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·专题论坛·

NLR及其在植物抗病中的调控作用

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摘要 为适应丰富多变的生存环境, 植物逐渐进化出一套复杂的免疫系统来抵抗病原菌的侵染。核苷酸结合的富含亮氨酸重复蛋白(NLR)作为植物体内普遍存在的一类抗性(R)蛋白, 对植物的抗病性具有重要调控作用。该文综述了NLR蛋白结构、信号转导以及对植物抗病的调控作用近几年的研究进展。

关键词 NLR, 植物免疫, 抗性蛋白, 信号转导

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植物病害常造成大范围的粮食减产, 培育抗病品种是抵御作物病害最为经济且环保的手段之一。1994年, 植物核苷酸结合的富含亮氨酸重复蛋白(nucleotide-binding leucine-rich repeat, NLR)基因首次被克隆, 分别为烟草(*Nicotiana tabacum*)的*N*基因(Whitham et al., 1994)和拟南芥(*Arabidopsis thaliana*)的*RPS2*基因(Bent et al., 1994; Mindrinos et al., 1994); 其中, N对烟草花叶病毒具有特异抗性, 而RPS2赋予了植物对*Pseudomonas syringae* pv. *glycinea* (具有AvrRPS2)的特异抗性。过去20年中, 一系列植物*R*基因及其对应的*Avr*基因(无毒基因)被克隆。与动物NLRs不同, 植物NLRs可识别快速进化的效应子, 动物NLRs则更倾向于响应保守的病原相关分子模式(pathogen-associated molecule pattern, PAMPs)。

1 NLR的结构与特性

典型的NLR分为3部分, 即非保守的N端结构域、位于序列中间的核苷酸结合的寡聚结构域(nucleotide-binding and oligomerization domain, NOD)以及C端LRR (leucine-rich repeat)结构域。N端序列通常由TIR (toll/interleukin-1 receptor like)或CC (coiled-coil)组成。根据N端结构域的不同, NLR可分为TNL和CNL两大类。

1.1 N端结构特性

与CC相比, TIR结构域更加保守, 且广泛存在于动、植物中(单子叶植物除外), 被认为与先天免疫系统的蛋白和蛋白互作相关。CC结构域的序列则较为多样化, 且常与其它结构域串联。例如, 番茄(*Solanum lycopersicum*)中的Sw-5b与SD序列相连(Peiró et al., 2014), 二者结合可在植物免疫中发挥更重要的作用。近年, 有关植物NLR蛋白N端的晶体结构解析表明, 其在整个免疫反应中具有重要作用。NLR蛋白可以发生自身二聚化或者多聚化。例如, 大麦(*Hordeum vulgare*) MLA10的CC结构域通过形成二聚体实现自激活, 阻止MLA10 CC结构域的二聚化可中断下游信号转导(El Kasmi and Nishimura, 2016)。与TNL被激活后才形成二聚体不同, 许多CNL在未被诱导的静息状态下, 同样处于二聚体形态, 如RPM1、RPS5、HRT、MLA10、Rp1-D和Sr50 (El Kasmi et al., 2017)。但与MLA10近源的Sr33及远源NLR Rx的CC端在静息状态时却仍为单体晶体结构(Casey et al., 2016; El Kasmi and Nishimura, 2016)。上述结果表明, N端二聚体的形成对于NLR的激活和下游的信号转导至关重要。

此外, 植物NLR的N端也参与特异性识别病原菌效应子和引起细胞死亡的过程。动、植物体内NLR蛋

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白被病原体诱导后, TIR结构域的NAD^+裂解活性被激活, 识别病原体并转化为细胞死亡信号, 从而引起免疫反应(Horsefield et al., 2019; Wan et al., 2019)

1.2 NB-ARC结构域特性

NB-ARC属于NLR蛋白保守结构域, 与哺乳动物的细胞凋亡蛋白酶激活因子Apaf-1 (apoptotic protease-activating factor-1)、植物*R*基因编码的抗病蛋白和秀丽线虫的细胞死亡蛋白CED-4 (caenorhabditis elegans death-4 protein)具有同源性(van der Biezen and Jones, 1998), 通常被认为具有“信号开关”功能。亚麻锈病抗病蛋白M的NB-ARC区域在结合ADP时处于静息状态, 结合ATP后才可被激活, 进而引发植物的超敏反应(Williams et al., 2011)。同为亚麻抗病蛋白的L6和L7, 因与ADP的结合程度不同诱发的免疫强度也不同。L7蛋白NB-ARC与ADP结合更紧密, 故其免疫反应稍弱于L6 (Bernoux et al., 2016)。近期, 我国科学家使用冷冻电镜技术揭示了拟南芥抗病蛋白ZAR1 (HopZ-activated resistance 1)的作用机制。他们发现, 当ZAR1-RKS1复合体与ADP结合时处于失活状态, 与被AvrAC诱导后尿苷酰化的$PBL2^{UMP}$结合, 形成ZAR1-RKS1-$PBL2^{UMP}$复合体时, 构象发生改变, 释放ADP而处于中间状态; 形成的复合体与dATP/ATP结合后诱发ZAR1寡聚结构域暴露, 导致ZAR1-RKS1-$PBL2^{UMP}$形成轮状五聚体免疫抗病小体, 进而激活超敏反应和抗病性(Wang et al., 2019a, 2019b)。进一步的生化和功能分析表明, 该漏斗状结构使得激活状态的抗病小体与质膜(plasma membrane, PM)结合, 而这一功能对于细胞死亡和抗病性不可或缺, 暗示抗病小体很可能通过质膜穿孔或形成离子通道发挥作用(夏石头和李昕, 2019)。

1.3 LRR结构域特性

LRR是整个蛋白结构中最为保守且简单的结构域, 处于NLR蛋白的C端, 由多个富含亮氨酸的重复序列组成。功能域互换实验表明, 即使蛋白的其它部分高度相似, 仅LRR不同, NLR蛋白识别的效应子也不同。例如, NAIP5 (neuronal apoptosis inhibitory protein 5)可通过其C末端的LRR结构域识别不同的细菌鞭毛蛋白(Yang et al., 2018)。但多数情况下, LRR并不直接与病原菌相互识别。例如, Prf的LRR区域就是通过与伴侣蛋白SGT1 (suppressor of the G2 allele of skp1)互作来感受病原菌, 进而引发一系列抗病反应(Kud et al., 2013)。也有研究表明, LRR不仅参与病原菌的相互识别, 还参与调节NLR蛋白的信号转导。拟南芥中RPS2、RPP1A和RPS5对信号转导具有抑制作用, 去除LRR结构域后表现出持续的激活状态, 引发抗病反应(Qi et al., 2012)。

2 NLR的识别与信号转导

2.1 NLR对病原体的识别

植物通过对病原菌效应蛋白的特异识别感知病原体的入侵, 进而激活快速、精准的免疫反应。识别方式包括2类: 一类为相对简单的直接识别(图1A); 另一类为复杂的间接识别。直接识别即NLR蛋白直接与病原菌效应蛋白互作, 进行特异识别。例如, 拟南芥RBA1 (response to the bacterial type III effector protein HopBA1)与细菌III型分泌系统效应蛋白HopBA1直接识别, 以调节细胞死亡过程(Nishimura et al., 2017); 烟草Roq1 (recognition of XopQ 1)蛋白可特异识别黄单胞杆菌XopQ及假单孢杆菌HopQ1两种不同效应子, 且免疫共沉淀实验显示, Roq1可与二者互作(Schultink et al., 2017)。此外, 在直接识别模式下, 又衍生出微量识别模式(图1D)。例如, 番茄Sw-5b可直接识别NSm, 进而诱导免疫反应, 当其CC端串联SD序列时, SD序列先与微量NSm结合, 增强Sw-5b与NSm的结合能力, 从而引起更强的抗病反应(Li et al., 2019)。

间接识别指NLR蛋白不直接与病原菌效应蛋白互作, 而是作用于某些目标蛋白, 通过感知目标蛋白(也称保卫蛋白)的变化引发免疫反应(图1B)。间接识别最初仅有保卫模式, 随后衍生出诱饵模式。在保卫模式下, 病原菌效应蛋白与保卫蛋白互作, 引起后者生理生化变化和结构改变, 被NLR蛋白感知后引发防御反应。例如, 拟南芥CRCK3 (calmodulin-binding receptor-like cytoplasmic kinase 3)作为磷酸化途径成员, 其磷酸化后被NLR蛋白SUMM2 (suppressor of mkk1 mkk2 2)识别, 进一步促进免疫反应发生(Zhang et al., 2017)。水稻(*Oryza sativa*)保卫蛋白APIP10 (AvrPiz-t and AvrPiz-t interacting protein 6)通过抑制NLR蛋白Piz-t维持植物的稳态。米曲霉

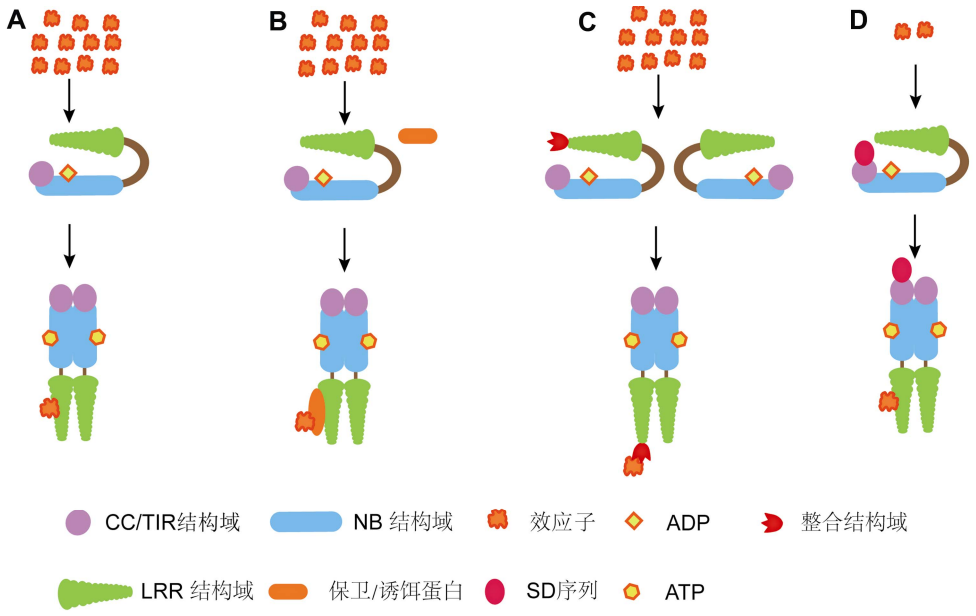

图 1 NLRs 的识别和激活模型
(A) 直接识别模型; **(B)** 保卫/诱饵模型; **(C)** 整合激活模型; **(D)** 微量识别模型

Figure 1 Models for recognition and activation of NLRs
(A) Direct interaction model; **(B)** Guard/decoy model; **(C)** Integrated activation model; **(D)** Microrecognition model

入侵时，APIP10被效应蛋白AvrPiz-t降解，从而解除对Piz-t的抑制，造成Piz-t的大量积累，继而引发ETI (effector-triggered immunity) 反应 (Park et al., 2016)。与保卫模式不同，诱饵模式下的保卫蛋白会根据与其互作的NLR蛋白有无，选择性地改变其特性。NLR存在时，保卫蛋白以最佳状态增强与病原菌效应蛋白的互作，以便被NLR蛋白识别；NLR不存在时，保卫蛋白则会尽量避免与效应子结合，以减轻其对植物的伤害。例如，水稻中的Exo70作为诱饵蛋白，被稻瘟菌效应蛋白Avr-Pii识别后形成大量复合物，继而被Pi-i识别并激发ETI，从而增强植株对病原的抗性 (Fujisaki et al., 2015)。

NLR本身也可成为诱饵蛋白，此类NLR含有特殊的整合结构域，可对病原微生物进行特异性识别(图1C)。水稻中RNG4/RNG5形成复合物时可特异识别稻瘟菌Avr-CO39和Avr-Pia两种效应子，进而引发下游的抗病反应，但二者单独存在时，却不诱发ETI (Ortiz et al., 2017)。拟南芥中, CHS3 (chilling sensitive 3)和CSA1 (constitutive shade-avoidance 1)是两个近源NLR，其中CHS3具有识别功能, CSA1则提供抗性，二者结合后才能激活完整的抗病反应(Xu et al., 2015)。高等植物中普遍存在类似两个NLR蛋白联合行使功能的抗病方式(Kroj et al., 2016)。Xie等(2019)研究发现，有些NLR在植物体内以辅助蛋白的形式存在，如最新克隆的水稻Pizh-2，其单独存在时不具有抗病功能，与Pizh-1结合后可增强后者对稻瘟菌的抗性。

2.2 NLR的激活与信号转导

NLR通常需要形成二聚体或者多聚体才被激活(El Kasmi and Nishimura, 2016; Wang et al., 2019a)。二聚体可以由NLR自身不同结构域之间结合形成(以N、SNC1和L6为代表的TNLs或以Prf、RPS5和MLA10为代表的CNLs都可自身结合形成二聚体而激活) (Qi and Innes, 2013)；也可是NLR两两配对形成，如马铃薯(*S. tuberosum*)，其SINRC4a与LeEIX2相互结合形成二聚体而激活表达(Leibman-Markus et al., 2018)。这些NLRs在染色体上的定位通常相邻(大部分前后相连)，但DM1 (DANGEROUS MIX 1)和DM2d (DANGEROUS MIX 2d)较为特别，虽然分别存在于不同的拟南芥生态型中，但仍可在体外烟草中共表达后形成二聚体(Tran et al., 2017)。ZAR1则需要形成五聚体后才可以被完全激活(Wang et al., 2019b)。由此推测，植物中可能还存在其它的NLR激活方式。

许多CNLs (包括RPS5、RPS2和RPM1)定位于质膜(Qi and Innes, 2013), 其膜定位对于它们的活性是必需的, 可能与其相应效应子(或保卫/诱饵蛋白)的质膜定位相关。大部分NLRs被激活后亚细胞定位发生改变。不同定位的同一蛋白行使的功能可能不同。SNC1和Rx1均存在于细胞核和细胞质中, 但仅定位于细胞核中的SNC1才具活性, 仅定位于细胞质中的Rx1 (resistance to *Potato virus X*)才发挥作用(Slootweg et al., 2010; Xu et al., 2014a)。定位于细胞质的NLRs可能主要与病原菌的识别相关, 或与引发MAPK磷酸途径和诱导ROS积累相关; 细胞核中的NLRs则可能更多与转录因子相互作用。

2.3 NLR与转录因子互作调控下游免疫信号转导

研究表明, NLRs可通过与转录因子直接互作来调控下游免疫信号转导。例如, 大麦CNL MLA10通过其CC结构域与两种相互拮抗的转录因子WRKY1和MYB6 (v-myb avian myeloblastosis viral oncogene homolog 6)直接互作, 进而调控植物免疫(Chang et al., 2013)。而SNC1可与两种(如TPR1和bHLH84)及以上不同的转录因子相互作用。TPR1是一种转录抑制因子, 抑制多种已知免疫负调节因子的表达; *tpr*三重突变体中*snc1*的自身免疫被完全抑制(Zhu et al., 2010)。bHLH84是一种转录激活因子, 可作为免疫正调节因子与SNC1和RPS4互作。bHLH84过表达可增强免疫力, 敲除bHLH84及其2个近缘旁系同源蛋白则抑制RPS4和SNC1活性(Xu et al., 2014b)。此外, 烟草转录因子SPL6 (squamosa promoter-binding protein like6)激活后可与细胞核中的N蛋白相互作用, SPL6调节一部分防御基因, 为N蛋白下游ETI所必需。与此类似, 拟南芥同源基因*AtSPL6*为RPS4下游ETI所必需(Padmanabhan et al., 2013)。近期, Zhai等(2019)研究发现, 水稻转录因子RRM (RNA-recognition motif)可直接与NLR PigmR互作以激活其防御, 进而特异抵抗稻瘟菌的入侵。

3 NLR的调节方式

3.1 表观遗传调控

植物具备多种表观遗传调控机制, 主要包括染色质重塑、DNA甲基化、组蛋白修饰和非编码RNA调控4类。染色质重塑因子SPLAYED (在筛选MUSE过程中得到)属于SWI/SNF家族, 其通过影响*SNC1*转录抑制由*SNC1*介导的免疫反应(Johnson et al., 2015)。*nrpc7-1* (RNA聚合酶III的亚基缺失突变体)也在MUSE的正向筛选过程中得到, 该突变在增强植物抗性的同时引起了一系列表型缺陷, 原因可能是突变体在小RNA水平发生了修饰(Johnson et al., 2016)。染色质重塑因子CHR5 (chromatin-remodeling factor 5) (与组蛋白单泛素酶HUB1 (HISTONE MONOUBIQUITINATION 1)互作)上调细胞内*SNC1*的表达, 并可与另一种染色质重塑基因*DDM1*产生拮抗(Zou et al., 2017)。此外, DNA甲基化也调节*SNC1*的转录。Espinas等(2016)研究发现, *mos1*和*ddm1*均负调控胞嘧啶甲基化, 但二者在*snc1*调节中相互拮抗。通常情况下, DNA甲基化缺陷导致防御反应增强, DNA甲基化程度上升则增强易感性。有研究表明, 在转录因子RPP7的第1个内含子中插入反转录转座子*COPIAR7*会导致转录提前终止, 对RPP7等转录因子的调节可影响组蛋白甲基化, 并且各种组蛋白甲基化突变体中RPP7的表达也受到影响(Tsuchiya and Eulgem, 2013; Le et al., 2015)。与此类似, *SNC1*以及*RPP4*的表达依赖于组蛋白赖氨酸甲基转移酶ATXR7 (*Arabidopsis* trithorax-related 7)及其相关蛋白MOS9 (modifier of snc1,9) (Xia et al., 2013)。

Halter和Navarro (2015)研究表明, miRNAs的产生对植物的抗病过程有显著影响, RNA的调节与植物免疫之间关系密切。NLR超量表达会对植物的生长发育造成不利影响, miRNAs通常作为NLR的负调控因子维持其在植物体内的稳态。从进化角度看, miRNAs高度靶向NLR保守序列, 以此减少其在植物体内积累造成的影响(Zhang et al., 2016)。

番茄miR482/2118超级家族与拟南芥miR472-RDR6为同源microRNA, 它们通过RNA诱导沉默方式在转录后水平调节多个CNLs (Boccara et al., 2014)。miR482/2118家族靶向调控NLR在番茄体内的积累, 从而调控其对卵菌和细菌病原体的抗性(Canto-Pastor et al., 2019)。miR9863家族通过特异调节大麦CNL MLA进而调控大麦的免疫反应及细胞死亡信号转导(Liu et al., 2014)。烟草miR6019以及miR6020靶向调节TNL N, 影响N蛋白在烟草中的表达及其介导的抗烟草花叶病毒反应(Deng et al.,

2018)。大豆(*Glycine max*) miR1510a/b靶向调节特异性防御大豆疫霉所需的TNL, 但在大豆疫霉菌感染期间其效应被抑制(Liu et al., 2014)。gma-miR151则靶向调节大豆中NB-LRR免疫受体基因*Glyma.16-G135500*, 在调节对大豆疫霉的抗性中起关键作用(Cui et al., 2017)。

3.2 可变剪接调控

可变剪接在调控NLR (特别是TLR)的过程中起重要作用, 其通过产生异常转录本/蛋白(或通过引发NMD (nonsense-mediated decay))的方式行使功能。转录组分析显示, 可变剪接广泛存在于具有多个内含子的NLR (特别是TNL)中。Xu等(2012)在MOS筛选过程中鉴定出多个突变体, 这些突变体大多属于*MOS4*相关复合物(MAC), 可调节NLR的拼接。若*SNC1*和*RPS4*的可变剪接发生改变, 将影响植物的基础免疫抗性(Xu et al., 2012)。但需要指出的是, MAC相关蛋白MOS2、CDC5和PRL1也参与miRNA的生物合成, 表明这两种系统间可能存在某种联系(Zhang et al., 2014)。与此不同的是, 参与miRNA剪接的小核糖核蛋白所需的甲基体复合物的突变可增强植物的免疫力, 这与其它剪接突变体的表型相反(Huang et al., 2016a)。Zhang等(2014)研究发现, NMD因子突变体表现出依赖于PAD4 (pHYTOALEXIN DEFICIENT4)和EDS1 (enhanced disease susceptibility 1)的自身免疫, 表明NMD与可变剪接密切配合, 在转录后水平调节NLR, 防止自身免疫并调节免疫应答。

3.3 翻译后修饰

翻译后修饰主要包括泛素化、磷酸化和糖基化等几类, 其中泛素化修饰的应用最为广泛, 可以在蛋白水平调节植物的免疫。Huang等(2014, 2016b)在MUSE突变体筛选过程中, 得到拟南芥唯一的与E4酶和2个E3酶相关的TRAF (TNF receptor associated factor)蛋白(MUSE13以及MUSE14)。Gou等(2012)研究发现, SNC1和RPS2通过F-Box E3 SCF^{CPR1}经由泛素-蛋白酶体途径直接调节植物的免疫反应。Wang等(2016)则发现RING型E3 MIR1调节大麦中的多种MLA NLRs; 其它E3s靶向非NLR免疫调节因子或影响对效应子的识别(Copeland et al., 2016a, 2016b; Tong et al., 2017); 免疫正调节因子E3泛素连接酶SAUL1 (senescence-associated E3 ubiquitin ligase 1)由TNL SOC3保卫(Tong et al., 2017)。

已有研究表明, RAR1-SGT1b-HSP90伴侣复合体各组分对由各种NLR介导的ETI都很重要(例如, RpiBLB2信号转导需要SGT1 (suppressor of the G2 allele of skp1), 但无须HSP90 (heat shock protein 90)或RAR1 (required for Mla12 resistance) (Oh et al., 2014)), 但作用机制相当复杂, 尚需要深入探究(Kadota and Shirasu, 2012)。

4 总结与展望

NLR作为植物抗病蛋白中最重要的一类, 自发现之日起便受到研究者的广泛关注。随着*NLR*基因的不断被克隆, 相关研究也在不断深入。冷冻电镜技术的应用, 尽管可使我们更加直观地观察到NLR的结构组成及激活方式, 但对其整个信号通路目前仍不完全清楚。此外, CRISPR技术的日渐成熟为培育抗病新品种提供了新思路。因此, 在未来可预见的一段时间内, 对NLR的探究仍然是植物抗性研究领域的热点。

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NLR and Its Regulation on Plant Disease Resistance

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Abstract In order to adapt to various living environments, plants have gradually evolved a complex immune system against the infections caused by pathogens. The nucleotide-bounding leucine-rich repeat proteins (NLRs) act as typical resistance (R) proteins which commonly exist in plants and play an important role in regulating plant disease resistance. In this paper, the research progress of NLRs is reviewed from the aspects of NLR protein structures, signal transductions and regulations of plant disease resistance.

Key words NLR, plant immunity, resistance protein, signal transduction

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