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·特邀综述·

植物NLR免疫受体的识别、免疫激活与信号调控

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摘要 高等植物进化出大量膜表面和胞内免疫受体以感知各种病原信号, 抵御病原物入侵。其中, 细胞表面的模式识别受体感知模式分子后激活基础免疫反应, 核苷酸结合和富亮氨酸重复蛋白(NLRs)则通过感知病原微生物分泌的效应蛋白激活特异免疫反应, 导致超敏反应与细胞死亡。该文主要综述了NLRs对效应蛋白的识别、植物免疫激活及下游信号调控的最新研究进展。

关键词 植物免疫, NLRs, 受体识别, 信号调控

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为区分病原微生物与共生微生物或有益微生物, 并在受到病原体攻击时适度激活防御反应, 植物进化出复杂的先天免疫系统。高等植物细胞表面和胞内有大量的免疫受体以感知病原体侵染相关的各种信号(Kourelis and Van Der Hoorn, 2018; Van De Weyer et al., 2019)。细胞表面免疫受体包括受体样蛋白(receptor-like proteins, RLPs)以及受体激酶(receptor-like kinases, RLKs), 通常被称为模式识别受体(pattern-recognition receptors, PRR), 可感知微生物相关分子模式(microbe-associated molecular patterns, MAMP)、病原体相关分子模式(pathogenassociated molecular patterns, PAMP)或者宿主产生的损伤相关分子模式(damage-associated molecular patterns, DAMP)。感知到分子模式后, PRR激活基础免疫反应PTI (pattern-triggered immunity), 包括活性氧(reactive oxygen species, ROS)的快速产生、Ca^{2+}内流和丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)级联反应的启动等(Couto and Zipfel et al., 2016; Jones et al., 2016; Van Der Burgh and Joosten, 2019), 从而抑制病原体的增殖。

病原体则利用多种机制促进宿主感染, 其中最重要的是分泌效应蛋白干扰宿主PRR蛋白的功能及其免疫相关过程。为克服效应蛋白引起的干扰并阻止病害发生, 植物进化出胞内核苷酸结合和富亮氨酸重复序列受体(nucleotide-binding domain leucine-rich repeat containing receptors, NLRs), 以感知病原体效应物并启动强烈特异性免疫反应ETI (effector-triggered immunity) (Dodds and Rathjen, 2010; Jones et al., 2016)。NLRs与PRR介导的免疫反应具有相似的分子特征但又有所不同, 并且常伴随超敏反应(hypersensitive response, HR)与细胞死亡现象(Yuan et al., 2021; Ofir et al., 2021)。近年来, 有关植物先天免疫, 特别是NLRs介导的植物免疫研究取得了一系列重大突破。本文以NLRs介导的免疫反应为重点, 综述了其对效应蛋白的识别、免疫激活及调控的最新研究进展。

1 NLRs及其与效应蛋白的识别

1.1 NLRs的结构与类型

高等植物中, NLRs对病原体效应物识别和免疫反应启动至关重要(Jones et al., 2016; Zhou and Zhang, 2020)。尽管NLRs也存在于包括哺乳动物在内的多种动物中, 越来越多的证据表明, 动、植物都是通过不同的方式进化出类似受体(Urbach and Ausubel, 2017)。植物和动物NLRs都包含1个位于中间的核苷

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酸结合(nucleotide-binding, NB)域和1个C端富含亮氨酸重复序列(leucine-rich repeats, LRR)区域。其中, NB结构域为受体寡聚化后形成更高级复合物(如高等植物中的抗病小体和哺乳动物中的炎症小体)以传递信号所必需; 高度可变的LRR区域通常参与自我抑制、蛋白质-蛋白质相互作用和效应子识别(Saur et al., 2021)。

根据N末端的不同, 典型的植物NLRs分为2种主要类型: TIR型和CC型。TIR型NLR (TIR-type NLRs, TNL)的特征是其N端具有Toll/白介素1受体(Toll/interleukin-1 receptor, TIR)结构域, 而CC型NLR (CC-type NLRs, CNL)具有卷曲螺旋(coiled-coil, CC)结构域(Tamborski and Krasileva, 2020; Saur et al., 2021) (图1A)。大多数动物中NLRs数目较少, 而高等植物中NLRs数目可达数百个, 除负责病原体效应蛋白识别的免疫受体(sensor NLRs, sNLR)外, 还存在对下游免疫信号起转导功能的辅助免疫受体(helper NLRs, hNLR), 如NRG1以及ADR1蛋白家族中的hNLR, 作用于TNL的下游(Peart et al., 2005; Bonardi et al., 2011; Dong et al., 2016)。由于ADR1以及NRG1的N端携带RPW8 (Resistance to Powdery Mildew 8)类CC域, 而不是典型的CC域(Jubic et al., 2019), 故也称为RNLs (RPW8-type NLRs)。

NLRs广泛分布于细胞质、细胞核、质膜(plasma membrane, PM)、液泡膜和内质网等亚细胞结构(Chiang and Coaker, 2014), 如大麦(*Hordeum vulgare*) CNLMLA10和拟南芥(*Arabidopsis thaliana*) TNLRPS4位于细胞核和细胞质中, 且这2个亚细胞定位均为抗性激活所必需(Shen et al., 2007; Bai et al., 2012)。而拟南芥CNL (RPM1)组成性地与质膜结合并识别膜靶向的假单胞菌效应蛋白(Nimchuk et al., 2000; Gao et al., 2011)。最新研究表明, 在大豆(*Glycine max*)中存在一种细胞壁定位的NLR Rsc4, 介导大豆对花叶病毒的抗性(Yin et al., 2021)。因此, NLRs不仅定位于不同的亚细胞结构, 而且在病原体效应蛋白识别机制上也呈现多样性。

1.2 NLRs识别效应蛋白的多种模式

1.2.1 直接识别模式

NLRs感知效应蛋白最简单且最直观的机制是直接结合识别模式(图1B)。直接识别的一个典型例子是亚麻(*Linum usitatissimum*) L位点编码的TNL L5、L6和L7对亚麻栅锈菌(*Melampsora lini*)效应蛋白AvrL567不同变体的感知(Dodds et al., 2006)。然而, 直接相互

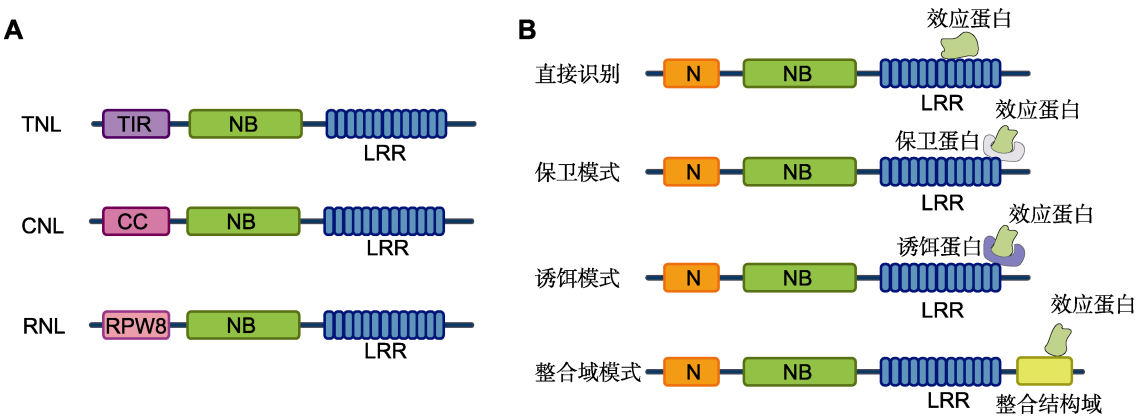

图1 NLRs的结构组成及与效应蛋白的识别模式(改自Duxbury et al., 2021)

(A) 植物NLRs的结构域分为3类, 包括中间部位的核苷酸结合域(NBD)和C端的富含亮氨酸重复序列(LRR)结构域以及N端的TIR、CC或类RPW8的CC结构域; **(B)** 植物NLRs识别效应蛋白的不同模式: 一些植物NLR直接与相应的效应蛋白结合, 或通过保卫蛋白或诱饵蛋白间接检测病原体效应蛋白; 此外, 一些植物NLRs具有特异的整合结构域(ID), 介导效应蛋白的识别。

Figure 1 Structural composition of NLRs and its recognition pattern to effector proteins (modified from Duxbury et al., 2021)

(A) The domains of plant NLRs are divided into three categories, including a central nucleotide-binding (NB) domain, a C-terminal leucine-rich repeats (LRR) region and N-terminal TIR, CC, or RPW8-like CC domain; **(B)** Different patterns of effector recognition by plant NLRs: Some plant NLRs directly bind to the corresponding effector proteins or indirectly detect the pathogen effector through the guardee or decoy proteins; Some plant NLRs have special integrated domains (ID) to mediate effector recognition.

作用的识别可能推动了*AvrL567*基因座多样化衍变，从而产生12个变体，其中5个变体具有逃避NLRs结合和阻碍NLRs识别的能力(Dodds et al., 2006)。这种协同进化的“军备竞赛”也推动了亚麻*L*基因座的多样化，该基因座含有13个等位基因，其中包括3个识别单个效应蛋白变体的NLR。同时，L蛋白LRR结构域的高度多态性赋予其对多个AvrL567变体的识别，因此L6 LRR中11个氨基酸的替换会导致其特异性识别效应蛋白数从多个减少至1个(Dodds et al., 2006; Rayamajhi et al., 2013)。

水稻NLR *Pik*基因家族通过直接结合的方式感知稻瘟病菌中多个AVR-Pik效应蛋白。例如，Pikm可识别3个AVR-Pik效应蛋白，而Pikp只能识别1个。通过分析Pik与AVR-Pik蛋白间的相互作用，研究人员利用结构导向工程(structure-guided engineering)创造了1个新的*Pik*等位基因，该基因结合了以前未被识别的AVR-Pik (De La Concepcion et al., 2019)。需要指出的是，大麦CNL抗白粉病基因座A (mildew resistance locus a, Mla)已进化出新的等位基因，该基因可直接识别白粉病真菌的结构相关序列无关效应蛋白(sequence-unrelated effectors) (Saur et al., 2019)。此外，新近2项冷冻电镜结构分析显示，烟草(*Nicotiana benthamiana*) TNL ROQ1 (Recognition of *Xanthomonas* outer protein Q1)与其效应蛋白XopQ，以及拟南芥TNL RPP1 (Recognition of *Peronosporaparasitica* 1)与其效应蛋白ATR1，均是通过LRR域直接结合。

1.2.2 间接识别模式

许多植物NLRs特异性识别时不与效应蛋白直接结合(图1B)，而是以一种间接的识别模式，该模式可分为2种。其一是保卫假说模式，是指通过间接配体-受体互作识别效应蛋白(Dangl and Jones, 2001)。NLRs监测效应蛋白(或受保护靶标蛋白)的完整性，并在感知到受保护蛋白结构或功能发生变化时激活免疫反应。拟南芥中，多个效应蛋白靶向RIN4 (RPM1-interacting protein 4)，而RPS2 (Resistance to *P. syringae* 2)和RPM1 (Resistance to *P. syringae* pv. *maculicola 1*)则通过蛋白互作监测RIN4，从而识别效应蛋白。丁香假单胞菌(*Pseudomonas syringae*)可通过其三型分泌系统(type III secretion system, T3SS)分泌蛋白酶AvrRpt2，随后AvrRpt2裂解RIN4从而触发RPS2依赖型免疫反应(Axtell and Staskawicz, 2003; Mackey et al., 2003)。此外，丁香假单胞菌效应因子AvrRpm1诱导RIN4腺苷二磷酸(ADP)核糖基化，进而导致宿主激酶对RIN4磷酸化，触发RPM1介导的免疫反应(Chung et al., 2011; Liu et al., 2011; Redditt et al., 2019)。近期的研究结果表明，病原效应蛋白HopZ5和AvrBst能对RIN4 T166保守位点乙酰化，从而触发RPM1依赖型免疫反应。需要指出的是，植物内源性去乙酰化酶SOBER1能够对RIN4 T166去乙酰化，从而抑制免疫反应的发生。这可能是植物为实现免疫反应的精细调控，避免免疫过度(Choi et al., 2021)。

其二是诱饵假说模式。该模式是保卫假说模式的衍生，指当受NLRs保护的诱饵蛋白除作为效应蛋白识别诱饵外，在免疫中不具备有其它功能(Zhou and Chai, 2008; Van Der Hoorn and Kamoun, 2008)。经典的植物诱饵蛋白如假激酶PBL2，为激活CNL ZAR1 (HopZ-activated resistance 1)所必需。ZAR1可间接识别多种效应物，包括野油菜黄单胞菌(*Xanthomonas campestris* pv. *campestris*)的效应器蛋白AvrAC和丁香假单胞菌的效应蛋白HopZ1a (Lewis et al., 2013; Wang et al., 2015; Seto et al., 2017; Schultink et al., 2019; Laflamme et al., 2020)。病原菌入侵过程中，通过分泌效应蛋白AvrAC尿苷酸化BIK1，或HopZ1a乙酰化MKK7，抑制植物的PTI反应以增强其致病力(Feng et al., 2012; Wang et al., 2015; Rufián et al., 2021)；而植物进化出诱饵蛋白PBL2，AvrAC使PBL2尿苷酰化(产生$PBL2^{UMP}$)，并激活ZAR1介导的免疫反应。但PBL2尿苷酰化不会增强AvrAC介导的致病力，因此，这些宿主蛋白被认为是诱饵蛋白，而不是受保卫蛋白(Lewis et al., 2013; Wang et al., 2015)。

然而，因功能冗余，有时很难区分诱饵蛋白和保卫蛋白。例如，RPS5监测蛋白激酶PBS1在质膜上的状态(Shao et al., 2003; Ade et al., 2007)，而PBS1在植物PTI中具有部分功能，当PBS1被质膜定位的效应蛋白AvrPphB水解时，RPS5通过识别切割位点从而激活免疫反应(Zhang et al., 2010)，表明PBS1充当了保卫蛋白的角色。但AvrPphB可靶向并水解至少8种PBL蛋白，如AvrPphB水解BIK1，从而抑制植

物的PTI反应。有研究推测, 大豆BIK1的直系同源物可能是AvrPphB的最佳底物。然而, 拟南芥PBS1在PTI中仅具有微弱功能(Zhang et al., 2010), 表明PBS1也可作为诱饵蛋白。因此, 植物可能通过冗余的调控模式灵活应对病原体入侵。

1.2.3 整合域识别模式

一些NLRs包含源于效应蛋白靶向的其它蛋白结构域, 被称为整合结构域(integrated domains, ID), 可识别相应的病原体效应蛋白(图1B)。为此, 一些ID直接与其匹配的效应蛋白互作, 甚至被效应蛋白酶所修饰。这与保卫模式识别类似, ID和NLR之间就像受保卫蛋白与NLR的融合。事实上, ID与NLR融合后导致的遗传连锁带来了诸多好处。例如, 在长期进化过程中, 受保卫蛋白与其对应NLR的突变或等位基因所造成的差异可能导致二者之间失去相容性, 而在整合的NLR-ID中, 这种可能性要小很多。说明NLR和ID的融合可能通过反转录转位或异位重组发生(Bailey et al., 2018)。目前, 已有几个通过NLR-ID模式识别效应蛋白的机制被揭示。研究得最为透彻的实例之一是RRS1 (Resistance to *Ralstonia solanacearum* 1)介导的青枯菌抗性, 其以C末端的WRKY结构域作为ID (Le Roux et al., 2015; Sarris et al., 2015)。青枯菌分泌的效应蛋白PopP2是一种乙酰转移酶, 能结合并乙酰化WRKY转录因子, 破坏其与DNA的结合以提高致病力(Le Roux et al., 2015; Sarris et al., 2015)。然而, 在超表达*RRS1*植株中, RRS1的WRKY结构域被PopP2乙酰化, 而WRKY结构域乙酰化足以激活RPS1及RPS4 (Resistance to *P. syringae* 4)介导的免疫应答(Le Roux et al., 2015; Sarris et al., 2015)。同时, RRS1还具有识别其它效应蛋白的功能。例如, 效应蛋白AvrRps4也能够结合RRS1的WRKY结构域(Sarris et al., 2015)。此外, 包括水稻NLRs RGA5和Pik-1的其它已知植物NLR-ID, 都含有重金属相关(heavy metal-associated, HMA)的整合结构域, 从而识别稻瘟病菌效应蛋白AVR-PikD (Césari et al., 2014)。

2 免疫激活与抗病小体的形成

2.1 CNL与抗病小体

植物NLRs被激活后形成寡聚复合物, 该结构类似动物中的炎症小体(Davis et al., 2011; Wang et al., 2019; Ma et al., 2020; Martin et al., 2020)。例如, CNL型抗病小体ZAR1包含了ZAR1、受体样胞质激酶(RLCKs) RKS1以及PBL2。来自野油菜黄单胞菌分泌的效应蛋白AvrAC通过其尿苷酰转移酶功能将PBL2转化为$PBL2^{UMP}$, ZAR1与RKS1形成复合物并招募$PBL2^{UMP}$形成有活性的PBL^{UMP}-ZAR1-RKS1复合体, 从而完成对AvrAC的特异性识别。该复合物组装成五聚轮状, 并在其平面上形成由5个CC域的N端α-螺旋构成的漏斗状结构(Wang et al., 2019), 用作通道孔开启植物的防御之门(图2A) (Wang et al., 2019; 夏石头和李昕, 2019)。然而, 天然ZAR1寡聚体是否也采用五聚体构型并在植物细胞膜上形成孔结构, 这种孔结构如何调节膜的通透性和底物的选择性, 以及这种活性如何触发细胞死亡和免疫反应目前尚不清楚。

最近的2项突破性研究表明, ZAR1、NRG1a和ADR1均可在质膜形成可供钙渗透的阳离子选择性通道(Bi et al., 2021; Jacob et al., 2021)。当野生型ZAR1与AvrAC、RKS1和PBL2在非洲爪蟾卵母细胞表达时, 在双电极电压钳记录分析实验中检测到施加电压后的强电流迹象, 这表明激活的ZAR1复合物具有通道活性。单通道记录实验进一步表明, 形成的孔状通道对包括Na^+、K^+、Cs^{2+}、Mg^{2+}和Ca^{2+}在内的阳离子具有渗透性, 且该通道的活性取决于位于通道孔中的保守酸性残基E11 (Bi et al., 2021)。在ZAR1抗病小体诱导的细胞死亡过程中, 植物细胞在相对较短的时间内经历了PM损伤和破裂, 类似于哺乳动物的细胞焦亡和坏死性凋亡(需要形成孔状通道) (Bi et al., 2021)。然而, 动物MLKL (mixed lineage kinase domain-like)阳离子通道(Xia et al., 2016)引发的坏死性凋亡中通常伴随渗透性肿胀, 而在ZAR1介导的植物细胞死亡中未检测到肿胀或PM突出(Bi et al., 2021)。因此, 由ZAR1抗病小体诱导的细胞死亡与细胞焦亡和坏死性凋亡并不完全相同。

2.2 TNL与抗病小体

已报道的多数植物TNL在细胞核中定位或发挥作用, TIR极少可能采用与CC结构域相同的机制诱导细胞死亡。通过对动物中含TIR结构域的SARM1 (sterile alpha and TIR motif-containing 1)进行研究, 发现SARM1的TIR结构域具有寡聚依赖性NADase活性。

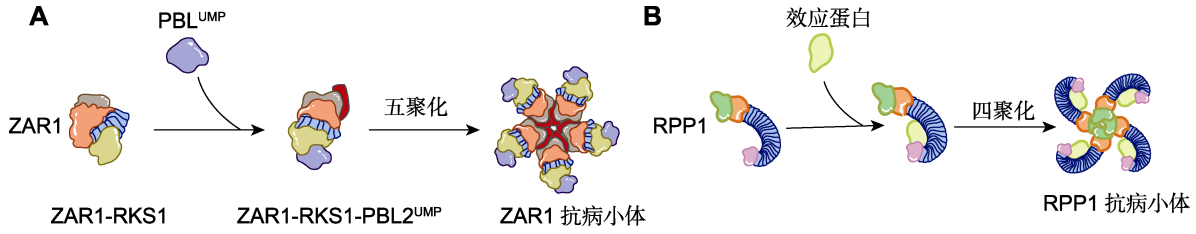

图2 两类NLRs的激活方式(改自Duxbury et al., 2021)

(A) CNL (ZAR1)抗病小体形成示意图。黄单胞菌效应蛋白AvrAC尿苷酸化拟南芥激酶PBL2。尿苷化的PBL2 (PBL2^{UMP})与胞内预先形成的ZAR1-RKS1二聚体结合，导致ZAR1的构象发生变化，并在ZAR1 NBD核苷酸结合位点以三磷酸腺苷或脱氧三磷酸腺苷((d)ATP)替换二磷酸腺苷(ADP)。最终，5个ZAR1-RKS1-PBL2^{UMP}单体形成1个五聚轮状ZAR1抗病小体。**(B)** TNL (RPP1)抗病小体形成示意图。胞内典型TIR-type NLR通过富亮氨酸重复序列(LRR)和羧基末端结构域(C-JID)直接识别病原体无病毒效应器，形成具有烟酰胺腺嘌呤二核苷酸糖水解酶(NADase)活性的四聚体结构。

Figure 2 Activation modes of two NLRs (modified from Duxbury et al., 2021)

(A) Schematic diagram of CNL (ZAR1) resistosome formation. The *Xanthomonas* effector AvrAC uridylates the *Arabidopsis thaliana* kinase PBL2. Uridylated PBL2 (PBL2^{UMP}) associates with the intracellular pre-formed ZAR1-RKS1 dimer. This leads to a conformational change of ZAR1 and replacement of adenosine diphosphate (ADP) by adenosine triphosphate or deoxyadenosine triphosphate ((d)ATP) in the nucleotide-binding site of the NBD of ZAR1. Ultimately, this results in the formation of a pentameric wheel-like ZAR1 resistosome, which is composed of five ZAR1-RKS1-PBL2^{UMP}protomers. **(B)** Schematic diagram of TNL (RPP1) resistosome formation. Direct recognition of a pathogen avirulence effector by the leucine-rich repeat (LRR) and carboxy-terminal domains (C-JID) of a canonical Toll/interleukin-1 receptor (TIR) domain-containing intracellular nucleotide-binding domain (NBD)-like receptor (TIR-type NLR) leads to the formation of a tetrameric structure with nicotinamide adenine dinucleotide glycohydrolase (NADase) activity.

体外生化分析中，纯化的SARM1 TIR结构域(SARM-1TIR)将NAD$^+$切割成二磷酸腺苷核糖(ADPR)、环状二磷酸腺苷核糖(cADPR)和烟酰胺(NAM) (Essuman et al., 2017)，为植物TNL研究奠定了重要基础。近期，一项突破性研究是利用杆状病毒表达系统在昆虫细胞中共表达TNL类抗病蛋白RPP1及其对应的效应蛋白ATR1，纯化并通过冷冻电镜单颗粒重构技术，成功解析了RPP1抗病小体的结构，发现RPP1-ATR1形成了四聚体(Ma et al., 2020) (图2B)。同时，另一研究组解析了ROQ1-XopQ类似的四聚体结构(Martin et al., 2020)，表明TNL可能存在共同的激活机制。这些抗病小体的结构解析为揭示RPP1和ROQ1直接识别其同源效应蛋白并四聚化以提高NADase活性，从而激活下游免疫反应的关键机制奠定了基础。有研究表明，在RPP1和ROQ1的羧基末端存在1个不同的结构域，称为C-JID (C-terminal jelly-roll and Ig-like domain) (Ma et al., 2020; Martin et al., 2020)。该结构域与LRR协同介导与效应蛋白的特异性结合，其残基的突变会破坏其与效应蛋白的互作结构区域，减轻ETI介导的宿主细胞死亡现象。对于RPP1和ROQ1而言，效应子与LRR和C-JID的直接结合可能会释放NBD, NBD构象发生变化并寡聚化为四聚体。四聚体环使TIR结构域紧密接触，形成具有NADase全酶活性的TNL抗病小体。

需要指出的是，与ZAR1类抗病小体通过结合ATP/dATP交换ADP激活不同，RPP1类抗病小体通过结合ADP交换ATP激活。在RPP1和ROQ1抗病小体中，NBD四聚化使4个TIR结构域靠近，形成2个头尾相连的二聚体，不对称TIR二聚体形成1个预测的NAD$^+$结合位点，而对称TIR二聚体可稳定复合物。激活后，在BB-loop的辅助下，2个二聚体以头尾相连的方式出现，并作为完整的NADase催化NAD$^+$水解，产生信号物质从而诱导宿主细胞死亡。研究发现，仅BB-loop内的氨基酸突变可改变RPP1和ROQ1 NADase活性及其在免疫中的作用。因此，TNL的TIR结构域四聚化形成全酶可能代表此类植物NLR的常见激活机制。RPP1和ROQ1结构的解析揭示了一种与CNL ZAR1不同的新型TNL抗病小体，为理解植物TNL的激活机制提供了助力。然而，目前尚不清楚TIR NADase活性激活下游免疫的具体机制。Jubicet等(2019)认为TNL的NADase活性需通过下游关键组分和辅助型hNLR (包括ADR1和NRG1)激活，并以一种

目前未知的方式进行免疫信号转导。此外，与ZAR1不同，RPP1和ROQ1抗病小体都包含直接识别其效应蛋白的结构域。因此，解析不同性质抗病小体的非典型结构将有助于更好地理解高等植物NLR的不同激活机制。

3 免疫执行及其信号调控

3.1 免疫执行

CNL型NLRs识别效应蛋白后，寡聚的ZAR1抗病小体结合到PM上并形成可供钙渗透的阳离子选择性通道，导致钙离子内流并进一步激活下游防御反应，包括细胞死亡等(Bi et al., 2021)。因此, ZAR1抗病小体既是病原体效应器的传感器，也是下游细胞死亡和信号转导的执行器。更重要的是，ZAR1抗病小体诱导ROS产生、PM完整性被破坏和HR均需残基E11，显示出ZAR1通道活性在导致细胞死亡和免疫下游事件执行中的关键作用(Bi et al., 2021)。然而，是否所有CNL均可作为Ca^{2+}内流通道需进一步研究。未来通过对具有不同N末端的其它CNL进行结构和功能分析，可进一步揭示CNL是否存在替代机制。

TIR型NLRs介导的免疫反应则依赖寡聚TIR复合物的NADase活性，以及EDS1 (enhanced disease susceptibility 1)家族的类脂肪酶蛋白和含HeLo样结构域的辅助型hNLRs。与ZAR1 E11类似，NRG1.1 E14和ADR1 D11残基对钙离子通道的形成和下游信号转导的诱导也至关重要。Jacob等(2021)通过对NRG1a变体的研究发现，当第485位的天冬氨酸突变为缬氨酸(D485V)时会发生免疫自激活现象，且在PM富集并寡聚化，从而触发Ca^{2+}内流依赖型细胞死亡。NRG1a (D485V) (残基1–124)的X射线晶体结构与ZAR1和MLKL的N端4个螺旋束(4-helical bundles, 4HBs)的高度相似(Jacob et al., 2021)，表明其N末端孔洞的形成可能与ZAR1类似。值得一提的是，虽然观察到NRG1a (D485V)的同源寡聚化，但是由hNLR形成的天然寡聚体通常需要与类脂肪酶蛋白EDS1-PAD4 (phytoalexin deficient 4)和EDS1-SAG101 (senescence-associated gene 101)相互作用(Sun et al., 2021; Wu et al., 2021)。因此, TNL可能通过寡聚化形成抗病小体获得NADase活性，产生信号分子，诱导EDS1家族的类脂肪酶蛋白和辅助型NLR形成复合体，从而触发细胞Ca^{2+}内流和细胞死亡。

3.2 免疫信号调控

CNL和TNL激活后，最终产生相似的转录表达，引发局部和系统抗性(Bartsch et al., 2006; Mine et al., 2018; Saile et al., 2020; Zhou and Zhang, 2020)。NLRs还与PRR协同作用，增强PTI，从而产生完整的免疫反应(Ngou et al., 2021; Yuan et al., 2021)。CNL类NLRs可以作为质膜上的阳离子通道介导Ca^{2+}内流，证明多个Ca^{2+}渗透通道可传导Ca^{2+}内流，并激活具有激酶活性的膜定位受体，从而触发免疫反应(Tian et al., 2019; Thor et al., 2020)。Hu等(2020)研究发现，通过$LaCl_3$处理可阻断钙通道活性且不影响ZAR1复合物的形成，并在很大程度上抑制细胞死亡，推测ZAR1通道引起的Ca^{2+}快速内流是ZAR1介导的植物免疫反应的下游信号，但不排除ZAR1抗病小体在免疫反应进程中也具有其它作用。

TNL类NLRs通过NADase活性将NAD^+裂解产生多种产物，包括烟酰胺腺嘌呤单核苷酸等(Wan et al., 2019)，这些产物及其衍生物可作为下游激活EDS1依赖性防御信号。大量实验证实, EDS1、PAD4和SAG101为TNL介导的免疫反应所必需。EDS1下游的信号转变为分别依赖SAG101和PAD4的两条平行通路(图3)。SAG101和PAD4与EDS1共享1个类脂肪酶结构域，它们与EDS1形成不同的复合物激活下游防御反应(Cui et al., 2015)。虽然这些复合物的作用方式目前尚不清楚，但异源二聚体之间形成的空腔可作为未知蛋白质或者激活免疫信号配体的结合部位(Bhandari et al., 2019)。

研究表明，NRG1与EDS1和SAG101在TNL诱导的HR激活中发挥作用(Qi et al., 2018; Gantner et al., 2019; Lapin et al., 2019)。拟南芥有2个冗余的NRG1直系同源物，NRG1a和NRG1b，二者均为TNL触发的HR和免疫所必需(Castel et al., 2019; Wu et al., 2019) (图3)。而拟南芥RNL ADR1、ADR1-L1和ADR1-L2作用于EDS1及PAD4的下游，促进由TNL和一些CNL触发的SA的生物合成(Bonardi et al., 2011; Dong et al., 2016; Wu et al., 2019) (图3)。不同的辅助型免疫受体与特定的类脂肪酶蛋白家族形成复合体，从而传递免疫信号。Sun等(2021)研究发现，TNL免疫受体的激活可诱导EDS1-SAG101与

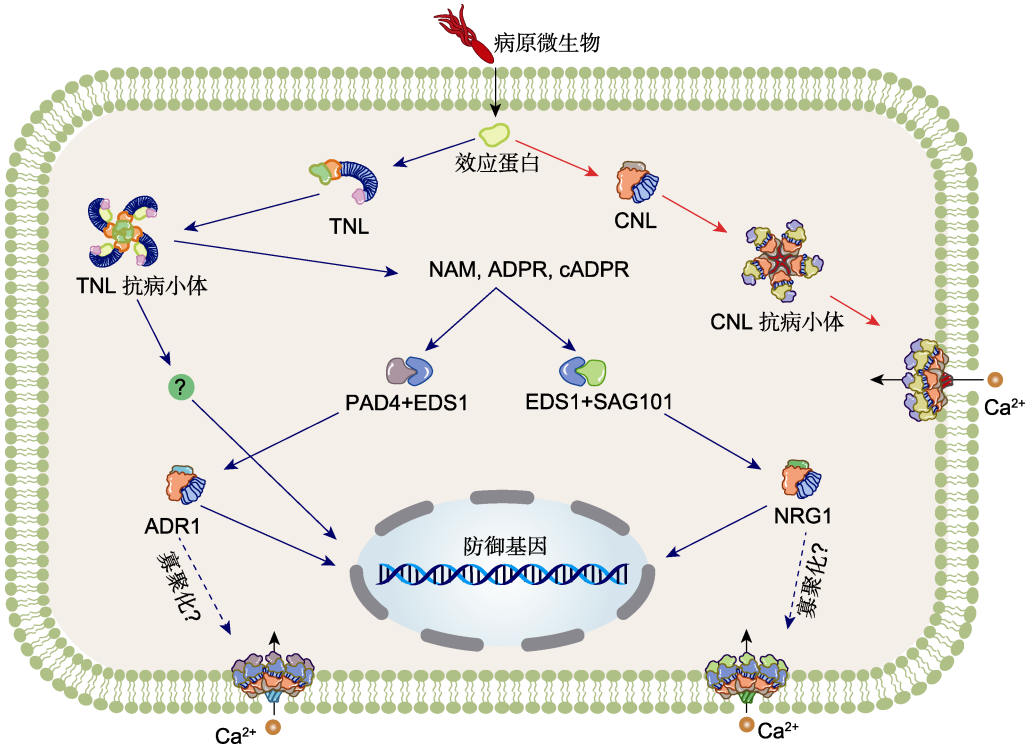

图3 高等植物NLRs介导的免疫反应工作模型(改自Liu et al., 2021)
当植物细胞被病原菌侵染时，一些病原菌可分泌效应因子以突破植物的免疫防线。在长期进化过程中，植物进化出许多胞内受体来识别这些效应因子，从而促发其对病原菌的抗性。CNLs通过感知效应蛋白触发其在质膜的五聚化和抗病小体形成(该模型以间接识别模式的ZAR1抗病小体示例)，并通过N端CC结构域中的α1螺旋形成的孔道作为Ca^{2+}内流通道，介导胞质Ca^{2+}浓度上升，开启细胞死亡和防御反应。TNLs感知效应蛋白后形成四聚抗病小体(该模型以直接识别模式的RPP1示例)，TNL抗病小体的形成导致TIR NADase激活，触发可能包含EDS1-PAD4-ADR1s或EDS1-SAG101-NRG1s低聚合物的组装。辅助型NLR的寡聚作用可形成CNL类似孔道，作为Ca^{2+}内流通道，介导下游免疫和细胞死亡。红色箭头表示CNL信号，蓝色箭头表示TNL和RNL信号。

Figure 3 Working models of activation of NLRs-mediated immunity in higher plants (modified from Liu et al., 2021)
Upon an infection of a plant cell by a pathogen, some pathogens can secrete effectors to break through the immune defense line of plants. In the process of long-term evolution, plants have evolved many intracellular receptors to recognize these effectors, so as to promote resistance to pathogens. CNLs triggers pentamerization and resistosome formation on the plasma membrane (PM) through sensing effector (The example here depicts a ZAR1 resistosome which indirect recognition effector assembly). The pore formed by the N-terminal CC α1 helices serves as a Ca^{2+} influx channel, mediating increase of cytosolic Ca^{2+} concentration and turning on cell death and defense responses. TNLs, upon perception of effectors (the model here depicts an example of direct effector-receptor recognition as with RPP1), formation of the TNL resistosome leads to activation of TIR NADase activity, triggering assembly of oligomeric complexes presumably containing EDS1-PAD4-ADR1s or EDS1-SAG101-NRG1s. The oligomerization of the helper NLRs enables a similar pore formation as CNLs, serving as Ca^{2+} influx channels to mediate downstream immunity and cell death.The red arrows indicate CNL signal, and the blue arrows indicate TNL and RNL signals.

NRG1形成蛋白复合体; Wu等(2021)则证实TNL免疫受体能促使ADR1与EDS1-PAD4形成多聚体。因此推断，TIR型NLR可能通过其TIR结构域的催化功能产生信号小分子，这些小分子激活EDS1及其家族成员并结合和激活辅助型NLR ADR1或NRG1 (图3)，也可能EDS1及其家族成员向TIR型NLR提供其它代谢底物，通过酶促转化为激活ADR1或NRG1的信号分子。

上述关于CNL ZAR1、hNLRs NRG1a和ADR1作为Ca^{2+}内流通道的研究丰富了植物免疫反应中NLR信号转导通路。Ca^{2+}内流作为细胞死亡的触发因素与之前的研究结果一致，即错误上调的Ca^{2+}积累可导致细胞死亡和自身免疫(Yoshioka et al., 2006; Zhao et al., 2021)，Ca^{2+}通道的调控处于NLR介导的植物免疫反应的核心地位(Xia et al., 2021)。

4 总结与展望

近年来, NLRs介导的植物免疫相关重大进展和里程碑式发现, 加深了人们对植物免疫系统感知和调控病原体的理解, 为作物抗病改良提供了新思路。NLRs结构和功能的解析也为揭示CC及TIR结构域的信号转导机制提供了新线索, 使人能更好地理解NLRs如何特异性识别效应蛋白, 及设计新颖特异的NLRs。尽管如此, 仍有许多悬而未决的问题需要进一步探索。例如, 其它CNL或TNL是否形成类似ZAR1、Roq1或RPP1抗病小体结构? 成对的NLRs (如RRS1/RPS4)或形成网络的NLRs是否也能形成类似抗病小体或炎症小体结构以激活免疫反应? 其它hNLR是否可被TNL激活形成类似ADR1/NRG1钙通道以介导细胞死亡和其它下游信号? 是否存在其它被忽视的机制? 另外, TNL-NADase活性如何与下游信号(如转录激活或辅助型NLR激活(如ADR1/NRG1))联系起来也不十分清楚。TNL和CNL激活可导致类似的转录重编程(Jacob et al., 2018; Ding et al., 2020; Saile et al., 2020), 但是如何汇聚到一起并产生类似转录程序尚不明确。最近发现NLR介导的抗病性依赖PRR, 反之, NLR激活增强PRR介导的免疫反应(Ngou et al., 2021; Yuan et al., 2021), 表明植物免疫反应的全面激活需要PRRs和NLRs信号的协同作用(王伟和唐定中, 2021), 因此, PTI和ETI并非是独立的免疫途径。未来研究应聚焦于细胞表面受体与胞内免疫受体之间介导的免疫反应的一般机制。例如, NLR介导的免疫通路如何接收PRR的信号? PTI和ETI的免疫协作是否广泛存在于植物与病原菌的互作中? 对PTI和ETI免疫协作潜在机制的研究将有助于全面了解植物的免疫系统, 为作物抗病育种工程和绿色农业发展奠定理论基础。

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Recognition, Immune Activation and Signal Regulation of Plant NLR Immune Receptor

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Abstract A large area of membrane surface and intracellular immune receptors have been evolved in higher plants to sense various pathogen signals and prevent pathogen invasion. Among them, pattern recognition receptors on the cell surface activate basic immune response after sensing pattern molecules, while nucleotide-bounding leucine-rich repeat proteins (NLRs) activate specific immune response by sensing effector proteins secreted by pathogenic microorganisms, resulting in hypersensitivity and cell death. In this review, the latest research progress of plant immunity is mainly reviewed from the aspects of NLRs on the recognition of effector proteins, plant immune activation and downstream signal regulation.

Key words plant immunity, NLRs, receptor recognition, signal regulation

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