特约综述 Invited Reviews

油菜素甾醇类激素的生物合成、代谢及信号转导
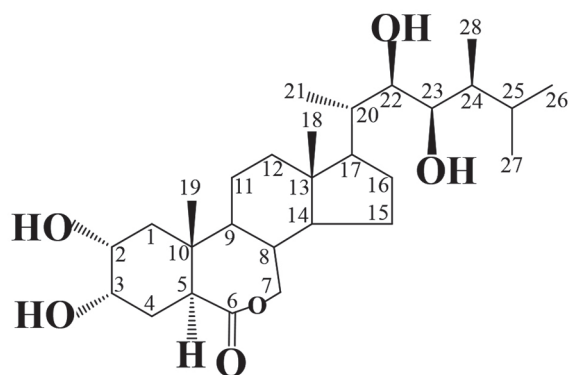
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摘要: 油菜素甾醇(brassinosteroids, BRs)是植物中的一类类固醇激素, 可调控许多至关重要的生长发育过程。BR特异的生物合成由CR (campesterol)起始, 经由多条途径, 受DWF4、CPD、DET2、ROT3/CYP90D1、PsDDWF1、BR6ox1/2等生物合成酶的催化最终形成生物活性最高的BR, 即油菜素内酯(brassinolide, BL)。其中不依赖于CN (campestanol)的一条八步合成途径, 在拟南芥中被证明是最主要的生物合成途径。BR代谢产物众多, 但目前已发现相关酶的代谢方式仅包括羟基化、糖基化、磺酰化、还原反应和酰基化。BR信号由细胞质膜上的受体BRI1和共受体BAK1的胞外结构域共同感知, 进而引起抑制因子BKI1从BRI1上解离, 并使BRI1和BAK1的激酶结构域相互作用并相互磷酸化激活, 激活的BRI1可磷酸化激活BSKs和CDGs, BSKs和CDGs再激活下游磷酸酶BSU1, BSU1可去磷酸化失活负调元件BIN2, 从而解除BIN2对下游转录因子BZR1和BES1的磷酸化抑制, 非磷酸化的BZR1和BES1进入细胞核正调或负调多种靶基因。

关键词: 油菜素甾醇; 油菜素内酯; 生物合成; 代谢; 信号转导; 类受体激酶

油菜素甾醇(brassinosteroids, BRs)是一类多羟基的类固醇植物激素。1970年, Mitchell等(1970)从油菜(*Brassica napus* L.)花粉的提取物中发现了一类新的植物生长调节物质, 其对植物的生长促进效应有别于其他植物激素, 故将此类物质命名为油菜素(brassin)。1979年, Grove等(1979)从蜜蜂采集的油菜花粉中分离出了4 mg的生物活性物质, 通过晶体衍射分析确定了该种物质的化学结构(图1), 该化合物被命名为油菜素内酯(brassinolide, BL)。三年后, 日本科学家又从板栗的虫瘿(chestnut insect galls)中分离出了另一种类似于BL的生物活性物质CS (castasterone), 并证明CS是BL的直接前体(Yokota等1982)。随后, 科学家们又从不同植物的

多种器官中分离出了七十多种BL和CS的类似物, 并将此类物质统称为油菜素甾醇类化合物(brassinosteroids, BRs) (Bajguz和Tretyn 2003; Fujioka和Yokota 2003; Bajguz 2007)。

自上世纪90年代起, 油菜素甾醇的相关研究进入了一个快速发展的时代。日本科学家们利用同位素标记的不同BR中间体饲喂长春花(*Catharanthus roseus*)悬浮细胞系或拟南芥(*Arabidopsis thaliana*)幼苗, 通过气相色谱-质谱联用(gas chromatography-mass spectrometry, GC-MS)技术分析其BR代谢产物, 逐步解析了油菜素甾醇主要的生物合成过程(Choi等1996; Fujioka等2000, 2002; Noguchi等2000)。随着油菜素甾醇生物合成通路突变体*det2*及*cpd* (Li等1996; Szekeres等1996)和信号通路突变体*bri1* (Clouse等1996; Li和Chory 1997)的克隆, 人们对油菜素甾醇调控的植物生长发育过程的重要性有了新的认识。因此, 油菜素甾醇被普遍认为是除生长素、乙烯、脱落酸、赤霉素、细胞分裂素之外的第六大类植物内源性激素。在随后近20年的研究中, 科学家们利用分子遗传学、生物化学、组学、生物信息学和结构生物学等多种技术手段围绕油菜素甾醇的生物合

图1 油菜素内酯的化学结构式
Fig.1 Chemical structure of brassinolide

成、代谢和信号转导过程进行了深入的探究, 目前已基本形成较为成熟的通路和调控网络, 本文主要根据近5到10年来的主要研究进展对上述过程进行阐述。

1 油菜素甾醇的生物合成

1.1 油菜素甾醇生物合成的主要过程

油菜素甾醇特异的生物合成途径是由CR (campesterol)开始的。CR首先经多步催化形成CN (campestanol), 可通过依赖于CN的途径(CN-dependent pathway)合成BL。CN可通过两条平行的途径形成BL的直接前体CS。这两条途径以BL中C-6发生氧化反应的早晚分别被命名为早C-6氧化途径(early C-6 oxidation pathway)和晚C-6氧化途径(late C-6 oxidation pathway)。在早C-6氧化途径中, CN首先在C-6发生氧化反应形成6-oxoCN (6-oxocampestanol), 随后C-22发生羟基化形成CT (cathasterone), 进而通过一系列催化形成CS。而在晚C-6氧化途径中, CN首先在C-22发生羟基化形成C-6非氧化形式的CT即6-deoxoCT (6-deoxocathasterone), 随后经过一系列与早C-6氧化途径相对应的催化步骤, 并在最后一步完成C-6的氧化反应形成CS (Fujioka和Yokota 2003) (图2)。

CR也可不形成CN, 直接通过更为简略的八步催化反应形成BL, 因此被称为不依赖于CN的合成途径(CN-independent pathway)。在该途径中, CR依次经DWF4、CPD、DET2、ROT3/CYP90D1、PsDDWF1、BR6ox1/2等多种酶的催化最终形成BL (图2)。就模式植物拟南芥而言, 不依赖于CN的合成途径是BL生物合成的主要途径(Fujioka等2002; Ohnishi等2012)。此外, BL生物合成途径中还存在多个分支旁路和可逆步骤, 部分步骤的催化酶和可逆反应意义还不明确, 有待进一步研究。

1.2 油菜素甾醇生物合成过程中的关键酶及其所编码基因受调控的机制

BL生物合成途径中的关键酶基本上都是通过相应遗传突变体的获得而被发现的, 目前大部分反应步骤中的关键酶已被解析, 且编码这些酶的基因受调控的机制也有一些报道, 下文以不依赖于CN的合成途径中酶的催化顺序进行详细阐述。

1.2.1 DWF4

DWF4是C-22位羟化酶。其首次被发现源于T-DNA插入缺失突变体dwf4表现出典型的BR缺陷

表型, 且这种缺陷表型能被BL所恢复, 而不能被其他植物激素恢复(Azpiroz等1998; Serrano-Cartagena等1999)。以C-22羟基化的BR中间体饲喂dwf4, 也可恢复其缺陷表型(Choe等1998)。DWF4可催化的步骤包括: 从CR转化为22-OHCR [(22S)-22-hydroxy-CR]、从4-en-3-one [(24R)-22-ergost-4-en-3-one]转化为22-OH-4-en-3-one [(22S, 22R)-22-hydroxy-4-en-3-one]、从3-one [(24R)-5a-ergost-3-one]转化为22-OH-3-one [(22S, 22R)-22-hydroxy-3-one]、从CN转化为6-deoxoCT以及从6-OxoCN转化为CT (图2)。

DWF4在植物中的mRNA水平相对较低, 但其所催化反应前体的含量远超出DWF4催化能力的范围, 且得到的产物通常较少(Fujioka等1995; Choe等2001; Bancos等2002; Guo等2010)。这可能是因为DWF4的蛋白量比较少, 没能达到转化所有前体分子所需要的量, 说明DWF4所催化的步骤是整个生物合成途径的限速步骤。超表达DWF4也可明显促进植物的生长, 可能是由于增加了BL的生物合成(Choe等2001)。研究表明, 植物中DWF4的转录表达量与有活性的BR含量呈正相关的趋势(Nomura等2001; Shimada等2003)。这些证据支持DWF4是BL生物合成过程中的限速酶的假说(Kim等2006)。

植物激素合成的特有抑制剂是研究植物激素功能和作用机制的重要工具。科学家们发现拟南芥幼苗在一种含有三唑类的化合物BRZ (brassinazole)的培养基中培养可导致典型的BR缺陷表型, 而施加BL可恢复这种缺陷表型(Min等1999; Asami等2000; Shimada等2001)。进一步的研究表明BRZ可直接与DWF4相互作用从而抑制BL合成(Asami等2001)。在油菜素甾醇稳态维持的调控网络中, 信号转导下游的转录因子抑制多个生物合成关键酶基因的表达, 该调控方式被称为负反馈抑制。研究表明, 限速酶DWF4可受到该种机制的调节, BR信号下游的核心转录因子BZR1可直接结合DWF4的启动子区并下调其表达(He等2005)。DWF4并不是唯一受BZR1负反馈调节的BL合成基因, CPD、ROT3和BR6ox1也是BZR1的靶基因并受其负调控(He等2005) (图3)。近期研究表明, HAT1也可识别并结合到DWF4的启动子区, 并联合BR信号下游的另一个核心转录因子BES1共同抑制DWF4的表达(Zhang等2013)。DWF4除受到下调表达调控外,
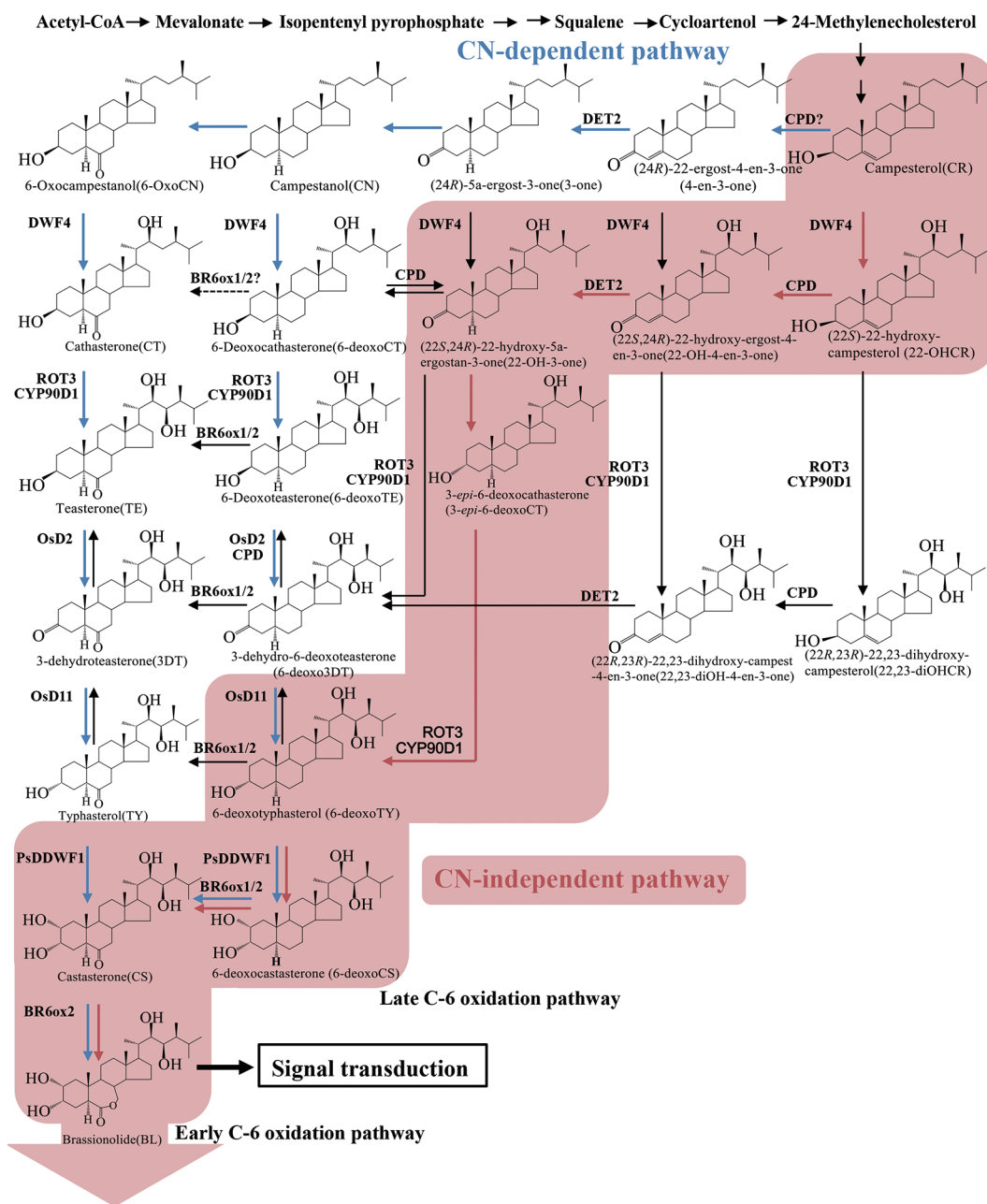
图2 油菜素甾醇的生物合成途径示意图

Fig.2 A simplified brassinosteroid biosynthetic pathway

　　油菜素甾醇最原始的合成前体是植物的初级代谢产物Acetyl-CoA, 但其特异的生物合成途径是从CR (campesterol)起始, 可经多条途径, 最终形成最具活性的BRs, 即油菜素内酯BL (brassinolide)及其前体CS (castasterone)。以C-6位发生氧化反应的早晚分为早C-6氧化途径(early C-6 oxidation pathway)和晚C-6氧化途径(late C-6 oxidation pathway)。以合成过程中是否生成CN (campestanol)分为依赖于CN的合成途径(蓝色箭头)和不依赖于CN的合成途径(红色箭头)。其中, 不依赖于CN的合成途径被认为是拟南芥中BR主要的合成途径。问号表示还未在植物中证实的步骤。此图据Zhao和Li (2012)的模型绘制。

同时还受到转录因子TCP1的上调表达(图3)。TCP1参与BR合成调节是通过激活标签法筛选*bri1-5*的遗传抑制子发现的, 超表达*TCP1*可部分恢

复*bri1-5*的缺陷表型(Guo等2010)。而TCP1的显性负效应*TCP1-SRDX*转基因植株表现出典型的BR缺陷表型, 且该缺陷型表型可被BL恢复, 说明TCP1
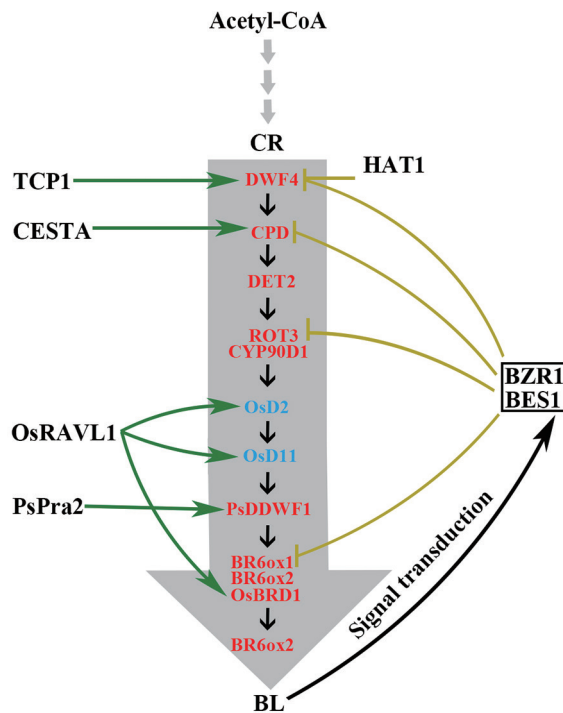
图3 油菜素甾醇合成基因转录调控示意图

Fig.3 Transcriptional regulation of the brassinosteroid biosynthetic genes

油菜素甾醇合成基因不仅受到BZR1和BES1的负反馈调控(黄线), 还受到多个转录因子或互作蛋白的正向调控(绿色箭头)。此图据Zhao和Li (2012)的模型绘制。

调控BL的合成(Guo等2010)。随后的ChIP (chromatin immunoprecipitation)和EMSA (electrophoretic mobility shift assay)实验证明TCP1可直接结合DWF4启动子区2个GGNCC的靶序列(Guo等2010; Gao等2015)。此外, 高温(29°C)可诱导DWF4的上调表达同时增加BL的合成(Maharjan等2011)。生长素也可诱导DWF4在拟南芥根中的表达量增加, 同时伴随着其催化产物22-OH-CR和22-OH-3-one的含量增加, 但这种增加并不依赖于BR合成或信号转导, 这其中具体的调控机制还需进一步研究(Chung等2011; Yoshimitsu等2011)。

1.2.2 CPD

CPD是C-3位氧化酶。与其他的BR缺陷突变体一样, CPD的T-DNA插入缺失突变体cpd和转座子技术构建的突变体cbb1均在暗培养条件下表现出去黄化的表型(Kauschmann等1996; Szekeres等1996)。在最早的研究中, 以C-23羟基化的TE (teast-

erone)饲喂cpd可恢复其缺陷表型, 因此人们认为CPD是一种可催化C-23羟基化的酶(Szekeres等1996)。但在后来的研究中发现ROT3和CYP90D1才是主要的C-23羟化酶(Ohnishi等2006)。而CPD真正催化的是C-3位的氧化反应这一更为上游的步骤。在cpd中, 22-OHCR积累的比CR更多, 这说明CPD主要的催化步骤是从22-OHCR转化为22-OH-4-en-3-one (图2) (Ohnishi等2012), 而这一步骤在早先的研究中却被认为是由SAX1催化的(Ephritikhine等1999a, b)。

CPD同样受到BL的负反馈抑制性调节, BZR1可识别并结合CPD的启动子区并下调其表达(He等2005)。此外, CPD还受到BRX1的上调表达, 且BRX1本身还受到生长素的正向调节和BL的负向调节(Mouchel等2006)。这意味着BL不仅通过BZR1负调CPD, 还通过BRX1正调CPD, 这两种截然相反的机制在维持其稳态中起重要作用。近期的研究表明, CPD还受到bHLH (basic helix-loop-helix)转录因子CESTA的正向调节(图3)。与TCP1相似, CESTA的显性负效应突变体表现出BR缺陷表型, 该缺陷可被BL恢复。ChIP和EMSA实验表明CESTA可直接结合在CPD的启动子区的G-box上起转录调控作用(Poppenberger等2011)。

1.2.3 DET2

DET2编码一种还原酶(Li等1996)。DET2的缺失突变体det2最先是在光信号通路的研究中筛选到的, 其在黑暗培养条件下表现出去黄化的表型(Chory等1991)。饲喂实验表明DET2可催化4-en-3-one转化为3-one, 22-OH-4-en-3-one转化为22-OH-3-one, 及22,23-diOH-4-en-3-one转化为6-deoxo3DT (3-dehydro-6-deoxoteasterone)的还原反应(图2) (Fujioka等1997, 2002; Noguchi等1999; Ohnishi等2012)。DET2不受BL负反馈机制的调节(Tanaka等2005), 调控DET2表达的转录因子目前还未见报道, 有待进一步筛选。

1.2.4 ROT3与CYP90D1

ROT3与CYP90D1是C-23位羟化酶。DWF1 (CYP90B1)、CPD (CYP90A1)、ROT3 (CYP90C1)和CYP90D1均为细胞色素P450家族的同源蛋白。早期的饲喂实验表明ROT3与CYP90D1可能催化不同的反应步骤(Kim等2005)。但后期研究发现只

有C23位羟基化的BR中间体才可以恢复*ROT3*和*CYP90D1*的双重缺失突变体的缺陷表型, 说明这两个酶共同催化C23位的羟基化步骤(图2) (Ohnishi等2006)。

虽然ROT3与CYP90D1催化的反应相同, 但在生物学功能的细节上也有不同之处, *ROT3*在叶片中大量表达且不受光调控, 而*CYP90D1*仅在叶维管束和叶柄中表达且在黑暗培养条件下上调(Kim等2005)。另外, *ROT3*和*CYP90D1*的表达量也受BL下调, 且有证据表明*ROT3*是BZR1的靶基因(图3) (He等2005; Ohnishi等2006)。

1.2.5 PsDDWF1

PsDDWF1是豌豆(*Pisum sativum*)中的C-2位羟化酶, 在拟南芥中催化该步骤的酶尚未被发现。饲喂实验证明PsDDWF1可催化TY (typhasterol)转化为CS和6-deoxoTY转化为6-deoxoCS (图2) (Kang等2001)。生理生化实验表明一个受暗培养诱导的小G蛋白PsPra2可与PsDDWF1相互作用并正调其酶活性(图3) (Kang等2001)。

1.2.6 BR6ox1、BR6ox2与OsBRD1

BR6ox1和BR6ox2是拟南芥中的的C-6位氧化酶, OsBRD1是水稻(*Oryza sativa*)中的C-6氧化酶。最早发现的C-6氧化酶DWARF是在番茄(*Solanum lycopersicum*)中得到的, 后又发现拟南芥中与DWARF同源的蛋白BR6ox1 (CYP85A1)和BR6ox2 (CY-P85A2)也可催化6-deoxoTE转化为TE、6-deoxo3DT转化为3DT (3-dehydroteasterone)、6-deoxoTY转化为TY、6-deoxoCS转化为CS的氧化反应(图2) (Bishop等1999; Shimada等2001)。*BR6ox1*单突变体无明显表型, 而*BR6ox2*的单突变体有较弱的BR缺陷表型, 且该缺陷可被BL和CS恢复, 进一步的分析表明BR6ox2催化BL合成通路的最后一步, 即从CS转化为BL (图2) (Kim等2005; Kwon等2005; Nomura等2005)。

*BR6ox1*和*BR6ox2*均受到BL的负反馈抑制性调节, 且有证据表明*BR6ox1*是BZR1的靶基因(图3) (Bancos等2002; He等2005; Tanaka等2005)。水稻中的C-6位氧化酶*OsBRD1*则被证明是OsRAVL1靶基因并受其上调表达(图3) (Je等2010)。

1.2.7 OsD2与OsD11

除了上述不依赖于CN合成途径中的合成酶, 在水稻中还发现了两个依赖于CN合成途径中的酶, 即OsD2及OsD11, 分别是C-3位脱氢酶和C-3位羟化酶。*OsD2*与*OsD11*的缺失突变体分别表现出水稻中BR缺陷表型, 且BL能恢复这种缺陷。饲喂实验表明OsD2可催化TE转化为3DT和6-deoxoTE转化为6-deoxo3DT的步骤, 而OsD11则催化3DT转化为TY和6-deoxo3DT转化为6-deoxoTY的步骤(图2) (Hong等2003; Tanabe等2005)。

近期的研究表明, 水稻中*OsD2*与*OsD11*可受到转录因子OsRAVL1的直接正向调节, *OsRAVL1*的超表达转基因植株表现出BR合成增加或信号增强的表型, 而其缺失突变体表现出BR缺陷的表型。生化实验表明OsRAVL1除直接调控外*OsD2*、*OsD11*和*OsBRD1*外, 还直接调控水稻中的BR受体*OsBRI1*的表达(图3) (Yamamuro等2000; Je等2010)。

2 油菜素甾醇的代谢

与其他植物激素相同, 具有活性的BR含量陡然增多或减少都会严重影响植物的生长发育。因此, 存在多种调控机制以维持植物体内的BR稳态。BRs除了通过负反馈机制抑制合成基因转录的调控方式外, 还可通过多种代谢酶使有活性的BRs失活以调节其含量。化学分析显示BRs具有多种代谢产物, 但科学家们目前仅发现了几种BR代谢相对应的催化酶, 对更多的代谢酶及代谢过程的调控机制还知之甚少, 有待进一步研究。

2.1 羟基化代谢

最先被发现的BR羟基化代谢酶是BAS1, 激活标签法筛选发现其能恢复光受体PHYB的突变体表型, 且其缺失突变体对外源BL超敏感(Neff等1999)。*BAS1*编码的酶属于P450家族成员, 即CY-P72B1 (又名CYP734A1) (Nelson等2004)。进一步的研究表明BAS1是一个C-26位羟化酶, 可催化具有生物活性形式的BRs即CS和BL转化为非活性形式的26-OHCS和26-OHBL (图4) (Neff等1999; Turk等2003)。

BL可上调BAS1的转录以实现对冗余BL的失活。近期的研究表明, 这种调节可通过转录因子LOB1实现, LOB1可直接结合BAS1启动子区并上调其表达(Bell等2012)。此外, BL还可通过抑制转录因子*ATAF2*从而负调节BAS1的含量, ATAF2可直接结合*BAS1*的启动子区并下调其表达(Peng等2015)。
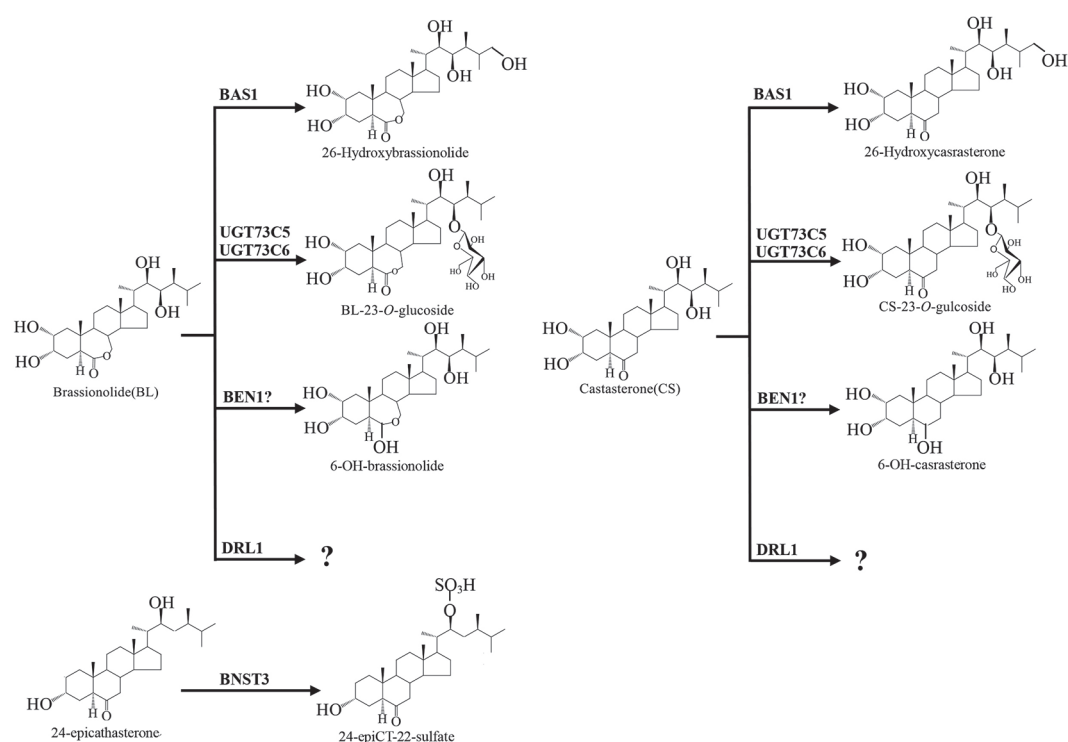
图4 油菜素甾醇的代谢反应

Fig.4 Catabolic reactions of brassinosteroids

油菜素甾醇的代谢产物众多, 但目前已发现相关酶的代谢方式仅有以上几种。问号表示尚未在植物中证实的酶或产物。

另一个羟基化代谢酶是由三个课题组在同一年发表的, 分别被命名为CHI2、SHK1和SOB7, 这三个酶实为同一个蛋白即CYP72C1, 该蛋白与BAS1 (CYP72B1)是同源物, 蛋白序列的一致性(identity)达36% (Nakamura等2005; Takahashi等2005; Turk等2005)。虽属同源, 但CYP72C1与BAS1并不催化相同的代谢步骤, 且有证据表明CYP72C1对TY和TE的亲和性高于CS和BL, 但具体的催化步骤和确切的底物还有待进一步发掘(Nakamura等2005; Takahashi等2005; Turk等2005; Thornton等2010)。此外, 研究发现ATAF2可直接结合*SOB7*启动子区并下调其表达(Peng等2015)。

2.2 糖基化代谢

通过糖基化修饰调控甾醇类激素的活性在哺乳动物中较为常见(Hum等1999; Lim和Bowles 2004)。在植物的相关研究中, 编码拟南芥中糖基转移酶的基因*UGT73C5*的超表达植株表现出BR缺陷表型且积累了糖基化的非活性CS和BL, 而其RNA干扰突变体则检测不到糖基化的BRs, 说明UGT73C5

可催化CS和BL在C-23位的糖基化失活过程(图4)(Poppenberger等2005)。近期的研究表明, 其同源蛋白UGT73C6也可催化相同的过程, 但这两个基因受BL上调的具体机制还有待研究(Husar等2011)。

2.3 磺酰化代谢

油菜中发现的BR磺酰基转移酶BNST3可催化C-23位的磺酰化反应, 但其对24-epiCT的亲和性高于24-epiBL (图4)。由于24-epiCT的生物活性低于24-epiBL, 因此可能导致了超表达*BNST3*并未产生BR缺陷表型(Rouleau等1999; Marsolais等2004)。此外, 油菜中还发现了另一个磺酰基转移酶BNST4可催化除了24-epiCT外的其他BRs (Marsolais等2004)。拟南芥中也发现了两个磺酰基转移酶AtS-T4a 和 AtST1, 可催化以24-epiCT为主的多个BR磺酰化反应(Marsolais等2007)。

2.4 还原反应代谢

通过激活标签法筛选*bri1-5*发现超表达*BEN1*可增强*bri1-5*的缺陷表型, 其单独超表达的转基因植物也表现出BR缺陷表型, 而其缺失突变体则表

现出BL合成增多或信号增强的表型, 这说明BEN1与BR代谢相关(Yuan等2007)。BEN1编码一个还原酶, 其很有可能将CS或TY催化为C-6位羟基化的无活性形式, 但这一猜想还需要直接的实验证据(图4) (Yuan等2007)。

2.5 酰基化代谢

　　近期的研究中, 五个课题组独立发表了五篇关于BL的两个酰基化代谢酶的文章, 即ABS1/BIA1和PIZ1/ DRL1/BAT1, 其中ABS1、BIA1和DRL1通过激活标签法筛选野生型拟南芥得到, 而PIZ1和BAT1则通过FOX (full-length cDNA overexpressor gene)技术获得(Roh等2012; Schneider等2012; Wang等2012; Choi等2013; Zhu等2013; Higuchi-Takeuchi和Matsui 2014)。这两个基因的超表达转基因植株或功能获得性(gain-of-function)突变体均表现出BR缺陷表型, 且这些突变体中多个BRs如TY、CS和BL的含量均有所下降, 但其确切的酰基化产物还未见报道(图4) (Choi等2012; Roh等2012; Schneider等2012; Wang等2012; Zhu等2013)。生长素信号通路下游的转录因子ARF19可直接调控*BAT1*的表达(Choi等2013)。BIA1可以被BL上调表达, 但具体分子机制还不清楚(Roh等2012)。

3 油菜素甾醇的信号转导

　　油菜素甾醇信号通路的第一个突变体*bri1-1*是1996年首次报道的(Clouse等1996)。随后的研究中, 科学家们利用甲基磺酸乙酯(ethyl methane sulfonate, EMS)诱变野生型拟南芥的种子并筛选对外源BL处理不敏感的突变体, 其中18个突变体经图位克隆发现其突变均发生在同一个基因中, 即油菜素甾醇的受体*BRI1* (Li和Chory 1997)。此后, 科学家们利用多种技术手段对BR信号转导的分子机制展开了大量研究。截至目前, BR信号从胞外被感知到胞内的级联放大再传递至下游响应基因的基本过程已被解析, 但更为细节的分子机制还在不断的研究中(图6)。

3.1 细胞质膜上的信号感知和信号途径的起始

　　油菜素甾醇的受体BRI1是一个单次跨膜的富含亮氨酸重复序列的类受体蛋白激酶(leucine-rich repeat receptor-like protein kinase, LRR-RLKs), 该蛋白含有多个功能性结构域(Li和Chory 1997)。在过去的二十年中, 已发现了20多个BRI1不同类型的突变体, 它们的表型强弱不一, 但均表现出叶片

变圆、叶柄缩短、育性下降、衰老延迟等BR缺陷的典型特点(图5) (Clouse等1996; Kwon和Choe 2005; Vert等2005)。其中多个弱突变体作为遗传筛选材料对BL合成、代谢和信号转导, 乃至与其他通路互作调控元件的发掘做出了重要贡献。例如, BAK1 (Li等2002)、BRS1 (Li等2001)、BRL1 (Zhou等2004)、BEN1 (Yuan等2007)、TCP1 (Guo等2010)和BSU1 (Mora-Garcia等2004)均是由激活标签法筛选*bri1-5*的遗传抑制子得到的; BES1是通过EMS诱变筛选*bri1-119*的点突变遗传抑制子发现的(Yin等2002); 还有内质网蛋白质量监控系统的多种元件EBS1至EBS7是近年来通过EMS诱变筛选*bri1-9*的遗传抑制子发现的(Jin等2007, 2009; Su等2011, 2012; Hong等2012; Liu等2015)。
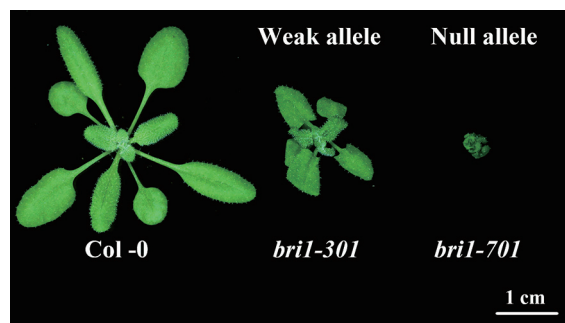

图5　典型的*bri1*弱突变体和强突变体的表型
Fig.5　Phenotypes of a typical weak *bri1* allele and a null *bri1* allele

　　BRI1的胞外结构域由25个LRR串联重复序列组成, 其中第21和22个LRR之间有一个约70个氨基酸残基组成的岛屿(island)结构域, 早先的遗传学分析和近期的晶体结构证据表明该结构域与BL的结合直接相关, 岛屿及其附近结构可形成一个刚好容纳BL的三维疏水口袋(Li和Chory 1997; Hothorn等2011; She等2011)。BRI1的胞内结构由近膜结构域、激酶结构域及一个负调控激酶活性的羧基末端组成(Li和Chory 1997; Wang等2001)。早先的生化实验证明BRI1可形成同源二聚体(Wang等2005), 而近期的晶体结构分析则认为BRI1的胞外结构域过于庞大, 并不利于同源二聚体稳定结构的形成, 而BRI1与BAK1的异源二聚体才适宜形成稳定结构(Hothorn等2011; She等2011; Santiago等2013; Sun等2013)。BRI1激酶具有丝/苏氨酸和酪氨酸双

重激酶活性, 其中很多磷酸化位点已通过质谱技术鉴定得到(Oh等2000, 2009; Wang等2005)。此外, BRI1的同源蛋白BRL1和BRL3也在BR信号转导中发挥功能, 但遗传学结果表明BRI1是主要的受体蛋白(Cano-Delgado等2004; Zhou等2004)。

　　在哺乳动物中, 甾醇类激素的受体可通过内吞作用受到调节(Miaczynska等2004)。在植物中, 也观察到了BRI1的内吞现象(Russinova等2004)。BRI1除定位在细胞质膜上外, 还定位在胞内体(endosome)上, 且这种定位会增强BR信号输出(Geldner等2007)。近年来, 通过对BRI1的两个弱突变体

bri1-5和bri1-9的细致研究, 发现大部分bri1-5或bri1-9蛋白不能通过内质网质量监控系统而滞留在内质网中, 仅有少量可成功运输至细胞质膜上, 这种膜上受体的不足导致了bri1-5和bri1-9的弱缺陷表型, 而滞留在内质网的BRI1最终被降解(Jin等2007, 2009; Su等2011, 2012; Hong等2012; Liu等2015)。甲基转移酶SBI1可通过甲基化激活磷酸酶PP2A, 激活后的PP2A可以去磷酸化BRI1从而使其降解或循环回到细胞质膜上(Di Rubbo等2011; Wu等2011)。而近期研究则认为, PP2A仅是将BRI1去磷酸化失活, 并不能引起降解(图6) (Zhao等2016)。
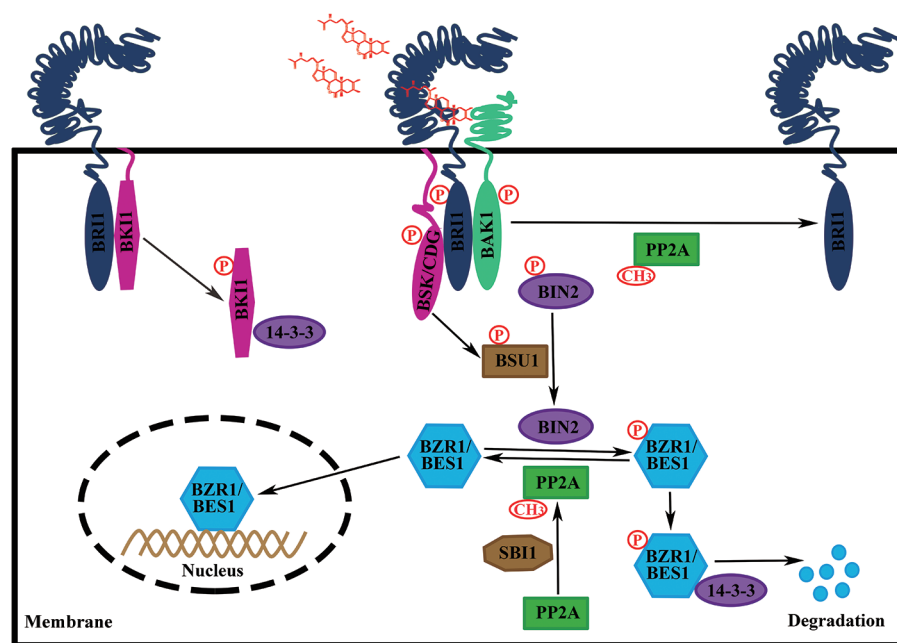

图6 油菜素甾醇信号转导模式图

Fig.6 A current model of brassinosteroid signal transduction

　　当油菜素甾醇信号缺失时, 其受体BRI1的激酶活性受负调因子BKI1的抑制。当油菜素甾醇存在时, 其可与BRI1和BAK1的胞外结构域结合, 引起BKI1从细胞质膜上解离, 进而导致BRI1与BAK1的激酶结构域相互磷酸化激活。激活后的BRI1磷酸化激活BSKs和CDGs。BSKs通过蛋白互作激活BSU1, 而CDG1/CDL1可磷酸化激活BSU1。激活的BSU1可去磷酸化失活负调元件BIN2, 从而解除BIN2对下游转录因子BZR1和BES1的磷酸化抑制, 非磷酸化的BZR1和BES1进入细胞核正调或负调成百上千个靶基因。此外, 14-3-3与细胞质中积累的磷酸化的BZR1和BES1相互作用并导致其降解, 而解离到细胞内的BKI1可竞争性的结合14-3-3以抑制这一过程。SBI1通过甲基化激活PP2A, 使其发挥去磷酸化BRI1、BZR1和BES1的功能。

　　BAK1作为共受体参与BR信号转导最先是由两个独立的研究组分别通过激活标签法筛选bri1-5的遗传抑制子和酵母双杂交筛选BRI1胞内域的互作蛋白这两种不同的技术手段同时发现的(Li等2002; Nam和Li 2002)。与BRI1相同, BAK1也属于LRR-RLK家族, 但其胞外仅有5个LRR (Li等2002)。

早先的各项分析表明BAK1可与BRI1直接相互作用从而正向调控BR信号转导。近期的研究表明, BAK1及其SERK家族的同源蛋白构成的三重缺失突变体serk1 bak1 bkk1在黑暗下已完全不响应外源BL的处理, 且外源施加BL处理前后, 此三突中均检测不到BRI1的苏氨酸磷酸化, 这些证据证实了

SERK家族成员在BR信号感知中起不可或缺的作用(Gou等2012)。近期的晶体结构分析也证明BRI1和BAK1的胞外结构域以及BL可直接形成稳定的异源复合物(Santiago等2013; Sun等2013)。除胞外结构域的直接结合外, BRI1和BAK1的胞内激酶结构域也可通过磷酸化作用相互激活(图6) (Wang等2008)。这些研究表明, BR信号很有可能通过与BRI1和BAK1的胞外结构域结合, 将两者距离拉近, 进而导致胞内激酶结构域的相互作用和相互激活。

　　早先的研究中, 科学家们也观察到了BAK1的内吞现象(Russinova等2004)。后续的研究发现MSBP1参与调控这一过程, MSBP1可与BAK1直接相互作用并增强其内吞同时使BAK1与BRI1的相互作用减弱, 从而负调节BR信号通路(Yang等2005; Song等2009)。近期的研究发现, 光信号通路中的转录因子HY5及其同源蛋白HYH均可识别并直接结合到*MSBP1*的启动子区并上调其表达, 为光信号通路和BR信号通路找到了一个连接点(Shi等2011)。BAK1除参与BR信号转导途径外, 还参与调控细胞死亡、花药的绒粘层发育、植物固有免疫、气孔发育及根发育等多项植物生长发育过程, 其功能的多样性和复杂性一直是近年来的研究热点(Li等2002; Nam和Li 2002; Albrecht等2005; Colcombet等2005; Chinchilla等2007; He等2007; Heese等2007; Li 2010; Yamaguchi等2010; Meng等2015; Ou等2016; Shinohara等2016; Song等2016)。

　　BKI1参与BR信号转导也是通过酵母双杂交筛选BRI1的胞内域互作蛋白发现的(Wang和Chory 2006)。BKI1是一个N末端定位于膜上的蛋白, 当BR信号缺失时, BKI1与BRI1相结合并抑制BRI1的激酶活性; 当BR信号与BRI1和BAK1的胞外结构域结合时, 可促使BKI1从BRI1上解离(图6) (Wang和Chory 2006)。近期的晶体结构分析表明, BRI1的胞内结构域可与BKI1的一个肽段直接结合(Wang等2014)。近期的遗传分析表明, BKI1的缺失突变体表现出类似BRI1超表达的表型(Wang等2001; Jiang等2015)。

　　BRI1被激活后, 可通过磷酸化不同的底物以实现信号的进一步传递。BSK1作为其中一个底物是通过蛋白质组学鉴定得到的, 是一个RLCK (receptor-

like cytoplasmic kinases)蛋白(Tang等2008)。近期的研究表明, 其所在亚家族中的8个成员均冗余的参与BR信号转导(Sreeramulu等2013)。除参与BR通路外, 其中一个成员BSK1还被发现参与调节植物的固有免疫(innate immunity) (Shi等2013)。近年来的研究还发现BRI1也可磷酸化CDG1和其同源蛋白CDL1, 这两个蛋白也属RLCK (Muto等2004)。与BSK1相似, CDG1和CDL1也可进一步激活BR通路的下一步元件(Kim等2011), 但BSK1及其同源蛋白被认为不具备激酶活性, 而CDG1和CDL1是有激酶活性的, BSK1究竟如何激活下游分子还有待进一步研究。

　　在BR信号转导的早期事件中, 还发现其他一些蛋白可与BRI1信号感知复合体互作, 如BRS1 (Li等2001; Zhou和Li 2005)、TRIP1 (Jiang和Clouse 2001; Ehsan等2005)、TTL1 (Nam和Li 2004; Pessoa等2010)、TWD1 (Chaiwanon等2016; Zhao等2016)等, 但它们在BR信号通路中具体作用机制还尚不清楚, 有待进一步研究。

3.2 细胞质内的信号级联放大

　　BR信号由BRI1和BAK1共同感知后经一系列磷酸化反应传至BSKs, 由此, 信号从细胞外传至细胞内, 并在细胞质中开始了一系列的信号放大过程。BSU1是激活标签法筛选*bri1-5*的遗传抑制子发现的, BSU1是一个磷酸酶, 早先的研究认为其功能是将BR信号下游转录因子BES1去磷酸化(Mora-Garcia等2004)。随后的研究否认了这一观点, 认为其在更上游处发挥功能, BSKs通过蛋白互作激活BSU1, 而CDG1/CDL1可磷酸化激活BSU1, 激活的BSU1可去磷酸化BR信号通路重要的负调因子BIN2并使其失活(图6) (Tang等2008; Kim等2009; Kim等2011)。近期的研究表明, BSU1与其家族成员可聚合在一起共同发挥功能(Kim等2016)。

　　最早发现BIN2负调节BR信号通路是筛选得到一个对BRs不敏感的功能获得性突变体*bin2-1*, 其表型类似于*bri1*突变体(Li等2001)。BIN2是哺乳动物GSK3 (glycogen synthase kinase 3)的同源物, 蛋白序列一致性(identity)达70% (Li和Nam 2002)。其在拟南芥中的多个同源蛋白共同参与BR信号通路(Yan等2009; Rozhon等2010)。下游核心转录因子BZR1是由于其功能获得性点突变体*bzr1-1D*对

BRZ的处理敏感性降低而筛选得到的(Wang等2002), 而它的同源蛋白BES1是通过筛选EMS诱变的*bri1-119*发现的, 其功能获得性点突变体*bes1-1D*可部分恢复*bri1-119*的表型(Yin等2002)。但目前尚没有对BZR1和BES1缺失突变体表型的报道, 它们的生物学功能有待于用遗传学手段进一步验证。当BR信号缺失时, 激酶BIN2处于磷酸化的激活状态, 可磷酸化转录因子BZR1和BES1, 使其不能进入细胞核调控下游靶基因并在细胞质中积累; 当BR信号传递至BSU1时, BSU1可去磷酸化失活BIN2, 从而解除了对BZR1和BES1的抑制作用, 使信号传递下去(图6) (Li和Nam 2002; Kim等2009; Peng等2010)。近年来的多项研究发现, BIN2除了磷酸化BZR1和BES1外, 还可磷酸化多个不同的底物, 如生长素信号通路中的ARF2 (Cho等2014), 调节气孔发育的YODA、MKK4和MKK5 (Kim等2012; Khan等2013), 调控下胚轴伸长的PIF4 (Bernardo-Garcia等2014), 调控根毛发育的EGL3和TTG1 (Cheng等2014)等, BIN2已成为植物生长发育调控网络中的一个整合点。这些研究结果表明, BR下游信号输出很有可能由多个转录因子调控, 不仅仅只有BZR1及BES1。

滞留在细胞质中磷酸化的BZR1和BES1可被14-3-3直接识别并通过26S蛋白酶体途径降解(Gampala等2007)。近期的研究表明, BKI1从 BRI1处解离进入细胞质后, 还可与14-3-3竞争性相互结合, 从而使更多的BZR1和BES1入核传递信号(图6)(Wang等2011)。磷酸酶PP2A除了去磷酸化BRI1抑制上游信号起始外, 还可去磷酸化BZR1促进下游信号的传递, 以双重调节的方式维持信号传输的稳态(图6) (Di Rubbo等2011; Tang等2011)。

3.3 下游信号响应

非磷酸化的BZR1和BES1进入细胞核可直接调控靶基因, 进而调节多项植物生长发育过程。由此, BR信号完成了从细胞外至细胞核内的传递。BZR1和BES1是同源物, 基因序列的一致性(identity)达88%, 均属bHLH转录因子, BZR1靶序列是BRRE (CGTGT/CG)而BES1的靶序列是E-box (CANNTG) (Sun等2010; Yu等2011)。BZR1和BES1不仅通过自身磷酸化状态的转变调控BR信号输出, 还可正调或负调多种靶基因的表达, 其中, BZR1

有900多个响应BR信号的靶基因, 而BES1至少有250个响应BR信号的靶基因(Ryu等2007, 2010; Sun等2010; Yu等2011)。

BZR1和BES1还联合其他转录调节因子或转录因子共同调控不同的生物学过程。如BES1和BIM1共同调控*SAUR-AC1* (Yin等2005), BES1与ELF6和REF6共同调控开花(Yu等2008), BES1与IWS1共同调控转录延伸(Li等2010), *MYB30*、*HAT1*、*MYBL2*受BES1的直接调控, 但其表达的蛋白又可与BES1共同调控其他靶基因(Li等2009; Ye等2012; Zhang等2013)。近期的研究表明, BES1与MAX2共同调控植物分蘖(Wang等2013), BZR1与TOPLESS共同抑制其靶基因的表达(Oh等2014)。

4 展望

油菜素甾醇的生物合成、代谢和信号转导一直是植物领域的研究热点。自油菜素内酯的结构被确定至今, 经过近30年不懈的努力, 科学家们对这些过程中涉及的重要元件及分子机制已有了较深入的认知。但不可否认的是, 这其中仍有许多知识盲点和细节需要进一步探究。首先, 由于油菜素甾醇不能长距离运输, 如何保持其在局部组织的稳态是植物维持其正常生长发育面临的重要问题。目前科学家们已发现植物可通过BR信号转导的下游转录因子负反馈调控合成基因的表达, 还发现了几种BR代谢方式, 但更多的调控方式和这些调控方式如何响应植物生存的内外环境变化还有待进一步的研究。其次, BR信号转导过程的主线虽已较为明确, 但其中一些过程还需要缺失突变体的遗传学证据支持。另外, 植物生长发育必然受到网络式的调控, 那么BR信号通路如何与其他通路相互连接和相互调节仍需大量的研究。随着实验技术的不断革新和大数据时代带来的便利, 相信BR领域还会有令人惊奇的发现。这些知识将为人们有效利用分子育种手段改良农作物提供理论指导。

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Biosynthesis, catabolism, and signal transduction of brassinosteroids

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Abstract: Brassinosteroids (BRs) are a class of steroidal phytohormones playing essential roles during normal plant growth and development. In Arabidopsis, an 8-step campestanol (CN)-independent pathway, starting from campesterol (CR) to the final and most active BR, brassinolide (BL), has been demonstrated as the predominant BR biosynthetic pathway. Reactions in this pathway are catalyzed by a series of enzymes including DWF4, CPD, DET2, ROT3/CYP90D1, PsDDWF1, and BR6ox1/2. Although dozens of BR catabolites have been identified, only a few enzymes corresponding to their formation have been discovered. These enzymes catalyze several catabolic reactions including hydroxylation, glycosylation, sulfonylation, reduction, and acylation. BRs are mainly perceived by their receptor BRI1 and co-receptor BAK1. Direct interaction between BRs and the extracellular domains of BRI1 and BAK1 triggers dissociation of a cytoplasmic inhibitory component BKI1 and association of BRI1 kinase domain with BAK1 kinase domain. The activated BRI1-BAK1 complex can then phosphorylate downstream receptor-like cytoplasmic kinases, BSKs and CDGs. The activated BSKs and CDGs can inhibit kinase activity of a negative regulator BIN2 via a protein phosphatase, BSU1. Dephosphoylation of BIN2 can result in the accumulation of two unphosphorylated transcription factors BZR1 and BES1 in the nuclei, which can directly mediate the expression of numerous BR responsive genes.
Key words: brassinosteroids; brassinolide; biosynthesis; metabolism; signal transduction; receptor-like kinase