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评 述

植物细胞外ATP释放、降解及其感知与下游信号通路研究进展

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摘要 细胞外ATP(extracellular ATP, eATP)作为一种胞外信号分子, 在调控植物生长发育、介导生物与非生物胁迫响应中发挥重要功能. 研究表明, eATP可以通过细胞损伤、胞吐作用、ABC转运蛋白(ATP-binding cassette transporter)及质膜腺嘌呤核苷酸转运蛋白1(plasma membrane adenine nucleotide translocator 1, PM-ANT1)等多条途径从胞内释放到胞外, 并与质膜受体P2K1(P2-type purinergic receptor kinase 1)和P2K2(P2-type purinergic receptor kinase 2)结合, 进而激活下游钙信号(Ca^{2+})、活性氧(reactive oxygen species, ROS)爆发、一氧化氮(nitric oxide, NO)产生, 以及丝裂原活化蛋白激酶(mitogen activated protein kinase, MAPK)级联反应, 调控植物生理应答. 最新研究发现, 叶锈病10号抗病位点类受体蛋白激酶样蛋白3(leaf rust 10 disease-resistance locus receptor-like protein kinase-like 3, LRK10L3)与油菜素类固醇不敏感1相关受体激酶1(brassinosteroid insensitive 1 associated kinase 1, BAK1)可在质膜形成复合体, 作为eATP感知新单元, 进一步完善了eATP的识别机制. 在eATP稳态调控方面, 腺苷三磷酸双磷酸酶(Apyrase, APY)通过降解ATP, 动态调节eATP浓度. 本文系统整合了植物eATP的释放与降解机制, 受体识别途径及下游信号组分, 总结了eATP受体介导的信号通路调控植物胁迫响应与生长发育的机制, 综述了该领域的最新研究进展, 并对当前尚未解决的问题与未来研究方向提出了展望.

关键词 植物, 细胞外ATP(eATP), 释放与降解, eATP识别, 信号通路

5′-三磷酸腺苷(adenosine 5′-triphosphate, ATP)普遍存在于所有生物体中, 是细胞内代谢调控与能量转换的核心分子. ATP主要参与细胞内复杂的代谢活动, 此外, 部分ATP被释放到细胞外基质, 形成胞外ATP (extracellular ATP, eATP), 作为一种信号分子而发挥功能[1,2].

哺乳动物中, eATP作为嘌呤类信号分子的关键配体, 广泛参与调控免疫应答、神经信号传递及组织修复等重要生理过程[3~5]. 近年来, 植物体内eATP的存在也得到了明确证实[6~8]. 研究表明, 植物中的eATP不仅可以作为信号分子调控生长发育的多个环节, 还能作为一种损伤相关分子模式(damage-associated molecu-

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lar pattern, DAMP), 在植物应对生物与非生物逆境胁迫响应过程中发挥重要功能[9,10].

随着植物eATP特异性受体P2K1/P2K2(P2-type purinergic receptor kinase 1/2)的分子鉴定, 以及受体下游信号通路的逐步解析[11,12], 植物eATP信号相关研究进入快速发展阶段. 尽管目前已有综述文献对eATP的生理功能进行过概括性总结[13], 但随着受体介导的下游信号通路研究的持续深入, 当前在eATP的释放、降解、识别与信号转导等方面, 仍缺乏系统性梳理与归纳. 基于此, 本文将围绕eATP的释放与降解机制、eATP的受体识别与信号感知、eATP下游关键信号组分, 以及eATP受体介导的信号通路调控植物胁迫响应和生长发育的机制, 整合最新研究进展进行系统性总结, 并对该领域未来研究方向进行展望, 旨在为深化植物eATP相关领域的研究与应用提供参考.

1 eATP的释放与降解

eATP作为一种关键的信号分子和DAMP, 其胞外浓度与稳态具有重要的生物学意义. eATP通过多种途径从胞内释放到胞外, 进而启动下游信号转导; 而胞外ATP的水解则有效调控eATP信号强度. 这种释放-降解的调控机制共同维持了eATP信号的时空特异性(图1).

1.1 eATP的释放机制

植物在正常生长发育过程中, 或受到生物与非生物胁迫下, 会将ATP释放到细胞外基质, 作为信号分子调控生长发育与胁迫反应[14~16]. 由于ATP带有负电荷, 无法自由跨膜扩散, 其释放依赖于特定的途径: 机械损伤等刺激下, 胞内ATP会被动泄漏至胞外[17]; 此外, 植物通过主动转运机制, 例如胞吐作用、ABC转运蛋白(ATP-binding cassette transporter)以及质膜定位的线粒体载体家族(mitochondrial carrier family, MCF)成员质膜腺嘌呤核苷酸转运蛋白1(plasma membrane adenine nucleotide translocator 1, PM-ANT1)将胞内ATP释放到胞外[18~20].

植物正常发育过程中, 胞外ATP通常维持在nmol水平[13]. 机械损伤或昆虫取食等会破坏细胞质膜的完整性, ATP通过受损伤的细胞膜直接释放到胞外环境, 使胞外ATP浓度增加[17,21]. 动物细胞中, ATP可以通过囊泡转运的方式释放到胞外[22]. 植物根毛是一种极性生长细胞, 其顶端富含分泌囊泡, 当采用囊泡转运抑制剂Brefeldin A处理苜蓿时, 发现可显著抑制其根毛释放eATP的过程, 提示通过胞吐作用释放ATP的途径在植物中同样存在[19]. 哺乳动物多药耐药家族(multidrug resistance, MDR)蛋白可以直接外排ATP[23,24]. 拟南芥ABC超家族(ATP binding cassette superfamily)成员P-糖蛋白1(P-glycoproteins 1, AtPGP1)是MDR1的同源蛋白, 其在酵母中异源表达时, 培养基中ATP浓度升高; 同时, AtPGP1过表达拟南芥株系的eATP释放增加, 表明AtPGP1具备ATP转运功能[18]. 此外, 研究还发现, 质膜定位的ANT1沉默后, 花粉胞外ATP下降而胞内ATP积累, 花药开裂受阻[20]. 综上, 植物通过细胞损伤、胞吐作用、ABC转运蛋白以及PM-ANT1等多条途径精细调控eATP的释放, 为胞外ATP信号的建立提供了基础.

1.2 eATP的降解机制

eATP的降解与稳态维持是植物正常生理活动的基础. 哺乳动物细胞中, 定位于细胞膜外侧的胞外腺苷三磷酸双磷酸酶(ecto-apyrase), 可以高效水解eATP而影响eATP稳态[25]. 植物Apyrase最早发现于马铃薯(*Solanum tuberosum*)[26], 随后在拟南芥(*Arabidopsis thaliana*)、小麦(*Triticum aestivum*)、水稻(*Oryza sativa*)、棉花(*Gossypium hirsutum*)、杨树(*Populus*)等多种植物中均发现了Apyrase的存在[27~31]. 大量研究已证实, Apyrase具有水解ATP/ADP的活性, 外源施加Apyrase可有效清除eATP[28,31~33].

根据亚细胞定位, Apyrases可分为内切型(endo-apyrase)和外切型(ecto-apyrase). 内切型Apyrase定位于高尔基体、内质网和囊泡等, 通过水解胞内ATP限制其向外运输. 研究发现, 拟南芥Apyrase家族含有7个成员(AtAPY1~7), 其中AtAPY1~6均表现出三磷酸腺苷双磷酸酶活性. AtAPY1, AtAPY2, AtAPY4, AtAPY5和AtAPY7均定位于高尔基体, 而AtAPY3定位于内体, AtAPY6定位于内质网. 因此, 在模式植物拟南芥中, 已鉴定的Apyrase家族成员均定位于细胞内膜系统, 通过调控胞内囊泡中的ATP, 间接参与胞外ATP浓度的调节[27]. 目前, 植物中外切型Apyrase的系统生物学研究仍较为薄弱, 相关功能分析与定位证据分散于少数物种的报道中. Deng等人[31]将胡杨(*Populus euphratica*) PeAPY2-eYFP融合蛋白与CFP标记的质膜特异性标记

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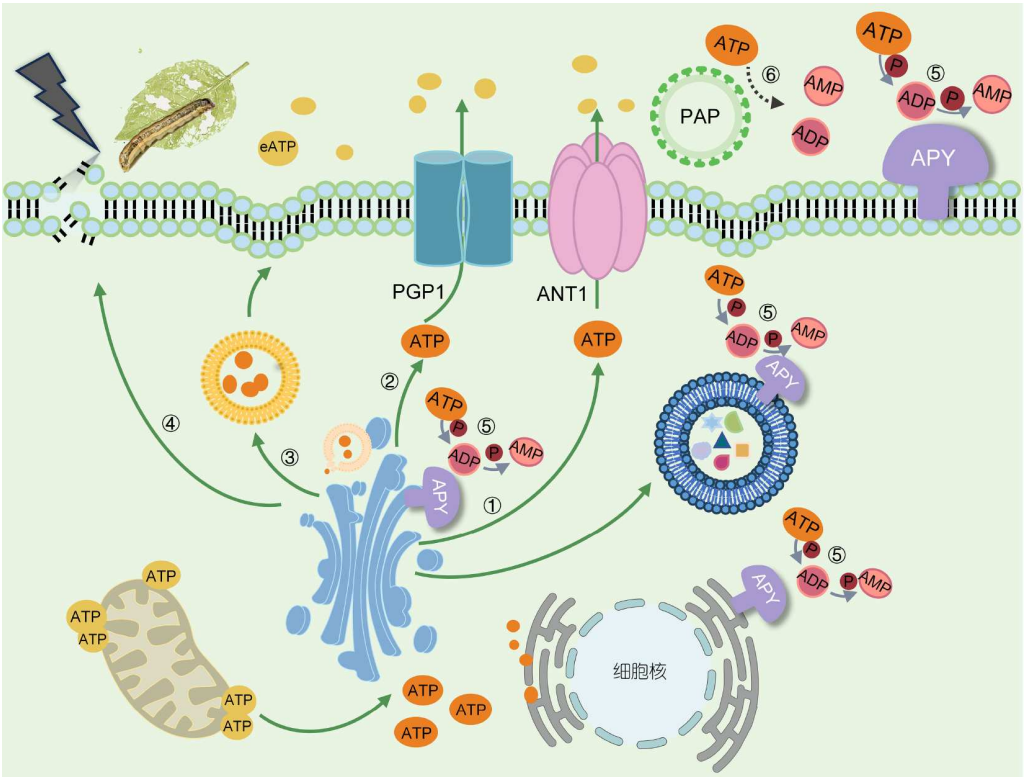


图 1　eATP的释放与降解机制. eATP通过如下四种方式释放到胞外: ① 通过质膜定位的ANT1释放到胞外; ② 通过哺乳动物多药耐药家族MDR在拟南芥中的同源蛋白PGP1释放到胞外; ③ 储存于细胞内囊泡中的ATP, 随胞吐作用而释放到胞外; ④ 机械损伤或昆虫咬食引起细胞膜完整性受损, 细胞内ATP通过损伤的质膜释放到胞外. eATP的降解主要通过两种途径: ⑤ Apyrase分布在质膜外侧和胞内囊泡、内质网、高尔基体上. 前者通过水解eATP降低其浓度, 后者则通过水解降低胞内ATP浓度, 从而减少向胞外释放的ATP分子. ⑥ 存在于胞外空间的PAP也可以水解ATP, 可能与Apyrase共同参与eATP稳态维持. ADP: 二磷酸腺苷; AMP: 单磷酸腺苷

Figure 1　Release and degradation mechanism of eATP. eATP is released through the following four primary mechanisms: ① eATP is released via plasma membrane-localized ANT1; ② eATP is exported through PGP1, an *Arabidopsis thaliana* homolog of the mammalian multidrug resistance (MDR) family protein; ③ ATP stored within intracellular vesicles is released via exocytosis; ④ mechanical damage or insect herbivory compromises plasma membrane integrity, leading to the release of intracellular ATP into the extracellular space. The degradation of eATP occurs mainly through two pathways: ⑤ Apyrase is localized on the extracellular face of the plasma membrane as well as on intracellular vesicles, the endoplasmic reticulum, and the Golgi apparatus. The Apyrase on the plasma membrane hydrolyzes eATP to reduce its extracellular concentration, while intracellular Apyrase diminishes the pool of ATP available for release by hydrolyzing ATP within the cell. ⑥ PAP localized in the extracellular space also exhibits ATP hydrolytic activity, thereby potentially contributing to the maintenance of eATP homeostasis in plants. ADP: adenosine diphosphate; AMP: adenosine monophosphate

蛋白AtPIP2A共转化拟南芥叶肉原生质体, 结果显示, PeAPY2-eYFP的荧光信号与AtPIP2A-CFP的质膜定位信号重叠, 证明PeAPY2主要分布于细胞质膜, 与外切型Apyrase的定位特征相符. 此外, 有研究报道大豆(*Glycine max*)GS52、双孢扁豆(*Vigna unguiculata* subsp. *Cylindrica*)DbLNP、豌豆(*Pisum sativum*)PsAPY1和马铃薯(*Solanum tuberosum*)StAPY3蛋白分别定位于细胞质膜、细胞表面、细胞壁和质外体空间[34~37], 但这些Apyrase家族成员是否真正具备外切型Apyrase的核心功能, 其亚细胞精确定位及生理功能特异性仍缺乏系统实验验证, 需要通过多学科手段进一步分析.

近年来, 植物分泌的紫色酸性磷酸酶(purple acid phosphatases, PAPs), 因其分泌性质和ATP水解活性而引起了关注. 植物分泌PAP到细胞外基质或者根际, 以降解有机磷酸盐, 释放无机盐而适应磷饥饿[38,39]. 研究表明, 大豆紫色酸性磷酸酶7a/7b(*Glycine max* purple acid phosphatase 7a/7b, GmPAP7a/7b)可以有效地水解eATP, 其过表达可显著增强大豆毛状根对eATP的利用能力. 因此, PAP可能与Apyrase在功能上形成协同, 通

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过参与eATP的水解过程共同调控植物eATP的稳态平衡[40].

2 eATP的识别与感知

eATP作为关键的胞外信号分子, 需要通过激活细胞质膜上的特异性受体, 方可将胞外信号传递到胞内, 进而启动下游信号级联反应. 1976年, 首个eATP受体在动物细胞中被发现, 该受体被命名为P2受体[41]. 依据结构和功能特征差异, P2受体可分为两类, 即配体门控离子通道受体P2X家族, 以及G蛋白偶联受体P2Y家族[42]. 值得注意的是, 植物基因组中并未发现P2受体的同源蛋白序列. 2014年, Choi等人[11]通过正向遗传学筛选策略, 并结合胞质钙离子内流检测体系, 首次鉴定到响应外源ATP处理而钙离子内流缺陷的拟南芥突变体, 将其命名为*dorn1*(does not respond to nucleotides 1). 进一步分析表明, DORN1编码凝集素类受体激酶-I.9(lectin receptor kinase, LecRK-I.9), 此后, 该蛋白被命名为P2K1[11]. 2020年, 同属LecRK受体激酶家族的LecRK-1.5也被证实具有eATP结合活性, 进而被确定为第二个植物eATP受体, 并被命名为P2K2[12]. 此外, 近来研究还发现, 由叶锈病10号抗病位点类受体蛋白激酶样蛋白3(leaf rust 10 disease-resistance locus receptor-like protein kinase-like 3, LRK10L3)与油菜素类固醇不敏感1相关受体激酶1(brassinosteroid insensitive 1 associated kinase 1, BAK1)组成的蛋白复合体, 可以作为eATP感知新单元参与胞外ATP信号的识别与转导[43].

2.1 P2K1受体

P2K1受体的N端具有信号肽, 由胞外L型凝集素结构域、单跨膜结构域和胞内丝/苏氨酸激酶结构域组成[44,45]. P2K1胞外凝集素结构域由Loop A, B, C, D及一个延伸环构成. Loop A~D四个环形成的腔具有多个保守残基(Gly98, His99, Thr117, Arg118, Ile143, Gly245,Thr246, Ala247), 这些保守残基可以通过氢键和疏水作用协同识别ATP的腺嘌呤和三磷酸基团[11,44], 其与ATP的结合常数K_d约(45.7±3.1) nmol/L.

P2K1胞内激酶结构域的活性是eATP诱导植物产生相关响应的前提. eATP与P2K1结合后, 可触发P2K1受体发生自磷酸化. 质谱鉴定与位点突变分析发现, 在P2K1胞内激酶结构域中, S391, S440和S451被明确为介导信号传递的关键磷酸化位点[45]. 将这些丝氨酸残基突变为丙氨酸, 模拟去磷酸化突变后, ATP诱导的Ca^{2+}内流与ROS爆发均被阻断; 而将这些丝氨酸突变为天冬氨酸, 模拟磷酸化突变回补*dorn1*突变体时, *dorn1*突变体的eATP信号响应能力可被恢复. 因此, S391, S440和S451的磷酸化是P2K1介导的eATP信号启动下游级联反应的必要开关[45].

2.2 P2K2受体

拟南芥基因组中包含45个LecRK家族成员[46], 鉴于LecRK-I.9(即P2K1)被明确鉴定为eATP受体, 科学家们推测该家族其他成员可能同样具备嘌呤类配体结合潜力. 为验证这一假设, Pham等人[12]以*dorn1-3*突变体为背景, 通过分别表达LecRK家族的所有成员进行功能筛选, 结果发现只有LecRK I.5能够部分恢复突变体中eATP诱导的胞质Ca^{2+}浓度升高缺陷表型. 进一步研究发现, LecRK I.5的功能缺失突变体对eATP触发的Ca^{2+}响应显著减弱. 此外, LecRK I.5体外具有结合ATP的能力. 基于此, LecRK I.5被确定为拟南芥中的第二个eATP受体, 并被命名为P2K2.

P2K2与P2K1在氨基酸序列水平上具有74%的相似性, 同样由胞外L型凝集素结构域、单次跨膜结构域和胞内丝/苏氨酸激酶结构域组成[12], 其胞外结构域亦包含Loop A, B, C, D和延伸环共5个主要环区. 同源建模与体外结合实验表明, H99, R144和S256是P2K2体外结合ATP的关键残基, 其与ATP的结合常数K_d为(44.5±15.73) nmol/L[47]. 此外, P2K2胞内激酶结构域也具有自磷酸化与转磷酸化活性[12].

P2K1可以磷酸化P2K2, 反之, P2K2则不可磷酸化P2K1. 在表达模式上, P2K1呈广泛分布, P2K2则主要定位于根尖, 根成熟区和叶肉细胞中转录水平显著降低. 遗传功能研究表明, 识别eATP后激活的P2K2和P2K1存在功能冗余, 通过启动胞质Ca^{2+}信号, 协同激活下游免疫信号, 介导植物对病原微生物的防御反应与系统性防御响应[12,48,49]. 相比P2K1, P2K2对ATP/ADP的结合特异性更高, 且在胁迫条件下表达上调, 共同构成植物eATP信号感知的多样化适应性机制.

2.3 LRK10L3- BAK1复合体

近来, Dong等人[43]在拟南芥的研究中发现, S-域

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受体激酶LRK10L3与共受体BAK1形成的蛋白复合体, 共同参与eATP信号的感知与传导. 表现为: *lrk10l3*或*bak1*的单突变体对eATP(0.5 mmol/L)的响应减弱, 而*lrk10l3*/*bak1*双突变体几乎完全丧失对eATP的响应能力. 分子互作与结合实验证实, LRK10L3与BAK1可以在质膜形成功能复合体, 且两者的胞外域均具备ATP结合活性. 进一步的磷酸化分析显示, ATP处理可显著影响野生型拟南芥中BAK1与LRK10L3的磷酸化水平: 处理15 min时, BAK1磷酸化水平即显著升高, 处理30 min时, LRK10L3磷酸化水平开始显著上升. 遗传实验表明, *bak1*突变体中, ATP处理无法诱导LRK10L3磷酸化水平的增强; 而*lrk10l3*突变体中, ATP虽然可诱导BAK1磷酸化, 但磷酸化水平显著低于野生型, 提示ATP介导的LRK10L3磷酸化依赖于BAK1的磷酸化.

结合常数测定结果表明, BAK1与ATP的结合常数K_d为(4.4±1.4) μmol/L, LRK10L3与ATP的结合常数K_d为(73.2±0.9) μmol/L; 当两者胞外域共同结合ATP时, 复合体与ATP的结合亲和力显著增强, K_d为(0.8±0.2) μmol/L. 值得注意的是, 同已知的P2K受体相比, LRK10L3-BAK1复合体对ATP的结合亲和力明显更低, 这表明其可能在感知并响应高浓度ATP方面发挥功能[43]. 综上, 双类受体激酶BAK1和LRK10L3复合体可以作为eATP的感知单元, 通过触发磷酸化级联反应, 进而介导根和下胚轴生长素不对称分布、茉莉酸(jasmonic acid, JA)合成相关基因表达以及生长抑制反应, 为解析植物eATP信号感知的分子网络提供了新视角.

3 eATP下游关键信号组分

eATP作为植物生长发育与胁迫应答中的关键胞外信号分子, 其功能的实现依赖于受体识别后的下游信号转导. 当eATP与细胞膜上的特异性受体结合后, 会迅速激活一系列下游信号分子, 通过Ca^{2+}、活性氧(reactive oxygen species, ROS)、一氧化氮(nitric oxide, NO)等第二信使, 以及丝裂原活化蛋白激酶(mitogen activated protein kinase, MAPK)级联反应等核心通路[9,11,21,50], 将胞外信号转化为胞内生理响应.

3.1 钙信号的激活

eATP介导的钙信号激活是植物eATP信号通路的核心环节. 在P2K1介导的信号通路中, P2K1已被证实可通过与两类环核苷酸门控通道(cyclic nucleotide-gated channel, CNGC)形成功能模块激活钙信号[51]. 首先, P2K1与CNGC2-CNGC4复合体直接互作, 静息状态下, 钙调蛋白7(calmodulin 7, CaM7)结合CNGC2-CNGC4并抑制其通道活性[51,52]; 当eATP结合P2K1后, 触发P2K1胞内域自磷酸化, 进而特异性磷酸化CNGC2的Ser705和Ser718位点, 解除CaM7抑制并介导Ca^{2+}内流. 其次, P2K1与CNGC19存在功能性互作, eATP诱导的Ca^{2+}升高在*cngc19*突变体中显著减弱, 且*p2k1*/*cngc19*双突变体的Ca^{2+}响应缺陷更为严重, 表明P2K1与CNGC19通过蛋白互作, 协同介导eATP诱导的钙信号激活[53]. 动物细胞中的研究表明, eATP可激活胞外Ca^{2+}内流, 并同时触发内质网等胞内钙库释放Ca^{2+}, 两者协同引起胞质Ca^{2+}浓度显著升高[50,54]. 植物中, P2K1可在eATP刺激下激活质膜CNGC通道, 从而介导胞外Ca^{2+}内流[51,53]; 然而, P2K1是否通过未知机制进一步诱导液泡、内质网等胞内钙库释放Ca^{2+}, 仍需借助亚细胞Ca^{2+}成像、钙库特异性抑制剂, 或借助突变体材料进行深入研究.

P2K2受体识别eATP信号后, 也可显著诱导胞质Ca^{2+}浓度升高. 遗传证据显示, *p2k2*单突变体对eATP的Ca^{2+}响应显著弱于野生型, 过表达P2K2则增强Ca^{2+}响应. P2K2受体具有自磷酸化和转磷酸化活性, 同时, P2K1与P2K2互作, 并可磷酸化P2K2[12], 因此, P2K2可能一方面通过自身激酶活性, 另一方面可能与P2K1协同调控钙信号相关蛋白, 共同介导eATP诱导的钙信号激活. 但目前尚无直接证据表明P2K2与特定CNGC家族成员存在互作或调控关系, 其下游介导钙信号激活的机制仍有待明确. 同样, 关于LRK10L3-BAK1复合体如何触发钙信号激活的分子机制, 仍有待深入解析.

3.2 活性氧的爆发

eATP与P2K1结合后触发下游ROS产生, 该过程依赖受体磷酸化与钙信号协同作用. 与野生型相比, *dorn1*(*p2k1*)突变体在ATP刺激下Ca^{2+}的内流缺失, ROS生成减少, 同时气孔无法正常关闭, 说明P2K1是eATP诱导ROS产生的关键调控因子[45].

Chen等人[45]利用激酶底物捕获(kinase client assay, KiC)及^{32}P标记体外磷酸化实验证实, P2K1可直接

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与呼吸爆发氧化酶同源蛋白D(respiratory burst oxidase homolog D, RBOHD)相互作用, 并催化其N端Ser22磷酸化, 从而激活RBOHD的酶活性, 并引发ROS爆发. 此外, eATP诱导的胞质Ca^{2+}升高可能通过两条途径进一步促进ROS产生: Ca^{2+}直接结合RBOHD的EF-hand基序, 增强酶活性[55]; Ca^{2+}信号激活钙依赖蛋白激酶(calcium-dependent protein kinases, CPKs)和类钙调磷酸酶B互作蛋白激酶(calcineurin-B-like protein interacting protein kinases, CIPKs), 这些激酶同样可以磷酸化并调控NADPH氧化酶[56~58]. 综上, P2K1识别eATP后, 通过直接磷酸化激活RBOHD, 以及Ca^{2+}-激酶网络双重机制, 协同放大ROS信号[45].

3.3 一氧化氮的产生

胞外ATP激活其受体, 触发胞质Ca^{2+}升高, 进而诱导NO的快速积累. 使用不可水解的ATP类似物ATPγS处理野生型拟南芥, 利用NO荧光探针DAF-2DA可在花粉及根毛中检测到NO水平的增加, 而硝酸还原酶双突变体(*nia1nia2*)中, 相同处理并不引起NO的积累, 表明eATP诱导的NO产生依赖于硝酸还原酶[59,60]. 尽管钙信号被认为在eATP信号与硝酸还原酶活化之间起桥梁作用, 但eATP受体与硝酸还原酶之间是否存在直接互作或者磷酸化级联反应, 仍有待于进一步探究.

作为信号分子, NO可通过多种机制影响下游信号通路: 一方面, 它可激活鸟苷酸环化酶, 生成环鸟苷酸(cyclic guanosine monophosphate, cGMP)[61]. 在动植物细胞中, cGMP可进一步转化为8-硝基cGMP[62,63], 后者已被证明能诱导气孔闭合[63], 但目前尚不清楚eATP诱导的气孔免疫是否有该途径参与. 另一方面, NO还可通过对钙调蛋白等靶蛋白进行S-亚硝基化修饰来调控其活性, 从而为NO与Ca^{2+}信号之间的串扰提供了一种分子机制[61]. 此外, 研究还表明, NO的生成可反馈调节胞质Ca^{2+}升高, 进一步强化了两者之间紧密的功能联系[61].

Ca^{2+}, ROS和NO均为eATP下游关键的第二信使, 研究表明, Ca^{2+}内流是eATP诱导ROS与NO生成的关键步骤, 利用Ca^{2+}通道阻断剂处理会显著抑制eATP诱导的NO和ROS的产生[64,65]. 不同植物中, ROS与NO之间的调控关系存在差异: 在丹参(*Salvia miltiorrhiza*)毛状根系统中, eATP诱导的NO能够促进ROS积累, 而ROS不能诱导NO的产生[64]; 相反, 在胡杨细胞培养物中, eATP诱导的NO则依赖于ROS的产生[66]. 这些不同的调控模式表明, 不同物种的信号整合方式可能存在差异.

3.4 MAPK级联反应的激活

P2K1可与Raf样的MAPK激酶的激酶(MAPK kinase kinase, MAPKKK)家族成员整合素受体激酶5(integrin receptor kinase 5, ILK5)互作, 且ATP处理可显著增强两者的互作. 体外激酶实验证实, P2K1催化ILK5的192位Ser磷酸化, 而该位点的磷酸化对ILK5的功能至关重要: 将Ser192突变为天冬氨酸(模拟磷酸化状态), 可显著增强ILK5的激酶活性; 突变为丙氨酸(模拟非磷酸化状态)则导致其酶活性大幅下降. 进一步研究揭示, 活化的ILK5可直接磷酸化下游丝裂原活化蛋白激酶的激酶5(MAPK kinase 5, MAPKK5或MKK5), 最终激活丝裂原活化蛋白激酶3/6(mitogen-activated protein kinase 3/6, MPK3/6)[67], 通过直接磷酸化胞质蛋白激酶以及激活转录因子, 启动防御相关基因表达两条途径, 级联放大免疫信号. 与P2K1类似, P2K2也能磷酸化ILK5. 但不同的是, P2K2对ILK5的磷酸化并不依赖Ser192位点[68]. 尽管其特异性磷酸化位点仍需要进一步分析, P2K2和P2K1对ILK5磷酸化位点的差异化选择, 表明二者虽然在功能上存在冗余, 但可能还具有精细分工, 使eATP介导的信号通路具备了功能分化的潜能.

此外, P2K1在体内还可以磷酸化另一Raf样MAPKKK家族成员整合素受体激酶4(integrin receptor kinase 4, ILK4). 实验证明, P2K1与ILK4存在互作, 且外源ATP处理可以显著增强两者的互作强度. 体外激酶实验表明, P2K1直接磷酸化ILK4的4个氨基酸位点(Thr326, Ser330, Ser331, Tyr334). 与ILK5类似, ILK4能与MKK4和MKK5发生互作[69]. 然而功能分析显示, *ilk4*突变体在外源ATP作用下, MPK3/6的磷酸化水平反而升高, 暗示ILK4作为P2K1的下游底物, 可能通过与ILK5竞争结合MKK5, 负向调控MPK3/6的激活[69]. 因此, P2K1和P2K2通过选择性磷酸化MAPKKK家族成员, 形成功能互补且精细调控的信号网络, 精准平衡MPK3/6的激酶活性.

4 eATP受体信号通路调控植物胁迫响应与生长发育的机制

eATP受体识别胞外ATP, 启动下游钙信号、ROS

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爆发、NO产生、MAPK级联反应等信号事件, 并进一步整合激素信号、代谢通路及转录调控网络, 在植物抗病防御、非生物胁迫响应及生长发育中发挥重要功能.

4.1　植物免疫

eATP受体介导的抗病机制是植物先天免疫的重要组成部分. 当植物遭受丁香假单胞菌(*Pseudomonas syringae*)侵染、真菌灰霉菌(*Botrytis cinerea*)寄生、植食性昆虫取食等胁迫时[53,70,71], 细胞会快速释放eATP, eATP随后被细胞膜上的特异性eATP受体识别并结合, 启动下游防御响应. 遗传学实验表明, P2K1功能缺失突变体*p2k1*对丁香假单胞菌*Pst* DC3000及土传真菌立枯丝核菌(*Rhizoctonia solani*)的敏感性均显著高于野生型[70], 然而其对坏死营养型真菌灰霉菌(*Botrytis cinerea*)的敏感性反而降低, 表现为叶片褪绿面积更小, 病害受损伤程度更轻[71]. 这一差异可能与病原菌致病生活方式及其与寄主互作模式的差异相关. P2K2功能缺失突变体*p2k2*同样表现出对丁香假单胞菌*Pst* DC3000的易感表型, 且*p2k1p2k2*双突变体的敏感性显著高于单突变体, 说明P2K1与P2K2在抗病防御中存在功能部分冗余, 二者协同强化eATP介导的防御信号[12]. 值得注意的是, *P2K1*的转录表达受JA-冠菌素不敏感蛋白1(coronatine insensitive 1, COI1)通路的调控. JA处理、病原菌攻击或昆虫取食均可显著诱导*P2K1*的表达, 而JA受体突变体*coi1-1*中*P2K1*的上调表达被抑制. 因此, 形成了胁迫诱导eATP释放, P2K1表达上调, eATP信号敏感性提升的调控模式, 利于抗病信号的快速启动和高效传导[53,72].

P2K2的点突变体*old12*(onset of leaf death 12)呈现自身免疫突变体的表型, 包括生长缺陷、细胞自发死亡, 防御基因激活以及早期衰老[73]. 研究进一步发现, *old12*突变体第407位保守的Cys变为Tyr, 该位点在P2K1中已被证明被S-酰化后, 通过负调控P2K1的自磷酸化抑制eATP受体介导的免疫反应[74]. 在P2K2中, C407Y的突变同样破坏其激酶活性, 不同的是, 该突变最终导致水杨酸(salicylic acid, SA)积累增加以及防御反应持续性激活[73].

eATP下游ROS信号介导的气孔免疫构成了植物免疫的物理防御屏障. P2K1-RBOHD通路激活的ROS爆发可诱导气孔快速关闭, 阻断了病原菌以气孔入侵的途径, 而*p2k1*, *rbohd*突变体因无法实现该响应(图2), 表现出显著的气孔免疫缺陷[45]. 此外, eATP介导的MAPK级联反应精准调控启动分子防御网络. P2K1通过差异化磷酸化ILK5和ILK4实现对MPK3/6活性的双向调控: 磷酸化ILK5的Ser192位点正向激活MKK5-MPK3/6级联反应, 磷酸化ILK4的Thr326和Ser330等位点则负向调控该级联反应[67,69], 避免防御信号过度放大对植物造成损伤, 维持免疫信号的动态平衡. P2K2也可直接磷酸化ILK5[68], 提示其可能与P2K1共同作用于MKK5-MPK3/6信号级联, 从而调控下游防御反应(图3).

eATP受体介导的信号可与JA和SA通路协同作用, 共同调控植物防御响应. 外源ATP处理在30 min内并不会刺激JA合成途径, 但可以通过增强COI1-茉莉酸ZIM结构域蛋白1(jasmonate ZIM-domain protein 1, JAZ1)的相互作用来促进JAZ1的泛素化降解, 解除JAZ1对于JA信号通路的抑制, 由此激活了JA信号通路, 从而快速启动JA介导的植物防御响应[53,75,76]. 当昆虫取食触发eATP释放后, P2K1受体感知信号并与CNGC19互作, 协同介导Ca^{2+}内流, Ca^{2+}信号进一步诱导JA合成相关基因表达及茉莉酸-异亮氨酸(jasmonic acid-isoleucine, JA-Ile)的积累, JA-Ile与受体COI1结合后可以进一步解除JAZ的抑制作用, 放大JA信号通路. 同时, JA信号通过其受体COI1介导的信号通路, 反馈诱导P2K1的转录表达[72,76], 维持了eATP信号的持续传递[53,75]. 这种eATP与JA信号通路的交叉对话, 使植物能够实现从胁迫感知到防御响应的快速转变, 显著增强对植食性昆虫的抗性(图4)[77]. 作为新型eATP感知单元, LRK10L3与BAK1通过调控JA合成基因的表达, 增强防御能力, *lrk10l3*/*bak1*双突变体中, eATP诱导的JA合成关键基因的表达上调受到抑制, JA含量显著降低, 说明LRK10L3-BAK1复合体在JA信号通路激活中具有关键作用[43].

eATP与SA信号通路通过相互拮抗靶向下游共同的效应蛋白, 精准调控防御强度与细胞命运. ATP可以诱导部分SA响应基因的表达, 但外源SA处理会显著降低烟草细胞的eATP水平, 外源ATP短期处理不影响SA含量, 长期处理则降低烟草SA水平[75,78]. 当eATP被耗减时, 烟草病程相关蛋白(pathogenesis related protin, PR)表达上调, 而添加外源ATP能够拮抗SA诱导的PR蛋白积累, 表明eATP作为SA介导的防御信号通路中

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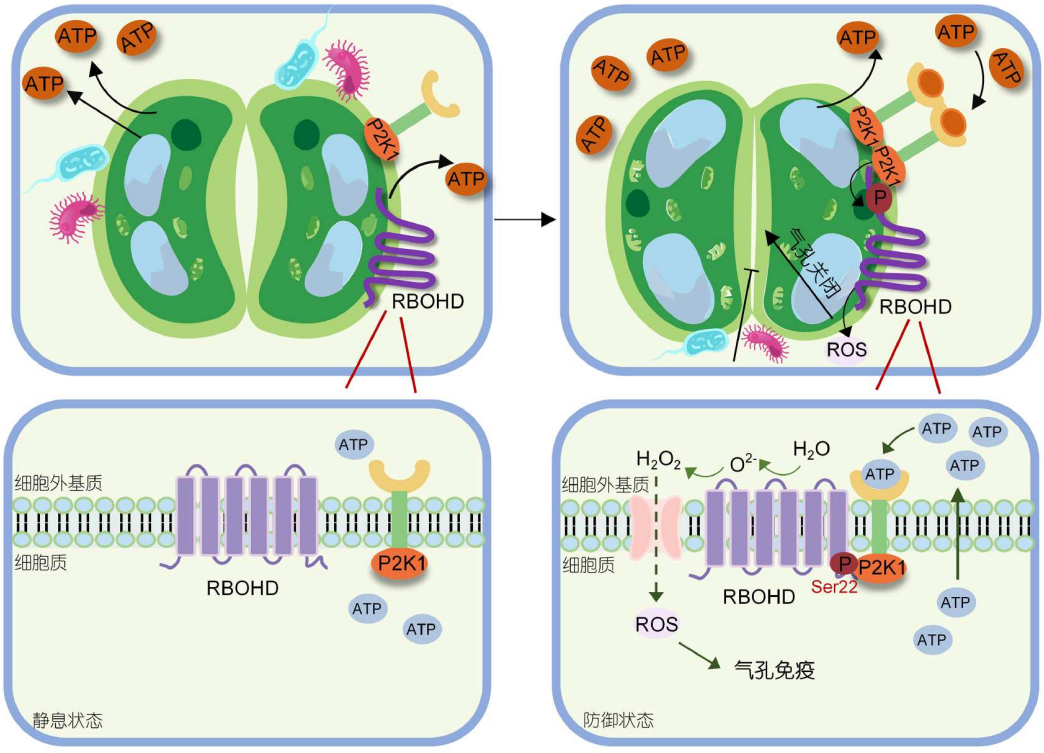


图 2 eATP受体介导的气孔免疫信号通路. 植物受到病原菌攻击时, 胞内ATP释放到胞外, 形成eATP. eATP结合并激活P2K1受体, P2K1与RBOHD互作并磷酸化RBOHD的Ser22残基, 被磷酸化激活的RBOHD催化产生活性氧, 从而诱导气孔关闭, 有效阻断病原菌的入侵通道

Figure 2 The eATP receptor-mediated stomatal immunity signaling pathway. Upon pathogen attack, intracellular ATP is released into the extracellular space, forming eATP. eATP is perceived by the receptor kinase P2K1, which subsequently interacts with and phosphorylates RBOHD at Ser22. This phosphorylation activates RBOHD, leading to the production of ROS. The resulting ROS burst induces stomatal closure, thereby blocking the pathogen's invasion route via stomata

的一个关键负调控因子发挥作用[78]. 此外, eATP通过抑制核糖核酸酶1(ribonuclease 1, RNS1)的积累, 缓解烟曲霉毒素B1(fumonisin B1, FB1)诱导的程序性细胞死亡. SA则促进RNS1转录表达, 增强FB1诱导的RNA降解, 从而加速细胞死亡[79]. 因此, RNS1是SA和eATP信号通路的共同下游效应蛋白, 两者通过反向调控RNS1的表达水平, 决定植物细胞的死亡命运. 此外, 与JA能够增强eATP诱导的胞质钙离子升高及下游基因转录不同, SA显著抑制eATP触发的胞质钙响应, 形成对eATP信号的拮抗调控[72].

此外, 甲羟戊酸(mevalonic acid, MVA)途径作为eATP信号与代谢调控的交叉节点, 在植物抵御生物胁迫的防御反应中发挥关键作用[80,81]. eATP受体P2K1可与MVA途径关键酶——甲羟戊酸激酶(mevalonate kinase, MVK)直接互作, 并通过特异性磷酸化其S329和T342位点激活MVK的酶活性. 激活后的MVK能驱动MVA途径高效运转, 显著促进下游代谢产物甲羟戊酸-5-磷酸(mevalonic acid-5-phosphate, MVP)、异戊烯基二磷酸(isopentenyl diphosphate, IPP)的合成与积累[82], 而这些代谢产物可通过调控胁迫响应相关信号分子的生成参与防御应答. 研究发现, *mvk*突变体表现出显著的eATP信号转导缺陷, 表现为eATP诱导的胞质Ca^{2+}内流减弱、MPK3/6磷酸化水平降低、eATP响应基因表达上调幅度减弱, 同时对丁香假单胞菌变种*Pst* DC3000的敏感性显著增强, 这充分证实了MVA途径在eATP介导的植物生物胁迫防御中的重要功能.

4.2 非生物胁迫应答

eATP受体通过整合环境信号与细胞内稳态调控网络, 在植物应对盐、干旱等非生物胁迫中发挥重要作用. 盐胁迫诱导eATP释放, 通过激活P2K1/P2K2受体, 一方面通过下游Ca^{2+}信号激活, 形成"Ca^{2+}波"向远

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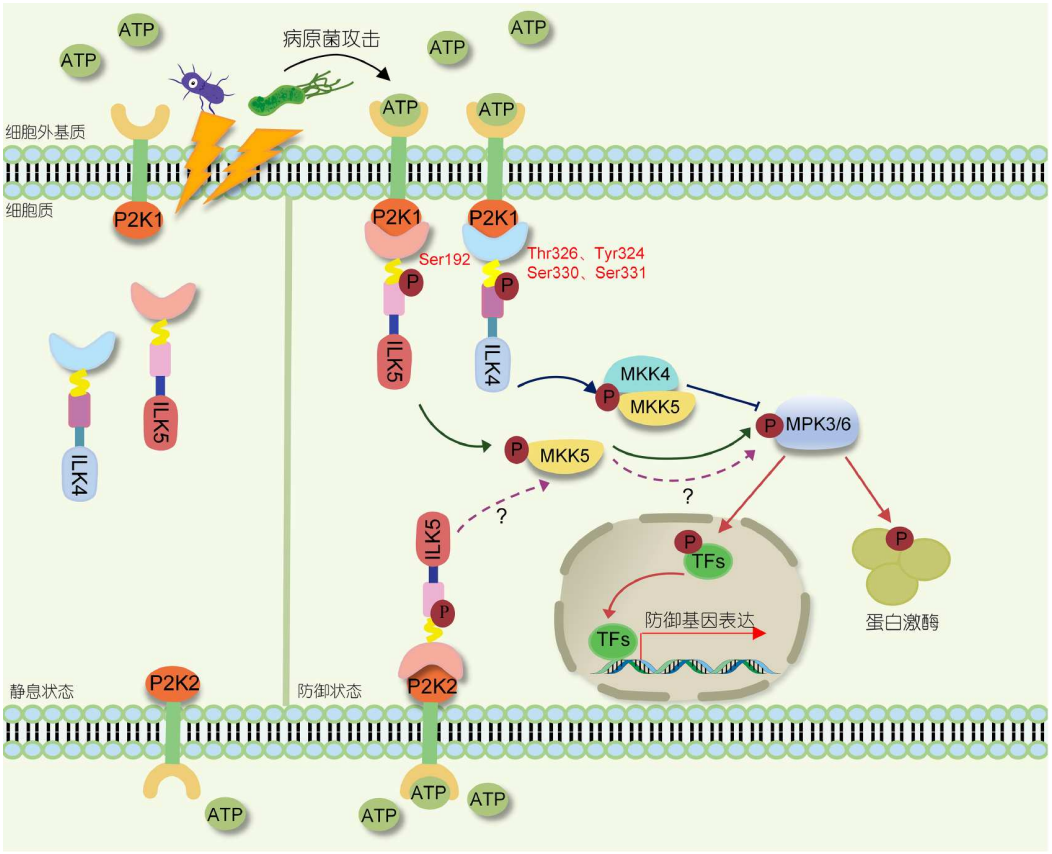


图 3　eATP受体通过磷酸化MAPKKK激酶调控植物免疫信号. 植物受到病原菌侵染时, 胞外ATP积累并激活P2K1受体. 激活的P2K1通过差异化磷酸化下游两类MAPKKK, 实现对MKK5-MPK3/6级联反应的双向调控: 磷酸化ILK4的Thr326和Ser330等位点负向调控MKK5-MPK3/6级联反应, 而磷酸化ILK5的Ser192位点则正向激活该级联反应. MPK3/6或通过磷酸化下游蛋白激酶等靶蛋白直接介导免疫应答, 或通过磷酸化激活转录因子, 启动防御基因表达, 从而放大免疫信号. 此外, P2K2也能磷酸化激活ILK5, 但其磷酸化位点及其下游通路尚不明确. TFs: 转录因子

Figure 3　Regulation of plant immune signaling by eATP receptors via MAPKKK phosphorylation. Upon pathogen attack, elevated eATP initiates immune signaling by activating the P2K1 receptor. Activated P2K1 exerts bidirectional control of the MKK5-MPK3/6 cascade through differential phosphorylation of two MAPKKKs: it suppresses the cascade by phosphorylating ILK4 at residues such as Thr326 and Ser330, while promoting activation by phosphorylating ILK5 at Ser192. The activated MPK3/6 amplifies the immune signaling either by directly mediating immune responses through phosphorylation of downstream target proteins (e.g., protein kinases), or by phosphorylating and activating transcription factors to initiate defense-related gene expression. In addition, P2K2 can also phosphorylate and activate ILK5, although its specific phosphorylation sites and downstream pathways remain to be elucidated. TFs: transcription factors

端传导; 另一方面通过P2K1-RBOHD通路诱导ROS局部积累并系统性扩散[83]. 尽管P2K受体介导的通路对于激活防御反应至关重要, *p2k1*突变体在盐胁迫下却表现出生长抑制减弱的表型. 遗传学证据表明, *p2k1*和*p2k2*单突变体均能显著减弱盐胁迫诱导的局部与系统性的ROS积累、Ca^{2+}波传递以及eATP早期响应基因的表达, 同时, 损害了局部盐胁迫激活的系统性光合保护能力. 与单突变体相比, 双突变体中过氧化氢(hydrogen peroxide, H_2O_2)积累和过氧化氢酶(catalase, CAT)活性也表现出进一步的缺陷, 此外, 在系统性ROS信号的强度以及光合保护方面, 双突变体缺陷更为显著[48,49], 表明P2K1与P2K2在介导系统性抗逆中存在功能冗余.

干旱胁迫诱导植物细胞释放eATP, 被P2K1感知后, P2K1磷酸化RBOHD的Ser22位点, 激活ROS生成, 进而诱导气孔关闭, 减少蒸腾失水[45]. 研究表明, 野生型植物经ATP处理后, 水分流失显著减少, 而*dorn1-3* (*p2k1*)和*rbohd*突变体水分流失率显著高于野生型, 同时二者仍能响应脱落酸(abscisic acid, ABA)诱导的气孔关闭, 表明eATP信号介导的气孔关闭独立于ABA信

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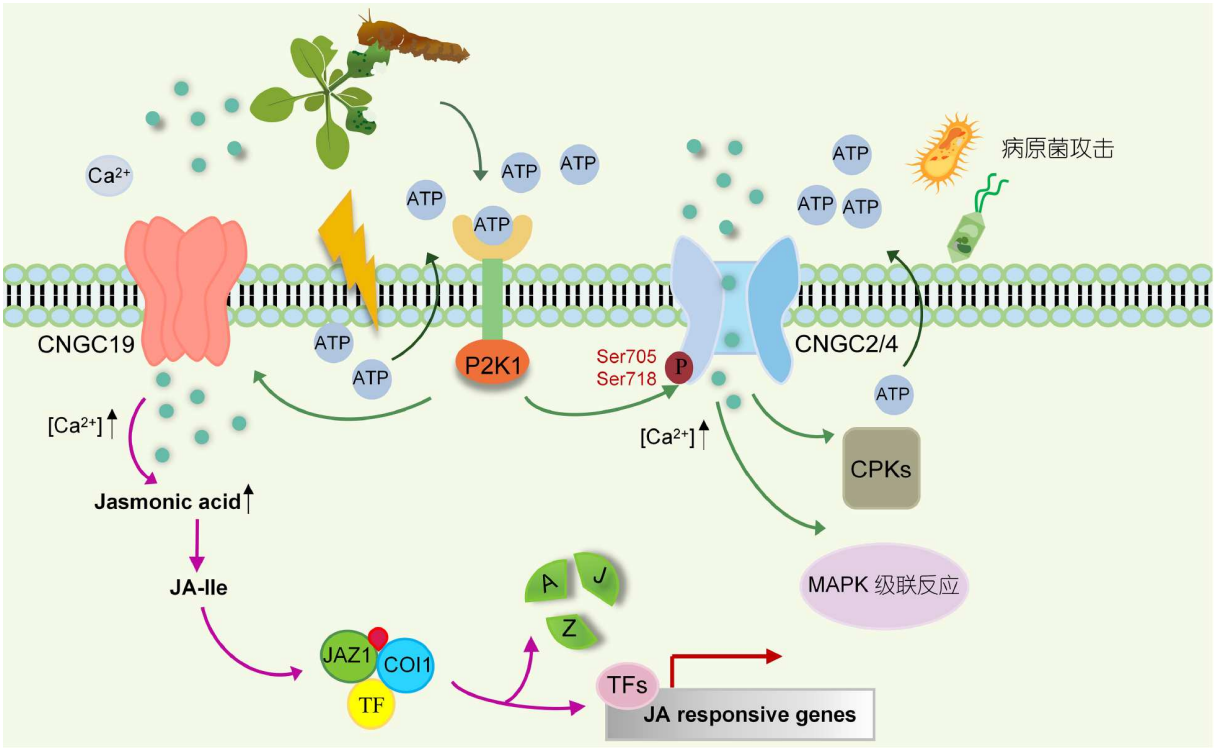


图 4 P2K1受体介导的eATP信号协同茉莉酸与钙信号调控植物免疫. 拟南芥图片修改自文献[77](开放获取). 当植物受到昆虫取食或机械损伤后, 释放到胞外的ATP结合并激活P2K1受体, 激活的P2K1与钙离子通道CNGC19互作, 引发胞质Ca^{2+}浓度升高, Ca^{2+}浓度升高可能也涉及其他钙通道的激活. 胞质增加的Ca^{2+}促进茉莉酸合成基因的表达, 增加活性形式茉莉酸-异亮氨酸的积累, JA-Ile与COI1结合后可以解除JAZ的抑制作用, 激活JA响应基因的表达. 此外, 当植物受到病原菌侵染时, 激活的P2K1可以与钙离子通道CNGC2/4互作, 并磷酸化CNGC2的Ser705/718位点, 解除钙调蛋白CAM7对通道的抑制作用, 进而诱导Ca^{2+}内流. Ca^{2+}进一步通过激活钙离子依赖性蛋白激酶和放大MAPK级联信号, 介导植物免疫防御反应

Figure 4 P2K1 receptor-mediated eATP signal coordinates JA and Ca^{2+} signaling to regulate plant immunity. Figure of *Arabidopsis thaliana* adapted from ref. [77] (Open Access). Upon insect herbivory or mechanical damage, plants release eATP, which binds to and activates the P2K1 receptor. Activated P2K1 interacts with the calcium channel CNGC19, triggering a rise in cytosolic Ca^{2+} concentration. This Ca^{2+} influx may also originate from the activation of other calcium channels. The increased cytosolic Ca^{2+} promotes the expression of JA biosynthesis genes, resulting in the accumulation of its active form, JA-Ile. JA-Ile binds to COI1, which subsequently degrades the JAZ repressors, thereby activating the expression of JA-responsive genes. Furthermore, upon pathogen attack, activated P2K1 interacts with the calcium channels CNGC2/4 and phosphorylates CNGC2 at Ser705 and Ser718. This phosphorylation relieves the inhibition imposed by the calmodulin CAM7 on the channel, thereby inducing Ca^{2+} influx. The elevated Ca^{2+} then mediates immune responses by activating CPKs and amplifying the MAPK cascade signaling

号通路[45]. 此外, 活化的P2K1还能够磷酸化钙感受器钙调磷酸酶B类似蛋白9(calcineurin B-like protein 9, CBL9)的第196位Thr[69], 该位点紧邻CBL9的功能关键位点(Ser201)[84], Ser201位点的磷酸化可直接影响CBL9与下游CIPK家族成员的互作[85,86]. 因此, P2K1对CBL9第196位Thr的磷酸化, 也可能间接影响其与下游CIPK的互作. 由于CBL9与CIPK家族成员(CIPK3, CIPK23等)形成的复合物, 已被报道通过调控ABA信号转导、钾离子通道活性及干旱响应相关转录因子, 直接参与气孔运动调控、细胞离子平衡维持及抗旱相关信号激活等生理应答, 是植物干旱适应的重要调控模块[85~87]; 鉴于P2K1可直接磷酸化CBL9的Thr196位点, 推测其可能通过调控CBL9与CIPK的互作, 使eATP信号与ABA抗旱通路偶联, 协同调控植物抗旱适应性. 然而, 该推测尚缺乏直接实验证据支持, 仍需进一步验证.

4.3 生长发育调控

eATP 作为植物生长发育的关键信号分子, 通过受体被感知后, 参与调控植物的根系发育、花粉管生长以及种子萌发等过程[32,88,89].

eATP受体及其互作蛋白共同参与植物根生长与

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构型的调控. 研究发现, P2K1可与钙离子依赖性蛋白激酶CPK28直接互作, eATP处理能诱导CPK28表达, 而CPK28过表达则会抑制eATP诱导的胞质钙内流. 在P2K1过表达植株中表达CPK28, 可缓解其eATP超敏表型, 同时, *cpk28-1*突变体中, 植物对eATP介导的根生长调控敏感性增强[71]. 因此, P2K1感知eATP后启动钙信号, CPK28被诱导表达并通过与P2K1互作, 负调控该通路以避免eATP信号过度放大, 从而平衡植物生长与防御; 此外, LRK10L3-BAK1复合体也参与了植物根的发育调控过程. *lrk10l3*/*bak1*双突变体对eATP介导的根弯曲生长完全不敏感, 主根伸长抑制减弱, 侧根生成无明显促进, 而通过转入*LRK10L3*或*BAK1*基因构建回补株系, 可恢复植株对eATP的响应表型, 表明LRK10L3与BAK1复合体在eATP调控根生长与构型中发挥重要功能[43].

eATP参与花粉萌发与花粉管生长的调控, 在花粉萌发和花粉管生长过程中, 花粉会向胞外释放一定浓度的ATP[90]. 体外实验表明, 低浓度eATP促进花粉萌发和花粉管伸长, 高浓度eATP则表现为抑制效应[90]. 膜片钳实验发现, eATP可显著增强花粉原生质体的K^+和Ca^{2+}内流, 而*dorn1-3*、异源三聚体G蛋白α亚基(G protein alpha subunit, GPA1)缺失突变体*gpa1-1*, *cngc2*或*cngc4* 突变体中, eATP诱导的离子内流、花粉萌发及花粉管生长均显著受损, 而相应的突变体回补*P2K1*, *GPA1*或*CNGC*基因后, 可恢复对eATP的响应表型[91]. 研究表明, GPA1, CNGC2/CNGC4均是P2K1的底物[51,69], eATP信号可能通过P2K1对这些靶蛋白的活性调控而影响离子内流, 而这些离子的动态变化对维持花粉管顶端生长所需的离子稳态和极性至关重要.

eATP还参与种子萌发过程的调控, 适宜浓度的eATP可显著提升拟南芥等多种植物的种子萌发率, 而高浓度的eATP则抑制种子萌发[89]. *dorn1-1*, *dorn1-3*和*lrk10l3*/*bak1*双突变体对eATP的萌发响应显著减弱, 相应的回补株系则可恢复对eATP的响应表型[43]. 低浓度eATP主要由P2K1受体感知, 通过激活CPK28和CNGC2/4介导的K^+和Ca^{2+}内流, 维持萌发所需的离子稳态与激素平衡, 进而促进种子萌发[51,71,91]; 高浓度eATP则由LRK10L3-BAK1受体复合体识别, 通过促进JA合成相关基因表达及介导生长素在根与下胚轴中的不对称分布, 改变幼苗生长速率和方向, 以帮助幼苗适应或规避局部不利环境[43].

5　展望

eATP作为植物中普遍存在的信号分子, 在调控植物生长发育、生物胁迫与非生物胁迫应答中发挥核心功能. 目前已在拟南芥中鉴定出两个eATP受体P2K1与P2K2, 并发现了LRK10L3-BAK1这一eATP感知新单元. 尽管已有研究初步揭示了eATP在植物免疫、气孔运动以及钙信号转导中的作用机制, 该领域仍存在诸多关键问题亟待深入探索.

首先, P2K1与P2K2氨基酸序列一致性达到74%, 两者同属于LecRK家族, 均可以形成同源二聚体并具备自磷酸化及底物磷酸化活性. 此外, 二者还可以相互作用, 提示两者可能在信号起始阶段组装成异源复合体传递信号. 然而, 迄今为止尚无直接证据揭示P2K1与P2K2异源复合体在免疫调控中的动态及功能意义. 因此, 借助超高分辨率显微镜及单分子技术, 在单个活细胞水平实时追踪eATP信号刺激后两者的聚合、解离及磷酸化动态, 可以系统地比较P2K1与P2K2在时空分布、停留时间及下游招募蛋白上的差异, 从而明确二者功能冗余与特异性的分子基础.

其次, 尽管目前已鉴定出P2K1下游的一些互作蛋白, 并初步解析了其相关的信号通路, 但对于P2K2下游互作蛋白的了解尚不足. 此外, P2K1和P2K2均可以ILK5为底物, 然而在磷酸化ILK5时修饰位点存在差异性. 因此, 两者是否存在各自特异的直接靶标、磷酸化底物及分支通路, 仍缺乏系统性的比较分析. 未来仍需要深入解析P2K1/P2K2对不同底物的磷酸化特异性及其生物学意义, 阐明不同信号分支如何被精确整合, 以响应多样化的环境胁迫.

再次, 诸多研究表明, 植物对于eATP存在剂量依赖性响应. 例如, 低浓度ATP促进拟南芥气孔开启, 而高浓度ATP则诱导气孔关闭[92]. 随着近期LRK10L3-BAK1这一eATP感知新机制的发现, 是否存在其他不同的eATP功能性受体, 并在不同组织或者生理条件下特异性介导eATP信号转导, 仍需通过遗传筛选、结构预测以及生理互补实验等进行系统研究.

最后, 目前有关eATP信号的相关研究主要集中于模式植物拟南芥, 尽管已有文献通过生物信息学方法挖掘了作物中潜在的eATP受体蛋白[93], 但作物与林木中的eATP信号通路仍知之甚少. 基于在拟南芥中已明

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确eATP参与调控生长发育及逆境响应等重要过程，在作物与林木中系统解析eATP及其信号组分的功能，并阐释其信号网络，将为作物与林木的遗传改良提供新策略.

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Research progress on the release, degradation, perception and downstream signaling pathways of plant extracellular ATP

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Extracellular ATP (eATP), as an extracellular signaling molecule, plays a crucial role in regulating plant growth and development, as well as mediating responses to biotic and abiotic stresses. Current studies have demonstrated that eATP can be released from intracellular compartments to the extracellular space through multiple pathways, including cell damage-induced leakage, exocytosis, ABC transporters and PM-ANT1 proteins mediated release. Once released, eATP binds to plasma membrane-localized receptors P2K1 and P2K2, thereby triggering downstream signaling such as calcium influx, reactive oxygen species (ROS) burst, nitric oxide (NO) production, and mitogen-activated protein kinase (MAPK) cascade activation, which collectively regulate plant physiological responses. Recent research has identified that two receptor-like kinases, LRK10L3 and BAK1, can form a complex on the plasma membrane. This complex functions as an eATP-sensing unit, which further improves and enriches the eATP perception network in plants. In terms of maintaining eATP homeostasis, Apyrases dynamically regulate extracellular eATP concentrations through the degradation of ATP. This review systematically integrates current knowledge on the mechanisms of ATP release and degradation, receptor-mediated recognition, and downstream signaling components in plants. Additionally, it summarizes how eATP-receptor-mediated pathways regulate stress responses and developmental processes, highlights recent advances in the field, and discusses the unresolved issues and future research directions.

plant, extracellular ATP (eATP), release and degradation, eATP perception, signaling pathways

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