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·研究报告·

磷酸肌醇激酶FAB1调控拟南芥根毛伸长

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摘要 FAB1/PIKfyve是介导PI(3,5)P_2 (磷脂酰肌醇3,5-二磷酸)生物合成的磷酸肌醇激酶。在动物和酵母(*Saccharomyces cerevisiae*)中, PI(3,5)P_2参与调控胞内膜运输, 但在植物中的研究较少。该文通过分析拟南芥(*Arabidopsis thaliana*) FAB1的T-DNA插入突变体的表型解析PI(3,5)P_2的生物学功能。拟南芥*FAB1*基因家族包含*FAB1A*、*FAB1B*、*FAB1C*和*FAB1D*四个基因。研究发现, *fab1a*/*b*呈现雄配子体致死的表型。利用遗传杂交获得*fab1b*/*c*/*d*三突变体, 发现*FAB1B*、*FAB1C*和*FAB1D*功能缺失导致根毛相比野生型变短, 经FAB1特异性抑制剂YM201636处理后的野生型中也观察到相似的短根毛表型。此外, *fab1b*/*c*/*d*三突变体中DR5转录水平降低。同时, 外源施加生长素类似物2,4-D和NAA能部分恢复*fab1b*/*c*/*d*植株短根毛的表型, 但*fab1b*/*c*/*d*突变体对生长素转运抑制剂(1-NOA和TIBA)的敏感性与野生型相似。此外, *FAB1B*/*C*/*D*功能缺失使根毛中ROS的含量减少且影响肌动蛋白的表达。上述结果表明, *FAB1B*/*C*/*D*通过调控生长素分布、ROS含量和肌动蛋白的表达影响拟南芥根毛伸长。

关键词 FAB1, 根毛, 生长素, 活性氧, 肌动蛋白, 拟南芥

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根毛是植物根系的重要组成部分, 具有吸收水分、矿物质营养以及与土壤微生物互作等功能(王立德等, 2004)。根毛的快速顶端伸长为研究细胞的极性生长机制提供了很好的模型(Griersona et al., 2014)。生长素是根毛顶端伸长的重要因素, 在根毛顶端伸长发育过程中必不可少。生长素信号转导缺陷双突变体*arf7*/*arf9* (*auxin response factor*, *ARF*)的根毛相比野生型更短、更少(Qin and Huang, 2018)。*rhd6* (*root hair defective 6*)突变体短根毛表型可以通过外源施加生长素IAA得以恢复(Cui et al., 2018)。生长素通过调节CrRLK1L激酶ERULUS来控制根尖伸长过程中细胞壁组分的合成(Schoenaers et al., 2018)。根毛伸长过程伴随着细胞核向根毛顶端的移动(Zhang et al., 2015), 而根毛中的核迁移依赖F-肌动蛋白(Nakamura et al., 2018)。微丝系统如果遭到破坏, 就会抑制根毛伸长(Ketelaar et al., 2003)。钙离子(Ca^{2+})和活性氧(ROS)是根毛伸长过程中2个主要的信号传感器(Mendrinna and Persson, 2015)。Ca^{2+}在细胞质中以连续的浓度梯度分布(Ishida et al., 2008), 其浓度变化需要ROS的产生并且需要增加细胞表面的pH来调节, 细胞表面ROS的产生又激活Ca^{2+}流入细胞, 维持胞内Ca^{2+}浓度梯度(Monshausen et al., 2007)。因此, 根毛顶端伸长受生长素、Ca^{2+}水平、微丝、pH和ROS等多种因素调控。

磷酸肌醇(phosphoinositides, PIs)在20世纪70年代被发现后, 即引起人们极大的研究兴趣(Balla, 2013)。虽然磷酸肌醇只占细胞磷脂的一小部分(Payrastre et al., 2001; Mueller-Roeber and Pical, 2002), 但它们几乎控制包括信号转导、细胞骨架重组、膜动力学和囊泡运输在内的各种细胞过程(McCartney et al., 2014)。PI(3,5)P_2在酵母(*Saccharomyces cerevisiae*)和哺乳动物中分别占总磷酸肌醇的0.1%和0.04%, 占总磷脂的0.05%–0.1% (Dove et al., 2009; McCartney et al., 2014; Hasegawa et al., 2017)。

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$PI(3,5)P_2$自1997年被发现以来, 虽作为丰度最低的磷酸肌醇, 但可控制内体到溶酶体过程中多个点的囊泡转运, 进而调节溶酶体内腔的大小、形状和酸度(Dove et al., 1997, 2009; De Craene et al., 2017)。在酵母和哺乳动物中, PI3P被唯一的FAB1/PIKfyve激酶磷酸化产生$PI(3,5)P_2$ (Gary et al., 1998)。在酵母中敲除FAB1导致$PI(3,5)P_2$完全缺失, 造成液泡急剧增大(Kirsch et al., 2018)。小鼠(*Mus musculus*)中PIKfyve功能缺失造成早期胚胎致死(Takasuga et al., 2013)。不同于酵母和哺乳动物细胞, 拟南芥(*Arabidopsis thaliana*)基因组中包含4个FAB1/PIKfyve激酶: FAB1A、FAB1B、FAB1C和FAB1D (Whitley et al., 2009)。反向遗传学研究表明, 同时敲除*FAB1B*和*FAB1D*影响花粉管伸长并使质膜内吞速度减慢(Serrazina et al., 2014); 而同时敲除*FAB1B*和*FAB1C*造成气孔关闭缺陷(Bak et al., 2013); *FAB1A*和*FAB1B*功能缺失则引起花粉致死(Whitley et al., 2009); 而下调*FAB1A*和*FAB1B*的表达造成多效性的表型缺陷, 如液泡变大、液泡酸性改变和根毛形态异常(Hirano et al., 2011, 2018; Bak et al., 2013)。上述结果充分表明, 在植物细胞中, FAB1同样具有重要的生物学功能。本研究利用遗传学、细胞生物学和药理学等分析方法, 首次证明了FAB1参与调控拟南芥根毛伸长。

1 材料与方法

1.1 植物材料与培养条件

拟南芥(*Arabidopsis thaliana* L.) Col-0 (Columbia-0)生态型、*DR5::GFP*、*DR5::GUS*和*ABD2::GFP*等材料均由本实验室保存。*FAB1*突变体*fab1b-2* (*SALK_066673*)、*fab1c-2* (*Cs1002310*)和*fab1d-2* (*SALK_047604*)购自拟南芥突变体库ABRC (*Arabidopsis* Biological Resource Center)。采用PCR方法鉴定T-DNA插入纯合突变体。所用引物序列见表1。

取适量拟南芥种子, 消毒后在黑暗条件下4°C春化3天。将种子接种于1/2MS培养基(含1.5% agar, 下同)上, 培养温度为22°C, 光周期为16小时光照/8小时黑暗, 相对湿度为80%, 光照强度为90 $\mu mol \cdot m^{-2} \cdot s^{-1}$。用Zeiss体式显微镜(Discovery V20, 下同)观察并拍照幼苗。用Zeiss荧光显微镜(Axio Imager 2)观察并拍照根毛表型。

生理学实验: 将在上述培养条件下培养3天的野生型和*fab1b*/*c*/*d*幼苗转移至分别含有10 $nmol \cdot L^{-1}$ 2, 4-二氯苯氧乙酸(2,4-dichlorophenoxyacetic acid, 2, 4-D)、0.1 $\mu mol \cdot L^{-1}$萘乙酸(1-naphthaleneacetic acid, NAA)、1 $\mu mol \cdot L^{-1}$ 2,3,5-三碘苯甲酸(2,3,5-triio-dobenzoic acid, TIBA)和20 $\mu mol \cdot L^{-1}$1-萘氧乙酸(1-naphthoxyaceticacid, 1-NOA)的1/2MS培养基上, 培养3天后用Zeiss体式显微镜观察记录。YM201636 (FAB1特异性抑制剂)处理: 将野生型拟南芥种子在含有10 $\mu mol \cdot L^{-1}$ YM201636的1/2MS培养基上培养5天。用ImageJ软件测定根毛长度。上述实验进行独立的3次生物学重复, 每次生物学重复设3次技术重复。

1.2 半定量RT-PCR

取1/2MS培养基上培养5天的野生型和*fab1b*/*c*/*d*突变体幼苗各30株, 采用RNAprep pure植物总RNA试剂盒(天根, Cat No.DP432)提取植物总RNA, 然后用ReverTra Ace反转录酶体系(TOYOBO, Cat No. FSQ-101)进行反转录得到cDNA。以*AtACTIN2*为对照, 用RT-PCR检测突变体中*FAB1B*、*FAB1C*和*FAB1D*是否被敲除。所用引物序列见表1。

1.3 根毛生长速率测定

把野生型和*fab1b*/*c*/*d*突变体的种子分别接种于涂有1/2MS培养基的载玻片上, 将载玻片固定在培养皿

表1 引物序列

Table 1 Primers used in this study

Primer name	Primer sequence (5'–3')	Purpose
F1	GGCGAGGGATATTGAGTTCAG	Genotyping of *fab1b-2* and RT-PCR
R1	GTCATACATGTGGGATCACCG	Genotyping of *fab1b-2* and RT-PCR
F2	TGGGAGAAAACAGCAATGAAC	Genotyping of *fab1c-2* and RT-PCR
R2	CACGACAACTTCCCCGAAGCACAA	Genotyping of *fab1c-2* and RT-PCR
F3	AGGTTGGGATGAATGGTTTTG	Genotyping of *fab1d-2* and RT-PCR
R3	AGGTCGTGCCGTATCTCTTTC	Genotyping of *fab1d-2* and RT-PCR
sgtDs3'-1	GGTTCCCGTCCGATTTCGACT	Genotyping of *fab1c-2*
LBb1.3	ATTTTGCCGATTTCGGAAC	Genotyping of *fab1b-2* and *fab1d-2*
AtACTIN-F	GTCGTACAACCGGTATTGTG	Internal control for RT-PCR
AtACTIN-R	GAGCTGGTCTTTGAGGTTTC	Internal control for RT-PCR

里，22°C培养3天。将载玻片置于Zeiss荧光显微镜(Axio Imager 2)下观察，挑选符合实验要求的根毛并每隔5分钟记录1次，连续观察50分钟。跟踪单个根毛的伸长过程，并用ImageJ软件测量根毛长度，分析伸长速率。实验中野生型和突变体各观察35株植物，每株植物观察2根根毛。

1.4 激光共聚焦显微镜参数

本研究使用的激光共聚焦显微镜型号为Nikon A1R+ Ti2-E，GFP设置为激发光波长488 nm，发射光波长510 nm；YFP设置为激发光波长550 nm，发射光波为570 nm；DCF-DA设置为激发光波长550 nm，发射光波长570 nm。相同实验均在相同设置的背景色、针孔大小和激光器电压下完成，并至少独立重复3次。

1.5 ROS探针染色

使用染料DCF-DA (2',7'-二氯二氢荧光素-二乙酸酯，分子探针)检测植物体中的ROS含量(Casimiro et al., 2003)。萌发5天的野生型和*fab1b*/*c*/*d*突变体幼苗用20 μmol·L^{-1} DCF-DA (溶于DMSO)溶液染色，4°C处理60分钟。然后用Wash buffer (0.1 mmol·L^{-1} KCl和0.1 mmol·L^{-1} $CaCl_2$，pH6.0)冲洗3次。最后在Wash buffer中孵育60分钟(22°C)。实验设3次独立的生物学重复，每次生物学重复分析野生型和突变体各10株。

2 结果与讨论

2.1 *fab1b*/*c*/*d*三突变体表现短根毛表型

FAB1是催化PI(3)P合成PI(3,5)P_2的磷脂激酶，几乎存在于所有真核生物中(Jin et al., 2016)。研究表明，拟南芥中有4个FAB1蛋白(FAB1A、FAB1B、FAB1C和FAB1D)与酵母和人类(*Homo sapiens*)中的FAB1蛋白序列同源(Whitley et al., 2009)。为深入解析FAB1在拟南芥生长发育过程中的生物学功能，我们分离鉴定了*fab1* T-DNA插入突变体，并分别获得各个单、双、三突变体(图1A)。其中*fab1a*/*b*双突变体表现雄配子体致死(Whitley et al., 2009)，*fab1b-2*、*fab1c-2*和*fab1d-2*的单突变体和双突变体的根毛表型与野生型相比无显著差异(图1E–L，N)，而*fab1b-2*/*fab1c-2*/*fab1d-2* (*fab1b*/*c*/*d*)三突变体呈现非常明显的短根毛表型(图1C)，根毛长度约为野生型的50% (图1D)。RT-PCR结果表明，在纯合的*fab1b*/*c*/*d*三突变体株系中未检测到*FAB1B*、*FAB1C*和*FAB1D*的表达，表明这3个基因均完全缺失(图1B)，暗示FAB1B、FAB1C和FAB1D之间存在功能冗余，这与前人的研究(拟南芥*FAB1*基因功能冗余)相一致(Whitley et al., 2009; Hirano et al., 2011)。为进一步验证*fab1b*/*c*/*d*三突变体根毛变短是否由FAB1的功能缺陷造成，我们使用FAB1特异性抑制剂YM201636进行药理学实验，发现含有YM201636平板上的拟南芥根毛明显短于对照组(图1M)。我们还发现*fab1b*/*c*/*d*三突变体的根毛形态也发生变化，出现了分叉和局部膨大的根毛(图1O)。在*fab1b*/*c*/*d*背景下，具有分叉表型的根毛约占25% (野生型中仅占不到1%) (图1P)。综上表明，FAB1B、FAB1C和FAB1D以功能冗余的方式参与根毛的生长发育。

2.2 FAB1B、FAB1C和FAB1D功能缺失抑制根毛伸长

根毛发育通常可分为生毛细胞命运决定、根毛起始、根毛伸长和成熟4个阶段(李林等，2016)。为了明确*fab1b*/*c*/*d*突变体影响根毛发育的具体阶段，我们首先分析了*fab1b*/*c*/*d*突变体中的根毛密度，发现*fab1b*/*c*/*d*与野生型的根毛密度无明显差异(图2D)，暗示突变体的根毛起始可能是正常的。随后，我们又观察了野生型和*fab1b*/*c*/*d*突变体的单个根毛的伸长情况(图2A)，发现在野生型中，根毛通过尖端以恒定的生长速率伸长(生长速率为0.93 μm·min^{-1}) (图2B, C)，而在*fab1b*/*c*/*d*突变体中，根毛的伸长速率要慢得多(生长速率为0.50 μm·min^{-1}) (图2B, C)。基于上述结果，我们推测FAB1可能参与调控根毛的顶端伸长。

2.3 FAB1影响生长素的分布

前人的研究表明，生长素参与根毛生长发育的多个过程(Cui et al., 2018; Nakamura et al., 2018; Qin and Huang, 2018; Schoenaers et al., 2018; Shibata and Sugimoto, 2019)。为了探究FAB1在生长素调控根毛发育过程中的作用，我们将萌发后3天的野生型和*fab1b*/*c*/*d*突变体幼苗转移到含有NAA或2,4-D的1/2MS培养基上培养3天。经过生长素类似物(NAA和

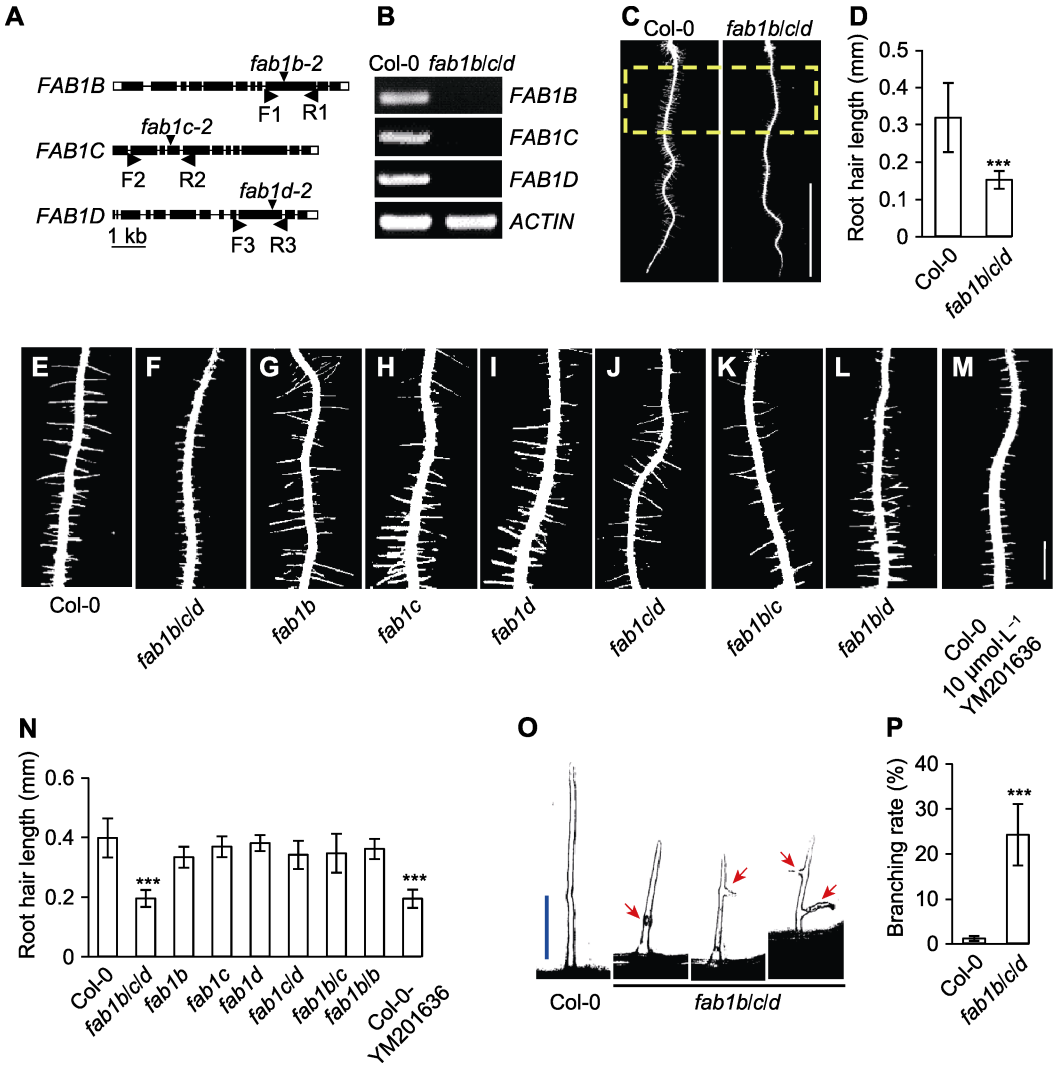

图1 *FAB1B*、*FAB1C*和*FAB1D*调控拟南芥根毛伸长

(A) *FAB1*基因结构和T-DNA插入位点; **(B)** RT-PCR检测*FAB1B*、*FAB1C*和*FAB1D*的表达; **(C)** 5天龄幼苗根毛长度(黄色虚线框代表定量区域) (Bar=5 mm); **(D)** 定量根毛长度; **(E)–(M)** FAB1单突变体、双突变体及YM201636 (FAB1特异性抑制剂)处理的野生型幼苗根毛表型(Bar=0.5 mm); **(N)** 定量**(E)–(M)**突变体根毛长度; **(O)** 根毛形态分析, 红色箭头表示根毛上的凸起和分叉(Bar=75 μm); **(P)** 定量分叉根毛占总根毛数量的百分比。*** P<0.001 (Student's *t*-test)

Figure 1 *FAB1B*, *FAB1C* and *FAB1D* regulate root hair growth in *Arabidopsis*

(A) *FAB1* gene structure and T-DNA insertion sites; **(B)** Analysis of gene expression of *FAB1B*, *FAB1C* and *FAB1D* by RT-PCR; **(C)** Assay of root hair length in 5-day-old seedlings (the yellow dotted box represents quantitative area) (Bar=5 mm); **(D)** Quantification of root hair length; **(E)–(M)** The root hair phenotype of the FAB1 single mutants, double mutants and YM201636 (FAB1-specific inhibitor) treatment of Col-0 seedling (Bar=0.5 mm); **(N)** Quantification of **(E)–(M)** mutant root hair length; **(O)** Images of the typical root hair morphologies, the root hair morphologies were categorized as swollen or branched (red arrow indicates) (Bar=75 μm); **(P)** Quantification of frequency of branched root hairs (%). *** P<0.001 (Student's *t*-test)

2,4-D)处理后, 野生型和*fab1b/c/d*突变体的根毛相比对照组均明显伸长(图3A–C)。有趣的是, 2,4-D处理后突变体的根毛长度与野生型之间无显著差异(图3B, D), 而NAA处理后三突变体与野生型之间的差异缩小(图3C, D)。上述结果表明, *fab1b/c/d*短根毛表型可能是生长素不足造成。DR5是生长素反应的强启动子, 通过分析*DR5-GFP*和*DR5-GUS* (*DR5-GFP*/*GUS*)的表达可间接反映生长素的水平与分布。由于直接检测

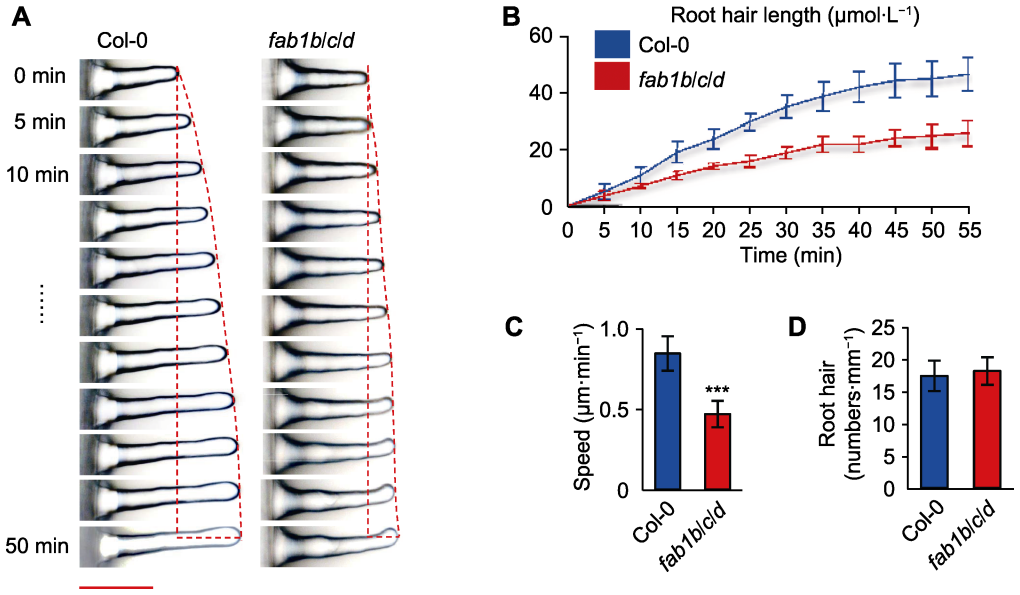

图2 拟南芥*fab1b/c/d*突变体根毛生长速率减慢

(A) 根毛伸长动力学分析(使用荧光显微镜连续50分钟记录野生型Col-0和*fab1b/c/d*突变体的动态生长趋势，并每隔5分钟拍照1次) (Bar=50 μm); **(B)** 单位时间根毛长度; **(C)** 根毛生长速率(*** P<0.001, Student's t-test); **(D)** 单位长度内根毛数量。

Figure 2 Slow growth of *Arabidopsis fab1b/c/d* mutant root hairs

(A) Growth dynamics of individual Col-0 and *fab1b/c/d* root hairs (fluorescence microscopy was used to assess root hair elongation, showing consecutive frames of growing root hairs for a period of 50 minutes, pictures were taken every 5 minutes) (Bar=50 μm); **(B)** Root hair length per unit time; **(C)** Root hairs growth speed (*** P<0.001, Student's t-test); **(D)** Average root hairs number.

根毛细胞中的*DR5-GFP/GUS*比较困难，因此为了测定FAB1在生长素分布中的作用，我们观察了拟南芥根尖*DR5-GFP/GUS*的分布，结果发现*fab1b/c/d*突变体中*DR5-GFP/GUS*表达水平下降(图4A, B)。这一结果同样暗示FAB1可能调控拟南芥根毛伸长过程中生长素的分布或影响生长素信号转导。

生长素运输调控拟南芥根毛的伸长发育(Jones et al., 2009)。我们验证了*fab1b/c/d*突变体和野生型对生长素输出抑制剂(TIBA)和输入抑制剂(1-NOA)的反应。其中, TIBA对*fab1b/c/d*根毛伸长的促进作用与野生型相似(图3E, G), 1-NOA对根毛伸长的抑制作用在野生型和突变体中也无明显差异(图3F, G)。因此，上述结果暗示FAB1并非通过生长素转运途径影响根毛的发育，而是对生长素分布产生影响，进而调控根毛伸长。

2.4 FAB1影响根毛ROS水平和微丝的分布

ROS是控制根毛顶端伸长的关键因子(Lee et al., 2008)。我们检测了*fab1b/c/d*三突变体根毛中的ROS水平。采用ROS探针DCF-DA (2',7'-二氯二氢荧光素-二乙酸酯，分子探针)染色处理5天的野生型、*fab1b/c/d*突变体和用YM201636处理过的野生型幼苗，并在激光共聚焦显微镜下观察(图5A)。结果显示，与野生型相比，*fab1b/c/d*突变体和YM201636处理过的野生型根毛中ROS水平显著降低(图5A, B)。但是, FAB1是否直接影响根毛中的ROS含量仍需深入研究。

Griersona等(2014)研究表明，肌动蛋白(actin)在调控根毛尖端伸长中发挥了重要作用。我们利用肌动蛋白Marker ABD2-GFP分析了FAB1B、FAB1C和FAB1D是否影响肌动蛋白的表达。共聚焦结果显示，*fab1b/c/d*突变体背景下ABD2-GFP荧光信号相比野生型明显减弱。同时, YM201636处理野生型的ABD2-GFP的荧光信号也显著减弱(图5C, D)。综上表明，*FAB1B*、*FAB1C*和*FAB1D*功能缺失可能影响根毛中ROS的分布和微丝的排布，进而影响根毛伸长。

2.5 讨论

研究表明，在酵母和动植物中PI(3,5)P_2作为FAB1的

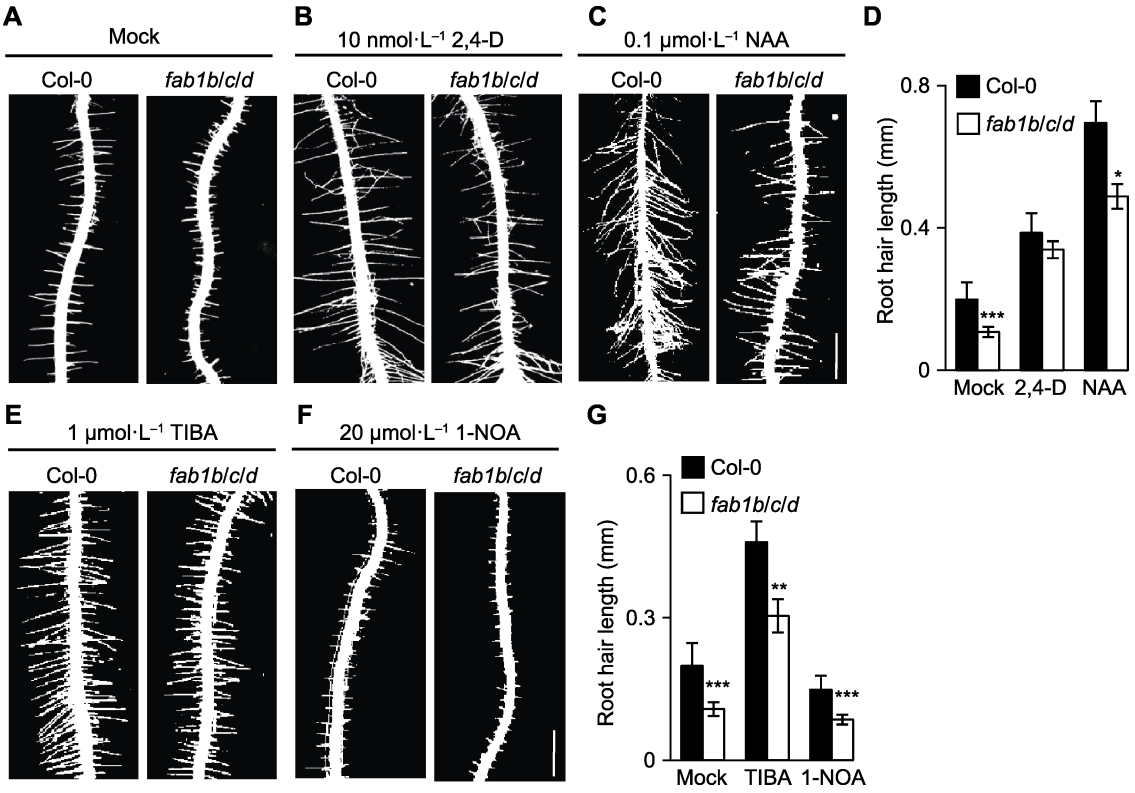

图3 外源生长素能部分恢复拟南芥*fab1b/c/d*突变体的短根毛表型

(A)–(C) 外源添加DMSO (Mock)、10 nmol·L^{-1} 2,4-D或0.1 μmol·L^{-1} NAA可部分恢复*fab1b/c/d*突变体根毛表型(Bar=0.5 mm); **(D)** 定量分析根毛长度; **(E), (F)** 生长素运输抑制剂TIBA (生长素输出抑制剂)和1-NOA (生长素输入抑制剂)处理后根毛表型(Bar=0.5 mm); **(G)** 定量分析根毛长度。* *P*<0.05; ** *P*<0.01; *** *P*<0.001 (Student's *t*-test)

Figure 3 Exogenous auxin application partially rescued the root hair defect of *Arabidopsis fab1b/c/d* mutants

(A)–(C) Root hairs of *fab1b/c/d* treated with DMSO (Mock), 10 nmol·L^{-1} 2,4-D and 0.1 μmol·L^{-1} NAA, respectively, the phenotype of root hairs was rescued partially (Bar=0.5 mm); **(D)** Quantification of root hair length; **(E), (F)** Col-0 and *fab1b/c/d* seedlings were transferred to plates containing TIBA (auxin efflux inhibitors) and 1-NOA (auxin influx inhibitor) (Bar=0.5 mm); **(G)** Quantitative analysis of root hair length. * *P*<0.05; ** *P*<0.01; *** *P*<0.001 (Student's *t*-test)

产物参与调控细胞内膜运输、细胞自噬和逆境响应信号转导(McCartney et al., 2014)。大多数真核生物(如小鼠、果蝇(*Drosophila melanogaster*)和线虫(*Caenorhabditis elegans*))中敲除唯一的*FAB1*基因造成胚胎致死(Takasuga et al., 2013)。然而, 拟南芥中却存在4个*FAB1*基因(*FAB1A–D*), FAB1的多拷贝暗示FAB1和PI(3,5)P_2在拟南芥中的功能可能更加丰富, 遗传学证据也支撑了这种推断。在拟南芥*fab1*单突变体中并未观察到明显可见的发育异常(图1G–I), 而*FAB1A*和*FAB1B*功能缺失都会导致雄配子体致死, *FAB1A*/*FAB1B*基因表达下调的突变体则表现生长缓慢、向地性反应异常、生长素敏感性改变以及花器官发育和根毛形态异常(Serrazina et al., 2014; Hirano et al., 2015, 2018); 敲除*FAB1B*和*FAB1C*导致气孔

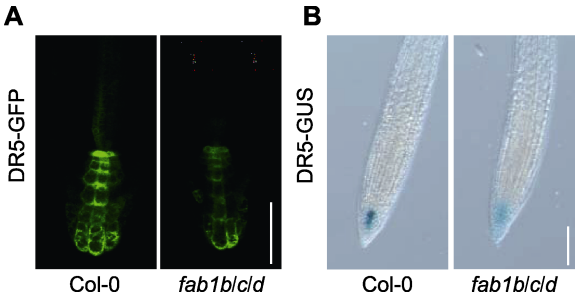

图4 FAB1影响拟南芥生长素的分布

(A) 利用*DR5-GFP*分析Col-0和*fab1b/c/d*根部的生长素分布变化(Bar=75 μm); **(B)** *DR5-GUS*在Col-0和*fab1b/c/d*中的表达情况(Bar=100 μm)

Figure 4 FAB1 affects auxin distribution in *Arabidopsis*

(A) The expression analysis of *DR5-GFP* for auxin distribution in the root (Bar=75 μm); **(B)** *DR5-GUS* expression in Col-0 and *fab1b/c/d* (Bar=100 μm)

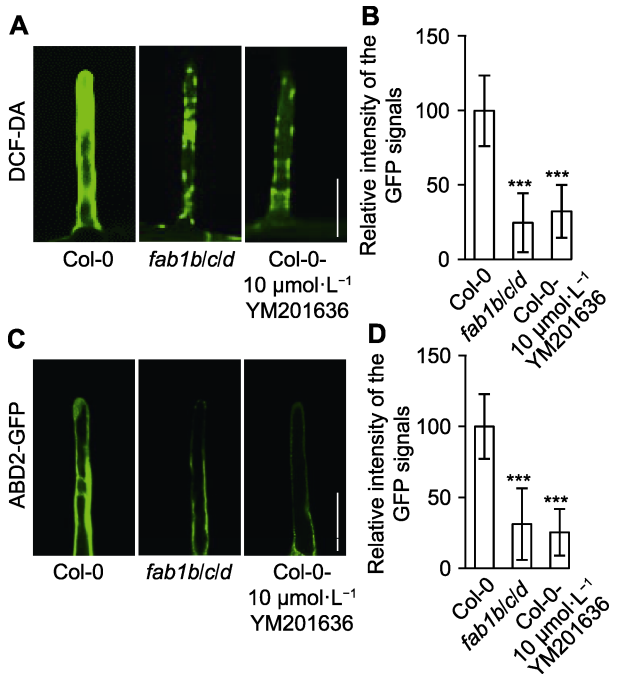

图5 拟南芥*fab1b*/*c*/*d*突变体根毛活性氧(ROS)含量及肌动蛋白稳定性改变

(A) 用DCF-DA (ROS染液)检测Col-0、*fab1b*/*c*/*d*和Col-0-YM201636根毛中的ROS含量(Bar=25 μm); **(B)** 定量分析DCF-DA荧光强度; **(C)** Col-0、*fab1b*/*c*/*d*和Col-0-YM201636的根毛肌动蛋白细胞骨架标记ABD2的分布(Bar=25 μm); **(D)** 定量分析ABD2-GFP荧光强度。*** P<0.001 (Student's *t*-test)

Figure 5 Reactive oxygen species (ROS) intensity and actin stability were altered in root hairs of *Arabidopsis fab1b*/*c*/*d* seedlings

(A) Total ROS generated by oxidation of DCF-DA in wild type, *fab1b*/*c*/*d* and Col-0-YM201636 root (Bar=25 μm); **(B)** Relative intensity of the GFP signals; **(C)** Distribution of actin cytoskeleton marker ABD2 in root hair of Col-0, *fab1b*/*c*/*d* and Col-0-YM201636 treatment (Bar=25 μm); **(D)** Average relative intensity of the ABD2-GFP signals. *** P<0.001 (Student's *t*-test)

关闭障碍(Bak et al., 2013); *fab1b*/*d*呈现花粉管形态异常等表型(Whitley et al., 2009)。但是, 在拟南芥FAB1的单突变体中并未观察到明显的缺陷表型(图1G–I), 说明FAB1之间存在功能冗余(Whitley et al., 2009)。本研究成功获得了*fab1b*/*c*/*d*三突变体纯合体、FAB1的单突变体(*fab1b*、*fab1c*和*fab1d*)和双突变体(*fab1b*/*c*、*fab1b*/*d*和*fab1c*/*d*) (图1F–L), 对5天龄幼苗的单、双突变体进行表型分析未见异常发育, 但在三突变体中发现FAB1调控根毛的伸长。在*fab1b*/*c*/*d*三突变体中, 根毛长度约为野生型的50% (图1D)。在FAB1特异性抑制剂YM201636处理下, 野生型幼苗的根毛长度受到抑制(图1M)。在单根根毛水平上, 利用显微镜成像技术, 我们发现*fab1b*/*c*/*d*植株中根毛的起始是正常的, 但不能完成随后的快速伸长(图2A)。已有研究表明, FAB1在真核生物中参与调控内膜运输及在植物中调控花粉管囊泡运输(Serrazina et al., 2014)。我们推测*FAB1B*、*FAB1C*和*FAB1D*功能缺失可能干扰了根毛细胞中的内膜系统转运途径, 进而调控根毛的尖端生长。虽然拟南芥FAB1缺失影响花粉管的膜泡运输和根尖PIN2的转运(Hirano et al., 2015), 但是根毛中的囊泡运输情况并没有直接被检测, 还有待深入研究证实。

对生长素信号元件和多种生长素转运体的研究表明, 根毛细胞的生长素信号转导和稳态对根毛伸长至关重要(Velasquez et al., 2016)。生长素信号受体(Transport inhibitor response 1)突变体*tir1*及其*afb1*、*afb2*和*afb3* (*auxin signaling f-box*, *AFB*)突变体均表现出根毛伸长缺陷(Dharmasiri et al., 2005), 而根毛特异性过表达*TIR1*可提高根毛伸长速率(Ganguly et al., 2010)。生长素抗性突变体*iaa7*、*iaa17*和*iaa28* (indole-3-acid protein, IAA)都表现出根毛伸长受抑制(Lee and Cho, 2013)。而且, 生长素信号突变体*arf7*/*arf19*也表现出短根毛表型(Velasquez et al., 2016)。此外, 生长素生物合成抑制剂yucasin处理会抑制拟南芥根毛伸长(Nishimura et al., 2014)。在*fab1b*/*c*/*d*三突变体中我们观察到根尖DR5的表达量降低, 说明*fab1b*/*c*/*d*三突变体中生长素分布受到影响(图4A, B)。外源施加生长素类似物(2,4-D和NAA)能部分恢复*fab1b*/*c*/*d*突变体根毛伸长的缺陷(图3B, C), 暗示FAB1可能通过影响根毛生长素分布或生长素信号转导途径调控根毛伸长。同时, 生长素只能部分恢复突变体的表型, 这增加了生长素运输缺陷的可能性。已有研究表明, AUX1介导非生毛细胞生长素运输维持根毛发育(Jones et al., 2009); 在生毛细胞中过表达6种PIN (Pin-formed)蛋白(PIN1–PIN4、PIN7和PIN8)和3种PGP (Phosphoglycoprotein)蛋白(PGP1、PGP4和PGP19)都极大地抑制根毛细胞的伸长, 这可能是由于它们的高生长素输出活性降低了生毛细胞的生长素水平(Lee and Cho, 2006; Ganguly et al., 2010); 此外, 生长素输出抑制剂TIBA和NPA通过抑制生长素的输出增加内源IAA的积累, 进而促

进根毛伸长(Niu et al., 2011)。反之，生长素输入抑制剂1-NOA则抑制根毛伸长(Rahman et al., 2002)。药理学实验结果表明，用TIBA和1-NOA分别处理对野生型和*fab1b*/*c*/*d*根毛伸长的影响相似(图3E, F)。上述结果表明，FAB1调控根毛发育并不是通过影响生长素运输，而是干扰了生长素的分布。同样，*fab1b*/*c*/*d*突变体生长素分布的变化(图4A, B)也可能是由于FAB1的缺失打破了生长素合成和降解的平衡，但还需要通过实验证实。

根毛伸长除了与生长素有关外，也与ROS密切相关(Shibata and Sugimoto, 2019)。*fab1b*/*c*/*d*突变体的ROS水平下降(图5A, B)，暗示FAB1对维持根毛细胞ROS水平至关重要。NADPH氧化酶RHD2 (ROOT HAIR DEFECTIVE 2)是根毛ROS产生的主要来源(Lee et al., 2008)，敲除RHD2或突变NADPH氧化酶的转录因子RSL4 (ROOT HAIR DEFFECTIVE SIX-LIKE 4)都表现短根毛表型(Jones et al., 2007; Vijayakumar et al., 2016; Mangano et al., 2017)。同时，外源施加ROS可以部分恢复*rhd2*突变体的根毛表型(Carol and Dolan, 2006)。有研究表明，在拟南芥中ROS调控PI(3,5)P_2后期在体内的积累(Hirano et al., 2017)。PI(3,5)P_2合成抑制剂处理拟南芥野生型产生短根毛表型(图1M)并降低根毛ROS水平(图5A, B)，这暗示PI(3,5)P_2和ROS之间的相互影响是根毛正常发育所必需的。

肌动蛋白细胞骨架参与根毛发育的所有阶段(根毛起始、伸长和成熟)，并在不同阶段呈现不同的排布(Ketelaar et al., 2003; Griersona et al., 2014)。此外，肌动蛋白结合蛋白介导磷酸肌醇调控细胞骨架(Pei et al., 2012)。抑制PI(4,5)P_2合成(抑制剂mastoparan处理)和敲除PI(4,5)P_2合成酶(*pip5k3*突变体)都造成根毛显著变短(Kusano et al., 2008; Stenzel et al., 2008)，其它磷脂(如PA、PI3P和PI4P)也参与根毛尖端伸长(Ishida et al., 2008)。在*fab1b*/*c*/*d*突变体和YM-201636处理的拟南芥中，肌动蛋白Marker (ABD2-GFP)表达量显著下降(图5C, D)，结合肌动蛋白在根毛发育中的作用，暗示FAB1可能调控微丝的动态解聚和聚合，进而介导根毛伸长。

综上所述，拟南芥根毛细胞中*FAB1B*、*FAB1C*和*FAB1D*的缺失严重影响生长素的分布、ROS的含量和肌动蛋白的表达。但是FAB1是如何调控这些因素的，FAB1是否也影响根毛伸长的其它关键因子(如pH、Ca^{2+}、囊泡运输和细胞壁重塑)，目前还不清楚。后续研究将进一步揭示FAB1在根毛伸长中的具体作用。

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A Role of *Arabidopsis* Phosphoinositide Kinase, FAB1, in Root Hair Growth

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Abstract Lipid kinase FORMATION of APLOID and BINUCLEATE CELLS 1 (FAB1/PIKfyve) is a key enzyme that generates PI(3,5)P_2. PI(3,5)P_2 plays an important role in regulating membrane trafficking in yeast and animal cells, but its function in plants remains poorly understood. Here we study the functional role of PI(3,5)P_2 via analysis of phenotypes of *Arabidopsis* FAB1 T-DNA knockout lines. The *Arabidopsis* FAB1 family contains four orthologous genes: *FAB1A*, *FAB1B*, *FAB1C*, and *FAB1D*. The *fab1a*/*b* double mutant showed a complete male gametophyte lethal phenotype. *fab1b*/*c*/*d* mutant was successfully isolated. Genetic analyses showed that the loss of *FAB1B*, *FAB1C* and *FAB1D* function disrupts root hairs elongation. Further pharmacological analysis showed that the FAB1-specific inhibitor YM201636 inhibited the root hairs growth. In addition, the transcription level of *DR5-GFP*, an indicator of auxin expression and distribution, was downregulated in single mutation of *FAB1B*, *FAB1C* and *FAB1D*. Moreover, triple mutant phenotypes (short root hairs) were partially rescued by exogenous application of auxin analog 2,4-D and NAA. However, the mutant's sensitivity to 1-NOA (auxin influx inhibitor) and TIBA (auxin efflux inhibitor) in root hair elongation assay is identical to that of the wild-type. Furthermore, loss of *FAB1B*, *FAB1C* and *FAB1D* function reduces the level of reactive oxygen species (ROS) and affects actin expression in root hairs. Taken together, the *FAB1B*/*C*/*D* affects *Arabidopsis* root hair elongation by regulating auxin distribution, levels of ROS, and actin expression.

Key words FAB1, root hair, auxin, ROS, actin, *Arabidopsis thaliana*

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