综述

BRI1受体的胞内运输及其在油菜素甾醇信号转导中的作用

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摘要 油菜素甾醇(brassinosteroids, BRs)作为一种重要的植物激素调控植物生长发育的诸多进程以及逆境胁迫响应, 而其信号转导途径的研究也一直是植物生物学研究的热点之一。BRs信号的识别起始于BRI1(brassinosteroid insensitive 1)受体对BRs的感知, 然后通过一系列下游信号组分进行转导实现对靶基因的表达调控。该文主要从细胞生物学的角度综述了新合成的BRI1通过内质网质量控制系统监控后运输到细胞膜上、BRI1的内吞作用和液泡运输、共受体BAK1(BRI1-associated kinase 1)影响BRI1的胞内运输以及BR信号的终止等内容, 并探讨了今后的研究方向及待解决的问题。

关键词 油菜素甾醇; BRs信号转导; BRI1; BAK1; 内吞; 液泡运输

Subcellular Trafficking of BRI1 Receptors and Its Role in Brassinosteroids Signal Transduction

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Abstract Brassinosteroids (BRs) are a class of important phytohormones that control many processes during plant development and also responsible to stress. Thus, studies in BRs signal transduction are one of the hotspots in the field of plant biology. The BRI1 (brassinosteroid insensitive 1) receptor perceives BRs signal to initiate signal transduction, which is subsequently propagated through a number of downstream components, resulting in transcriptional changes of BRs-responsive genes. From the perspective of cell biology, the aim of this review is to summarize recent advances on the following aspects: (1) newly synthesized BRI1 receptors trafficking to the plasma membrane after being monitored by endoplasmic reticulum quality control; (2) endocytosis and vacuolar trafficking of BRI1; (3) the co-receptor BAK1 (BRI1-associated kinase 1)-mediated regulation of BRI1

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trafficking; (4) termination of BR signaling, and highlight the open questions and potential research directions.

Keywords brassinosteroids; BRs signal transduction; BRI1; BAK1; endocytosis; vacuolar trafficking

油菜素甾醇(brassinosteroids, BRs)是一类具有高生理活性的甾醇类植物激素, 参与调控植物生长发育的许多过程, 包括细胞的分裂和伸长、维管束的分化、植株的衰老、雄性育性和光形态建成, 同时在植物响应生物和非生物胁迫中发挥作用[1-3]。近年来, 利用遗传学、生物化学、基因组学、蛋白质组学等手段研究BRs信号转导的分子机理, 并取得了一系列重要进展。BRs信号首先被细胞膜上一个小基因家族所感知, 其成员编码富亮氨酸重复序列受体激酶(leucine-rich repeat receptor kinase, LRR-RK)。BRI1(brassinosteroid insensitive 1)是该家族中的主要成员, 负责大部分的BRs结合活性[4-7]。BRs与BRI1的胞外结构域(extracellular domain, ECD)相结合激活BRI1的胞内激酶活性[8-11], 并促使抑制因子BKI1(BRI1 kinase inhibitor 1)从BRI1上解离[12-13]。BKI1的解离促使BRI1与其共受体BAK1/SERK3(BRI1-associated kinase 1/somatic embryogenesis receptor-like kinase 3, 下文简称BAK1)的相互作用并相互磷酸化激活, 接着通过中间途径的激酶和磷酸酶的顺序作用, 将BRs信号继续传递下去[14-18]。随后, BRs信号通路中的关键转录因子BZR1(brassinazole-resistant 1)和BES1(BRI1-EMS-suppressor 1, 也被称为BZR2)被去磷酸化而处于激活状态, 进入细胞核调控BRs响应基因的表达[18-20]。尽管BRs信号转导的机制在过去二十年里被详细研究, 但是BRs领域中的一些关键问题才刚刚得到研究者的关注。比如, BRI1受体是如何在细胞内进行运输、其运输受到哪些机制调控, 以及BRI1的胞内运输是如何影响BRs信号转导的。因此, 本文从细胞生物学层面对上述问题作一综述, 并对今后的研究方向进行了展望。

1 新合成的BRI1通过内质网质量控制系统监控后运输到细胞膜上

内质网内蛋白质的折叠过程受到内质网质量控制系统(endoplasmic reticulum quality control, ERQC)的监控, 错误折叠的蛋白质将滞留在内质网内并通过内质网相关的降解途径(ER-associated degradation, ERAD)进行降解。对BRI1的两个弱突变体*bri1-5*和*bri1-9*的研究表明, 这两种突变形式的BRI1蛋白质不能通过ERQC而被滞留在内质网中, 最终通过ERAD降解(图1), 从而阻碍了BRs信号转导[21-22]。折叠正确的有功能的BRI1经过高尔基体(Golgi)和反式高尔基体网络/初级内体(trans-Golgi network/early endosome, TGN/EE)后, 到达细胞表面[23-24](图1)。在BRs的诱导下, BRI1在细胞表面与BAK1形成异二聚体, 启动BRs信号转导[18,25]。

来自细胞外和细胞内两方面的因素拮抗地调节BRs受体复合物的形成。首先, 细胞外的配体BRs作为一种“分子胶水”将BRI1和BAK1二者的ECD结合到一起[9,26-27]。然而, Bücherl等[28]报道, 一部分BRI1-BAK1异二聚体的形成并不依赖于BRs, 而是在细胞膜上提前形成。其次, BRs信号通路的抑制因子BKI1从细胞内部调控BRs受体复合物的形成[12-13]。BKI1的C-端尾巴(C-terminal tail)是由20个氨基酸残基组成的肽段, 它与BRI1的激酶区结合, 从而阻止了共受体BAK1与BRI1的接近, 因此不能形成有活性的BRs受体复合物[12-13]。在感知BRs以后, 被激活的BRI1激酶在至少三个位点(Tyr211、Ser270和Ser274)快速磷酸化BKI1, 从而使BKI1从细胞膜上解离, 启动BRs信号转导[13,29]。

2 BRI1的内吞作用和液泡运输

研究发现, 当增加内体(endosome)中BRI1的含量时, 可以激活BRs信号通路, 暗示植物细胞中存在着与动物类似的内体信号系统(endosome-mediated signaling), 内体可能为BRs信号转导提供平台[23]。但近年来有越来越多的研究表明, BRs信号转导主要起始于细胞表面, 并且细胞膜上BRI1的含量受到其胞内运输的调控[30-32]。细胞膜上的BRI1经过内吞作用(endocytosis)进入细胞后, 在TGN/EE进行分拣, 分拣之后的BRI1可以经过多囊泡体/液泡前体(multivesicular body/prevacuolar compartment, MVB/PVC)被定向运输到液泡进行降解, 也可以被重新运回细胞表面[23-24,33](图1)。Luo等[31]研究发现, 当编码V-ATPase C亚基的基因*DET3*(*De-etiolated 3*)发生突变时, TGN/EE的pH值升高, 从而使BRI1从TGN/EE运回细胞表面的速率降低, *det3*突变体植株的BRs信

号通路受到抑制。值得注意的是, BRI1的亚细胞定位以及在细胞中的运输过程并不依赖于与配体BRs的结合[23,33]。

调控BRI1内吞作用的机理并不完全清楚。网格蛋白介导的内吞(clathrin-mediated endocytosis, CME)是BRI1的内吞途径之一(图1)。在植物细胞学研究中, 化学药物TyrA23(Tyrphostin A23)常用作CME途径的抑制剂[34-36]。Irani等[30]开发了一种荧光标记的具有生物活性的BRs类似物AFCS(alexa fluor 647-castasterone), 并在植物细胞中实现了对受体–配体复合物的观察。使用TyrA23可以有效地阻止BRI1-AFCS进入细胞, 让处于激活状态的受体–配体复合物保留在细胞表面, 从而增强BRs信号转导[30]。表达显性负性突变体(dominant-negative)形式的网格蛋白HUB1同样可以阻碍AFCS的内吞以及增强BRs信号通路[30]。细胞膜上网格蛋白包被囊泡(clathrin-coated vesicles)的形成需要异四聚体AP-2(adaptor protein complex-2)的参与[37]。研究表明, BRI1的内吞途径依赖于AP-2[37]。通过遗传手段抑制AP-2的功能, 削弱了BRI1的内吞, 同时增强了BRs信号转导[37]。Gadeyne等[38]鉴定了由八个核心组分构成的TPLATE复合体, 证明了该复合体在植物中负责驱动CME。TPLATE复合体在细胞膜上与AP-2、动力相关蛋白(dynamin-related proteins)、网格蛋白协调行动[38]。利用人工微RNA(artificial micro-RNA, amiR)介导的基因沉默方法抑制TPLATE复合体亚基TML(TPLATE muniscin-like)的表达可以增加BRI1在细胞膜上的含量, 并且抑制AFCS的内吞[38]。

Wang等[39]发现, 除了CME外, BRI1从细胞表面进入细胞内还能利用网格蛋白非依赖型内吞途径(clathrin-independent endocytosis, CIE)(图1)。AtFlot1(At-Flotillin1)参与细胞膜微结构域(membrane microdomains)的形成, 因此可以用作膜微结构域的标记蛋白[40-41]。BRI1与AtFlot1在细胞膜上不仅共定位而且共扩散[39]。使用干扰细胞膜微结构域的化学药物或者采用amiR介导的基因沉默方法抑制AtFlot1的表达都能削弱BRI1的内吞, 增加细胞表面BRI1的含量[39]。这项研究还表明, 施加外源BRs可以显著性地提高BRI1与AtFlot1的共定位, 提示BRs促使BRI1被募集到细胞膜微结构域并增强膜微结构域介导的BRI1内吞途径[39]。

Martins等[32]发现, BRI1在体内可以通过K63多聚泛素化进行翻译后修饰。BRI1的泛素化修饰不仅促进BRI1的内吞, 而且对BRI1在TGN/EE的识别过程至关重要, 识别后的BRI1被运输到液泡中进行降解(图1)。将BRI1胞内结构域中的25个赖氨酸突变为精氨酸, 则BRI1不能被泛素化, 导致BRI1在细胞膜上的含量增加, 并使植株出现BRs超敏表型, 进一步证明了BRs信号转导主要开始于细胞表面。这种不能被泛素化修饰的突变形式的BRI1仍

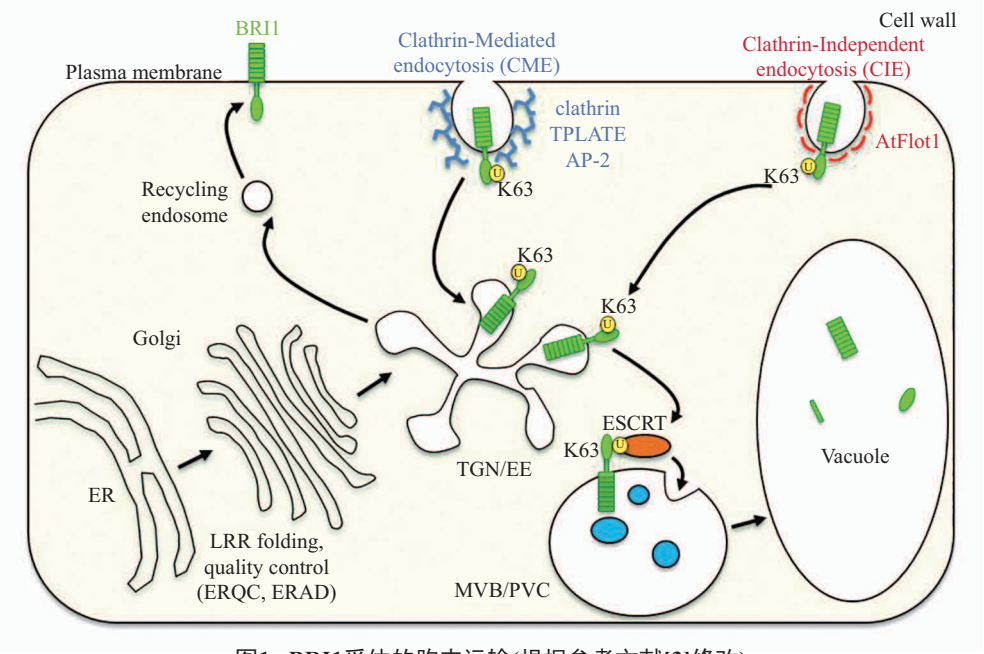

图1 BRI1受体的胞内运输(根据参考文献[3]修改)

Fig.1 Subcellular trafficking of BRI1 receptors (modified from reference [3])

然能缓慢地从细胞表面进入细胞, 提示泛素化非依赖型内吞途径也同时存在。最近, Yang等[42]报道了水稻中的类受体蛋白ELT1(enhanced leaf inclination and tiller number 1)定位于细胞膜上, 并与BRI1直接相互作用, 通过互作抑制了BRI1的泛素化及其介导的内吞, 导致BRI1大量积累, 同时增强BRs信号通路。此外, Cardona-López等[43]研究表明, 转运必需内体分拣复合物(endosomal sorting complex required for transport, ESCRT)可能负责对被泛素化修饰的BRI1进行分拣并送入到MVB/PVC的腔内囊泡(intraluminal vesicles, ILVs)中(图1), 因为当ESCRT-III结合蛋白ALIX(apoptosis-linked gene-2 interacting protein X)功能缺失时, BRI1的液泡运输被阻断。如果无法对BRI进行泛素化修饰或者不能将BRI1正确地分拣到ILVs中, 都会迫使BRI1被运回细胞表面。

FLS2(flagellin-sensitive 2)是已作充分研究的另一个LRR-RLK, 负责识别病原菌鞭毛蛋白(flagellin) N-端中由22个氨基酸残基组成的肽段并激活下游抗病信号转导[44-46]。最近的研究证明, BRI1和FLS2在细胞表面位于不同的受体簇(receptor clusters)中, 这两个LRR-RLK介导的信号转导的特异性可能是由二者不同的时空分布引起的[47]。尽管如此, Mbengue等[48]在烟草中共表达BRI1和FLS2时发现, BRI1与被活化的FLS2在内吞囊泡中共定位, 说明二者胞内运输途径可能一样。

3 共受体BAK1影响BRI1的胞内运输

在早先的研究中, Russinova等[33]观察到, BRI1的共受体BAK1也存在内吞现象。后续的研究证明, MSBP1(membrane steroid-binding protein 1)参与调控该过程。MSBP1能与BRs结合, 而且以不依赖于BRs的方式在细胞膜上与BAK1的ECD相互作用, 导致BAK1的内吞增强同时减弱BRI1与BAK1的相互作用, 进而抑制BRs信号转导[49-50]。对BAK1的亚细胞定位研究表明, BAK1定位于细胞膜、胞内小室(intracellular compartments)以及液泡膜(tonoplast)上[28,51-52]。通过施加外源BRs来激活BRs信号通路, 将提高BRI1与BAK1的共定位, 并促进受体与共受体在细胞膜上的异源寡聚化, 然而大部分BRI1与BAK1的共定位并不依赖于BRs[28]。并且, 细胞膜上大约7%的BRI1组成型地与BAK1形成异源寡聚体[28]。BRI1对蛋白质转运抑制剂布雷非德菌素(Brefeldin A, BFA)高度敏感, 而BAK1对BFA处理则表现出抗性, 提示BRI1与BAK1在细胞内的运输过程可能利用了不同的分拣机制[28]。利用原生质体瞬时表达系统发现, BRI1在细胞膜上形成同二聚体, 而在内体中BRI1和BAK1更倾向于形成异二聚体, 内吞囊泡(endocytic vesicle)中可单独含有BRI1或者BAK1, 也可以二者皆有[33]。共表达BRI1和BAK1会加速BRI1的内吞, 而且可能促进BRI1的液泡运输及降解, 因此, 提高共受体BAK1的含量能够影响BRI1的胞内运输[33]。但是在*bak1*突变体背景中, BRI1的内吞并没有受到影响[33], 这可能是因为BAK1与其他SERK家族成员在BRs信号通路中存在功能冗余[53]。

4 BRs信号的终止

大多数情况下, 细胞表面的受体经配体诱导后被激活, 然后通过内吞进入细胞并最终在溶酶体或者液泡中进行降解, 从而使受体失活[54]。Geldner等[23]研究表明, 植物液泡是BRI1受体的降解场所; BRI1的半衰期(half-life)大约为5 h, 外源施加BRs并不影响BRI1的降解速度。目前, BRs从BRI1-BAK1异源二聚体中脱离出来这一过程是否促使BRs信号终止, 还不清楚。在前文中已经提到, BRI1与BRs类似物AFCS在细胞膜上结合, 证明配体BRs与受体结合发生在细胞表面[30]。一旦进入到内体, 结合BRs的ECD就会暴露在内体轻度的酸性环境中(pH6.0~7.0)[55]。体外实验表明, BRs优先在与植物细胞壁类似的极酸性环境中(pH4.0~5.7)与BRI1结合[9-10,27]。因此, BRI1通过内吞途径由细胞表面进入细胞后, 由于所处环境的pH值突然变化, 可能使BRs结合口袋(BR binding pocket)中(或者周围)的质子化状态发生改变, 导致配体和受体之间发生解离。在动物中, 上述机理已经得到验证[56]。低密度脂蛋白(low-density lipoprotein, LDL)作为配体与其受体LDLR(low-density lipoprotein receptor)在细胞表面(pH7.4)结合后, 以复合物的形式进入内体[57-58]。内体中的酸性环境(pH6.0~6.5)导致配体从受体复合物中脱离出来[58]。LDLR在卸载配体后被重新运回细胞膜上, 而配体LDL以及装载配体的LDLR则被运往溶酶体中降解[56-57]。

Wu等[59]筛选获得了抑制*bri1-5*表型的突变体*sbi1*(*suppressor of bri1*), 该突变体中被BRs激活的BRI1受体在膜区室中积累。进一步研究发现, BRs

可以诱导*SBI1*的转录; *SBI1*编码一个亮氨酸羧甲基转移酶(leucine carboxylmethyltransferase, LCMT), 其底物为PP2A(protein phosphatase 2A)的催化亚基。SBI1对PP2A的甲基化导致PP2A定位在膜区室中, 甲基化的PP2A进一步与活化的BRI1受体结合, 促使BRI1的去磷酸化和降解, 从而实现了对BRs信号的终止。最近的研究发现, BIL4(Brz-insensitive-long hypocotyl 4)是一个在高等生物中进化保守的含有七次跨膜结构域的蛋白质, 它通过调控BRI1的亚细胞定位来影响BR信号通路, BIL4功能缺失将促使BRI1被运往液泡进行降解[60]。在拟南芥中, KAPP(kinase-associated protein phosphatase)作为受体激酶的下游调节因子, 负调控众多受体激酶介导的信号转导[61-64]。比如, KAPP可以与BRI1及BAK1相互作用, 并负调控BRs信号通路[64]。有趣的是, 当SERK家族成员SERK1(somatic embryogenesis receptor-like kinase 1)与KAPP在原生质体中共表达时, SERK1进入到内吞囊泡中, 提示KAPP参与调控植物受体的内吞途径[65]。此外, Oh等[66]证明, 位于BRI1的ATP结合结构域中的Ser-891自磷酸化(autophosphorylation)也是BRs信号终止的机制之一。

5 结语与展望

近年来, BRs信号转导的分子机理是植物生物学研究的一个热点, 从细胞表面BRs信号的感知到细胞核内的转录调控等一系列过程都取得诸多进展, 但是仍有大量的问题并不清楚[18,67]。本文主要综述了调控BRI1受体胞内运输的机理, 以及BRI1的胞内运输是如何影响BRs信号转导, 并提出以下一些亟待解决的重要问题。(1)BRI1从TGN/EE运回细胞表面的速率可以影响细胞表面BRI1受体的含量。在*det3*突变体中, 由于TGN/EE的pH值升高, 导致BRI1从TGN/EE运回细胞表面的速率降低, 从而抑制了BRs信号转导[31]。未来的工作可以研究内膜系统(endomembrane system)pH值的改变影响BRI1胞内运输的机理。(2)ARF-GEFs是小G蛋白ADP核糖基化因子(ADP-ribosylation factor, ARF)的鸟苷酸交换因子(guanine-nucleotide exchange factor, GEF), 调控着细胞囊泡运输, 从而控制着蛋白的分拣和转运[68-69]。在拟南芥的ARF-GEFs中, GNOM及其最近的同源蛋白GNL1(GNOM-like 1)被研究得最为深入, BRI1的内吞依赖于GNOM和GNL1[30]。后续的工作需要在CME和CIE的背景下, 研究GNOM和GNL是如何调控BRI1的内吞途径。表皮生长因子受体(epidermal growth factor receptor, EGFR)的研究工作使人们认识到了细胞内吞、胞内运输与信号转导之间存在的错综复杂的关系[70-71]。在低浓度的表皮生长因子(epidermal growth factor, EGF)的条件下, CME为主要的内吞途径, 此时大部分的EGFR被运回细胞表面, 只有少量的EGFR被降解, 因此延长了EGFR介导的信号转导; 在高浓度EGF的条件下, CIE发生并导致大量的EGFR以一种依赖于泛素化的方式被运往溶酶体进行降解[71-73]。在植物细胞中, 应对胞外不同的BRs生理浓度时, 是否也存在类似的双重机制来调控BRI1的亚细胞动态, 目前尚无明确的结论。(3)共受体BAK1可以调控BRI1的胞内运输, 提高共受体BAK1的含量能促进BRI1的内吞和液泡运输, 但具体作用机理还有待于进一步研究。此外, 与BRI1相比, BAK1的胞内运输机制几乎是未知的, 同时BAK1的亚细胞定位也存在着争议[28,51-52], 阐明这些问题对解释BAK1的胞内运输与BRs信号转导之间的关系具有重要意义。(4)BRI1受体被BRs激活后, 通过内吞途径最终在液泡中降解, 使受体活性丧失, 从而实现了对BRs信号输出的精细调控。在未来的工作中, 鉴定更多BRs信号途径的负调控因子将有助于阐述植物细胞是如何对BRs信号进行终止的。(5)BRs合成过程中的一些关键酶位于细胞内部, 尤其是与内质网相结合, 提示BRs在细胞内合成[74]。但是感知BRs的部位却在细胞表面的外侧(exterior cell surface)。因此, 细胞合成的BRs需要从细胞内部运输到细胞外部, 然后再被同一个细胞或者相邻细胞所感知, 从而启动BRs信号转导[74]。BRs运输到细胞外的速率可能影响BRs信号转导, 所以探讨细胞中BRs的合成与运输机制可能是未来研究的方向。(6)目前科学家们已发现一些植物激素或它们的衍生物存在于液泡中[75-78]。Irani等[30]观察到具有生物活性的BRs类似物AFCS在液泡中积累, 提示液泡中也可能含有BRs。未来的研究工作可以分离纯化出植物液泡, 并采用代谢组学的方法验证BRs是否存在于液泡中, 同时解析液泡如何调控细胞内BRs的水平以及稳态。

相信在后续的研究中, 随着实验技术的不断革新, 以上问题将得到解答, 从而以细胞生物学的角度丰富和完善BRs信号转导的调控机理, 为农作物的

产量和品质改良提供更多的理论依据。

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