植物学报 *Chinese Bulletin of Botany* 2019, **54** (2): 168–184, www.chinbullbotany.com
doi: 10.11983/CBB18219

·特邀综述·

蛋白磷酸化修饰在植物-病原微生物互作中的作用研究进展

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摘要 蛋白磷酸化修饰是植物细胞信号调控的普遍机制。植物-病原微生物互作过程中, 关键调控蛋白的磷酸化状态影响免疫信号的激活。多种病原微生物通过干扰宿主蛋白的磷酸化状态攻击免疫系统, 以提高致病性。该文对植物免疫调控过程中关键元件的磷酸化修饰及其在免疫信号中的调控作用进行了综述。研究植物-病原菌互作过程中关键蛋白的磷酸化修饰, 有助于深入探讨植物-病原微生物互作的分子机理。该文将为寻找广谱抗病的新途径提供理论依据。

关键词 蛋白磷酸化修饰, 蛋白激酶, 蛋白磷酸酶, 植物-病原微生物互作

刘雅琼, 侯岁稳 (2019). 蛋白磷酸化修饰在植物-病原微生物互作中的作用研究进展. 植物学报 **54**, 168–184.

植物在漫长的进化过程中形成至少2种免疫途径来抵抗自然界中各种病原微生物的入侵。一种是由定位于细胞质膜的受体识别病原菌关联的分子模式(pathogen-associated molecular patterns, PAMPs)激活的防御反应, 称为PAMP触发的免疫(PAMP-triggered immunity, PTI)。病原微生物分泌效应蛋白进入植物细胞, 干扰宿主免疫激活并增强致病性。另一种免疫途径是通过植物抗病蛋白直接或间接识别效应蛋白后激活, 即效应子触发的免疫(effector-triggered immunity, ETI) (Dangl and Jones, 2001; Jones and Dangl, 2006; Dodds and Rathjen, 2010)。蛋白磷酸化修饰作用是调控细胞信号转导的主要机制, 这一过程依赖于蛋白激酶和蛋白磷酸酶。在植物-病原微生物互作的复杂过程中, 蛋白激酶和蛋白磷酸酶通过调节多种蛋白的磷酸化状态调控植物免疫途径。

1 蛋白激酶和蛋白磷酸酶

模式植物拟南芥(*Arabidopsis thaliana*)的基因组编码大约1 100种蛋白激酶以及约150种蛋白磷酸酶(Schweighofer and Meskiene, 2015)。根据磷酸化修饰的氨基酸残基不同, 蛋白激酶主要分为两大类, 即丝氨酸/苏氨酸蛋白激酶和酪氨酸蛋白激酶。此外, 有些激酶同时具有丝/苏氨酸蛋白激酶活性和酪氨酸蛋白激酶活性。目前, 在植物免疫途径中研究相对较多的主要有4种激酶, 即跨膜的受体激酶(receptor like kinase, RLK)、胞质内受体激酶(receptor-like cytoplasmic kinase, RLCK)、丝裂原活化激酶(mitogen-activated protein kinase, MAPK)以及钙依赖蛋白激酶(calcium-dependent protein kinase, CPK)。根据结构、催化方式以及底物特异性的不同蛋白磷酸酶分为3种类型, 即蛋白丝氨酸/苏氨酸磷酸酶、蛋白酪氨酸磷酸酶以及双特异性磷酸酶(Luan, 2003)。在拟南芥中, 约96%的蛋白磷酸化作用发生在丝氨酸/苏氨酸残基上。催化丝氨酸/苏氨酸残基去磷酸化的磷酸酶包括2个家族, 即丝氨酸/苏氨酸蛋白磷酸酶家族(phosphoprotein phosphatase, PPP)和金属离子依赖的蛋白磷酸酶(metal-dependent protein phosphatases, PPM)。PPP类蛋白磷酸酶的催化亚基结合不同的调节亚基组成的全酶具有不同的底物特异性。PPM家族蛋白磷酸酶主要由PP2C组成, 其本身同时具有催化活性和调节功能, 不需要单独的调节亚基进行调控(Schweighofer and Meskiene, 2015)。

蛋白质经磷酸化修饰后, 其稳定性、活性以及亚细胞定位等发生变化, 参与调控各种生物学过程(Bigeard et al., 2014)。例如, 部分蛋白经磷酸化修饰后构象发生变化, 导致酶活性改变; 而有些蛋白的磷

收稿日期: 2018-10-20; 接受日期: 2018-12-29
基金项目: 国家自然科学基金(No.31870251)和农业部转基因生物新品种培育重大专项(No.2016ZX08009-003-002)
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酸化修饰影响与其它蛋白的相互作用; 此外, 有的蛋白需经磷酸化修饰后才能结合到特定的部位发挥功能。因此, 蛋白的可逆磷酸化修饰需要受到精细调控, 以保障各种生物学过程有序进行。

2 受体激酶对免疫信号的识别和激活

植物中跨膜的受体类激酶和受体类蛋白(receptor like protein, RLP)识别胞外信号并将其转化为胞内信号。这些RLK或RLP又被称为模式识别受体(pattern recognition receptors, PRRs)。典型的RLK由胞外域、单次跨膜的结构域以及胞质内的激酶结构域组成, RLP则不具有胞内激酶结构域。RLK或RLP的胞外域主要有4种类型: 亮氨酸重复序列(leucine-rich repeat, LRR)、赖氨酸基序(lysine motif, LysM)、凝集素样结构域(lectin domain)以及类表皮生长因子(epidermal growth factor, EGF)结构域(Couto and Zipfel, 2016)。目前已报道多种受体激酶或受体蛋白参与植物免疫信号的识别和激活(Tang et al., 2017)。

2.1 LRR类受体激酶和受体蛋白调控免疫激活

2.1.1 LRR受体激酶对信号的识别和激活

LRR类PRRs主要识别蛋白或多肽序列(图1)。例如, LRR类受体激酶FLS2 (FLAGELLIN SENSING 2)和EFR (ELONGATION FACTOR-Tu RECEPTOR)分别识别并结合病原菌鞭毛蛋白保守的22个氨基酸残基组成的肽flg22, 以及延伸因子Tu (EF-Tu) N末端的保守氨基酸序列elf18 (Chinchilla et al., 2006; Zipfel et al., 2006); PEPR1/2 (PEP RECEPTOR)识别植物响应病原菌分泌的内源可溶性前体肽pep (propeptide) (Yamaguchi et al., 2006); 番茄(*Solanum lycopersicum*)中FLS3识别细菌flgII-28并增强对细菌的免疫响应(Hind et al., 2016); 水稻(*Oryza sativa*) LRR-类受体激酶XA21识别水稻白叶枯菌(*Xanthomonas oryzae* pv. *oryzae*, *Xoo*)中保守的蛋白RaxX等(Pruitt et al., 2015)。

拟南芥LRR类受体激酶SERKs (SOMATIC EMBRYOGENESIS RECEPTOR KINASEs)作为FLS2/EFR/PEPRs等PRRs的共受体参与免疫信号的识别和激活。PRR接受病原信号后与BAK1/SERK3 (BRI1-ASSOCIATED RECEPTOR KINASE 1)结合, 并且胞内激酶结构域发生自磷酸化修饰。与此同时, PRR与BAK1的激酶域相互磷酸化修饰, 迅速激活免疫信号(Gómez-Gómez and Boller, 2000; Yamaguchi et al., 2010; Yamada et al., 2016b)。水稻中, OsSERK2正调控XA21、XA3以及OsFLS2介导的免疫响应(Chen et al., 2014)。

由于缺少胞内激酶结构域, LRR-RLP识别配体后通过结合RLKs激活胞内免疫信号。LRR类受体激酶SOBIR1 (SUPPRESSOR OF BIR1-1 1)结合多种LRR-RLP调控防御反应的激活(Gust and Felix, 2014)。拟南芥RLP23识别细菌、真菌以及卵菌分泌的坏死/乙烯诱导的多肽蛋白nlp20 (NECROSIS AND ETHYLENE-INDUCING PEPTIDE 1-LIKE PROTEINS 20)后结合SOBIR1, 并招募BAK1形成复合体激活免疫信号(Albert et al., 2015)。RLP30结合SOBIR1和BAK1, 识别死体营养型真菌蛋白激发子SCFE1 (SCLEROTINIA CULTURE FILTRATE ELICITOR 1)并激活下游免疫反应(Zhang et al., 2013)。番茄中, Cf-4、Ve1和EIX1 (ETHYLENE-INDUCING XYLANASE 1)等RLPs结合SOBIR1激活真菌诱导的免疫途径(Liebrand et al., 2013)。番茄受体激酶ELR (ELICITIN RESPONSE)结合免疫共受体BAK1/SERK3, 通过识别多种疫霉菌激肽蛋白(elicitin proteins)激活广谱且持久的抗病性(Du et al., 2015)。

2.1.2 免疫受体和共受体活性的调控

免疫受体和共受体的活性受到蛋白激酶和蛋白磷酸酶的调控(图2)。无病原菌时, 拟南芥LRR-RLK BIR2 (BAK1-INTERACTING RECEPTOR-LIKE KINASE 2)结合BAK1, 防止其与FLS2等PRRs结合而导致PTI途径不必要的激活(Halter et al., 2014)。PP2A类蛋白磷酸酶负调控PRR复合体的激活。无PAMP诱导时, 由A1、C4以及B’η/ζ亚基组成的PP2A全酶结合BAK1防止其被磷酸化激活; 配体诱导后, PP2A全酶复合体的活性迅速降低, 导致BAK1被磷酸化激活(Segonzac et al., 2014)。拟南芥质膜定位的磷蛋白MKKK7结合FLS2, 受鞭毛蛋白诱导时, MKKK7的多个丝氨酸残基被不同程度地磷酸化修饰, 负调控鞭毛蛋白激活的信号以及基础免疫响应。MKKK7通过直接调控FLS2复合体, 抑制FLS2介导的ROS产生

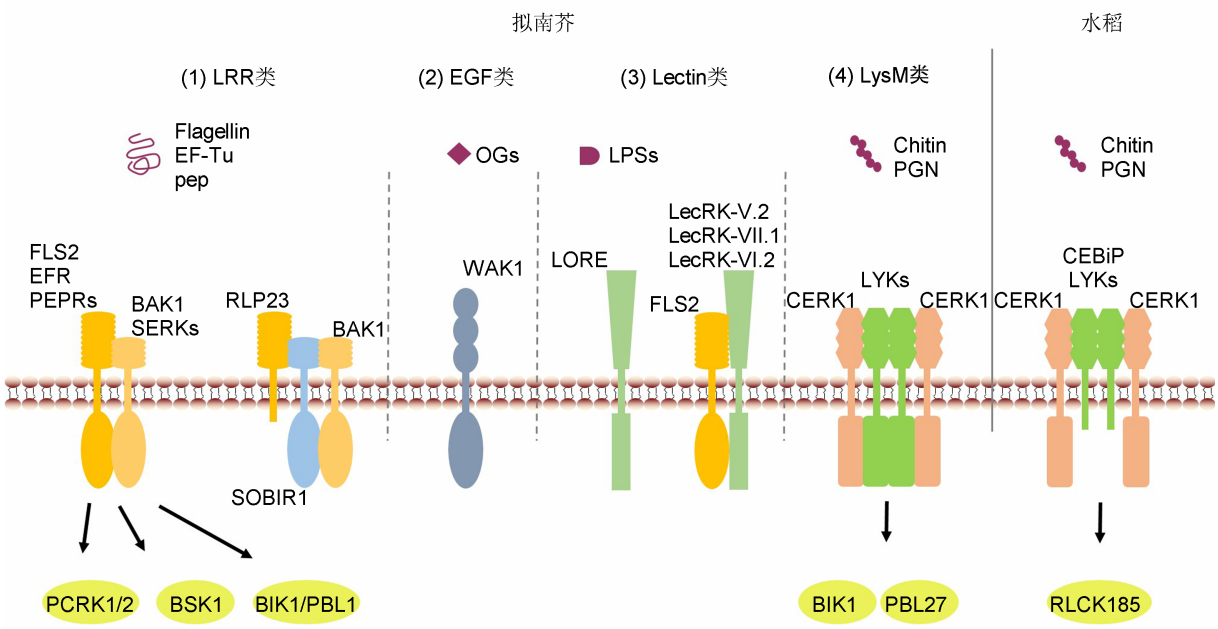

图1 受体激酶和受体蛋白对免疫信号的识别

(1) 拟南芥中, LRR类受体激酶和受体蛋白识别病原微生物蛋白或多肽序列; (2) 拟南芥EGF类受体激酶WAK1识别寡聚半乳糖醛酸; (3) Lectin类受体激酶参与免疫信号识别; (4) 拟南芥和水稻LysM类受体激酶或受体蛋白识别几丁质和肽聚糖。

Figure 1 Recognition of the immune signals by receptor kinases and receptor proteins

(1) In Arabidopsis, LRR-type receptor kinase and receptor protein recognize pathogenic protein or polypeptide; (2) EGF-type receptor WAK1 recognize oligogalacturonides (OGs); (3) Lectin-type receptor kinase participate in immune signaling recognition; (4) LysM-type receptor kinase or receptor protein recognize chitin and peptidoglycan (PGN) signal in Arabidopsis and rice.

(Mithoe et al., 2016)。此外, 水稻PP2C类蛋白磷酸酶XB15 (XA21 BINDING PROTEIN 15)结合并去磷酸化修饰XA21, 负调控XA21介导的免疫激活(Park et al., 2008)。

2.2 LysM类和EGF类受体激酶对免疫信号的识别和激活

LysM类和EGF类PRRs主要识别包含碳水化合物的分子, 如真菌几丁质(chitin)、细菌肽聚糖(peptidoglycan, PGN)和丛枝菌根菌分泌的脂质几丁寡糖(lipochitooligosaccharides, LCOs)或者植物细胞壁衍生的寡聚半乳糖醛酸(oligogalacturonides, OGs)等(图1)。拟南芥中的3个LysM类受体激酶CERK1 (CHITIN ELICITOR RECEPTOR KINASE 1)、LYK4 (LysM-CONTAINING RECEPTOR-LIKE KINASE 4)和LYK5共同调控几丁质的识别及免疫信号的激活(Miya et al., 2007; Wan et al., 2012; Cao et al., 2014); LYK1和LYK3参与对不同结构的PGN信号的识别, 在AtCERK1存在的条件下激活PGN诱导的防御信号(Willmann et al., 2011)。水稻LysM类RLK OsCERK1胞外的LysM结构域不能直接结合几丁质, LysM类受体蛋白OsCEBiP (CHITIN OLIFOSACCHARIDE ELICITOR BINDING PROTEIN)作为受体结合几丁质, 随后结合OsCERK1激活几丁质诱导的防御反应(Kaku et al., 2006; Shinya et al., 2015)。拟南芥EGF类受体激酶WAK1 (WALL-ASSOCIATED KINASE 1)接受OGs信号并激活防御反应(Brutus et al., 2010)。水稻免疫信号激活过程中, OsWAK1发生自磷酸化并磷酸化下游转录因子OsRFP1。此外, 水稻稻瘟菌侵染、机械损伤及水杨酸和茉莉酸甲酯处理均可以激活OsWAK1转录水平的表达(Li et al., 2009)。

2.3 Lectin类受体激酶参与植物防御反应

根据胞外凝集素结构域的不同, 凝集素样受体激酶(lectin receptor kinase, LecRK)又分为3种类型: G (GNA-related or S-locus)-、C (calcium-dependent)-以及L (legume)-类(Bouwmeester and Govers, 2009)。目前已经鉴定到的L-类LecRK有45个, 它们的功能大部分尚属未知。越来越多的研究表明, L-类LecRK广泛参与植物免疫调控。病原菌或PAMPs诱导*LecRK-I.9*、*LecRK-V.5*、*LecRK-VI.2*以及*LecRK-IX.2*激活表达; 这些LecRKs功能缺失表现出对不同病原菌的敏感性, 过表达则对病原菌抗性增强(Bouwmeester et al., 2011; Singh et al., 2012; Wang et al., 2014b; Luo et al., 2017)。LecRK-V.5抑制病原菌侵染后的气孔关闭过程; *lecrk-V.5*缺失突变体对疫霉菌属的抗病性减弱, 对丁香假单胞菌(*Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000)的抗病性增强(Desclos-Theveniau et al., 2012; Wang et al., 2014)。L-类LecRK-V.2和LecRK-VII.1结合FLS2, 正调控气孔免疫途径(Yekondi et al., 2018)。LecRK-VI.2结合FLS2, 正调控PTI的响应, 参与对丁香假单胞菌和果胶杆菌(*Pectobacterium carotovorum*)的抗性(Singh et al., 2012; Huang et al., 2014)。LecRK-IX.2招募CPKs并磷酸化修饰RBOHD (respiratory burst oxidase homolog protein D), 正调控依赖于RBOHD的水杨酸(salicylic acid, SA)积累以及PTI信号途径(Luo et al., 2017)。此外, 有研究表明, 拟南芥Lectin类受体激酶LORE识别细菌脂多糖(lipopolysaccharides, LPSs)并激活下游免疫信号(Ranf et al., 2015)。

2.4 细胞内免疫信号的传递依赖于蛋白磷酸化修饰

2.4.1 RLCK参与免疫信号的传递

胞质内的受体激酶结合RLK/RLP, 共同调控植物的生长发育、激素信号以及生物和非生物胁迫等过程。研究表明, 拟南芥RLCK-VII家族的PBS1 (AvrPphB SUSCEPTIBLE 1)、BIK1 (BOTRYTIS-INDUCED KINASE 1)、PBL1 (PBS1- LIKE 1)、PBL2、PBL27、PCRKs (PTI COMPROMISED RECEPTOR-LIKE CYTOPLASMIC KINASE)以及RLCK-XII家族成员BSK1 (BRASSINOSTEROID-SIGNALING KINASE 1)参与植物免疫信号传递(图1)。例如, FLS2、EFR、PEPRs以及CERK1等多种PRR被激活后磷酸化修饰BIK1, 导致BIK1与PRRs复合体分离并进入细胞质激活下游免疫信号(Lu et al., 2010; Yang et al., 2010; Liu et al., 2013)。PCRK1和PCRK2结合FLS2并在PAMPs诱导后被磷酸化激活, 冗余地诱导转录因子SARD1 (SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1)和CBP60g (CALMODULIN-BINFING PROTEIN 60g)的表达, 促进SA的合成(Kong et al., 2016)。水稻RLCK-VII家族的OsRLCK185和OsRLCK176结合CERK1, 传递PGN和几丁质激活的免疫信号(Yamaguchi et al., 2013; Ao et al., 2014)。OsRLCK185在拟南芥中的同源蛋白PBL27被CERK1磷酸化激活后, 正调控几丁质诱导的防御反应(Shinya et al., 2014)。

2.4.2 RLCK对底物的磷酸化修饰

RLCK经PRRs激活后, 一方面招募钙离子依赖的蛋白激酶(CPKs)促进产生活性氧(ROS); 另一方面激活下游MAPK信号级联反应(图2)。植物中ROS主要由NADPH氧化酶RBOHD催化生成。目前已报道多种RLCKs调控RBOHD的活性。例如, 被flg22诱导激活的BIK1一方面通过磷酸化修饰RBOHD的多个氨基酸位点激活RBOHD的活性; 另一方面通过磷酸化植物Gα蛋白XLG2 (EXTRA LARGE G-PROTEIN 2)促进ROS的产生(Kadota et al., 2014; Li et al., 2014b; Liang et al., 2016)。GTP酶促进蛋白(RGS1)维持FLS2结合的G蛋白不活跃状态。被flg22诱导激活的BIK1/PBL1磷酸化RGS1的S428和S431位点, 促进RGS1与FLS2-G蛋白复合体分离, 从而正调控免疫信号的激活(Liang et al., 2018)。BSK1结合FLS2调控ROS的生成。*bsk1*缺失突变体中, flg22诱导的ROS爆发减弱, 病原菌诱导的SA积累受到阻碍并且对白粉菌、丁香假单胞菌和卵菌的敏感性增强(Shi et al., 2013)。在无病原菌侵染时, PBL13通过结合RBOHD负调控植物对致病菌的先天免疫途径(Lin et al., 2015)。

PRRs复合体接受免疫信号后激活MAPK信号级联反应, 但是PRR复合体与MAPKs之间信号是如何传递的, 一直以来都鲜有研究。最近, 有研究表明, RLCKs直接连接PRRs复合体与MAPK激酶。

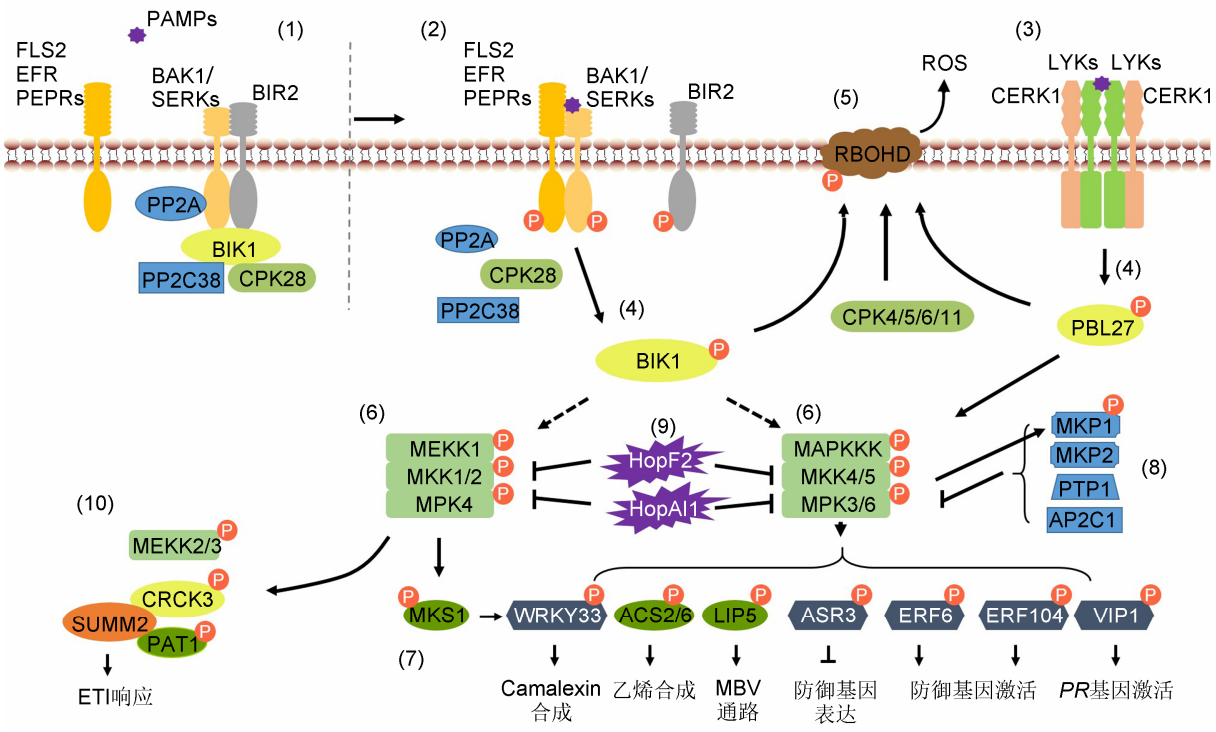

图2 植物中防御相关蛋白的磷酸化修饰调控免疫信号途径

(1) 拟南芥中, 未接受病原信号时, PP2A负调控BAK1的激活, 同时BIK1的活性受到PP2C38和CPK28的共同抑制。(2), (3) 病原菌侵染时, LRR类PRRs识别PAMPs, 结合BAK1后被磷酸化激活, LysM类受体激酶CERK1结合LYKs激活免疫信号。(4) 激活的PRRs磷酸化激活胞质内受体激酶(BIK1和PBL27)等。(5) BIK1与CPKs共同磷酸化激活RBOHD, 促进ROS的产生。(6), (7) 激活的RLCKs将信号传递至MAPK信号级联, 导致MAPK激酶被激活。激活的MAPK磷酸化修饰不同的下游底物, 调控不同的防御应答。(8) MKP1、MKP2、PTP1以及AP2C1等磷酸酶负调控MAPKs的活性。(9) 病原菌分泌的效应蛋白HopF2和HopAI1抑制MAPK信号级联途径。(10) 抗病蛋白SUMM2通过监测MPK4对底物MEKK2、CRCK3以及PAT1的磷酸化修饰, 适时地激活ETI途径。

Figure 2 Phosphorylation of defense related proteins in plant immune signaling pathway

(1) In Arabidopsis, PP2A negatively regulates the activation of BAK1, meanwhile PP2C38 and CPK28 negatively regulate the phosphorylation of BIK1 without pathogen infection. (2), (3) LRR-type PRRs combine with BAK1 and LysM-type RLK CERK1 combine with LYKs to activate immune signaling after perception of pathogen attack. (4) Activated PRRs phosphorylate BIK1 or PBL27. (5) BIK1 and CPKs phosphorylate RBOHD to promote ROS generation. (6), (7) MAPK cascades are activated and then phosphorylate different substrates to regulate different defense responses. (8) MKP1, MKP2, PTP1 and AP2C1 negatively regulate the activity of MAPKs. (9) Bacterial pathogens inject effector proteins HopF2 and HopAI1 to inhibit the activation of MAPK cascades. (10) Resistance protein SUMM2 activate ETI pathway in time by monitoring the phosphorylation statue of MEKK2, CRCK3 and PAT1, the substrates of MPK4.

Yamada等(2016a)报道, 拟南芥RLCK PBL27在质膜上同时结合CERK1和三级激酶MAPKKK5。PBL27在chitin诱导后被CERK1激活, 随后磷酸化修饰MAPKKK5进而激活下游MAPK信号途径。唐定中研究组最近发现, BSK1对MAPKKK5第289位丝氨酸残基的磷酸化修饰促进MAPKKK5调控的对细菌和真菌的抗性, 但不影响MAPKKK5介导的细胞死亡(Yan et al., 2018)。此外, 还有研究显示, BIK1被EFR激活后直接进入细胞核, 结合WRKY转录因子参与JA和SA的调控(Lal et al., 2018)。

2.4.3 RLCK活性的调控

RLCK的活性和稳定性受到严格调控。没有PAMP诱导时, 蛋白磷酸酶PP2C38结合并去磷酸化胞质内受体激酶BIK1, 抑制其对RBOHD的磷酸化修饰及下游免疫途径的激活。经PAMP诱导后, PP2C38与FLS2/

EFR-BIK1复合体分离，解除对BIK1活性的抑制。此外，BIK1对PP2C38第7位丝氨酸残基的磷酸化修饰促进二者分离(Couto et al., 2016)。钙离子依赖的蛋白激酶CPK28一方面磷酸化修饰U-box蛋白PUB25/26，增强其泛素蛋白酶活性，进而促进BIK1的降解；另一方面CPK28直接磷酸化BIK1，负调控多种PAMPs诱导后BIK1介导的免疫途径。XLG2/XLG3 (Gα)和AGB1 (Gβ)等G蛋白则通过抑制PUB25/26的活性稳定BIK1 (Monaghan et al., 2014; Liang et al., 2016; Wang et al., 2018a, 2018b)。

2.5 受体激酶酪氨酸残基的磷酸化修饰

目前已报道的参与免疫调控的受体激酶主要是丝氨酸/苏氨酸类蛋白激酶。近年来，越来越多的研究表明，这些受体激酶的酪氨酸残基的磷酸化修饰同样在植物免疫调控中发挥重要作用。例如, BAK1第403位酪氨酸残基的磷酸化修饰对于BAK1的功能以及免疫信号的激活非常重要，该位点在多种植物SERKs家族中非常保守。大多数LRR-RK中存在Y403的类似位点Tyr-Via，这一位点的磷酸化修饰同样影响多种受体激酶对配体的识别和信号激活(Macho et al., 2014; Perraki et al., 2018)。EFR介导的对丁香假单胞菌的免疫途径依赖于其Tyr-Via位点Y836的磷酸化。丁香假单胞菌分泌具有酪氨酸磷酸酶活性的效应子HopAO1，可减少EFR的磷酸化并抑制随后的免疫响应(Macho et al., 2014)。拟南芥CERK1识别几丁质后，对下游信号的激活依赖于其第428位的酪氨酸残基的自磷酸化修饰。Chitin信号激活后，CERK1招募预测的丝氨酸/苏氨酸蛋白磷酸酶CIPP1 (CERK1-INTERACTING PROTEIN PHOSPHATASE 1)，对Y428去磷酸化修饰，关闭CERK1介导的免疫信号，以防止防御反应被持续激活(Liu et al., 2018)。此外，BAK1通过磷酸化修饰BIK1的酪氨酸残基调控免疫信号的激活, BIK1第150、243及250位酪氨酸位点的磷酸化修饰影响BIK1在免疫调控中的功能(Lin et al., 2013, 2014)。

3 MAPKs在植物-病原菌互作过程中的重要作用

MAPK信号级联由MAPK、MAPKK和MAPKKK三个相互关联的激酶组成。MAPK是信号级联最下游的激酶，其活性区域包含1个Thr和1个Tyr残基，经MAPKK磷酸化后激活。MAPKK的活性环包含2个Ser/Thr残基，MAPKKK通过磷酸化修饰这2个Ser/Thr残基调控MAPKK的活性。目前植物中已经报道了60多个MAPKKK、11个MAPKK以及20余个MAPK，不同MAPKKK激活的MAPKK发挥不同的功能(Meng and Zhang, 2013)。

3.1 MAPKs的激活

拟南芥中已报道2种MAPKs信号级联参与PTI途径，分别是MAPKKK-MKK4/5-MPK3/6和MEKK1-MKK1/2-MPK4。最近，周俭民研究组发现，拟南芥中MAPKKK3和MAPKKK5至少在4种PRRs的下游激活MPK3/6，调控对细菌和真菌的抗病性(Bi et al., 2018)。MAPKKK5对MPK3/6以及下游防御反应的激活，依赖于RLCK VII对MAPKKK5第599位丝氨酸残基的磷酸化修饰。同时，激活的MPK6磷酸化修饰MAPKKK5第682和692位的丝氨酸残基，增强MAPKKK5调控的抗病反应。此外, RLCK VII和MPK4磷酸化修饰MEKK1第603位的丝氨酸残基，增强PAMP诱导的MEKK1途径的激活(Bi et al., 2018)。

通过对烟草(*Nicotiana benthamiana*)和番茄的研究发现，MAPKs除在PTI途径中发挥功能外，也是ETI途径的组成元件。烟草和番茄中丝/苏氨酸蛋白激酶Pto与丁香假单胞菌分泌的效应子AvrPto/AvrPtoB相互作用，激活抗病蛋白Prf及ETI响应。此过程中，MAPKKKα和MAPKKKε正调控Pto-介导的ETI途径(del Pozo et al., 2004; Oh and Martin, 2011)。在表达抗病蛋白Cf-9的转基因烟草中，真菌效应子Avr9激活烟草中的MAPK激酶SIPK (SALICYLIC ACID-INDUCED PROTEIN KINASE)和WIPK (WOUND-INDUCED PROTEIN KINASE)，并导致WIPK蛋白积累(Romeis et al., 1999; Pedley and Martin, 2005; Meng and Zhang, 2013)。烟草花叶病毒(tobacco mosaic virus, TMV)在烟草抗病蛋白N的作用下磷酸化SIPK和WIPK，并增加WIPK的mRNA丰度以及蛋白含量(Zhang and Klessig, 1998)。此外, AvrRpt2对拟南芥MPK3和MPK6也有激活作用，与野生型*Pst* DC3000相比，携带AvrRpt2效应蛋白的*Pst* DC3000对MPK3和MPK6的激活能力增强(Eschen-Lippold et

al., 2016)。

3.2 MAPKs磷酸化修饰不同底物参与免疫应答

激活的MPK3、MPK4和MPK6通过磷酸化修饰不同的底物调控多种免疫途径(图2)。目前已鉴定的免疫调控过程中MAPK的底物包括ACS (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID SYNTHASE)、乙烯应答因子ERF104 (ETHYLENE RESPONSE FACTOR 104)、TZF9 (TANDEM ZINC FINGER 9)、VIP1 (VirE1-INTERACTING PROTEIN 1)、WRKY33、ASR3 (ARABIDOPSIS SH4-RELATED 3)、MKS1 (MPK4 SUBSTRATE 1)以及包含VQ-motifs的相关蛋白，如ERF6、PHOS32以及LIP5 (LYST-INTERACTING PROTEIN 5) (Sheikh et al., 2016)。

MAPKs磷酸化修饰某些WRKY类转录因子，调控转录重编程。烟草SIPK和WIPK识别并直接磷酸化I类WRKY转录因子WRKY8，增强WRKY8与W-box的结合和反式激活活性，进而激活HMGR2 (3-HYDROXY-3-METHYLGLUTARYL CoA REDUCTASE 2)的表达，产生类异戊二烯植物抗毒素，促进植物的免疫反应(Ishihama et al., 2011)。拟南芥中，与烟草WRKY8同源的转录因子WRKY33与MPK4以及MKS1在细胞核内形成复合体；受到flg22或者病原菌刺激时，MPK4磷酸化修饰MKS1并促使WRKY33-MKS1与MPK4分离，磷酸化的WRKY33随后结合植物抗毒素(camalexin)合成相关基因*PAD3*启动子区的W-box，激活*PAD3*的表达并促进camalexin的合成(Andreasson et al., 2005; Qiu et al., 2008; Lenzoni et al., 2018)。WRKY33还参与MPK3/6调控的camalexin的合成过程。灰霉菌诱导时，MPK3/6磷酸化修饰WRKY33，促进相关基因转录重编程(Mao et al., 2011; Li et al., 2012)。MPK6结合并磷酸化ERF104调控防御相关基因的表达(Bethke et al., 2009)。拟南芥中ASR3作为转录抑制因子负调控PAMP诱导的免疫响应。PAMP处理后MPK4磷酸化ASR3第189位苏氨酸，磷酸化的ASR3与DNA结合能力增强，从而抑制flg22诱导的防御基因的表达(Li et al., 2015)。

MAPKs对底物蛋白的磷酸化修饰影响底物蛋白的稳定性。拟南芥中，MPK3/MPK6通过磷酸化修饰ACS2/ACS6调控乙烯产生。泛素蛋白酶降解复合体结合未被磷酸化修饰的ACS6的C末端非催化区域，导致ACS6经泛素化途径降解。MPK3/MPK6对ACS6的磷酸化修饰在其C末端引入了负电荷，抑制ACS6的泛素化降解，增强其蛋白的稳定性，进而促进乙烯生成(Joo et al., 2008; Han et al., 2010)。MPK3/MPK6磷酸化修饰ERF家族成员ERF6，增强其蛋白稳定性，激活*PDF1.1* (*plant defensin 1.1*)、*PDF1.2a*、*PDF1.2b*和*ChiB*等多种防御相关基因的表达(Meng et al., 2013)。受到PAMP诱导后，MPK3磷酸化修饰WRKY46的S168和S250位点，促进WRKY46的降解(Sheikh et al., 2016)。LIP5参与病原菌诱导的内吞作用和囊泡运输，MPK3和MPK6磷酸化修饰LIP5，增强LIP5的蛋白稳定性，从而正调控多泡体(multivesicular bodies, MVB)通路，影响免疫应答过程中相关因子的重定位(Wang et al., 2014a)。E3泛素连接酶PUB22是拟南芥中PTI响应的抑制因子。MPK3对PUB22的磷酸化修饰抑制其自泛素化，从而导致PUB22积累，抑制免疫信号激活(Furlan et al., 2017)。

MAPKs通过磷酸化修饰底物蛋白改变底物蛋白的亚细胞定位。激活的MPK3对bZIP类转录因子VIP1的磷酸化修饰促进其从细胞质进入细胞核，激活病程相关基因(*pathogenesis-related* gene, *PR* gene)的表达(Djamei et al., 2007)。

R蛋白监控MAPKs对底物蛋白的磷酸化修饰(图2)。多种病原菌分泌效应蛋白攻击宿主细胞内MAPKs信号级联组成元件，抑制MAPKs调控的防御反应(Zhang et al., 2007, 2012; Wang et al., 2010)。拟南芥抗病蛋白SUMM2 (SUPPRESSOR OF MKK1 MKK2 2)在MAPKs信号的下游，监控MPK4底物PAT1 (PROTEIN ASSOCIATED WITH TOPOISOMERASE II)、CRCK3 (CALMODULIN-BINDGIN RECEPTOR LIKE CYTOPLASMIC KINASE 3)以及MEKK2的磷酸化状态，从而调控免疫应答。拟南芥*PAT1*编码mRNA脱帽装置的组成元件，与MPK4结合形成复合体。PAMP诱导后，PAT1被MPK4磷酸化并在细胞溶质中聚集成离散的点状物。*pat1*缺失突变体表现出依赖于SUMM2的免疫自激活(Roux et al., 2015)。CRCK3编码钙离子结合的胞质内受体激酶，

结合MPK4并被MPK4磷酸化修饰。CRCK3缺失可恢复*mekk1*、*mkk1*/*mkk2*双突变体以及*mpk4*中SUMM2介导的免疫自激活。此外, CRCK3和PAT1均可以与SUMM2相互作用, 表明SUMM2可能通过监控CRCK3和PAT1的磷酸化状态, 从而确定MEKK1-MKK1/MKK2-MPK4信号通路的完整性, 并适时启动免疫应答(Bi and Zhou, 2017; Zhang et al., 2017)。

3.3 磷酸酶对MAPK信号级联的调控

MAPK的持续磷酸化会导致免疫反应被持续激活, 因此MAPK的活性需要受到严格控制。目前已报道多种磷酸酶在植物免疫过程中负调控MAPK的激活。这些磷酸酶主要包括蛋白酪氨酸磷酸酶(PTPs)、双特异性磷酸酶(DSPs)以及PP2C类蛋白磷酸酶。不同的磷酸酶对MAPK的去磷酸化修饰导致不同的下游信号事件。拟南芥蛋白酪氨酸磷酸酶PTP1 (PROTEIN TYROSINE PHOSPHATASE 1)以及双特异性磷酸酶MKP1 (MAP KINASE PHOSPHATASE 1)和MKP2参与对MPK3/MPK6活性的调控。MKP1通过负调控MPK6途径抑制PAMP激活的免疫应答(Anderson et al., 2011); PTP1结合MPK6与MKP1, 协同抑制MPK6调控的防御反应激活。MKP2与MPK3/MPK6结合并且可在体外去磷酸化修饰MAK3和MPK6 (Lee and Ellis, 2007)。真菌激发子诱导的过程中, MKP2抑制MPK6介导的超敏反应(hypersensitive response, HR), 从而调控氧化胁迫以及对青枯病病原菌(*Ralstonia solanacearum*)的抗性(Lumbreras et al., 2010)。此外, MKP1的活性也受到MPK6的调控。PAMP诱导后MKP1蛋白迅速积累, 而这一过程依赖于MPK6对MKP1的磷酸化修饰(Jiang et al., 2017)。拟南芥PP2C类蛋白磷酸酶(AP2Cs蛋白磷酸酶)通过激酶结合基序(kinase interaction motif, KIM)与MAPK相互作用(Fuchs et al., 2013)。AP2C1负调控植物免疫应答过程中MAPK信号的激活, 过表达AP2C1抑制损伤诱导的乙烯产生以及对死体营养型真菌灰霉菌的抗性。AP2C1功能缺失增强PAMP诱导的MAPK激活、胼胝质积累以及对细菌的抗病性(Schweighofer et al., 2007; Galletti et al., 2011; Sidonskaya et al., 2016; Shubchynskyy et al., 2017)。

4 其它植物免疫相关功能蛋白的可逆磷酸化修饰

4.1 免疫调控的中心蛋白RIN4及其结合蛋白的磷酸化

拟南芥包含NOI结构域的蛋白RIN4在陆生植物中非常保守, 其并不具有类似已知的蛋白酶类的特征。二级结构预测发现, RIN4蛋白表现出高度的、固有的无序性, 不能形成二级和三级结构, 是植物中固有的无序蛋白(Lee et al., 2015)。无序蛋白在与客户蛋白结合时转变成高度折叠状态, 松散并特异地与多种客户蛋白结合(Dyson and Wright, 2005)。研究表明, RIN4是AvrRpt2、AvrRpm1、AvrB、HopF2以及AvrPto等多种病原菌效应子蛋白在宿主内攻击的靶标, 表明RIN4在植物-病原微生物互作中具有重要作用(Mackey et al., 2002, 2003; Axtell and Staskawicz, 2003; Luo et al., 2009; Wilton et al., 2010)。

RIN4蛋白不同位点的磷酸化修饰具有不同的功能(Nühse et al., 2007; Chung et al., 2014)。有研究表明, RIN4第47和141位的丝氨酸是受flg22诱导后被磷酸化修饰的可能位点(Nühse et al., 2004, 2007)。Chung等(2014)发现, RIN4第141位的丝氨酸残基的磷酸化修饰影响flg22诱导对病原菌生长的抑制, 导致胼胝质沉积、ROS爆发以及防御基因表达上调。当RIN4第141位丝氨酸突变为不能被磷酸化修饰的丙氨酸时, flg22诱导的PTI响应受到抑制。RIN4蛋白第166位的苏氨酸残基的磷酸化修饰, 发挥与S141位点磷酸化修饰相反的作用。拟南芥的胞质内类受体激酶RIPK磷酸化修饰RIN4蛋白的T21、S160和T166位点。T166位点被磷酸化修饰后, PTI途径的激活受到抑制。与S141位点磷酸化相比, T166位点被磷酸化修饰后发挥的功能具有上位性。

4.2 钙离子依赖的蛋白激酶调控植物防御反应

钙离子依赖的蛋白激酶(CPK)调控RBOHD及转录因子的激活。拟南芥基因组编码34个CPKs (Cheng et al., 2002; Bigeard et al., 2015), 广泛参与各种胁迫响应的调控。拟南芥CPK5、CPK6和CPK11功能同时缺失可抑制乙烯合成酶ACS2/ACS6的积累及乙烯的合成, 从而影响对死体营养型致病菌灰霉菌的抗病

性(Gravino et al., 2015)。Gao等(2013)研究表明，拟南芥抗病蛋白RPS2和RPM1被激活后，CPKs通过磷酸化修饰不同的底物参与调控多种抗病蛋白依赖的免疫激活。CPK1/2/4/11以及CPK5磷酸化质膜定位的NADPH氧化酶促进ROS的生成；激活的CPK4/5/6/11特异地磷酸化修饰WRKY转录因子WRKY8/28/48，协同调控转录重排，参与抗病蛋白介导的对病原菌的抗性; CPK1/2/4/5则在超敏反应过程中调控程序性细胞死亡的起始(Dubiella et al., 2013; Gao et al., 2013)。此外, CPK5参与非典型抗病蛋白TN2激活的抗性。拟南芥胞外复合体亚基EXO70B1缺失突变体中CPK5被过度激活，参与抗病蛋白TN2介导的免疫自激活过程，过表达CPK5导致依赖于TN2的免疫自激活以及抗病性增强。TN2结合并且稳定CPK5的活性，从而调控防御反应(Liu et al., 2017)。

4.3 植物免疫过程中NPR1的磷酸化修饰

拟南芥水杨酸信号通路中，NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELAED GENE 1)作为转录共激活因子调控多种免疫相关基因的表达。其蛋白稳定性受到泛素蛋白酶体途径的调控，动态地调节免疫相关基因的转录激活或抑制。研究表明，NPR1不同位点的磷酸化修饰对其泛素化产生不同的影响。S11和S15位点的磷酸化修饰促进NPR1与SUMO3的结合，从而促进NPR1的降解。而NPR1的S55和S59位点的磷酸化修饰则抑制NPR1的SUMO类泛素化，增强NPR1蛋白的稳定性(Spoel et al., 2009; Saleh et al., 2015)。此外, SnRK (SNF1-RELATED PROTEIN KINASE)激酶PSK5通过磷酸化修饰NPR1调控转录因子WRKY38和WRKY62的表达(Xie et al., 2010)。

4.4 蛋白磷酸酶在植物免疫调控中的作用

除了调控MAPK活性的磷酸酶，还有多种蛋白磷酸酶参与植物免疫途径。丁香假单胞菌三型效应子AvrRPM1诱导PP2C类蛋白磷酸酶PIA1 (PP2C INDUCED BY AvrRpm1)积累，调控对AvrRPM1的抗性。*pia1*缺失突变体中, *PR5*和*PDF1.2*等防御相关基因被激活，但*PR1*和*PR2*的表达以及SA积累减弱，最终表现出对*Pst* DC3000 (AvrRPM1)的抗病性增强(Widjaja et al., 2010)。拟南芥*IBR5* (*INDOLE-3-BUTYRIC ACID RESPONSE 5*)编码双特异性MAPK蛋白磷酸酶，与分子伴侣HSP90和SGT1b形成复合体，共同结合并稳定TIR类抗病蛋白CHS3 (CHILLING SENSITIVE 3); IBR5缺失抑制低温条件下CHS3蛋白的积累(Liu et al., 2015)。此外, IBR5还参与*SNC1*、*RPM1*和*RPS4*等抗病基因调控的防御反应。MAMP诱导时，拟南芥CDKC (CYCLIN-DEPENDENT KINASE C)磷酸化RNA聚合酶II C末端(C-terminal domain, CTD); 磷酸酶CPL3 (CTD PHOSPHATASE-LIKE 3)通过去磷酸化修饰CTD第2位丝氨酸残基，负调控免疫相关基因的表达(Li et al., 2014a)。植物激素脱落酸(abscisic acid, ABA)诱导PP2C类蛋白磷酸酶*HAI1* (*highly ABA-induced 1*)、*HAI2*和*HAI3*激活表达，HAI磷酸酶通过去磷酸化修饰作用参与ABA诱导的MPK3和MPK6的失活，进而抑制宿主的免疫途径。最新研究表明，丁香假单胞菌可激活宿主内ABA信号，诱导HAI表达。此外，丁香假单胞菌分泌的冠毒素(coronatine, COR)通过激活宿主转录因子MYC2诱导HAI磷酸酶表达。激活的HAI磷酸酶进一步通过抑制MPK3和MPK6的活性负调控宿主细胞的免疫应答(Mine et al., 2017)。

5 展望

近年来，植物免疫调控研究取得了重要进展(毕国志和周俭民, 2017; 闫佳等, 2018)。其中，蛋白磷酸化修饰作为细胞内信号调控的开关，在植物免疫途径中发挥重要作用。最近，陈学伟研究组发现，水稻转录因子IPA1的磷酸化修饰可平衡水稻的产量和抗病反应，进一步表明研究蛋白磷酸化修饰的重要意义(Wang et al., 2018a)。目前，已报道多种蛋白激酶广泛参与植物免疫调控，但是相关磷酸酶的研究还有很大空白。因此，蛋白磷酸酶对植物免疫的调控还有待深入研究。病原菌的效应蛋白通常会选择植物防御途径中的关键蛋白激酶和蛋白磷酸酶作为攻击的靶标。例如，效应蛋白通过干扰植物免疫信号中关键蛋白的磷酸化水平抑制植物免疫激活; 有些效应蛋白模拟植物激素或者利用植物蛋白磷酸酶等免疫负调控因子来增强致病毒力。因此，全面了解植物-病原微生物互作过程中关键调控蛋白的磷酸化修饰，有助于深入探究植物免疫调控和病原菌致病机理，寻找植物广谱抗

病的新途径。

尽管蛋白磷酸化修饰在植物防御途径，尤其是在PTI途径中的作用已取得巨大研究进展，但是该领域依然存在许多未解之谜。例如，受到不同的免疫信号刺激后，激活的MAPK如何特异地选择不同的底物而激活相应的免疫响应？ETI途径中，抗病蛋白作为胞内免疫受体识别效应蛋白后对下游信号的激活是否依赖于磷酸化修饰作用？没有病原菌侵染时，磷酸化修饰作用是否参与抗病蛋白活性的调控等。这些问题还需要进一步研究，以期更全面地揭示植物免疫调控机制。

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Research Progress in Protein Phosphorylation in Plant-pathogen Interactions

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Abstract Reversible protein phosphorylation is a common mechanism regulating plant signaling pathways. Phosphorylation of key components in plant-pathogen interactions affects the activation of defense signaling. Many pathogens attack the plant immune system and enhance pathogenic toxicity by disturbing the phosphorylation status of defense regulators. In this review, we summarize the phosphorylation of regulators in plant defense responses and its regulating effect in plant immunity. Understanding the phosphorylation of key regulators in the plant-pathogen interaction may help to explore new mechanism of plant immune regulation. This review may provide support and a basis for studying new approaches of broad-spectrum disease resistance.

Key words protein phosphorylation, protein kinase, protein phosphatase, plant-pathogen interaction

Liu YQ, Hou SW (2019). Research progress in protein phosphorylation in plant-pathogen interactions. *Chin Bull Bot* **54**, 168–184.

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