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·热点评·

独脚金内酯信号感知揭示配体-受体作用新机制

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摘要 植物激素在调控细胞与细胞及细胞与环境的相互作用中起着至关重要的作用。作为一种信号分子, 植物激素如何被植物细胞感知一直是植物生物学研究的热点。与底物-酶相互作用的结果不同, 激素分子与受体结合后会触发信号转导, 但激素分子一般不会被受体修饰, 信号转导起始后激素分子通常会从复合体中释放出来被重新利用或降解。近期, 我国科学家通过对独脚金内酯及其受体复合体(AtD14-D3-ASK1)的结构学解析, 发现独脚金内酯的生物活性分子CLIM (covalently linked intermediate molecule)是独脚金内酯被其受体水解后得到的中间分子。研究表明, CLIM与受体AtD14的催化中心以共价键相结合, 进而激活其信号转导。该研究揭示了一种全新的“底物-酶-活性分子-受体”激素识别机制。这种配体-受体作用新机制的发现为植物激素研究开拓了新的视野。

关键词 独脚金内酯, 配体-受体相互作用, D14, CLIM

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植物激素是指植物自身产生, 并自产生部位运输到作用部位, 在极低浓度下就有明显生理效应的一类微量有机物质。除传统六大类激素——生长素、细胞分裂素、赤霉素、脱落酸、乙烯和油菜素内酯外, 独脚金内酯、水杨酸及茉莉酸等也被公认为是植物激素。甚至植物中广泛存在的分泌型小肽分子也被认为是一类植物的多肽激素(Motomitsu et al., 2015)。植物激素在细胞伸长与分裂、组织与器官分化、开花与结实、成熟与衰老、休眠与萌发以及离体组织培养等方面分别或相互协调地调控植物的生长发育及逆境响应。早期对植物激素及其受体相互作用的研究表明, 已知的植物激素都是以非共价键的形式可逆地与受体相互作用, 进而启动下游信号转导, 以发挥其调节功能(Tan et al., 2007; Murase et al., 2008; Santiago et al., 2009; Sheard et al., 2010; Hothorn et al., 2011a, 2011b) (图1A)。这也正是与“底物-酶”相互作用的不同之处(Fang and Chen, 2017)。最近, 清华大学谢道昕研究组发现独脚金内酯信号感知有别于其它已知激素与其受体的相互作用方式(Yao et al., 2016) (图1B)。

独脚金内酯是一类天然的独脚金醇类化合物的总称。最初是从棉花(*Gossypium hirsutum*)根分泌液中分离出来的, 被认为是独脚金属(*Striga* spp.)植物种子萌发的刺激物, 因此被命名为独脚金内酯(Cook et al., 1966, 1972)。研究表明, 独脚金内酯能诱导寄生植物种子的萌发(Yoneyama et al., 2010), 促进丛枝真菌菌丝分支及营养吸收(Akiyama et al., 2005, 2010; Akiyama and Hayashi, 2006), 并与生长素和细胞分裂素协同调节植物侧枝发生(Umehara et al., 2008; Gomez-Roldan et al., 2008; Ferguson and Beveridge, 2009; Hayward et al., 2009; Xu et al., 2015)。

通过对独脚金内酯信号转导途径的研究, 发现拟南芥(*Arabidopsis thaliana*)和水稻(*Oryza sativa*)中D14 (DWARF14)蛋白的突变植株对体外施加独脚金内酯不敏感(Arite et al., 2009; Waters et al., 2012), 表明D14蛋白在独脚金内酯的信号感知过程中起重要作用。进一步研究发现, AtD14可招募F-box蛋白MAX2形成SCF复合体(在水稻中对应的同源蛋白分别为OsD14和D3) (Stirnberg et al., 2007; Nelson et al., 2011), 再通过泛素化介导的蛋白酶体降解途径, 促进抑制因子D53/D53-like SMXLs的泛素化降解(Jiang et al., 2013; Zhou et al., 2013; Wang et al., 2015), 从而解除其对下游基因表达的抑制。尽管独

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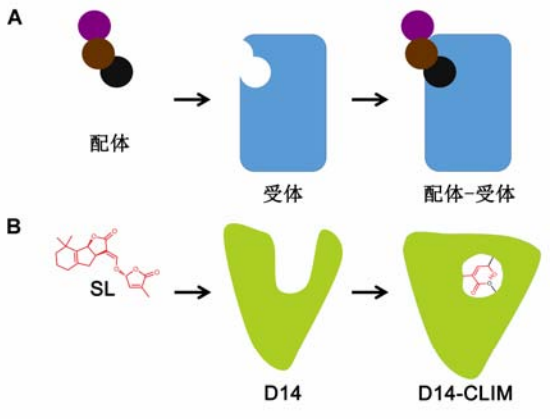

图1 植物激素的配体-受体相互作用模式

(A) 有关植物激素的经典研究认为激素分子(配体)与受体结合后可激活受体的信号转导，但激素分子自身通常不会被受体修饰或降解；**(B)** 独脚金内酯被受体D14识别并被降解为中间活性分子CLIM，且CLIM与D14以共价键相结合，进而起始独脚金内酯的信号转导

Figure 1 The interaction pattern of ligand-receptor for phytohormones

(A) Classical plant hormones research suggests that hormone (ligand) was perceived by the receptor to initiate cellular signaling, but the ligand was not changed usually; **(B)** Strigolactone (SL) was hydrolysed by the open state D14 into an intermediate molecule (CLIM), which is covalently sealed inside the catalytic centre of D14 (in closed state) to trigger SL signal transduction

脚金内酯信号转导途径的研究已经取得了一定的进展，但对其信号感知仍有几大问题没有解决。如独脚金内酯的活性分子形式是什么？它是如何与受体D14相互作用的？受体D14在与其配体结合后又是如何发挥生物学功能的?

在对独脚金内酯的受体进行研究时，发现水解酶D14可将独脚金内酯的人工合成类似物(GR24)水解为最终产物羟甲基丁烯内酯(D环，D-OH)和三环内酯ABC环(ABC-OH)。ABC-OH不能发挥与GR24相似的功能——抑制拟南芥腋芽的发生，表明ABC-OH不是独脚金内酯的活性分子(Hamiaux et al., 2012)。D-OH在低浓度下不能替代GR24抑制水稻侧枝发生，也不能替代GR24诱导OsD14与赤霉素信号抑制因子SLR1的相互作用，表明其不具有独脚金内酯的生物学功能，所以D-OH也不是独脚金内酯的活性分子(Nakamura et al., 2013)。因此，对于独脚金内酯最终活性分子的分离鉴定成为这一新型植物激素研究的热点和难点。只有在分离到独脚金内酯的活性分子之后，才能探究它与受体D14是如何相互作用，也才能在分子水平上揭示受体D14与其配体结合后是如何与D3相互作用，进而形成受体复合体，将信号传递到下游信号元件。

为了揭示独脚金内酯信号感知的分子机理，谢道昕研究组对受GR24诱导的受体复合体AtD14-D3-ASK1进行了蛋白纯化及结晶。他们发现在复合体AtD14-D3-ASK1中存在1个小分子化合物——CLIM (covalently linked inter mediate molecule), CLIM被包裹在AtD14的催化中心，而且被共价固定在AtD14的H247和S97氨基酸残基上。CLIM并不直接与D3相互作用，通过对AtD14水解GR24的酶活性进行分析，证明D3的存在有助于AtD14将GR24水解的中间产物CLIM包裹在其催化中心，而不是进一步水解为最终产物D-OH。通过对GR24诱导的受体复合体AtD14-D3-ASK1的质谱分析，结合同位素标记实验，证明CLIM是由GR24或其活性类似物的D环在AtD14催化下衍生而来的$C_5H_5O_2$化合物。而且CILM的产生能够诱导受体复合体AtD14-D3的形成，外施GR24或同源类似物能够诱导植物体内CLIM的积累。通过不同实验方法获得的结果均表明：在植物体内CLIM是独脚金内酯由受体D14催化产生并与受体蛋白共价结合进而诱导信号转导的活性分子。

为了阐明AtD14与CLIM的作用方式及AtD14对CLIM的感知过程，谢道昕研究组对比分析了AtD14和受GR24诱导后AtD14-D3复合体中的AtD14蛋白三维结构。结果表明，AtD14在受GR24诱导前后经历了开放-闭合的蛋白结构转变。开放状态下的AtD14蛋白存在1个可与GR24等大分子相结合的开放型空腔，而AtD14-D3复合体中闭合型的AtD14只包含1个仅可容纳CLIM大小的小型闭合型空腔，在AtD14蛋白从开放到闭合的过程中，通过其自身蛋白结构的转变使其催化中心的空腔由大变小，并形成1个适合D3蛋白结合的作用界面。AtD14的催化中心由其催化三联体(S97-H247-D218)构成，该催化三联体与其它氨基酸残基一起形成可包裹CLIM的疏水性空腔。但是在GR24诱导的AtD14-D3复合体中AtD14的D218氨基酸残基并不与CLIM相互作用，而且发生了不规律的偏移。他们推测，在AtD14开放-闭合过程中D218偏移

对催化活性的破坏使得由GR24水解产生的CLIM得以保留在AtD14的催化中心，而未被水解为没有生物活性的D-OH，同时这一结构的改变可能有利于AtD14/OsD14与抑制因子D53/SMXLs的结合。经过进一步分析AtD14与D3蛋白相互作用的结构学基础，发现D3蛋白通过其羧基端的亮氨酸富集重复序列与AtD14相互作用。同时，运用生化实验证明，突变后的D3即使在GR24的诱导下也不能与AtD14相互作用形成受体复合体。

遗传学实验证明，AtD14第158位的甘氨酸残基突变为谷氨酸后的拟南芥植株表现出严重的独脚金内酯信号阻断表型，即下胚轴的伸长和腋芽的生长对体外施加GR24不敏感及分枝明显增多。而结构学分析显示，第158位甘氨酸残基突变为谷氨酸后影响了闭合状态下AtD14与D3的相互作用。生化实验证明，即使存在GR24的诱导，突变后的AtD14 (G158E)也不能与MAX2/D3相互作用。但是突变后的AtD14在GR24的诱导下可以与抑制因子SMXL6相互作用。同时酶活性分析显示，AtD14 (G158E)可以水解GR24最终生成D-OH，而且水解速率更快，表明AtD14 (G158E)的水解酶活性没有受到影响。由于突变后的AtD14 (G158E)在闭合状态下不能与D3/MAX2相互作用，导致生成的中间体CLIM无法被完全包裹，进而被水解为D-OH的速率比正常的AtD14快。通过对AtD14 (G158E)的遗传、生化及酶学分析，证明AtD14可将独脚金内酯水解为可与其共价结合的活性分子CLIM或者最终产物D-OH，从而发挥其酶和受体的双重作用。上述研究揭示了一种全新的“底物-酶-配体-受体”的植物激素识别机制。

结合蛋白晶体结构、质谱分析、遗传学和酶活性分析等手段，研究人员最终阐明独脚金内酯信号感知过程。开放状态的AtD14催化中心与各种不同结构式的独脚金内酯分子相结合，水解独脚金内酯并生成可与AtD14催化中心共价结合的D环衍生中间产物CLIM，进而使AtD14蛋白构象转变为可与D3/MAX2相互作用的形式，形成AtD14-D3/MAX2受体复合体，同时D3/MAX2的结合能够稳定闭合状态AtD14的蛋白构象，从而激活独脚金内酯信号转导。

近期，谢道昕研究组揭示了独脚金内酯在促进寄生植物(如独脚金)种子萌发过程中的分子感知机理。独脚金内酯及其人工合成类似物可被独脚金中与AtD14同源的ShHTL7蛋白所感知，并被其水解为可以与ShHTL7共价结合的中间活性分子CLIM，从而启动下游信号转导(Yao et al., 2017)。

这种新型的配体-受体相互作用方式在植物激素研究中尚属首次报道，为该领域研究开辟了新的途径，也为研究其它小分子化合物与受体的结合提供了新的参考。如从野火烟(*Nicotiana attenuata*)中发现一类能促进种子萌发的小分子化合物Karrikins (Baldwin et al., 1994)。自2004年首次从烟中分离鉴定到以来，对Karrikins的结构及信号转导途径的研究已取得较大进展(Flematti et al., 2004)。Karrikins的结构与独脚金内酯相似，而且与D14同源的α/β水解酶家族成员KAI2的功能缺失突变体对Karrikins不敏感(Waters et al., 2012; Guo et al., 2013)。研究表明，Karrikins的下游信号途径也是通过D3/MAX2实现的(Nelson et al., 2011)，说明无论是其分子结构、生物学功能还是信号的传递途径都与独脚金内酯有极大的相似性。因此，人们认为其信号分子的感知过程可能与独脚金内酯类似。

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Plants Use an Atypical Strategy to Perceive Strigolactones

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Abstract Phytohormones, as signaling molecules, play critical roles in regulating cell-to-cell and cell-to-environment communications. The mechanisms plant cells use to perceive phytohormones remain hot research topics in plant biology. Previous studies indicated that most plant hormones are perceived by non-covalent physical interactions with their corresponding receptors. After signaling pathways are initiated, the ligands usually dissociate with their binding receptors, which can interact with other receptor molecules or go through a degradation pathway. Therefore, ligand-receptor interaction is distinct from substrate-enzyme association. Recently, Xie and colleagues resolved a 3D structure of a strigolactone-induced AtD14-D3-ASK1 receptor complex. Strigolactones could be cleaved into a covalent-linked intermediate molecule in the reaction center of AtD14, the receptor of strigolactones. Further analyses revealed detailed molecular mechanisms of strigolactone-induced ligand-receptor complex formation and subsequent signaling initiation. Such a mechanism has never been reported in plants. These results provide significant insights into our better understanding of cellular signaling in plants.

Key words strigolactones, ligand-receptor interactions, D14, CLIM

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