



蛋白质磷酸化修饰与种子休眠及萌发调控

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摘要 种子休眠与萌发是截然不同而又紧密联系的两个生理过程, 也是植物生命周期中的关键阶段, 对自然状态下的植物物种繁殖与地理分布以及农业生产均具有重要意义, 且两个过程受不同内源激素和环境信号之间的精确互作调控。大量研究表明, 蛋白质磷酸化修饰作为一种重要的翻译后修饰方式, 参与调控种子休眠与萌发以及植物逆境胁迫响应等过程并发挥重要作用。该文简要介绍了蛋白质磷酸化、去磷酸化修饰过程及其功能, 系统总结了蛋白质磷酸化修饰在种子休眠与萌发过程中的调控作用, 并展望了未来的研究方向。

关键词 种子, 休眠, 萌发, 蛋白质磷酸化, 植物激素

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种子萌发是指种胚突破胚乳和种皮的物理限制而向外生长, 是种子从休眠状态恢复到活跃生理状态的过程(Nonogaki et al., 2010; Wang et al., 2014)。种子休眠及萌发是高等植物生活史中的重要过程, 对于植物繁殖和地理分布至关重要。该过程需精确协调多种外部环境和内部因素的动态变化, 如光照、温度、水分和内源植物激素(Finch-Savage and Leubner-Metzger, 2006; Finkelstein et al., 2008; Rajjou et al., 2012; Nonogaki, 2017)。在农业生产系统中, 及时萌发和整齐出苗是决定作物高产稳产的重要因素之一(Chen et al., 2020)。因此, 深入研究调控种子休眠及萌发的分子机制具有重要的理论和实际意义。

种子休眠与萌发受到内源激素与外界环境因子的精细互作调控。在诸多植物激素中, 脱落酸(abscisic acid, ABA)与赤霉素(gibberellins, GA)对种子休眠与萌发的影响最为重要(Bewley, 1997; Gubler et al., 2005; Finkelstein et al., 2008)。ABA与GA拮抗调控种子休眠与萌发。ABA促进种子休眠, 抑制种子萌发; 而GA促进种子萌发, 抑制种子休眠(Guan et al., 2014; Luo et al., 2021)。除ABA和GA外, 还有多种植物激素也参与调控种子休眠与萌发, 包括生长素(auxin, AUX)、细胞分裂素(cytokinin, CTK)、乙烯

(ethylene, ETH)、油菜素甾醇(brassinosteroids, BR)、水杨酸(salicylic acid, SA)、茉莉酸(jasmonic acid, JA)和独脚金内酯(strigolactones, SL)。这些激素通过与ABA/GA合成以及信号转导等通路中的重要基因发生互作, 间接调控种子休眠与萌发(Shu et al., 2016)。除植物激素外, 环境因子也参与调控种子休眠与萌发。例如, 光信号通过调控内源ABA和GA的生物合成及信号转导进而调控种子休眠与萌发(杨立文等, 2019)。红光通过抑制ABA合成基因转录促进种子萌发, 而远红光通过诱导ABA合成基因表达延缓种子萌发(Seo et al., 2006, 2009)。当种子处于高温或低温的萌发环境时, 种子休眠程度加深, 萌发速率降低(Gu et al., 2006; Biddulph et al., 2007; Bodrone et al., 2017)。水分也是影响种子休眠与萌发的关键因素, 在缺水条件下, 种子萌发会严重受阻(Liu et al., 2019)。

蛋白质磷酸化是指由蛋白激酶(protein kinases, PKs)催化的, 将三磷酸腺苷(ATP)的磷酸基团转移到底物蛋白特定氨基酸残基上的过程, 广泛参与植物几乎所有生命过程的调节, 是蛋白质翻译后修饰的主要方式之一(Humphrey et al., 2015)。蛋白质磷酸化主要发生在3类氨基酸上, 其中以丝氨酸最多, 苏氨酸次之, 第三类是酪氨酸(Olsen et al., 2006; Schwartz

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and Murray, 2011)。去磷酸化则是磷酸化反应的逆反应, 即把加在蛋白质特定氨基酸残基上的磷酸基团水解、还原成羟基的过程。这2个过程分别由PKs和蛋白磷酸酶(protein phosphatases, PPs)催化。蛋白质磷酸化与去磷酸化作为一种重要的蛋白质翻译后修饰方式, 直接或间接影响蛋白质自身的活性、稳定性以及亚细胞定位(Bigeard et al., 2014), 从而广泛参与细胞内信号传递以及植物生长发育过程(朱丹等, 2020)。

蛋白质磷酸化修饰在调控植物根系生长、开花时间、种子休眠与萌发以及生物/非生物胁迫响应等方面发挥重要作用。本文简要介绍了蛋白质磷酸化修饰、去磷酸化修饰过程及其功能, 系统综述了近年来蛋白质磷酸化修饰在调控种子休眠与萌发过程中的主要进展, 重点总结了磷酸化相关基因在该过程中的分子功能与调控通路, 并展望了未来的研究方向。

1 蛋白质磷酸化与去磷酸化的分子过程及功能

PKs、类受体激酶(receptor-like kinases, RLKs)和PPs在蛋白质磷酸化和去磷酸化修饰过程中扮演至关重要的角色(张静和侯岁稳, 2019)。PKs将底物蛋白的特定定位点磷酸化, 使底物蛋白的分子构象发生改变, 进而使其活性缺失或获得。PPs则将被磷酸化的底物蛋白特定氨基酸残基上的磷酸基团去除, 恢复磷酸化之前的蛋白活性。而RLKs是一大类特殊的激酶, 作为特定的跨膜蛋白参与磷酸化修饰。

PKs是催化蛋白质磷酸化过程的关键酶(Jha et al., 2017)。目前, 已经在拟南芥(*Arabidopsis thaliana*)、大豆(*Glycine max*)和水稻(*Oryza sativa*)等多种植物中分离出大量PKs。在细胞信号转导和细胞周期调控等过程中, PKs形成了纵横交错的调控网络(Shen et al., 2005)。这类酶通过磷酸化修饰调节蛋白活性, 使其发挥相应的生理功能。PKs的种类较多, 根据其底物蛋白被磷酸化的氨基酸残基种类, 可将其分为5类, 分别为丝氨酸/苏氨酸蛋白激酶、酪氨酸蛋白激酶、组/赖/精氨酸蛋白激酶、半胱氨酸蛋白激酶以及天冬氨酰基/谷氨酰基蛋白激酶(Hanks and Hunter, 1995)。目前已发现的植物蛋白激酶大多是前3类。

RLKs是PKs家族中重要而特殊的一类, 同时在植物中也是较大的基因家族之一(Ye et al., 2017), 具有独特的蛋白结构。RLKs是定位于细胞质膜上的跨膜蛋白, 主要由包含胞外结构域、跨膜结构域和胞内激酶域三大结构域和一段信号肽序列组成(Shiu and Bleecker, 2003)。在信号转导过程中, 胞外结构域首先识别受体, 感知细胞外信号, 跨膜结构域将该信号传递至细胞质一侧, 胞内激酶结构域与下游底物蛋白相互作用, 启动磷酸化等一系列生化反应, 最后将信号传递到细胞核内, 调控下游基因表达, 使其进行信号输出(Ye et al., 2017), 从而帮助生物体适应外界环境变化。

PPs是催化蛋白质去磷酸化过程的酶, 与PKs相对应存在, 共同构成磷酸化和去磷酸化这一重要的蛋白质活性开关系统(Luan, 2003)。PPs通过水解磷酸基团将对应底物蛋白去磷酸化, 其效应与PKs的作用正好相反。根据去磷酸化的氨基酸残基的不同, PPs可分为丝氨酸/苏氨酸磷酸酶、酪氨酸磷酸酶和双特异性磷酸酶(Schweighofer and Meskiene, 2015)。

2 PKs和RLKs参与种子休眠与萌发

在种子休眠与萌发调控过程中, PKs可直接发挥作用, 或通过影响ABA/GA等激素信号转导间接调控种子休眠与萌发以及逆境响应等生物学过程。目前, 在种子休眠与萌发方面研究相对较多的主要有4种激酶, 即丝裂原活化的蛋白激酶(mitogen-activated protein kinases, MAPKs)、钙依赖性蛋白激酶(calcium-dependent protein kinases, CDPKs)、蔗糖非发酵型1相关蛋白激酶(sucrose non-fermentation 1-related protein kinase, SnRKs)和RLKs(表1)。

2.1 MAPKs参与种子休眠与萌发

MAPK级联由3类酶组成, 分别为MAPK、MAPKK(mitogen-activated protein kinases kinases)和MAPKKK(mitogen-activated protein kinases kinases kinases), ABA信号转导通路也与此级联反应相关(Nakagami et al., 2005)。同时, 该级联反应也涉及多个生物学过程, 如调控植物生长发育及响应生物/非生物胁迫(Colcomb et al., 2008)。拟南芥*mpk8*突变体种子比野生型休眠程度深, 表现出延

表1 蛋白质磷酸化修饰过程中参与种子休眠与萌发的主要调节基因

Table 1 The main regulatory genes involved in seed dormancy and germination during protein phosphorylation modification

酶	大类	关键基因	分子功能	参考文献
蛋白激酶	MAPKs	<i>MPK8</i>	通过与GA反应的转录因子TCP14相互作用磷酸化TCP14, 增强其转录活性, 调控从休眠到萌发的转换。	Zhang et al., 2019
		<i>MKK1</i> 、 <i>MPK6</i>	参与ABA和糖调节的种子萌发, 通过增强ABA的合成与信号强度, 维持种子休眠, 抑制种子萌发。	Xing et al., 2009
		<i>Raf10</i> 、 <i>Raf11</i>	过表达 <i>Raf10</i> 和 <i>Raf11</i> , 使种子对ABA敏感性增强, <i>ABI3</i> 和 <i>ABI5</i> 表达上调, 从而延缓种子萌发。	Lee et al., 2015
		<i>MKK3</i>	<i>MKK3-A</i> 高表达促进小麦种子休眠释放, 且MKK3可以与Qsd2-AK相互作用, 调控大麦种子休眠。	Nakamura et al., 2016; Torada et al., 2016
	CPKs	<i>CPK4</i> 、 <i>CPK11</i>	过表达 <i>CPK4</i> 和 <i>CPK11</i> , 种子表现出对ABA敏感表型, 萌发受到明显抑制。	Zhu et al., 2007
		<i>CPK12</i>	CPK12通过与ABI2相互作用磷酸化ABI2, 并下调 <i>ABF1</i> 和 <i>ABF4</i> 表达, 负调控ABA信号, 促进种子萌发。	Zhao et al., 2011a, 2011b
		<i>CPK32</i>	CPK32通过与ABF4相互作用磷酸化ABF4, 增强其转录活性, 促进ABA信号转导, 抑制种子萌发。	Choi et al., 2005
	SnRKs	<i>SnRK2.2</i> 、 <i>SnRK2.3</i>	ABA信号通路复合物PYLs-ABA-PP2C通过激活SnRK2.2和SnRK2.3激酶活性, 激活下游转录因子, 诱导种子对ABA的响应。	Finkelstein et al., 2008; Fujii and Zhu, 2009
		<i>SAPK2</i>	ABA受体PYL/RCAR5作用于SAPK2上游, 激活SAPK2激酶活性, 通过OREB1介导ABA信号转导, 激活ABRE启动子活性, 负调控种子萌发。	Kim et al., 2012
	类受体激酶	RLKs	<i>GRACE</i>	编码膜蛋白, 在干种子中高表达, 其表达水平受ABA诱导, 进而延缓种子萌发, 维持种子休眠。
<i>RPK1</i>			<i>RPK1</i> 基因表达受ABA诱导, 通过正调控ABA信号传导, 抑制种子萌发。	Hong et al., 1997; Osakabe et al., 2005
<i>CRK28</i>			CRK28能够上调 <i>ABI3</i> 和 <i>ABI5</i> 的表达, 使ABA反应增强, 削弱种子萌发。	Pelagio-Flores et al., 2019
<i>CRK45</i>			过表达 <i>CRK45</i> 能够上调ABA合成及反应基因表达水平, 正调控ABA信号转导, 延缓种子萌发。	Zhang et al., 2013
<i>CARK1</i> 、 <i>CARK6</i>			CARK1和CARK6通过与ABA受体RCAR11-14相互作用, 使其磷酸化, 促进ABA信号转导, 最终抑制种子萌发。	Zhang et al., 2018; Wang et al., 2019
<i>OsLecRK</i>			<i>OsLecRK</i> 表达受萌发信号刺激而上调, 进而与OsADF相互作用, 并进一步转导萌发信号, 上调 α -淀粉酶基因表达, 增强种子活力, 促进种子萌发。	Cheng et al., 2013
磷酸酶	PP2Cs	<i>AHG1</i> 、 <i>AHG3</i>	DOG1与AHG1/AHG3相互作用, 形成PP2C磷酸酶复合物, 抑制其磷酸酶活性, 进而增强ABA信号, 促使种子休眠。	Née et al., 2017; Nishimura et al., 2018
		<i>PP2C-a10</i>	PP2C-a10与TaDOG1L1和TaDOG1L4互作, 促进小麦种子萌发; 也可与ABA反应基因(<i>ABI3</i> 、 <i>ABI4</i> 、 <i>ABI5</i> 、 <i>EM1</i> 和 <i>EM6</i>)互作, 降低其表达水平, 促进拟南芥种子萌发。	Yu et al., 2020
		<i>ABI1</i> 、 <i>ABI2</i>	在ABA存在条件下, ABA与PYR1/PYL/RCAR受体结合, 抑制ABI1和ABI2活性, 激活SnRK2s的激酶活性, 并磷酸化下游转录因子, 使ABA信号向下传递, 抑制种子萌发。	Merlot et al., 2001; Raghavendra et al., 2010
		<i>RDO5</i>	RDO5是种子特异性表达的休眠积极调控因子, 独立于ABA对种子休眠的调控, 通过抑制 <i>APUM9</i> 和 <i>APUM11</i> 的转录水平调节种子休眠。	Xiang et al., 2014
		<i>FsPP2C1</i>	过表达 <i>PP2C1</i> 种子萌发率高, 对ABA敏感性低, 且能够在不利的条件(如甘露醇和盐)下萌发, 通过负调控ABA信号转导, 促进种子萌发。	González-García et al., 2003; Saavedra et al., 2010
		<i>HON</i>	HON通过上调GA合成和响应基因的表达, 下调ABA响应基因和GA分解基因表达, 激活GA信号, 抑制ABA信号, 使种子解除休眠, 向萌发过渡。	Kim et al., 2013
	脂质磷酸酶	<i>LPP2</i>	后熟可激活 <i>LPP2</i> 的转录活性, 使其表达上调, 抑制种子对ABA的敏感性, 使种子能够完成萌发。	Carrera et al., 2008; Barrero et al., 2009
	线粒体蛋白磷酸酶	<i>SLP2</i>	SLP2与AtMIA40互作, 形成AtSLP2-AtMIA40蛋白质复合体, 通过抑制GA相关过程负调控种子萌发。	Uhrig et al., 2017
	肌醇多聚磷酸1-磷酸酶	<i>FRY1</i>	<i>FRY1</i> 突变使IP3大量积累, 导致ABA的诱导和内源 <i>RD29A</i> 及其它胁迫响应基因的表达显著增强, 负调控ABA和逆境信号, 促进种子萌发。	Xiong et al., 2001

迟萌发表型, 且后熟和外源赤霉素处理均无法有效缓解这种深度休眠, 但其具体机制尚待进一步探究(Zhang et al., 2019)。TCP14 (Teosinte branched1/Cycloidea/Proliferating cell factor)是种子萌发过程中参与GA反应的转录因子, 也是MPK8的底物蛋白, 可被MPK8磷酸化, 增强其转录活性, 进而调控从休眠到萌发的转换(Tatematsu et al., 2008), 且*mpk8/tcp14*双突变体种子的休眠程度比各自单突变体种子深; 进一步研究发现, *mpk8*、*tcp14*及*mpk8/tcp14*双突变体种子中GA合成基因(*GA3ox1*和*GA3ox2*)和响应基因(*CP1*、*GASA4*和*GASA14*)的转录水平均有所下降, 且突变体种子均呈现出对GA合成抑制剂多效唑敏感的表型(Resentini et al., 2015)。因此, MPK8经由转录因子TCP14介导负调控种子休眠(Zhang et al., 2019)。

AtMKK1和AtMPK6是拟南芥中参与ABA和糖调节种子萌发过程的关键分子(Xing et al., 2009)。在未层积化处理情况下, *mkk1/mpk6*双突变体种子显示出比野生型更高的萌发率, 且*mpk6*和*mkk1/mpk6*突变体对ABA和葡萄糖处理不敏感, 而过表达MKK1或MPK6种子则对ABA和葡萄糖超敏感; 此外, 葡萄糖能够通过上调*NCED3*和*ABA2*的表达诱导ABA合成, 但这种上调在*mkk1/mpk6*双突变体中被阻断(Xing et al., 2009)。因此, MKK1和MPK6是种子萌发过程中葡萄糖信号的下游调节因子, 葡萄糖通过MKK1和MPK6促进ABA合成, 从而抑制种子萌发(Xing et al., 2009)。同样, 水稻OsMPK6也通过增强ABA的合成与信号强度, 实现对种子休眠的维持与萌发的抑制(Xu et al., 2018; Zhang et al., 2019)。此外, 在MAPKKK途径中, Raf10和Raf11激酶正调控种子休眠(Lee et al., 2015)。与野生型相比, *raf10*和*raf11*突变体种子的休眠程度和对ABA的敏感性较低, 而过表达则导致种子萌发延迟, ABA的敏感性增强; 进一步研究发现, 在*Raf10*和*Raf11*过表达种子中, ABA信号正调控基因*ABI3*和*ABI5*的表达均有所上调(Nguyen et al., 2019); 并且Raf10和Raf11可以发生自磷酸化, 其激酶活性被MAPKKK抑制剂BAY 43-9006抑制(Lee et al., 2015), 从而影响其对种子休眠的调控。

MKK3位于MAPKK途径上, 在控制谷物种子休眠中发挥重要作用(Nakamura et al., 2016)。小麦(*Triticum aestivum*) *TaMKK3-A*位于4A染色体上, 是

种子休眠位点*Phs1*的候选基因(Martinez et al., 2020); 小麦品系*MEL29*和*MEL31*显示出不同的休眠水平, *MEL29*种子萌发率比*MEL31*高。*TaMKK3-A*基因在*MEL29*种子中表达水平高于*MEL31*, 而较高的*TaMKK3-A*表达促进了休眠释放(Torada et al., 2016)。而大麦(*Hordeum vulgare*)在5H染色体上有2个主要的种子休眠数量性状位点*SD1*和*SD2* (Gong et al., 2014), 其中*SD2*所处的*Qsd2-AK*位点决定了不同品种间种子休眠的差异; 有意思的是, MKK3可以与*Qsd2-AK*相互作用, 进而调控种子休眠。此外, N260作为影响MKK3激酶活性的重要氨基酸, 该等位基因中的N260T替代会降低MKK3激酶活性, 导致休眠加深, 从而延迟种子萌发(Nakamura et al., 2016)。然而, ABA是否以及如何影响MKK3激酶的作用, 目前还不清楚。

2.2 CPKs参与种子休眠与萌发

钙是植物细胞信号转导的主要调节剂, 已被证明是参与ABA信号转导的重要第二信使(Finkelstein et al., 2002; Hepler, 2005)。植物钙调蛋白和CDPKs等可作为钙传感蛋白, 其中CDPKs是植物中最典型的钙信号之一(Cheng et al., 2002; Luan et al., 2002)。

CDPKs被认为与ABA信号有关, 参与调节种子萌发及植物发育(Yu et al., 2006)。拟南芥CDPK超家族的不同成员CPK4、CPK11和CPK12通过在ABA信号转导中发挥拮抗作用而构成一个调节环(Zhao et al., 2011a)。CPK4和CPK11是ABA信号转导途径2个重要的正调节因子(Zhu et al., 2007)。*cpk4*和*cpk11*突变体种子表现出萌发加快和ABA/盐不敏感表型; *cpk4/cpk11*双突变体种子比各自单突变体种子具有更强的ABA不敏感和盐响应表型, 其过表达种子则表现出相反表型, 萌发受到明显抑制, 但详细的调控机制尚不清楚。而CPK12在种子萌发和萌发后生长过程中是ABA信号的负调节因子(Zhao et al., 2011a)。与野生型相比, *CPK12-RNAi*种子在萌发期间对ABA敏感; CPK12通过与ABA信号通路的负调节蛋白ABI2相互作用磷酸化ABI2, 使ABA响应转录因子ABF1和ABF4磷酸化, 并下调其表达(Zhao et al., 2011b), 从而正调控种子萌发。拟南芥CDPK超家族的另一个成员CPK32也参与ABA介导的种子萌发。*CPK32*过表达导致ABA超敏表型, 种子萌发受到抑制; 进一步研

究表明, CPK32通过与ABF4相互作用磷酸化ABF4, 增强其转录活性, 促进ABA信号转导, 进而抑制种子萌发(Choi et al., 2005)。

2.3 SnRKs参与种子休眠与萌发

SnRKs是植物中特异性表达的激酶家族, 由SnRK1、SnRK2和SnRK3三个亚家族共同组成(Hrabak et al., 2003), 在种子休眠与萌发方面研究较多的主要是SnRK2亚家族。SnRK2亚家族的10个成员根据其结构可分为3个亚类, 其中亚类III中的2个成员(SnRK2.2和SnRK2.3)作为ABA信号通路的正调控因子(Boudsocq et al., 2004; Fujita et al., 2009)参与ABA诱导的种子萌发调控。*snrk2.2*和*snrk2.3*突变体种子与野生型相比无显著差异, 而*snrk2.2/snrk2.3*双突变体在种子萌发中表现出很强的ABA不敏感表型(Nakashima et al., 2009)。因此, SnRK2.2和SnRK2.3的功能冗余(Fujii et al., 2007)。此外, 随着ABA的积累, ABA信号通路复合物PYLs-ABA-PP2C通过激活SnRK2.2和SnRK2.3的激酶活性激活下游转录因子(Fujii and Zhu, 2009), 包括ABA响应元件ABRE的结合因子ABF (ABF1和ABF2)、ABI5、ABI3和ABI4, 从而诱导种子对ABA的响应, 削弱种子萌发(Finkelstein et al., 2008)。

有意思的是, 水稻含有10个SnRK2激酶(SAPK1–10) (Kobayashi et al., 2004), 也可分为3个亚类。SAPK2是水稻SnRK2亚类II家族的成员, *sapk2*突变体种子在萌发和萌发后阶段表现出ABA不敏感表型, 但外源ABA处理并不上调*SAPK2*的表达。ABA受体PYL/RCAR5在SAPK2上游起作用, 并激活SAPK2的激酶活性, 进而通过ABRE结合因子OREB1介导ABA信号转导, 诱导ABA依赖的ABRE启动子活性, 从而负调控种子萌发及萌发后的幼苗生长(Kim et al., 2012)。

2.4 RLKs参与种子休眠与萌发

RLKs是植物中最重要的感官蛋白之一, 在感知环境信号中起主要作用(Walker and Zhang, 1990; Walker, 1993)。通过磷酸化级联反应, RLKs将胞外信号传递至胞内, 以调节生长发育(Shiu and Bleecker, 2001b; Ye et al., 2017)和响应生物/非生物胁迫。LRR-RLK是拟南芥中最大且研究最充分的RLK亚家族(Shiu

and Bleecker, 2001a)。GRACE是该亚家族中编码膜蛋白的成员之一, 在干种子中高表达且具有维持种子休眠的功能(Wu et al., 2017)。外源ABA处理能够显著上调*GRACE*表达, 但其与ABA互作调控种子休眠的分子机制仍需要进一步研究。同样, 从拟南芥中分离得到的*RPK1*基因也属于该亚家族, 其表达受ABA诱导(Hong et al., 1997)。*RPK1*突变体(*rpk1-1*和*rpk1-2*)在种子萌发过程中对ABA不敏感, 且*antisense-RPK1*转基因种子表现出相同的表型; 进一步研究发现, 该表型是由于*RPK1*表达下降引起(Osakabe et al., 2005), 但其具体机制尚待进一步研究。

CRK28是一种富含半胱氨酸的类受体激酶(CRKs), 在种子萌发期间正调控ABA信号(Pelagio-Flores et al., 2019)。与野生型相比, *crk28*突变体种子萌发率无显著差异, 而*35S:CRK28*过表达的种子萌发率较低, 且表现出对ABA超敏感表型; 后续研究发现, CRK28上调*ABI3*和*ABI5*的表达, 从而导致ABA反应增强(Pelagio-Flores et al., 2019)。CRKs家族的另一个成员CRK45也参与种子萌发期间对ABA的响应(Zhang et al., 2013)。在无ABA的情况下, 野生型、*crk45*和*35S:CRK45*的种子萌发率相似; 但在ABA存在的情况下, *crk45*表现出对ABA不敏感的表型, 而*35S:CRK45*表现出相反的表型; 进一步研究发现, CRK45过表达上调了ABA合成基因(*NCED3*、*NCED5*、*ABA1*、*ABA2*和*AAO3*)及ABA响应基因(*ABF1-4*和*MYC2*)的表达水平, 从而正调控ABA信号转导, 延缓种子萌发(Zhang et al., 2013)。

CARK1和CARK6是一类胞质类受体激酶(RLCKs), 属于RLCK VIII亚家族, 在ABA信号转导中发挥积极作用(Wang et al., 2019)。与野生型相比, *cark1*和*cark6*突变体种子对ABA不敏感, 其过表达种子对ABA更敏感; 且CARK1和CARK6与ABA受体(RCAR11、RCAR12、RCAR13和RCAR14)均能相互作用, 使受体蛋白磷酸化, 进而促进ABA信号转导(Zhang et al., 2018; Li et al., 2019), 最终削弱种子萌发。OsLecRK是从水稻中分离出来的G型凝集素类受体激酶, 在种子萌发和植物免疫中具有双重作用(Cheng et al., 2013)。在种子萌发过程中, 萌发信号(如生长因子)会刺激*OsLecRK*表达, 使被激活的OsLecRK激酶结构域与OsADF (actin-depolymerizing factor)结合, 导致 α -淀粉酶合成基因表达上

调, 从而增强种子活力, 促进种子萌发(Cheng et al., 2013)。因此, 在未来的研究中, ABA是否以及如何影响OsLecRK激酶活性将是一个重要课题。

3 蛋白磷酸酶参与种子休眠与萌发

蛋白磷酸酶2C (PP2C)是一类丝氨酸/苏氨酸蛋白磷酸酶, 是高等植物中存在的最大的蛋白磷酸酶家族(Singh et al., 2010)。目前, 已经在植物中发现了多种PP2C类磷酸酶, 它们中的大多数都参与ABA通路的信号转导(翁华等, 2003)。

AHG1 (ABA-hypersensitive germination 1)和AHG3是PP2C分支A的2个成员, 负调节种子休眠且功能冗余(Yoshida et al., 2006; Nishimura et al., 2007)。值得注意的是, 该分支的多数成员是ABA信号通路的负调控因子(Rubio et al., 2009; Raghavendra et al., 2010)。在有ABA时, 该分支的多数磷酸酶活性被ABA受体PYR/PYL/RCARs家族抑制(Antoni et al., 2012)。而DOG1 (delay of germination 1)是种子休眠过程中关键的正调控因子(Cyrek et al., 2016; Breeze, 2019), 其突变体种子表现出非休眠表型(Bentsink et al., 2006; Nakabayashi et al., 2012)。在种子中, DOG1需要借助PP2C控制种子休眠, DOG1通过与AHG1和/或AHG3结合, 抑制其磷酸酶活性, 进而增强ABA信号(Née et al., 2017; Nishimura et al., 2018)。*dog1/ahg1*和*dog1/ahg3*双突变体是非休眠的, 而*dog1/ahg1/ahg3*三突变体表现出非常强的休眠表型。上述结果表明, 磷酸酶AHG1和AHG3功能冗余, 且二者位于DOG1的下游, 为DOG1发挥功能所必需(Nishimura et al., 2018)。后续研究发现, ABA和DOG1调控的休眠途径在PP2C磷酸酶分支A处汇聚, 通过降低共同以及单独的PP2C磷酸酶活性调节种子休眠(Née et al., 2017)。与之一致的是, AHG1亚家族的另一个成员PP2C-a10也可以通过DOG1L调节种子萌发(Yu et al., 2020)。在小麦中, TaPP2C-a10与TaDOG1L1和TaDOG1L4相互作用, 促进种子萌发。同样, 在拟南芥中, PP2C-a10的异源表达也能够促进种子萌发, 降低种子对ABA的敏感性。PP2C-a10能够通过ABA响应基因(*ABI3*、*ABI4*、*ABI5*、*EM1*和*EM6*)相互作用, 降低其表达水平, 进而促进种子萌发(Yu et al., 2020)。

ABI1和ABI2是PP2C分支A中另外2个ABA信号通路的负调控因子(Merlot et al., 2001), 其隐性突变体表现出种子休眠及对ABA敏感的表型; 在ABA存在条件下, ABA与其受体PYR1/PYL/RCARs结合, 导致ABI1和ABI2蛋白磷酸酶失活, 进一步激活SnRK2激酶活性, 并磷酸化下游转录因子ABFs/AREBs和ABI5, 使ABA信号向下传递(Raghavendra et al., 2010)。

RDO5 (reduced dormancy 5)属于PP2C磷酸酶家族, 是种子中特异性表达的休眠正调控因子。它不与A分支磷酸酶聚集在一起, 独立于ABA对种子休眠的调控(Xiang et al., 2014)。与野生型相比, *rdo5*突变体种子休眠显著减弱, 但对ABA的敏感性不变(Xiang et al., 2016), 而下调*APUM9* (*Arabidopsis PUMILIO 9*)和*APUM11*的表达可以恢复其休眠减弱表型; 因此, RDO5通过抑制*APUM9*和*APUM11*的转录水平调节种子休眠, 且RDO5介导的调控通路不同于ABA信号通路(Xiang et al., 2014), 其具体机制需要深入研究。与之不同, FsPP2C1是一种在山毛榉(*Fagus sylvatica*)中特异表达的功能性PP2C磷酸酶, 其表达受ABA调控(Lorenzo et al., 2001; Saavedra et al., 2010)。在拟南芥中, 35S:*FsPP2C1*转基因种子休眠程度较低, 对ABA不敏感, 且能够在不利条件(如甘露醇和盐)下萌发, 其过表达拟南芥种子也表现出ABA不敏感表型(González-García et al., 2003); 然而, FsPP2C1如何被激活以调控ABA信号转导, 从而促进种子萌发, 尚属未知。另一种PP2C蛋白HON是种子休眠的负调控因子, 在ABA存在条件下能够与PYR/PYL/RCARs结合, 降低HON的PP2C磷酸酶活性(Kim et al., 2013)。与野生型相比, *hon*突变体休眠程度加深, 但其过表达种子休眠程度减弱; 此外, HON通过下调ABA响应基因(*EM1*和*EM6*)和GA分解代谢基因*GA2ox2*的表达, 上调GA响应基因(*CP1*和*EXP1*)和GA合成基因(*GA3ox1*和*GA3ox2*)的表达, 抑制ABA信号而激活GA信号, 进而使种子解除休眠, 向萌发过渡(Kim et al., 2013)。

与PP2C磷酸酶不同, 脂质磷酸酶LPP (lipid phosphate phosphatase)家族成员在后熟诱导的种子休眠解除中起重要作用(Barrero et al., 2009)。在拟南芥和大麦中, *lpp2*突变体在萌发过程中表现出ABA超敏表型(Katagiri et al., 2005; Barrero et al., 2009),

且后熟能够激活 *LPP2* 基因转录, 使其表达上调 (Carrera et al., 2008), 进而抑制种子对 ABA 的敏感性, 使种子能够完成萌发。SLP2 (*shewanella*-like protein phosphatase 2) 是一种线粒体蛋白磷酸酶, 位于线粒体膜间隙, 能够与 AtMIA40 (mitochondrial oxidoreductase import and assembly protein 40) 互作, 形成 AtSLP2-AtMIA40 蛋白质复合体, 通过抑制 GA 相关过程负调控种子萌发 (Uhrig et al., 2017)。*atslp2-2* 突变体萌发表型与内源 GA 水平有关; 同时, 在无 AtSLP2 的情况下, GA 水平升高, GA 诱导的 AtSLP2 表达水平与 GA3 氧化酶基因 (*GA3ox*) 和 *GID1A* 表达呈负相关, 而与 GA 合成基因下游的 DELLA 转录因子 (*RGA1* 和 *RGL2*) 表达呈正相关 (Uhrig et al., 2017), 但其底物蛋白与详细机制尚不清楚, AtSLP2 如何负调控 GA 相关过程值得深入探究。此外, 肌醇多聚磷酸1-磷酸酶 *FRY1* 能够通过负调控 ABA 信号转导抑制种子休眠 (Xiong et al., 2001)。与野生型相比, *fry1-1* 突变体种子萌发期间表现出对 ABA 和渗透胁迫敏感的表型; 并且在低温、渗透胁迫或 ABA 处理下, *FRY1* 突变使第二信使 IP₃ (inositol(1,4,5)-triphosphate) 大量积累, 导致 ABA 的诱导和 *RD29A* 及其它胁迫响应基因 (如 *KIN1*、*COR15A*、*HSP70* 和 *ADH*) 的表达显著增强, 促进种子休眠 (Viswanathan and Zhu, 2002) (表1)。

4 总结与展望

大量研究表明, 各种内源和环境因素 (如光照、温度、水分状况以及植物激素 (如 ABA 和 GA)) 均调节休眠与萌发之间的平衡。而蛋白质磷酸化修饰作为重要的翻译后修饰方式之一, 也参与种子休眠与萌发的平衡调节。然而, 着眼于蛋白质磷酸化修饰与种子休眠及萌发调控的总结却极少。本文主要综述了蛋白质磷酸化修饰调节种子休眠与萌发的重要基因及调控通路 (表1)。目前, 有几个悬而未决的问题值得进一步研究并有望取得突破。

首先, 蛋白质磷酸化修饰在 ABA 信号转导通路中具有重要作用, 大多数研究集中在磷酸化修饰通过介导 ABA 信号进而调控种子休眠与萌发, 但对 GA 合成及信号转导以及其它激素通路的调节机制尚所知有限。因此, 后续要深入探究蛋白质磷酸化修饰与其它

植物激素通路互作调控种子休眠与萌发的分子机理, 进一步丰富对种子休眠与萌发调控的认识。

其次, 氧气与水分是种子萌发过程中不可或缺的元素, 而蛋白质磷酸化修饰与植物氧气/水分利用效率与途径的关系值得深入研究。因此, 系统揭示蛋白质磷酸化修饰与氧气/水分通路的关系, 将极大地拓展和加深我们对于种子休眠与萌发过程调控机制的理解, 该领域也是种子生物学领域非常重要的研究方向之一。

最后, 目前大多数研究均着眼于磷酸化相关基因调控非生物胁迫下的植物发育, 而调控种子休眠与萌发的精确分子机制很大程度上仍然未知, 且目前对蛋白磷酸酶的结构、机理和功能的了解大部分来自动物和真菌。因此, 深入研究植物蛋白激酶和磷酸酶的分子功能, 解析其蛋白结构, 对于系统认识蛋白质磷酸化相关基因调控种子休眠与萌发的分子机制至关重要。

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Protein Phosphorylation and Its Regulatory Roles in Seed Dormancy and Germination

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Abstract Seed dormancy and germination are two distinct but closely related physiological processes, which are also key stages during plant life-cycle and have great significance to agricultural production, plant species reproduction, and geographical distribution. These processes are precisely regulated by interactions between different endogenous phytohormones and environmental signals. A large number of studies have shown that protein phosphorylation, plays an important role in regulating seed dormancy and germination, as well as plant response to stresses. This review paper briefly introduces the procedures and functions of protein phosphorylation and dephosphorylation modification, and summarizes the regulatory roles of protein phosphorylation modification in seed dormancy and germination. Finally, some future research directions are prospected.

Key words seed, dormancy, germination, protein phosphorylation, phytohormone

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