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ARF-GTPase activating protein mediates auxin influx carrier AUX1 early endosome trafficking to regulate auxin dependent plant development

Cheng Du[1,2] and Kang Chong[1,*]

[1]Key Laboratory of Photosynthesis and Molecular Environmental Physiology; Institute of Botany; Chinese Academy of Sciences; [2]College of Life sciences; Beijing Normal University; Beijing, China

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Polar auxin transport (PAT) plays a critical role in the regulation of plant growth and development. Auxin influx carrier AUX1 is predominantly localized to the upper side of specific root cells in Arabidopsis. Overexpression of OsAGAP, an ARF-GTPase activating protein in rice, could induce the accumulation of AUX1. But the mechanism is poorly known. Here we reported that overexpression of ARF-GAP could reduce the thickness and bundling of microfilament (MF) which possibly could greatly interfere with the endocytosis of AUX1 early endosome; but not the exocytosis of AUX1 recycling endosome. Therefore, AFR-GAP overexpression suppressed-MF bundling is likely involved in regulating endocytosis of Auxin influx carrier AUX1 and in mediating auxin dependent plant development.

Auxin is the first discovered hormone in plant. Auxin distribution is mediated through two distinct transport pathways: non-polar transport in the phloem and polar auxin transport in various tissues.[1] There are two kinds of polar auxin transport carriers: auxin efflux carriers [PIN-FORMED (PIN) family] and auxin influx carriers [AUXIN-RESISTANT 1/LIKE AUX1 (AUX1/LAX) family]. Plasma membrane (PM)-localized auxin carriers are delivered to and recycled from the PM via endosomes.[1] Fungal toxin brefeldin A (BFA) is well known to block trafficking from recycling endosomes to the PM and induce endosomes to accumulate into so-called BFA compartments.[2] In contrast with PIN1 completely accumulated in the root cells after BFA treatment, prolonged BFA treatments did not affect AUX1 localization at the PM, suggesting that AUX1 recycling endosomes are delivered to the PM in a BFA-independent manner.[3] PAT carrier dynamics rely on microfilament organization.[3,4] However, AUX1 is more dependent on the intact microfilament (MF) organization than PIN1.[3] Latrunculin B (LatB)-mediated MF disruption led AUX1 accumulating in the root cells; but did not alter PIN1 localization.[3] It is less known on its regulation mechanism. Here, our data suggest that ARF-GTPase-activating proteins (GAPs) could regulate AUX1 endosomes dynamics through affecting MF organization.

The ARF-GAPs are a family of proteins that could inactivate GTPase of small G protein ARF through inducing hydrolysis of GTP-bound ARF to GDP-bound ARF.[5] Overexpression of *OsAGAP* could reduce the thickness and bundling of MF in rice and Arabidopsis root cells as compared with wild type.[6] And microtubules were not affected by OsAGAP overexpression.[6] Consistently, AUX1 localization and trafficking rely on intact microfilaments instead of microtubules. And plant ARF-GAPs are similar to animal ARF-GAPs, which also regulate MF organization.[7]

Trans-Golgi network (TGN) was proposed to act as an early endosome in plant.[8] When the TGN marker, GONST1,[9,10] was transformed into rice and Arabidopsis protoplasts, GONST1-GFP accumulation was increased approximately 30% in protoplasts overexpressing OsAGAP compared with controls.[6] The monoclonal antibody JIM 84 can recognize a carbohydrate residue in a small family of glycoproteins, and it was widely used as plant Golgi maker, and sometimes it was used as TGN marker.[10-12] Golgi apparatuses, which were detected by monoclonal antibody JIM 84, accumulated slightly either in transgenic rice root cells or transgenic Arabidopsis (**Fig. 1**). Lipophilic dye FM4-64 is widely used as endocytic tracer in living cells and mainly stained early endosomes within 30 min in plant.[13] Endocytic trafficking of FM4–64-labeled early endosomes was promoted in the MF-disrupted root cells.[6] And AUX1-YFP could partially colocalize with FM4-64-labeled vesicles of its internalization.[6] This result suggested that the endocytosis of AUX1 early endosomes may also be stimulated by MF disruption.

Fluorescence recovery after photobleaching (FRAP) experiment was performed to examine any pattern change in exocytosis of AUX1 in the MF-disrupted cells. The recovery rate of fluorescence of AUX1-YFP in MF completely disrupted cells (20 μM)

*Correspondence to: Kang Chong; Email: chongk@ibcas.ac.cn
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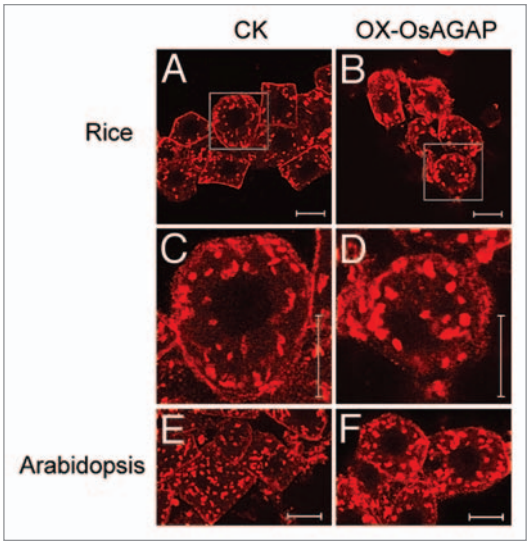

Figure 1. Golgi apparatuses and TGN in OsAGAP-overexpressed transgenic rice and Arabidopsis root cells. The monoclonal antibody JIM 84 was widely used as Golgi maker, and sometimes it was used as TGN marker in plant. Immunofluorescent assay shows that Golgi apparatuses and TGN, which were detected by anti-JIM 84 antibody (Undiluted), accumulated in transgenic rice (A–D) and Arabidopsis (E and F) root cells. (C and D) show the magnification in the boxes of (A and B). OX-OsAGAP, OsAGAP-overexpressed transgenic line; CK, wild type. Bars = 10 μm.

was only a little faster than in control. And lower concentration of LatB at 2 μM was not sufficient to promote the exocytosis.[6] Therefore, exocytosis of the AUX1 recycling endosome is not sensitive to MF disruption.

Based on these results, we propose that MF acts a barrier to vesicle motility. And AUX1 early endosome was a novel trafficking pathway distinct from the AUX1 recycling endosome (**Fig. 2**). MF could greatly interfere with the transport of AUX1 early endosomes. When MFs in root cells were disrupted by LatB treatment or OsAGAP overexpression, the endocytosis velocity of AUX1 early endosomes is much faster than that of AUX1 recycling endosomes. Thus, we can see the AUX1 accumulation in MF disrupted cells. Also, this model could explain why actin stabilization by the auxin transport inhibitor TIBA impairs vesicle motility in and out of cells.[14] When MFs are thicker and more bundled after TIBA treatment, the created barrier may be high enough to impair AUX1 trafficking. Considering PIN1 localization was not sensitive to MF disruption and the exocytosis of RLK-GFP was dramatically promoted by RIC3-mediated actin depolymerization,[15] we suggest that sensitivities to the MF organization of different organelles are different. ARF-GAP could mediate AUX1 endosome trafficking in an actin-dependent manner to regulate auxin mediated plant development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Michniewicz M, Brewer P, Friml J. Polar Auxin Transport and Asymmetric Auxin Distribution. The Arabidopsis Book 2007; DOI:10.1199/tab.0108.
2. Geldner N, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, et al. The arabidopsis gnom arf-gef mediates endosomal recycling, auxin transport and auxin-dependent plant growth. Cell 2003; 112:219-30; PMID:12553910; DOI:10.1016/S0092-8674(03)00003-5.
3. Kleine-Vehn J, Dhonukshe P, Swarup R, Bennett M, Friml J. Subcellular trafficking of the arabidopsis auxin influx carrier aux1 uses a novel pathway distinct from pin1. Plant Cell 2006; 18:3171-81; PMID:17114355; DOI:10.1105/tpc.106.042770.
4. Geldner N, Friml J, Stierhof YD, Jurgens G, Palme K. Auxin transport inhibitors block pin1 cycling and vesicle trafficking. Nature 2001; 413:425-8; PMID:11574889; DOI:10.1038/35096571.
5. Donaldson JG, Jackson CL. Regulators and effectors of the Arf GTPases. Curr Opin Cell Biol 2000; 12:475-82; PMID:10873831; DOI:10.1016/S0955-0674(00)00119-8.
6. Du C, Xu Y, Wang Y, Chong K. ARF-GAP stimulates the transport of AUX1 from plasma membrane to trans-Golgi network which relies on actin cytoskeletal organization in rice root development. J Integr Plant Biol 2011; 53:698-709; PMID:21631728.
7. Randazzo PA, Hirsch DS. Arf gaps: Multifunctional proteins that regulate membrane traffic and actin remodelling. Cell Signal 2004; 16:401-13; PMID:14709330; DOI:10.1016/j.cellsig.2003.09.012.
8. Viotti C, Bubeck J, Stierhof YD, Krebs M, Langhans M, Berg W, et al. Endocytic and Secretory Traffic in Arabidopsis Merge in the Trans-Golgi Network/Early Endosome, an Independent and Highly Dynamic Organelle. Plant Cell 2010; 22:1344-57; PMID:20435907; DOI:10.1105/tpc.109.072637.
9. Baldwin TC, Handford MG, Yuseff MI, Orellana A, Dupree P. Identification and characterization of Gonst1, a golgi-localized gdp-mannose transporter in arabidopsis. Plant Cell 2001; 13:2283-95; PMID:11595802.
10. Tse YC, Mo B, Hillmer S, Zhao M, Lo SW, Robinson DG, et al. Identification of multivesicular bodies as prevacuolar compartments in nicotiana tabacum by-2 cells. Plant Cell 2004; 16:672-93; PMID:14973159; DOI:10.1105/tpc.019703.
11. Satiat-Jeunemaitre B, Hawes C. Redistribution of a Golgi glycoprotein in plant cells treated with Brefeldin A. J Cell Sci 1992; 103:1153-66.
12. Horsley D, Coleman J, Evans D, Crooks K, Peart J, Satiat-Jeunemairre B, et al. A monoclonal antibody, JIM 84, recognizes the Golgi apparatus and plasma membrane in plant cells. J Exp Bot 1993; 44:223-9.
13. Bolte S, Talbot C, Boutte Y, Catrice O, Read ND, Satiat-Jeunemaitre B. Fm-dyes as experimental probes for dissecting vesicle trafficking in living plant cells. J Microsc 2004; 214:159-73; PMID:15102063; DOI:10.1111/j.0022-2720.2004.01348.x.
14. Dhonukshe P, Grigoriev I, Fischer R, Tominaga M, Robinson DG, Hasek J, et al. Auxin transport inhibitors impair vesicle motility and actin cytoskeleton dynamics in diverse eukaryotes. Proc Natl Acad Sci USA 2008; 105:4489-94; PMID:18337510; DOI:10.1073/pnas.0711414105.
15. Lee Y, Szumlanski A, Nielsen E, Yang Z. Rho-gtpase-dependent filamentous actin dynamics coordinate vesicle targeting and exocytosis during tip growth. J Cell Biol 2008; 181:1155-68; PMID:18591430; DOI:10.1083/jcb.200801086.

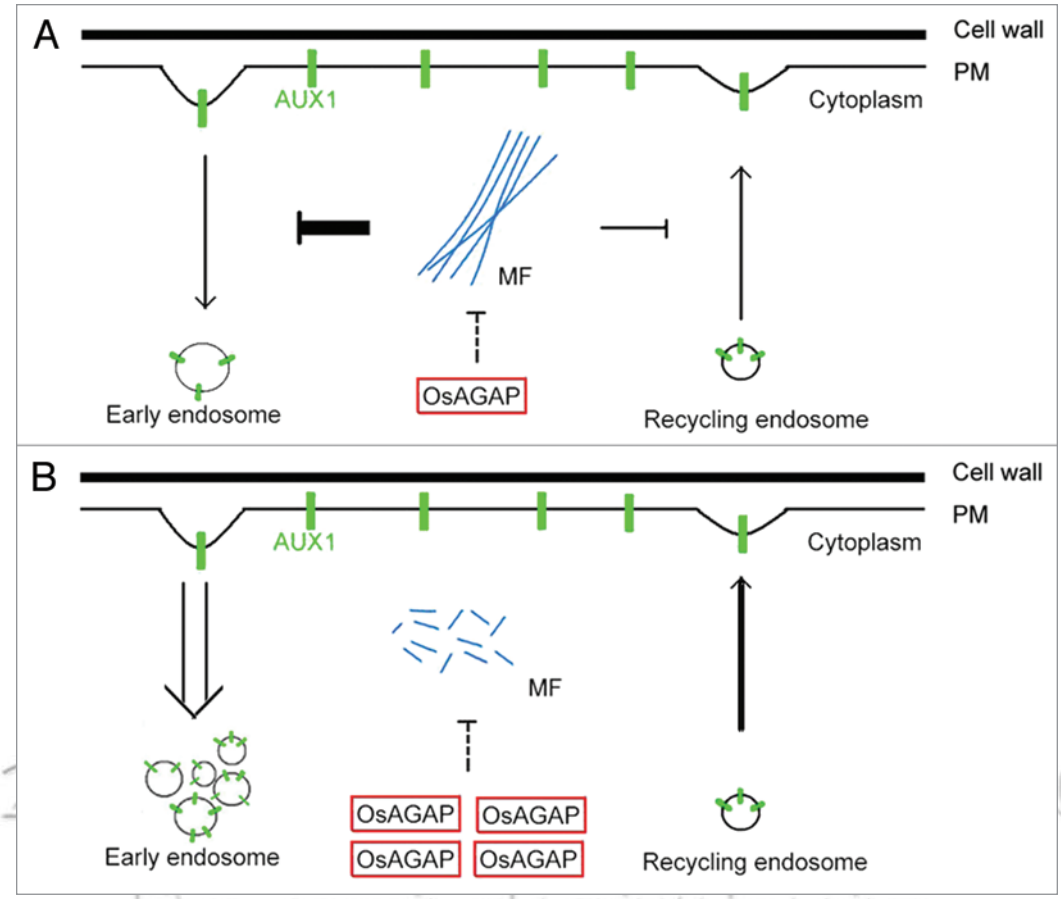

Figure 2. Working models for ARF GTPase-GAP mediated AUX1 endocytosis. Under normal condition (A) microfilaments (MFs) interfere with the endocytosis of the auxin influx carrier AUX1 into early endosome, but exocytosis of the AUX1 via the recycling endosome is only slightly inhibited. OsAGAP regulates the organization of MF through reducing the thickness and bundling. When the MFs were disrupted by OsAGAP overexpression or by actin depolymerizer LatB (B), delivery of AUX1 early endosome from the PM to cytoplasm was greatly promoted; but AUX1 recycling endosome trafficking was only slightly affected. These combined actions result in induced aggregation of AUX1 in root cells.