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植物激素脱落酸受体偶联途径研究进展

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摘要: 脱落酸(ABA)是传统的“五大”植物激素之一。目前已知ABA受体偶联途径主要由受体PYR1/PYLs/RCARs蛋白、A分支蛋白磷酸酶PP2C、蛋白激酶SnRK2组成, 主要通过激活或抑制SnRK2, 实现ABA信号输出的“开”和“关”。在没有ABA时, PP2C去磷酸化并抑制SnRK2。胁迫诱导ABA产生后, ABA与受体形成复合体, 结合并抑制PP2C, 使SnRK2从抑制状态释放出来。近年发现的B亚组RAF蛋白激酶可以磷酸化并重新激活SnRK2, 启动下游ABA信号途径。本文主要综述了ABA受体偶联核心信号途径组分及其调控机制, 介绍了近年这一研究领域的主要进展。

关键词: 脱落酸; PYL; SnRK2; PP2C; RAF

Recent advances in phytohormone abscisic acid receptor–coupled pathway

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Abstract: Abscisic acid (ABA) is one of the five “classical” plant hormones. The ABA receptor–coupled core signaling pathway mainly composes ABA receptor PYR1/PYLs/RCARs, clade A PP2C protein phosphatases, and protein kinase SnRK2s. Mainly though activating or inhibiting SnRK2 kinase, these components turn “on” or “off” downstream ABA response. In the absence of ABA, PP2Cs bind to and inhibit SnRK2s. Stress induces the accumulation of ABA, and ABA binds to its receptors and inhibits PP2Cs, releasing SnRK2s from inhibition. Recent studies suggest that B subgroup RAF kinases phosphorylate and reactivate the dephosphorylated inactive SnRK2s and initiate ABA signaling. In this review, we summarize core components in ABA receptor–coupled core signaling pathway and mainly focus on the recent progress in this field.

Key words: abscisic acid; PYL; SnRK2; PP2C; RAF

脱落酸(abscisic acid, ABA)是传统的“五大”植物激素之一, 参与了植物种子萌发、休眠、气孔关闭、胁迫响应以及生长发育等许多生理过程的调控。2009年以来, 随着ABA受体PYR1 (pyrabactin resistance 1)/PYLs (PYR1-likes)/RCARs (regulatory

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components of ABA receptors) (以下简称PYL)的发现, ABA受体偶联转导途径才逐渐清晰(图1)。

目前已知ABA受体偶联途径主要由ABA受体PYL蛋白、A分支PP2C (clade A protein phosphatase 2C, 简称PP2C-A)蛋白磷酸酶、蔗糖非发酵相关的蛋白激酶2 (sucrose non-fermenting-1-related protein kinase 2, SnRK2)及其下游底物如ABA应答元件结合蛋白(ABA-responsive-element binding factor, ABF)等组成(图1)。SnRK2蛋白激酶是ABA信号通路中的主要功能组分, 通过磷酸化转录因子ABF等介导下游的生理过程, 而PP2C是这一信号通路的主要负调控组分, PP2C通过去磷酸化并结合抑制SnRK2的活性。ABA受体PYL蛋白本质上是PP2C的抑制子(inhibitor), 在ABA存在的情况下, PYL结合并抑制PP2C, 将SnRK2从抑制的状态释放出来。近两年发现的B亚组RAF蛋白激酶(B subgroup RAF-like protein kinase, 简称B-RAF)则是SnRK2的激活子(activator)。PP2C解离后, B-RAF通过磷酸化重新激活处于去磷酸化失活状态的SnRK2, 启动下游ABA信号途径(图1)。

1 SnRK2蛋白激酶

SnRK在植物中高度保守, 根据其蛋白序列和结构特征分为3个亚家族: SnRK1、SnRK2和SnRK3。SnRK2是植物特有的一类蛋白激酶, 是哺乳动物AMP激活的蛋白激酶(AMP-activated protein kinase, AMPK)和酵母蔗糖非发酵相关蛋白1 (sucrose non-fermenting-1, SNF1)的同源蛋白(Halford等2003; Hrabak等2003)。SnRK1是由α催化亚基和β、γ调节亚基组成的异源三聚体的丝氨酸/苏氨酸激酶, 参与糖和能量代谢等。SnRK3也被称为钙调磷酸酶B蛋白互作的蛋白激酶(calcineurin B-like protein- interacting protein kinase, CIPK), 和钙离子受体蛋白(calcineurin B-like proteins, CBLs)共同参与植物对各种离子的响应过程, SnRK2激酶家族则特异地应答植物对ABA和渗透胁迫的响应过程(Zhu 2016)。

Anderberg和Walker-Simmons (1992)利用合成的激酶保守区段探针从ABA处理的小麦(*Triticum aestivum*)种胚cDNA文库中杂交筛选到了一个ABA诱导转录水平上调的蛋白激酶PKABA1 (abscisic

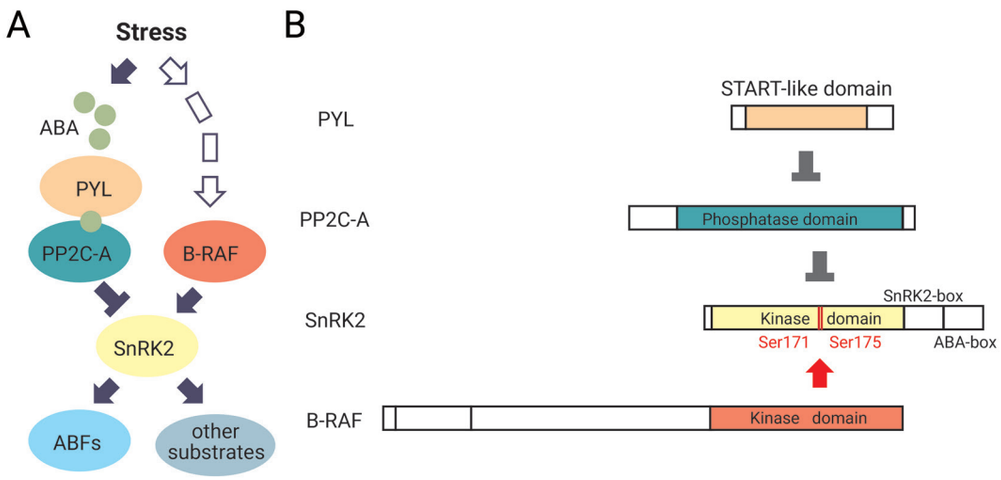

图1 ABA受体偶联核心信号途径模式

Fig. 1 Model of ABA receptor–coupled core signaling

A: ABA受体偶联核心信号途径主要由ABA受体PYL蛋白、蛋白磷酸酶PP2C-A、蛋白激酶SnRK2以及SnRK2激活子RAF蛋白激酶组成。B: PYL、PP2C-A、SnRK2、B-RAF蛋白的结构域(以PYR1、ABI1、SnRK2.6/OST1以及RAF3为例)。ABA: 脱落酸; ABFs: ABA应答元件结合蛋白; Kinase domain: 激酶结构域; Phosphatase domain: 磷酸酶结构域; START-like domain: 类固醇生成急性调节蛋白-相关脂质转移结构域; Stress: 逆境胁迫。实心箭头代表激活作用, T形箭头代表抑制作用, 空心箭头代表目前尚不清楚的调控机制。

acid-responsive protein kinase mRNA 1), 这是ABA信号通路中最早报道的蛋白激酶。凝胶激酶实验鉴定到了蚕豆(*Vicia faba*)保卫细胞中受ABA特异激活的丝氨酸/苏氨酸蛋白激酶AAPK (ABA-activated protein kinase), 它控制着保卫细胞ABA诱导的气孔关闭(Li和Assmann 1996; Li等2000)。2002年, 不同研究组在拟南芥(*Arabidopsis thaliana*)中利用正向遗传学筛选、凝胶激酶实验等方法鉴定到了参与ABA信号通路的SnRK2蛋白激酶(Merlot等2002; Yoshida等2002)。小麦PKABA1和蚕豆AAPK蛋白激酶都是拟南芥SnRK2激酶的同源蛋白, 它们在不同物种中均调控ABA的生理反应, 体现了SnRK2在进化中的功能保守性。

模式植物拟南芥SnRK2家族共有10个成员, 被称作SRK2A~J或SnRK2.1~10 (以下称为SnRK2.1~10; Yoshida 等 2002; Boudsocq 等 2004)。其中SnRK2.2/3/6特异地被ABA强烈激活, SnRK2.7和SnRK2.8能被ABA微弱激活(Boudsocq等2004)。除SnRK2.9外, 其他9个SnRK2可以被渗透胁迫处理激活。SnRK2家族在水稻(*Oryza sativa*)中有10个成员, 命名为SAPK (stress/ABA-activated protein kinase) 1~10。根据蛋白序列和对ABA及渗透胁迫的响应将拟南芥SnRK2和水稻SAPK分为3个亚组(subclass), 其中SnRK2.2/3/6以及SAPK8~10属于Subclass III; Subclass II包含SnRK2.7/8以及SAPK1~3; Subclass I包括SnRK2.1/4/5/9/10以及SAPK4~7 (Boudsocq等2004; Kobayashi等2004)。

拟南芥SnRK2 Subclass III中的*SnRK2.6*也被称作*Open Stomata 1* (*OST1*), 主要在保卫细胞中表达。*snrk2.6*/*ost1*单突变体气孔持续开放且对ABA处理不敏感, 水分散失快, 叶片温度较低, 但*snrk2.6*/*ost1*的种子萌发对ABA的敏感性与野生型类似(Mustilli等2002; Yoshida等2002)。随后的研究发现SnRK2.6通过调控钾离子(K^+)通道KAT1、慢速阴离子通道SLAC1 (slow anion channel-associated 1)、快速阴离子通道QUAC/ALMT等, 参与气孔运动的调节过程(Geiger等2009; Sato等2009; Imes等2013)。SnRK2.6还磷酸化RBOHF, 调节保卫细胞活性氧的产生(Sirichandra等2009)。后续的研究工作还证实SnRK2.6参与了病原菌侵染过程的气孔调节(Melotto等2006)。二氧化碳(CO_2)是调节气孔运动的另一个主要因素, 高浓度的CO_2促进气孔关闭。在*snrk2.6*突变体中, 高浓度CO_2诱导的气孔关闭响应受到影响(Mustilli等2002; Merilo等2013)。尽管保卫细胞对高浓度CO_2响应需要ABA-SnRK2信号途径参与(Zhang等2020), 但SnRK2的活性不被CO_2处理激活(Hsu等2018), ABA受体PYL4和PYL5对于CO_2诱导的气孔关闭也不是必需的(Dittrich等2019)。SnRK2参与高浓度CO_2诱导的气孔关闭的机理仍需要进一步解析。

与SnRK2.6不同, 拟南芥SnRK2.2/3在植物中广泛表达。*snrk2.2*和*snrk2.3*单突变体在萌发过程中只有微弱的ABA不敏感表型, *snrk2.2/3*双突变体可以在5 μmol·L^{-1} ABA处理条件下正常萌发与生长(Fujii等2007), 但*snrk2.2*、*snrk2.3*以及*snrk2.2/3*双突变体在叶片失水和气孔运动方面与野生型相当。*snrk2.2/3/6*三突变体对ABA极不敏感, 可在300 μmol·L^{-1} ABA处理下正常萌发(Fujii和Zhu 2009; Fujita等2009), 其气孔的ABA应答以及ABA诱导的基因表达也被完全阻断。这说明SnRK2.2/3/6是ABA信号通路中的核心组分, 以功能冗余方式介导了ABA应答过程。除对ABA极不敏感外, *snrk2.2/3/6*三突变体还表现出早花、育性降低、植株矮小等表型, 表明ABA和SnRK2在植物生长发育调控过程中有重要作用(Fujii和Zhu 2009; Fujita等2009; Wang等2013)。

外源ABA能微弱地激活拟南芥SnRK2.7和SnRK2.8 (Boudsocq等2004)。SnRK2.7和SnRK2.8的双突变体中, 一些ABA诱导基因表达受到抑制(Mizoguchi等2010), 表明SnRK2.7/8也参与ABA信号途径, 但*snrk2.1/4/5/7/8/9/10*七突变体并没有明显的ABA不敏感表型(Fujii等2011)。SnRK2.8磷酸化转录因子NTL6, 促进其核质定位调节过程(Kim等2012; Lee等2015a)。SnRK2.8还能磷酸化代谢途径的多个酶, 调控代谢过程(Shin等2007)。*SnRK2.8*的表达还受病原菌的诱导, 并参与植物获得性免疫过程(Lee等2015a; Lei等2020)。SnRK2.8对植物免疫应答的核心蛋白NPR1磷酸化介导了NPR1从细胞质到细胞核的定位过程(Lee等2015a)。SnRK2.8磷酸化假单孢菌激发子AvrPtoB, 对于AvrPtoB介导的FLS2降解是必需的(Lei等2020)。

除SnRK2.9外, 其余所有的SnRK2均被渗透胁迫迅速激活。*snrk2.1/2/3/4/5/6/7/8/9/10*十突变体对渗透胁迫敏感, 且不能完成正常的生活史(Fujii等2011)。值得注意的是, 渗透胁迫对SnRK2的激活不依赖于ABA信号通路(Vlad等2010; Zhao等2018)。

水稻SAPK蛋白激酶的研究中, 发现OsSAPK8/9/10被ABA强烈激活并且在ABA信号转导中起重要作用(Kobayashi等2004; Kobayashi等2005)。*OsSAPK10*的过表达水稻对ABA敏感, OsSAPK10通过磷酸化bZIP72增加其稳定性, 介导种子萌发过程(Wang等2020b)。OsSAPK10还磷酸化OsbZIP86正向调控*OsNCED3*的表达, 调节ABA的生物合成(Gao等2022)。OsSAPK1和OsSAPK2正调控水稻耐逆性(Lou等2018; Wang等2020b)。OsSAPK8磷酸化并激活环核苷酸门控通道OsCNGC9, 引发细胞钙水平升高, 激活水稻低温胁迫应答基因的表达, 参与水稻的冷胁迫应答(Wang等2021a)。这些研究显示水稻SAPK与拟南芥SnRK2蛋白激酶类似, 参与水稻ABA信号途径和多种环境胁迫应答过程。

如图1-B所示, SnRK2蛋白激酶N端是一个典型的丝氨酸/苏氨酸蛋白激酶结构域(kinase domain, KD)。SnRK2 Subclass III成员相比其他亚组SnRK2, 在激酶结构域前具有约18个氨基酸的序列, 这一段序列缺失会导致SnRK2.6活性降低, 但不影响ABA和山梨醇对SnRK2.6的激活(Yoshida等2006)。在C末端, 有SnRK2 box (也被称作domain I)和富含酸性氨基酸的ABA box (也被称作domain II)。SnRK2.6缺失ABA box时在体内不能被ABA激活, 也不能回补*ost1*的气孔缺陷表型, 但仍可以被渗透胁迫激活(Belin等2006; Yoshida等2006)。推测ABA box与PP2C-A富含正电荷的氨基酸相互作用, 促进SnRK2与PP2C-A的结合(Soon等2012)。玉米(*Zea mays*)中的CK2通过磷酸化OST1的ABA box的多个位点, 增强其与PP2C-A的结合并促进Zm-OST1降解(Vilela等2015)。SnRK2 box缺失会导致SnRK2活性降低, 不能回补*ost1*的缺陷表型(Belin等2006; Yoshida等2006)。

磷酸化与去磷酸化是SnRK2活性调控的主要方式。SnRK2.6的多个位点(Ser7、Ser18、Ser29、Ser43、Ser171、Ser175、Thr176、Ser179、Ser182等)在体内均会发生磷酸化(Boudsocq等2004, 2007; Belin等2006; Vlad等2010)。ABA处理后, 位于SnRK2.6激酶激活环(activation loop)的Ser171和Ser175磷酸化水平升高, Ser175的点突变导致SnRK2.6激酶活性丧失, 原核表达的SnRK2.6^{Ser171Ala}虽然维持一定的激酶活性, 但内源SnRK2.6^{Ser171Ala}活性完全丧失, 这两个位点的磷酸化对于SnRK2.6的激活是必需的(Vlad等2010)。

生物化学和结构生物学研究发现, SnRK2.6蛋白的Ser175是PP2C-A的靶位点(Soon等2012), PP2C-A通过在Ser175位点去磷酸化抑制SnRK2活性, 但Ser175位点的磷酸化即SnRK2的激活过程在很长时间内并不清楚。基于大肠杆菌(*Escherichia coli*)原核表达(已被高度磷酸化)的SnRK2.6的研究结果显示, Ser175是SnRK2.6自磷酸化位点; PP2C-A离开后, SnRK2.6可能通过自磷酸化激活(Ng等2011)。但最近结果显示, B-RAF蛋白激酶磷酸化这一保守的磷酸化位点, 启动SnRK2.6的激活过程, 对于SnRK2的重新激活是必需的(见后文)。Shang等(2016)报道BAK1 (BRI1-associated receptor kinase 1)可能通过磷酸化SnRK2, 介导SnRK2的激活过程, 但未进一步鉴定磷酸化位点和调控方式。最近巩志忠研究组发现BAK1磷酸化SnRK2.6的Thr146位点, 并证实BAK1通过磷酸化Thr146抑制其活性, 而不是促进SnRK2激活(Deng等2022)。在SnRK2.6的激活环上还存在保守的Thr179位点。拟南芥糖原合成酶激酶(glycogen synthase kinase 3, GSK3)家族成员BIN2 (brassinosteroid insensitive 2)磷酸化SnRK2.2的Thr181和SnRK2.3的Thr180位点(对应SnRK2.6的Thr179), 参与SnRK2.2/2.3的激活过程, 但BIN2可能并不磷酸化SnRK2.6 (Cai等2014)。由于SnRK2家族成员高度保守, BIN2如何特异地磷酸化SnRK2.2/3尚不清楚。已知BAK1、BIN2都是油菜素甾醇(brassinosteroid, BR)信号途径的核心组分, BAK1、BIN2等调控SnRK2的活性, 介导了ABA与BR信号途径的交互(crosstalk)。前面提到, SnRK2.6还存在Ser7/Ser18/Ser29等磷酸化位点, 但磷酸化这些位点的蛋白激酶以及生物学功能尚不清楚(Belin等2006)。

除磷酸化调控外, SnRK2的活性还受氧化还原调控。生物化学结果显示一氧化氮(nitric oxide, NO)和过氧化氢(H_2O_2)都能在体外抑制SnRK2.6的活性(Zhu等2014; Wang等2015)。SnRK2.6蛋白Cys137点突变能解除NO对其活性的抑制。ABA诱导产生的NO通过诱导Cys137位点的S-亚硝基化(S-nitrosylation), 抑制SnRK2.6的激酶活性, 可能作为脱敏机制(desensitization)负反馈调节ABA信号途径(Wang等2015)。还有报道Cys131和Cys137位点可以发生硫巯基化(persulfidation)增强SnRK2.6的活性(Chen等2020), 可能在ABA信号更早期正反馈调节SnRK2的活性。一些泛素E3连接酶如PP2-B11/HOS15等通过介导SnRK2.3/6泛素化, 调控其稳定性(Cheng等2017; Ali等2019)。

2 PP2C-A磷酸酶

PP2C-A磷酸酶是ABA信号途径的负调控组分, 通过结合并抑制SnRK2, 阻断ABA信号途径。以ABA抑制的种子萌发为筛选标志, Leung等(1997)筛选获得了两个ABA不敏感显性突变体*abi1-1* (*ABA insensitive1-1, abi1*^{G180D})和*abi2-1* (*abi2*^{G168D})。有趣的是, *abi1*和*abi2*其他等位突变体在萌发等过程中对ABA更敏感(Gosti等1999; Merlot等2001)。ABA受体发现后, 研究者才明确G180D和G168D突变导致ABI1/2与PYL蛋白的结合受到抑制, 使ABI1^{G180D}和ABI2^{G168D}的活性不受ABA信号调控, 导致*abi1-1*和*abi2-1*对ABA不敏感(Park等2009)。

PP2C磷酸酶是Mg^{2+}/Mn^{2+}依赖的丝氨酸/苏氨酸磷酸酶。拟南芥基因组编码了约80个PP2C, 按照序列相似性可分为13个分支(A~L; Kerk等2002; Xue等2008)。其中PP2C-A有9个成员, 除了ABI1和ABI2外, 还包括HAB1 (hypersensitive to ABA 1)、HAB2、HAI1 (highly ABA induced 1)、HAI2、HAI3、AHG1 (ABA hypersensitive germination 1)、AHG3/PP2CA。已知*pp2ca*、*hai1*、*ahg1*、*ahg3*等突变体均对ABA的敏感性增强, 过表达植株则对ABA不敏感(Yoshida等2006; Nishimura等2007)。*abi1/hab1/abi2*以及*abi1/hab1/pp2ca*等多突变体对ABA超敏感, ABA诱导的SnRK2活性增加, 甚至在没有ABA处理的情况下也可以检测到SnRK2的激活(Fujii等2009; Rubio等2009; Bhaskara等2012; Li等2016; Miao等2021)。很早已经知道ABI1能与SnRK2.6相互作用(Yoshida等2006), 对HAB1靶位点的筛选发现HAB1去磷酸化SnRK2.6/OST1蛋白激活环Ser175位点, 抑制SnRK2.6/OST1的活性, 揭示了PP2C-A和OST1相互作用的基本模式(Vlad等2009)。Soon等(2012)获得了SnRK2.6和HAB1复合体, 通过结构生物学方法解析PP2C-A抑制SnRK2的分子机制, 发现HAB1可以直接结合并去磷酸化Ser175位点, 去磷酸化的SnRK2.6失去大部分活性; 另外SnRK2.6富含负电荷的ABA box与HAB1表面结合, 完全抑制SnRK2.6的活性。此外, 结构生物学结果显示ABA box和HAB1的结合方式与ABA受体PYL2-ABA和HAB1结合的空间特征类似。

ABI1和ABI2还可以去磷酸化蛋白激酶GHR1 (guard cell hydrogen peroxide-resistant 1)、CKL2 (casein kinase 1-like protein 2)等, 参与ABA对气孔运动的调节过程(Hua等2012; Shi等2022)。已知CKL2磷酸化ADF4 (actin depolymerizing factor 4)参与气孔运动过程中细胞骨架的调节(Zhao等2016a)。在ABA信号途径中, CKL2和SnRK2可能以平行的方式调控下游生物学过程(Wang等2020a; Shi等2022)。值得注意的是, CKL2还可以磷酸化ABI1/2, 调控ABI1/2的稳定性(Shi等2022)。GHR1是调控气孔运动的核心蛋白激酶。*ghr1*突变体在气孔运动过程中对ABA、H_2O_2、CO_2、渗透胁迫等不敏感(Hua等2012; Sierla等2018; Hsu等2021)。体内富集的GHR1能磷酸化SLAC1, 调控SLAC1的活性(Hua等2012; Sierla等2018)。GHR1还参与了ABA和H_2O_2诱导的胞内钙离子(Ca^{2+})浓度升高(Hua等2012)。有趣的是, GHR1蛋白激酶结构域HRD和DFG两个关键区域存在突变导致其本身并不具有激酶活性(Sierla等2018)。ABI2能抑制GHR1对于SLAC1的激活(Hua等2012), 但分子机制可能不同于其对Sn-RK2和CKL2的调控。

除此之外, PP2C-A还可以去磷酸化MPKKK18 (Mitula等2015)、BIN2 (Wang等2018a)、FERONIA (FER; Yu 等 2012; Chen 等 2016)、CIPK23/CIPK26 (Lyzenga等2013)、CPK21/CPK23 (Geiger等2010)、CPK11 (Lynch等2012)、RDK1 (Kumar等2017), 介

导ABA信号途径以及ABA和其他激素的互作(cross-talk)。除PP2C-A外, E分支的EGR2也被报道能抑制SnRK2 (Xue等2008; Bhaskara等2017), 可能作为另外的负反馈调节, 防止SnRK2等过度激活。

I型蛋白磷酸酶(type I protein phosphatase, TOPP)成员也被证实参与了SnRK2的活性调控, 在模式植物拟南芥TOPP家族中有9个成员(TOPP1~TOPP9)。Hou等(2016)报道TOPP1能结合并去磷酸化SnRK2.6, TOPP1及其调节蛋白Inhibitor I-2 (I-2)也与ABA受体PYL蛋白相互作用, 模拟PP2C-A的功能, 参与ABA信号途径。除TOPP1外, TOPP2/5/3/7也参与ABA信号途径。*topp1/2/5/3/7*在萌发、气孔运动过程中对ABA敏感(Hu等2022)。丁香假单胞菌(*Pseudomonas syringae*)效应因子AvrE/hopM1通过直接抑制TOPP1的活性, 调控ABA信号途径, 介导水渍产生的过程(Hu等2022)。PP2A家族成员也被报道参与调控SnRK2活性, 参与ABA信号途径(Kelner等2004; Pernas等2007; Takahashi等2013)。

目前已知ABA和PYL通过直接结合并抑制磷酸酶活性, 是调控PP2C-A活性最主要的方式。另外, EAR1蛋白以及多个蛋白激酶参与了调控PP2C-A的活性。EAR1是一个未知功能的蛋白, 与ABI1、ABI2、HAB1、HAB2、AHG1以及AHG3的N末端的自抑制结构域(inhibition domain)结合, 并促进PP2C-A的活性, *ear1*突变体对ABA超敏感(Wang等2018b)。受体激酶PR5K2磷酸化ABI1/2磷酸酶结构域, 增强ABI1/2的活性(Baek等2019a)。生长素信号途径的核心蛋白激酶TMK1 (trans-membrane kinase 1)磷酸化ABI1磷酸酶结构域的T321位点, 抑制ABI2的活性(Yang等2021)。有趣的是TMK家族的另一个成员TMK4磷酸化ABI2的S139、S140和S266位点, 促进ABI2的活性(Li等2021)。TMK家族不同成员与PP2C-A的关系值得进一步深入研究。类受体激酶RDK1 (receptor dead kinase 1)介导ABI1在质膜上的募集, 对ABA信号转导起着正调控作用, 但RDK1本身不具有激酶活性, 其调控PP2C-A活性的分子机制并不清楚(Kumar等2017)。受体激酶FER也被报道磷酸化并促进ABI2的活性, 负调控ABA信号途径(Yu等2012)。ABI2等又通过去磷酸化FER, 负反馈调控FER的活性(Chen等2016)。

很早已经知道H_2O_2能抑制ABI2的活性, 还原剂二硫苏糖醇(dithiothreitol, DTT)则促进ABI2的活性(Meinhard等2002)。Miao等(2006)报道谷胱甘肽氧化酶与ABI2结合, 可以作为活性氧信号的传感器(transducer), 将氧化信号传递给ABI2, 调控ABI2的活性。磷脂酸(phosphatidic acid, PA)也可以结合并抑制ABI1的活性; ABA通过影响*PLDα1*的表达, 调控PA合成(Zhang等2004)。

ABA通过影响*PP2C-A*基因的表达以及蛋白稳定性调控体内PP2C-A含量。*ABI1*、*ABI2*、*HAI1*的表达受ABA诱导, 是标志性的ABA应答基因。PP2C-A蛋白含量也受到严格调控。拟南芥泛素E3连接酶COP1、PIR1/2、RGLG1/5、Cullin3-RING等调控了PP2C-A的稳定性(Wu等2016; Baek等2019b; Belda-Palazon等2019; Julian等2019; Chen等2021)。

已知水稻PP2C-A磷酸酶OsABIL2 (OsABI-LIKE2)的过表达植株对ABA不敏感, 影响气孔密度和根系发育, *OsPP2C49*的过表达也会减弱植株对ABA的敏感性, 导致植物迅速失水(Li等2015; Zong等2016)。OsPP2C51作为ABA信号的负调控因子, 参与水稻种子萌发(Bhatnagar等2017)。与拟南芥PP2C-A类似, OsPP2C09可在体外去磷酸化OsSAPK, 参与ABA信号途径并调控水稻的耐旱性(Miao等2020)。

3 PYR1/PYLs/RCARs受体

ABA的受体PYL是最后一个被鉴定的“五大”植物激素受体。在PYL受体被发现前, FCA、叶绿体蛋白CHLH/ABAR、G蛋白偶联受体GCR2、GPCR类G蛋白也曾被认为是植物激素ABA的受体, 但由于缺乏明确的生理学和结构生物学证据支持ABA依赖的活性调控, 以及无法与经典的ABA信号组分关联, 这些受体并未被广泛认可(Shen等2006; Gao等2007; Liu等2007; Pandey等2009)。ABA受体长期难以发现的原因在于PYL以功能高度冗余的基因家族形式存在, 单个成员的突变并不显著影响突变体对ABA的应答。S. Cutler研究组利用化学遗传学方法, 筛选获得了一个在萌发阶段对ABA类似物pyrabactin不敏感的突变体, 将其命名为*Pyrabactin resistant 1* (*pyr1*; Park等2009)。PYR1及其同

源蛋白PYL能强烈抑制HAB1的活性, 且这一抑制依赖于ABA (Park等2009)。E. Grill研究组通过酵母双杂交系统筛选发现了与PP2C-A磷酸酶互作的蛋白RCAR也发挥着ABA受体的作用(Ma等2009)。

拟南芥PYL家族共有14个成员, 其中*pyr1/pyl1/2/4*四突在萌发过程中对ABA不敏感(Park等2009)。六突变体*pyr1/pyl1/2/4/5/8*可以在含50 μmol·L^{-1} ABA的培养基上正常萌发, 其对ABA不敏感与*snrk2.2/3/6*相当(Gonzalez-Guzman等2012)。ABA诱导的SnRK2的激活在*pyr1/pyl1/2/4/5/8*中完全消失(Gonzalez-Guzman等2012)。Zhao等(2018)通过基因编辑构建了PYL的多突变体, 其中十二突变体*pyr1/pyl1/2/3/4/5/7/8/9/10/11/12*可以在含毫摩尔级别ABA的培养基上正常萌发, 气孔运动对ABA完全不敏感, 表明PYL在感受ABA中的核心作用, 也暗示了拟南芥可能并不存在其他的ABA受体。与*snrk2.2/3/6*三突变体类似, *pyr1/pyl1/2/3/4/5/7/8/9/10/11/12*也表现出植株矮小、花器官发育异常等表型。有趣的是*pyl3/7/9/11/12*多突变体并没有明确的ABA不敏感表型(Zhao等2018)。文章提到作者无法获得*PYL*的更高阶突变体, 可能是由于ABA及其受体除介导胁迫应答过程外, 对植株的生长发育过程也是必需的, PYL完全缺失会影响正常生命周期的完成。

发现PYL后, 多个研究组迅速解析了PYL蛋白以及PYL-PP2C复合体的结构(Melcher等2009; Miyazono等2009; Nishimura等2009; Santiago等2009; Yin等2009)。研究发现, PYR1、PYL1/2/3在体外可以形成二聚体, ABA结合位点隐藏在二聚体受体内部。ABA进入PYL的中央疏水区域后, 诱导表面CL2环结构发生构象重排, 并创建一个PP2C-A结合表面抑制PP2C-A磷酸酶活性(Melcher等2009; Yin等2009)。ABI1、ABI2、HAB1、PP2CA等存在下, PYL与ABA的亲和力可以增加近100倍(Ma等2009), 因此PP2C-A可被认为是ABA的共受体(Ma等2009)。PYL4/5/6/8/9/10主要以单体形式存在, 在体外这些PYL对PP2C-A的抑制可以不依赖于ABA (Melcher等2010; Hao等2011; Dorosh等2013)。PYL13蛋白在ABA结合区域的几个氨基酸发生突变, 结构生物学和遗传学结果都证实PYL13失去了结合ABA的能力, 但可以以不依赖ABA的方式与PP2CA持续结合, 抑制PP2CA活性, 仍参与ABA信号通路(Li等2013; Zhao等2013; Fuchs等2014)。

拟南芥有14个PYL蛋白, 暗示这些蛋白存在强烈的功能冗余, 一些PYL在特定组织、器官、发育阶段以及生理过程中有独特的作用。例如, *PYR1/PYL1/2/4/5/8*在气孔中高表达, 其中PYL2主导ABA诱导的气孔关闭, 而PYL4/5介导了高浓度CO_2对气孔的关闭(Dittrich等2019)。研究*pRD29A::PYL9*转基因株系, 发现PYL9调控了ABA诱导的叶片衰老(Zhao等2016b)。这些不同组合的PYL-PP2C复杂又精细地调控细胞内ABA的感知和信号的起始(Fujii等2009; Hao等2011; Zhao等2013; Tischer等2017)。同时, 一些PYL还可以作为ABA氧化代谢产物phaseic acid (PA)的受体, 参与PA信号并参与干旱胁迫应答(Weng等2016)。

已知PYL的转录水平和蛋白活性、稳定性、二聚体形成以及定位等受到多层次的复杂调控。植物通过这些调控精确地调节胁迫应答水平和生长发育过程, 最大程度地适应复杂的环境变化。在种子萌发过程中, ABA激活的转录因子ABI5可以直接结合*PYL11/12*基因的启动子区域, 在转录水平对其进行调控(Zhao等2020)。Wang等(2018)利用磷酸化蛋白组学, 发现PYL蛋白存在一个高度保守的磷酸化位点(对应于PYL1蛋白Ser119位点), ABA处理后这一位点的磷酸化水平下降或消失。这一位点的磷酸化导致PYL蛋白不能结合ABA, 失去感受和传递ABA信号的能力。控制细胞能量代谢、生长和发育过程的关键蛋白激酶雷帕霉素靶蛋白(target of rapamycin, TOR)能特异地磷酸化这一位点。非胁迫条件下, TOR通过磷酸化失活PYL蛋白, 促进生长发育并抑制胁迫应答。同时, TOR复合体中的调节组分Raptor B (regulatory-associated protein B of mTOR)与SnRK2相互作用且是SnRK2的底物(Wang等2018c; Zhu等2022)。胁迫条件下SnRK2通过磷酸化Raptor B蛋白, 促进TOR复合体解离, 抑制TOR活性(Wang等2018c; Zhu等2022)。这一研究发现了ABA受体的磷酸化调控机制, 并发现TOR蛋白激酶复合体和ABA受体偶联途径组成的调节环。植物通过这一机制介导了胁迫应答和生长发育的平衡。值得注意的是PYL1 Ser119对应的

位点在所有陆地植物中高度保守, TOR对PYL的磷酸化调控可能是高等植物共有的保守机制(Wang等2018c)。但目前去磷酸化Ser119位点和Raptor B的磷酸酶尚未见报道, 并且Raptor B蛋白存在多个磷酸化位点; 除SnRK2外, 哪些蛋白激酶磷酸化这些位点, 以及这些位点的功能仍需要进一步研究。

除TOR以外, CARK1 (cytosolic ABA receptor kinase 1; Zhang等2018; Li等2019, 2022)、CEPR2 (C-terminally encoded peptide receptor 2; Yu等2019; Zhang等2021)、AEL (Chen等2018)、WNK8 (Waadt等2019)和BAK1 (Shang等2022)等蛋白激酶也能磷酸化PYL蛋白, 参与ABA信号途径。CARK1磷酸化RCAR11/PYR1 (Thr78位点)、RCAR3/PYL8、RCAR12、RCAR13、RCAR14等, 可能通过促进PYL蛋白形成二聚体, 促进PYL结合ABA和抑制PP2C-A活性(Zhang等2018; Li等2019; Li等2022)。蛋白激酶CEPR2磷酸化PYL4的Ser54位点, 促进PYL的降解(Yu等2019)。CEPR2还磷酸化NRT1.2/NPF4.6, 促进ABA向内转运(import)的活性(Zhang等2021)。拟南芥酪蛋白激酶AEL也可以通过磷酸化PYL1的S136和S182位点, 促进其泛素化降解, 进而调控ABA信号输出(Chen等2018); 酪蛋白激酶CKL2也可以磷酸化PYL上相同位点(Shi等2022), 可能和AEL共同调控PYL蛋白的稳定性。此外酪蛋白激酶参与ABA信号通路和BR信号通路的转换过程, CKL2通过磷酸化BRI1参与植物胁迫后生长恢复过程的调节(Zhao等2023)。最近报道, BAK1还可能磷酸化PYR1末端的T137和S142位点, 可能通过调控功能PYL1二聚体的形成, 正反馈ABA信号途径(Shang等2022)。BAK1可能通过磷酸化多个ABA信号组分, 参与ABA信号途径的多个阶段及不同下游响应过程的调控(Shang等2016, 2022; Deng等2022; Pei等2022)。

膜锚定的C2-domain ABA-related (CAR)蛋白与PYL相互作用, 介导PYL蛋白的膜定位(Rodriguez等2014)。WD40蛋白ABT能与PYL以及ABI相互作用, 抑制ABA信号途径(Wang等2020c)。PYL蛋白稳定性也受到严格控制, RSL1 (single-subunit ring-type E3 ubiquitin ligase)泛素化并促进PYL4和PYR1降解(Bueso等2014; Irigoyen等2014; Yu等2016; García-León等2019)。Castillo等(2015)报道PYL蛋白的多个位点还可以发生酪氨酸硝基化(tyrosine nitration)和半胱氨酸的巯基亚硝酰化(S-nitrosylation), 其中的Y120和Y143位点酪氨酸硝基化会导致PYR1蛋白失活。ABA诱导产生的NO可能通过酪氨酸硝基化失活PYL蛋白, 负反馈调控ABA信号途径。

干旱、高盐及低温胁迫严重影响作物产量。多个研究组尝试利用化学和遗传学方法, 改造ABA-PYL途径, 提高植物抗逆性。ABA类似物pyrabactin可被用来作为抗蒸腾剂, 提高作物耐旱性(Park等2009; Puli和Raghavendra 2012)。Vaidya等(2019)发现与PYR1蛋白亲和力更强的ABA受体激动剂opabactin可显著增强番茄(*Solanum lycopersicum*)、小麦的抗寒性, 在改良植物抗旱、植物生理学研究和农业生物技术应用中具有潜在的价值。朱健康研究组筛选获得多个ABA类似物, 如AM1 (ABA mi-mic 1)、AMF等, 化学性质稳定, 成本低, 在促进气孔闭合和诱导应激反应基因的表达方面具有长久效果, 在拟南芥和大豆(*Glycine max*)中应用能提高耐旱性(Cao等2013, 2017; Cheng等2016)。通过改造PYR1多个位点, 促进PYR1单体的形成, 可以控制ABA的响应及提高转基因作物的抗旱性(Park等2015)。Zhao等(2016b)利用胁迫应答基因启动子增强*PYL9*在胁迫调节下的表达, 能增加拟南芥和水稻的抗旱性。敲除水稻PYL1/4/6在不显著影响水稻抗逆性的同时, 能大幅提高基因编辑水稻的产量(Miao等2018)。超表达小麦*PYL*基因可以提高水分利用效率, 增加小麦产量(Mega等2019)。

4 SnRK2的下游底物

SnRK2是ABA信号通路输出的核心组分, SnRK2通过磷酸化下游底物, 控制基因表达、离子通道、生长发育等几乎所有ABA应答过程。与所有SnRK以及CPK蛋白激酶类似, SnRK2主要识别并磷酸化LxRxxS/T基序(motif)。在SnRK2已知底物中, 碱性亮氨酸拉链(basic leucine zipper, bZIP)家族转录因子是ABA调控基因表达的核心元件(Guiltinan等1990)。A亚组bZIP转录因子, 包括ABF1~4、ABI5、CBF4 (CRT/DRE binding factor 4)等参与了

ABA诱导基因的表达。*areb1/areb2/abf3*三突变体耐旱性和ABA敏感性降低, ABA及胁迫响应基因表达水平降低(Yoshida等2010)。ABF1~ABF4蛋白包含多个SnRK2的磷酸化位点, 这些位点突变后, SnRK2对ABF2的磷酸化能力下降, ABF2介导ABA应答基因表达的功能消失(Fujii等2009)。ABF2片段还被用来作为SnRK2的底物, 以检测SnRK2的活性(Fujii等2009; Wang等2018c)。ABI5则是控制植物种子萌发的核心蛋白, ABI5功能缺失以后, 植物种子的萌发对ABA处理不敏感。除bZIP家族转录因子外, SnRK2还可以磷酸化bHLH家族转录因子AKS1~4 (也被命名为FBH1~3)、RAV1 (related to ABA-insensitive 3/VP1)、VOZ1等来调控衰老、开花等过程(Takahashi等2013; Feng等2014; Chong等2022)。除磷酸化转录因子外, SnRK2还可以磷酸化转录调节因子BRM (CHR chromatin-remodeling ATPase BRAHMA)、miRNA合成调控蛋白SERRATE和HYL1、RNA的剪接相关蛋白BTR1L以及表观修饰相关蛋白HD2B等, 参与转录调控的多个阶段(Peirats-Llobet等2016; Yan等2017)。有趣的是, 组蛋白(histone)多年来被用作为检测SnRK2激酶活性的底物, 但SnRK2如何调控组蛋白的功能尚未见报道。

除转录调控蛋白外, 已知SnRK2的底物还包括阳离子通道KAT1、慢速阴离子通道SLAC1/2、快速阴离子通道QUAC1/ALMT等(Geiger等2009; Sato等2009; Imes等2013; Maierhofer等2014; Grondin等2015)。SnRK2通过直接调控这些通道蛋白的活性调控ABA诱导的气孔关闭过程。SnRK2还可磷酸化糖转运蛋白SWEET, 通过调控源库间的运输, 调控干旱条件下植物根的发育(Chen等2022)。SnRK2也被认为调控了SnRK1, 对植物生长发育有双重作用, 但详细的分子机制并不清楚(Belda-Palazón等2020)。

多个研究组利用磷酸化蛋白组学方法, 通过比较ABA处理前后*snrk2.2/3/6*突变体和野生型中ABA诱导的蛋白磷酸化的变化, 鉴定了数十个潜在的SnRK2底物(Umezawa等2013; Wang等2013)。Wang等(2020)还利用更高效的KALIP2方法, 发现SnRK2.6在体外可以磷酸化超过1 500个蛋白, 显示SnRK2通过对底物的广泛调控, 介导多种生物学过程(Wang等2020a)。

5 B-RAF蛋白激酶

早期基于原核表达蛋白的研究认为, 从PP2C-A的抑制中释放后, SnRK2可以通过自磷酸化位于激活环中的两个保守氨基酸Ser171和Ser175后重新激活(auto-activation; Ng等2011)。但最近的研究结果表明, 去磷酸化状态下的SnRK2不能自激活, B-RAF蛋白激酶磷酸化SnRK2蛋白激活环上对应于S171/S175的位点, 介导SnRK2的重新激活过程。

丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)是一类高度保守的丝氨酸/苏氨酸类蛋白激酶, 主要介导细胞外信号到细胞内的传递过程。典型的MAPK级联途径通常由三级激酶级联组成: MAPKKK接受上游信号并磷酸化MAPKK, MAPKK继而磷酸化MAPK, MAPK磷酸化下游底物并调控细胞内的生物学过程。拟南芥MAPKKK家族共有80个成员, 其中46个属于RAF亚家族(Ichimura等2002)。在22个B亚组RAF中, RAF1/CTR1是乙烯受体偶联组分, 并被报道参与ABA信号途径(Saruhashi等2015; Stevenson等2016; Islam等2021)。RAF10/11也通过磷酸化SnRK2在ABA信号通路中发挥调控作用, RAF11的过表达转基因植株在种子萌发上对ABA超敏感, 说明RAF10/11通过正调控参与ABA的信号转导(Lee等2015b)。RAF5/SIS8、RAF2/EDR1也被报道参与了ABA信号途径。

研究人员在苔藓植物小立碗藓(*Physcomitrella patens*)中筛选获得一个ABA不敏感突变体*AR7*, 进一步解析发现B亚组RAF蛋白激酶ARK (ABA and abiotic stress-responsive Raf-like kinase)的突变导致其ABA不敏感的表型。PpARK可以通过磷酸化正调控PpSnRK2的激酶活性(Saruhashi等2015)。小立碗藓B-RAF家族只有6个成员, 其中PpARK (另两项研究命名为PpCTR1/PpANR)同时介导了ABA和乙烯的信号途径(Saruhashi等2015; Yasumura等2015; Stevenson等2016)。尽管拟南芥*RAF3/4/5*等*ARK*同源基因可以回补*AR7*突变体的ABA不敏感表型(Saruhashi等2015), 但高等植物中B-RAF蛋白激酶在SnRK2的激活过程以及ABA信号途径中的

作用并不清楚。

2020年, 4个不同的研究组先后报道了B-RAF磷酸化并介导SnRK2激活的分子机制(Katsuta等2020; Lin等2020; Soma等2020; Takahashi等2020)。J. Schroeder研究组早期利用miRNA文库发现沉默多个B亚组RAF基因会导致转基因材料ABA不敏感的表型(Hauser等2013)。基于这一发现, 他们构建了B3亚组RAF3/4/5 (也被称作M3Kδ1/δ6/δ7)多突变体。该多突变体在种子萌发、气孔运动过程中对ABA不敏感, ABA诱导的SnRK2活性降低。与ARK类似, RAF3/4/5能够磷酸化SnRK2.2/3/6 (Takahashi等2020)。Katsuka等(2020)根据RAF4/RAF5/RAF3互补ARK情况, 将其命名为AtARK1/2/3, 并发现三突变体*atark1/2/3*在萌发过程中对渗透胁迫不敏感, 失水较快, ABA应答基因表达受到抑制。王鹏程研究组通过磷酸化蛋白组学发现B4以及B2/3亚组RAF蛋白能被渗透胁迫迅速激活, 将其命名为OK^{130}和OK^{100} (Lin等2020)。通过CRISPR-Cas9基因编辑技术构建B亚组RAF多突变体, 发现在B2和B3的多突变体*OK^{100}-oct* (*raf3;raf4;raf5;sis8;raf7;raf8;raf9;raf10;raf11*)和*OK^{100}-nonu* (*raf3;raf4;raf5/sis8;raf6;raf7;raf8;raf9;raf10;raf11*)中, ABA诱导的SnRK2.2/3/6的激活几乎完全消失(Lin等2021)。*OK^{100}-oct*和*OK^{100}-nonu*可以在含有25 μmol·L^{-1} ABA的培养基上萌发, 是除了*snrk2.2/3/6*和*pyl*多突变体之外已知的对ABA最不敏感的突变体(Lin等2021), 进一步证明了B亚组RAF蛋白激酶在ABA信号转导途径中的核心作用。有趣的是, 尽管B2和B3亚组RAF共同参与ABA信号转导, 它们的作用似乎并不完全一样。突变体*OK^{100}-B3*植株矮小, 生长发育受到抑制, *OK^{100}-B2*则与野生型类似(Lin等2021)。*OK^{100}-B3*在含蔗糖的培养基上对ABA不敏感, 这一不敏感性在不含蔗糖的培养基上消失, 而*OK^{100}-B2*的ABA不敏感性似乎与是否有糖无关。值得注意的是*OK^{100}-oct*和*OK^{100}-nonu*在种子萌发和气孔运动的ABA不敏感程度仍不能与*snrk2.2/3/6*和*pyl*多突相比, 这显示*OK^{100}-oct*和*OK^{100}-nonu*中剩余的RAF1/RAF2/RAF12或除B2和B3以外的蛋白激酶也参与了ABA信号途径。最近, 有研究揭示了RAF6在气孔运动过程中的核心作用(Hsu等2021), 也有报道B1亚组RAF成员RAF13和RAF15也能磷酸化SnRK2.6, 参与ABA诱导的气孔运动过程(Wang等2021b)。

与B1/2/3亚组RAF参与ABA信号途径不同, B4亚组RAF只调控了不依赖ABA的SnRK2的激活。渗透胁迫诱导的SnRK2.1/4/5/10在多突*OK^{130}-null*中完全消失。*OK^{130}-null*也表现出对渗透胁迫敏感、渗透胁迫应答基因表达受抑制等表型(Lin等2020)。Soma等(2020)报道鉴定到B4亚组RAF成员与ABA不依赖的SnRK2相互作用, 参与了渗透胁迫对SnRK2的激活。B4亚组RAF40/HCR1还被发现参与低氧胁迫下K^+的响应, 与植物水分转运能力相关(Shahzad等2016)。

上述研究还进一步揭示了RAF蛋白激酶调控SnRK2的分子机制。完全去磷酸化的SnRK2并没有自激活的活性。B2/3亚组的RAF通过磷酸化SnRK2.6的S171和S175位点, 激活SnRK2.6。Lin等(2021)还利用腺嘌呤核苷三磷酸(ATP)类似物和点突变的SnRK2蛋白, 区分RAF对SnRK2的磷酸化以及SnRK2的自磷酸化, 结果进一步证实RAF对SnRK2的磷酸化是失活态SnRK2重新激活所必需的。SnRK2被RAF激活后, 活化的SnRK2能磷酸化其他尚未活化的SnRK2, 迅速放大SnRK2的激活过程。这一机制从一定程度上支持了基于原核表达SnRK2的结构生物学结果(Ng等2011), 即磷酸化激活的SnRK2.6可以通过磷酸化活化其他Sn-RK2.6; 在植物体内, RAF对于完全失活的SnRK2的激活是必需的。与渗透胁迫迅速激活B亚组RAF蛋白激酶活性不同, ABA处理并不能激活B2/3亚组RAF, 但B2/3的本底活性可能足以激活SnRK2 (Lin等2021)。这一“起始-放大”机制保证了ABA存在时, SnRK2被RAF激活后, 能通过激活其他未激活的SnRK2迅速放大ABA信号途径(Lin等2021)。但尚不清楚这一“起始-放大”机制是RAF-SnRK2激酶级联途径独有机制, 还是所有蛋白激酶级联的共有机制。

从已有的结果来看, 与典型的MAPKKK-MAPKK-MAPK三级级联途径不同, 植物利用了一套非典型的RAF-SnRK2级联途径介导了ABA和渗透胁迫对SnRK2的激活过程, 但目前对于RAF蛋白激酶本身的激活方式尚不清楚。在*reduced hyperosm-*

olality-induced [Ca^{2+}]$_i$ increase (*osca*)多突变体中, RAF和SnRK2的激活过程也不受影响, 推测RAF的激活可能不依赖于OSCA途径(Yuan等2014; Lin等2020)。仅有的结果显示ARK等RAF蛋白激酶多个位点的磷酸化受渗透胁迫处理诱导, 显示RAF的激活仍依赖于磷酸化过程, 但介导RAF激活的上游蛋白激酶尚未鉴定。与ABA不激活拟南芥B-RAF不同, 在小立碗藓中ABA处理可以直接增强PpARK蛋白激酶的磷酸化水平(Islam等2021), 表明苔藓和拟南芥B-RAF在进化过程中产生了功能差异。最近的研究发现苔藓中组氨酸激酶PpHK在ABA诱导的PpARK的磷酸化和SnRK2的激活中发挥作用(Toriyama等2022), 但拟南芥的组氨酸激酶是否参与ABA的信号转导仍不清楚。

Lin等(2020)的结果显示, 在SnRK2的十突变体*snrk2-dec*中, 渗透胁迫诱导的RAF的激活相对于野生型降低, 表明SnRK2以未知的方式反馈RAF的激活过程。除了B-RAF外, C亚组的RAF也被报道参与了ABA信号途径。C亚组RAF22/36被SnRK-2.6/OST1磷酸化激活, 参与ABA信号途径(Kamiyama等2021; Sun等2022)。Sun等(2022)还报道RA-F22磷酸化ABI1蛋白Ser416残基, 促进ABI1的活性, 作为负反馈机制, 精细调控(fine-turn) ABA信号的激活强度。RAF蛋白激酶除磷酸化并激活Sn-RK2以外, 是否还磷酸化其他蛋白激酶或底物, 参与ABA信号途径, 也有待进一步研究。

6 总结与展望

从*abi1/abi2*的筛选以及ABRE/EmBP1发现开始(Guiltinan等1990), 研究者用超过30年的时间, 逐步阐明了ABA信号转导的分子机制。PYL受体的发现是近年来植物生物学领域最重要的发现之一, 首次将ABA感受与核心信号组分关联起来, 阐明了ABA激活SnRK2的分子机制。B亚组RAF的发现则说明SnRK2的激活仍依赖于更多的上游信号, 对原有的ABA信号通路做出了重要修订。RAF上游调控组分的鉴定和功能解析, 是今后这一研究领域亟待解决的重要生物学问题之一。传统植物生理学和细胞生物学发现Ca^{2+}、三磷酸肌醇(inositol triphosphate, IP_3)、磷脂酶C (phospholipase C, PLC)/磷脂酶D (phospholipase D, PLD)、G蛋白等也是ABA信号途径的关键组分, 但这些组分很难放进现有PYL-PP2C-SnRK2核心途径中, 这些组分是否通过直接或者间接作用影响RAF的功能, 以及如何调控ABA信号通路也需要进一步研究。

目前对于ABA信号通路, 尤其是通过SnRK2调控基因转录、种子萌发、气孔运动的分子机制有较多的研究, 但对于ABA调控的其他重要生物学过程研究较少。例如, PYL和SnRK2的多突变体都表现出早花和育性降低的表型, 但详细的分子机制仍不清楚, SnRK2磷酸化组蛋白的生物学功能也未阐明。

从进化的角度来看, ABA受体偶联途径是植物从水生到陆生过程中适应陆地缺水和复杂多变环境的核心信号转导途径。高等植物PYL、PP2C-A、SnRK2、ABF以及B-RAF都以多基因家族形式存在, 超过50多个蛋白组成了一个复杂的调控网络, 介导不同组织、不同发育阶段、不同环境条件下ABA信号通路的精细调控。对这一复杂调控网络的解析, 不仅需要利用单细胞转录组/蛋白组解析特定细胞类群的信号转导特异性, 也需要通过系统生物学和数学建模的方法在系统和植株水平上理解这一调控网络的运作方式。此外, ABA是否影响植物与环境微生物互作也需要进一步解析。

尽管通过遗传学和化学方法筛选ABA类似物、对PYL受体蛋白的改造, 以及调控气孔运动等方式来改良植物抗逆性, 已经展现了通过ABA受体偶联信号途径来提高作物逆境适应方面的潜力。但由于ABA对生长的抑制, 以及气孔关闭在减少水分蒸发的同时会造成CO_2固定速率的降低等问题, 目前这些方法难以在农业生产中应用。如何将ABA信号途径促进逆境适应能力与抑制植物生长解偶联, 在提高作物逆境适应能力的同时不影响作物的产量和品质, 是ABA信号转导与植物逆境生物学领域面临的最大挑战。

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