NOD-Like Receptors: A Tail from Plants to Mammals Through Invertebrates

Alessandra Pontillo[1,*] and Sergio Crovella[2]

[1]*Laboratory of Immunogenetics, Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil;* [2]*Department of Genetics., Federal University of Pernambuco (Recife., Brazil)*

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Abstract: NOD Like Receptors (NLRs) are the most abundant cytoplasmic immune receptors in plants and animals and they similarly act sensing pathogen invasion and activating immune response. Despite the fact that plant and mammals NLRs share homology.; with some protein structure differences.; for signalling pathway.; divergent evolution of the receptors has been hypothesized. Next generation genome sequencing has contributed to the description of NLRs in phyla others than plants and mammals and leads to new knowledge about NLRs evolution along phylogeny. Full comprehension of NLR-mediated immune response in plant could contribute to the understanding of animal NLRs physiology and/or pathology.

Keywords: NLR, plant, invertebrate, mammals, LRR, domains.

INTRODUCTION

NOD-Like Receptors (NLRs) are the most abundant cytoplasmic immune receptors in plants and animals and, despite the evolutionary distance and some differences in the domain' structure, they similarly act sensing pathogen invasion and activating immune response. Originally described in plants and mammalians, recently NLRs were discovered also in some invertebrates' phyla. In this review we summarise recent findings on plants and invertebrates NLRs.; focusing on structure and mechanism of action, similarities with mammals NLRs.; and evolutionary hypotheses about plant and animal NLRs.

The structure of a typical NLR consists in three domains, a C-terminal leucine rich repeat (LRR) domain, a central nucleotide binding and oligomerization domain (NB or NOD), and an N-terminal domain that mediates the receptor specific effector function. The LRR domain is commonly indicated as the receptor motif for pathogen's conserved molecular patterns (Pathogen Associated Molecular Patterns/PAMPs and/or Danger Associated Molecular Pattern/DAMPs).-LRRs can sense pathogens by directly binding or indirectly.; through pathogen-induced alterations of host proteins. The central domain (known as NACHT in mammals and NB-ARC in plants) mediates the activation of the receptor in an ATP-dependent way (ADP/inactive state.; ATP/activated state). In animals it mediates NLRs homo or hetero-oligomerization leading to the assembly of multimeric complex responsible for intracellular signal transduction, such as inflammasome or NODsome [1]. NB-ARC seems to be important also for plant receptor dimerization or oligomerization, however, larger molecular complex have not yet been described [2]. NLR N-terminal domains are generally involved in downstream signalling and immune response, such as enzymatic activation of effector molecules and/or activation of transcription factors, finally leading to inflammation/immune response and/or programmed cell death (PCD). Several types of N-terminal domains have been described: death fold domains (DEATH, CARD and DED) present in plants and animals, coiled-coil (CC) and TOLL/interleukin 1 (TYR) domains in plants, peptidase and kinase domains in invertebrates, pyrin and Baculovirus Inhibitor of apoptosis protein Repeat (BIR) domains in mammals.

NLRs have been thoroughly studied in humans where mutations in specific genes are causative of rare genetic diseases (i.e., *MHC2TA*/CIITA in Bare Lymphocyte Syndrome, *NLRP3* in Cryopyrin Associated Periodic Syndrome/CAPS, *NLRC4* in Autoinflammation with infantile enterocolitis/AIFEC) as well as polymorphisms in several *NLR* genes have been associated with disorders characterized by inflammatory component [3, 4]. The 22 humans NLRs were classified into 5 categories according to their N-terminal domain: NLRA, NLRB, NLRC, NLRP and NLRX (Fig. **1**). NLRs with a pyrin N-terminal domain (NLRP1-14) are involved in NF-kB regulation (NLRP6, NLRP12) and/or in the assembling of inflammasome (NLRP1, NLRP3, NLRP6, NLRP7, NLRP12), an intracellular complex formed by the adaptor protein ASC and the inflammatory caspase-1, that activates the production of the pro-inflammatory cytokines IL-1ß and IL-18, and in some cases an inflammatory cell

*Address correspondence to this author at the Avenida Prof. Lineu Prestes, 1730 – ICB IV, 05508-900 Cidade Universitaria, Sao Paulo, Brazil;
Tel: + 55 11 3091 7367; E-mail: alepontillo@usp.br

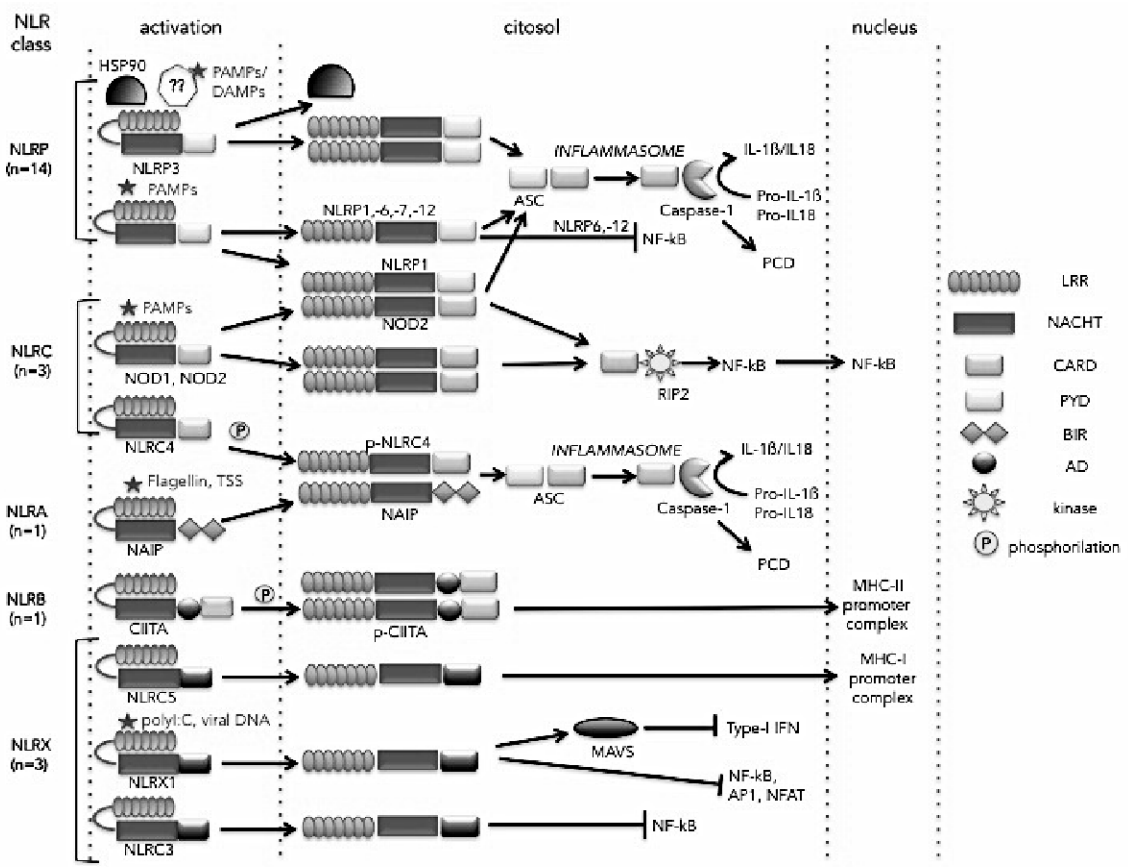

Fig. (1). Human NLRs. Protein domain structure and mechanism of activation and cell signal transduction were summarized.

death, called pyroptosis [5]. CIITA and NAIP are the only members of the two sub-families, NLRA and NLRB, respectively. Both are non-classical NLRs: CIITA does not recognize pathogens but it is a regulator of MHC-II expression, whether NAIP is a "helper" NLR and contribute to the binding of pathogen by other NLRs (i.e., NLRC4). The NLRC subfamily comprises members with an N-terminal caspase-recruitment domain (CARD): NLRC1 (NOD1) and NLRC2 (NOD2), which triggers a RIP2-dependent protein kinase and activates the inflammatory transcription factor NF-kB [6], NLRC4, which recognizes flagellate bacteria and mounts an inflammasome-mediated response [5]. Three NLRs have an N-terminal domain that has not been fully characterized. NLRC3 is a regulator of NF-kB activation. NLRC5 is a co-activator of MHC-I transcription. NLRX1 display an N-terminal mitochondrial-targeting sequence that mediates the trafficking to the mitochondrial membrane and the interaction with MAVS (mitochondrial anti-viral signal protein). It seems to inhibit anti-viral responses and NF-kB activation [7].

Human *NLR* family is distributed along all the human genome with three main clusters (chromosome 11, 16 and 19) comprising 17 out of 22 genes, compatible with gene duplication origin.

Although there are diverse and complex evolutionary events underlying *NLR* gene composition in distant phyla, the NLR-mediated immune signalling appears to be generally conserved along taxonomy. In several plants and invertebrates the repertoire of NLR genes is notably huge compared to vertebrates. Within plants, angiosperms present variable, but often largest, number of NLRs, in comparison to bryophytes, lycophytes and gymnosperms (Table **1**). Similarly it can be observed in metazoan, where organisms exist with a large repertoire of NLRs, such as sponge and sea urchin, as well as arthropods, as *D melanogaster*, lacking NLRs, and moreover mammals with a reduced number of receptors [8].

The evolution of NLRs through phylogeny is still largely discussed and strictly depending on the definition of NLR itself (considering only the central nucleotide binding domain, or the NB-LRR, or NB-LRR with a N-terminal domain, or NACHT versus NB-ARC). However some points appeared to be largely accepted and contribute to our understanding on inter- and intragenic variation of NLRs and species-specific NLR repertoires [9-11]. In this review, we considered NLRs with NB, LRR and N-terminal domains.

NLRs are present in clusters along the genome. Homogenous clusters (formed by the same type of NLRs) are generated by tandem duplication, whereas more heterogeneous clusters have been hypothesized as arising from duplication of a larger segment of genome followed by local rearrangement. Local and large-scale duplications and gene contraction mechanisms are responsible for NLR genes expansion and reduction in specific lineage observed along the phylogeny, as well as for the refresh of the NLRs repertoire. Evolutionary pressure handled NLR domains in opposite direction: the central nucleotide binding domain appeared to be under purifying selection, as it was expected due to its

Table 1. The number of *NLR* genes in plants and animal species (modified from by Jacob *et al.* [8], Robertson *et al.* [10], Yuen *et al.* [12]).

Species	Common name	NLRs		
Plants			TNLs	CNLs
Arabidopsis thaliana	Thale cress	151	94	55
Arabidopsis lyrata	Lyre-leaved rock-cress	138	103	21
Brachypodiumdistachyon	Brachhypodium	212	0	145
Brassica rapa	Mustard	80	52	28
Carica papaya	Papaya	34	6	4
Chlamydomonas reinhardtii	Chlamydomonas	0	0	0
Cucumis sativus	Cucumber	53	11	17
Glycine max	Soybean	319	116	20
Medicago truncatula	Barrel medic	270	118	152
Oryza sativa	Rice	458	0	274
Physcomitrella patens	Moss	25	8	9
Populus trichocarpa	Poplar	317	91	119
Sorghum bicolor	Sorghum	184	0	130
Solanum tuberosum	Potato	371	55	316
Selaginella moellendorffii	Spike moss	2	0	NA
Vitis vinifera	Wine grape	459	97	215
Zea mays	Maize	95	0	71
Invertebrates				
Amphimedon queenslandica	Sea sponge	62		
Hydra magnipapillata	Sea anemone	290		
Strongylocentrotud purpuratus	Sea urchin	206		
Capitella teleta	Polychaete worm	55		
Lottia gigantea	Owl limpet	1		
Crassostrea gigas	Pacific oyster	1		
Pinctada fucata	Akoya pearl oyster	45		
Strigamia maritima	Coastal European centipede	2		
Nasonia vitripennis	Jewel wasp	1		
Branchiostoma floridae	Amphioxus	92		
Vertebrates				
Danio rerio	Zebrafish	201		
Takifugu rubripes	Puffer fish	70		
Mus musculus	Mouse	30		
Homo sapiens	Human	22		

structural role, whether LRRs that are very polymorphic within species and lineages, under positive diversifying selection [11].

According to Yuen *et al.* [12], considering that, despite similarity in structure and function.; plant NB-ARC and animal NACHT domains are different NTPase, plant and animal NLRs appeared to have originated independently but with a convergent evolution of general mechanisms.

PLANTS NLRS

NLRs mediate plants defence through the recognizing of microbial proteins that enter the cytoplasmic compartment (virulence effector proteins) and the consequent activation of ion fluxes, oxidative burst, NO synthesis, activation of kinases signalling, salicylic acid (SA) accumulation at the site of infection, resulting in the so called effector-triggered immunity (ETI). ETI leads to the elimination of the pathogen or its containment and can be eventually followed by programmed host cell death (PCD) as a hypersensitivity reaction (HR) [13-17].

Plants present a large number of NLRs classified in two main sub-families identified by their N-terminal domain, the TOLL/interleukin 1 receptor (TYR)-type NLRs (TNL) and Coiled-coil (CC)-type NLRs (CNLs) (Table **2** and Fig. **2**). TNL and CNL receptors mediate resistance through different intracellular signalling [13].

Whether the involvement of specific NLR in defence response against pathogens is often known, the exact mechanism of recognition, through LRR domain or other motifs, as well the interacting molecules that mediate signalling and defence mechanism are still not well defined.

Example of plants NLRs are discussed below and resumed in Table **2** and Fig. **2**.

Plants LRR domains are very polymorphic as it was expected due to their role in effector recognition, however, direct interaction between LRR and pathogen effector proteins has been demonstrated only for some NLRs, such as flax L protein and fungal AvrL567 [18, 19], Arabidopsis RPP1 and oomycete ATR1 [20, 21], tomato Tm-2 and Tomato Mosaic Virus (ToMV), however, in the last case it seems that the stabilizing protein NbMIP1 (MP Interacting Protein 1) is also involved in the recognition [22]. A direct interaction between the N-domain and the virulence effector was reported in some cases such as in rice, *Magnaporthe oryzae*: Avr-Pik is recognized by the NLR Pik, through its CC domain [23]. More often plants NLRs recognize pathogens indirectly through virulence effector-induced modifications of other cell proteins [24-26]. Tobacco TLN recognizes Tobacco Mosaic Virus (TMV) through NRIP1 (N Receptor Interacting Protein 1), a chloroplast localized receptor. During TMV infection, NRIP1 moves from chloroplast to cytoplasm where it binds TMV p50 and interacts with N TIR domain. Potato Rx interacts with RanGAP2 (Ran GTPase Activating Protein 2) through its CC domain to respond against Potato Virus X (PVX) [26-28]. In Arabidopsis three CNLs, RPS2, RPS5 and RPM1, detect *P syringae* effectors in an indirect way. Pathogen induces modifications in host proteins.; RIN4 and PSB1, which can be recognized by NLR CC domain. RPS5 monitors conformational modification in PSB1 induced by AvrPphB [29]. *P syringae* AvrB and AvrRpm1 interact with RPM1 through specific modification in RIN4 [30]. Another virulence effector of *P syringae*, the cysteine protease AvrRPt2 induces the cleavage of RIN4 making host RPS2 able to recognize the insult [31]. In this way NLRs can recognize several pathogen effectors just monitoring "self" proteins.

In mammals it is well established that some NLRs, such as NOD2 or NAIP5, present a relative specific PAMP recognition (bacterial muramyl dipeptide/MDP or flagellin, respectively), whether NLRP3 is activated in response to various pathogens due to its ability to sense modification in cell milieu (Ca^{2+}, K^{+} ion fluxes, ATP/ADP ratio, ROS production) and/or in host proteins (lysosomal cathepsin G, TXNIP, GBP5, PKR) [5, 32].

Beyond its role in pathogen recognition.; LRR is important in maintaining plant NLR proteins in a "close" and inactive conformation, in the absence of virulence factors.; through the interaction with the NB-ARC domain [33-36]. How this interaction happens is still unclear. In Rx an acidic loop in the NB-ARC domain has been found to associate with basic residues in LRR and this intra-molecular interaction keep it in an inactive state [37]. Recently the mouse NLRC4 protein crystal structure was obtained [38] revealing that there exists more than one area of LRR-NB domains interaction.; but likely not all of them can be present in plants taking in account the molecular structure of the NB-ARC domain [34].

Similarly to mammals.; the central NB-ARC domain determines the activation state of plant NLRs. Plant NB-ARC consists in three sub-domains.; NB.; ARC1 and ARC2. NB is the catalytic core, containing the phosphate-binding loop (P-loop or Walker A motif). ARC1 mediates intra-molecular interactions with LRR, and ARC2, specifically the methionine-histidine-aspartate (MHD) motif, regulating the inactive/active state of the NLR [39, 40]. P-loop and MHD motives seem to be essential for plants NLR function.; however it was reported that in rice the CNL panicle blast-resistance Pb1 lacks the P-loop motif suggesting that in some NLR the activation mechanism may involve other sequences [40]. Plants lack the ARC3 motif that is essential for animal NLRs oligomerization formation of large molecular complex [39, 40].

Pathogen sensing leads to ADP/ATP substitution within the P-loop domain of NB-ARC and the consequent change from a closed/inactive to an open/active NLR conformation [41-44]. Activated NLRs may associate with other molecules of the same type (self- or homo-oligomerization) and/or with "helper" NLRs (hetero-oligomerization) to form complexes that transduce downstream signalling. This model of multi-protein complex was demonstrated for specific NLRs, such as flax TNL L6 and barley CNL MLA10 [14, 41], whether in other plants the N-terminal domain was sufficient to activate a cell signalling (i.e., potato Rx) [35].

It was reported that many pathogens.; with the aim to escape from plant complex immune machinery, produce potent suppressors of RNAi leading to up-regulation of the transcript levels of targeted NLRs. The main targets of CC-NLRs appeared to be the highly conserved P-loop sequence

Table 2. Examples of plant NLRs are reported together with their cognate pathogen effector.; known interactor proteins and activated transcription factors.

Plant	NLR	NLR class	Pathogen (effector)	Interactors	Transcription Factors
Arabidopsis	HRT	CNL	*Turnip Crinkle Virus*		
	RCY1	CNL	*Cucumber Mosaic Virus Y*		WRKY70
	RPS2	CNL	*Pseudomonas syringae (*AvrRPt2*)*	ADR1.; CPKs	
	RPS4	CNL	*P. syringae* (AvrRPS4) *Ralstonia solanacearum (*PopP2) *Colletotrichum higginsianum* (unknown)	RRS1.; SNC1.; EDS1	
	RPS5	CNL	*P. syringae* (AvrPphB)	PSB1	
	RPS6	CNL	*P. syringae* (AvrPphB)	SNC1.; EDS1	
	RPM1		*P. syringae* (AvrB and AvrRpm1)	RIN4.; CPKs	
	RPP1	TNL	Oomycete (ATR1)		
	RPP2	TNL	Oomycete (ATR1)	ADR1	
	RPP4	TNL	Oomycete (ATR1)	ADR1	
	RRS1		*P. syringae* (AvrRPS4)		
Barley	MLA10	CNL	*Blumeria graminis f. sp. Hordei*		WRKY.; HvMYB6
Flax	L		Fungus (AvrL567)		
	M		Fungus		
Potato	Rx1	CNL	*Potato Virus X*	RanGAP2.; SGT1	
	Rx2	CNL	*Potato Virus X*		
	GPA2	CNL	*Globodera pallida*	RanGAP2	
	Y-1	TNL	*Potato Virus X*		
Rice	Pik	CNL	*Magnaporthe oryzae* (Avr-Pik)		
	Pb1		*Magnaporthe oryzae*		WRKY45
Soybean	RSV1	CNL	*Soybean Mosaic Virus*		
Tobacco	N	TNL	*Tobacco Mosaic Virus*	NRIP1.; NGR1.; SGT1	SPL6
Tomato	Tm-2	CNL	*Tomato Mosaic Virus*	NbMIP1	
	Sw-5	CNL	*Tomato Mosaic Virus*		

in NB domain. In tobacco, micro RNA 6019 (miR6019) and miR6020 (miR6020) are involved in N-mediated resistance against TMV [45-47].

In the inactive state, some plants NLRs bind "stabilizing" proteins, such as the co-chaperonin SGT1. This mechanism is quite conserved, and, also in mammals, SGT1 and another protein, known as HSP90, contribute to maintain, at least, NLRP3 in a stable and inactive form [48].

Plants present different N-domain structures within the same lineage and between phylogenetic different lineages. During evolution TIR domain was lost in monocot lineages or reduced (i.e., Magnoliids). On the other hand.; the term "CC domain" indicates at least three different domains: (a) the properly called CC domain; (b) the CC domain characteristic of ADR1 (Activated Disease Resistance 1) family of plant NLRs (CC_R), that is highly conserved in monocot and dicot species; and (c) a CC domain with the EDVID motif (CC_{EDVID}) important for intra-molecular interactions that is not present in ADR1 receptors [2].

In mammals it was demonstrated that some "sensor" NLRs need "helper" NLRs to mediate a response against pathogens. Mouse Nlrc4 forms hetero-oligomers with Naip2 or Naip5 in response to flagellate bacteria type-III secretion

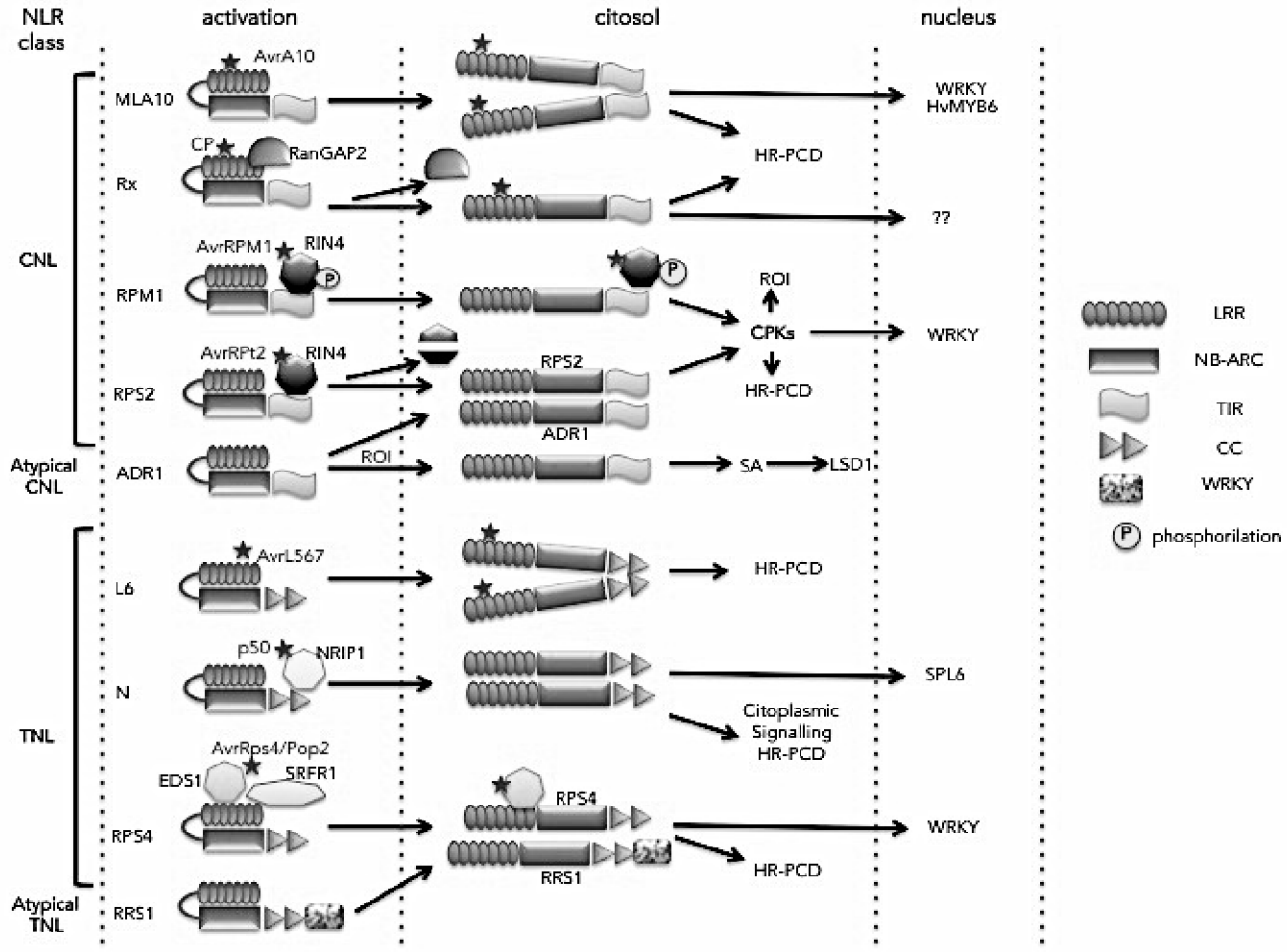

Fig. (2). Plant NLRs. Protein domain structure and mechanism of activation and cell signal transduction were summarized for selected plant receptors and virulence effector proteins: barley MLA10 and *B. graminis* AVrA10.; Potato Rx and Potato vírus CP; Arabidopsis RPM1 and *P. syringae* AvrRPM1.; RPS2 and *P. syringae* AvrRPt2; the atypical CNL Arabidopsis ADR1 and reactive oxygen intermediates (ROI); flax L6 and fungal AvrL567; tobacco N and *Tobacco Mosaic Viru*s p50; Arabidopsis RPS4 and *P. syringae* (AvrRPS4) and *R. solanacearum* (PopP2); the atypical TNL RRS1 of Arabidopsis.

MLA10 directly sense AvrA10 through its LRR domain and dimerize through its N-terminal domain to migrate into the nulceus and activate transcription factors WRKY and HvMYB6 or to induce programmed cell death and hypersensitivity reaction (HR-PCD). When CP LRR of Rx.; the RanGAP2 stabilizing protein is loss and Rx can migrate to the nucleus or induce HR-PCD. Its nuclear function is still unknown. RPM1 senses AvrRMP1 indirectly throug the phosporilated protein RIN4 and it activates a signalling cascade that involved calcium dependente protein kinases (CPKs).; ROI production.; HR-PCD. CPKs migrate into the nulceus to activate WRKY. RPS2 sense indirectly AvrRPt2 through the protrein RIN4. This activation leads to the cleavage of RIN4 and the interaction of RPS2 with ADR1.; which seems to be necessary for the consequent signalling. ADR1 can be also activated by ROI to mediate salicylic acid (SA) production and LSD1 activation. L6 directly sense AvrL567 through its LRR domain. Activate L6 dimerizes through its N-terminal domain to induce HR-PCD. Activation of N is mediated by NRIP1 protein and lead to homodimerization and signalling transduction and HR-PCD as well as migration into the nucleus and actoivation of the transcription factor SPL6. The inactive form of RPS4 binds EDS1 and SRFS1. When virulence factors bind EDS1.; SRFR1 was removed and RPS4 can interact with RRS1 to transduce defence response.

system or flagellin, respectively [49, 50]. Nod2 and Nlrp1 also cooperate in bacterial MDP (muramyldipeptide) sensing [6].

In a similar way some plant NLRs need a second NLR to activate an effector-triggered immunity and a defence response against pathogens. *Arabidopsis* RPS4 requires RRS1 to respond against *P syringae* AvrRPS4 [51, 52]. Moreover.; *Arabidopsis* atypical CNL ADR1 cooperates with CNL RPS2 and with the TNLs RPP2 and RPP4 [37]. NGR1, a homologue of ADR1, interacts with the TNL N in tobacco and promotes the signalling after TMV recognition [53]. Whether phylogenetic analysis revealed that it does not exist TNL without ADR1 gene family.; indicating that the signalling of TNL needs the help of those proteins.; this is not true for CNLs [24]. In rice two CNLs.; RGA4 and RGA5 interact to mediate resistance response against *Magnaporthe oryzae*: RGA5 acts as a receptor, while RGA4 is necessary for PCD [54]. Such "helper" NLRs can be involved also in non-NLR effector-triggered immunity, as tomato NRC1 which is necessary for the R protein Cf-4-mediated disease resistance [55].

To date proteins and mechanisms involved in NLRs-mediated resistance response are largely unknown. In *Arabidopsis* the L-family lipase EDS1, together with other partners such as PAD4 and SAG101, is required for TNL but not CNL-mediated resistance to bacterial and oomycete pathogens [56, 57]. This signalling transduction complex seems to be involved in SA as well as in ROI production [56]. More recently SRFR1.; a negative regulator of TNL-mediated response.; has been discovered as participating to EDS1 complex. SRFR1 regulates the stability of TNL/EDS1 complex, directly or through the binding with the co-chaperone SGT1, and the consequence activation of TNL-mediate response [58-60]. Moreover, *Arabidopsis* RPS2 and RMP1 sense RIN4 modifications induced by *P syringae* effectors and trigger a cytoplasmic influx of calcium related with the activation of multiple calcium-dependent protein kinases, CPK1-2-4-5-6-11. Distinct phosphorylation substrate specificity drives different ETI outcomes (respiratory burst.; PCD or gene transcription) [61]. In rice the Rac-GTPase OsRac1 plays a key role in immune response. Upon fungus infection, OsRac1 is necessary for the CNL Pit to activate oxidative burst and PCD [62].

Even if signalling pathways mediating by NLR are not elucidated at all, it is well known that TNLs and CNLs control defence genes expression by regulating transcription factors [63]. The complex ESD1-SRFR1 function as a repressor of plant defence gene expression, the TNL binding of virulence effectors disrupt this complex permitting expression of SNC1 and defence response [64]. As mentioned above, CPKs act on transcription factors to modulate plant transcriptional profile during ETI. The WRKY family of plant specific transcription factors play a role in several physiologic processes, including immune response in *Arabidopsis* and barley. It was showed that CPK-4, -5 -6 and -11 directly activate WRKY-8, -28 and -48, a sub-group of WRKYs crucial for ETI [61].

TNLs and CNLs are able to modulate gene expression participating in a short signalling pathway.; through the re-localization into the nucleus and direct interaction with transcription factors. This is the case of barley MLA10 interacting with HvMYB6 transcription factor.; and of rice Pb1 with WRKY45 [65]. Similarly it was demonstrated that, during antiviral response, tobacco N interacts with SPL6 transcription factor, through its CC domain [66] as well as *Arabidopsis* RCY1 with WRKY70, through its CC domain [66].

In mammals NLR activation lead to anti-viral and/or inflammatory response through the activation of IRFs and/or NF-KB transcription factors and commonly involved a signalling pathway. NOD1 and NOD2 upon bacterial PAMPs sensing.; through the RIP2-cIAPs-XIAP complex.; activate the effector kinase TAK1 that determines the activation of NF-kB [6]. However NLR-mediated direct transcriptional regulation also has been reported for mammals CIITA and NLRC5 that regulates the expression of MHC-I and -II by recruiting transcription factors elements [7, 67]. Of note, recently also NLRP3 has been demonstrated to play a role as transcription co-factor in CD4+ T cells [68].

Subcellular localization of plant NLRs and partners is essential for protein functions. NLRs are cytoplasmic proteins, however, they may localize in different compartment of plant cell to exert specific actions. It is very common that, once activated, plants NLRs translocate to the nucleus to mediate directly or indirectly the activation of defence transcription factors. The mechanism of cytoplasm-nucleus translocation is still unclear as plants NLRs lack a known nuclear localization signal [66, 67, 69, 70]. The activation of potato Rx and tobacco N in the cytoplasm precedes the nuclear translocation to efficiently mount a defence resistance [71, 72]. The phosphorylation status of the co-chaperone SGT1 regulates their subcellular localization [72]. Besides SGT1, RanGAP2 also has been described to regulate Rx translocation to the nucleus [73, 74]. For N, no single domain has been implicated in nuclear localization [75]. On the other side only for few plants NLRs a direct interaction with transcription factors have been reported, as for barley CNL MLA10 and the transcription factors WRKY and MYB6 [76]. Arabidopsis TNL RPS4 goes into the nucleus to activate a resistance defence [77]. Other NLRs need to re-localize to plasma membrane (PM), such as RPS5 and RPM1 [78, 79], or to other endomembranes to keep in contact with their cognate pathogen effector proteins.; such as flax L6 to Golgi apparatus and M to tonoplast [80, 81]. Moreover.; rice Pit is associated in a palmitoylation-dependent manner to PM and this localization is fundamental for its action over OsRac1 and the consequent immune response [62]. Sub-cellular dynamic is important also for proteins involved in NLR-mediated signalling.; such as CPKs.; which translocate from cytosol to nucleus upon NLR activation [60].

NLR-activated response leads often to programmed cell death and local hypersensitive response to limit pathogen infection [82], nevertheless the mechanism of PCD remains still obscure. PCD mediated by RPM1 and RPS2 is preceded by fusion of vacuolar and PM membranes and the consequent release of vacuolar proteins into the extracellular space [83]. The resulting extracellular fluid possesses both antibacterial activity and inducing activity towards cell death [83]. Mammalian apoptotic caspases are absent in plants, however, proteases with cysteine-dependent activity, such as metacaspases (i.e., *Arabidopsis* AtMC1) [84, 85], or aspartate-dependent activity, such as phytaspases [86], mediate HR and PCD, respectively, in plants [84-86].

Tobacco phytaspase is involved in N-mediated resistance against TMV.; promoting PCD. Intriguingly phytaspase is constitutively secreted in the apoplast, but during PCD it is re-located into the cytoplasm where it possibly cleaves substrates related to cell death [86]. As it was discussed in [86], this unusual mechanism of PCD may recall cytotoxic cell death mediated by NK and CD8+ lymphocytes but with the inverse delivery of cytotoxic material: “inside (self) delivery” PCD in plants, and “outside delivery” PCD in animals. By the way granzyme B display the same aspartate specific cleavage of phytaspase [86]. Moreover Franklin BS *et al.* [87] recently showed that ASC-caspase-1 complexes (ASC specks) may be released extracellularly after pyroptose (inflammatory cell death) and are still able to cleave caspase-1 specific substrates.

The number of NLR genes in plants is large when compared to mammals and vary within groups.; species and isolates, without a clear correlation to the phylogeny. It appears that natural selection has driven plants to develop NLRs.;

through either loss or expansion of *NLR* genes, to be able to fight new effectors pathogens. The elevated fitness costs related to the maintenance of those large NLRs repertoire has been hypothesized to be in part compensated by transcriptional control mediated by microRNAs with NLR target [88]. Recently Zhang *et al.* proposed a model of evolution for resistance (R) genes hypothesizing a divergent origin for the large and differentiated plethora of plant NLRs [89].

INVERTEBRATE NLRs

As the genomic analysis of the experimental model of arthropod *Drosophila melanogaster* and nematode *Caenorhabditis elegans* did not revealed any *NLR* genes [90, 91], it has been supposed that invertebrates did not present NOD-like receptors; however.; recent studies have demonstrated the presence of a large number of NLRs in more than one phylum (Porifera, Cnidarian, Annelida, Molluscs).

Despite the lack of thorough characterization of invertebrate NLRs, it was shown that they usually present the typical tripartite structure, with the LRR and central NOD domain, but often with a N-terminal death domain (DD) that it is not present in mammalian orthologous. (Fig. **3**) [12, 92]. By the way a huge number of genes containing only NOD in combination with other domains has been also described in invertebrate and reviewed in [15, 93, 94].

In the genome of the sponge *Amphimedon queenslandica* have been identified 135 putative NLRs but only 48 with the tripartite structure including a N-terminal CARD or Death domain (AqNLRs) [12]. As described for plants [1], AqNLRs show divergent LRR domains possibly originated from a recent rapid expansion and diversification within clade [12]. It has not been described whether AqNLRs recognize pathogen directly or indirectly. Several proteins containing CARD.; DD and DED alone or in combination with effectors domains.; such as nucleotide phosphorilase (PNP_UDP_1), peptidase_C14 and protein tyrosine kinase (PTKs), were identified suggesting that sponge NLRs may associate with those potential adaptors and effectors proteins to mount intracellular complexes for downstream signalling and antimicrobial compound production [12, 94, 95].

NLRs were described in animals belonging to Cnidarian lineage.; *Hydra magnipapillata* and two anthozoas *Nematostella vectensis* and *Acropora millepora.* Classical NLRs were encountered in *N vectensis* and *A millepora* but to date not in *H magnipapillata*. Hydra genome contains 290 NDB loci, 130 NACHT-type and 160 NB-ARC type. Proteomics data revealed that Hydra expressed at least 16 proteins with a NACHT and a N-terminal Death domain.; such as the so-called HyNLR type 1 (HyNLR1). Endodermic epithelial cells appeared to be the local of prominent NLRs expression, and it is interesting to notice that these cells are responsible also for antimicrobial compounds secretion upon pathogen insult [12, 93, 94, 96]. The link between NLRs and AMPs was previously described in mammals [97, 98] and plants [99].

Very limited information is available about the role of invertebrates NLRs in host defence; however.; it is possible

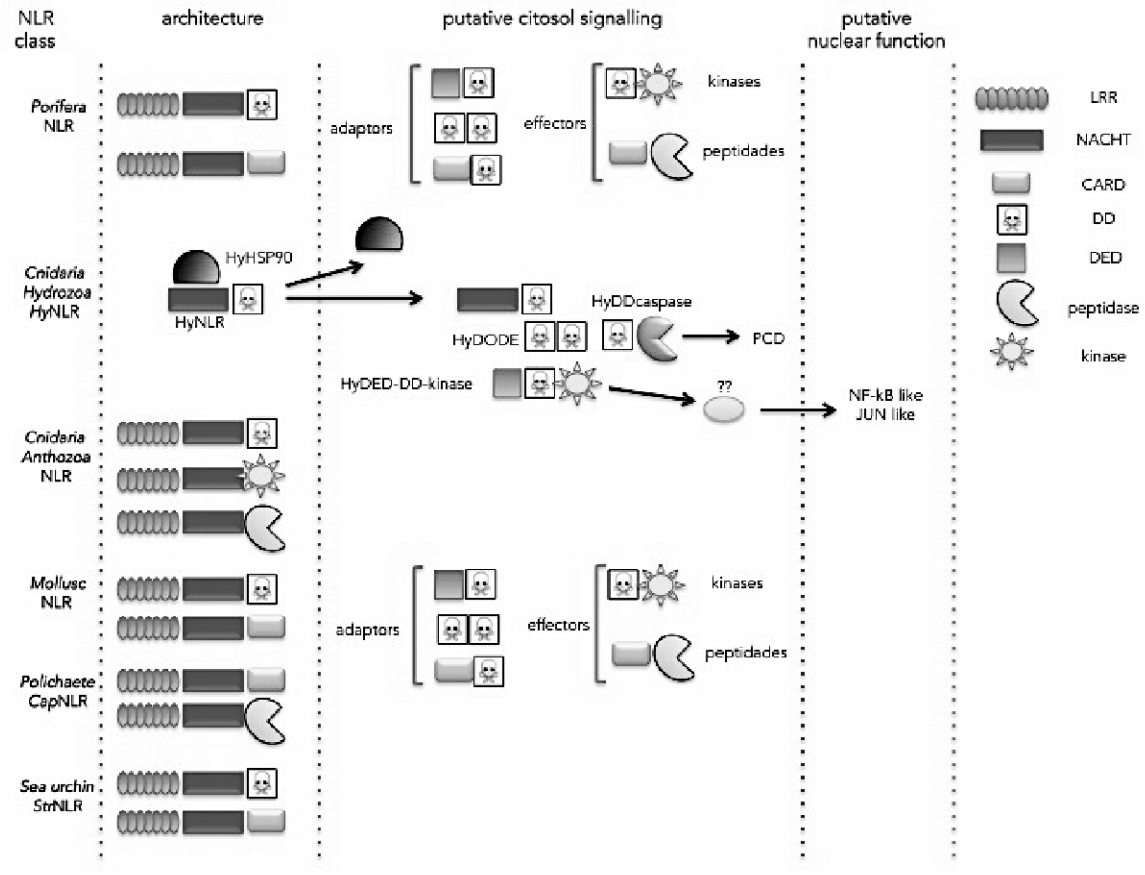

Fig. (3). Invertebrate NLRs. Protein domain structure and putative activation mechanisms were summarized.

that upon pathogen recognition they might be able to oligomerize and to mediate a short or more complex signalling. In *H magnipapillata* several potential HyNLR1 interactors have been described: (a) orthologous of chaperonin HSP90 and co-chaperonin Chp-1 and SGT1, (b) a protein with two Death domains, HyDODE, that could be an adaptor protein; (c) two caspases with a N-terminal DED domain (HyDED-caspase) or DD domain (HyDD-caspase). In the absence of a LRR domain it is not clear how these HyNLRs are able to sense pathogens and to be activated. However some authors hypothesized that HyNLR could be able to assemble a complex through HyDODE and to recruit a kinase (HyDED-DD-Kinase 1) that possibly links the HyNLRs to a not well-defined gene transcription pathway [93, 96].

Genomic sequencing of mollusca *Mytilus edulis* did not revealed any NLRs or NACHT domain-containing proteins, neither main constituents of the NLR pathway, such as ASC, RIP2 or caspase-1 suggesting that mollusca may use other intracellular receptors instead of NLR [100]. However Yuen *et al.* [12] recently identified NLR genes in several invertebrates including mollusca (*Lottia gigantean, Crassotrea gigas* and *Pinctada fucata*), polychaetes (*Capitella telata*) and arthropods (*Strigamia maritime* and *Nasonia vitripennis*). Discrepancy in results may be due to the limited sequencing data available for invertebrates to date.

Sea urchin *Stringylocentrotus purpuratus* presents a high number of NLRs with the typical tripartite structure but in some cases the N-terminal domain presents a death domain similarly with other invertebrates. *S purpuratus* NLRs are supposed to form Rip2-like complex that activate signalling pathways leading to NF-kB activation. NLRs expression was found at the level of gut epithelium.; at the boundary with external milieu [92, 100-102].

NLRs and NF-kB pathway are also been described in the cephalochordate amphioxus *Branchistoma floridae* but deeper investigation is needed to further characterize the receptors and their signalling [103-106].

Several questions remain open about the presence and the functionality of NLRs in invertebrates; however.; on-going investigations are trying to elucidate whether invertebrate NLRs are derived from plants hortologues or precursor of actual mammals ones. In a recent published phylogenetic analysis, based only on NACHT NLRs, Yuen *et al.* [12] showed as NLRs in sponge, human NLRP and NLRC belong to a unique clade, while echinoderm, mollusca, cnidarian, *C telata* NLRs as well as human NAIP and IPAF to a different one. Hydra non-common NLRs do not enter in this classification, however, all the NLR-interacting genes discovered in *H magnipapillata* genome have hortologues representative in clade 1. NLRs expansion occurs in differential way in the two clades. The invertebrate NLRs expansion predominantly occurs in clade 2 and vertebrate in clade 1. To note the genome of zebrafish (*Danio rerio*) presents a large number of NLRs (Table **1**) belonging to clade 1 and lacks of homologous from clade 2 (NAIP and IPAF).; underlining a separate line of evolution of NLRs from the two phylogenetic clades [12].

CONCLUSION

Despite protein structure differences.; plants and animals NLRs shared elevated homology for signalling pathway suggesting a converged evolution of the receptors.

Interestingly, the so-called NLR-mediated sensitivity has been demonstrated in plants for some pathogens that exploit plant NLR-mediated immunity for their survival (i.e., Arabidopsis LOV1 and pathogenic fungus *Cochliobolus victoriae*) as well as in animals (i.e..; Mouse NOD2 and intestinal pathogenic bacteria *Yersinia pseudotuberculosis*) [8], underlining once more common mechanisms.

Fully comprehension of NLR-mediated immune response in plants could support the use of NLR genes in genetic modified organism engineering contributing to the development of broad-spectrum disease resistance, but, on the other hand, could also contribute to the knowledge about animal NLRs physiology and/or pathology.

Next generation of genome sequencing contributes to the description of NLRs in phyla others than plants and mammals and leading to new knowledge about NLRs evolution along phylogeny. However functional investigation will be needed to better understand how conserved could be the mechanism of action of these evolutionary distant NLRs of invertebrates compared to mammals ones.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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