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植物钙依赖性蛋白激酶及其相关蛋白激酶(CDPKs/CRKs)的研究进展

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摘要　作为细胞第二信使,Ca^{2+}协调着植物对各种生理反应的感知。钙离子传感器向下游传递钙信号并引发级联反应,调控植物生长发育以及对环境的响应等过程。钙依赖性蛋白激酶在Ca^{2+}介导的信号转导中起重要作用。综述了近年来植物 CDPKs/CRKs 相关研究进展,包括分子结构和作用机制、表达模式、亚细胞定位和生物学功能,旨在为 CDPKs/CRKs 相关研究提供参考。

关键词　钙依赖性蛋白激酶;钙依赖性蛋白激酶相关蛋白激酶;调控机制;生物学功能
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Research Progress of Plant Calcium-dependent Protein Kinases(CDPKs) and CDPK-related Kinases(CRKs)

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Abstract　As a second messenger in cell, Ca^{2+} coordinates the perception of plant for a variety of physiological reaction. The calcium ion sensor transmits calcium signal and triggers a cascade reaction to regulate plant growth and development. Calcium dependent protein kinases play important roles in Ca^{2+}-mediated signal transduction. This paper reviewed the related research progress in plant CDPKs and CRKs, including molecular structure and mechanism, expression patterns, subcellular localizations and biological functions. Hence, the purpose of this review is to provide reference for related research on CDPKs/CRKs.

Key words　Calcium-dependent protein kinases;CDPK-related kinases;Regulation mechanism;Biology function

Ca^{2+}作为第二信使,协调植物对内外界多种生理反应的感知[1]。Ca^{2+}传感器接受钙信号,将化学信号转化为细胞的生理生化反应。植物主要有 4 种Ca^{2+}传感器:钙调素(Calmodulin,CaM)及其类似蛋白(CaM-like proteins,CMLs)、钙依赖性蛋白激酶(Calcium-dependent protein kinases,CDPKs or CPKs)及其相关的蛋白激酶(CDPK-related kinases,CRKs)、钙调磷酸酶 B 类似蛋白(Calcineurin B-like protein,CBL)、钙和钙调素依赖性蛋白激酶(calcium-and calmodulin-dependent protein kinases,CCaMKs)[2]。目前对 CDPKs 的研究较为深入,研究表明 CDPKs/CRKs 存在复杂的调控模式,在植物发育及多种胁迫响应中均扮演重要角色。笔者综述了近年来植物 CDPKs/CRKs 相关研究进展,旨在为 CDPKs/CRKs 相关研究提供参考。

1　CDPKs/CRKs 的分子结构和作用机制

CDPKs 存在于植物、绿藻和单细胞原生生物中[3]。拟南芥、水稻、玉米和棉花中分别含有 34、31、40 和 41 个 *CDPK* 基因[4-8];拟南芥、水稻、白杨、番茄和辣椒中分别含有 8、5、9、6 和 5 个 *CRK* 基因[4,9-12]。对于 CDPKs 和 CRKs 分子结构以及激酶活性调控的研究主要集中在拟南芥。

CDPKs 蛋白包含 4 个结构域:N 端可变结构域(variable N-terminal domain,VNTD)、Ser/Thr 蛋白激酶域(Ser/Thr protein kinase domain,PKD)、自抑制连接域(junction domain,JD)和 C 端具有 EF 手型结构的类钙调素调控结构域(CaM-like regulatory domain,CaMLD)[13-15]。CRKs C 端的 EF 手型结构已经退化,其他结构与 CDPKs 相似[16]。CDPKs/CRKs 的蛋白结构见图 1a。

CDPKs 的 N 端具有较高比例的脯氨酸、谷氨酰胺、丝氨酸和苏氨酸(PEST)序列,这些序列可以进行快速的蛋白降解[14]。VNTD 区氨基酸序列多样,长度各异,保守性低。拟南芥 CDPKs 的 VNTD 区序列长度最短只有 25 个氨基酸,最长达 180 个氨基酸[17]。VNTD 区参与底物特异性识别过程。NtCDPK1 与 RSG 互作,而 AtCPK9 不与 RSG 互作,但含有来源于 NtCDPK1 的 VNTD 区的重组 AtCPK9 可与 RSG 互作,这表明 NtCDPK1 的 VNTD 区在特异性识别底物 RSG 的过程中发挥了重要作用[18]。研究发现多数 CDPKs N 端具有参与蛋白膜结合的豆蔻酰化或棕榈酰化位点,暗示 VNTD 区参与 CDPKs 膜结合过程[19]。

CDPKs 蛋白的 PKD 区保守性高,含有 Ser/Thr 磷酸化位点,关键氨基酸突变往往会使激酶失活[4]。PKD 区分为 2 个球形结构,分别是 N-lobe 和 C-lobe,前者对Ca^{2+}的亲和性低于后者[13]。CRKs 除含有 Ser/Thr 激酶活性,还具有极高的酪氨酸自磷酸化活性且可以磷酸化底物的酪氨酸位点[20]。

CDPKs 上的 JD 区保守性高,主要作为假底物结合在 CDPKs 的激酶区[14]。

正常情况下,JD 区与 C-lobe 的催化位点结合,作为假底物维持 CDPKs 处于低基础激酶活性状态。当植物受到光照、低温或高温、高盐、干旱、激素甚至病原体等刺激时,细胞内形成特异性Ca^{2+}信号,Ca^{2+}与 EF 手型结构直接结合,改变了 CDPKs 的构象,暴露出激酶活性位点,激活激酶活性(图 1b);此外,PKD 区结合 ATP 或 GTP 并转移 γ-磷酸基团到受体羟基残基,激活底物,从而引发植物多种生理反应[15,21-22]。近年研究显示在部分 CDPKs 上具有 CaM 结合位点并在体外试验中证明了二者的相互作用,体外激酶试验显示 CaM 可抑制这一类 CDPKs Ca^{2+}依赖性的激酶活性[23]。

大部分 CRKs C 端的 EF 手型结构退化,因此不受Ca^{2+}直接调控,但由于部分 CRKs 具有 CaM 结合区,所以 CRKs 的

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激酶活性会受到 Ca^{2+} 和 CaM 的协调控制[24-25]。

磷酸化作用是影响 CDPKs/CRKs 激酶活性的另一个重要因素。AtCPK28 第 228 和 318 位的丝氨酸位点突变使其不能发生自磷酸化作用,降低了激酶活性。除典型的Ser/Thr磷酸化位点,CRKs和第Ⅳ类CDPKs还具有Tyr磷酸化位点[23,26]。CDPKs/CRKs 可以被泛素化修饰。Teng 等[27]、樊莉娟等[28]、邓亚男等[29]研究结果显示,拟南芥中 CRK5 的蛋白水平通过 DWD 蛋白介导被泛素化降解;AtTR1 可以对 AtCPK28 和 AtCPK32 进行体外多泛素化修饰;过表达 E3 泛素连接酶 ABRv1 通过单泛素化 AtCPK3,可以提高拟南芥耐旱性[27-29]。

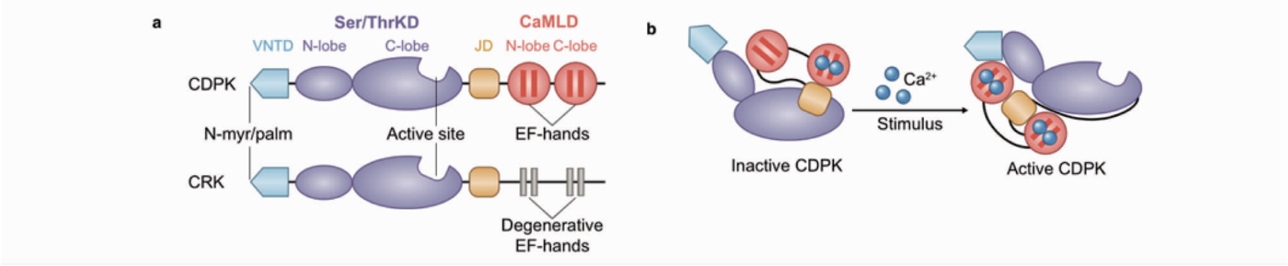

图 1　CDPKs/CRKs 的蛋白结构(a)和激活机制(b)[15]

Fig.1　Structure and active mechanism of CDPKs/CRKs

2　CDPKs/CRKs 的表达模式和亚细胞定位

转录组分析结果显示 CDPKs 具有不同的表达模式,表明 CDPKs 在植物细胞中功能的多样性[6]。一些 CDPKs 呈现泛表达模式,而另一些 CDPKs 则特异性表达在植物的某些器官或组织中。如 *NtCDPK*1 在烟草根、茎、花中表达,叶中不表达[30];*AtCPK*12 在拟南芥大部分组织包括根、茎、叶等均有表达,但在种子中无法检测到[31];*AtCPK*17/20/34 仅在花粉中表达,调控花粉管生长[32-33];*AtCPK*3/6 则在保卫细胞中大量表达,调节气孔运动[34];*BnCDPK*1 主要在油菜叶片中表达,油菜花中表达量很低,种子等其他组织表达量适中[35]。CRKs 的表达模式也因其种类而异。番茄 *LeCRK*1 在叶、茎、花中均有表达,但在成熟果实中表达量最高[36]。qRT-PCR 结果表明 *AtCRK*5 在花中表达量最高,茎中表达量最低[37]。

CDPKs/CRKs 在细胞中的定位与其功能密切相关。CDPKs 的亚细胞定位模式多样,包括细胞质、细胞核、细胞膜、内质网、过氧化物酶体、脂质体等,暗示其功能的多样性[6,38]。多数 CDPKs 在细胞膜处均有分布,与其 N 端的酰基化修饰有关,其中棕榈酰化位点与细胞膜形成可逆的稳定结合,豆蔻酰化位点形成的则是不可逆的松散结合[39]。豆蔻酰化或棕榈酰化位点的突变会改变 CDPKs 的膜定位。AtCPK16 定位于细胞膜,当发生 G2A 突变后,阻断了该蛋白发生豆蔻酰化修饰,AtCPK16 则定位于叶绿体;当该蛋白发生 C4S 突变阻断其发生棕榈酰化修饰后,则表现为核定位[40];AtCPK3 定位于细胞膜,G2A 位点突变阻断其发生豆蔻酰化修饰,在细胞质中也可以检测到点突变的 AtCPK3[41]。部分 CRKs 是细胞核或细胞质定位,其他均为膜定位。目前研究表明所有 AtCRKs 均具有 N 端酰基化位点[6],除 AtCRK6 的亚细胞定位尚不清楚,其他 7 个 AtCRKs 在细胞膜处均有分布[42-43]。

3　14-3-3 蛋白对 CDPKs/CRKs 的调控

CDPKs 可以与脂质或 14-3-3 蛋白相互作用来调节激酶活性。脂质可以增加 CDPKs 对底物的亲和性[44],而 CDPKs 与 14-3-3 蛋白的作用关系更为复杂。一方面,14-3-3 蛋白可以直接调节 CDPKs 激酶活性,调控其稳定性。研究表明 14-3-3 蛋白可以在体外促进 AtCPK1/21/23 的激酶活性但不影响其钙敏感性,同时抑制 AtCPK3 在细胞内的降解[45-47]。另一方面,14-3-3 蛋白也可以作为 CDPKs 的底物发挥作用[48-49]。

4　CDPKs 的生物学功能

4.1　CDPKs 调控植物生长发育　研究表明 CDPKs 参与调控植物根、茎、叶的发育。MtCDPK1 对于苜蓿根毛的正常生长必不可少,抑制 *MtCDPK*1 基因表达会导致根长和根毛长度变短[50];*OsCDPK*5 和 *OsCDPK*13 在根皮层细胞大量表达,诱导 OsRBOHH 介导的 ROS 生成,保证水稻在淹水条件下根系通气组织正常形成[51]。同时沉默 *NaCDPK*4 和 *NaCDPK*5 导致转基因植株茎发育异常,生长受阻[52];*AtCPK*28 在维管和分生组织大量表达,参与调控茎基部节间次生生长和木质部发育,*cpk*28 突变体严重矮化[26]。敲除烟草 *NtCDPK*1 基因导致新生叶片细胞形态异常和过早死亡[53];AtCPK3 磷酸化的 RhoGDI1 通过调节 ROP 信号通路来调控拟南芥幼苗形态和叶表皮细胞发育[54]。

CDPKs 参与调控植物开花、花粉萌发及花粉管生长、种子发育等过程。*AtCPK*33 与 bZIP 转录因子 FD 相互作用并磷酸化 FD 是成花素复合体形成的关键,AtCPK33 功能缺陷导致开花延迟[55]。AtCPK2/4/6/11/14/16/17/20/24/26/32/34 在花粉中表达[15,56-57],其中 AtCPK11/24 通过抑制 SPIK 介导的 K^{+}内流抑制花粉管生长[58];AtCPK2/6/20 通过激活 SLAH3、ALMT12/13/14 介导的 NO$_3^-$ 和苹果酸盐外流促进花粉管生长[33,59];*Atcpk*17/*Atcpk*34 双突变体花粉管顶端极化生长存在缺陷[32,60];过表达 AtCPK32 引起花粉管尖端 Ca^{2+}浓度增加并伴随花粉管尖端膨胀[61]。*OsCPK*21-RNAi 转基因植株在花药发育第 10 期时花粉发育严重缺陷,花粉细胞死亡[62]。*OsCPK*31 过表达转基因植株种子灌浆期提前,成熟期缩短,表明 *OsCPK*31 对水稻种子的灌浆和成熟有重要作用[63];*OsCDPK*1 在种子发育中期大量表达,负调控直链淀粉含量、胚乳透明度和种子大小[64]。

4.2　CDPKs 参与调控植物激素信号通路　CDPKs 参与调控赤霉素(Gibberellin,GA)生物合成或信号通路。CDPKs 参

与 GA 合成主要是通过影响 GA20-氧化酶（GA20ox）和 GA3-羟基化酶（GA3ox）来调控活性 GA 的合成[65]。AtCPK28 促进 GA3ox1 合成，增加活性 GA 水平，正调控 GA 稳态[26]。NtCDPK1 使 bZIP 转录因子 RSG 失活，下调 GA20ox1，负调控 GA 稳态[66-67]。OsCDPK1 负调控 GA20ox1 和 GA3ox2 的表达，抑制 GA 合成[68]。NtCDPK1 与 14-3-3 蛋白的非磷酸化基序结合，调节叶鞘生长[18]。此外，外源 GA 处理后，OsCDPK13、NtCPK4、IiCDPK2 表达均上调[69-71]。

CDPKs 参与生长素转运过程。AtCPK3/4 磷酸化马铃薯糖蛋白相关磷脂酶 AtPLA IVA 和 IVB 调控生长素信号通路[72]。体外试验表明 StCDPK1 可以磷酸化生长素运输载体 StPIN4 从而调控生长素水平[73]。

CDPKs 响应乙烯信号并影响乙烯生物合成。StCDPK5 可以被乙烯诱导表达，在番茄花梗脱落过程中发挥重要作用[74]。AtCDPK16 通过磷酸化 ACC 合成酶 AtACS7，参与调控拟南芥根的向重力性[75]。

CDPKs 响应茉莉酸（Jasmonic Acid，JA）信号并参与调控 JA 生物合成。NtCDPK4 和 NtCDPK5 响应 JA 信号，影响 JA 早期生物合成 [76]。*Atcpk*28 突变体生长缓慢，JA 相关基因表达量升高，JA 代谢物增加[77]。

CDPKs 参与 ABA 诱导的气孔运动和响应 ABA 信号。AtCPK13 主要在保卫细胞表达，响应 ABA 信号，通过磷酸化并抑制 2 个 K^+ 内流通道：AtKAT1 和 AtKAT2，使气孔开放[78]。AtCPK6 通过磷酸化 ABA 响应元件结合因子，正调控 ABA 信号和耐旱性[79]。AtCPK9 通过调控离子通道活性，负调控 ABA 介导的气孔运动[80]。OsCPK9/12/21 已被证实可以响应 ABA 信号[81-83]。

4.3　CDPKs 参与植物生物胁迫反应　植物进化出了有效的免疫系统来抵御各种病原微生物的攻击。在第一层防御中，病原微生物信号（microbe-associated molecular patterns，MAMPs）被植物细胞膜上的模式识别受体（pattern recognition receptors，PRRs）感知，引起机体免疫反应（pattern-triggered immunity，PTI）：ROS 的增加或诱导致病相关基因表达等[84-85]。在 PTI 信号通路中，PRRs 感知 MAMPs 会引发激酶介导的复杂信号反应，包括 MAPK 级联反应和 CDPKs 介导的信号转导[86-87]。

过表达 *AtCPK*1 可诱导水杨酸积累和水杨酸调控的防御抗病基因的组成型表达，从而对病原体感染具有广谱保护作用[88]。当病原体感染拟南芥时，AtCPK5 被快速激活并磷酸化 NADPH 氧化酶 AtrbohD，激活植物防御机制；AtCPK5 在 exo70B1 介导的自身免疫中也发挥重要作用[89-90]。OsCPK18-OsMPK5 通路抑制防御相关基因表达，负调控水稻对稻瘟病的抗性，过表达 *OsCPK*4 可以提高水稻抗病性[91-92]。

4.4　CDPKs 参与植物非生物胁迫反应　研究表明，干旱、高温、低温、盐胁迫均可引起 CDPKs 特异性表达[93]。通过对寒冷、干旱和盐胁迫条件下的水稻进行微阵列分析，发现 6 个 *OsCDPK* 基因（OsCPK4/10/12/13/15/21）表达上调，1 个（*OsCPK*1）表达下调[5,94]。

干旱引发的植物细胞反应包括通过调节保卫细胞来诱导 ABA 介导的气孔运动、通过积累渗透物质调节渗透压、通过 ROS 稳态调节氧化损伤[95-96]。干旱条件下，*OsCPK*9 过表达转基因植株，通过调节渗透压诱导气孔关闭，提高花粉活性和小穗育性[82]；OsCPK10 通过调节过氧化氢（H_2O_2）酶的积累和脂质过氧化水平，保护了细胞膜的完整性，提高了水稻对 H_2O_2 的解毒能力，增强了水稻的耐旱性[97]。

盐胁迫通过积累 Na^+ 和 Cl^+ 导致胞内离子不平衡，使植物发生氧化应激反应，同时还会增加植物体内 ROS 的产生[98]。AtCPK27 和 AtCPK12 通过调节离子稳态和 ROS 稳态提高植株耐盐性[99-100]。OsCPK21 与 OsGF14e 相互作用并在 Tyr-138 位点磷酸化 OsGF14e，通过对 OsGF14e 的转录后调控来响应 ABA 信号和盐胁迫[101]。ZmCPK11 通过调节 Na^+ 和 K^+ 的体内平衡以及稳定光系统Ⅱ来提高转基因拟南芥植株的耐盐性[102]。

CDPKs 参与植物响应冷胁迫和热激反应。水稻 OsCPK17 以蔗糖合酶和质膜固有蛋白为底物，是冷应激反应所必需的[103]；OsCPK24 通过 Ca^{2+} 信号通路，抑制谷氨酰胺多辛（OsGrx10），使其维持较高的谷胱甘肽水平和磷酸化状态，正调控水稻对冷胁迫的响应[104]。香蕉 MaCDPK7 是热激诱导的果实成熟和冷胁迫的正调控因子[105]。

5　CRKs 的生物学功能

CRKs 在调控植物生长发育、响应生物和非生物胁迫中发挥重要作用。

拟南芥 *crk*5 突变体根长和侧根数目减少，根的向重力性改变。研究表明 AtCRK5 通过磷酸化 PIN2 参与根的向重力性生长[37]。此外，研究发现连续光照使拟南芥 *crk*1 突变体出现严重的表型缺陷（侏儒症和萎黄病），暗示拟南芥 CRK1 可能参与了光调控的植物生长发育[42]。SlCRK6 正调控番茄对 Pst DC3000 和核盘菌（Sclerotinia sclerotiorum）的调控[106]。

6　结语

截至目前，CDPKs 的作用机制以及生物学功能等研究已相对成熟，但其底物的多样性还有待进一步发现。研究发现 CDPKs 与 14-3-3 蛋白间的调控关系十分复杂，但二者的交叉磷酸化调节机制以及这种调节作用对下游靶基因的影响还需要进一步探究；同时，关于二者是如何保持信号的特异性并引起特异性应答反应，目前也知之甚少，有望成为今后科学工作者致力研究的方向。

与 CDPKs 相比，CRKs 生物学功能的信息非常有限。鉴于 CRKs 和 CDPKs 高度同源，希望该研究对 CDPKs 研究进展的总结，能够为相关科研工作者研究 CRKs 的功能提供思路和方向。

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同,移栽基质的配比往往决定了组培苗的粗壮程度、长势以及根系的生长情况。

表 4　不同基质配方成活率比较

Table 4　Survival rate of seedlings with different matrix formulations

配方 Formula	根数 Root number	根长 Root length//cm	移栽成活率 Transplant survival rate//%
CK	5 cdCD	4.38±0.04 eF	85.50±0.27 cdC
T_1	4 dD	4.30±0.05 eF	75.62±0.54 hF
T_2	6 cdBCD	5.16±0.04 dE	82.56±0.27 fD
T_3	6 cdBCD	5.52±0.14 cdCDE	85.03±0.27 dC
T_4	6 cdBCD	5.30±0.12 dE	83.64±0.27 eD
T_5	7 bcABC	5.80±0.60 bcBCD	81.17±0.26 gE
T_6	8 abAB	6.13±0.05 abAB	73.92±0.27 hG
T_7	7 bcABC	5.96±0.07 bABC	87.96±0.47 bB
T_8	9 aA	6.34±0.10 aA	92.90±0.54 aA
T_9	6 cdBCD	5.39±0.04 dDE	85.96±0.53 cC
T_{10}	6 cdBCD	5.16±0.04 dE	83.49±0.96 eD

注:同列不同大写字母表示差异极显著($P<0.01$);不同小写字母表示差异显著($P<0.05$)

Note:Different capital letters in the same column indicated extremely significant difference($P<0.01$),and different lowercase letters indicated significant difference($P<0.05$)

该研究选配的各个基质配方中,T_1、T_2 和 T_{10} 持水孔隙不满足要求;T_6 和 T_7 的 EC 值偏高(大于 3.0 ms/cm),不满足要求。经过比较分析后发现 T_4、T_5、T_8 和 T_9 符合作物栽培理想基质的要求。经不同基质配方生长指标和成活率比较分析,T_8 以泥炭土∶珍珠岩∶黄土=2∶1∶1为非洲菊新品种“明卉傲阳”最佳育苗基质配方。

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