植物学报 *Chinese Bulletin of Botany* 2020, **55** (5): 541–550, www.chinbullbotany.com
doi: 10.11983/CBB20058

·特邀综述·

植物类LORELEI糖基磷脂酰肌醇锚定蛋白研究进展

李思佳[1], 张咏雪[1], 贾明生[2], 李莹[1], 戴绍军[2*]

[1]东北林业大学生命科学学院, 东北盐碱植被恢复与重建教育部重点实验室, 哈尔滨 150040
[2]上海师范大学生命科学学院, 上海植物种质资源工程技术研究中心, 上海 200234

摘要 类LORELEI糖基磷脂酰肌醇锚定蛋白(LLG)定位于细胞质膜外表面, 作为CrRLK1L家族类受体激酶的分子伴侣, 参与其转运和胞外信号转导, 从而调控植物生殖发育以及免疫与逆境应答等过程。LLG2/3与ANX和BUPS互作, 调控花粉管顶端生长与爆裂。LLG1与FER (FERONIA)互作, 调控下游的NADPH氧化酶产生活性氧(ROS), 促进根部细胞伸长和根毛生长。此外, LLG1作为FER的共受体, 与快速碱化因子(RALFs)互作, 调节G蛋白β亚基(AGB1)和质膜H^+-ATPase功能、胞内ROS稳态以及Ca^{2+}瞬变, 引起根部和气孔的盐应答反应。LLG1与FLS2和EFR互作激活下游RbohD, 调节ROS产生, 调控植物免疫应答。该文综述了植物LLG的相关研究进展, 可为深入理解LLG的生物学功能提供重要信息。

关键词 LLG, RALF, 花粉管, 根, 免疫与盐应答

李思佳, 张咏雪, 贾明生, 李莹, 戴绍军 (2020). 植物类LORELEI糖基磷脂酰肌醇锚定蛋白研究进展. 植物学报 **55**, 541–550.

植物糖基磷脂酰肌醇锚定蛋白(glycosylphosphatidylinositol-anchored protein, GPI-AP)定位于细胞膜外表面, 作为CrRLK1L家族类受体激酶的分子伴侣, 参与调节细胞生殖、生长以及免疫与盐碱胁迫应答等多种生物学过程(Yu et al., 2013; Cheung et al., 2014; Zhang et al., 2020)。植物GPI-AP家族包括COBRA、ENODL和LRE (LORELEI)三个亚家族, 分别调节细胞壁纤维素生物合成(Liu et al., 2013)、花粉管接受(Hou et al., 2016)和双受精作用(Feng et al., 2019)。拟南芥(*Arabidopsis thaliana*)中有3个类LORELEI糖基磷脂酰肌醇锚定蛋白(LORELEI-like GPI-AP, LLG), 其中LLG1在根和叶片等多个器官中表达, LLG2和LLG3在花粉粒和花粉管中表达(Ge et al., 2019)。LLG参与FER (FERONIA)等多种类受体激酶(receptor-like kinase, RLK)从内质网向质膜的转运及其质膜定位, 并作为这些RLK的共受体感知外部信号和快速碱化因子(rapid alkalinization factor, RALF)等配体(Li et al., 2015), 调节RLK的功能, 参与花粉管生长与爆裂、根与根毛生长以及免疫与盐胁迫应答等过程(Li et al., 2015; Shen et al., 2017; Feng et al., 2018; Yu et al., 2018; Zhao et al., 2018; Ge et al., 2019)。

1 LLG参与RLKs的转运与定位

LLG的氨基酸序列包括N端信号肽(含23个高度保守的氨基酸残基)、中央区域、C端构象可变区以及C端GPI锚(约20个氨基酸残基) (图1A)。LLG有8个保守的半胱氨酸, 形成4对二硫键, 参与蛋白质3D结构形成(图1B) (José-Estanyol et al., 2004; Liu et al., 2016; Shen et al., 2017)。LLG形成过程中, GPI-AP前体在内质网中添加预组装的GPI锚, C末端被脂质化修饰, 然后通过高尔基体转运, 分泌到细胞膜外小叶, 主要分布于富含鞘脂和胆固醇的膜微区(Zurzolo and Simons, 2016)。LLG与FER等RLKs跨膜结构域N末端的胞外近膜区(juxtamenbrane region, exJM)结合, 并将这些RLKs从内质网转运到质膜上(Li et al.,

收稿日期: 2020-04-05; 接受日期: 2020-05-20
基金项目: 黑龙江省自然科学基金(No.JJ2019ZZ0235)和上海市科委科研计划(No.17391900600)
* 通讯作者。E-mail: daishaojun@hotmail.com

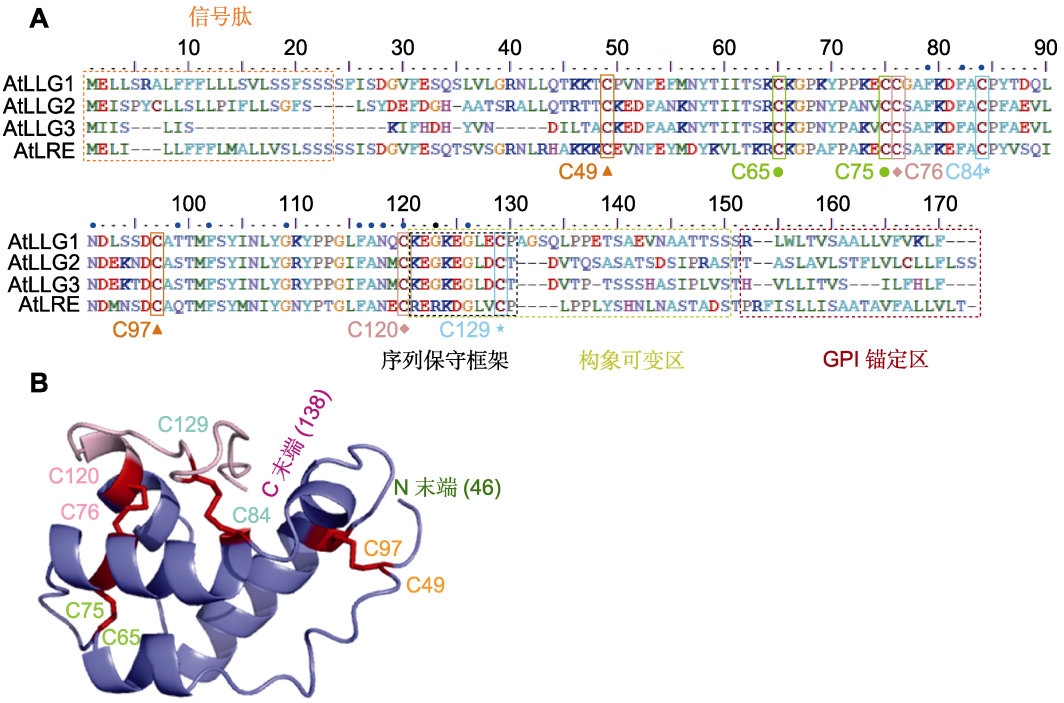

图1 拟南芥LLG氨基酸序列与3D结构模型

(A) LLG氨基酸序列(彩色框标注的分别是N端信号肽、序列保守框架、C端构象可变区及C端GPI锚定区；三角形、绿色圆点、菱形和五角星表示可形成4对二硫键的8个Cys位点，蓝色圆点表示保守氨基酸位点)；**(B)** LLG1的46–138位氨基酸的3D结构(α螺旋、β折叠、N末端(46)、C末端(138)及8个保守Cys位点形成4对二硫键)

Figure 1 *Arabidopsis* LLG amino acid sequences and 3D structure model

(A) LLG amino acid sequence (The colored boxes indicate the N-terminal signal peptide, sequence conservative frame, C-terminal conformational flexible region, and C-terminal GPI anchor region, respectively; the triangles, green dots, diamonds and pentagonal stars represent 8 Cys sites that can form 4 pairs of disulfide bonds, and the blue dots represent conservative amino acid sites); **(B)** The 3D structure of amino acids 46–138 of LLG1 (α-helix, β-sheet, N terminal (46), C terminal (138), and conserved 8 Cys sites formed 4 pairs of disulfide bonds)

2015)。LLG作为RLKs的共同受体，感知细胞外部信号或配体，调节下游各种信号通路。在拟南芥*llg1*突变体中，FER-GFP滞留在内质网和细胞质，而在*llg1*突变体中回补表达LLG1可以减少FER-GFP在细胞质中的滞留，恢复FER的质膜定位。*llg1*突变体早期生长发育表型与*fer*的表型相似，如对RALF1的敏感性降低、表皮细胞形状改变以及根毛生长缺陷(Li et al., 2015)。LLG1/2/3与其配体RALFs保守的N端(4–17位的14个氨基酸)结合，并与FER胞外结构域(FER^{ECD})互作，形成LLG-RALF-FER复合物，调控下游信号通路(Xiao et al., 2019)。

2 LLG2/3调节花粉管顶端生长与爆裂

植物花粉管快速生长进入胚珠的过程受到精细调控(Johnson et al., 2019)。定位在花粉管顶端的多种RLKs感知外部信号，调控花粉管生长(Li and Yang, 2016; Zhong and Qu, 2019)。ANX1/2 (ANXUR 1/2)和BUPS1/2 (Buddha's paper seal 1/2)是花粉管顶端质膜定位的RLKs，两者形成受体激酶复合物，通过响应RALF小肽信号调节花粉管生长和精细胞释放(Ge et al., 2017, 2019; Franck et al., 2018; Li and Yang, 2018)。花粉特异表达的LLG2/3可以与ANX和BUPS的exJM区互作，调节其质膜定位，缺失exJM区的ANX/BUPS滞留在胞内(Ge et al., 2019)。LLG2/3作为分子伴侣，协助ANX和BUPS从内质网分泌到花粉管顶端的质膜，形成受体-共受体复合物，共同感受胞外RALF信号(图2A)；通过感受花粉管自分泌的RALF4和RALF19小肽，维持花粉管的完整性和顶端生长(Mecchia et al., 2017; Feng et al., 2019)；通过识别胚珠分泌的RALF34小肽，控制花粉管爆裂并释放精细胞(Ge et al., 2017)。RALF4和

RALF19显著增强LLG2/3与ANX/BUPS的相互作用, RALF4的C端与LLG结合, 其N端(包括YISY motif)与LLG微弱互作, 而与ANX/BUPS强烈互作(Ge et al., 2019)。Xiao等(2019)解析了FER^{ECD}、ANX1、ANX2、LLG1以及RALF23-LLG2-FER^{ECD}复合体的晶体结构(Xiao et al., 2019)。结果表明, FER^{ECD}与$ANX1^{ECD}$和$ANX2^{ECD}$的晶体结构非常相似, RALF23的N端α螺旋与LLG2表面大沟结合, 且FER^{ECD}与RALF23和LLG2互作后, 其构象无明显变化(Xiao et al., 2019)。以上研究结果为认识RALF4/19/34与LLG2/3和ANX/BUPS之间的互作关系提供了参考。

在感受胞外RALF4/19后, LLG2/3-ANX1/2-BUPS1/2复合体通过与GDP-ROP1相互作用, 将其激活为GTP-ROP1, 进而激活下游花粉特异表达的NADPH氧化酶RbohH/J, 产生活性氧(ROS), 导致ROS在花粉管顶端积累, 调节花粉管生长, 降低花粉管的爆裂率(图2A) (Kaya et al., 2014; Mangano et al., 2016; Feng et al., 2019)。拟南芥*LLG2*/*3* RNAi植株的花粉管ROS含量降低, 生长迟滞, 爆裂率较高。*LLG2*/*3*的缺乏引起花粉管细胞壁成分改变, 甲酯化果胶质在花粉管顶端区域积累, 去甲酯化果胶质在花粉管亚顶端和shank区域积累, 花粉管中的胼胝质含量降低, 引起花粉管生长变缓(Feng et al., 2019)。外源施加H_2O_2能够以剂量依赖的方式恢复*LLG2* RNAi植株花粉管的长度, 降低花粉管爆裂率, 这与*anx1*$^{-/-}$/*anx2*$^{+/-}$突变体花粉管表型能被外源施加H_2O_2恢复相似(Duan et al., 2010; Feng et al., 2019)。由此可见, ROS稳态对于RALF-LLG-ANX/BUPS调节花粉管生长与爆裂十分重要。

3 LLG1调控根与根毛生长

LLG1通过与FER的exJM结合, 协助FER定位于根部细胞质膜, 进而共同调控根与根毛生长(图2B) (Duan et al., 2010; Li et al., 2015)。拟南芥*llg1*突变体与*fer-4*突变体有相似的根毛缺陷表型(Li et al., 2015)。*fer*突变体的营养生长(Keinath et al., 2010)、根毛生长(Duan et al., 2010; Huang et al., 2013)以及下胚轴伸长均受到抑制(Guo et al., 2009; Deslauriers and Larsen, 2010), 根毛出现卷曲或异常分枝(Duan et al., 2010; Li et al., 2015)。在*llg1*与*fer*突变体中分别转入*LLG1*和*FER*, 可以恢复其野生型表型(Duan et al., 2010; Li et al., 2015)。

LLG1与FER相互作用共同感受RALF1, 形成LLG1-RALF1-FER复合物并激活FER, 进而调控根生长。外源施加RALF1抑制野生型幼苗根的生长, 但并不影响*llg1*与*fer-4*突变体的根长。激活的FER与RopGEFs互作, 将RAC/ROP从与GDP结合的无活性状态转变为与GTP结合的活化状态。*fer*突变体与几种*rac*/*rop*突变体表型相似, 表明*fer*与*rac*/*rop*之间有调控关系(图2B) (Li et al., 2015)。活化的RAC/ROP调节NADPH氧化酶产生ROS, 进而调控根生长(Duan et al., 2010, 2014; Li et al., 2015)。*llg1*与*fer-4*突变体根中的ROS水平显著降低(Swanson and Gilroy, 2010), 导致细胞壁丧失完整性、细胞质外渗及细胞塌陷(Li et al., 2015)。同时, 激活的FER^{KD}引起AHA2磷酸化并失活, 导致质外体pH值升高, 引起细胞壁硬化, 抑制根部细胞伸长和根毛生长(图2B) (Haruta et al., 2014; Li et al., 2015; Xiao et al., 2019)。反之, 低pH值会导致细胞因膨胀紊乱而爆裂(Monshausen et al., 2007)。由此表明, LLG1和FER复合体通过与胞外小肽RALF1互作, 动态调节质外体ROS与pH稳态, 从而调控根与根毛生长。

LLG1可能与FER共同参与脱落酸(ABA)、生长素、乙烯及油菜素内酯(BR)等激素信号转导过程, 从而调控根与根毛生长(Guo et al., 2009; Duan et al., 2010; Deslauriers and Larsen, 2010; Huang et al., 2013)。*llg1*与*fer-4*突变体对生长素和ABA有相似的响应。*fer*突变体根毛伸长对生长素不敏感(Duan et al., 2010; Li et al., 2015), 根伸长对ABA的抑制敏感(Yu et al., 2012; Li et al., 2015), 下胚轴伸长对乙烯和BR敏感(Deslauriers and Larsen, 2010)。当ABA存在时, FER通过与GEF1/4/10和GTP-ROP11互作, 激活A-type PP2C磷酸酶ABI2 (ABA insensitive 2)的活性, 抑制SnRK2活性, 从而抑制ABA信号通路, 同时抑制SLAC1通道和NADPH氧化酶介导的ROS产生, 负调控根生长(Yu et al., 2012)。

4 LLG1调控盐胁迫应答

盐离子干扰植物根部细胞壁结构, 导致细胞壁完整性降低, 抑制细胞极性生长, 引起细胞爆裂(Dinneny

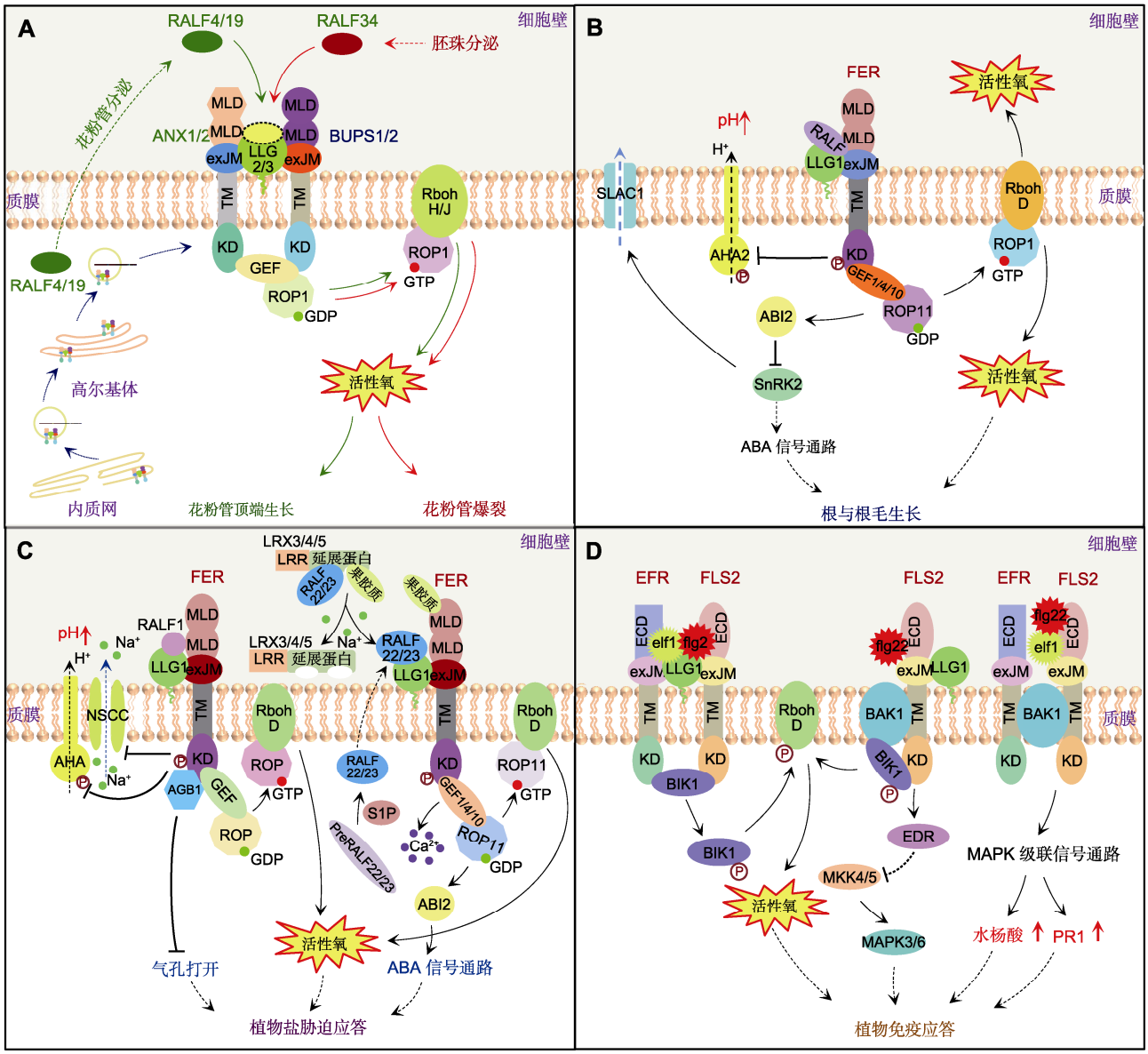

图2 LLG与RLK家族蛋白不同成员互作调控花粉与根发育以及盐胁迫与免疫应答过程

(A) LLG2/3与ANX1/2和BUPS1/2互作调控花粉管生长与爆裂; **(B)** LLG1与FER互作调控根与根毛生长; **(C)** LLG1与FER互作调控盐胁迫应答; **(D)** LLG1与FLS2和EFR互作调控免疫应答。ABA: 脱落酸; ABI2: A型 PP2Cs磷酸酶; AGB1: 异源三聚体G蛋白β亚基; AHA2: 质膜H^+-ATPase 2; ANX1/2: ANXUR1/2; BAK1: 油菜素受体激酶; BIK1: Botrytis诱导激酶1; BUPS1/2: 佛祖之金字亚贴1/2; ECD: 胞外结构域; EDR1: 负调控抗病蛋白1; EFR: 延伸因子Tu受体; elf18: 细菌延伸因子Tu N端18个氨基酸小肽; exJM: 胞外近膜结构域; FER: FERONIA; flg22: 细菌鞭毛蛋白N端22个氨基酸小肽保守基序; FLS2: 鞭毛蛋白传感蛋白2; GDP: 鸟苷二磷酸; GEF1/4/10: 鸟嘌呤核苷酸交换因子1/4/10; GTP: 鸟苷三磷酸; KD: 激酶结构域; LLG1/2/3: 类LORELEI糖基磷脂酰肌醇锚定蛋白1/2/3; LRR: 富含亮氨酸重复序列; LRX: 富含亮氨酸重复序列的延展蛋白; MAPK: 丝裂原活化蛋白激酶; MKK: 丝裂原激活的蛋白激酶激酶; MLD: 类Malectin结构域; NSCC: 非选择性阳离子通道; PR1: 病程相关因子1; RALF: 快速碱化因子; Rboh: 呼吸爆发氧化酶; ROP1/11: 植物Rho相关小G蛋白1/11; S1P: 位点-1蛋白酶; SLAC1: 慢阴离子通道蛋白1; SnRK2: 丝氨酸/苏氨酸蛋白激酶SnRK2D; TM: 跨膜结构域。实线表示直接调控过程, 虚线表示间接调控过程或物质转运。箭头表示促进, T表示抑制。

Figure 2 Interaction between different members of the LLG and RLK family proteins regulates pollen and root development and salt and immune response processes

(A) LLG2/3 interacts with ANX1/2 and BUPS1/2 to regulate pollen tube growth and burst; **(B)** LLG1 interacts with FER to regulate root and root hair growth; **(C)** LLG1 interacts with FER to regulate salt stress response; **(D)** LLG1 interacts with FLS2 and

EFR to regulate the immune response. ABA: Abscisic acid; ABI2: ABA insensitive 2; AGB1: Heterotrimeric G-protein β-subunit; AHA2: Plasma membrane H^+-ATPase 2; ANX1/2: ANXUR1/2; BAK1: Brassinosteroid insensitive 1-associated receptor kinase 1; BIK1: Botrytis-induced kinase 1; BUPS1/2: Buddha's paper seal1/2; ECD: Extracellular domain; EDR1: Enhanced disease resistance 1; EFR: EF-Tu receptor; elf18: 18 amino acid peptide of EF-Tu N-terminus; eXJM: Extracellular membrane domain; FER: FERONIA; flg22: 22 amino acid peptide of bacterial flagellin N-terminus; FLS2: Flagellin sensing 2; GDP: Guanosine diphosphate; GEF1/4/10: Guanine nucleotide exchange factor1/4/10; GTP: Guanosine triphosphate; KD: Kinase domain; LLG1/2/3: LORELEI-like GPI-anchored protein1/2/3; LRR: Leucine-rich repeat; LRX: Leucine-rich repeat extensin-like protein; MAPK: Mitogen-activated protein kinase; MKK: Mitogen-activated protein kinase kinase; MLD: Malectin-like domain; NSCC: Non-selective cation channels; PR1: Pathogenesis-related factor 1; RALF: Rapid alkalinization factor; Rboh: Respiratory burst oxidase homolog; ROP1/11: Rho-related GTPase1/11 from plants; S1P: Site-1 protease; SLAC1: Slow anion channel 1; SnRK2: Serine/threonine-protein kinase SnRK2D; TM: Transmembrane domain. The solid line represents the direct regulation process, the dotted line represents the indirect regulation process or material transport. The arrow represents promotion, and the T represents inhibition.

et al., 2008; Feng et al., 2018)。盐胁迫引起的细胞壁果胶质交联变化可以被FER胞外2个串联的MLD (malectin-like domain) A和B感知，引起胞内激酶活性变化，激发胞质Ca^{2+}浓度瞬变，启动盐应答信号通路(图2C) (Feng et al., 2018)。

LLG1作为FER的共受体参与盐应答过程。拟南芥*fer2*和*fer4*突变体根的生长存在缺陷，并且对Na^+敏感，但对甘露醇和山梨醇引起的渗透胁迫不敏感(Feng et al., 2018)。与之相似，*llg1*突变体的根也对Na^+敏感。用浓度高于100 mmol·L^{-1} NaCl处理可引起*llg1*和*fer4*突变体根部伸长区细胞丧失活力；而对于*sos1*和*sos2*突变体而言，50 mmol·L^{-1} NaCl即可导致其根部从伸长区至根尖大范围的细胞死亡。这表明LLG1和FER可能参与调控与SOS通路不同的盐胁迫应答途径(Feng et al., 2018)。

LLG1通过与RALFs和FER互作形成三元复合物，调控下游信号通路。三者的关键氨基酸位点可能影响其互作关系(Xiao et al., 2019)。LLG1通过其C端构象变化调控其与RALFs保守的N端结合，进而与FER^{ECD}互作(Xiao et al., 2019)。LLG1/2/3和LRE中12个保守氨基酸在其与RALF23互作中发挥关键作用(图1A)。与RALF23结合时，LLG1的129–138位氨基酸区域的构象发生变化。同时，LLG1/2/3的^{121}KEGKEGLD^{128}区域非常保守，其中G123R突变会减弱LLG1与RALF23互作(Xiao et al., 2019)。*llg1-3*突变体发生的G114R替换也降低了LLG1与RALF23的体外互作，减弱了RALF23对幼苗生长的抑制作用(Xiao et al., 2019)。但是，LLG1有些位点的突变(如N91A、T99R、A117Y和N118Y)并未影响其与RALF23和FER的互作(Xiao et al., 2019)。此外，RALF23关键位点突变(I6A、I6Y、L11Y和N14A)会影响其与LLG1的互作，进而消除其对elf18诱导的ROS产生与幼苗生长的抑制作用(Xiao et al., 2019)。FER氨基酸位点突变也会影响RALF23对其的调节作用。与野生型相比，在*fer-4*背景下回补表达点突变的FER (G257A)和FER (N303Y)，幼苗生长的抑制明显受到RALF23诱导(Xiao et al., 2019)。

在盐胁迫应答过程中，RALF22/23诱导FER通过胞吞途径内化，从而负调控FER的功能(图2C) (Zhao et al., 2018)。正常状态下，细胞壁富含亮氨酸重复序列延展蛋白(leucine-rich repeat extensin, LRX) 3/4/5 N端的富含亮氨酸重复序列(leucine-rich repeat, LRR)与配体RALF22/23结合，C端高度糖基化的延展蛋白与细胞壁成分(果胶质)交联；而在盐胁迫条件下，盐诱导的细胞壁交联变化被LRX3/4/5感知，释放出与其结合的RALF22/23。同时，盐胁迫也诱导依赖于SITE-1肽酶(site-1 protease, S1P)催化的成熟RALF-22的积累。盐诱导的RALF22和RALF23的增加，促进了其与FER的互作，导致FER内化(图2C)。拟南芥*llg1*突变体、*lrx3/4/5*三突变体、*fer4*突变体以及*RALF22*和*RALF23*过表达植株都表现出相似的生长迟滞和盐敏感表型(Zhao et al., 2018)。也有研究表明，FER^{ECD}的malectin基序可以与细胞壁中的果胶质相互作用，感知盐引起的细胞壁软化，触发FER介导的Ca^{2+}瞬变，调节细胞壁完整性，以防盐胁迫下根细胞在生长过程中发生爆裂(Feng et al., 2018)。

在叶片中，FER与G蛋白β亚基(heterotrimeric G-protein β, AGB1)相互作用，抑制气孔开放(图2C) (Yu et al., 2018)。*agb1*和*fer*均表现出盐敏感表型，而*agb1*的表型相对温和(Yu and Assmann, 2015; Yu et

al., 2018)。有研究表明, AGB1通路和FER通路协同作用, 参与盐胁迫应答, 且这种作用可能受到蒸腾条件的影响(Yu et al., 2018)。在蒸腾条件下, *agb1*突变体气孔导度较大, 而*fer*突变体大量气孔关闭, 这导致两者蒸腾拉力有较大差异; 两者Na^+从根向地上部转运的效率不同, *agb1*表现为全株盐敏感, 且Na^+在地上部大量积累, 但*fer*突变体无显著的Na^+积累(Yu et al., 2018)。在非蒸腾条件下, 植株细胞内Na^+含量主要取决于Na^+通过非选择性阳离子通道(non-selective cation channels, NSCC)内流和Na^+/H^+反转运子外排。*fer*突变体的AHA活性较高, 导致质外体酸化驱动的Na^+/H^+反转运子活跃, 促进Na^+外排, 相对增强耐盐性(Yang et al., 2010)。然而, *fer*的盐敏感表型可能是由于*fer*不能持续激活AHA (Yu et al., 2018)。*fer*突变体中大量Na^+积累可能主要由于根部表皮和皮层细胞完整性丧失所致(Feng et al., 2018)。

RALF1通过诱导FER磷酸化将其激活(Haruta et al., 2014; Chen et al., 2016), 进而通过磷酸化AHA的Ser^{899}, 抑制其质子转运能力(Haruta et al., 2014), 降低质外体酸化和Na^+外排, 阻止细胞延长和根生长, 且对盐敏感(图2C) (Yu et al., 2018)。此外, RALF1处理会引起根部FER依赖的胞质Ca^{2+}增加(Haruta et al., 2008, 2014)。外源施加1 μmol·L^{-1} RALF1可以抑制拟南芥野生型和*agb1*突变体根的生长, 但不影响*fer2*、*fer4*和*agb1-2*/*fer2*的表型, 这表明RALF1通过FER对根生长发挥抑制作用(Yu et al., 2018)。

盐诱导质膜定位的NADPH氧化酶RbohD产生ROS, 并受到AGB1和FER的协同调控(图2C) (Yu et al., 2018)。盐胁迫影响*agb1*和*fer*的ROS稳态, *agb1*/*fer*双突变体表现出比各自单突变体更严重的ROS失衡表型, 且这种ROS失衡在不同浓度和时间的盐处理以及不同器官(叶片和根)中存在差异(Yu et al., 2018)。

盐胁迫引起ABA含量升高进而激活FER, 调控下游通路; 同时, FER也通过GEF1/4/10-ROP11途径增强ABI2的活性, ABI2直接与FER互作将其去磷酸化而失活, 从而抑制ABA响应。虽然*fer*突变体对ABA和盐胁迫非常敏感, 但是FER调控并非全部依赖ABA, 其它信号通路也可能参与其中, 具体机制尚不清楚(Chen et al., 2016)。

对盐生牧草小花碱茅(*Puccinellia tenuiflora*)盐碱应答转录组的分析表明, FER及其相关基因*HERK1*、*NOTIA*和*MARIS*, 以及FLS2及其互作蛋白BAK1、EFR、BIK1、SCD1、BSK1和GRP7协同作用, 调节G蛋白介导的Ca^{2+}信号通路、ABA信号通路以及MAPK级联信号通路和ROS稳态, 从而调控盐碱应答过程(Zhang et al., 2020)。

5 LLG1调控免疫应答

在植物应答病原菌侵染过程中, 质膜模式识别受体(pattern recognition receptors, PRRs)参与激活免疫系统。多数植物PRRs是具有胞外配体识别结构域、跨膜结构域和胞质激酶结构域的类受体激酶, 如富含亮氨酸重复序列受体激酶FLS2 (flagellin sensing 2)和EFR (EF-Tu receptor)。LLG1作为分子伴侣, 与EFR和FLS2的exJM区互作调节其质膜定位, 进而调控植物免疫应答过程(Shen et al., 2017)。

当植物受到病原菌侵害时, FLS2和EFR迅速与其共受体BAK1 (brassinosteroid insensitive 1-associated receptor kinase 1)形成复合体, 激活下游MAPK级联信号通路, 调控防御基因的表达, 促进水杨酸(SA)等防御激素的积累, 提高植株的抗病性(Sun et al., 2013)。MAPK级联信号通路中的EDR1 (enhanced disease resistance 1)是一种Raf-like MAP3K, 通过调节MKK4/5-MPK3/6通路负调控植物免疫, 且拟南芥*edr1*突变体具有抗病性(Frye et al., 2001)。Shen等(2017)在*edr1*背景下将LLG1的Gly^{114}突变为Arg^{114}获得双突变体*edr1*/*llg1-3*, 并将T-DNA插入获得的*llg1-2*突变体与*edr1*杂交获得双突变体*edr1*/*llg1-2*。研究表明, *edr1*/*llg1-3*和*edr1*/*llg1-2*都对白粉病菌(*Golovinomyces cichoracearum* UCSC1)敏感。*llg1-3*通过抑制*edr1*突变体中免疫标记基因*PR1*的表达和SA的积累削弱*edr1*对白粉病的抗性。*edr1*/*llg1-3*对活体营养型卵菌(*Hyaloperonospora arabidopsidis* Noco2)和植物病原细菌(*Pseudomonas syringae* pv tomato (*Pto*) DC3000)等多种病原体敏感。*llg1-2*和*llg1-3*突变体对上述3种病原体都敏感, 且突变体体内SA和*PR1*表达水平都受到*G. cichoracearum*侵染的抑制。在*llg1-2*和*llg1-3*突变体中转入*LLG1*可以恢复其野生型表型, 表明LLG1参与对多种病原体的免疫应答过程(图2D) (Shen et al., 2017)。

LLG1不同位点突变对其与FER、FLS2和EFR互作的调控存在差异。*llg1-2*具有明显的生长缺陷表型(Li et al., 2015), 而*llg1-3* (LLG1^{G114R})中LLG1与FER的互作及质膜定位不受影响, 无生长发育缺陷表型, 这表明LLG1^{G114R}点突变仅失去免疫功能, 对植物生长发育无影响(Shen et al., 2017)。酵母双杂交和CO-IP实验证明LLG1与EDR1不能互作, 这暗示LLG1对免疫的调节可能与EDR1信号通路无关。LLG1与FLS2和EFR的互作不受flg22处理及LLG1^{G114R}点突变的影响, 并且在flg22诱导下可以与BAK1互作(图2D) (Shen et al., 2017)。与野生型相比, FLS2的积累、质膜定位以及flg22诱导的降解在*llg1-2*中均降低, 但在*llg1-3*中无变化。这表明LLG1具有的分子伴侣与信号转导功能可能是相互独立的, LLG1^{G114R}点突变并未影响其与FLS2互作, 但可能影响LLG1和FLS2的构象, 从而导致FLS2调控的下游信号通路改变(图2D) (Shen et al., 2017)。LLG1与FLS2和EFR形成的复合体调控胞内BIK1 (botrytis-induced kinase 1)磷酸化, 激活下游的RbohD产生ROS, 参与免疫应答反应(图2D) (Shi et al., 2013; Li et al., 2014)。*llg*突变体中flg22诱导的FLS2和BAK1互作未受影响, 但*llg1-2*和*llg1-3*原生质体中flg22诱导的BIK1磷酸化水平降低, *llg1-2*和*llg1-3*突变体中受flg22和elf18诱导的ROS水平低于野生型(Shen et al., 2017)。

6 结论与展望

LLG作为质膜定位的RLKs (如FER和FLS2)的分子伴侣, 负责将其从内质网运送到质膜, 继而在RALFs等配体和胞外信号的调节下, 动态调控RLKs的质膜定位与活性。拟南芥*llg1*和*fer*突变体的多样化发育与逆境应答表型, 暗示LLG与FER等RLKs之间的互作可能不是简单的开关, 而是存在精细的调节机制。对拟南芥LLG构象的解析及其与RALFs和FER互作位点的研究表明, LLG可能通过构象变化调节三者的互作关系, 从而精细调控胞外信号感知, 招募不同的RLKs完成胞内信号转导(Xiao et al., 2019)。然而, 在特定发育(如叶表皮细胞形状、花粉管生长与爆裂、根部伸长区与根毛细胞生长)与逆境(如病原体、盐碱、温度、低氮、低磷以及碳水化合物)应答过程中, LLG如何感受质外体信号(如ROS水平及不同配体)调整自身构象, 如何调节其与不同配体(如RALFs)结合, 如何招募不同的RLKs并激活其下游信号通路(Huang et al., 2013; Yang et al., 2015; Yeats et al., 2016; Yin et al., 2018; Xu et al., 2019), 以及LLG的4对保守的半胱氨酸位点在ROS信号感知和蛋白质构象调节方面是否具有功能(Duan et al., 2020; Yu et al., 2020)等, 这些科学问题都有待深入研究。

参考文献

Chen J, Yu F, Liu Y, Du CQ, Li XS, Zhu SR, Wang XC, Lan WZ, Rodriguez PL, Liu XM, Li DP, Chen LB, Luan S (2016). FERONIA interacts with ABI2-type phosphatases to facilitate signaling cross-talk between abscisic acid and RALF peptide in *Arabidopsis*. *Proc Natl Acad Sci USA* **113**, E5519–E5527.

Cheung AY, Li C, Zou YJ, Wu HM (2014). Glycosylphosphatidylinositol anchoring: control through modification. *Plant Physiol* **166**, 748–750.

Deslauriers SD, Larsen PB (2010). FERONIA is a key modulator of brassinosteroid and ethylene responsiveness in *Arabidopsis* hypocotyls. *Mol Plant* **3**, 626–640.

Dinneny JR, Long TA, Wang JY, Jung JW, Mace D, Pointer S, Barron C, Brady SM, Schiefelbein J, Benfey PN (2008). Cell identity mediates the response of *Arabidopsis* roots to abiotic stress. *Science* **320**, 942–945.

Duan QH, Kita D, Johnson EA, Aggarwal M, Gates L, Wu HM, Cheung AY (2014). Reactive oxygen species mediate pollen tube rupture to release sperm for fertilization in *Arabidopsis*. *Nat Commun* **5**, 3129.

Duan QH, Kita D, Li C, Cheung AY, Wu HM (2010). FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. *Proc Natl Acad Sci USA* **107**, 17821–17826.

Duan QH, Liu MCJ, Kita D, Jordan SS, Yeh FLJ, Yvon R, Carpenter H, Federico AN, Garcia-Valencia LE, Eyles SJ, Wang CS, Wu HM, Cheung AY (2020). FERONIA controls pectin- and nitric oxide-mediated male-female interaction. *Nature* **579**, 561–566.

Feng HQ, Liu C, Fu R, Zhang MM, Li H, Shen LP, Wei QQ, Sun X, Xu L, Ni B, Li C (2019). LORELEI-LIKE GPI-ANCHORED PROTEINS 2/3 regulate pollen tube growth as chaperones and coreceptors for ANXUR/BUPS receptor kinases in *Arabidopsis*. *Mol Plant* **12**, 1612–1623.

Feng W, Kita D, Peaucelle A, Cartwright HN, Doan V, Duan QH, Liu MC, Maman J, Steinhorst L, Schmitz-

Thom I, Yvon R, Kudla J, Wu HM, Cheung AY, Dinneny JR (2018). The FERONIA receptor kinase maintains cell-wall integrity during salt stress through Ca^{2+} signaling. *Curr Biol* **28**, 666–675.

Franck CM, Westermann J, Boisson-Dernier A (2018). Plant malectin-like receptor kinases: from cell wall integrity to immunity and beyond. *Annu Rev Plant Biol* **69**, 301–328.

Frye CA, Tang DZ, Innes RW (2001). Negative regulation of defense responses in plants by a conserved MAPKK kinase. *Proc Natl Acad Sci USA* **98**, 373–378.

Ge ZX, Bergonci T, Zhao YL, Zou YJ, Du S, Liu MC, Luo XJ, Ruan H, García-Valencia LE, Zhong S, Hou SY, Huang QP, Lai LH, Moura DS, Gu HY, Dong J, Wu HM, Dresselhaus T, Xiao JY, Cheung AY, Qu LJ (2017). *Arabidopsis* pollen tube integrity and sperm release are regulated by RALF-mediated signaling. *Science* **358**, 1596–1600.

Ge ZX, Cheung AY, Qu LJ (2019). Pollen tube integrity regulation in flowering plants: insights from molecular assemblies on the pollen tube surface. *New Phytol* **222**, 687–693.

Guo HQ, Li L, Ye HX, Yu XF, Algreen A, Yin YH (2009). Three related receptor-like kinases are required for optimal cell elongation in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* **106**, 7648–7653.

Haruta M, Monshausen G, Gilroy S, Sussman MR (2008). A cytoplasmic Ca^{2+} functional assay for identifying and purifying endogenous cell signaling peptides in *Arabidopsis* seedlings: identification of *AtRALF1* peptide. *Biochemistry* **47**, 6311–6321.

Haruta M, Sabat G, Stecker K, Minkoff BB, Sussman MR (2014). A peptide hormone and its receptor protein kinase regulate plant cell expansion. *Science* **343**, 408–411.

Hou YN, Guo XY, Cyprys P, Zhang Y, Bleckmann A, Cai L, Huang QP, Luo Y, Gu HY, Dresselhaus T, Dong J, Qu LJ (2016). Maternal ENODLs are required for pollen tube reception in *Arabidopsis*. *Curr Biol* **26**, 2343–2350.

Huang GQ, Li E, Ge FR, Li S, Wang Q, Zhang CQ, Zhang Y (2013). *Arabidopsis* RopGEF4 and RopGEF10 are important for FERONIA-mediated developmental but not environmental regulation of root hair growth. *New Phytol* **200**, 1089–1101.

Johnson MA, Harpe JF, Palanivelu R (2019). A fruitful journey: pollen tube navigation from germination to fertilization. *Annu Rev Plant Biol* **70**, 809–837.

José-Estanyol M, Gomis-Rüth FX, Puigdomènech P (2004). The eight-cysteine motif, a versatile structure in plant proteins. *Plant Physiol Biochem* **42**, 355–365.

Kaya H, Nakajima R, Iwano M, Kanaoka MM, Kimura S, Takeda S, Kawarazaki T, Senzaki E, Hamamura Y, Higashiyama T, Takayama S, Abe M, Kuchitsu K (2014). Ca^{2+}-activated reactive oxygen species production by *Arabidopsis* RbohH and RbohJ is essential for proper pollen tube tip growth. *Plant Cell* **26**, 1069–1080.

Keinath NF, Kierszniowska S, Lorek J, Bourdais G, Kessler SA, Shimosato-Asano H, Grossniklaus U, Schulze WX, Robatzek S, Panstruga R (2010). PAMP (pathogen-associated molecular pattern)-induced changes in plasma membrane compartmentalization reveal novel components of plant immunity. *J Biol Chem* **285**, 39140–39149.

Li C, Yeh FL, Cheung AY, Duan QH, Kita D, Liu MC, Maman J, Luu EJ, Wu BW, Gates L, Jalal M, Kwong A, Carpenter H, Wu HM (2015). Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in *Arabidopsis*. *eLife* **4**, e06587.

Li HJ, Yang WC (2016). RLKs orchestrate the signaling in plant male-female interaction. *Sci China Life Sci* **59**, 867–877.

Li HJ, Yang WC (2018). Ligands switch model for pollen-tube integrity and burst. *Trends Plant Sci* **23**, 369–372.

Li L, Li M, Yu LP, Zhou ZY, Liang XX, Liu ZX, Cai GH, Gao LY, Zhang XJ, Wang YC, Chen S, Zhou JM (2014). The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. *Cell Host Microbe* **15**, 329–338.

Liu LF, Shangguan KK, Zhang BC, Liu XL, Yan MX, Zhang LJ, Shi YY, Zhang M, Qian Q, Li JY, Zhou YH (2013). Brittle Culm1, a COBRA-like protein, functions in cellulose assembly through binding cellulose microfibrils. *PLoS Genet* **9**, e1003704.

Liu XL, Castro C, Wang YB, Noble J, Ponvert N, Bundy M, Hoel C, Shpak E, Palanivelu R (2016). The role of LORELEI in pollen tube reception at the interface of the synergid cell and pollen tube requires the modified eight-cysteine motif and the receptor-like kinase FERONIA. *Plant Cell* **28**, 1035–1052.

Mangano S, Juárez SPD, Estevez JM (2016). ROS regulation of polar growth in plant cells. *Plant Physiol* **171**, 1593–1605.

Mecchia MA, Santos-Fernandez G, Duss NN, Somoza SC, Boisson-Dernier A, Gagliardini V, Martínez-Bernardini A, Fabrice TN, Ringli C, Muschietti JP, Gros-

sniklaus U (2017). RALF4/19 peptides interact with LRX proteins to control pollen tube growth in *Arabidopsis*. *Science* **358**, 1600–1603.

Monshausen GB, Bibikova TN, Messerli MA, Shi C, Gilroy S (2007). Oscillations in extracellular pH and reactive oxygen species modulate tip growth of *Arabidopsis* root hairs. *Proc Natl Acad Sci USA* **104**, 20996–21001.

Shen QJ, Bourdais G, Pan HR, Robatzek S, Tang DZ (2017). *Arabidopsis* glycosylphosphatidylinositol-anchored protein LLG1 associates with and modulates FLS2 to regulate innate immunity. *Proc Natl Acad Sci USA* **114**, 5749–5754.

Shi H, Shen QJ, Qi YP, Yan HJ, Nie HZ, Chen YF, Zhao T, Katagiri F, Tang DZ (2013). BR-SIGNALING KINASE 1 physically associates with FLAGELLIN SENSING 2 and regulates plant innate immunity in *Arabidopsis*. *Plant Cell* **25**, 1143–1157.

Sun YD, Li L, Macho AP, Han ZF, Hu ZH, Zipfel C, Zhou JM, Chai JJ (2013). Structural basis for flg22-induced activation of the *Arabidopsis* FLS2-BAK1 immune complex. *Science* **342**, 624–628.

Swanson S, Gilroy S (2010). ROS in plant development. *Physiol Plant* **138**, 384–392.

Xiao Y, Stegmann M, Han ZF, DeFalco TA, Parys K, Xu L, Belkhadir Y, Zipfel C, Chai JJ (2019). Mechanisms of RALF peptide perception by a heterotypic receptor complex. *Nature* **572**, 270–274.

Xu GY, Chen WJ, Song LM, Chen QS, Zhang H, Liao HD, Zhao GQ, Lin FC, Zhou HN, Yu F (2019). FERONIA phosphorylates E3 ubiquitin ligase ATL6 to modulate the stability of 14-3-3 proteins in response to the carbon/nitrogen ratio. *J Exp Bot* **70**, 6375–6388.

Yang T, Wang L, Li CY, Liu Y, Zhu SR, Qi YY, Liu XM, Lin QL, Luan S, Yu F (2015). Receptor protein kinase FERONIA controls leaf starch accumulation by interacting with glyceraldehyde-3-phosphate dehydrogenase. *Biochem Biophys Res Commun* **465**, 77–82.

Yang YQ, Qin YX, Xie CG, Zhao FY, Zhao JF, Liu DF, Chen SY, Fuglsang AT, Palmgren MG, Schumaker KS, Deng XW, Guo Y (2010). The *Arabidopsis* chaperone J3 regulates the plasma membrane H^+-ATPase through interaction with the PKS5 kinase. *Plant Cell* **22**, 1313–1332.

Yeats TH, Sorek H, Wemmer DE, Somerville CR (2016). Cellulose deficiency is enhanced on hyper accumulation of sucrose by a H^+-coupled sucrose symporter. *Plant Physiol* **171**, 110–124.

Yin YL, Qin KZ, Song XW, Zhang QH, Zhou YH, Xia XJ, Yu JQ (2018). BZR1 transcription factor regulates heat stress tolerance through FERONIA receptor-like kinase-mediated reactive oxygen species signaling in tomato. *Plant Cell Physiol* **59**, 2239–2254.

Yu F, Qian LC, Nibau C, Duan QH, Kita D, Levasseur K, Li XQ, Lu CQ, Li H, Hou CC, Li LG, Buchanan BB, Chen LB, Cheung AY, Li DP, Luan S (2012). FERONIA receptor kinase pathway suppresses abscisic acid signaling in *Arabidopsis* by activating ABI2 phosphatase. *Proc Natl Acad Sci USA* **109**, 14693–14698.

Yu JJ, Li Y, Qin Z, Guo SY, Li YF, Miao YC, Song CP, Chen SX, Dai SJ (2020). Plant chloroplast stress response: insights from thiol redox proteomics. *Antioxid Redox Signal* **33**, 35–57.

Yu SC, Guo ZW, Johnson C, Gu GF, Wu QY (2013). Recent progress in synthetic and biological studies of GPI anchors and GPI-anchored proteins. *Curr Opin Chem Biol* **17**, 1006–1013.

Yu YQ, Assmann SM (2015). The heterotrimeric G-protein β subunit, AGB1, plays multiple roles in the *Arabidopsis* salinity response. *Plant Cell Environ* **38**, 2143–2156.

Yu YQ, Chakravorty D, Assmann SM (2018). The G protein β-subunit, AGB1, interacts with FERONIA in RALF1-regulated stomatal movement. *Plant Physiol* **176**, 2426–2440.

Zhang WT, Liu J, Zhang YX, Qiu J, Li Y, Zheng BJ, Hu FH, Dai SJ, Huang XH (2020). A high-quality genome sequence of alkaligrass provides insights into halophyte stress tolerance. *Sci China Life Sci* **63**, 1269–1282.

Zhao CZ, Zayed O, Yu ZP, Jiang W, Zhu PP, Hsu CC, Zhang LR, Tao WA, Lozano-Durán R, Zhu JK (2018). Leucine-rich repeat extensin proteins regulate plant salt tolerance in *Arabidopsis*. *Proc Natl Acad Sci USA* **115**, 13123–13128.

Zhong S, Qu LJ (2019). Peptide/receptor-like kinase-mediated signaling involved in male-female interactions. *Curr Opin Plant Biol* **51**, 7–14.

Zurzolo C, Simons K (2016). Glycosylphosphatidylinositol-anchored proteins: membrane organization and transport. *Biochim Biophys Acta* **1858**, 632–639.

Advances of LORELEI-like Glycosylphosphatidylinositol-anchor (LLG) Proteins in Plants

Sijia Li[1], Yongxue Zhang[1], Mingsheng Jia[2], Ying Li[1], Shaojun Dai[2*]

[1]Key Laboratory of Saline-alkali Vegetation Ecology Restoration, Ministry of Education, College of Life Sciences, Northeast Forestry University, Harbin 150040, China; [2]Shanghai Plant Germplasm Resources Engineering Technology Research Center, College of Life Sciences, Shanghai Normal University, Shanghai 200234, China

Abstract The outer surface of plasma membrane (PM)-localized LORELEI-like glycosylphosphatidylinositol-anchor (LLG) proteins, as the molecular chaperone of CrRLK1Ls family of receptor-like kinase, are involved in the transport of CrRLKs and extracellular signal transduction, regulating plant reproduction, development, as well as immune and stress responses. LLG2/3 interacting with ANX and BUPS regulates pollen tube growth and rupture. LLG1 interacted with FER activates the ROPGEF1-ROP2-NADPH oxidase pathway for ROS production, and then promotes root cell elongation and root hair growth. Besides, LLG1, as co-receptor of FER, interacts with RALFs, and then regulates G protein β (AGB1), PM H^+-ATPase activity, as well as the homeostasis of intracellular ROS and Ca^{2+}, for modulating stomata and roots in response to salinity. For immune response, LLG1 interacts with FLS2 and EFR, activating the downstream RbohD for ROS production. This review provides important information for understanding LLG biological functions.

Key words LLG, RALF, pollen tube, root, immune and salinity responses

Li SJ, Zhang YX, Jia MS, Li Y, Dai SJ (2020). Advances of LORELEI-like glycosylphosphatidylinositol-anchor (LLG) proteins in plants. *Chin Bull Bot* **55**, 541–550.

---

* Author for correspondence. E-mail: daishaojun@hotmail.com

(责任编辑：白羽红)