Plant Biotechnology 32, 103–108 (2015)
DOI: 10.5511/plantbiotechnology.15.0106a

Note

Arabidopsis LAZY1 is a peripheral membrane protein of which the carboxy-terminal fragment potentially interacts with microtubules

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Received October 28, 2014; accepted January 6, 2015 (Edited by K. Kato)

Abstract LAZY1 is a protein involved in gravity signaling of shoot gravitropism of rice, maize and Arabidopsis. Although the *lazy1* mutants have been well-characterized, the function of the LAZY1 protein is still largely unknown. In this study, we used fluorescence microscopy to examine the subcellular localization of Arabidopsis LAZY1 (AtLAZY1) and its truncated proteins fused to GFP in tobacco leaves. We found that AtLAZY1 localizes to the plasma membrane through the C-terminal region, suggesting that the putative trans-membrane domain in the N-terminal half is not required for localization. Next, we took a biochemical approach to investigate the membrane association of AtLAZY1. Transiently expressed AtLAZY1 in transgenic Arabidopsis was fractionated in an insoluble fraction that contained membranous compartments. AtLAZY1 was solubilized by a non-ionic detergent or at a high pH condition, suggesting that AtLAZY1 is a peripheral membrane protein. We also found that when expressed in tobacco the C-terminal part of AtLAZY1 co-localized with microtubules. A microtubule binding assay showed that the C-terminal half of AtLAZY1, which localized to the plasma membrane, interacted with microtubules in vitro. These results suggest that AtLAZY1 may function with microtubules at the periphery of the plasma membrane in the gravity signaling process.

Key words: Shoot gravitropism, microtubule, plasma membrane.

Gravitropism is one of many fundamental responses of plants to external stimuli. Shoots generally grow upwards to get more light energy for photosynthesis, while roots grow downwards to obtain water and nutrients (Morita 2010). In agriculture, shoot gravitropism of trees and crops is important for managing plantations (Dardick et al. 2013) and root gravitropism is important for overcoming drought stress in the field (Uga et al. 2013). Hence a better understanding and modification of gravitropism are likely to improve yields of crops and other agricultural products.

Although shoots and roots grow in opposite directions, their gravitropic responses both require amyloplasts (Morita 2010). Amyloplasts sediment to the lower side of gravity sensing cells (statocytes): endodermal cells and columella cells of shoots and roots, respectively. The downward movement of amyloplasts is thought to be transduced to physiological signals, which ultimately causes an asymmetric growth (Baldwin et al. 2013). However, the molecules and mechanisms of this signal transduction process remain to be elucidated. The LAZY1 protein has been initially identified as a key regulator in gravity perception of rice shoots (Li et al. 2007; Yoshihara and Iino 2007). These proteins are conserved in a range of higher plant species (Dardick et al. 2013). *lazy1* mutants of rice (Li et al. 2007; Yoshihara and Iino 2007), maize (Dong et al. 2013) and Arabidopsis (Yoshihara et al. 2013) show impaired shoot gravitropic responses, indicating that LAZY1 is involved in a conserved mechanism of gravitropism. *lazy1* mutants show enhanced polar auxin transport and are defective in lateral auxin redistribution in coleoptiles (Dong et al. 2013; Li et al. 2007; Yoshihara and Iino 2007). In the rice *lazy1* mutant amyloplast sedimentation is normal (Abe et al. 1994), suggesting that the LAZY1 protein plays an essential role somewhere in the subsequent steps of gravitropic signaling. However, the molecular activity of LAZY1 protein remains unknown, in part because its amino acid sequence reveals no known functional domains. As an alternative, knowledge of subcellular protein localization can provide crucial information as to its function. So far, Yoshihara et al. (2013) have shown that when expressed in leaf epidermal cells of *Nicotiana benthamiana*, Arabidopsis LAZY1 (AtLAZY1) fused to GFP localizes to the nucleus and the plasma membrane and when expressed in Arabidopsis hypocotyls it

This article can be found at http://www.jspcmb.jp/
Published online February 11, 2015

localizes to the nucleus and cell periphery. They further show that the nuclear localization of AtLAZY1 is not essential for its function. We have also examined the subcellular localization of AtLAZY1-GFP, in *N. benthamiana* cells, but our results differed from those of Yoshihara et al. (2013). In the study reported here we used truncated versions of AtLAZY1 to investigate more closely the subcellular localization of AtLAZY1, and have found that the protein has a capacity to bind with microtubules (MTs).

We first examined the subcellular localization of AtLAZY1-GFP transiently expressed in leaf epidermal cells of *N. benthamiana*, using fluorescent microscopy. In our experiments AtLAZY1-GFP did not localize to the nucleus, but only to plasma membrane (Figure 1B). This localization pattern was verified by plasmolysis assays (Supplementary Figure S1). These results differ from those reported by Yoshihara et al. (2013). The difference may be due to particular constructs used by the two groups. We fused AtLAZY1 to the N-terminal of GFP, whereas Yoshihara et al. inserted GFP between amino acid residues 308 and 309 of AtLAZY1. The GFP fusion of Yoshihara et al. functionally complemented the *atlazy1-1* mutant (Yoshihara et al. 2013), while our GFP fusion was not expressed in Arabidopsis, and thus its functionality remains to be examined. Although we did not observe nuclear localization of AtLAZY1-GFP, our protein was indeed localized to the plasma membrane.

To examine which sequence was responsible for this subcellular localization, we divided the amino acid sequence of AtLAZY1 into N- and C-terminal halves (Figure 1A) and each sequence was fused to the N-terminus of GFP using gateway binary vectors (Nakagawa et al. 2007). We found that the N-terminal half of AtLAZY1 diffused throughout the cell including cytoplasmic strands and nucleus. On the other hand, the C-terminal half was restricted to the plasma membrane (Figure 1B, Supplementary Figure S1). This result was surprising because rice LAZY1 has a putative transmembrane domain (TMD) in the N-terminal half of the protein, which has been reported to be essential for plasma membrane localization (Li et al. 2007), and a homologous sequence of the domain is also found in the N-terminal half of AtLAZY1 (Figure 1A). In contrast, our result suggests that the C-terminal half of AtLAZY1 is required for plasma membrane localization. Next, we further divided the C-terminal half of AtLAZY1 into three parts, based on GlobPlot analysis (http://globplot.embl.de). Two globular regions were predicted in the C-terminal half of AtLAZY1, which we named C1 and C3, and an intermediate disordered sequence between them, named C2 (Figure 1A). C2C3 protein localized to the plasma membrane, and small unknown particles or vesicles were also observed in the C2C3 sample (Figure 1B). By contrast, C1C2 showed more diffused localization than C2C3.

We then examined the subcellular localization of C3. C3 did not appear to be localized to the plasma membrane (Figure 2A, Supplementary Figure S2), suggesting that both C2 and C3 were necessary for plasma membrane localization. Instead of localizing to the plasma membrane, C3 showed a fibrous distribution pattern in the cell (Figure 2A), suggesting that it might bind to MTs. To investigate whether the fibrous structure was caused by association with MTs, we co-expressed C3-GFP with MICROTUBULE ASSOCIATED PROTEIN (MAP) 65-1 (Lucas et al. 2011) fused to mRFP. These proteins colocalized well, suggesting that C3-GFP associated with MTs (Figure 2A). Treatment with a MT destabilizing drug, oryzalin, abolished fibrous localization pattern of C3, confirming that the localization pattern depends on MTs (Supplementary Figure S3). We also found that C3 without C-terminal 48 residues did not show the fibrous localization pattern (Supplementary Figure S4), indicating that C-terminal 48 amino acids, containing conserved region V, are essential for the fibrous localization pattern.

To confirm the interaction between AtLAZY1 and MTs, we performed an in vitro MT co-sedimentation assay (Shoji et al. 2004). Unfortunately, when separated on a SDS-PAGE gel, full-length AtLAZY1 was indistinguishable from tubulins due to similar electrophoretic mobility, and thus the amount of AtLAZY1 was difficult to measure, even when western blotting was used for more specific detection. Therefore we examined whether or not the C-terminal half, which localized to the plasma membrane in vivo (Figure 1B), bound to MTs. The purified C-terminal half of AtLAZY1 was mixed with taxol-stabilized MTs at 25°C. In the absence of MTs, the C-terminal half of AtLAZY1 partly self-aggregated, but 23% of the protein remained in the supernatant fraction of a sample after centrifugation (Figure 2B, C). When MTs were added, however, all of the C-terminal half was fractionated in the pellet (Figure 2B, C), suggesting a significant interaction between the C-terminal half of AtLAZY1 and MTs. We also conducted this co-sedimentation assay between C3 and MTs, and found no interaction between them (data not shown). Although speculative, these results suggest that C1C2 enhances both plasma membrane localization and interaction with MTs, and that plasma membrane localization is dominantly observed in the pavement cells.

We have shown that AtLAZY1 can be localized to the plasma membrane without the putative TMD in the N-terminal half. This suggests that AtLAZY1 is likely to be a peripheral membrane protein that localizes to the membrane through protein–protein interaction or through membrane anchoring. To further investigate this localization, we generated a transgenic Arabidopsis

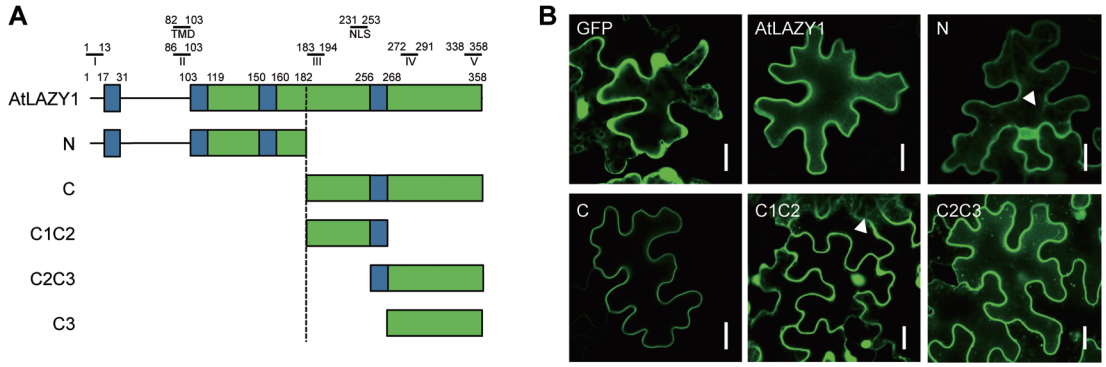


Figure 1. Subcellular localization of AtLAZY1 and its fragments. A: Schematic diagram of AtLAZY1 and its fragments used in this study. In the structural model shown at the top, potential globular and disordered domains as predicted with GlobPlot are displayed in green and blue boxes, respectively. Five short regions, I to V, which are conserved in rice and Arabidopsis LAZY1 families are also shown, as well as a putative nuclear localization signal (NLS) of AtLAZY1 (Yoshihara et al. 2013) and a transmembrane domain (TMD) predicted in rice LAZY1 (Li et al. 2007). B: Fluorescent images of the pavement cells of *N. benthamiana* that transiently expressed AtLAZY1 or its truncated proteins fused to GFP by 35S promoter by using the agro-infiltration method (Kapila et al. 1997). Arrowheads indicate cytoplasmic strand. Bar=20 μm.

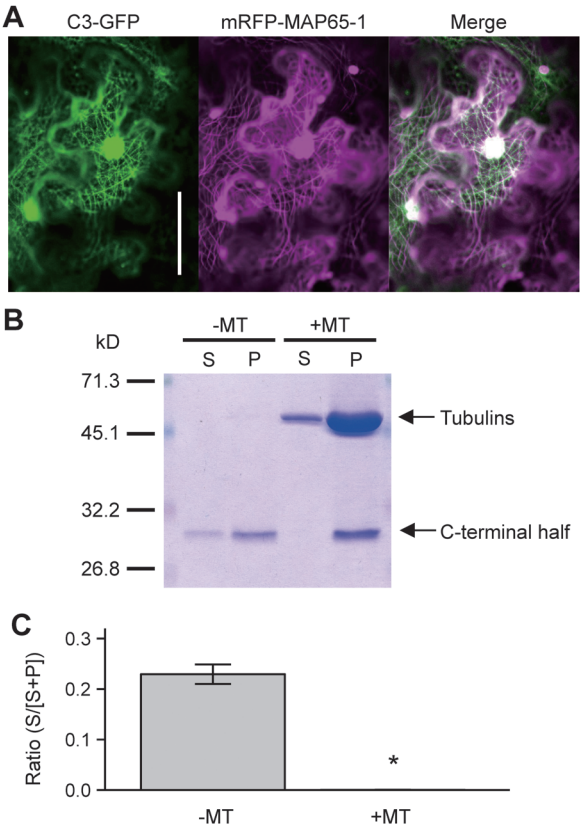


Figure 2. The C-terminal fragments of AtLAZY1 binds to microtubules (MTs). A: Fluorescent images of the pavement cells of *N. benthamiana* co-expressing C3-GFP and mRFP-MAP65-1. The focal plane was set on surface of the cells. White color in the merged image indicates colocalization. Bar=50 μm. B: Cosedimentation of taxol-stabilized MTs and the C-terminal half of AtLAZY1. The porcine tubulin (HTS format, Cytoskeleton) was polymerized in PME buffer (100 mM PIPES, pH 6.9, 0.5 mM $MgSO_4$, 1 mM EGTA, 20 μM taxol) with 1 mM GTP at 37°C for 60 min, and then polymerized MTs were washed once with PME buffer. The C-terminal half of AtLAZY1 (0.03 mg ml^{-1}), expressed and purified from *Escherichia coli*, was incubated in PME buffer with (+MT) or without (−MT) 0.4 mg ml^{-1} polymerized MTs at 25°C for 3 min and then centrifuged at 29,000 *g* for 10 min. The supernatants (S) and pellets (P) were separated on an SDS-PAGE gel and stained with Coomassie blue. C: Quantitative examination of association of the C-terminal half with MTs. Intensities of the band of the C-terminal half in MT cosedimentation assays shown in B were quantitated and the ratios of the band intensities in S to those in S plus P are shown. The data represent the mean±SD of three independent samples. Asterisk represents statistical significance between samples with or without MT ($p<0.005$, Student's *t*-test).

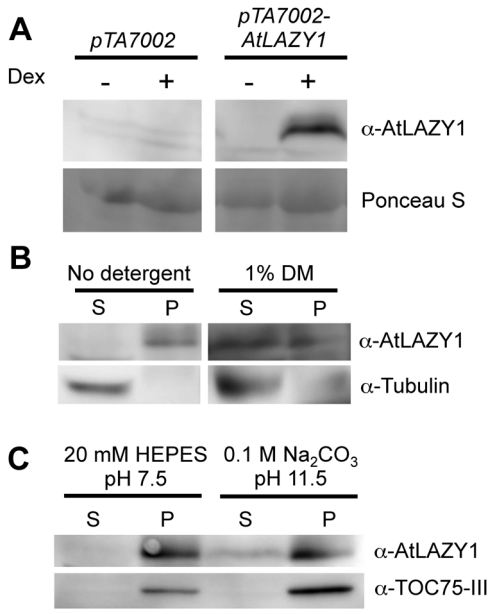


Figure 3. Immunoblot analyses of AtLAZY1 in protein extracts of transgenic *atlazy1-2* carrying the pTA7002-AtLAZY1 construct. A: Immunoblot analysis of AtLAZY1 in total protein extracts from 4-day-old seedlings treated with 10 μM dex for 160 min. *pTA7002* represents an empty vector. Total proteins were subjected to immunoblot analyses after SDS-PAGE. Polyclonal anti-AtLAZY1 antibody was prepared against AtLAZY1 protein expressed and purified from *E. coli*. B: Effects of 1% *n*-dodecyl-β-D-maltoside (DM) on fractionation of AtLAZY1 and α-tubulin in cell extracts. Five 7-day-old seedlings of the transgenic plants were homogenized in 50 μl of 20 mM HEPES (pH 7.5) plus 150 mM NaCl with or without 1% DM. After incubation for 5 min on ice, the homogenates were divided into the supernatant (S) and the pellet (P) by centrifugation at 25,000 *g* for 10 min. Tubulin was detected using monoclonal anti-α-tubulin antibody (DM1A, Sigma-Aldrich). C: Effects of 0.1 M sodium bicarbonate (pH 11.5) on fractionation of AtLAZY1 and TOC75-III in cell extracts. S and P were derived after extraction with 20 mM HEPES (pH 7.5) or 0.1 M sodium bicarbonate (pH 11.5). Anti-TOC75 antibody was obtained from Agrisera.

harboring dexamethasone (dex)-inducible AtLAZY1 with no tags (Aoyama and Chua 1997), and examined its solubility in the presence of non-ionic detergent or under high pH, using an anti-AtLAZY1 antibody. After dex induction for ca. 3 h, AtLAZY1 was detected in the crude protein extracts (Figure 3A). We then fractionated the protein extract by centrifugation to give supernatant (S) and pellet (P) fractions. Consistent with the membrane localization of AtLAZY1 in Figure 1B, AtLAZY1 was fractionated to the pellet, and was solubilized to the supernatant when the tissues were extracted in the presence of a non-ionic detergent, *n*-dodecyl-β-D-maltoside (DM) (Figure 3B). However, AtLAZY1 association with the membrane was sensitive to alkaline carbonate buffer (pH 11.5), and a significant amount of AtLAZY1 was observed in the supernatant after extraction with the alkaline buffer (Figure 3C). In contrast, in both neutral and high pH buffers a β-barrel transmembrane channel TRANSLOCON AT THE OUTER ENVELOPE MEMBRANE OF CHLOROPLASTS (TOC) 75-III (Baldwin et al. 2005) was found only in the pellet, confirming that transmembrane proteins can not be solubilized in alkaline buffer. These results suggest that AtLAZY1 is not a transmembrane protein but is a peripheral membrane protein.

According to these results, AtLAZY1 is likely to be localized to the plasma membrane through interaction with other membrane proteins. This unknown interaction of AtLAZY1 may be an important factor in gravity perception. Maize LAZY1 (ZmLAZY1) interacts with an IAA17 transcriptional repressor protein and a protein kinase catalytic domain (PKC) (Dong et al. 2013), although the functional significance of the interactions remains elusive. ZmLAZY1 and PKC are reported to interact on the plasma membrane, and that interaction does not require the N-terminal 100 amino acids of ZmLAZY1 (Dong et al. 2013). Consistently, we showed that the C-terminal half of AtLAZY1 contributed to the plasma membrane localization. Thus AtLAZY1 possibly localizes to the plasma membrane through interaction with PKC. However, it should be noted that ZmLAZY1 localizes to the plasma membrane via TMD in the N-terminal half. This difference might reflect the species-specific function of the *LAZY1* genes. In fact, ZmLAZY1 is involved in tassel development as well as gravitropism (Dong et al. 2013) while AtLAZY1 does not seem to be (Yoshihara et al. 2013). In contrast, LAZY1 is involved in shoot gravitropism in all the plant species so far examined (Dardick et al. 2013).

The C3 region, which bound to MTs in planta, contains two highly conserved sequences (IV and V in Figure 1A). Unfortunately, the C-terminal half and C3 did not successfully express in Arabidopsis as well as the full-length AtLAZY1, and thus functionality of the fragments remains to be examined. However, these two sequences may be important for the MT binding of AtLAZY1. We showed that C-terminal 48 amino acids were likely to be essential for colocalization with MTs in planta (Supplementary Figure S4). *LAZY1* belongs to the *IGT* gene family that contains *TILLER ANGLE CONTROL 1* (*TAC1*) as well as *LAZY1* genes. While LAZY1 acts positively in gravitropism, TAC1 affects gravitropism negatively, and lacks the ca. 40 amino acid-long C-terminal region (including region V) that is more or less conserved in LAZY1 subfamily (Dardick et al. 2013). How these structural differences contribute to their contrasting function is poorly understood. However, although speculative, it is possible that the lack of the V sequence causes loss of the MT binding, and thus TAC1 may be able to competitively inhibit the LAZY1 function.

Why the full-length AtLAZY1 did not co-localize with MTs is an interesting question. One possibility is that the N-terminal half, or the C1 and C2 regions, negatively

regulate its binding affinity for MTs. In the case of a Rho GTPase, ROP11, its plasma membrane localization and co-localization with cortical MTs are observed only when MICROTUBULE DEPLETION DOMAIN 1 (MIDD1) is co-expressed with it (Oda and Fukuda 2012). Similarly MT binding of AtLAZY1 might be derepressed when a specific interactor is co-expressed with it. AtLAZY1 expresses in endodermal cells of shoots in a natural context (Yoshihara et al. 2013), thus its subcellular localization will have to be examined in the endodermal cells. However, we have not succeeded in observation of endogenous AtLAZY1, and further challenge is required for this important problem.

In conclusion, we have demonstrated that AtLAZY1 is a peripheral membrane protein which has the potential to associate with MTs; the biological significance of this association though remains unknown. Our results and previous studies strongly indicate that interaction between plasma membrane and cytoskeleton is part of the signaling pathway in gravitropic responses. ALTERED RESPONSE TO GRAVITY 1 (ARG1) is a peripheral membrane protein involved in gravity perception of root, hypocotyl and stem (Boonsirichai et al. 2003; Sedbrook et al. 1999; Kumar et al. 2008), and has been shown to associate with actins (Harrison and Masson 2008). ARG1 can also be solubilized by alkaline buffer and detergents (Boonsirichai et al. 2003) similarly to AtLAZY1. Hence, AtLAZY1 appears to share some characteristics with ARG1. The 'tethered model' for the molecular mechanism of gravity perception hypothesizes that amyloplast sedimentation disrupts the organization of cytoskeletons anchored to membranes. This disruption may be transmitted to the membrane, thus activating mechanosensitive channels (Volkmann and Baluska 1999). It is possible that AtLAZY1 and ARG1 are involved in this hypothetical mechanism. Moreover, MTs have been supposed to influence tropism by positioning of regulatory proteins on the plasma membrane as well as cell expansion during organ bending (Bisgrove 2008). The relationship between AtLAZY1 function and MT organization should be addressed further to elucidate the molecular mechanism of gravity signaling.

Acknowledgements

We are grateful to Dr. Tsuyoshi Nakagawa (Shimane University) for GATEWAY binary vector pGWBs. This work was supported in part by a Grant-in-Aid from Ministry of Education, Culture, Sports, Science and Technology, Japan to K.T.Y. (19060008).

References

Abe K, Takahashi H, Suge H (1994) Localization of cells containing sedimented amyloplasts in the shoots of normal and lazy rice seedlings. *Biol Sci Space* 8: 221–225

Aoyama T, Chua NH (1997) A glucocorticoid-mediated transcriptional induction system in transgenic plants. *Plant J* 11: 605–612

Baldwin A, Wardle A, Patel R, Dubley P, Park SK, Twell D, Inoue K, Jarvis P (2005) A molecular-genetic study of the Toc75 gene family. *Plant Physiol* 138: 715–733

Baldwin KL, Strohm AK, Masson PH (2013) Gravity sensing and signal transduction in vascular plant primary roots. *Am J Bot* 100: 126–142

Bisgrove SR (2008) The roles of microtubules in tropisms. *Plant Sci* 175: 747–755

Boonsirichai K, Sedbrook JC, Chen R, Gilroy S, Masson PH (2003) ALTERED RESPONSE TO GRAVITY is a peripheral membrane protein that modulates gravity-induced cytoplasmic alkalinization and lateral auxin transport in plant statocytes. *Plant Cell* 15: 2612–2625

Dardick C, Callahan A, Horn R, Ruiz KB, Zhebentyayeva T, Hollender C, Whitaker M, Abbott A, Scorza R (2013) PpeTAC1 promotes the horizontal growth of branches in peach trees and is a member of a functionally conserved gene family found in diverse plants species. *Plant J* 75: 618–630

Dong Z, Jiang C, Chen X, Zhang T, Ding L, Song W, Luo H, Lai J, Chen H, Liu R, et al. (2013) Maize LAZY1 mediates shoot gravitropism and inflorescence development through regulating auxin transport, auxin signaling, and light response. *Plant Physiol* 163: 1306–1322

Harrison B, Masson PH (2008) ARG1 and ARL2 form an actin-based gravity-signaling chaperone complex in root statocytes? *Plant Signal Behav* 3: 650–653

Kapila J, DeRycke R, VanMontagu M, Angenon G (1997) An *Agrobacterium*-mediated transient gene expression system for intact leaves. *Plant Sci* 122: 101–108

Kumar NS, Stevens MH, Kiss JZ (2008) Plastid movement in statocytes of the *arg1* (*altered response to gravity*) mutant. *Am J Bot* 95: 177–184

Li P, Wang Y, Qian Q, Fu Z, Wang M, Zeng D, Li B, Wang X, Li J (2007) LAZY1 controls rice shoot gravitropism through regulating polar auxin transport. *Cell Res* 17: 402–410

Lucas JR, Courtney S, Hassfurder M, Dhingra S, Bryant A, Shaw SL (2011) Microtubule-associated proteins MAP65-1 and MAP65-2 positively regulate axial cell growth in etiolated Arabidopsis hypocotyls. *Plant Cell* 23: 1889–1903

Morita MT (2010) Directional gravity sensing in gravitropism. *Annu Rev Plant Biol* 61: 705–720

Nakagawa T, Kurose T, Hino T, Tanaka K, Kawamukai M, Niwa Y, Toyooka K, Matsuoka K, Jinbo T, Kimura T (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *J Biosci Bioeng* 104: 34–41

Oda Y, Fukuda H (2012) Initiation of cell wall pattern by a Rho- and microtubule-driven symmetry breaking. *Science* 337: 1333–1336

Sedbrook JC, Chen R, Masson PH (1999) *ARG1* (altered response to gravity) encodes a DnaJ-like protein that potentially interacts with the cytoskeleton. *Proc Natl Acad Sci USA* 96: 1140–1145

Shoji T, Narita NN, Hayashi K, Asada J, Hamada T, Sonobe S, Nakajima K, Hashimoto T (2004) Plant-specific microtubule-associated protein SPIRAL2 is required for anisotropic growth in Arabidopsis. *Plant Physiol* 136: 3933–3944

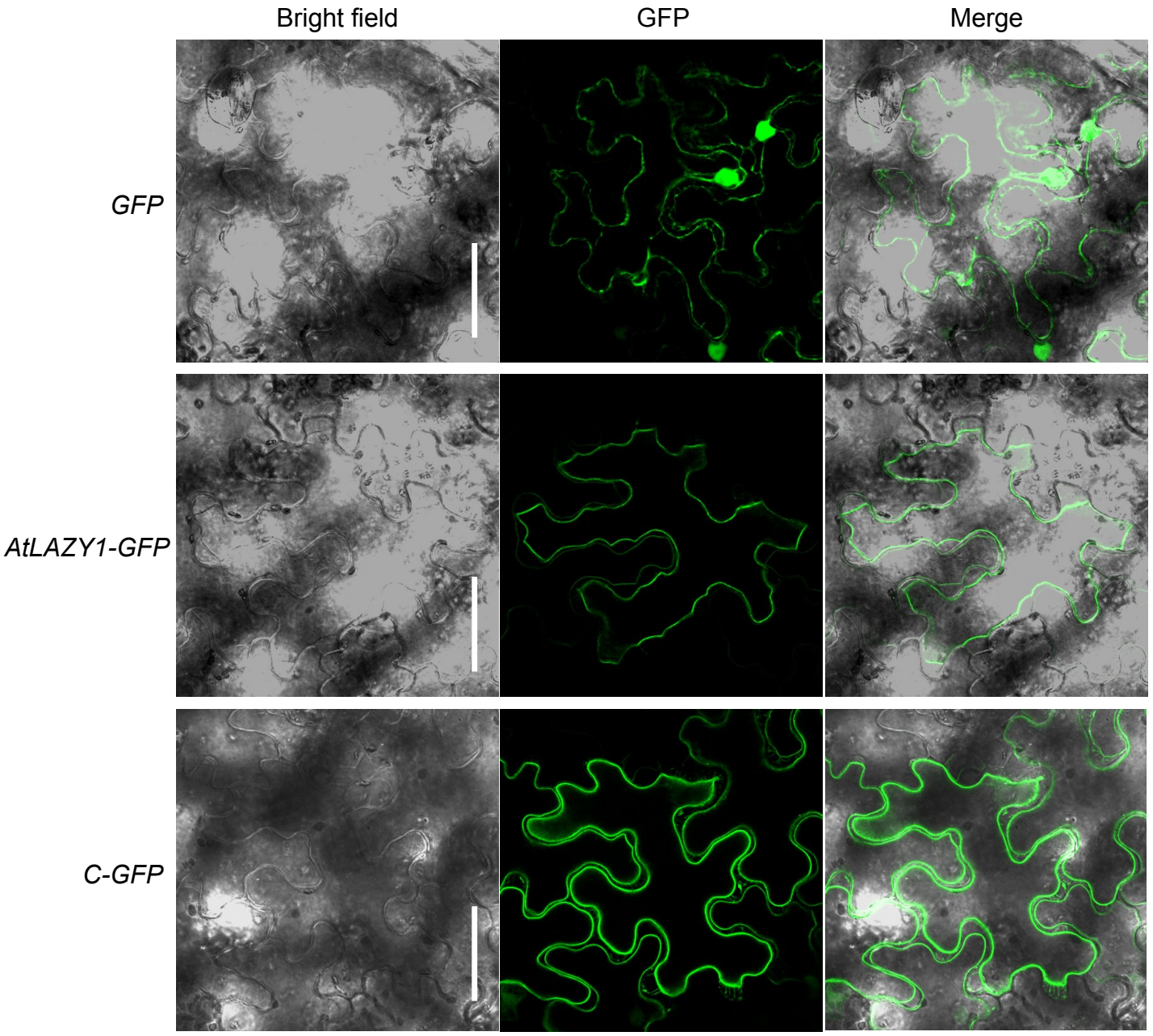
Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, et al. (2013) Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions. *Nat Genet* 45: 1097–1102

Volkmann D, Baluska F (1999) Actin cytoskeleton in plants: From

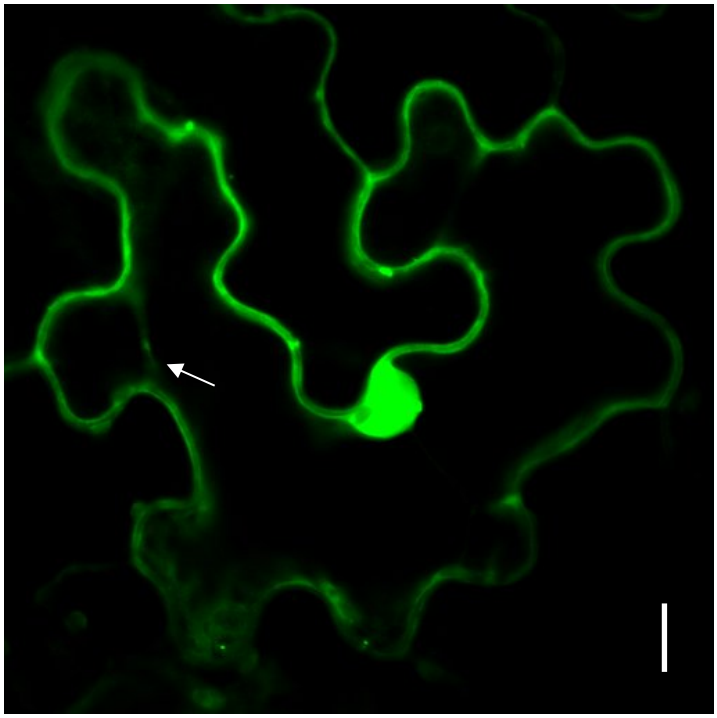
transport networks to signaling networks. *Microsc Res Tech* 47: 135–154

Yoshihara T, Iino M (2007) Identification of the gravitropism-related rice gene *LAZY1* and elucidation of LAZY1-dependent and -independent gravity signaling pathways. *Plant Cell Physiol* 48: 678–688

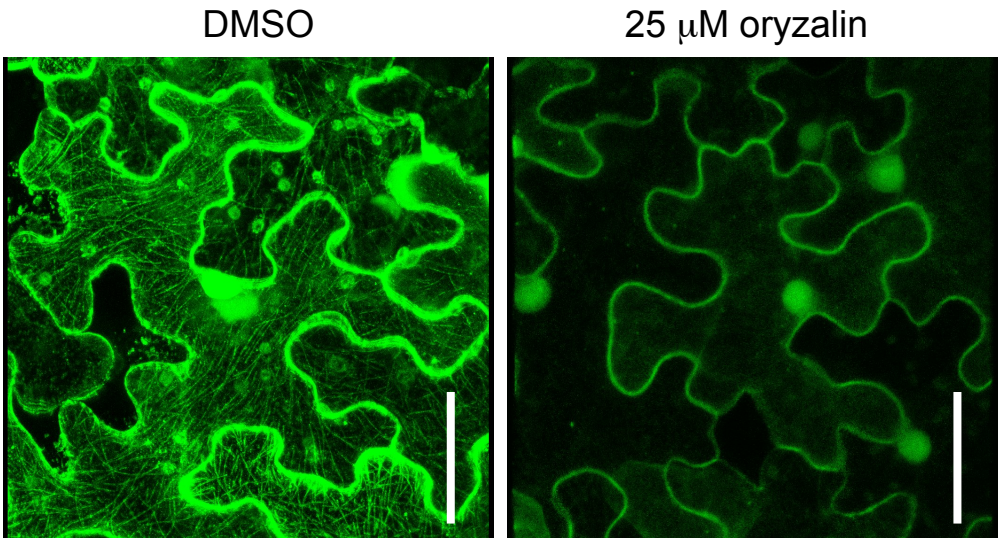
Yoshihara T, Spalding EP, Iino M (2013) AtLAZY1 is a signaling component required for gravitropism of the *Arabidopsis thaliana* inflorescence. *Plant J* 74: 267–279



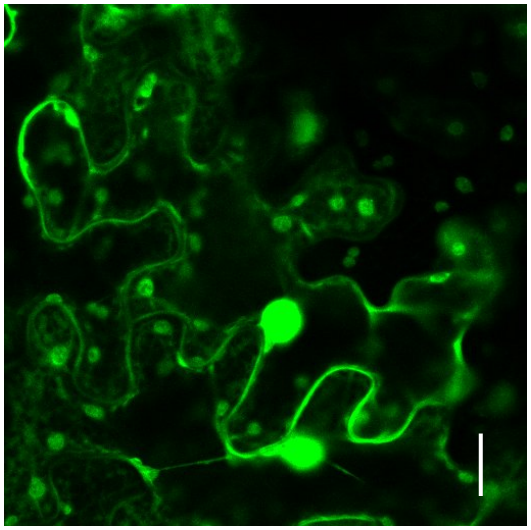
Supplementary Figure S1. Plasmolysis assays of AtLAZY1-GFP and C-GFP induced by 700 mM sucrose solution. GFP was used as a control. Each protein was transiently expressed in *N. benthamiana* pavement cells under the control of CaMV 35S promoter. Bar = 50 μm.



Supplementary Figure S2. Fluorescent image of the pavement cells of *N. benthamiana* leaf expressing GFP fusion protein of C3. The focal plane was set on the middle of nucleus. Arrow indicates cytoplasmic strand. Bar = 20 μm.



Supplementary Figure S3. Stacked fluorescent images of optical sections of *N. benthamiana* pavement cells expressing GFP fusion protein of C3. Agrobacterium harboring *p35S::C3-GFP* vector was co-infiltrated with mock (DMSO) or MT-depolymerizing drug (25 μM oryzalin) and then incubated for 2 days. The fibrous localization pattern was abolished in the oryzalin-treated cells. Bar = 50 μm.



Supplementary Figure S4. Fluorescent image of the pavement cells of *N. benthamiana* leaf expressing GFP fusion protein of C3 without C-terminal 48 amino acids. The fibrous localization pattern was not observed. Bar = 20 μm.