domain with an effector potentially results in conformational changes to NLR-ID of the pair (the 'sensor'), which in turn are sensed from its paired NLR (the 'executor'). These paired NLRs are usually genetically and physically linked [15,16,18]. Recent reports also indicate that many plant genetically unlinked NLRs cooperate with each other in order to function [20,39–41], while some of these NLRs appear to have a helper role in NLR-mediated defense, indicating the presence of specialized NLR networks in plants [40–43].

Parallels can be drawn with the mammalian NLRC4/NAIP cooperation and the related inflammasome construction (see below), as well as with the cooperation of NOD2/NLRP1 for the recognition of the PAMP muramyl dipeptide [44]. Furthermore, a mechanism similar to the plant integrated sensor/decoy model has been reported in mouse NLRP1b [21,22,45] and human NLRX1 [23]. NLRP1b carries at its N terminus an integrated cleavage site for the lethal factor (LF) metalloprotease of *Bacillus anthracis*. When this site is cleaved off by the LF, an inflammation response is activated. Similarly, the NLRX1, the only human NLR family member with a mitochondrial targeting sequence, contains an LC3-interacting region (LIR), which directly associates with LC3 through the LIR. The pathogenic bacterium *Listeria monocytogenes*, through the virulence factor listeriolysin O (LLO), induces oligomerization of NLRX1 to promote binding of its LIR motif to LC3 for the induction of mitophagy [23].

Lastly, and concerning the subcellular localization of the animal and plant NLRs, animal NLRs are mainly cytosolic while plant NLRs localize in various cellular compartments (cytosol, nucleus, Golgi, tonoplast, etc.) [46]. This probably relates to the different mechanisms by which animal and plant NLRs sense the corresponding stimuli but also to the different interacting components that need to activate the immune responses. While many of the mechanisms of animals NLRs activation and signaling transduction have been elucidated, the signaling transduction pathway(s) underlying the plant NLRs still remain obscure, leaving an open question regarding the role of the plant NLRs' differential subcellular localization. What has been made clear from various studies so far, is that proper localization is necessary for plant NLRs to function [47–50].

Altogether, NLR receptors from the plant and animal kingdom have similar architectures but seem to follow largely different modes of stimuli perception.

lipopolysaccharides, etc.) and are recognized by innate immune receptors [pattern recognition receptors (PRRs)], leading to immune responses.

Programmed cell death (PCD): a general term used to describe the variety of genetically controlled pathways ultimately leading to cell death. PCD is essential for proper development and defense against pathogens, operating in both the animal and plant kingdom, and is classified in different groups (e.g., apoptosis, pyroptosis, hypersensitive response, autophagy, etc.), depending on defining features that participate in each pathway.

Resistosome: multimeric protein complex formed in plant cells in response to pathogens, leading to cell death (hypersensitive response).

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Box 1. Animal Caspases and Caspase-like in Plants

The primary role of the apoptosome/inflammation platforms is the activation of constitutively synthesized zymogen molecules, termed procaspases giving rise to the active enzymes, the cysteine-aspartic proteases (caspases) [87,88]. Caspases belong to the family of cysteine proteases that usually cleave their substrate after the aspartic acid residue. In their inactive form they contain an amino terminal peptide of variable length, a feature sometimes used to make a rough classification into two groups, the initiator and executioner caspases. The initiator caspases have a long prodomain involved in protein–protein interactions [death effector domain (DED) or caspase recruitment domain (CARD)] [2], with molecules functioning upstream of the pathway (e.g., Apaf-1), while the executioner caspases have shorter prodomains and when activated, usually by the initiator caspases, cleave multiple cellular substrates and ultimately lead to immune responses such as apoptosis. The first cloned caspase is the mammalian interleukin 1β-converting enzyme (ICE), now known as caspase-1 [89,90]. To date, 11 distinct caspase genes have been identified in the human genome, while a large number has been characterized for several other mammalian genomes [91]. Five of these caspase genes have been connected to inflammatory processes (caspase-1, -4, -5, -11, -12), while at least seven other mammalian caspase genes have been connected to apoptotic pathways (caspase-2, -3, -6, -7, -8, -9, -10). It is noteworthy that some caspases may participate in both the apoptotic and the inflammatory processes (e.g., caspase-4), making unclear the boarders between apoptosis and inflammation [3].

Although plant genomes code for hundreds of proteases with various roles [92], caspase orthologs are absent in plants, with the closest relative being metacaspases (cysteine proteases that cleave after arginine or lysine); however, these do not have caspase activities [93]. The term caspase-like proteases has been widely used to describe the corresponding activities observed in plants. Such activities have been detected indirectly with the use of caspase-specific peptide inhibitors and caspase substrates or with the identification of a number of plant proteases involved in various types of plant PCD [94–96]. In all cases, evidence for a *bona fide* caspase activity is missing, which either suggests redundancy in plant proteases with caspase-like activities or that the operation of PCD mechanisms in plants are different from the corresponding mechanisms in animals. Unfortunately, the signaling events downstream of signal perception still remain a black box, making it even more difficult to decipher a role of caspase-like proteases.

Macromolecular Structures Related to Innate Immunity

The APAF-1 Apoptosome

The apoptosome is a macromolecular structure formed during the 'intrinsic' apoptotic pathway [51], while the 'extrinsic' pathway leads to the formation of another macromolecular structure, the death-inducing signaling complex (DISC) [52]. Both structures serve as 'docking' platforms for the activation of specific effector molecules, the apoptotic caspases (Box 1). The intrinsic pathway is also termed the mitochondria-mediated apoptotic pathway, due to the key role mitochondria play in apoptosis [53], with the mitochondrial release of cytochrome-c being the initiator of apoptosome formation in mammals (Figures 1–3) [54].

The best studied mammalian apoptosome is the APAF-1 apoptosome (Figures 1–3). The *Apaf-1* gene is the human homolog of the *C. elegans ced-4*, which together with *ced-3* are the first apoptotic proteins identified [55]. Apaf-1 is a cytoplasmic protein that forms a central hub in the mammalian apoptosis regulatory network and contains a CARD domain at the N terminus, a central ATPase domain (NB-ARC), few short helical domains, and several copies of the WD-40 domain at the C terminal part (Figure 1). Each of these domains is involved in the autoinhibition, activation, and oligomerization of the protein. More specifically, in the absence of stimuli (cytochrome-c), inactive Apaf-1 retains a monomeric folded form with dATP bound in its NB-ARC domain [56,57]. This folded form is the result of the association of the WD-40 domain with the N terminal part of the protein [58]. The first step in Apaf-1 activation is the interaction of the WD-40 with cytochrome-c. This interaction leads to release of the close conformation of the Apaf-1 protein [58,59]. Although WD-40 seems to be the 'lock' for opening the apoptosome formation pathway, its interaction with cytochrome-c is not sufficient to initiate the pathway [60]. This is regulated by the core ATPase domain activity of Apaf-1, the NB-ARC domain (Box 2). Following the first conformational changes of Apaf-1, the ATPase activity of NB-ARC drives the hydrolysis of the bound dATP to dADP that again is

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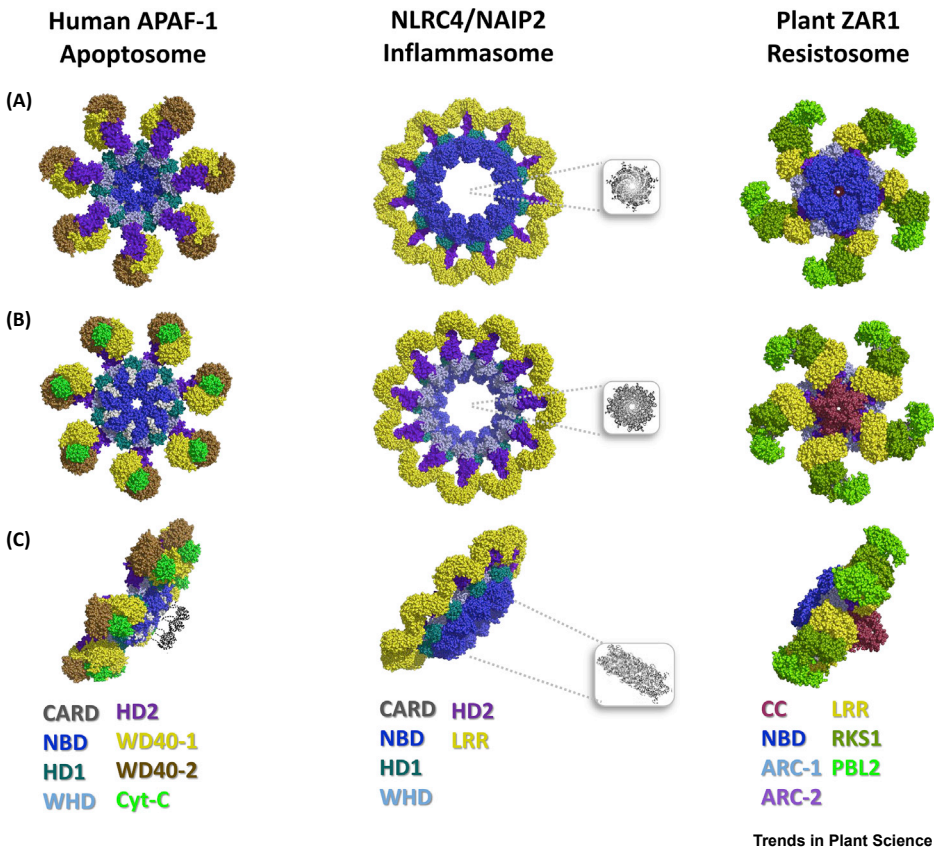

Figure 2. Three-Dimensional Models of Active Human Apoptosome, Human Inflammasome, and Plant Resistosome.

Each macromolecular structure is depicted from different angles; top view (A), bottom view (B), and side view (C). Each of these three structures form wheel-shaped complexes with different types of symmetry (sevenfold symmetry for APAF-1 apoptosome, 11-fold for the NLRC4/NAIP inflammasome, and pentameric for the plant resistosome). The structures were obtained from the Protein Data Bank (PDB) (ZAR1, PDB code: 6J5T; APAF-1 PDB code: 3JBT; NLRC4/NAIP inflammasome, PDB code: 3JBL). The figure was generated using the 2010 PyMOL Molecular Graphics System, Version 2.0.4. CARD domains of APAF-1 and NLRC4 structures have been added manually since they were not included in the accessions of PDB codes used. Abbreviations: APAF-1, Apoptotic protease-activating factor-1; CARD, caspase recruitment domain; CC, coiled coil domain; HD, helical domain; NAIP, NLR family apoptosis inhibitory protein; NBD, nucleotide-binding domain; NLR, nucleotide-binding domain and leucine-rich repeat receptors; PBL2, PBS1-LIKE PROTEIN 2; WHD, winged helix domain; ZAR1, HOPZ-ACTIVATED RESISTANCE 1.

not sufficient for the apoptosome formation [56,60]. The (d)ADP/(d)ATP exchange acts as the final check point for unlocking Apaf-1, thus preventing accidental formation of the apoptosome upon a signal not matching a certain threshold level [51]. If this threshold is reached, Apaf-1 attains a more open conformation, which on one hand allows access of the amino terminal CARD domain for recruiting the caspase-9 (the effector protease of this pathway) and on the other leads to Apaf-1 oligomerization [61,62]. This results in Procaspase-9 protein cleavage for the release of its mature activated form, which stimulates the subsequent cascade leading to the cell apoptosis.

According to data from cryo-electron microscopy (cryo-EM) studies [57,63], the activated APAF-1 apoptosome forms a wheel-shaped heptameric complex with a sevenfold symmetry structure (Figures 2 and 3). This wheel-like structure has seven spokes and a central hub with Apaf-1 oriented in an extended fashion. The caspase-9 molecules bind the CARD domains at the central hub, forming

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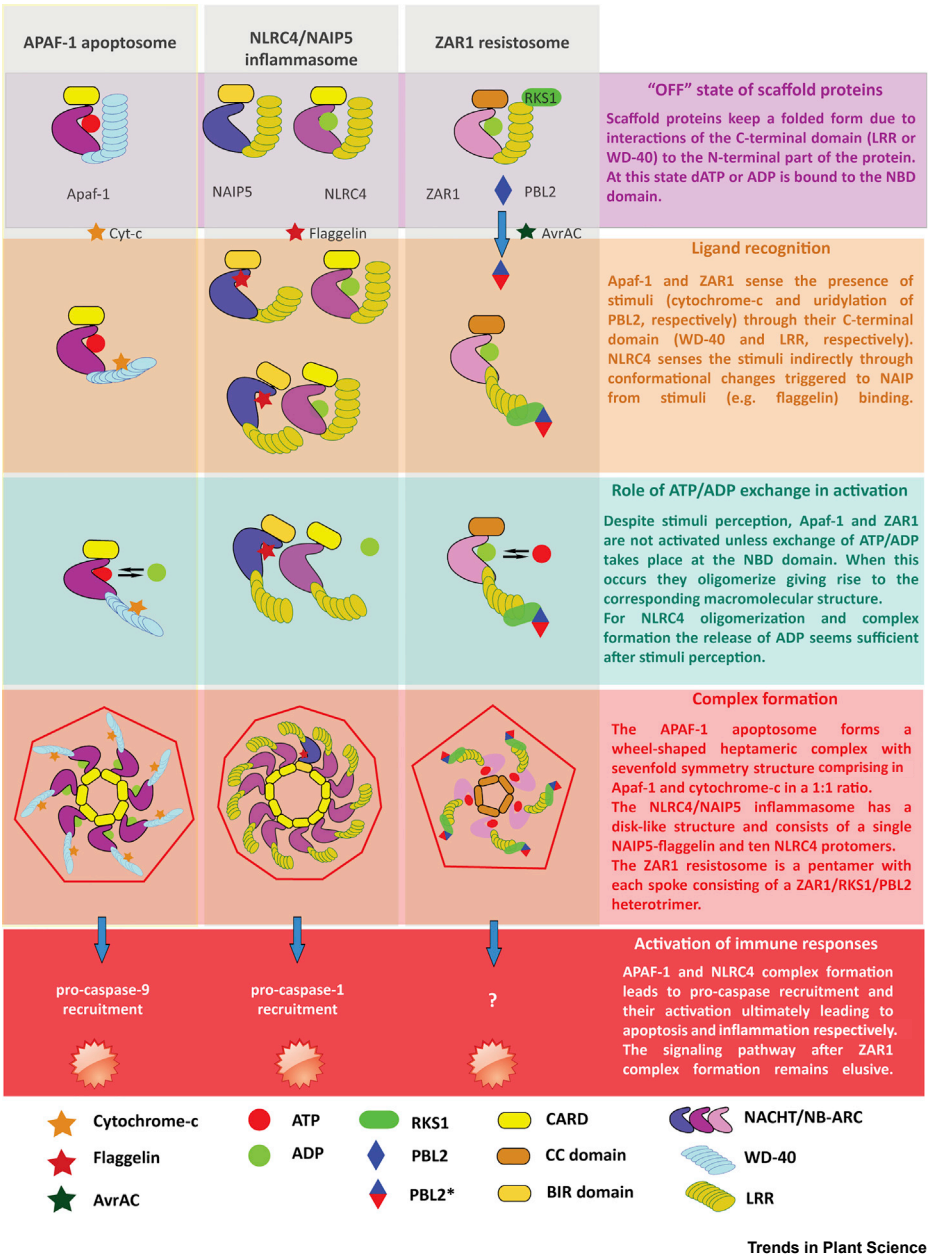

Figure 3. Overview of APAF-1 Apoptosome, Plant Resistosome, and NLRC4/NAIP Inflammasome Activation Pathway.

Apoptosome formation is triggered by the release of cytochrome-c from the mitochondria in response to an internal or an external cell death stimulus. Upon binding of dATP and cytochrome-c to the C terminus of APAF-1, the cytosolic protein APAF-1 forms an oligomeric apoptosome. The activated APAF-1 apoptosome forms a wheel-shaped heptameric complex with a sevenfold symmetry structure. NLRC4/NAIP5 inflammasome formation is triggered by the detection of bacterial stimuli (e.g., flagellin), which is sensed by NAIP5. As a result, NAIPs co-oligomerize with NLRC4, leading to the formation of a disk-like structure, similar to that of the apoptosome, containing 11 monomers. The trigger for ZAR1 resistosome formation is the uridylation of PBL2 by the AvrAC effector of *Xanthomonas campestris* pv. *campestris*. The uridylated PBL2 (PBL2*) is recognized by the

(Figure legend continued at the bottom of the next page.)

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Box 2. Role of ADP/ATP Equilibrium in Activation of Immunity Complexes

Animal NLR receptors are thought to function as binary molecular switches, with the ADP-bound form corresponding to the monomeric 'off state' and the ATP-bound form to the oligomeric 'on state' [2]. In both NLRC4 and APAF-1 proteins, an ADP molecule is buried at the interface formed by NBD, HD1, and WHD domains (Figure 1). Furthermore, the majority of the amino acids of the NBD and HD1 domains involved in recognition of ADP-forming the P-loop domain are conserved among APAF-1 and other AAA+ ATPases, including the plant NLRs [2,97]. Interestingly, the APAF-1 amino acid residue His438 and the related NLRC4 amino acid residue His443 are uniquely conserved in APAF-1 and NLRs from both mammals and plants, while the targeted mutagenesis of the NLRC4 H443L results in a constitutively active NLRC4 [3,67].

A similar role for ATP has been reported in plant NLR activation. Mutational studies in the nucleotide-binding domain point to its role as activation domain of the protein, with nucleotide binding and ATP hydrolysis being the regulatory parameters for turning from the off to the on state [98], although this might not always be the case [99]. This seems also to stand true for ZAR1 resistosome activation, since, in the absence of ATP, the complex persists as a heterotrimer of ZAR1/RKS1/PBL2* [80].

a dome-shaped structure [64]. Regarding the WD-40 repeats, they have been reported to reach up to 15 in number, composed of a seven-bladed beta-propeller and an eight-bladed beta-propeller [60,63].

Apoptosome complex structures from other organisms have many similarities, but are of quite different sizes and numbers of subunits, with the cytochrome-c not always required for the assembly of apoptosome in non-mammalian species, such as worms and fruit flies. Thus, the fruit fly system, called Dark, has a ring of eight subunits [65], while the nematode apoptosome, called CED-4, is also octameric but much smaller in size and it does not include the regions that would bind cytochrome-c [66].

The NLRC4 Inflammasome

Another macromolecular structure of the animal innate system is the inflammasome, which serves as an effective defense mechanism against infections. Like apoptosomes, inflammasomes are intracellular multiprotein platforms necessary for the activation of inflammation-related caspases (Box 1). Most of the inflammasomes studied to date contain an NLR scaffold protein, such as NLRP1, NLRP3, and NLRC4.

The best studied mammalian inflammasome so far is the one formed by the human NLRC4 (NOD-, LRR-, and CARD-containing 4; also known as IPAF or CARD12) encoded by the *NLRC4* gene. The NLRC4 protein appears to be conserved among a number of mammalian species and it reveals homology to the *C. elegans* CED4 protein. A truncated murine NLRC4 protein was the first member of this family whose crystal structure was reported [67]. The protein family of NLRC4 also includes the transcriptional coactivator CIITA and the inflammasome protein NLRP3. NLRC4 contains an N terminal CARD domain, a central NBD/NACHT domain, and a C terminal LRR domain (Figure 1). As mentioned above, NLRC4 cooperates with NAIP proteins in order to sense the stimuli and form the oligomer. These NLR proteins contain BIR domains at their N termini. In the human genome, only one NAIP gene has been annotated as coding for at least two isoforms, however in mice genome

preformed cytoplasmic ZAR1/RSK1 complex, leading to an ADP release from ZAR1, thus priming but not activating ZAR1. The activation seems to be a result of ATP or dATP binding to the ADP-depleted form of ZAR1, leading to oligomerization of the ZAR1/RKS1/PBL2* complex into a larger form, a pentamer with a funnel-like structure in the center. Abbreviations: APAF-1, Apoptotic protease-activating factor-1; BIR, baculovirus inhibitor of apoptosis protein repeat; CARD, caspase recruitment domain; CC, coiled coil domain; LRR, leucine-rich repeat; NAIP, NLR family apoptosis inhibitory protein; NBD, nucleotide-binding domain; NLR, nucleotide-binding domain and leucine-rich repeat receptors; PBL2, PBS1-LIKE PROTEIN 2; ZAR1, HOPZ-ACTIVATED RESISTANCE 1.

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at least four distinct NAIP genes have been identified [68]. The different NAIP/NLRC4 interactions determine the ligand specificity [69]. This includes the detection of the *Salmonella* type III secretion system (T3SS) component PrgJ, or the bacterial flagellin, by NAIP2 or NAIP5 in mouse, respectively [70] while the same signals are detected by the two human NAIP isoforms [71,72].

At its inactive form the NLRC4 resides in the cytosol in an autoinhibited conformation, as revealed by the crystal structure of the of the truncated mouse NLRC4 lacking the CARD domain [67]. Similar to Apaf-1, the auto-inhibition of NLRC4 is partly achieved by interdomain interactions among NBD, HD1, winged helix domain (WHD), and HD2, which is stabilized by the binding of an ADP molecule. Noteworthy, the relative domain organization among NBD/HD1/WHD is the same between APAF-1 and NLRC4, supporting the theory of a conserved mechanism of autoinhibition [3] (Figure 1). Another NLRC4 domain contributing to the autoinhibitory state of the protein is the C terminal LRR domain. The deletion of the LRR domain leads to NLRC4 activation [67], an aspect not observed in the corresponding truncated form of Apaf-1 [60]. For NLRC4 activation, NAIPs sense the corresponding ligand (flagellin for NAIP5) through the NB domain [73] and not through the LRR domain, as anticipated. Flagellin binding relaxes the closed conformation of NAIP5 by displacing the LRR and HD2 domains. This change exposes NBD and WHD, which in turn binds to NLRC4, initiating a new round of conformational changes of NLRC4 [73]. The oligomerization site of NLRC4 (WHD) becomes exposed, releasing the bound ADP, thus leading to NLRC4 polymerization and formation of the inflammasome [67,73,74]. At this point it should be noted that the release of ADP *per se* might be sufficient for NLRC4 activation without the need of ATP exchange, as it is the case for Apaf-1 activation. The activated NLRC4/NAIP complex, consistent with APAF-1, recruits and activates the inflammatory caspase, caspase-1, which initiates the immune responses. Recruitment of procaspase-1 (the inactive form of caspase-1) often requires or involves a cytosolic adapter called apoptotic speck protein containing a CARD (ASC) [75].

According to cryo-EM data, the NLRC4/NAIP5 inflammasome has a disk-like structure and consists of a single NAIP5-flaggelin and ten NLRC4 protomers [73], a structure similar to the NAIP2/NLRC4 complex [74] (Figure 2). This structure shows pronounced similarities to apoptosomes but also presents a major difference that relates to the stoichiometry of the ligand in each complex. In the apoptosome, the ratio between ligand and scaffold protein is 1:1, while in the inflammasome only one such ligand is present in each complex, pointing to a more sensitive immunosurveillance system [76,77]. The safety lock in this case relates to the separation of sensor/executional function, which in inflammasomes is implemented by different molecules.

Plant Resistosome

Although the plant NLR receptors were discovered in the early 1990s, before the animals NLRs, the potential formation of macromolecular structures similar to apoptosomes or inflammasomes was an open question for the plant kingdom. Since the first molecular characterization of an NLR-coding gene 25 years ago [78,79], no structural or functional data to demonstrate the formation of multicomponent structures by plant NLRs were available, and how plant NLRs function has long been a matter of speculation.

Recently, the definition of the structure and the mechanism of activation of an *A. thaliana* NLR, the HOPZ-ACTIVATED RESISTANCE 1 (ZAR1), has been reported [80,81]. ZAR1 is a conserved *Arabidopsis* CC-NLR, which guards several RLCK class XII pseudokinases, with the decoy kinase PBS1-LIKE PROTEIN 2 (PBL2) being one of them [82]. Among the modifications imposed by various effectors on these pseudokinases is the uridylation of PBL2 by AvrAC of *Xanthomonas campestris* [80,81]. This results in its association with RKS1 (a RLCK class XII pseudokinase) and to a complex formation with ZAR1 that activates innate immunity responses [82].

The cryo-EM analysis of coexpressed ZAR1/RKS1 in insect cells revealed the structure of the ZAR1 resistosome in 'off state' (ADP bound) and 'on state' (ATP bound). The complex appears to be heterodimeric. The RKS1 interacts exclusively with the LRR domain of ZAR1, with the amino acid residues

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that participate in this interaction to be conserved in the other class XII pseudokinases. The association of uridylylated PBL2 (PBL2*) with RKS1 leads to a large conformational change on ZAR1 that triggers the allosteric exchange of ADP from ATP at the binding domain of its NB-ARC [81] (Box 2). An interesting observation is that in the absence of ATP, the complex persists as a heterotrimer of ZAR1/RKS1/PBL2*.

Upon activation, the ZAR1/RKS1/PBL2* complex oligomerizes into a larger form, a pentamer, that triggers the proximity of the amino terminal region of ZAR1 that contains four α-helices in a CC-like domain (Figures 2 and 3). During activation of the ZAR1, the α-helix of the amino terminus changes and is replaced by another α-helix [80]. Activated ZAR1 relocalizes to the plasma, while the α-helices at the N terminus are elevated above the pentamer, forming a funnel-like structure. It is noteworthy that the ZAR1 resistosome could act as the analog of the membrane pores and ion channels that are formed during pyroptosis and necroptosis PCD in mammals [83–85]. Targeted mutagenesis in amino acids of this funnel-like structure alters ZAR1 functions in cell death activation, but not its oligomerization. Negatively charged amino acid residues from the interior of the funnel-structure are required for ZAR1 function but not for its oligomerization, which suggests that the interior of the funnel provides functional specificity to ZAR1.

The structural similarities between the NLR complexes of plants and animals could reflect a common evolutionary origin for these receptors, even if the independent convergence has also been proposed [86]. The key steps from ligand perception to complex formations of each of the above described macromolecular structures are depicted in Figure 3, highlighting the differences/similarities between them.

Outstanding Questions

How does ZAR1 resistosome induce HR? Does its action resemble the apoptosome or inflammasome cell death pathway or does it adopt another mode of action?

Are other components essential for cell death induction through the ZAR1 resistosome?

Are all plant NLRs, including CC-NLRs and TIR-NLRs, or NLRs that are activated after direct or indirect interaction with the ligand, able to form similar macromolecular structures? If so, do they all function by means of ADP/ATP-dependent oligomerization of the NB-ARC domain?

Are plant proteases responsible for caspase-like activities and, if so, how do they relate, if at all, to animal caspases?

Concluding Remarks and Future Perspectives

The immune system of plants and animals seems to be quite different. Animals have an adaptive circulating immune system, while in plants each cell must detect and respond to invading pathogens in order to prevent pathogen spread. Despite this difference, experimental evidence emerging from both fields highlights the striking similarities concerning key features of innate immunity mechanisms that exist between the two kingdoms. Thus, for years it has been shown that plants and animals code for intramembrane (pattern recognition receptors) and intracellular (NLRs) receptors with similar domain architecture and functions. Recently, the identification of the first plant NLR-based resistosome revealed new similarities between plant and animal innate immunity responses. Thus, progress made in one field can help advance our understanding in the other and vice versa.

Furthermore, with the resolution of the first plant NLR complex, plant immunologists finally found 'Ariadne's thread', which will help them find their way out of the labyrinth of plant innate immunity responses to pathogens and ultimately help them answer the longstanding question: 'How is signal transduced after pathogen perception?' Before this, a series of questions closely related to plant resistosomes are waiting to be answered (see Outstanding Questions), opening up a new field in plant immunity that will provide useful guidelines in engineering immune receptors with new recognition capacities for durable crop disease resistance.

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