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·热点评·

解析植物冷信号转导途径：植物如何感知低温

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摘要 低温胁迫(冷害和冻害)严重影响植物的生长发育和地理分布，是制约作物产量和品质的主要因素之一。在自然界，植物通过感知低温信号并启动一系列响应机制来抵御冷冻伤害。MAP蛋白激酶家族在植物响应逆境胁迫信号过程中发挥重要作用，但其是否参与冷冻胁迫信号传递仍不清楚。最近，朱健康、杨淑华和种康研究团队先后报道了拟南芥(*Arabidopsis thaliana*)和水稻(*Oryza sativa*)通过MAPK级联反应途径参与冷冻胁迫应答反应，通过磷酸化ICE1来调控其稳定性，并阐明了ICE1提高植物抗冷冻能力的分子机制。他们的研究完善了ICE1介导的低温应答网络，是植物低温应答研究领域的重要突破，并为未来的作物分子设计育种提供了强有力的理论依据。

关键词 低温应答，磷酸化，MAPK级联反应，ICE1，OsTPP1

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CBF (C-repeat-binding factor)是一类转录因子，它能激活下游多个冷胁迫响应基因的表达，进而增强植物抗冻性(Jia et al., 2016; Zhao et al., 2016; 刘静妍等，2017)。早在2003年，朱健康实验室利用荧光素酶(luciferase，LUC)建立了一个生物荧光报告筛选系统，结果发现了*CBF*基因的上游转录因子ICE1 (inducer of *CBF* expression 1) (Chinnusamy et al., 2003)。近年来的研究表明，ICE1的稳定性对植物低温应答至关重要(Dong et al., 2006; Miura et al., 2007; Ding et al., 2015)，但是调控ICE1稳定性的分子机制仍未研究清楚。最近，朱健康、杨淑华和种康研究组分别在拟南芥(*Arabidopsis thaliana*)和水稻(*Oryza sativa*)系统中，先后阐述了植物中可能存在的多种低温应答机制及相互协同的调控网络(图1)，建立了低温条件下MPK激活与ICE1稳定性之间的信号通路。他们的研究结果是植物低温应答调控领域的重要突破(Zhao et al., 2017; Li et al., 2017; Zhang et al., 2017)。

在拟南芥系统中，朱健康研究组通过定量磷酸化蛋白组学分析首先发现：低温处理下，MPK3、MPK4和MPK6蛋白磷酸化水平明显升高(Zhao et al., 2017)；同时，杨淑华研究团队利用酵母双杂交系统证实，MPK3和MPK6蛋白能与ICE1互作(Li et al., 2017)。这些结果暗示，MPK3和MPK6可能通过作用于ICE1在植物低温应答过程中发挥重要作用。为了验证这一推测，他们分析了*mpk3*和*mpk6*单突变体以及化学诱导型*mpk3*/*mpk6*双突变体*MPK6SR* (可回复*mpk3*/*mpk6*胚胎致死表型)在冷冻处理下的表型。结果发现，与野生型植株相比，突变体存活率高、离子渗漏水平低，而MKK5的组成型激活材料*MKK5*^{DD}在冷冻胁迫下表现出与突变体相反的表型。这些实验结果均表明，MPK3和MPK6是低温应答过程中的负调控因子。为了揭示MPK负调控作用的分子机制，他们分别利用体内和体外磷酸化实验证明，ICE1是MPK3和MPK6的磷酸化底物。生物信息学分析表明，ICE1具有6个可能的MPK激酶磷酸化位点(S94P、S203P、T366P、T382P、T384P和S403P)。利用点突变材料，他们证实了Ser94、Thr366和Ser403是MPK3/6磷酸化的位点，参与ICE1稳定性的调控(Zhao et al., 2017; Li et al., 2017)。前期研究表明，低温胁迫诱导ICE1降解(Dong et al., 2006; Ding et al., 2015)，那么MPK3/6是否通过磷酸化ICE1并诱导其降解，从而在植物低温应答过程中发挥负调控作用？朱健康和杨淑华研究团队分别利用组成型激活材料*MKK5*^{DD}、超表达ICE1的*mpk3*、*mpk6*和*MPK6SR*突变体以及磷酸化位点突变的*ice1*等多种突变体证明，低温胁迫下MPK3/6由MKK5激活后磷酸化ICE1，磷酸化促进了ICE1降解，

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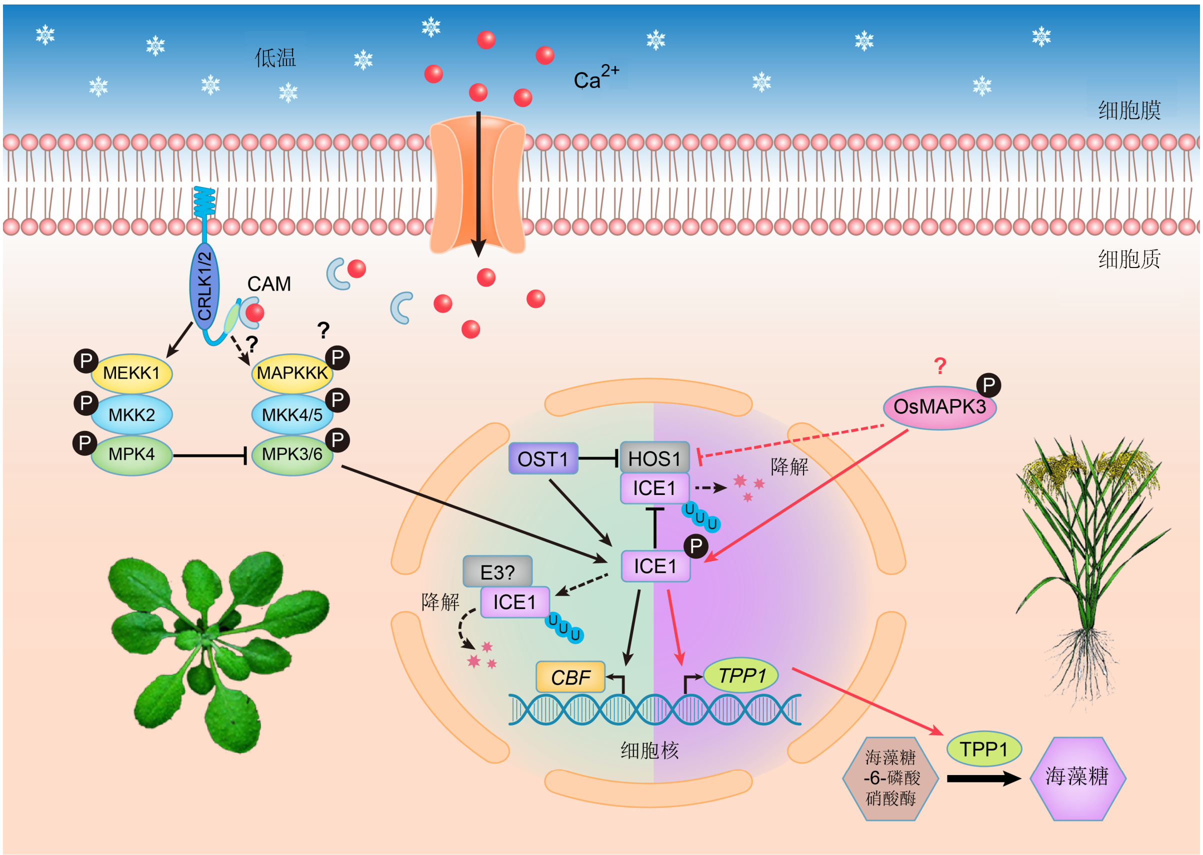

图1　MAP激酶级联反应调控拟南芥和水稻低温应答的工作模型

低温刺激诱导细胞质钙信号的变化。在拟南芥中，作为钙调蛋白的类受体激酶CRLK1和CRLK2被快速激活，启动MEKK1-MKK2-MPK4信号级联，抑制了MPK3/6活性，正调控植物抗冷反应。另一个信号通路MKK4/5-MPK3/6同样被冷胁迫快速诱导，在MKK5存在的情况下，MPK3/6被激活，磷酸化ICE1，促进其降解，CBFs转录水平下降，负调控植物抗冷反应。MPK3/6诱导的ICE1降解，与HOS1泛素化降解过程无关。同时，OST1也可磷酸化ICE1，但其磷酸化作用抑制HOS1对ICE1的泛素化降解，从而起到正调控作用。在水稻中，冷胁迫诱导OsMAPK3激活，而后磷酸化OsICE1。同时，OsMAPK3抑制OsHOS1和OsICE1的结合，进而抑制OsICE1的降解，维持了OsICE1的稳定。被磷酸化的OsICE1激活*OsTPP1*的转录，促进海藻糖的累积，增强水稻耐冷能力。(箭头代表激活，短横线代表抑制，虚线代表机制尚不清楚。红色线代表拟南芥内信号通路，黑色线代表水稻内信号通路)

Figure 1　A proposed working model shows the roles of MAP kinase cascades during the cold response in Arabidopsis and rice

Low temperature induces changes in cytoplasmic calcium signaling. In Arabidopsis, calcium/calmodulin regulated receptor-like kinase 1 and 2 (CRLK1 and CRLK2) are activated by cold signal, and then initiate MEKK1-MKK2-MPK4 cascade, which suppresses cold-induced activation of MPK3/6, thus positively regulating cold tolerance. MKK4/5-MPK3/6 cascades can also be rapidly activated in cold stress in parallel with MEKK1-MKK2-MPK4. Constitutively activated MPK3/6 by MKK5 phosphorylate ICE1 to promote degradation of ICE1, which decreases the transcriptional activity of CBFs, thus negatively regulating cold tolerance in Arabidopsis. OST1 also phosphorylates and affects the stability of ICE1, and positively regulate cold tolerance. In rice, cold signal induces the activation of OsMAPK3 that phosphorylates the OsICE1 and inhibits OsICE1 degradation by interrupting the interaction of OsHOS1 and OsICE1 to maintain the stability of OsICE1. Phosphorylated OsICE1 activates the transcription of *OsTPP1* to promote the accumulation of trehalose, and enhances the cold tolerance of rice. (Arrows represent activation, and bars represent inhibition, dotted lines represent unknown mechanisms. Red lines represent signal pathways in Arabidopsis, and black lines represent signal pathways in rice)

进而降低*CBF*基因的表达，最终负调控植物对冷胁迫的响应(Zhao et al., 2017; Li et al., 2017)。结合前期的研究成果(Ding et al., 2015)，可以推断ICE1的稳定性可能受MPK3/6 (负调控)和OST1 (正调控)动态

调控，利用这种机制，植物可能会更好地适应环境温度的变化。朱健康研究团队发现, MKK5-MPK3/6级联反应通过ICE1的3个磷酸化位点(Ser94、Thr366和Ser403)促进ICE1的降解，该过程由26S蛋白酶体途径介导(Zhao et al., 2017)。杨淑华研究团队利用泛素抗体证明，冷处理下MPK3/6促进ICE1泛素化降解，并且该降解过程与HOS1无关(Li et al., 2017)。但是他们的研究都没有涉及调控ICE泛素化降解的E3泛素连接酶的鉴定，此外，也没有验证MPK3/6介导的ICE1的磷酸化是否发生在细胞核内。

除了MPK3/6, 朱健康研究团队还揭示了MPK4与ICE1互作的生理学意义。近年来研究表明，冷胁迫下CRLK1 (calcium/calmodulin regulated receptor-like kinase)结合并且磷酸化MEKK1，通过MEKK1-MKK1/2-MPK4级联反应诱导*COR*基因表达，从而增强植物抗冻性(Teige et al., 2004; Yang et al., 2010; Furuya et al., 2013)。他们发现, CRLK1和CRLK2抑制MPK3/6激酶活性，并且CRLK1和CRLK2的存在是正常情况下稳定ICE1蛋白所必需的。另外，*mpk4*和*mekk1*单突变体以及*mkk1*/*mkk2*双突变体中，MPK3和MPK6激酶活性升高，甚至*mpk4*突变体中MPK3和MPK6蛋白质的累积也增加，且都表现出冷冻敏感表型。以上结果说明, MEKK1-MKK1/2-MPK4级联反应抑制MPK3/6活性，正调控植物冷胁迫响应。但在*mkk1*和*mkk2*单突变体中，MPK3/6的活性均不受影响，而*mkk2*中MPK4失去活性，说明MKK1/2不是抑制MPK3/6活性的上游，而MKK2是冷诱导MPK4激活所必需的。最新研究显示，MKK1/2与MEKK1相互作用，共定位于细胞质膜，MKP4在细胞质基质及细胞核内均发挥作用(Li et al., 2015)。这些结果清晰地阐明了一条与MPK3/6平行的植物低温应答的正调控途径，即冷胁迫诱导CRLK1/2磷酸化激活MEKK1，进而通过MAPK级联反应将冷信号逐级传递，抑制MPK3/6激酶活性，维持ICE1蛋白的稳定性，最终正调控*CBF*基因的表达(图1)。

非常有意思的是，种康研究团队发现水稻OsMAPK3在调控冷胁迫响应方面扮演着与拟南芥MPK3相反的角色。他们发现OsMAPK3磷酸化并维持OsbHLH002 (也称为OsICE1)的稳定，磷酸化的OsbHLH002促进OsTPP1 (trehalose-6-phosphate phosphatase 1)转录，诱导大量海藻糖产生，从而增强水稻的抗冷能力(Zhang et al., 2017)。已有研究表明，植物体内海藻糖由TPS (trehalose-6-phosphate synthase)和TPP介导合成，在植物响应非生物胁迫(如干旱)过程中扮演重要角色(Wingler, 2002; Li et al., 2011; Nuccio et al., 2015)。但是海藻糖代谢是否参与调控植物冷信号传递尚未见报道。为了阐明OsbHLH002与OsTPP1的关系，Zhang等(2017)利用多种生物化学手段证实: OsbHLH002结合至*OsTPP1*启动子–708– –727 bp区域的E-box基序上，冷处理后OsbHLH002可使*OsTPP1*转录水平上升，提高海藻糖的合成从而增强水稻耐冷能力。

以往的大量研究表明，水稻低温应答过程与拟南芥相似，在遭遇低温胁迫时，COLD1 (chilling tolerance divergence 1-RGA1 (rice G-protein a subunit1, RGA1)等膜蛋白复合体感知冷信号并激活钙离子信号转导，从而激活CBF转录因子，增强水稻抗冷能力(Ma et al., 2015)。已知OsbHLH002的同源蛋白OsRAI1是水稻OsMAPK3的底物(Kim et al., 2012)。Zhang等(2017)利用体外和体内实验证明: OsbHLH002与OsMAPK3在细胞核内存在互作关系，且OsMAPK3可被冷信号激活，从而磷酸化并增强OsbHLH002转录激活活性。与拟南芥HOS1功能类似，水稻泛素化E3连接酶OsHOS1结合OsbHLH002并介导其降解(Lourenco et al., 2013)。但是, OsMAPK3拮抗OsbHLH002与OsHOS1的结合，并且特异地抑制冷胁迫下OsHOS1介导的OsbHLH002降解。因此，种康研究团队揭示了一种新的植物低温应答分子机制，即OsMAPK3-OsbHLH002-OsTPP1途径，冷信号激活OsMAPK3磷酸化OsbHLH002，并且减少OsHOS1介导的OsbHLH002泛素化降解，磷酸化的OsbHLH002激活*OsTPP1*转录，进而诱导渗透保护剂海藻糖累积，最终增强水稻抵御低温胁迫的能力(Zhang et al., 2017) (图1)。

对水稻和拟南芥的相关研究均揭示了MAPK级联信号在植物低温应答调控中的重要作用，有助于解析冷信号的感知、传递及信号网络协同交叉的响应途径，同时提出了一些非常有趣的科学问题。首先，冷胁迫条件下，拟南芥MPK3/6介导ICE1磷酸化，促进了ICE1的降解，而OsMAPK3磷酸化OsbHLH002或OsMAPK3本身抑制了OsHOS1与OsbHLH002的结合，减少了OsbHLH002的泛素化降解，增加了Osb-

HLH002的稳定性。这种现象说明：在不同物种中，冷胁迫信号转导和响应途径之间存在差异。这种差异是否由于OsbHLH002被磷酸化的位点与ICE1不同，或者OsMAPK3介导了一个未知调节蛋白的磷酸化，从而影响了OsHOS1对OsbHLH002的泛素化降解，有待进一步阐明。其次，在冷胁迫下水稻OsbHLH002可以通过调控*OsTPP1*的表达，增加体内海藻糖含量来增强植株抗冻能力。这一新机制有别于拟南芥，可能在决定水稻的低温耐受性方面起主导作用。目前，通过基因工程的方法提高海藻糖的含量，已成功应用于增强植物的抗逆性。例如，超表达*TPP1*可增强水稻抗盐和抗旱能力(Li et al., 2011)。因此，有关水稻的研究结果也为未来利用海藻糖创制优质高产的抗冷冻作物新品种奠定了理论基础。

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Making Sense of Cold Signaling: ICE is Cold or not Cold?

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Abstract Cold (chilling or freezing) stress affects the growth and geographical distribution of plants, and it is one of the main factors that restricts crop yield and quality. Plants respond to cold signals by activating a series of effectors to adapt to cold stress. MAP protein kinase family plays a crucial role in plant response to environmental stresses, but it remains unclear whether they are directly involved in perception, transduction or/and networks in cold signaling. Recently, three research groups in China highlight the important role of MAP kinase in cold signaling transduction in *Arabidopsis thaliana* and rice, respectively. Low temperature activates MPK kinase that phosphorylates the ICE1 protein. Stability of ICE1 is controlled by MAP kinase mediated ICE phosphorylation, thus regulating freezing and chilling tolerance in plants. Their studies have advanced our understanding of the ICE1-mediated network of plant cold responses, which is an important breakthrough in the field. The outcome of these studies would provide a powerful theoretical basis for future molecular design breeding in crops.

Key words cold response, phosphorylation, MAPK cascades, ICE1, OsTPP1

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