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Review

Functional diversification of structurally alike NLR proteins in plants

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ABSTRACT

In due course of evolution many pathogens alter their effector molecules to modulate the host plants' metabolism and immune responses triggered upon proper recognition by the intracellular nucleotide-binding oligomerization domain containing leucine-rich repeat (NLR) proteins. Likewise, host plants have also evolved with diversified NLR proteins as a survival strategy to win the battle against pathogen invasion. NLR protein indeed detects pathogen derived effector proteins leading to the activation of defense responses associated with programmed cell death (PCD). In this interactive process, genome structure and plasticity play pivotal role in the development of innate immunity. Despite being quite conserved with similar biological functions in all eukaryotes, the intracellular NLR immune receptor proteins happen to be structurally distinct. Recent studies have made progress in identifying transcriptional regulatory complexes activated by NLR proteins. In this review, we attempt to decipher the intracellular NLR proteins mediated surveillance across the evolutionarily diverse taxa, highlighting some of the recent updates on NLR protein compartmentalization, molecular interactions before and after activation along with insights into the finer role of these receptor proteins to combat invading pathogens upon their recognition. Latest information on NLR sensors, helpers and NLR proteins with integrated domains in the context of plant pathogen interactions are also discussed.

1. Introduction

Plants and animals convey innate immunity to disease causing pathogens by using both cell surface and intracellular host receptors [1,2]. However, the intracellular recognition ability mostly belongs to NLR [Nucleotide binding domain (NBD) and leucine rich repeat (LRR)] superfamily proteins [3,4]. These immune receptors are found in plant systems starting from primitive unicellular alga to present day angiosperms and gymnosperms, and even in bryophytes and liverworts [5]. The diversities in animal kingdom are comparable to the plants, ranging from primitive chordates to the highly evolved mammals [6,7,8]. Plant and animal NLR proteins evolved independently from distinct derivatives of common prokaryotic ancestral ATPases [9,10,11]. The basic function of NLR protein appears to recognize the pathogen derived virulence factors [12]. NLR proteins may directly or indirectly recognize pathogen deployed effectors, which results in the induction of plant defense responses. In plants, effector recognition triggers oxidative burst with redox alteration of regulatory proteins [13,14]. NADPH oxidases such as respiratory burst oxidase homologs (RBOHs) primarily regulate reactive oxygen species (ROS) production in plants. RBOHs expression and ROS generation regulates hypersensitive response or HR induced cell death, which in turn limits the spread of the pathogen [15,16]. However, in animals, this results in secretion of soluble mediators that recruits immune active cells immediately at the sites of infection [17].

NLRs are multidomain proteins with conserved architecture including a C-terminal leucine-rich-repeat (LRR) domain, a central nucleotide-binding domain (NBD) and a variable N-terminal domain that recognizes the pathogen derived effectors either directly or indirectly [18,19]. Although, analyses at structural level have contributed significantly to our understanding of NLR protein functioning as a molecular switch that allows the pathogen recognition and defense activation, however, the underlying molecular mechanisms are still needs to be describe in a more comprehensive manner [20,21]. Recognition of pathogen has been explained by various models, like 'direct recognition', 'indirect recognition', 'decoy/guardee' and 'integrated decoy' [18,22,23]. In this review, we describe recent advances on NLR protein functioning in plants and how different domains confer recognition of virulence factors to trigger hyper-immune signaling. The study will also highlight how the knowledge gained from this rapidly growing field of research can be exploited to reduce crop damage.

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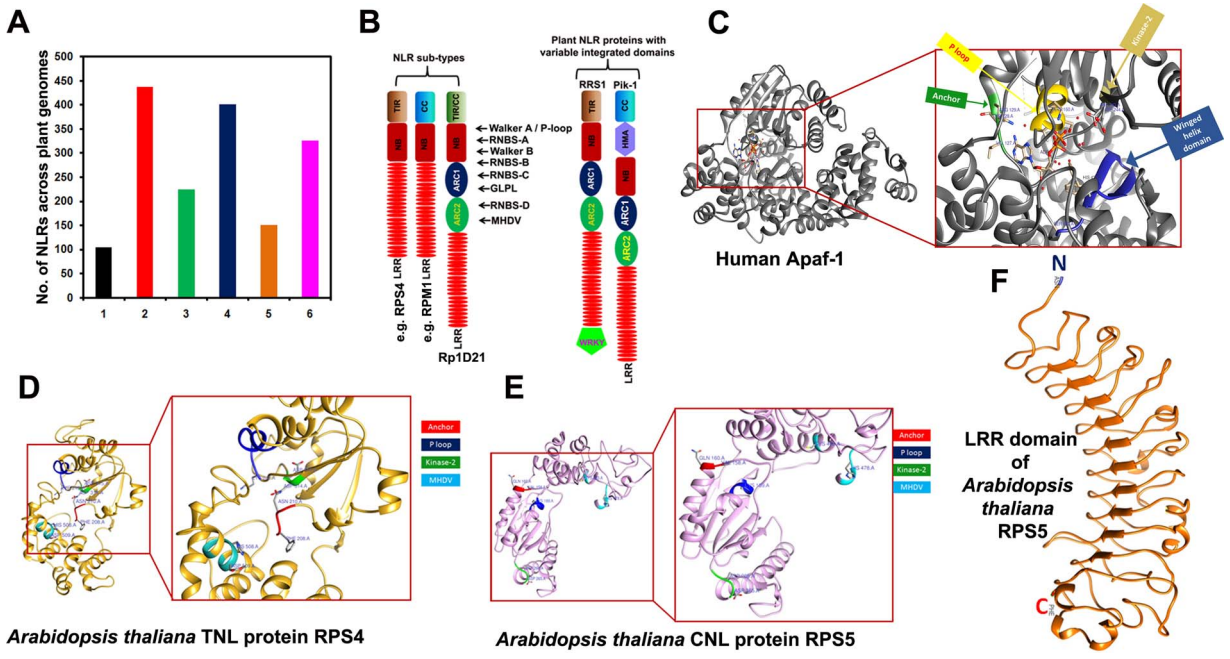

Fig 1. Structural apprehension of NLR proteins. (A) Bar diagram representing the number of NLR proteins present across the plant genomes. *Zea mays* (1); *Oryza sativa* (2); *Hordeum vulgare* (3); *Triticum aestivum* (4); *Arabidopsis thaliana* (5); *Solanum lycopersicum* (6). (B) Schematic representation of plant NLR proteins highlighting the major domains along with the integrated domains. (C) Human APAF-1, chain a; (PDB id: 1z6t). ADP and ATP interacting pockets are enlarged to show the NBS domain that coordinates the adenine residue of ADP or ATP, P-loop responsible for binding α and β- phosphates and winged-helix sub-domain residing MHDV motif that coordinates through sugar and β- phosphate moiety of ADP molecule. The kinase2 motif interacts with γ-phosphate of ATP. (D) Molecular structure of *Arabidopsis thaliana* TIR-NB-LRR (TNL) protein RPS4. NBS domain of *Arabidopsis thaliana* RPS4 and the highlighted residues are responsible for phosphate binding. (E) Predicted structure of *Arabidopsis thaliana* CC-NB-LRR (CNL) protein RPS5. NBS domain of *Arabidopsis thaliana* RPS5 showing the conserved motifs likewise APAF-1. (F) The predicted structure of an LRR domain of *Arabidopsis thaliana* RPS5 generated using bovine decorin (1xku PDB id) as the template. Cartoon representation of the predicted RPS5 LRR domain shows 'horseshoe' shaped arrangements of β-sheets which are represented as arrows, forming the concave side. Homology model of *Arabidopsis thaliana* RPS4 and RPS5 are generated using the SWISS-MODEL homology modelling server (https://swissmodel.expasy.org/) and UCSF Chimera (https://www.cgl.ucsf.edu/chimera/) has been used for the purpose of visualization of the molecular structures of the proteins.

2. NLR protein architecture and compartmentalization

Based on the N terminal diversity, plant NLR proteins are classified into two major classes: coiled-coil (CC)-NLR or CNL and Toll interleukin-1-receptor (TIR-NLR) or TNL proteins. Both classes of NLR proteins are present across dicot species (Fig. 1A, B), whereas, the group of canonical TNL type immune receptors were lost during evolution in monocots [24]. NBD and LRR domains share similar modular organization across plant and animal kingdom [9]. The other NLR subtypes include NB-ARC (Nucleotide-binding adaptor shared by Apaf1 resistance proteins, CED-4) domain containing proteins (Fig. 1C). Plant NLR proteins are structurally quite similar to NLR-like apoptosome protein Apaf1. Another distinctly related domain (Pfam domain TIR2) containing protein is present in both monocots and dicots, and the numbers of family members in above two groups are restricted to 2–5. TIR2 sequences, which belong to an ancient gene family, are evolutionarily distinct from the TIR sequences present in dicots [25]. Non-canonical TIR2 here contributes to most of the aspects associated with activation, pathogen sensing and signal transmission [26]. In contrast to plants, animals NBD subtype has NACHT (NAIP, CIITA, HET-E and TPI) domains. Plant genomes also harbour a large set of truncated NLR-like proteins. It has been seen that Arabidopsis genome carries 58 genes encoding NLR-like proteins lacking the LRR or even NB-ARC sequences [27,28]. A study showed, Arabidopsis Resistance to Powdery Mildew 8 or RPW8 protein consisting of a CC domain and lacking NB-ARC and LRR sequences, to confer broad-spectrum resistance against powdery mildew causing pathogens [29]. NBD of NLR protein is believed to exchange ADP for ATP in response to effector proteins and/or virulence factors. Although the exact NLR protein regulation is unclear, it is believed that ATP hydrolysis inactivates the NLR proteins [9,30,11,31,22]. In many cases single NLR protein can both recognize the pathogen and activate the signals, however, many NLR proteins work as pairs to confer resistance to the pathogen [32].

Our current knowledge about activation and function of NLR proteins comes from analysis of N-terminal domains [21]. Till date, six crystal structures of TIR domains have been solved [33,34,35,21]. The crystal structure of TIR domain of wild grapevine *Muscadinia rotundifolia* RPV1 (Resistance to *Plasmopara viticola* 1) was resolved as dimer, which is primarily mediated through residues in αA and αE helices [21]. The study also demonstrated that multiple TIR domain surfaces control cell death function of RPV1. CC domain structures of barley NLR Mildew locus A 10 (MLA10) protein, potato NLR Rx protein and wheat NLR Sr33 protein have also been successfully resolved [36,37,38]. The structure of Sr33 CC looks alike to the distantly related Rx than its true ortholog, MLA10 [39]. Interestingly, the length of CC domain was found to be critically important for determining the functions of the proteins and it was demonstrated that short $MLA10_{1\text{-}120}$ or $Sr33_{1\text{-}120}$ constructs were unable to induce autoactive cell death, whereas, long CC fragments (160 aa) comprising the C-terminal α-helix were found to be essential for autoactivity and cell death [40].

One of the most important features in plant immunity is intracellular movement of different proteins between the cytoplasm and nucleus. Several plant NLR proteins have been shown to accumulate in the nucleus upon activation by effectors [41]. The nuclear accumulations of the Arabidopsis TNL protein RPS4 (Fig. 1D) is required for immune signaling on recognition of *Pseudomonas syringae* effector protein AvrRps4 [41,42]. Similarly, Arabidopsis TNL protein Resistance to *Ralstonia solanacearum* or RRS1 conferred disease resistance to the bacterial pathogen *Ralstonia solanacearum* through the recognition of cognate type III effector PopP2 that carries a bipartite nuclear localization signal and is specifically targeted to host cell nuclei [43]. In both the cases nuclear pool was shown to be important for disease resistance signaling but the actual process of translocation was not demonstrated. Recent studies on NLR protein mediated disease resistance