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# 保卫细胞中ABA信号调控机制研究进展

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**摘要** 脱落酸(ABA)具有调节植物快速响应逆境的重要功能。植物细胞中ABA核心信号通路由ABA受体PYR1/PYLs/RCARs、A类碱性蛋白磷酸酶PP2Cs和Snf1相关蛋白激酶SnRK2s组成。活性氧(ROS)和Ca<sup>2+</sup>是保卫细胞中的重要第二信使, 调控ABA诱导的气孔关闭。该文对保卫细胞中核心ABA信号蛋白的调控以及ROS和Ca<sup>2+</sup>介导的ABA信号转导等最新研究成果进行综述, 旨在阐明保卫细胞中ABA信号调控机制。

**关键词** 脱落酸, 活性氧, 钙信号, 气孔调节

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脱落酸(abscisic acid, ABA)具有诱导气孔关闭、减少蒸腾作用水分散失并提高植物抗旱性的重要功能。自从ABA受体PYR1/PYLs/RCARs (pyrabactin resistance 1/pyr1-like proteins/regulatory components of ABA receptors)被成功鉴定以来, 植物ABA核心信号蛋白相继得到确认, 包括ABA受体PYR1/PYLs、A类碱性蛋白磷酸酶PP2Cs (protein phosphatase 2Cs)和Snf1相关蛋白激酶SnRK2s (Snf1-related protein kinase 2s) (Ma et al., 2009; Park et al., 2009; Fuji et al., 2009; Cutler et al., 2010)。近年来, 植物内源ABA积累和ABA信号调控受到广泛关注并取得重要进展(陈唯等, 2019; 张静和侯岁稳, 2019; Chen et al., 2020a; Hsu et al., 2021)。本文将从保卫细胞中ABA核心信号蛋白的调控、活性氧(reactive oxygen species, ROS)和Ca<sup>2+</sup>介导的ABA信号转导3方面进行综述(图1), 旨在阐明保卫细胞中ABA信号调控机制, 并为植物抗旱性研究提供有益参考。

## 1 保卫细胞中ABA核心信号蛋白的调控

当植物受到水分胁迫时, 保卫细胞中积累的ABA被受体蛋白PYR1/PYLs识别并与PP2Cs形成ABA-PYR1/PYLs-PP2Cs复合体, 解除PP2Cs对SnRK2s的抑制,

SnRK2s磷酸化激活或抑制下游靶蛋白, 快速引发气孔关闭。

### 1.1 ABA受体PYR1/PYLs

拟南芥(*Arabidopsis thaliana*) PYR1/PYLs家族有14个成员(PYR1/PYL1–13或RCAR1–14), 各成员的ABA亲和性与基因表达模式有明显差异, 且它们的遗传功能存在冗余。根据与ABA结合的构型差异, PYR1/PYLs可分为单体型(PYL4–6, 8–10)和二聚体型(PYR1, PYL1–2)。高浓度ABA条件下, 所有受体均可抑制PP2Cs并产生相似的ABA反应; 但缺少ABA时仅单体型受体能够抑制PP2Cs活性(Hao et al., 2011; Tischner et al., 2017)。基因表达分析表明, PYR1、PYL1、2、4、5和8在保卫细胞和维管束中表达, 其中PYL2主要负责ABA诱导的气孔关闭, 而PYL4和5则参与CO<sub>2</sub>诱导的气孔关闭, 且ABA抑制PYL2、4和5的表达(Dittrich et al., 2019)。与野生型相比, PYR1/PYLs多重基因缺失突变体的气孔开度和叶片失水率显著增大, 且突变体的气孔对ABA敏感程度与基因缺失数量呈负相关(Nishimura et al., 2010; Gonzalez-Guzman et al., 2012; Zhao et al., 2018)。

研究发现PYL9可在质膜脂质纳米结构域附近直接抑制ABI1 (ABA-insensitive 1), 推测可溶性受体

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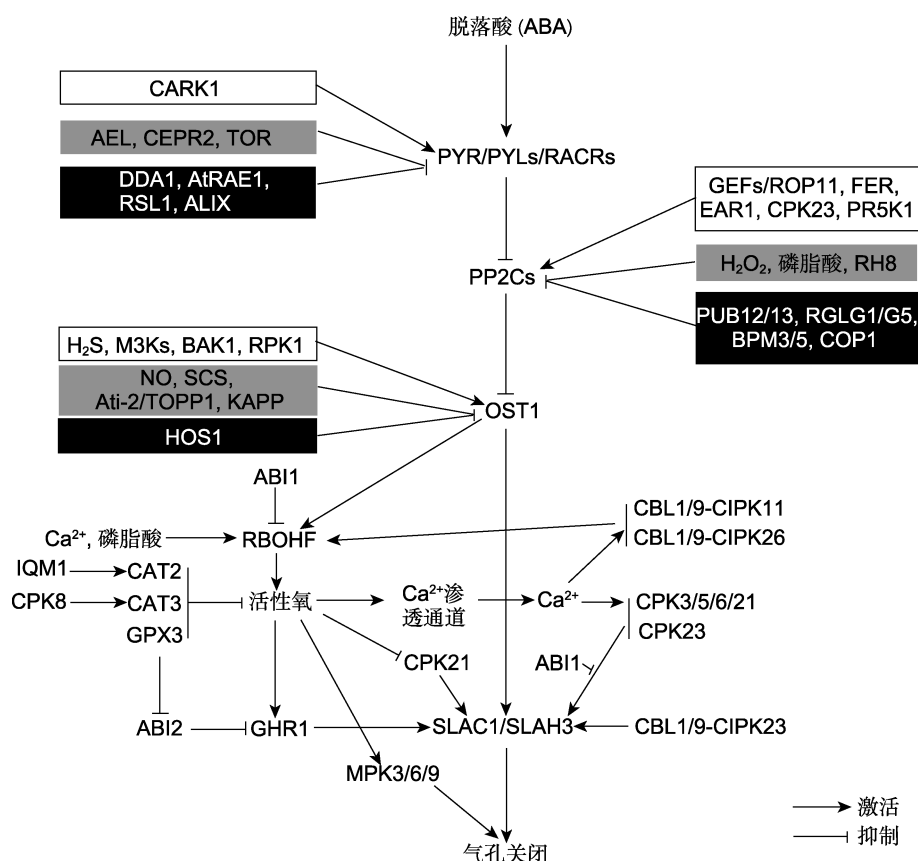


图1 保卫细胞中ABA信号转导机制模型

Figure 1 Schematic model of ABA signaling mechanism in guard cells

PYR1/PYLs是以某种机制与质膜结合后识别胞外ABA (Demir et al., 2013)。随后, 研究表明植物特异的小分子外周膜蛋白CAR (C2-domain ABA-related protein)可以协助PYR1/PYLs定位于质膜。水分胁迫使细胞质中 $\text{Ca}^{2+}$ 浓度上升到 $\mu\text{mol}\cdot\text{L}^{-1}$ 量级,  $\text{Ca}^{2+}$ 与膜蛋白CAR结合引起质膜产生的正向弯曲充当PYR1/PYLs招募点(Rodriguez et al., 2014; Diaz et al., 2016)。

研究表明, 拟南芥多个家族的蛋白激酶参与调控PYR1/PYLs的受体功能。例如, 细胞质受体激酶CARK1 (cytosolic ABA receptor kinase 1)磷酸化RCARs蛋白中 $\beta_3$ -loop的保守氨基酸残基(RCAR-3<sup>Thr77/11</sup>Thr78/12<sup>Thr105/13</sup>Thr101/14<sup>Ser81</sup>), 增强RCARs受体功能, *cark1*突变体气孔缺失正常的ABA反应(Zhang et al., 2018; Li et al., 2019)。然而, 酪蛋白激酶AEL (*Arabidopsis* EL1-like)磷酸化PYR1<sup>Ser152</sup>和PYL1<sup>Ser182</sup>残基, 能够降低PYR1和PYL1蛋白的稳定

性(Chen et al., 2018)。质膜受体样激酶CEPR2 (C-terminally encoded peptide receptor 2)的磷酸化可促进PYL2/PYL4蛋白降解, 但ABA抑制CEPR2的作用; 遗传分析表明, *CEPR2*与其同源基因*PXY* (*phloem intercalated with xylem*)和*PXL2* (*PXY-like 2*)共同负调控ABA信号(Yu et al., 2019)。蛋白激酶TOR (target of rapamycin)可以破坏ABA-PYR1/PYLs-PP2Cs复合体, 阻止ABA信号识别。然而, 当植物受水分胁迫时, ABA激活的SnRK2s可通过磷酸化抑制TOR活性, 表明TOR反馈调节植物细胞平衡生长和胁迫应答(Wang et al., 2018a)。

在正常生长或水分胁迫结束后, PYR1/PYLs蛋白泛素化降解可以衰减ABA信号, 植物恢复正常代谢和生长。拟南芥DDA1 (Det1/DDB1-associated 1)是CRL4 (cullin 4-Ring E3 ligase)与底物的连接蛋白, 介导CRL4泛素化降解PYL4、8和9; *DDA1*过表达可促进PYL8的降解并降低植物对ABA的敏感性(Irigoyen et

al., 2014)。CRL4-DDB1 (DNA-damage binding 1) 的底物受体AtRAE1 (RNA export factor 1 in *Arabidopsis*)促进RCAR1泛素化降解, *AtRAE1*过表达降低了气孔对ABA的敏感性, 导致叶片失水率显著高于野生型(Li et al., 2018)。RING-型E3泛素化连接酶RSL1 (Ring finger of seed longevity 1)泛素化质膜受体PYR1/PYL4并通过液泡途径降解, 而ALIX (ALG-2 interacting protein-X)协助泛素化受体的转运, *alix-1*缺失突变体ABA诱导的气孔关闭增强(Bueso et al., 2014; García-León et al., 2019)。

总之, ABA受体PYR1/PYLs正向调控保卫细胞ABA信号转导, 促进ABA诱导的气孔关闭。细胞质可溶性受体PYR1/PYLs通过Ca<sup>2+</sup>-CAR复合体定位在质膜上并识别胞外ABA; 而PYR1/PYLs的蛋白磷酸化调控机制比较复杂, 既有CARK1磷酸化增强又有TOR抑制PYR1/PYLs活性, 并且AEL和CEPR2磷酸化促进受体降解。水分胁迫结束后, 保卫细胞中PYR1/PYLs的泛素化降解途径可以衰减ABA信号并使植物恢复正常生长。

## 1.2 蛋白磷酸酶PP2Cs

拟南芥A类碱性磷酸酶PP2Cs家族的ABI1、ABI2、HAB1 (hypersensitive to ABA1)和PP2CA是保卫细胞中ABA信号的关键负调控因子。植物正常生长时, PP2Cs与SnRK2s结合, 通过去磷酸化抑制后者酶活性; 水分胁迫下, 保卫细胞迅速积累的ABA与PYR1/PYLs及PP2Cs形成复合体, 解除PP2Cs对SnRK2s的抑制, 触发ABA信号转导, 最终引发气孔关闭(Brandt et al., 2015; Hsu et al., 2021)。

正常生长的植物细胞中PP2Cs基因表达维持在基础水平, 水分胁迫诱导PP2Cs基因表达。现已证明MYB型转录因子AtMYB44是PP2Cs的转录抑制因子, *AtMYB44*过表达植株的气孔对ABA表现过敏感反应, 且盐胁迫诱导的ABI1、ABI2、HAB1和PP2CA基因表达也受到抑制; ChIP实验证明AtMYB44结合ABI1/ABI2启动子并抑制转录(Jung et al., 2008; Nguyen and Cheong, 2018)。由于外源ABA和水分胁迫显著增强PP2Cs基因表达, 诱导转录因子ABFs (ABRE binding factors)转录本和蛋白积累; 而ABFs结合ABI1/ABI2启动子并促进转录, 表明ABFs促进PP2Cs基因表达是植物细胞存在的负反馈调节(Xue

et al., 2008; Wang et al., 2019)。

保卫细胞中PP2Cs酶活性受小分子化合物和多种蛋白因子的复杂调控。H<sub>2</sub>O<sub>2</sub>、磷脂酸(PA)和RNA螺旋酶RH8 (RNA helicase-like 8)直接抑制PP2Cs活性, 正向调控ABA信号(Meinhard and Grill, 2001; Meinhard et al., 2002; Zhang et al., 2004; Baek et al., 2018)。然而, 拟南芥Ca<sup>2+</sup>依赖性蛋白激酶CPK23 (Ca<sup>2+</sup>-dependent protein kinase 23)和受体样激酶PR5K (PR5 receptor-like kinase)通过磷酸化激活ABI1/ABI2, 负调控保卫细胞中ABA信号及植物耐旱性(Geiger et al., 2010; Baek et al., 2019); ROP (Rho GTPase of plants)及激活因子GEF (guanine nucleotide exchange factor)可提高ABI1/ABI2的活性, 当细胞缺乏ABA时, ROP11-GEF1保护ABI1不受PYL9抑制, 而ABI1亦能增强GEF1的稳定性, 受体激酶FER (FERONIA)通过结合GEF1、4和10来增强ROP11和ABI2的活性(Li et al., 2012, 2016; Yu et al., 2012)。此外, 拟南芥未知功能蛋白EAR1 (enhancer of ABA co-receptor 1)与多个PP2Cs蛋白N端互作, 增强PP2Cs的活性; *ear1*缺失突变体有包括气孔在内的多种ABA过敏感表型, 表明EAR1通过增强PP2Cs的活性负调控ABA反应(Wang et al., 2018b)。

保卫细胞中PP2Cs降解直接参与ABA信号调控。拟南芥U-box型PUB12/PUB13泛素化降解ABI1, 与野生型相比, *pub12*、*pub13*和*pub12/pub13*突变体的气孔对ABA不敏感, 叶片失水率增大, 耐旱性减弱(Kong et al., 2015)。RING型RGLG 1 (Ring domain ligase 1)/RGLG5泛素化降解PP2CA, *RGLG1/5*表达下调使内源性PP2CA蛋白含量和叶片失水率增高, 干旱敏感性增强(Wu et al., 2016)。多亚基型CRL3-BPM3 (BTB/POZ and MATH domain protein)/BPM5泛素化降解细胞核中ABI1、ABI2、HAB1和PP2CA, *BPM3/BPM5*表达下调增大叶片失水率和气孔导度并降低叶表温度(Julian et al., 2019)。研究表明, 光信号蛋白COP1 (constitutively photomorphogenic 1)泛素化降解ABI1、ABI2、HAB1、HAB2和PP2CA, 正调控ABA诱导的气孔关闭(Chen et al., 2021)。

综上, PP2Cs是保卫细胞ABA信号的重要负调控蛋白, PP2Cs的基因转录与蛋白修饰在保卫细胞ABA信号转导调控中发挥重要作用。

### 1.3 Snf1相关的蛋白激酶SnRK2s

普遍认为, 拟南芥SnRK2s家族的OST1/SnRK2.6是ABA诱导气孔关闭的关键因子, OST1缺失破坏了ABA激活的S-型阴离子通道和Ca<sup>2+</sup>渗透通道以及ROS的合成, 在ost1突变体中, ABA不能诱导气孔关闭(Mustilli et al., 2002; Acharya et al., 2013)。

植物正常生长时, 保卫细胞中OST1与PP2Cs相互作用, PP2Cs使OST1蛋白激活环中多个Ser/Thr去磷酸化, 从而直接抑制OST1的活性; 植物受水分胁迫时, ABA-PYR1/PYLs解除了PP2Cs对OST1的抑制, OST1磷酸化激活或抑制下游靶蛋白, 引起快速的气孔关闭和持久的植物抗逆性(Umezawa et al., 2009; Soon et al., 2012)。水分胁迫时, 保卫细胞中OST1被重新激活是ABA诱导气孔关闭的关键。异源表达的OST1可通过自磷酸化而被激活, OST1激活对其激活非常关键的环中Ser<sup>175</sup>残基和C端SnRK2-box (Belin et al., 2006)。研究表明, 拟南芥M3Ks (mitogen activated protein kinase kinase kinases)家族中δ1、δ6和δ7对解除抑制的OST1并使其重新激活是必需的, M3Kδ1/6/7磷酸化OST1<sup>Ser171</sup>残基进而激活OST1 (Takahashi et al., 2020)。研究表明, 在ABA信号转导初期, 通过自磷酸化或转磷酸化2种途径可解除抑制的OST1。

拟南芥OST1活性和蛋白稳定性受多种调控因子的抑制。例如, 一氧化氮(NO)、Ca<sup>2+</sup>结合蛋白SCS (SnRK2-interaction calcium sensor)、蛋白磷酸酶TOPP1 (type one protein phosphatase 1)和KAPP (kinase-associated protein phosphatase)可抑制OST1的活性, 阻止ABA诱导的气孔关闭。NO催化OST1<sup>Cys137</sup>残基产生S-亚硝基化, 抑制OST1的活性; TOPP1去磷酸化抑制OST1, 而AtI-2 (At inhibitor-2)增强TOPP1对OST1的抑制(Wang et al., 2015; Hou et al., 2016; Tarnowski et al., 2020; Lu et al., 2020)。当细胞缺乏ABA时, CRL4-DDB1底物受体HOS15 (high osmotic stress 15)促进OST1快速降解, 负调控保卫细胞ABA信号(Ali et al., 2019)。

除上述抑制因子外, 质膜受体样激酶BAK1 (BRI1-associated kinase 1)和RPK1 (receptor-like protein kinase 1)在质膜附近先后与OST1形成BAK1-OST1与RPK1-OST1复合体, ABA促进2种复合体形成, BAK1磷酸化增强OST1的活性, 但ABI1拮

抗BAK1作用, RPK1与OST1相互磷酸化, ABI1同时抑制RPK1和OST1的活性, 表明OST1活性受到磷酸化和去磷酸化双重调控(Shang et al., 2016, 2020)。最新发现OST1存在一种新的蛋白翻译后调控机制, 即硫化氢(H<sub>2</sub>S)催化OST1/SnRK2.6产生过硫化作用, 增强ABA信号和气孔关闭。研究表明, ABA诱导保卫细胞中L-半胱氨酸脱硫水解酶DES1 (L-cysteine desulphydrase 1)催化合成H<sub>2</sub>S, H<sub>2</sub>S使OST1蛋白分子表面靠近激活环的Cys<sup>131/137</sup>残基过硫化, 进而增强OST1活性, 促进OST1与ABF2的结合。遗传分析也证明SnRK2.6<sup>C131S</sup>、SnRK2.6<sup>C137S</sup>或SnRK2.6<sup>C131SC137S</sup>不能互补ost1-3突变体表型(Chen et al., 2020b)。

综上, 保卫细胞中OST1活性和蛋白稳定性受到精细而复杂的调控。例如, Cys<sup>137</sup>残基的S-亚硝基化作用抑制OST1活性, 而Cys<sup>131/137</sup>残基的过硫化作用则增强OST1活性。不同家族的蛋白磷酸酶(ABI1、TOPP1和KAPP)均可去磷酸化抑制OST1的活性, 但是M3Kδ1/6/7、BAK1和RPK1等蛋白激酶能够磷酸化激活或增强OST1的活性。

## 2 ROS介导的ABA信号转导

水分胁迫下, 保卫细胞积累的ABA刺激质膜NADPH氧化酶合成ROS, ROS激活质膜Ca<sup>2+</sup>渗透通道, 胞质Ca<sup>2+</sup>水平升高并触发Ca<sup>2+</sup>信号转导; 同时, ROS通过弥散或水通道蛋白进入细胞, 参与调控ABA诱导的气孔关闭(Qi et al., 2018)。

### 2.1 NADPH氧化酶RBOHs

植物NADPH氧化酶RBOHs (respiratory burst oxidase homologs)负责胞外ROS合成, RBOHs蛋白有6个跨膜结构域、C端有1个FAD-结合域、细胞质一侧的N端有2个EF-hand基序(Suzuki et al., 2011)。早期研究证明, 拟南芥RBOHF是ABA诱导ROS合成的关键酶, 且ABA、干旱和盐胁迫均可上调RBOHF基因的表达(Kwak et al., 2003)。

研究表明, 拟南芥RBOHF酶活性受到蛋白磷酸化和Ca<sup>2+</sup>共同调控, 且磷酸化是初始触发事件。最初, ABA激活的OST1磷酸化RBOHF<sup>Ser13/174</sup>位点, 刺激RBOHF合成胞外ROS; 随后, ROS诱导的胞质Ca<sup>2+</sup>与RBOHF的N端EF-hand基序结合, 进一步增强了

RBOHF活性,但ABI1直接抑制RBOHF活性,而磷脂酸则增强其活性(Ogasawara et al., 2008; Sirichandra et al., 2009; Zhang et al., 2009; Kimura et al., 2012)。同时,胞质 $\text{Ca}^{2+}$ 与 $\text{Ca}^{2+}$ 传感蛋白CBL1 (calcineurin B-like 1)/CBL9结合,进而与互作蛋白CIPK11 (CBL-interacting protein kinase 11)/CIPK26形成复合体,激活的CIPK11/CIPK26磷酸化RBOHF<sup>Ser13/130/132</sup>残基并增强RBOHF活性(Drerup et al., 2013; Han et al., 2019)。水分胁迫时,拟南芥肌管素蛋白MTM1 (myotubularin 1)/MTM2及其产物磷酸肌醇5-磷酸酯(PtdIns5P)维持保卫细胞ROS稳态并调控气孔运动,但是MTM1与MTM2作用相反(Nagpal et al., 2018)。

## 2.2 ROS信号识别和转导

在保卫细胞中,ROS氧化靶蛋白的关键Cys残基,造成蛋白氧化损伤或构象改变,进而调控保卫细胞中ABA信号转导。例如,拟南芥SKOR (stelar  $\text{K}^+$  outward rectifier)通道蛋白位于质膜外侧的S3  $\alpha$ -螺旋的Cys<sup>168</sup>残基对 $\text{H}_2\text{O}_2$ 非常敏感,且 $\text{H}_2\text{O}_2$ 以电压依赖形式激活SKOR (García-Mata et al., 2010);质膜受体样激酶GHR1 (guard cell hydrogen peroxide-resistant 1)的C端位于质膜外侧,C端Cys<sup>57/66</sup>残基对于GHR1功能是必需的,而ROS可以激活GHR1 (Hua et al., 2012; Sierla et al., 2018)。

保卫细胞外的ROS通过弥散或水通道蛋白PIP2;1 (plasma membrane intrinsic protein 2;1)和PIP1;4进入细胞,OST1和BAK1磷酸化增强PIP2;1<sup>Ser121</sup>的活性(Grondin et al., 2015; Tian et al., 2016; Rodrigues et al., 2017)。进入保卫细胞的ROS氧化谷胱甘肽氧化酶GPX3 (glutathione peroxidase 3)生成氧化态GPX3,后者氧化并抑制ABI2活性(Miao et al., 2006);ROS还氧化抑制CPK21活性,但硫氧还蛋白可有效恢复CPK21活性(Ueoka-Nakanishi et al., 2013)。ROS诱导的NO还可抑制OST1和RBOHF的活性,表明ABA刺激的ROS合成存在反馈抑制机制(Wang et al., 2015)。

在保卫细胞ROS信号转导过程中,MPK (mitogen activated protein kinase)可能在ROS下游调控ABA诱导的气孔关闭,外源ABA可诱导拟南芥MPK3反义突变体合成ROS,但不能诱导其气孔关闭;ABA

和 $\text{H}_2\text{O}_2$ 可增强MPK3和MPK12的活性,但不能诱导mpk9/mpk12双突变体气孔关闭和阴离子通道的激活(Gudesblat et al., 2007; Jammes et al., 2009)。研究表明,拟南芥U-box型E3泛素化连接酶PUB18/PUB19介于ROS和 $\text{Ca}^{2+}$ 之间,负调控保卫细胞的ABA信号, $\text{H}_2\text{O}_2$ 可以但 $\text{Ca}^{2+}$ 不能诱导pub18-2/pub19-3双突变体的气孔关闭(Liu et al., 2011; Seo et al., 2012)。

过氧化氢酶(catalase, CAT)催化 $\text{H}_2\text{O}_2$ 降解,调控ABA诱导的气孔关闭。拟南芥cat2突变体保卫细胞中ROS组成性增加,ABA诱导的气孔关闭和ROS合成以及胞质 $\text{Ca}^{2+}$ 振荡均显著高于野生型(Jannat et al., 2011)。钙调素结合蛋白IQM1 (IQ motif-containing protein 1)缺失突变体保卫细胞中ROS组成性增加,且iqm1突变体的气孔对ABA和 $\text{H}_2\text{O}_2$ 不敏感,进一步研究发现IQM1与CAT2结合增强了CAT2活性(Zhou et al., 2012; Lv et al., 2019)。蛋白激酶CPK8磷酸化激活CAT3,促进保卫细胞中 $\text{H}_2\text{O}_2$ 降解(Zou et al., 2015)。

综上,植物受水分胁迫后,质膜RBOHF酶产生ROS受 $\text{Ca}^{2+}$ 不依赖性激酶OST1、 $\text{Ca}^{2+}$ 结合和 $\text{Ca}^{2+}$ 依赖性激酶CIPK11/CIPK26等多重调控,其中OST1的磷酸化激活是初始事件,ABI1直接抑制RBOHF活性; $\text{Ca}^{2+}$ 结合和CIPK11/CIPK26磷酸化作用进一步增强了RBOHF活性。因此推测RBOHF是保卫细胞中ABA、ROS和 $\text{Ca}^{2+}$ 信号交叉调控的重要节点。细胞中的ROS具有强氧化性,可通过氧化失活靶蛋白或改变靶蛋白构象来增强保卫细胞中的ABA信号,但是ROS介导ABA信号的调控机制有待阐明。

## 3 $\text{Ca}^{2+}$ 介导的ABA信号转导

$\text{Ca}^{2+}$ 是植物细胞重要的第二信使,ROS可刺激胞外 $\text{Ca}^{2+}$ 和钙库中的 $\text{Ca}^{2+}$ 进入细胞质,引起胞质 $\text{Ca}^{2+}$ 振荡,从而触发ABA诱导的 $\text{Ca}^{2+}$ 信号。已知多个 $\text{Ca}^{2+}$ 依赖性蛋白激酶和CBL互作蛋白激酶CIPK参与保卫细胞中ABA信号调控,调节ABA诱导的气孔关闭(Munemasa et al., 2015)。

### 3.1 $\text{Ca}^{2+}$ 依赖性蛋白激酶CPK

拟南芥基因组中有20个CPKs基因在保卫细胞中表达,其中CPK3、4、5、6、8、10、11、21和23促进

ABA诱导的气孔关闭,而CPK9和33则抑制ABA信号转导。已知拟南芥CPK5与CPK6、CPK21与CPK23的蛋白序列相似性最高,CPK3、5、6、21和23均可磷酸化激活保卫细胞质膜S-型阴离子通道SLAC1和SLAH3 (SLAC1 homology 3),正向调控ABA诱导的气孔关闭。在拟南芥*cpk3/cpk6*双突变体中,ABA和Ca<sup>2+</sup>既不能激活保卫细胞质膜Ca<sup>2+</sup>渗透通道和S-型阴离子通道,也不能诱导气孔关闭;但ABA可激活*cpk5*突变体S-型阴离子通道;生化实验证明CPK3与GHR1结合后磷酸化激活SLAC1,ABI2抑制GHR1-CPK3的作用;CPK6磷酸化SLAC1<sup>Ser59</sup>残基,增强SLAC1活性,而ABI1抑制CPK6的激活作用(Mori et al., 2006; Brandt et al., 2012, 2015; Sierla et al., 2018)。CPK21和CPK23功能缺失提高植物抗渗透与耐旱/盐能力,*cpk23*突变体气孔开度明显小于野生型,CPK21和CPK23均可磷酸化激活SLAC1/SLAH3,ABI1抑制CPK21/23的激活作用,然而CPK23核心活性并不依赖Ca<sup>2+</sup>的存在(Ma and Wu, 2007; Geiger et al., 2010; Franz et al., 2011)。

在保卫细胞中,CPKs可以磷酸化激活某些转录因子、过氧化氢酶、热激蛋白以及阳离子通道,促进ABA诱导的气孔关闭。例如,同源蛋白CPK4和CPK11磷酸化激活转录因子ABF1/4,正向调节ABA信号。*cpk4/cpk11*双突变体气孔对ABA不敏感程度显著大于*cpk4*和*cpk11*单突变体,说明CPK4和CPK11作用通路可能不同(Zhu et al., 2007)。ABA和Ca<sup>2+</sup>不能抑制*cpk10*突变体的内向钾离子电流,CPK10与热激蛋白HSP1互作,调节ABA和Ca<sup>2+</sup>诱导的气孔关闭(Zou et al., 2010)。*cpk8*突变体气孔关闭不能被ABA、H<sub>2</sub>O<sub>2</sub>和Ca<sup>2+</sup>诱导,CPK8通过磷酸化激活CAT3降低保卫细胞H<sub>2</sub>O<sub>2</sub>水平,说明CPK8同时介导保卫细胞Ca<sup>2+</sup>和ROS信号,调控ABA诱导的气孔关闭(Zou et al., 2015)。CPK9与CPK33高度同源,CPK33磷酸化激活质膜GORK (guard cell outward rectifying K<sup>+</sup> channel)活性,*cpk33*突变体中Ca<sup>2+</sup>诱导的气孔关闭受阻,但与野生型相比,*cpk9*、*cpk33*和*cpk9/cpk33*突变体ABA诱导的气孔关闭和S-型阴离子流增大,且双突变体的ABA敏感性显著高于单突变体,表明CPK9和CPK33通过不同通路负调控ABA信号(Corratgé-Faillie et al., 2017; Chen et al., 2019)。

### 3.2 CBL互作蛋白激酶CIPK

研究表明,植物CBL-CIPK激酶可以调节质膜/液泡膜上离子通道的活性,抑制保卫细胞ABA信号转导。拟南芥CBL1/9-CIPK23磷酸化质膜内向K<sup>+</sup>通道,与野生型相比,*cbl1/cbl9*和*cipk23*突变体的叶片蒸腾失水减少且气孔对ABA过敏感(Cheong et al., 2007)。在卵母细胞中,CBL1/9-CIPK23共表达可激活SLAC1/SLAH3;CBL1-CIPK11和CBL1/CBL9-CIPK26共表达则促进RBOHF合成ROS (Drerup et al., 2013; Maierhofer et al., 2014; Han et al., 2019)。研究发现,拟南芥PAT10 (protein S-acyl transferase 10)介导CBL2/CBL3定位在液泡膜上,PAT10-CBL2/3-CIPK-9/17复合体可激活液泡膜上NHX (K<sup>+</sup>/H<sup>+</sup> antiporter)的活性,促进K<sup>+</sup>进入液泡,维持胞质中Ca<sup>2+</sup>的稳定,从而抑制保卫细胞ABA信号(Song et al., 2018)。

综上,ABA和ROS刺激细胞质中Ca<sup>2+</sup>浓度迅速升高,Ca<sup>2+</sup>与下游的Ca<sup>2+</sup>传感蛋白相结合,参与调控保卫细胞的ABA信号转导。如前文所述,OST1和CBL1/9-CIPK11/26可磷酸化激活或增强RBOHF合成ROS,IQM1结合CAT2共同调控保卫细胞中的ROS水平,OST1和CPK3、5、6、21、23以及CBL1/9-CIPK23磷酸化激活SLAC1/SLAH3。上述研究表明,ABA诱导的气孔关闭存在Ca<sup>2+</sup>依赖性和Ca<sup>2+</sup>不依赖性2种途径,而质膜上的RBOHF和SLAC1/SLAH3是ABA核心信号、ROS信号和Ca<sup>2+</sup>信号的交叉调控点。

## 4 总结与展望

近10年来,模式植物拟南芥保卫细胞中ABA信号调控机制研究取得了许多重要进展,通过揭示ABA核心信号蛋白的可逆性磷酸化作用、泛素化降解以及OST1蛋白Cys残基的过硫化和亚硝基化作用阐明了保卫细胞ABA信号精细且复杂的调控机制(Coego et al., 2021; Hsu et al., 2021)。ABA刺激保卫细胞ROS积累和Ca<sup>2+</sup>浓度增加进一步放大了ABA诱导的气孔关闭反应,保卫细胞中Ca<sup>2+</sup>依赖性蛋白激酶(CIPK11/26、CIPK23和CPK3/5/6/21)和Ca<sup>2+</sup>不依赖性蛋白激酶(OST1和CPK23)通过磷酸化作用激活质膜RBOHF和SLAC1/SLAH3,促进ROS合成以及Cl<sup>-</sup>和NO<sub>3</sub><sup>-</sup>等阴离子流出细胞,最终引起气孔关闭(图1)。

研究表明,保卫细胞质膜的RBOHF和SLAC1/SLAH3是植物ABA核心信号、ROS信号和Ca<sup>2+</sup>信号的交叉调控点。

水资源缺乏使农作物生产受到严峻挑战,加剧未来全球粮食短缺。因此,为确保粮食安全和提高农业生产效率,作物育种应以提高作物抗旱性和水分生产力为目标。被子植物ABA信号通路具有高度保守性(Hsu et al., 2021),在拟南芥中建立的保卫细胞ABA信号调控机制可为其它作物抗旱育种及增产增收提供通用策略。前人研究表明,ABA受体基因PYR/PYLs过表达可增强白杨(*Populus trichocarpa*)、银灰杨(*P. canescens*)、小麦(*Triticum aestivum*)及水稻(*Oryza sativa*)的抗旱性、水分利用效率和水分生产力(Tian et al., 2015; Yu et al., 2017; Papacek et al., 2019; Mega et al., 2019; Bhatnagar et al., 2020; Mao et al., 2022)。除PYR/PYLs外,拟南芥PP2Cs基因缺失双突变体和三突变体均表现对外源ABA过敏感、叶片水分流失减少和抗旱性增强(Rubio et al., 2009; Antoni et al., 2012; Yang et al., 2019)。因此,利用作物特定的ABA受体PYR/PYLs和共受体PP2Cs来调节作物的ABA反应,实现在不影响作物生长和产量的前提下提高水分利用效率将是未来的育种目标。

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## Research Progress on the Regulatory Mechanisms of ABA Signal Transduction in Guard Cells

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**Abstract** ABA plays important roles in regulating the rapid responses of plants to stresses. The ABA core signal pathway consists of ABA receptors PYR1/PYLs/RCARs, clade A protein phosphatases PP2Cs and protein kinases SnRK2s in plant cells. Reactive oxygen species (ROS) and  $\text{Ca}^{2+}$  are the vital second messengers in guard cells that involved in the stomatal closure controlled by ABA. In this review, we focus on summarizing the latest research progresses on the regulations of core ABA signaling proteins in guard cells, ROS- and  $\text{Ca}^{2+}$ -mediated guard cell ABA signal transductions, and attempt to elucidate the mechanisms of ABA signal transduction in guard cells.

**Key words** abscisic acid, reactive oxygen species, calcium signal, stomatal regulation

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