转基因改良水稻磷吸收效率的策略

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摘要: 土壤中由于磷的固定造成作物可以吸收的有效磷较低, 培育磷高效吸收的水稻新品种能够有助于解决缺磷所造成的农作物减产问题, 为实现农业的可持续发展打下良好的基础。本文综述了水稻磷信号机制的最新研究进展, 并总结了转基因改良水稻磷吸收效率的成功案例。这些改良策略对其他作物的转基因改良提供了重要的依据。

关键词: 水稻; 磷吸收; 磷酸盐转运体; 转基因改良

The Strategyes on Engineering Improvement of Phosphorus Uptake Efficiency in Rice

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Abstract: The low mobility of phosphorus in soil cause low concentration of its assimilable form phosphate (Pi), which is often a limiting factor for crop growth in agriculture. Developing cultivars with improved Pi uptake efficiency is essential for the yield and sustainability of agriculture. In this review, we presented the mechanisms on phosphate signaling and homeostasis. Based on this understanding, we further introduced several successful examples in engineering improvement of phosphorus uptake efficiency in rice, which supplied very important cues for other crop improvement.

Key words: rice; phosphorus uptake; phosphate transporter; engineering improvement

磷是植物生长发育必需的大量元素, 由于磷酸盐Pi (PO_4^{3-})在土壤中的强烈固定作用, 使得土壤中可以被植物吸收利用的有效磷浓度要大大低于其他大量元素。因此在很多土壤中, Pi水平的高低是植物生长和产量的一个主要决定因子。长期大量施用磷肥虽然促进了农产品的增产, 但同时也导致了磷矿资源逐渐趋于耗竭、生态环境的污染, 影响农业可持续发展。

植物在长期的进化过程中形成了各种适应缺磷胁迫的机制, 包括刺激根毛生长与根构型适应, 生理代谢适应以及与菌类共生等等, 以此来提高对可溶性磷的吸收和利用效率以及对固定态磷的的活化与吸收能力。磷饥饿信号途径是介导各种缺磷胁迫适应反应的分子基础, 由于充分解析磷信号传导机制是改良作物磷吸收效率的基础, 有助于解决缺磷所造成的农作物减产问题并保持农业的可持续发展, 因此一直受到广泛关注。

1 磷信号途径与磷稳态平衡的研究进展

通过对拟南芥、水稻的研究, 已有一批磷信号途径成员得到解析(图1) (Zhang等2014)。拟南芥中MYB类转录因子AtPHR1 (PHOSPHATE STARVATION RESPONSE)及其水稻中同源的OsPHR2处于整个磷信号网络的中心环节(Rubio等2001; Zhou等2008)。该类转录因子能够识别结合P1BS顺式元件(GNATATNC) (Rubio等2001; Ruan等2015)。受AtPHR1及OsPHR2直接调控的一系列靶基因已得到解析, 如其对磷酸盐转运体PT家族成员的调控(Chiou和Lin 2011; Liu等2010), 定位于质膜上的磷酸盐转运体PTs也叫PHT1家族, 负责磷的吸收转运。AtPHR1及OsPHR2还直接调控miR399的转录, miR399与PHO2的5′UTR序列互补从而对PHO2进行转录后水平调控(Fujii等2005; Bari等2006; Chiou等2006)。拟南芥AtIPS1/At4与水稻的OsIPS1/2也是PHR的直接靶基因(Burleigh和Harrison 1999; Liu等1997; Hou等2005), 有趣的是它们作为非编码RNA, 能与miR399的序列发生

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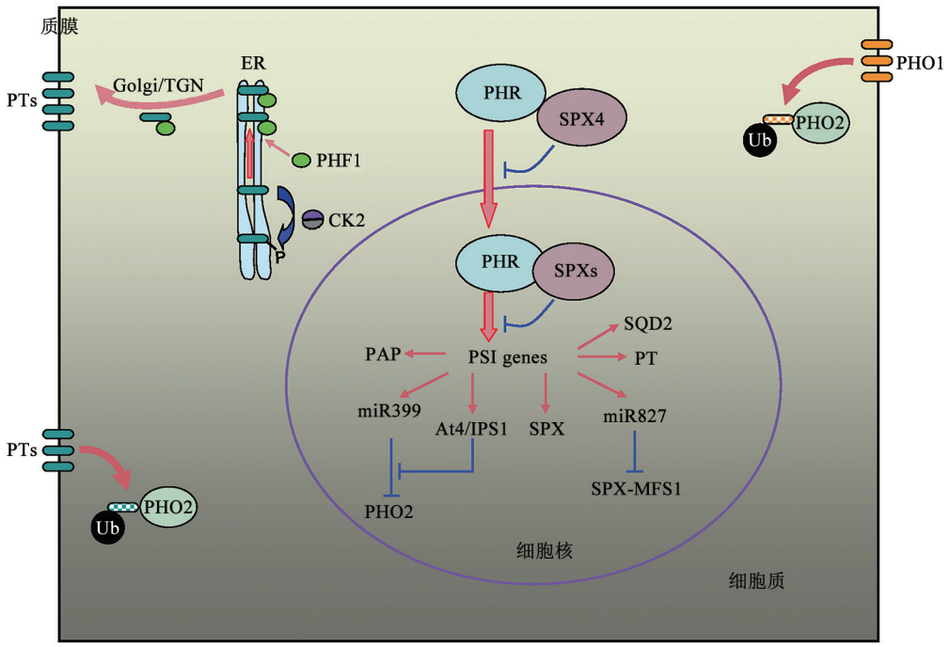

图1 植物根细胞中磷信号网络

Fig.1 Pi signaling network in plant root cells

MYB类转录因子PHR处于整个磷信号网络的中心环节, 调控着一系列下游磷饥饿响应(PSI)基因。SPX能与PHR2互作, 使PHR2丧失与下游靶基因(如*PAP*、*miR399*、*At4*/*IPS1*、*SPX*、*SQD2*、*PT*和*miR827*)启动子结合, 并且SPX对PHR2活性抑制是直接依赖于细胞Pi浓度的。酪蛋白激酶II (CK2)负责将PTs磷酸化, 磷酸化后的PTs丧失了与其伴侣因子PHF1的互作, 造成PTs滞留内质网。在翻译后水平PTs家族成员通过泛素降解途径成员PHO2和NLA调控其蛋白稳定性。

互补, 对miR399起到拮抗作用, 从而减轻miR399对PHO2 mRNA的降解(Franco-Zorrilla等2007)。PHO2作为一种泛素缀合酶E2 (ubiqutin-conjugating E2 enzyme, UBC24), 通过降解PHO1蛋白而起作用, PHO2功能缺失的拟南芥(Aung等2006; Bari等2006; Liu等2012)和水稻*pho2*突变体(本实验室未发表资料)均表现出植株地上部分磷超积累。PHO1则通过介导Pi输出到木质部而参与Pi从根部运输到地上部分(Stefanovic等2011), 拟南芥和水稻*pho1*突变体均表现出地上部分Pi含量降低而根部则维持正常含量(Hamburger等2002; Rouached等2011; Stefanovic等2011; Secco等2010)。

在磷信号网络机制的揭示中, 近年对SPX家族成员的功能有了突破性认识。SPX家族成员与磷信号网络的中心调控转录因子PHR互作, 使PHR丧失与下游靶基因启动子上的P1BS顺式元件结合。对水稻OsSPX4的研究表明, 该蛋白主要位于细胞质中, 在缺磷下受到泛素降解。缺磷下OsSPX4的降解造成更多OsPHR2释放进入细胞核内, 从而发挥其转录激活的功能(Lv等2014)。对水稻与拟南芥SPX1/2的研究表明, SPX对PHR2活性抑制是直接依赖于细胞Pi浓度的。由于在体外实验中直接证明了无磷条件下SPX与PHR2有很强的结合能力, 而提高缓冲液中磷浓度时, 两者的结合能力下降。此外, 除了提高缓冲液中的Pi浓度外, 当加入磷信号分子亚磷酸盐也有此效应(Wang等2014; Puga等2014), 由于亚磷酸盐在体内不能象磷酸那样被代谢, 但亚磷酸盐在结构上与磷酸盐类似, 一直被人们作为磷酸信号分子起作用(Carswell等1996; Ticconi等2001), 这些结果表明SPX参与植物Pi浓度感应(Wang等2014; Puga等2014)。这在磷信号网络分子机制揭示中是一个突破。

由于磷信号中心转录因子PHRs的转录水平在缺磷下仅有微弱改变, 因此缺磷条件下PHRs的蛋白丰度、稳定性及翻译后修饰很可能是未来研究的一个热点。已有报道的翻译后修饰主要涉及对AtPHR1的SUMO化泛素修饰(Miura等2005)。在拟南芥中AtSIZ1是一个植物小泛素(SUMO) E3连

接酶。*siz1*突变体中Pi饥饿信号被放大了, 尽管AtPHR1在体外可以被AtSIZ1进行SUMO化泛素修饰(Miura等2005), 但植物体内SUMO化的PHRs功能仍有待进一步阐明。

除了PHRs外, 其他参与Pi饥饿信号调控的转录因子也陆续得到解析。如拟南芥MYB62 (Devaiah等2009)与水稻OsMYB2P-1 (Dai等2012)被报道参与调控Pi饥饿响应基因表达以及根系结构的缺Pi响应。拟南芥中3个WRKY转录因子, WRKY75、WRKY6和WRKY42也被报道参与调控Pi饥饿响应(Devaiah等2007a; Chen等2009)。WRKY75的基因表达受Pi饥饿所诱导, 在Pi缺乏条件下, WRKY75 RNAi干涉植株表现出Pi饥饿诱导基因表达下降及Pi吸收减少(Devaiah等2007a)。WRKY6和WRKY42通过结合到PHO1启动子抑制其表达从而在Pi饥饿响应途经中承担负调控的作用。在Pi缺乏条件下WRKY6蛋白会发生由26S蛋白酶体介导的蛋白降解从而消除对PHO1表达的抑制(Chen等2009)。两个bHLH (basic HELIX-LOOP-HELIX)转录因子(拟南芥bHLH32与水稻OsPTF1)在调控Pi饥饿响应中的作用得到鉴定(Yi等2005; Chen等2007)。*bhlh32*突变体内花色苷积累、Pi饥饿诱导基因表达提高、总Pi含量与根毛形成均显著提高, 因此尽管*bHLH32*的基因表达受Pi饥饿诱导, 但其实际是一个Pi饥饿响应的负调控因子(Chen等2007)。与拟南芥*bHLH32*基因在根与冠部均受到缺Pi诱导不同的是, 水稻*OsPTF1*基因在冠部是组成型表达, 只在根中受缺Pi诱导。*OsPTF1*超表达植株表现出耐低磷的表型(Yi等2005)。此外, 一个C2H2 (cysteine-2/histidine-2)锌指转录因子*ZAT6* (zinc finger of *Arabidopsis thaliana* 6)受缺Pi诱导(Devaiah等2007b)。ZAT6 RNAi干涉对植物是致死的, 表明其对植物生长发育有着重要作用。*ZAT6*超表达植株表现出不依赖于Pi供应水平的生长减弱和根系结构改变。在缺Pi条件下, 跟野生型相比, *ZAT6*超表达植株在发育初期阶段体内花色苷积累、Pi含量下降、Pi饥饿诱导基因表达下降, 表明ZAT6在调控根系发育与Pi稳态平衡中发挥作用(Devaiah等2007b)。

磷酸盐转运体PTs活性的提高是一个极其重要的Pi饥饿响应(Raghothama 1999; Nussaume等2011)。除了受到转录水平的调控, 质膜上定位的PTs活性也受到转录后水平的调控。PHF1在调控PTs从内质网到质膜的运输过程中发挥着伴侣的重要作用, 在*phf1*突变体中PTs会滞留内质网, 造成植物磷吸收大大降低(Gonzalez等2005; Chen等2011)。此外, 在翻译后水平PHT1家族成员通过泛素降解途径成员PHO2和NLA调控其蛋白稳定性(Huang等2013; Lin等2013; Park等2014)。之前的研究表明PTs可能通过磷酸化而负调控PTs从内质网输出(Nuhse等2004; Hem等2007; Bayle等2011)。最近有报道表明酪蛋白激酶II (CK2)负责将PTs磷酸化, 磷酸化后的PTs丧失了与其伴侣因子PHF1的互作, 造成PTs滞留内质网, 从而抑制了磷的吸收(Chen等2015)。

染色质构型改变调控Pi饥饿响应是近年发展起来的一个新领域。一个保守的与肌动蛋白actin有关的蛋白质, ARP6是SWR1染色质构型改变复合体组份, 通过将组蛋白变体(histone variant) H2A.Z装载入染色质而调控转录(Choi等2005; Deal等2007)。染色体免疫共沉淀实验结果表明, 在Pi充足的野生型中, Pi饥饿诱导基因区域有较高含量的H2A.Z, 而*arp6*突变体中则不存在这一H2A.Z的富集。更重要的是, 缺Pi条件下, 这个Pi饥饿诱导基因区域的H2A.Z富集则消失了(Smith等2010)。这方面的研究提供了一个通过ARP6介导H2A.Z装载入特定基因区域的染色质从而调控Pi饥饿诱导基因的工作模型。此外, PER2 (Pi deficiency root hair defective 2)编码一个同源域(homeodomain)蛋白AL6 (ALFIN-LIKE6), AL6属于PHD指(PHD finger)蛋白小家族成员, 是一类含有植物同源域PHD (plant homeodomain)的核定位蛋白。PHD指蛋白能与H3K4me3和H3K4me2结合并显示出“阅读”组蛋白代码的功能(Santos-Rosa等2002; Schneider等2003; Bernstein等2005)。*per2*突变体根系结构上的Pi饥饿响应存在缺陷, 并且在花色苷积累与Pi含量上均下降了。这一研究为染色质构型调控Pi饥饿响应提供了又一个例子。可以预期, 未来的研究很可能会揭示DNA甲基化和组蛋白修饰在调控Pi饥饿响应中所发挥的重要作用。

2 转基因改良水稻磷吸收效率的研究实例

尽管前人的研究表明磷信号途径成员*PHO2*、*SPXs*、*CAX1/2*与*NLA1*突变后以及*OsPHR2*与*Os-*

*miR827*超表达后均能引起拟南芥或水稻磷吸收的提高造成体内磷积累(Aung等2006; Bari等2006; Wang等2009; Liu等2010, 2011; Lin等2013; Shi等2014; Lv等2014)。但是以上植物在缺磷条件下并没有更好的生长表现, 很有可能在这些植株中有未知的不利于生长的因子存在。

PHF1编码磷酸盐转运体的伴侣蛋白, 并参与调控了植物中的磷酸盐转运体PTs家族蛋白从内质网上膜的运输过程(Gonzalez等2005; Bayle等2011; Chen等2011)。*phf1*突变体会造成PTs过多地滞留于内质网(Gonzalez等2005; Bayle等2011; Chen等2011), 相反, *OsPHF1*超表达水稻植株能够提高磷酸盐转运体在质膜的定位并提高磷吸收利用效率(Wu等2013; 刘慧丽2013)。田间试验发现, 低磷(不施磷肥)和中磷[施磷肥225 kg (过磷酸钙)·hm^{-2}]条件下, 2个*OsPHF1*基因超表达株系的有效穗、生物量和产量较野生型均显著提高, 正常供磷条件下[正常施磷肥450 kg (过磷酸钙)·hm^{-2}]差异不显著(刘慧丽2013)。这表明对磷酸盐转运体进行转录后调控可以作为提高磷吸收的一种策略。

最近我们实验室发现与Pi信号中心转录因子OsPHR2结合的P1BS顺式元件(GNATATNC)中的2个可变碱基分别为A与T时(GaATATtC, 称为A-T-type P1BS), 它与OsPHR2之间有最高的亲合力。当我们用串联2次的A-T-type P1BS驱动下游基因时, 显示出了对缺Pi响应的最强诱导。由于*OsPHF1*的转录受缺Pi诱导的响应非常弱(Chen等2011), 因此我们将OsPHF1启动子含有的P1BS顺式元件改造成了A-T-type P1BS。结果表明在温室条件下的溶液培养与盆栽试验均显示出, 将OsPHF1启动子改造成了A-T-type P1BS后, 可以有效提高磷吸收以及植株的生长(Ruan等2015)。

此外, 我们还发现, 与OsPHR2超表达植株表现出不利于生长的情况不同的是, 当将其同源基因*OsPHR3*超表达后植株并未呈现出生长抑制, 并且在低磷条件下显示出了磷吸收的加强, 植株也长得更为壮实(Guo等2015)。另一条促进磷利用的策略是对分泌型磷酸酶进行超表达。在缺Pi条件下, 植物会促进分泌磷酸酶到根际土壤, 以催化有机磷中水解出无机磷。水稻紫色酸性磷酸酶OsPAP10a (purple acid phosphatase 10a)超表达植株可以增强对胞外有机磷的利用(Tian等2012)。

最近有报道表明酪蛋白激酶II (CK2)负责将PTs磷酸化, 磷酸化后的PTs丧失了与其伴侣因子PHF1的互作, 造成PTs滞留内质网。相反将PT8上的磷酸化修饰位点基因突变后的转基因水稻在低磷条件下体内有效磷浓度和生物量均得到了提高(Chen等2015)。后续可根据这一机制挖掘优良等位基因。也可以利用定点CRISPR/Cas (clustered regulatory interspaced short palindromic repeats/CRISPR associated proteins)技术筛选出不影响PT的磷酸转运功能但缺失磷酸化位点的新型突变基因, 在转基因后代中可以将引发此次DNA编辑的载体序列完全分离掉, 以此创建出的遗传改良作物无需担忧转基因序列存在所带来的风险, 因此利用磷信号机制的知识进行作物遗传改良育种有着极大的应用前景。

3 结语

在深入了解磷吸收转运调控的信号途径分子机制的基础上, 运用安全转基因技术, 获得没有筛选标记基因的磷高效作物新品种, 可以极大地促进农业的可持续发展, 对生态和农业经济都具有重要意义。

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