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植物应答低温胁迫的分子调控网络

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摘要 植物在生长发育过程中会遭受各种生物与非生物胁迫的影响. 低温是主要的非生物逆境之一, 严重影响农作物的生长发育、产量品质及地理分布. 在长期适应环境的过程中, 植物进化出了一系列抵御低温逆境的冷响应方式, 如冷锻炼(cold acclimation). 低温信号被植物感受后, 能够迅速激活多条信号通路, 调控下游冷响应基因的表达, 从而调节植物细胞的生理生化状态, 进而增强植物对低温的耐受能力. 本综述探讨了植物感知及应答低温信号的作用机理, 并着重概述了CBF/DREB1(C-repeat binding factor/dehydration-responsive element binding factor 1)信号通路在调控植物/作物应答低温过程中的作用.

关键词 低温胁迫, 冷驯化, 低温信号感知, 低温信号转导, CBF信号通路

植物在自然环境中常面临季节性温度变化, 而近年来气候变化导致的温度波动已对全球农作物产量造成显著影响[1]. 低温胁迫(cold stress)是制约植物生长发育和地理分布的重要环境因子之一. 根据温度的阈值, 研究人员将低温胁迫分为冷胁迫(chilling stress, 0~15°C和冻胁迫(freezing stress, <0°C[2]. 不同地理起源的植物对低温的耐受能力存在显著差异, 据此可以分为冷敏感植物和耐寒植物. 每类植物的生长都有其特定的最高、最佳和最低温度范围[3]. 冷敏感植物主要起源于热带和亚热带地区, 包括玉米(*Zea mays*)和水稻(*Oryza sativa*)等主要农作物. 其生长发育通常需要较高温度, 一般15°C以下的低温即可造成不利影响. 相比之下, 耐寒植物如拟南芥(*Arabidopsis thaliana*)和冬小麦(*Triticum turgidum*)多生长于温带地区, 甚至能够耐受零下低温.

在自然条件下, 随着秋季温度逐渐降低, 许多草本和木本植物会通过一系列生理生化反应增强对冬季低温的适应能力[4]. 这一过程被称为冷驯化(cold acclimation), 即植物在经历非致死低温后获得更强耐冻能力的过程[5]. 冷驯化过程中, 植物在形态、生理、生化和分子水平上发生显著变化, 包括生长抑制、光合效率降低、抗冻蛋白积累及渗透调节物质合成等[6]. 深入研究植物对温度的感知和响应机制, 将为利用现代生物技术提高植物低温抗性提供理论依据[7]. 本文综述了近年来植物低温感知及应答机制的研究进展, 以期为该领域的未来研究提供参考和启示.

1 植物低温信号感知机制

在应对环境变化的过程中, 感知是生物感受器通过改变自身结构或与其他分子相互作用, 将外界刺激解码并触发下游反应的关键第一步[8]. 在低温胁迫下, 植物通过潜在的低温感受器(cold sensor)识别低温信号, 并通过不同的信号转导通路将信号传递到细胞核, 调控一系列冷响应(cold responsive, *COR*)基因的表达, 从而增强植物的耐低温能力. 最近研究表明, 跨膜蛋白介导的胞内小分子内流以及蛋白质液-液相分离在低

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温感知及信号传递中发挥重要作用[9].

1.1 第二信使

质膜是一种温度敏感的结构，其流动性能够灵敏地反映环境温度的变化[10,11]. 传统研究认为，细胞膜是低温感知的初始位点，低温下细胞膜脂肪酸的组成及不饱和程度的改变与膜流动性及植物的低温耐受性密切相关[12,13]. 随着研究的深入，越来越多的证据表明，低温下细胞膜定位的膜结合蛋白也参与温度信号的感知过程. 这些蛋白能够介导低温诱导的细胞质钙离子(Ca^{2+})、环腺苷酸(cyclic adenosine monophosphate, cAMP)、肌醇三磷酸(inositol 1,4,5-trisphosphate, IP3)等第二信使分子浓度的迅速升高，形成低温特异信号，从而启动下游信号通路.

2015年，中国科学院植物研究所种康课题组发现水稻OsCOLD1(chilling tolerance divergence 1)编码一种G蛋白调节因子，定位于质膜和内质网. 研究表明OsCOLD1通过与G蛋白α亚基相互作用，激活钙离子通道并促进钙内流，从而正调控水稻耐低温能力[14]. 此外，该课题组也鉴定到其他钙信号调控相关蛋白，如水稻OsCRT3(calreticulin 3)以及OsCIPK7(CBL-interacting protein kinase 7). 内质网定位的OsCRT3 蛋白在低温刺激下发生构象变化，促进其与OsCIPK7 的相互作用并增强OsCIPK7的激酶活性，从而介导低温诱导的钙离子内流[15,16]. 最近的研究发现，OsCOLD6与冷诱导的OsOSM1(osmotin-like 1)形成低温感知复合物，通过提高 2′,3′-cAMP水平增强水稻的耐冷性[17]. 这些研究表明，膜蛋白激活的胞内第二信使对水稻耐冷性起重要作用(图1(a)).

本课题组鉴定了拟南芥钙离子通道CNGC20(cyclic nucleotide-gated channel 20)[18]以及潜在的钙离子转运体ANN1(annexin 1)[19]参与低温诱导的钙离子内流. 此外，钙依赖的蛋白激酶CPK28(calcium-dependent protein kinase 28)在低温诱导的钙信号的感知中起关键作用. CPK28定位于细胞膜上，其活性能够被低温快速激活，磷酸化转录因子NLP7(NIN-like protein 7)，使其从胞质进入细胞核调控冷响应基因的表达[20](图1(a)). 然而，这些蛋白是否直接介导植物低温信号的早期感知过程仍需进一步验证.

1.2 蛋白质液-液相分离

温度是调控相分离(phase separation)的重要因子之一，能够影响相分离体系的熵驱动作用，从而调控凝聚体的形成. 研究表明，蛋白分子能够通过液-液相分离(liquid-liquid phase separation, LLPS)直接感受温度变化. 例如，拟南芥ELF3(early flowering 3)蛋白在温度升高时发生相分离，从而感知环境温度的变化[21]. ELF3 是拟南芥生物钟夜间复合体(evening complex，EC)的组分之一，参与调控植物生长发育. 在较低的温度下(5~15°C)，ELF3与ELF4及LUX形成转录抑制复合体，在细胞核内弥散分布，抑制开花基因*FT*的表达. 温度升高时，ELF3发生相分离形成凝聚体，解除对下游基因的转录抑制，促进植物生长和开花[21,22]. ELF3蛋白包含一段富含polyQ(polyglutamine)重复序列的PrD(prion-like domain)结构域. 体外实验表明，ELF3 PrD结构的体外重组蛋白能够在温度升高时发生液-液相分离形成液滴，而在温度降低时液滴逐渐消失，表明该蛋白能够直接感知温度变化[21].

此外，光敏色素phyB(phytochrome B)通过光控可逆构象转变与温控液-液相分离，实现植物对光照与温度两种关键环境因子的同时感知[23]. 研究表明，phyB的NTE(intrinsically disordered N-terminal extension)结构域能够直接感知温度变化并发生相分离形成凝聚体(图1(b)). 隐花色素CRY2(cryptochrome 2)是重要的蓝光受体，能够在蓝光刺激下发生相分离形成凝聚体[24]. 我们之前的研究发现，低温能够增强CRY2蛋白的稳定性，从而正调控拟南芥的耐冻性[25]. 然而，CRY2是否同样作为潜在的感受器通过相分离感知温度变化仍需进一步探究.

2 植物低温信号转导

当植物感知低温后，细胞会在多个层面发生响应性变化，包括染色质修饰、转录、转录后加工和翻译后修饰等(图1(c, d))，这些变化反映了低温下基因表达调控的复杂性. 目前，植物响应低温的转录调控网络研究最为深入[26]. 通过对拟南芥低温处理多个时间点的基因芯片数据进行系统分析，发现基因的转录上调或下调呈现多种表达模式[27,28]. 研究表明，低温下大部分转录上调的基因主要在增强胁迫耐受性中发挥作用[29]，包括编码转录因子、糖和脯氨酸等渗透物合成酶、蛋白激酶、解旋酶、伴侣蛋白以及抗冻蛋白的基因. 这些转录变化的基因通常被称为冷响应(*COR*)基因[30].

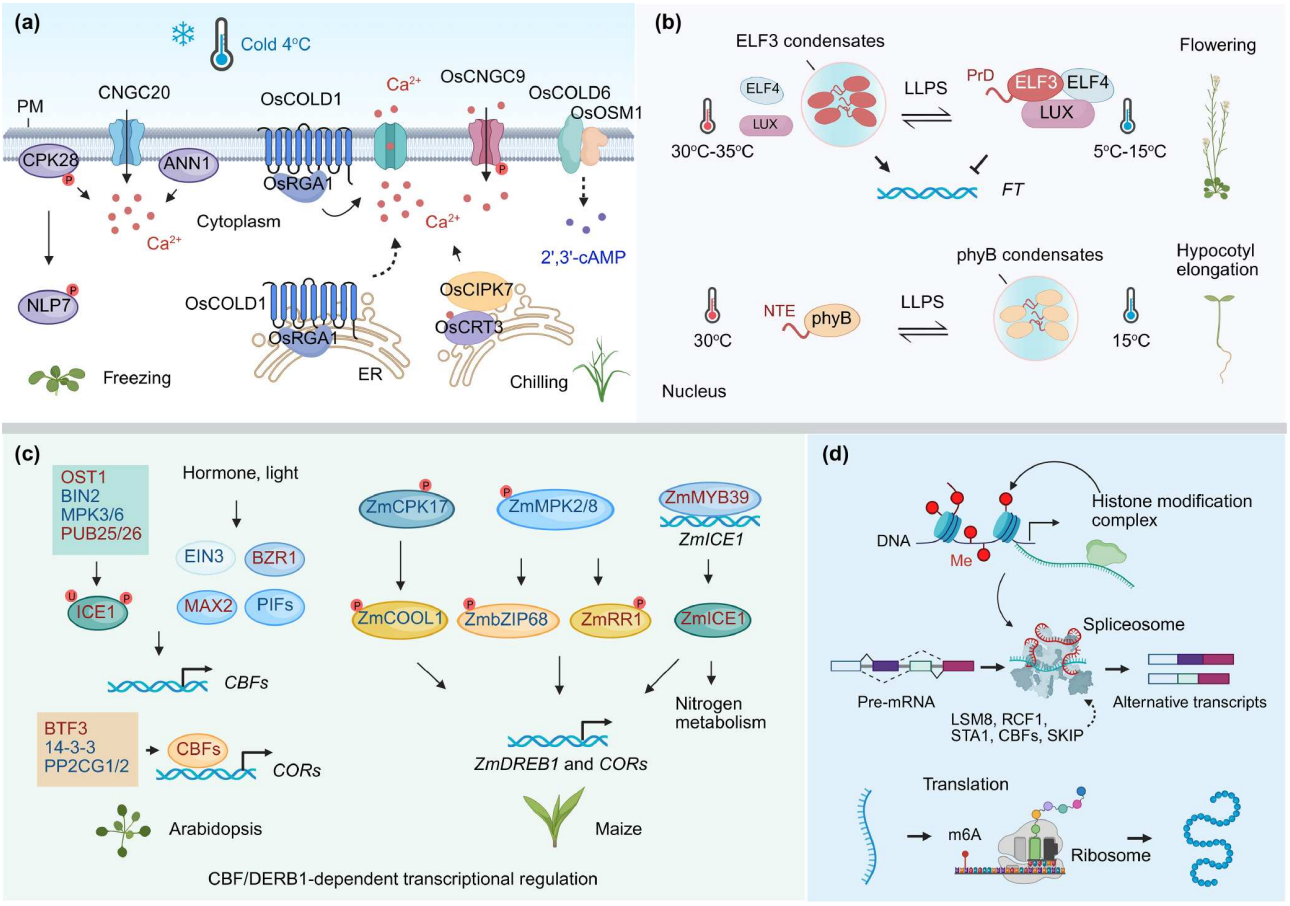

图 1 植物感知及响应低温的机制示意图. (a) 膜蛋白在低温信号感知和转导中的作用; (b) 细胞核定位的蛋白通过液-液相分离感知温度信号; (c) 依赖于CBF/DREB1的转录调控网络, 图中红色/蓝色字体分别表示基因正/负调控植物耐冷性; (d) 植物细胞通过表观遗传、可变剪接及翻译等多层次调控响应低温信号(使用BioRender.com制作)

Figure 1 Schematic representation of plant perception and response mechanisms to cold stress. (a) The role of membrane-located proteins in cold signal perception and transduction. (b) The mechanism by which nuclear-localized proteins sense temperature signals through liquid-liquid phase separation. (c) The CBF/DREB1-dependent transcriptional regulatory network, with red and blue fonts indicating genes that positively or negatively regulate plant cold tolerance, respectively. (d) The multi-layered celluar responses to cold signals, including epigenetic modifications, alternative splicing, and translational regulation, highlighting the complexity of plant cold stress adaptation (created with BioRender.com)

2.1 依赖及不依赖于CBF/DREB1 的低温信号通路

尽管近年来科研人员在转录水平、转录后水平及翻译后水平的调控机制上开展了大量研究, 但目前最为清楚的是依赖于CBF/DREB的低温信号通路(图1(c))[31]. CBF是一类ERF/AP2(ethylene-responsive/apetala 2)类型转录因子, 拟南芥中*CBF*基因包括四个成员, 分别为*CBF1~4*. 其中, *CBF1*、*CBF2*、*CBF3*(以下简称*CBF*s)串联排列在拟南芥基因组第4号染色体8.7 kb的区域内[32,33]. 研究发现, *CBF1*、*CBF2*、*CBF3*的转录水平被低温迅速诱导, 并翻译成蛋白在细胞核内大量积累[5]. CBFs蛋白能够识别并结合下游*COR*基因序列中的CRT/DRE(C-repeat/dehydration-responsive element)元件, 激活这些基因的表达, 从而介导植物低温应答[34,35].

*CBF*s是调控植物耐冻性的正调控因子[36,37]. 目前已鉴定到大量低温响应组分, 如转录因子、蛋白激酶、E3 泛素连接酶等, 能够直接或间接调控CBFs的转录或蛋白稳定性, 从而参与植物的低温应答过程[9]. 例如, 早期研究发现的转录因子ICE1(inducer of *CBF* expression 1)和CAMTAs (calmodulin binding transcription activators)能够激活*CBF*s的表达, 是植物耐冷性的正调控因子[38~40]. 此外, *CBF*s的转录还受到激素信号、光信号通路中多种调控因子的调控[9]. 例如, 光信号通路中的

PIF3(phytochrome-interacting factor 3)和乙烯信号通路中的EIN3(ethylene insensitive 3)负调控*CBF*s的表达, 而独脚金内酯信号通路中的MAX2(more axillary growth 2)和油菜素甾醇信号通路BZR1/BES1(brassinazole-resistant 1/BRI1-EMS suppressor 1)则正调控*CBF*s的表达[41~44].

CBFs信号通路中关键组分的翻译后修饰对其低温下的转录调控同样重要. 我们的研究表明, *CBF*s的上游调控因子ICE1 蛋白的泛素化修饰及磷酸化修饰影响其在低温下的活性和稳定性. 例如, 低温下PUB25/26(plant U-box)通过不同的泛素化修饰动态调控ICE1 的蛋白稳定性[45]; 蛋白激酶OST1(open stomata 1)、BIN2(brassinosteroid insensitive 2)和MPK 3/6(mitogen-activated protein kinase 3/6)通过磷酸化调控ICE1的蛋白稳定性[46~48]. 这些组分通过介导ICE1的翻译后修饰间接影响*CBF*s的表达, 从而调控植物的耐冻性. 此外, 本课题组还发现BTF3(basic transcription factor 3)、14-3-3以及PP2CG1/2(protein phosphatase 2C G group 1)能够直接参与CBFs蛋白水平的调控, 影响*COR*基因的表达[49~51]. CBFs还能整合光温信号, 例如低温下CBFs蛋白与PIF3相互作用, 抑制phyB的蛋白降解[52]. 光刺激条件下, CBF1通过促进PIF4/5 的蛋白积累调控低温下(17°C)拟南芥下胚轴的生长[53].

CBF/DREB1依赖的信号通路对于作物耐低温能力也起重要作用. 本课题组在玉米中鉴定到多个组分参与*CBF*/*DREB1*基因的转录调控. ZmICE1不仅激活*ZmDREB1*基因, 还抑制关键的谷氨酸/天冬氨酸生物合成基因(如*ZmASs*)的表达, 从而调控线粒体活性氧(reactive oxygen species, ROS)的产生和玉米耐冷性. ZmICE1启动子-465位点的自然变异影响其与*ZmMYB39*的结合能力, 从而调控*ZmICE1*的表达和玉米耐冷性[54]. 此外, 两个C亚族MPK成员(ZmMPK2 和ZmMPK8)负调控玉米耐冷性[55,56]. ZmMPK8在冷下被激活, 磷酸化并促进玉米响应调节子ZmRR1(response regulator 1)的降解, 抑制下游*ZmDREB1*及编码纤维素合成酶的基因*ZmCesA*的表达[56]. ZmRR1^{HapA}单倍型包含一个与玉米耐冷性高度相关的自然变异(InDel-35), 其保守的磷酸化位点(Ser15)能够防止其被ZmMPK8磷酸化进而被泛素化降解[56]. 此外, ZmMPK8还磷酸化转录因子ZmbZIP68, 增加其蛋白稳定性从而抑制*ZmDREB1*的表达[55]. 这些研究表明, MPK信号在玉米冷胁迫响应调控中发挥重要作用. 本课题组最新的研究鉴定出一个负调控玉米耐冷性的基因*ZmCOOL1*(*cold-responsive operation locus 1*). 该基因的自然变异能够通过增强低温耐受性促进其对高纬度地区的适应性. 该研究首次揭示了玉米适应高纬度低温气候的分子机制. 研究还发现, 低温激活ZmCPK17的激酶活性, 促进其从细胞质转移到细胞核并磷酸化ZmCOOL1, 增强其蛋白稳定性, 从而抑制*DREB1*及*TPS*(*trehalose-6-phosphate synthase*)的表达[57]. 这些研究均表明, CBF/DREB1依赖的转录调控网络对玉米耐冷性具有至关重要.

转录组数据分析表明, 拟南芥CBFs仅调控了约10%的冷响应基因[35], 因此还存在不依赖于CBFs的信号通路. 例如, 拟南芥转录因子HSFC1(heat shock transcription factor C 1), ZAT12/RHL41(responsive to high light 41), SZF2(salt-inducible zinc finger 2), 以及中介体复合体(mediator complex)相关亚基MED2(mediator 2)、MED14、MED16也参与了冷响应基因的调控[58]. CRY2通过独立于CBFs通路的转录因子BBX7/8(B-box domain protein)调控冷响应基因的表达[25]. 此外玉米ZmbZIP68转录因子直接作用于热激转录因子*ZmHSF21*的上游, 抑制其基因表达. ZmHSF21通过调控脂质代谢稳态而非依赖于ZmDREB1, 在幼苗和发芽阶段增强玉米的耐冷性[59,60]. 最近一项研究发现, 高原粳稻的HD-ZIP型转录因子CTB5(cold tolerance at the booting stage 5)在介导高原粳稻冷适应性生长与驯化中起重要作用. CTB5与另外一个HD-ZIP类转录因子OsHox12相互作用, 调控赤霉素信号通路中关键靶基因的表达, 从而影响水稻耐冷性[61]. 上述研究说明不依赖于CBF/DREB1通路的信号组分在调控植物耐冷性中也发挥重要的作用.

2.2 转录后水平的可变剪接调控

Pre-mRNA的剪接是转录后加工的关键步骤, 包含组成型剪接(constitutive splicing)和可变剪接(alternative splicing). 组成型剪接将内含子完全从mRNA前体中去除, 产生一种成熟的mRNA, 而可变剪接则通过不同的剪接方式产生多个mRNA剪接异构体, 导致转录本和蛋白的结构和功能呈现多态性[62,63]. mRNA前体的剪接由剪接体(spliceosome)完成, 剪接位点的正确识别是移除内含子的关键步骤, 当剪接位点错误识别时, 可变剪接事件就会发生[62,63].

低温胁迫会诱导大量冷响应基因发生可变剪接变化, 从而影响植物的低温响应. 例如, 拟南芥低温处理

后的转录组时间序列分析表明, 低温处理 6 h后基因的转录本形式变化与转录量变化同时达到顶峰, 暗示可变剪接在植物低温应答中的重要性[28]. 近期研究人员结合水稻不同品种低温胁迫下的泛转录组、重测序数据以及分子实验, 发现可变剪接在冷应答中的重要作用, 并鉴定出水稻低温适应性驯化的关键基因*OsRS33*和*OsRS2Z38*[64]. 低温下基因的可变剪接能够显著提高植物的低温适应性. 例如, 马铃薯(*Solanum tuberosum* L.)转化酶抑制基因*INH2* 的剪接变化参与低温储藏过程中蔗糖含量的调控[65]. 此外, 低温与生物钟调控相关基因的可变剪接也密切相关[66]. 目前已鉴定到多个参与冷响应基因可变剪接的组分, 包括LSM8(sm-like 8)、STA1(stabilized 1)和RCF1(regulator of *CBF* gene expression 1)[67~69]. 本课题组最新的研究表明拟南芥CBFs也参与冷响应基因的可变剪接. 低温下CBFs能够促进剪接因子SKIP(SKI-interacting protein)形成核凝聚体, 富集特定的冷响应基因转录本, 从而提高其剪接效率[70].

3 展望

本综述总结了近年来植物感受及响应低温信号的研究进展, 这些研究极大地深化了我们对植物耐低温的分子机制的理解, 但仍有许多未解之谜值得深入探索. 例如, 在植物感知温度信号的机制研究中, 部分研究表明膜结合蛋白比如钙离子通道以及类受体激酶可能参与低温信号的感知[9,14,50]. 细胞膜作为细胞与外界环境的分界屏障, 研究膜结合蛋白在温度感知中的作用重要的理论和实践意义. 然而, 目前尚缺乏充分的生化证据证明这些组分能够直接感知低温信号. 此外, 近期多项研究表明, 蛋白分子液-液相分离能够直接感知环境温度变化[21,71~73]. 这些组分主要定位于细胞核, 暗示植物细胞可能通过多种方式感知低温信号. 因此, 温度作为一种物理信号, 其感知机制仍需在未来的研究中系统解析.

植物细胞感知到温度变化后, 会在多个层面发生响应. 本综述重点阐述了mRNA调控在低温响应中的作用, 特别是蛋白编码基因的转录及可变剪接在植物耐冷性调控中的功能. 然而, 越来越多的研究表明, 基因的表观修饰变化以及RNA翻译效率的变化同样对植物耐冷性具有重要影响(图1(d)). 例如, REIL (rei1-like)蛋白能够调控冷诱导的核糖体重塑(ribosome remodeling)过程, 促进胞质核糖体亚基的积累[74], 并通过增强拟南芥*CBF*s的翻译提高植物的耐冻性[75]. MTA1 (methylase A)通过介导m^{6}A(N^6-methyladenosine)修饰, 提高二酰基甘油酰基转移酶*DGAT1*(*diacylglycerol acyltransferase 1*)的翻译效率, 从而调控低温下拟南芥的生长[76]. 在16°C条件下, H3K36me3(histone H3 lysine 36 tri-methylation)修饰的变化会影响RNA的可变剪接事件, 进而调控拟南芥的开花过程[77,78]. 此外, RNA的结构变化(如G四联体的形成)也被证明有助于植物适应低温环境[79]. 除了蛋白编码mRNA以外, 低温还能诱导非编码RNA (ncRNA)的产生. 例如, *FLC*(*flowering locus C*)基因反义链转录产生的长链非编码RNA(lncRNA) *COOLAIR*能够影响FRIGIDA蛋白的凝聚, 调控*FLC*的表达, 从而调控拟南芥在低温下开花[80]. 因此, 未来对mRNA结构、翻译效率、表观修饰以及非编码RNA的研究将有助于更全面理解植物如何应答低温胁迫.

此外, 低温下代谢物质的变化对植物耐冷性调控也具有重要意义. 本课题组发现氮代谢和脂代谢途径中的关键酶参与调控玉米的耐冷性[54,60]. 之前研究表明, 拟南芥CBF与糖代谢过程密切相关, 但其具体调控机制尚不清楚[81,82]. 因此, 在未来进一步探究代谢调控通路在植物耐冷性中的作用将是一个重要的研究方向. 通过整合分子、代谢和表观遗传层面的研究, 我们将能够更全面地揭示植物适应低温胁迫的复杂调控网络, 为作物抗逆育种提供新的理论依据和技术支持.

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Summary for “植物应答低温胁迫的分子调控网络”

The molecular regulatory network governing plant response to cold stress

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Cold stress is one of the most important abiotic stresses that can severely limit plant growth, development, and agricultural productivity. To adapt to cold stress, plants have developed sophisticated mechanisms to perceive, transduce, and respond to cold signals. This review focuses on the molecular mechanisms underlying plant responses to cold stress, with particular attention to cold stress signal perception, signal transduction pathways, and regulatory mechanisms that modulate cold stress tolerance.

The first step in plant cold stress response is the perception of cold stress. Plants sense temperature changes through alterations in cellular structures such as membranes, the cytoskeleton, and organelles, leading to the activation of specific signaling pathways. Recent studies have highlighted the role of second messengers, such as calcium ions (Ca^{2+}), reactive oxygen species (ROS), and inositol phosphates, in this early response. These molecules function as key intracellular signals that trigger downstream signaling cascades, activating the plant’s cold stress response mechanisms. A novel aspect of cold stress signal perception involves protein liquid-liquid phase separation (LLPS). LLPS enables certain cold-responsive proteins to form biomolecular condensates that spatially and temporally regulate the activity of signaling molecules, enhancing the cold stress response. Following the initial perception of cold stress, signal transduction pathways are activated to coordinate gene expression changes necessary for cold acclimation. The CBF/DREB1 (C-repeat binding factor/Dehydration responsive element binding protein 1) pathway is one of the most studied cold stress-responsive pathways. Upon cold exposure, the transcription factors CBF/DREB1 are rapidly induced and bind to C-repeat (CRT)/dehydration-responsive elements (DRE) in the promoters of cold-responsive (COR) genes, leading to the activation of these genes. The expression of COR genes is critical for improving cellular tolerance to cold stress by enhancing membrane stability, modulating osmotic balance, and detoxifying reactive oxygen species. The regulation of CBF genes involves complex transcriptional control, including the involvement of upstream regulators such as ICE1 (Inducer of CBF expression 1) and various other regulators.

In addition to the CBF/DREB1-dependent pathway, plants also rely on CBF-independent signaling mechanisms to respond to cold stress. These alternative pathways involve various transcription factors, including the ZAT, HSFC1, and CAMTA families, which regulate cold-responsive gene expression through different molecular mechanisms. Hormonal signaling pathways, particularly those involving abscisic acid (ABA), ethylene, and jasmonic acid, also play crucial roles in modulating the plant’s cold stress response. The integration of these signaling networks provides a flexible response to fluctuating cold stress, allowing plants to optimize their survival strategies. Moreover, post-transcriptional regulation, especially alternative splicing (AS), has emerged as a key mechanism in modulating cold stress responses. Alternative splicing of pre-mRNAs leads to the generation of distinct isoforms of key regulatory proteins, thereby increasing the diversity of the proteome under cold stress. This flexibility allows plants to fine-tune their cold stress responses by producing functionally distinct protein variants that may have enhanced or specialized activities under cold stress conditions.

Together, these insights highlight the complexity and multi-layered nature of plant responses to cold stress. Understanding the molecular basis of cold stress perception and signal transduction can provide valuable information for developing strategies to improve cold tolerance in crops, particularly through genetic engineering and breeding approaches.

cold stress, cold acclimation, cold signal perception, cold signal transduction, CBF signaling pathway

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