烟草TTG2蛋白质对NPR1介导的抗病防卫反应与ARF8影响的生长发育进行交叉调控的机制

韩冰, 朱茜, 葛军, 徐曼宇, 董汉松*
南京农业大学植物保护学院, 南京210095

摘要: NPR1蛋白是水杨酸信号和系统获得性抗性的转录调节因子, 它的功能受蛋白质降解酶体CUL3-E3的控制。植物的发育主要受生长素信号通路的控制, 生长素反应因子(ARF)参与生长素信号转导转录调控。植物转录因子NPR1和ARF8分别在蛋白质降解酶体CUL3-E3与CUL1-E3控制下, 调控抗病防卫与生长发育。烟草TTG2促进ARF8从细胞质向细胞核转运及其转录调控作用, 因此促进生长发育; 相反, TTG2把NPR1扣留在细胞质, 阻止它对防卫反应基因的转录调控作用, 从而抑制抗病性。TTG2与NPR1或ARF8并不直接互作, 说明存在协助因子。

关键词: TTG2; 交叉调控; 抗病防卫; 生长发育; 信号转导

Molecular Mechanisms Underlying the Roles of the TTG2 Protein in Modulating NPR1-Regulated Pathogen Defenses and ARF8-Engaged Development in Tobacco

HAN Bing, ZHU Qian, GE Jun, XU Man-Yu, DONG Han-Song*
College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China

Abstract: The NPR1 (nonexpressor of pathogenesis-related genes 1) protein is a transcription regulator of salicylic acid (SA) signaling and systemic acquired resistance, and the protein functions under the control of the cullin 3-based E3 ligase (CUL3-E3) proteasome in plants. In frequent crosstalk with the resistance, plant development is largely regulated by the auxin signaling pathway, which depends on auxin response factor (ARF) proteins to regulate auxin responses of plants. The transcriptional function of ARFs is repressed when binding to Aux/IAA proteins, and activated by the CUL1-E3 proteasome, which degrades Aux/IAA to release ARFs. The defensive role of NPR1 and the developmental role of ARF8 were oppositely regulated by the TRANSPARENT TESTA GLABRA 2 (TTG2) protein in tobacco. TTG2 suppresses resistance to viral and bacterial pathogens by sequestering NPR1 from the nucleus but promotes the developmental role of ARF8 by facilitating its localization to the nucleus. However, TTG2 does not directly interact with NPR1 or ARF8, suggesting that potential proteins assist TTG2 in modulating the nucleocytoplasmic transports. Starting with identifying the postulated assistant factors, this report aims to elucidate the mechanism of TTG2 modulating the nucleocytoplasmic trafficking of NPR1 and ARF8 and the following impact on E3 proteasome.

Key words: TTG2; crosstalk; pathogen defense; development; signal transduction

1 引言

植物抗病防卫反应与生长发育交叉调控的意义类似于人类“战争与和平”的关系, 防卫反应服从生长发育, 应该“召之即来挥之即去”。植物在遭受病原物侵染时, 能够快速调动防卫反应机制, 有效抵御病原物侵染与致病。

这种变化过程涉及多种植物激素与非激素信号转导通路的相互作用(Robert-Seilaniantz等2011; Desaki等2012; Spaepen和Vanderleyden 2011; Van der Does等2013), 其中, 植物激素水杨酸与生长素信号转导之间的对抗关系至关重要(Robert-Seilaniantz等2011; Li等2012)。在这一对抗关系中, 植物TRANSPARENT TESTA GLABRA 2 (TTG2)蛋白质作为交叉调控因子, 发挥十分关键的作用(Li等2012)。

早期文献把TTG界定为调节表皮毛与种子发育的蛋白质(Larkin等1994; Leon-Kloosterziel等1994; Szymanski等2000), 陆续阐释了这种功能的生理与分子机理(Vetten等1997; Petroni和Tonelli

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* 通讯作者(E-mail: hsdong@njau.edu.cn; Tel: 025-84399006)。

2011; Bouyer等2008)。TTG通过影响花青素生物合成途径, 改变花和种皮的颜色(Vetten等1997; Petroni和Tonelli 2011); 通过蛋白质-蛋白质互作, 调控表皮毛发育(Bouyer等2008)。最近研究发现, TTG还有调控植物抗病及抗逆防卫反应的功能(Li等2012; Jiang等2012; Nguyen等2013; Wang等2009)。这个新发现为深入探讨植物防卫反应与生长发育交叉调控的机制提供了新视野(Li等2012; Wang等2009), 抗病防卫反应调控因子的行止动态, 执行功能或闲置备用, 实际上受到细胞信号转导正反两种力量的制约(Robert-Seilaniantz等2011; Van der Does等2013; Li等2012; Wang等2013), 以保证植物防卫反应与生长发育之间的转变, 用最低的适应性代价完成对病原物侵染与致病性的有效抵御, 并迅速恢复生长发育进程。

2 水杨酸与生长素信号转导机制和相关问题

水杨酸介导的系统性获得抗性(systemic acquired resistance, SAR)是各种植物广谱抗病性最重要的机制, 可以有效抵御各种病原物致病性的发生发展(Dong等1999; Dong 1998; Ryals等1996)。水杨酸在正常生长的植物体内处于基础水平, 防卫反应机制闲置; 植物受病原物侵染后, 体内水杨酸含量升高, 引发一系列抗病防卫反应(Dong等1999; Ryals等1996)。水杨酸信号由NPR (nonexpressor of pathogenesis-related genes)蛋白质NPR3或NPR4识别, 转变为细胞内信号转导过程, 调动多种调控因子参与防卫反应(Fu等2012; Shi等2012)。WRKY (WRKYGQK motif-binding protein)转录因子调控*NPR1*基因表达水平, 决定NPR1蛋白质产生的量(Eulgem和Somssich 2007)。NPR1与TGA (TGACG motif-binding factor)转录因子互作, 共同调控防卫反应基因(如*pathogenesis-related 1*, *PR1*)的表达(Cao等1997; Fan和Dong 2002; Ryals等1997)。转录调控呈现一次性效应(图1), 一个NPR1分子启动*PR1*一轮转录之后, 便由依赖cullin (CUL)蛋白质CUL3的E3泛素连接酶体(CUL3-E3酶体)进行降解, 下一轮转录调控由新的NPR1分子另行担当(Spoel等2009)。因此, 每个NPR1分子在*PR1*一轮转录完成之后即行降解, 每轮转录调控都需要更换新的NPR1分子, 避免组成性防卫反应的发生(Mukhtar等2009; Spoel等2009)。

如图1所示, NPR1功能调控过程还有多个未知环节, WRKY有待鉴定(Eulgem和Somssich 2007), Adp-A和Adp-B属何种物质, 目前也不清楚(Mukhtar等2009; Kinkema等2000)。在未发生SAR的细胞质内, Adp-A把未磷酸化的NPR1引领到CUL3-E3酶体上接受降解; 在发生了SAR的细胞内, 磷酸化的NPR1与CUL3-E3酶体之间的互作需要Adp-B, 它能提高互作程度, 保证NPR1有效降解。与CUL3-E3对NPR1的作用不同, CUL1-E3酶体负责降解Aux/IAA (auxin/indole-3-acetic acid)蛋白质, 释放生长素反应因子(auxin response factor, ARF), ARF得以参与生长素信号转导转录调控(Tada等2008)。这种差别可能是水杨酸与生长素信号转导对抗关系的原因之一。

IAA是植物天然合成的生长素, 有调控植株顶端优势与向性生长以及细胞分裂、伸长与分化等多重功能(Tada等2008; Wiermer等2010), 影响多种器官的生长发育, 如胚胎形成(Mou等2003)、根茎叶的生长(Quint和Gray 2006; Vanneste和Friml 2009)、花器官建成(Julliard等1992)以及种子发育(Holm和Key 1969)。参与这些发育过程的调控因子分3类: (1)生长素受体TIR1 (F-box protein transport inhibitor response 1)和ABP1 (auxin-binding protein 1) (Wilmoth等2005; Julliard等1992); (2) ARF蛋白质家族的转录因子(Calderón等2012; Hayashi 2012; Guilfoyle等1998; Tiwari等2003; Ulmasov等1999); (3) Aux/IAA蛋白质家族的转录抑制因子(Guilfoyle等1998; Tiwari等2003)。这些蛋白质按转录调控与非转录调控两种方式发挥作用(Hayashi 2012; Strader和Nemhauser 2013), 影响细胞内信号转导过程(图2)。如图2所示, 转录调控机制实际上是一个很短但功能强大的细胞核内信号转导过程: 生长素与TIR1结合, 诱导CUL1-E3酶体(SCF^{TIR1})的活性, 使Aux/IAA降解, ARF得以释放(Tada等2008; Wilmoth等2005; Hayashi 2012; Guilfoyle等1998; Tiwari等2003), 转而调控生长素反应(auxin-responsive)基因的表达(Julliard等1992; Nitsch 1952; Calderón等2012)。ARF分转录激活子与抑制子两类, 特征分别是序列中部富含谷氨酸(glutamine, Q)与丝氨酸(serine, S) (Tada等2008; Nitsch 1952), 这种功能分化是生长素信号转导能够调控植物不同生长发育过程的重要原因(Tada等2008; Julliard等1992; Hayashi 2012; Tiwari等2003;

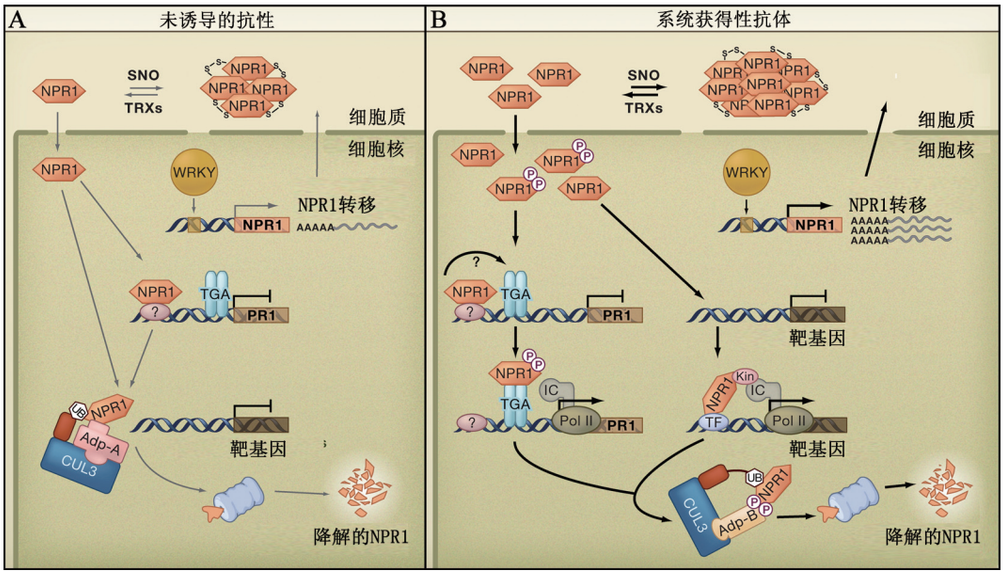

图1 NPR1降解与植物防卫反应

Fig.1 NPR1 degradation and plant defense responses

根据文献(Mukhtar等2009)改画。根据植物生长的条件, NPR1经历形态与核质定位的改变(Dong 2004; Spoel等2009)。在未发生SAR的细胞内, NPR1由*S*-亚硝基硫醇(*S*-nitrosothiol, SNO)介导, 借助二硫键形成低聚体, 并停留在细胞质。在发生了SAR的细胞质内, 硫氧还蛋白质(thioredoxin, TRX)调控细胞氧化还原状态, 使NPR1低聚体解离为单体, 大量NPR1单体转移到细胞核。进入细胞核后, NPR1对靶标基因(如*PR1*)的转录调控作用受蛋白质分解酶体控制。A: 在未发生SAR的细胞内, 时常有少量NPR1单体进入细胞核, 与一个未知蛋白质互作, 结合到*PR1*基因启动子上。但此时NPR1并不能与TGA结合, *PR1*也不能转录。相反, NPR1通过一个未知的底物接头(Adp-A)与CUL3-E3酶体结合, 通过泛素(ubiquitin, Ub)介导进行降解。B: 在发生了SAR的细胞内, 大量NPR1单体进入细胞核, 在作用于靶标基因之前被磷酸化。磷酸化与未磷酸化的NPR1都能与TGA结合。NPR1与TGA一旦结合, *PR1*基因转录便能启动。未磷酸化的NPR1也能作用于靶标基因, 但需要一个未知的、不同于TGA的转录因子来合作, 使RNA聚合酶II (RNA polymerase II, Pol II)起始复合体(IC)得以装配, 随后启动靶标基因转录。在这一反应中, 与Pol II结合的一个蛋白激酶(Kin)可能担当NPR1磷酸化的功能。磷酸化的NPR1与CUL3-E3酶体之间需要底物接头Adp-B, 它能提高CUL3-E与NPR1的亲和性, 保证高度互作, 使NPR1在完成一次转录调控任务之后旋即降解, 下一轮转录调控由新的NPR1分子另行承担。

Ulmasov等1999)。

因植物种类而不同, ARF蛋白质家族成员有23~29个不等, 何种ARF调控哪种发育过程还不完全清楚(Gray等2001; Liscum和Reed 2002; Strader和Nemhauser 2013; Rushton等2008)。烟草(*Nicotiana tabacum*) ARF家族至少有12个成员得到鉴定(Rushton等2008), 但目前仅对ARF1参与烟碱合成的功能有所了解(Remington等2004)。可见, 研究特定ARF与特定生长发育过程的关系, 还是一项艰巨的任务, 现在研究主要关注影响抗病性以及生长素与水杨酸信号转导交叉调控的ARF种类。

3 水杨酸与生长素交叉调控植物抗病性与生长发育的机制和相关问题

水杨酸与生长素对植物生长与抗病性呈对抗关系, 表现为两个方面(Remington等2004; Wu等2011; Rushton等2008)。一方面, 水杨酸通过抑制生长素信号转导来加强植物防卫反应能力, 提高抗病性(Rushton等2008)。在拟南芥(*Arabidopsis thaliana*)中, 水杨酸不仅削弱TIR1对生长素的识别能力, 而且抑制CUL1-E3的活性, 保护Aux/IAA免受降解, 因此阻止ARF释放与转录调控作用的发生。在此情况下, 拟南芥生长变弱, 但防卫反应基因表达水平提高, 病原物繁殖能力与致病性受到抑制。另一方面, 生长素通过抑制水杨酸信号转导来抑制抗病防卫反应(Wu等2011; Rushton等2008)。拟南芥过表达*TIR1*基因(Navarro等2006), 或者用IAA或NAA (人工合成的生长素萘乙酸)(Rushton等2008)处理以后, 对病原物的抗性都会减弱。相反, 削弱拟南芥对生长素的敏感性, 则可提高抗病能力(Rushton等2008)。表达恶臭假单胞

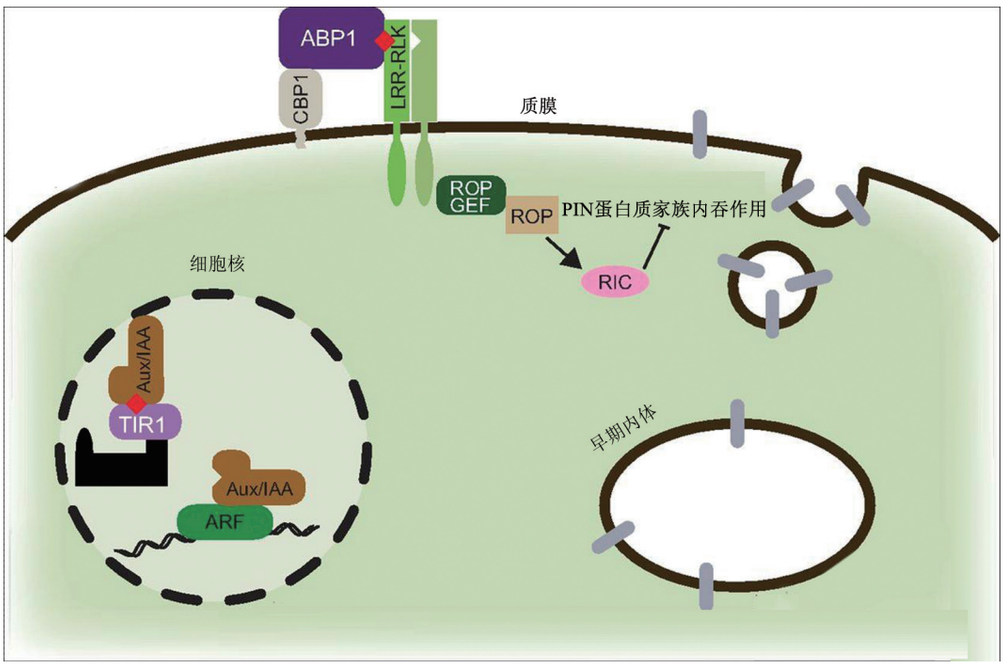

图2 生长素信号转导转录调控与非转录调控模式

Fig.2 Model of transcriptional and non-transcriptional auxin responses

根据文献(Hayashi 2012; Strader和Nemhauser 2013)改画。红色菱形表示生长素。ABP1识别生长素信号, 诱导非转录调控。ABP1由CBP1 (glycosylphosphatidylinositol-anchored protein)绑定在细胞膜上, 可能受LRR-RLK (leucine-rich repeat receptor-like protein kinase)激活, 与生长素结合, 促进ROP (Rho-related protein from plants)的活性。ROP转而激活RIC (ROP-interactive CRIB-containing protein), RIC加强F-纤维型肌蛋白质在细胞膜上的集结作用, 防止PIN蛋白质家族生长素运输载体的内吞作用。转录调控机制在正文叙述。

菌(*Pseudomonas putida*)水杨酸水解酶基因*NahG*的转基因植物不能积累水杨酸, SAR随之丧失(Li等2012; Todd等2010)。如果把与*NahG*转基因植物与失去生长素敏感性(auxin response, axr)的突变体*axr2-1*杂交, 杂交后代对生长素也不敏感, 对病原物的抗性随之加强, 说明对生长素敏感性的缺失可以弥补由于缺少水杨酸而引起的抗病性丧失(Rushton等2008)。这些研究说明, 抑制生长素信号转导是水杨酸对SAR进行调控的一个要素。

但也有相反的报道。拟南芥突变体*aux1*和*axr4*失去生长素极性运输能力, 但SAR水平不是降低, 而是提高, 说明SAR需要生长素信号(Truman等2010)。这项发现的研究人员比较了自己与他人(Navarro等2006; Wang等2007)相悖的试验结果, 解释是: SAR在诱发早期需要生长素信号, 随发展进程, 可能需要由生长素信号介导转变为水杨酸信号介导, 水杨酸介导、NPR1调控的防卫反应机制使植物早有准备, 因而能够快速实现信号调控的转变(Todd等2010)。这至少说明, 不同激素信号之间的交叉调控不仅复杂而且精细。关于生长素抑制(Wu等2011; Rushton等2008)或促进(Todd等2010)抗病防卫反应的机理及其与水杨酸信号的关系, 目前了解甚少, 研究工作只是刚刚开始。

还有一个问题是, 水杨酸与生长素的对抗关系在植物抗病防卫与生长发育交叉调控机制中的份量。仅从抗病防卫反应信号转导不同通路来说, 交叉调控并不限于生长素与水杨酸之间, 其他激素也有重要作用(Robert-Seilaniantz等2011; Spaepen和Vanderleyden 2011; Van der Does等2013; Dong 1998; Navarro等2006; Wang等2007; Gaffney等1993)。公认的观点是: 无论是以调控植物生长发育为基本功能的植物生长激素(乙烯、生长素、赤霉素、细胞分裂素), 还是以抑制植物生长发育或调控逆境反应为基本功能的植物逆境激素(如脱落酸), 都是通过影响水杨酸与茉莉酸或乙烯信号转导交叉调控而影响植物的抗病性(Robert-

Seilaniantz等2011; Spaepen和Vanderleyden 2011; Van der Does等2013; Wang等2007; Gaffney等1993)。绝大多数研究结果显示, 水杨酸在抗病防卫反应调控过程中与所有其他激素相对抗(Robert-Seilaniantz等2011; Spaepen和Vanderleyden 2011; Van der Does等2013; Dong 1998; Navarro等2006; Wang等2007; Gaffney等1993)。1990~2000年间, 至少有3个研究小组采用不同诱变方法, 对*NPR1*位点进行遗传标记与图位克隆(Ryals等1996; Truman等2010), 先后报道了关于突变体与基因克隆的研究结果(Cao等1994), 其中2个小组使用了基因异名*NIM1* (non-expressor of immunity 1) (Ryals等1997; Delaney等1995)和*SAI1* (salicylic acid-insensitive 1) (Delaney 1997)。"殊途同归"的一个原因在于, *NPR1*是调控抗病性的一个强势基因, 易于诱导, 诱导的转录本丰度高, 为遗传标记、突变体筛选与图位克隆带来了方便(Cao等1997; Ryals等1997; Mauch-Mani和Mauch 2005; Delaney等1995; Delaney 1997)。由于水杨酸-NPR1信号转导通路功能强大, 植物必须预备多种机制予以制衡, 避免防卫反应失控, 减少生长发育为此付出的代价(Mukhtar等2009; Spoel等2009; Cao等1994)。植物需要强有力的交叉调控因子, 在可以终止防卫反应、回到正常生长发育程序的时候发挥作用。近十多年来, 关于植物激素信号转导交叉调控的研究文献与日俱增, 但文献库(如NCBI PubMed)显示, 个例研究大都偏重防卫反应的一种机制抑制另一种, 如茉莉酸或乙烯介导的抗虫性对抗水杨酸介导的抗病性(Robert-Seilaniantz等2011; Spaepen和Vanderleyden 2011; Van der Does等2013; Dong 1998; Rushton等2008; Todd等2010; Ghanashyam和Jain 2009; Navarro等2006; Wang等2007; Gaffney等1993)。这种情况益发凸显对制衡水杨酸-NPR1信号转导通路、交叉调控生长发育的因子进行研究的重要性, NtTTG2就是这样一种因子。

4 NtTTG2在烟草中抑制抗病性和促进生长发育的作用与问题

研究表明, 烟草NtTTG2有抑制抗病性(Li等2012)、促进生长发育的功能。我们先选用普通烟烤烟型品种'NC89'进行研究, 因为它是烤烟优质品种, 抗病性差, 有助于SAR诱导(Delaney等1995; Shah等1997; Yu等1998; 董汉松1995); 花在发育过程中花色由白变红, 有利于从花青素代谢途径与遗传基础两方面研究调控机制(Chen等2008a; Sun等2010; Wu等2010)。

我们克隆了'NC89'的*NtTTG2*基因, 预测蛋白符合植物TTG的结构特征(Tanaka等2010), 即含WD40功能域(Li等2012; Larkin等1994; Vetten等1997; Petroni和Tonelli 2011; Bouyer等2008; Jiang等2012; Nguyen等2013; Wang等2009; Tanaka等2010; Todd等2010; Xu和Min 2011; Peng等2003)。通过发卡结构介导的基因沉默, 获得了*NtTTG2*基因表达水平下调、NtTTG2蛋白产量微弱的4个'NC89'转基因系(*TTG2RNAi* 1~4号)。把花椰菜花叶病毒35S启动子、*NtTTG2*与红色荧光蛋白(red-fluorescent protein, RFP)基因(*rfp*)构建成融合基因, 转化'NC89', 产生了过表达*NtTTG2*基因、NtTTG2蛋白产量大幅度提高的4个转基因系(*35S/TTG2/rfp* 1~4号)。把只含*rfp*的构建引入'NC89', 产生了转基因系*WT/rfp*, 用作对照。与*WT/rfp*相比, *TTG2RNAi*抗烟草花叶病毒(tobacco mosaic virus, TMV)、黄瓜花叶病毒(cucumber mosaic virus, CMV)和大白菜软腐病毒(*Pectobacterium carotovorum* subsp. *carotovorum*, Pcc)的能力显著提高, *PR1*和*PR2*表达水平提高4~7倍; *35S/TTG2/rfp*感病, *PR1*和*PR2*表达水平降低2~5倍。这说明, NtTTG2是一个抗病性抑制因子(Li等2012)。

普通烟东方型(香料烟)品种'Xanthi'及其表达*NahG*基因、不积累水杨酸的转基因系被广泛用来研究水杨酸介导的抗病性(Li等2012; Ryals等1996; Gaffney等1993; Shah等1997; Yu等1998; 董汉松1995)。我们分别构建了*NtTTG2*与*NPR1*同时过表达或同时沉默的'Xanthi'转基因系, 并把*NtTTG2*基因过表达与沉默分别引入'Xanthi' *NahG*。与*NPR1*或*NtTTG2*单独过表达的转基因系相比, 同时过表达这两种基因的转基因系感病性增加; 相反, 无论*NPR1*沉默还是*NahG*表达, 都能弥补*NtTTG2*过表达引起的抗病性损失。这表明NtTTG2通过抑制水杨酸信号转导和NPR1的功能而抑制抗病性。

与抑制抗病性的作用相反, NtTTG2促进烟草生长发育。*NtTTG2*基因表达受生长素诱导, 无器官特异性, 在根、茎、叶、花和果的组织内都能

表达, 说明*NtTTG2*可能全程参与烟草生长发育。*NtTTG2*基因沉默以后, 烟草营养生长与细胞伸展素基因*EXP1*、*EXP2*的表达都受到显著抑制, 但*NtTTG2*过表达则促进烟草生长, 提高*EXP*表达水平。细胞伸展受包括生长素在内的植物激素诱导而产生(Pang等2003; 王元琮等2010), 帮助细胞松弛以便于伸展(Peng等2009), 促进植物生长(Lee和Kende 2001; Chen等2008b)。因此, NtTTG2通过促进烟草细胞伸展素基因表达而促进烟草植株营养生长。但是目前对NtTTG2的功能链尚没有一个比较完整的认识, 还有一些问题须解决, 如: ARF8仅是烟草ARF蛋白质家族的成员之一(Rushton等2008), 其他成员是否介入NtTTG2对烟草生长发育的调控作用, 不同成员是否有功能冗余性; 另外, *GH3*、*SAUR*、*Aux/IAA*各自构成基因家族(Cox等2004; Cosgrove 1998; Lee和Kende 2001; Chen等2008b), 而生长素反应基因在烟草上只鉴定了3种(Cosgrove 1998; Lee和Kende 2001; Chen等2008b), 烟草是否还有其他的生长素反应基因, 它们的表达是否也受NtTTG2与ARF8调控等等。

5 NtTTG2交叉调控NPR1抗病功能与ARF8发育功能的可能机制

NtTTG2对烟草抗病性与生长发育交叉调控有十分重要的作用, 也是影响生长素与水杨酸信号转导交叉调控的重要因子(Li等2012)。NtTTG2把NPR1扣留在细胞质, 阻止它向细胞核转移, 防卫反应转录调控作用无从发生, 烟草抗病性因此受到抑制(Li等2012)。相反, NtTTG2促进ARF8从细胞质转入细胞核, ARF8对*GH3*表达的调控作用得以实现, 烟草生长发育受到促进。因此, 应先从蛋白质结构与功能的关系(构效关系)入手, 研究NtTTG2对ARF8与NPR1核质转运的调控机制; 然后根据生长素与水杨酸信号在细胞核内转导的关键环节, 研究NtTTG2通过影响CUL1-E3 (图2)与CUL3-E3 (图1)蛋白质降解酶体, 对ARF8与NPR1的功能进行调控的机制。

先看NtTTG2构效关系。它与NtTTG1类似, 含典型的WD40功能域(Li等2012; Wang等2009; Tanaka等2010)。WD40是各类生物多种蛋白质普遍保守的蛋白质-蛋白质互作功能域(Li等2012; Larkin等1994; Leon-Kloosterziel等1994; Bouyer等2008; Tanaka等2010; Todd等2010; Xu和Min 2011; Petroni和Tonelli 2011; Bombarely等2012; Hagen和Guifoyle 2002; Dargeviciute等1998), 最早的定义是含色氨酸(tryptophan, W)与天冬氨酸(aspartic acid, D)二缩肽(WD)、40个左右氨基酸残基的序列(Xu和Min 2011), 最新定义为44~60个氨基酸的短肽, 其前半段长11~24个氨基酸, 含甘氨酸(glycine, G)与组氨酸(histidine, H)二缩肽(GH), 后半段是WD40 (Petroni和Tonelli 2011) (图3-A)。在不同蛋白质序列上, WD40以不同数目的重复单元出现, 重复单元之间形成β-螺旋桨(β-propeller)结构, 成为互作蛋白质之间稳定或可逆结合的平台(Petroni和Tonelli 2011; Bombarely等2012)。WD40可适时变换互作对象, 作为底物受体或分子接头, 与性质各异的蛋白质结合(Li等2012; Larkin等1994; Guilfoyle和Hagen 2007; Kumar等2012; Lund等1997)。因此, 含WD40功能域的蛋白质既能与信号转导调控因子互作, 直接介入细胞信号转导, 也能充当分子接头, 为其他蛋白质互作牵线搭桥, 借此影响细胞信号转导(Li等2012; Bouyer等2008; Tanaka等2012; Todd等2010; Hagen和Guilfoyle 2002; Dargeviciute等1998)。

再看NPR1构效关系。它含一个BTB功能域和一个IκB功能域, BTB在N-端(Fan和Dong 2002; Kinkema等2000), IκB位于中部(Cao等1997; Ryals等1997)。含BTB功能域的蛋白质既可充当CUL3-E3酶体底物接头, 又可能是酶体的直接底物(Roux和Perrot-Rechenmann 1997; Xu和Min 2011), 所以NPR1能结合到CUL3-E3酶体上(Kinkema等2000)。IκB既是磷酸化功能域(Kinkema等2000), 也是WD40的互作对象(Roux等1998; Xu和Min 2011)。

虽然NtTTG2、NPR1 (Li等2012)、ARF8都有蛋白质互作功能域, 但前者与后两者都不能直接互作, 说明另有协助因子产生影响。如果存在协助NtTTG2把NPR1扣留在细胞质(Li等2012)的蛋白质cNPR1d (cytosolic NPR1 detaining protein), 那么, 滞留在细胞质的NPR1就不仅有同源低聚体(Dong 2004; Spoel等2009), 还有异源低聚体或多聚体。与同源低聚体相比, 异源低聚体或多聚体对NPR1的功能动态(Mukhtar等2009; Kinkema等2000)可能

更有制约力。

分子核输入依靠核输入载体(importin, IMP)。IMP有α和β两种类型, 以“底物-IMPβ”或“底物-IMPα-IMPβ”复合体的方式执行运输功能, 后一种方式有助于提高底物运输的专化性(Runne和Chen 2013)。由于IMPα和IMPβ都有穿越核孔的能力, “底物-IMPα-IMPβ”复合体可以变换接头, IMPα充当运输载体(Runne和Chen 2013)。ARF8可能以类似“底物-IMPα-IMPβ”复合体的方式向细胞核转移, 某种IMPα或类似蛋白质充当ARF8的运输载体ARF8C (ARF8 carrier protein); NtTTG2处在IMPβ的位置上, 借助WD40螺旋桨结构(Petroni和Tonelli 2011; Bombarely等2012), 提高ARF8与ARF8C结合的稳定性。

与ARF8相似, NPR1也必须依靠核输入载体(NPR1 carrier protein, NPR1C), 才可能进入细胞核。特别是在细胞水杨酸含量上升(Mukhtar等2009; Kinkema等2000; Mou等2003)、NtTTG2低于基础水平(Li等2012)的情况下, 大量NPR1单体进入细胞核(图1) (Mukhtar等2009; Kinkema等2000), 要求NPR1C有很高的运输效率。NtTTG2对ARF8与NPR1在细胞核内的功能动态可能也有不同影响。

先看ARF8。ARF与其束缚蛋白质Aux/IAA之间存在密切的功能关联。大多数Aux/IAA蛋白质序列上都有I、II、III、IV功能域(Tada等2008; Wilmoth等2005; Nitsch 1952), I与II在N-端, I是转录抑制位点; II是降解子, 把Aux/IAA引到CUL1-E3酶体SCF^{TIR1}上接受降解, 因此也是TIR1与Aux/IAA对生长素信号的识别位点; III和IV位于C-端, 都是互作功能域。大多数ARF蛋白质都有这两个互作功能域, 序列N-端另有DNA结合位点(nucleotide-binding site, NBS), 转录激活子与抑制子序列中段分别富含谷氨酸和丝氨酸(Tada等2008; Nitsch 1952; Calderón等2012; Hayashi 2012; Guilfoyle等1998; Tiwari等2003)。功能域的保守性为NtTTG2介入CUL1-E3酶体SCF^{TIR1}提供了可能。已知CUL4-E3酶体使用含WD40功能域的蛋白质作底物接头(Xu和Min 2011), SCF^{TIR1}通过含WD40功能域的蛋白质FBX2来调控植物对低磷胁迫的反应(Stimimann等2010; Yan和Willis 2013)。这说明含WD40功能域的蛋白质有可能替换F-box蛋白质, 充当SCF^{TIR1}的底物接头(Xu和Min 2011), 介入SCF^{TIR1}对Aux/IAA的降解作用, 促进ARF释放与转录调控的发生。

再看NPR1。在水杨酸含量上升(Mukhtar等2009; Kinkema等2000; Mou等2003)或NtTTG2低于基础水平(Li等2012)的细胞核内, NPR1受CUL3-E3酶体制约, 在调控防卫反应基因表达的同时, 抑制生长素信号转导(Todd等2010)。低含量ARF8可能仍然有作用, 借助CUL1-E3酶体而释放并行使转录调控功能, 以保持植物生长发育。因此, CUL3-E3作为水杨酸抑制生长素信号转导的一个要素, 与CUL1-E3可能有功能对抗关系。

已知交叉环节有生长素信号识别(TIR1)与转导(AXR2) (Todd等2010), ARF作为生长素反应基因的转录因子(Wilmoth等2005; Julliard等1992; Nitsch 1952; Calderón等2012; Hayashi 2012), 也必然介入。水杨酸这方, 受体(NPR3、NPR4) (Fu等2012; Shi等2012)与调控NPR1核质动态的因子(如WRKY) (Eulgem和Somssich 2007)和Trx (Yu等1998)), 都可能参与水杨酸对生长素信号转导的抑制作用。这个问题涉及NtTTG2-ARF8与TTG2-NPR1功能链的构成, 也关系烟草抗病性与生长发育交叉调控的运行机制。

6 结语

本文的一个核心问题是, NtTTG2如何通过核质转运与蛋白质降解机制, 对ARF8与NPR1的功能动态进行调控, 从而交叉调控植物抗病性与生长发育。围绕这个核心问题进行研究, 期望通过这一个例, 揭示作物抗病防卫反应与生长发育交叉调控以合乎自然规律的方式而运作的机制: 作物在病害循环过程中, 如何有效实现生长发育与防卫反应之间的转变, 用最低的适应性代价完成对病原物侵染与致病性的有效抵御, 并及时终止防卫反应, 迅速恢复生长发育进程。

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