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·热点评·

开启防御之门: 植物抗病小体

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摘要 NLR蛋白是存在于植物和动物中的一个免疫受体大家族, 具有核苷酸结合域并富含亮氨酸重复序列。植物NLR通过识别病原菌特异效应子开启免疫信号转导。第1个植物NLR抗性蛋白于25年前克隆, 但其激活机制仍不清楚, 至今仍未获得一个完整的NLR蛋白结构。最近, 柴继杰、周俭民和王宏伟实验室合作解析了第一个植物完整NLR ZAR1激活前后的结构, 研究成果以两篇论文形式发表在“科学”杂志上, 填补了NLR介导的免疫信号转导研究领域的空白。该文简要总结了相关研究进展, 讨论了NLR免疫信号转导研究领域尚需解决的问题。

关键词 植物免疫, NLR, ZAR1, 变构激活, 抗病小体

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自人类开始应用育种技术改良农作物以来, 培育抗病品种一直备受重视。抗病基因及其编码蛋白(R蛋白)的发现和应用, 无疑对作物的抗病性改良与环保高效生产具有重要意义。二十多年前, 人们应用分子生物学技术成功克隆了第一批抗病蛋白基因, 其编码的蛋白大部分属于具有核苷酸结合域并富含亮氨酸重复序列(nucleotide-binding domain, leucine-rich repeat)的NLR家族。过去20年间, 尽管科学家们付出了巨大努力, 但迄今仍未解析出一个完整的植物NLR蛋白结构。最近, 清华大学柴继杰研究组和王宏伟研究组以及中国科学院遗传与发育生物学研究所周俭民研究组合作对该问题进行了探索, 并取得了突破性进展。

NLR受体蛋白是存在于动植物细胞中的一个免疫受体大家族(Jones and Dangl, 2006; Maekawa et al., 2011)。植物中的NLR可以直接或间接地感知进入细胞的病原效应蛋白, 在植物免疫信号调控中起关键作用(Dodds and Rathjen, 2010; Duxbury et al., 2016)。NLR蛋白为具有多结构域的信号转导ATP酶(signal transduction ATPases with numerous domains, STAND)的成员, 包含1个非保守的N端域、1个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(Lukasik and Takken, 2009)。由于位于序列中间的结构域保守存在于最初发现的3个NLR成员(Apaf-1、抗性(R)蛋白和Ced-4)中, 故被称为NB-ARC域(Jones and Dangl, 2006; Maekawa et al., 2011; Duxbury et al., 2016)。ZAR1 (HOPZ-ACTIVATED RESISTANCE 1)是拟南芥(*Arabidopsis thaliana*) (Lewis et al., 2010)和烟草(*Nicotiana benthamiana*) (Schultink et al., 2019)共有的典型CC-NLR (CC (coiled-coil)-NLR)蛋白, 其通过与受体样胞质激酶(RLCKs)亚家族XII-2的多个成员互作形成特异响应的免疫受体复合物, 每个复合物特异响应不同的效应蛋白并触发ETI (effector-triggered immunity)抗病反应(Lewis et al., 2013; Wang et al., 2015; Seto et al., 2017)。例如, ZAR1与RLCK XII家族成员RKS1形成的复合物, 特异识别野油菜黄单胞菌(*Xanthomonas campestris*)的效应蛋白AvrAC。这一识别过程中, 首先需要AvrAC对PBL2 (拟南芥的另一个激酶)进行尿苷酸修饰形成PBL2^{UMP}, 之后PBL2^{UMP}通过与RKS1相互作用被ZAR1-RKS1招募, 完成拟南芥对黄单胞菌AvrAC的识别, 进而激活免疫反应(Wang et al., 2015)。激活后的NLR通过诱导超

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敏反应(hypersensitive response, HR)等多种防御机制阻止病原体增殖, 从而将病原体限制在感染部位(Chisholm et al., 2006; Cui et al., 2015)。

NLR作为AAA+ATPase的成员, 常被认为通过寡聚方式发挥作用。然而, 植物NLR在活化后是否也通过寡聚方式形成类似人体和动物凋亡小体(apopotosome)及炎症小体(inflammasomes)的大蛋白复合物仍不清楚。此外, 尽管已经进行了多年的深入研究, 但人们对植物NLR的生化功能仍知之甚少, 有关其自抑制、病原效应子识别和免疫激活模型虽然已得到植物实验结果(Rairdan and Moffett, 2006; Qi et al., 2012)的支持, 但在很大程度上也是根据人体APAF-1 (Riedl et al., 2005; Reubold et al., 2011; Zhou et al., 2015)和动物NLR (Hu et al., 2013; Hu et al., 2015; Zhang et al., 2015; Maekawa et al., 2016)中NOD的结构提出的。植物NLR中的NB-ARC被认为是一种具有ADP-和ATP-结合形式, 指示NLR信号“关闭”和“打开”状态的分子开关(Tameling et al., 2002; Williams et al., 2011; Bernoux et al., 2016), 但其内在分子机制仍然未知。柴继杰、周俭民和王宏伟团队通过结构生物学、生化、遗传以及生物学功能研究, 发现尿苷酰化的PBL2^{UMP}作为配体结合ZAR1-RKS1复合物后诱导ZAR1-NB结构域的构象变化, 促进ADP释放, 进入中间状态; 中间状态的ZAR1结合ATP后诱发ZAR1寡聚结构域暴露, 致使ZAR1-RKS1-PBL2^{UMP}形成轮状五聚体免疫抗病小体; 该抗病小体通过新形成的漏斗状结构定位于质膜, 激活超敏反应和抗病性(Wang et al., 2019a, 2019b)。

三位科学家团队利用冷冻电镜手段(cryo-electron microscopy), 在体外组装并解析了3个ZAR1复合物结构(Wang et al., 2019a, 2019b)。正常生长条件下, 植物NLR受到严格调控, 以免因免疫失控导致植物生长受阻和受损。通过解析ZAR1与假激酶RKS1形成的复合物结构, 他们一方面揭示了NLR被维持在非激活状态的机制(Wang et al., 2019a); 另一方面揭示了ZAR1与不同RLCK XII成员结合的机制。在ZAR1-RKS1复合物中, ZAR1的LRR结构域通过分子内互作, 遮盖了ZAR1中的寡聚化结构域, ADP的结合进一步稳定了非激活状态。当病原物入侵时, 植物NLR则转换为激活状态。他们对第2个复合物的结构以及功能进行解析, 发现了ZAR1-RKS1特异招募PBL2^{UMP}的分子机制(Wang et al., 2019a)。ZAR1-RKS1中RKS1蛋白的独特基序与PBL2^{UMP}中的UMP基团相互作用, 形成ZAR1-RKS1-PBL2^{UMP}复合物。PBL2^{UMP}的结合使本来松散的RKS1激活片段稳定下来, 后者与ZAR1中的NBD结构域发生空间碰撞, 导致ZAR1^{NBD}向外旋转约60度, ADP从ZAR1中释放出来, 从而使得ZAR1-RKS1-PBL2^{UMP}复合物进入随时可结合ATP的中间状态(Wang et al., 2019a) (图1)。由配体结合导致的NLR^{NBD}结构域构象变化, 对理解植物中其它NLR受体的激活机制具有重大意义。

在后续研究中, 他们将ZAR1-RKS1-PBL2^{UMP}复合物(中间状态)与dATP(或者ATP)共同孵育, 获得了分子量约为900 kDa的寡聚复合物, 称之为“ZAR1抗病小体”。进一步通过冷冻电镜分析, 他们获得了该抗病小体的高分辨率(3.4 Å)结构。ZAR1抗性小体为轮状五聚体, 其寡聚化由ZAR1介导。ZAR1的所有结构域(包括CC、NB结构域(NBD)、螺旋结构域1(HD1)、翼螺旋结构域(WHD)和LRR结构域)均参与了ZAR1抗性小体的五聚体化, 并被dATP进一步稳定(Wang et al., 2019b) (图1)。该结构与NLRC4炎症体(或APAF-1凋亡体)有较大差异。突变分析和功能研究证实, 抗病小体的形成导致了ZAR1的激活、HR细胞死亡和抗性启动。此外, 抗病小体还形成了一个由CC结构域组成的新功能结构。ZAR1寡聚化后, CC域的氮端α-螺旋组成了一个双性的漏斗状结构, 在五聚轮状结构的平面上形成一个突出结构(Wang et al., 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。

综上所述, 柴继杰、周俭民和王宏伟团队基于结构、生化、遗传和功能多重数据, 首次完成了植物NLR蛋白复合物组装、结构和功能分析, 揭示了NLR作用的关键分子机制。该研究是植物免疫领域的里程碑发现, 也是继国内学者近年在多个领域取得突破(施怡婷和杨淑华, 2016; 于倩倩等, 2018)后又一项重要原创性成果。这两项工作不仅为理解植物中其它NLR蛋白作用方式提供了结构模型, 还为人工改造NLR和发展广谱抗病新技术打下基础。

植物体内的另一大类NLR, 其N端为TIR (Toll/

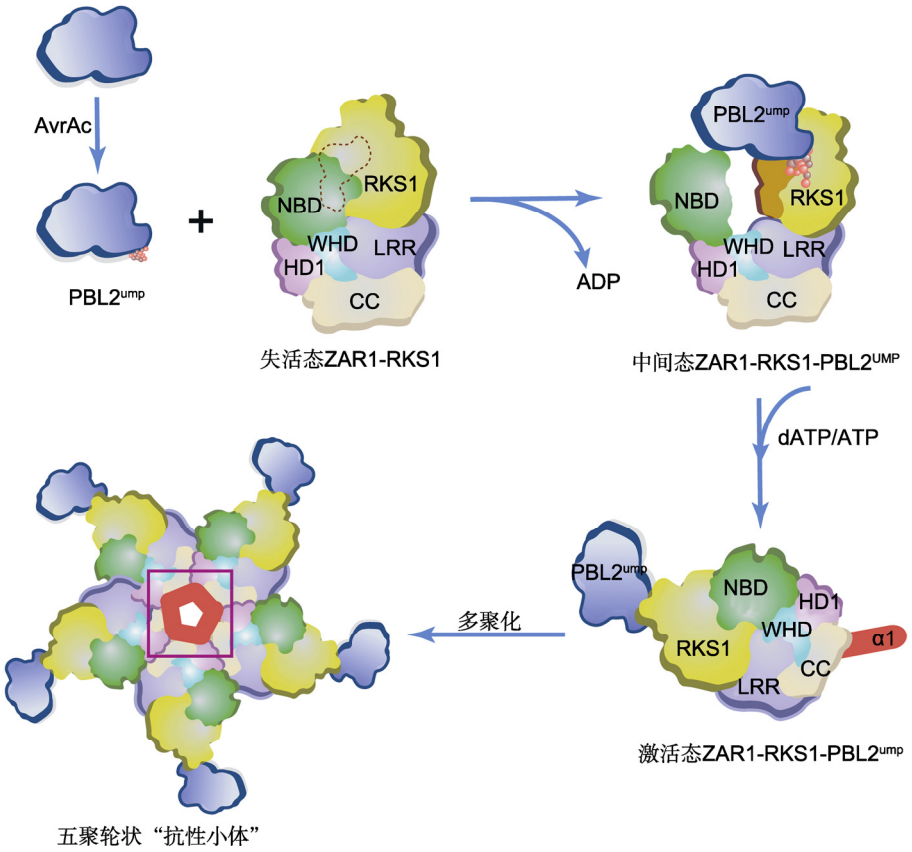

图1 PBL2^{UMP}诱导的ZAR1抗性小体的激活与装配

野油菜黄单胞菌的效应蛋白AvrAC以尿苷酰化修饰拟南芥PBL2激酶，尿苷酰化的PBL2^{UMP}作为配体通过与RKS1互作而被ZAR1-RKS1复合物招募。RKS1的激活片段在与PBL2^{UMP}的2个尿苷基部分(球形)相互作用后变得稳定(橙色表面)，并与ZAR1^{NBD}结构域发生立体碰撞，导致后者向外旋转，从而释放ADP，形成可与dATP/ATP结合的中间状态ZAR1-RKS1-PBL2^{UMP}复合体。该复合物结合dATP/ATP后，诱导ZAR1结构重塑和折叠转换，隐藏在非活性ZAR1-RKS1复合物中的ZAR1的N顶末端α-螺旋(α1，红色)暴露在溶剂中，导致ZAR1完全激活(激活态ZAR1-RKS1-PBL2^{UMP})，继而通过多聚化形成五聚轮状结构的ZAR1抗性小体(紫色方框内突出显示形成的漏斗状结构)。CC、NBD、HD1、WHD和LRR为ZAR1的不同结构域。

Figure 1 PBL2^{UMP}-induced activation and assembly of the ZAR1 resistosome

Arabidopsis PBL2 is modified by uridylyl transferase AvrAC, which is an effector protein from *Xanthomonas campestris*. The uridylylated PBL2 (PBL2^{UMP}) as a ligand is then recruited by the ZAR1-RKS1 complex through interaction with the pseudokinase RKS1. The activation segment of RKS1 becomes stabilized (orange surface) after interacting with the two uridylyl moieties (in sphere) of PBL2^{UMP}, and sterically clashes with ZAR1^{NBD}, causing the latter to rotate outward and consequently release ADP, forming an intermediate ZAR1-RKS1-PBL2^{UMP} complex which allows it to bind dATP/ATP. Binding of dATP/ATP induces structural remodeling and fold switching of ZAR1. The very N-terminal helix (α1, red) of ZAR1 buried in the inactive ZAR1-RKS1 complex becomes solvent-exposed in the activated ZAR1-RKS1-PBL2^{UMP} complex, forming a ZAR1 resistosome pentameric structure through polymerization (a funnel-shaped structure highlighted within the purple frame). CC, NBD, HD1, WHD and LRR are different structural domains of ZAR1.

interleukin receptor)域。TIR-NLR是否也通过类似CC-NLR蛋白的方式介导免疫信号目前尚不清楚。已有研究表明，有几种CC-NLR作用于TIR-NLR的免疫信号下游(Castel et al., 2018; Qi et al., 2018; Wu et al., 2018)。例如，CC-NLR NRG1 (N requirement gene 1) (Peart et al., 2005)和ADR1 (activated disease resistance 1) (Bonardi et al., 2011; Dong et al., 2016)属于RPW8分支族，对拟南芥和烟草多种TIR-NLR的抗性有重要调控作用。由此推测，这种新的免疫分子开关机制可为解释TIR-NLR免疫信号转导提供参考模型。但是TIR-NLR免疫信号转导与CC-NLR是否有所不同？植物体内NLR是否还存在其它

的调控机制? 对上述问题的回答将有助于我们深入理解免疫信号转导的分子机理。

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Open a Door of Defenses: Plant Resistosome

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Abstract Nucleotide binding, leucine-rich repeat (NLR) immune receptors are a major family of plant resistance (R) proteins, which are also found in animals. NLRs turn on immune signaling by recognizing pathogen-specific effectors in plants. Although the first few plant NLR *R* genes were cloned more than 25 years ago, the activation mechanism remained elusive. No structure is available for the full-length plant NLRs despite attempts over the last 2 decades. Recently, studies from the Chai, Zhou and Wang labs, published in *Science*, solved the structure of zygote arrest 1 (ZAR1) before and after effector recognition, which fills a huge gap in NLR biology. This mini review briefly summarized these findings and related progresses, and highlighted further challenges in NLR-mediated immune signaling field.

Key words plant immunity, NLR, ZAR1, allosteric activation, resistosome

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