RESEARCH PAPER

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An ABC transporter B family protein, ABCB19, is required for cytoplasmic streaming and gravitropism of the inflorescence stems

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Abbreviations: ABC transporter; ATP-binding cassette transporter; ABCB19; ATP BINDING CASSETTE SUBFAMILY B19; MDR1; MULTIDRUG RESISTANCE PROTEIN1; PGP19; P-GLYCOPROTEIN19; PIN; PIN-FORMED

A significant feature of plant cells is the extensive motility of organelles and the cytosol, which was originally defined as cytoplasmic streaming. We suggested previously that a three-way interaction between plant-specific motor proteins myosin XIs, actin filaments, and the endoplasmic reticulum (ER) was responsible for cytoplasmic streaming.[1] Currently, however, there are no reports of molecular components for cytoplasmic streaming other than the actin-myosin-cytoskeleton and ER-related proteins. In the present study, we found that elongated cells of inflorescence stems of *Arabidopsis thaliana* exhibit vigorous cytoplasmic streaming. Statistical analysis showed that the maximal velocity of plastid movements is 7.26 μm/s, which is much faster than the previously reported velocities of organelles. Surprisingly, the maximal velocity of streaming in the inflorescence stem cells was significantly reduced to 1.11 μm/s in an *Arabidopsis* mutant, *abcb19-101*, which lacks ATP BINDING CASSETTE SUBFAMILY B19 (ABCB19) that mediates the polar transport of the phytohormone auxin together with PIN-FORMED (PIN) proteins. Polar auxin transport establishes the auxin concentration gradient essential for plant development and tropisms. Deficiency of ABCB19 activity eventually caused enhanced gravitropic responses of the inflorescence stems and abnormally flexed inflorescence stems. These results suggest that ABCB19-mediated auxin transport plays a role not only in tropism regulation, but also in cytoplasmic streaming.

Introduction

Plant tropism has attracted significant attention over the years. Nearly every organ is able to bend in response to a variety of environmental stimuli including gravity and light.[2, 3] According to the classical Cholodny–Went model, gravitropic and phototropic curvature of roots and stems occurs by a lateral auxin gradient that promotes differential cell elongation.[2] Gravistimulation induces asymmetrical distribution of the major endogenous auxin indol-3-acetic acid in rice and corn coleoptile, which causes differential growth of cells on either side of the organ to establish the organ curvature.[4,5]

The molecular mechanisms underlying tropisms have been demonstrated in *Arabidopsis thaliana*. *Arabidopsis* plants sense gravity in endodermal cells of shoots[6] and in columella cells of roots.[7] The main components of inducing a lateral auxin redistribution are PIN FORMED (PIN) protein PIN3[8] and ATP BINDING CASSETTE SUBFAMILY B19 (ABCB19).[9] ABCB19 is also known as MULTIDRUG RESISTANCE PROTEIN1 (MDR1) and P-GLYCOPROTEIN 19 (PGP19). ABCB19 has an ability to transport auxin through plasma membrane and interacts with the auxin efflux carrier proteins PINs (PIN1 and PIN2), suggesting a complicated relationship between these transporters and auxin distribution.[10] *Arabidopsis* mutants with defects in the *ABCB19* gene showed not only abnormal auxin distribution but also abnormal tropisms: enhanced gravitropism and phototropism in hypocotyl and enhanced gravitropism in roots.[9,11-13]

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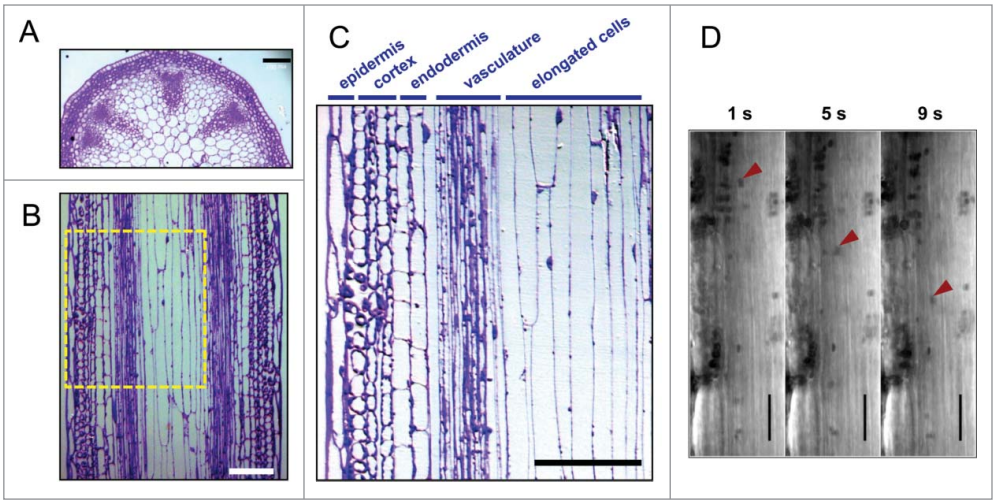

Figure 1. Active cytoplasmic streaming in elongated cells of the inflorescence stem. (A–C) Cross section **(A)** and transverse section (B and C) of upper part of the inflorescence stem were stained with Toluidine Blue. Bars = 100 μm. Boxed area in **(B)** was magnified in **(C)**. **(D)** Time course of the plastid movement in the elongated cells of the inflorescence stem (upper part). Arrow heads indicate the plastids showing maximal velocity in the time-lapse sequence. Bars = 20 μm. See also Supplementary Movie 1.

In the present study, we found that ABCB19 is required for gravitropic response of inflorescence stems and organelle movements in the stem cells. Cytoplasmic streaming was first described in 1774.[14] Unidirectional actin filament bundles and organelle-associated myosin XIs, a plant-specific class of myosin motors, are thought to cause bulk flow in the cell.[15,16] Previously, we demonstrated that the endoplasmic reticulum (ER) dynamics are driven primarily by the ER-associated myosin XI-K and that myosin XI deficiency affects organization of the ER network and orientation of the actin filament bundles. We suggested a model whereby dynamic three-way interactions between ER, F-actin, and myosin XIs determine the architecture and movement patterns of the ER thick strands, and cause cytosol hauling traditionally defined as cytoplasmic streaming.[1] However, the molecular mechanism and physiological meanings of cytoplasmic streaming are largely unknown.

Results

An inflorescence stem of *Arabidopsis* comprises radially patterned tissue layers of epidermis, cortex, endodermis, vasculature, and the inner tissues (**Fig. 1A and 1B**). Each layer has specific functions: the epidermis acts as an interface between the plant and the environmental stresses; the endodermis plays a role of sensing gravity;[17] and the vasculature consisting of phloem and xylem functions in the nutrient and water flow. The inner tissues comprise elongated cells with lengths up to 1 mm (**Fig. 1C**). However, the physiological function of the elongated cells is not known. To investigate the function of these elongated cells, hand-cut vertical sections of the inflorescence stem were prepared and the elongated cells of the inner tissues were observed in the living state. We found that the typical organelles plastids in the elongated cells rapidly streamed along the longitudinal axis of the cells (**Fig. 1D**). Statistical analysis revealed that the maximal velocity of the plastid movements was 7.26 μm/s (**Table 1**), which is much higher than the velocities of organelle movements reported before.[18,19] For example, the maximal velocity of the ER and the cytosol in petiole cells was reported to be ~3.5 μm/s.[1] These movements of organelles and cytosol correspond to cytoplasmic streaming. Hence, the elongated cells of the inflorescence stems exhibit active cytoplasmic streaming.

Quite unexpectedly, we discovered that cytoplasmic steaming was drastically impaired by depletion of *ABCB19* (**Fig. 2A**). The maximal velocity of plastid movements in *abcb19-101* was determined to be 1.11 μm/s (**Table 1**), indicating that a deficiency of ABCB19 caused a dramatic reduction of cytoplasmic streaming activity by up to 85% (**Fig. 2B**). Moreover, most of the plastids streamed long distances with almost constant velocities in the wild-type cells, whereas they streamed short distances in a discontinuous manner and frequently stopped moving (Supplemental Movie 1). These results suggest that ABCB19 plays a role in regulating cytoplasmic streaming of plant cells and that ABCB19 is related to the actin-myosin XI cytoskeleton. Until now, no components other than the actin-myosin XIs and ER-related proteins[19] were reported as being involved in cytoplasmic streaming. Furthermore, despite extensive studies on involvement of ABCB19/MDR1/PGP19 in auxin distribution and tropisms in hypocotyls and roots, no ABCB19 functions at the subcellular levels have been reported. Therefore, the discovery of a role of

Table 1. Maximal velocity of plastids in the fiber cells of *abcb19-101*. Absolute values and statistical analysis of the data presented in Fig. 2. *n*, number of data points in the data set; SD, standard deviation; SE, standard error.

	maximal velocities (μm/sec)				
	average	**SD**	**SE**	***n***	***P* value by *t*-test (wild type *versus fiz1*/+)**
wild type	7.26	1.07	0.38	8	
abcb19-101	1.11	0.58	0.14	17	2.04E-15

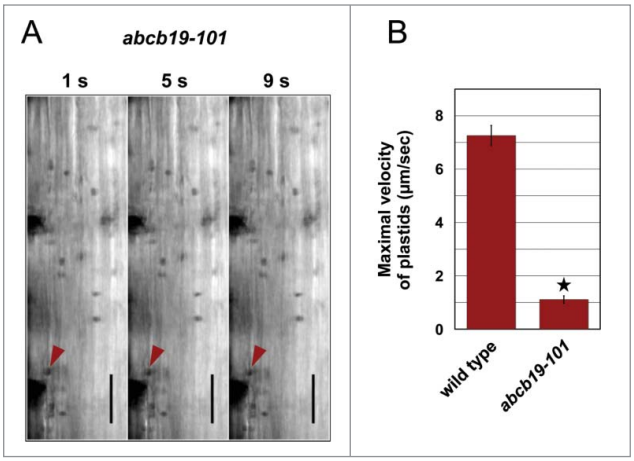

Figure 2. Defects of cytoplasmic streaming in elongated cells of the *abcb19* inflorescence stem. (A) Time course of the plastid movement in the elongated cells of the *abcb19-101* inflorescence stem (upper part). Arrow heads indicate the plastids showing maximal velocity in the time-lapse sequence. Bars = 20 μm. See also Supplementary Movie 1. **(B)** Maximal velocity of plastids in the elongated cells of wild type and *abcb19-101*. Error bars indicate SE. *, $P < 0.001$ by the Student's *t* test. See also **Table 1**.

ABCB19 provides novel insights into the field of cytoplasmic streaming in plants.

Next, we examined the gravitropic response of the wild-type and the *abcb19-101* mutant by focusing on the inflorescence stems, in which ABCB19 is expressed.[20] Plants with growing primary inflorescence stems were placed horizontally for 6 h. In the initial response phase, the stem of the wild-type bent 90° to become vertical (**Fig. 3A**). Subsequently, the wild-type continued growing vertically. Conversely, *abcb19-101* first overshot in one direction up to 120° and then overshot in the other direction (Figure 3A). The precise movements of the inflorescence stems of the wild-type and *abcb19-101* are shown in Supplementary Movie 1. Figure 3B shows the superimposed time-lapse images during the course of gravitropic response of inflorescence stems, showing the overshooting stem of *abcb19-101*. Statistical analysis of gravitropic bending of the stems (**Table 2**) and kinetics of gravitropism of the stems (**Fig. 3C**) clearly revealed that a deficiency of ABCB19 caused an enhanced gravitropic response of the inflorescence stems. This result is consistent with the enhanced bending of hypocotyls in response to gravity.[11] ABCB19 may be involved in adjusting the bending of the stems in response to gravity. The *abcb19-101* plants exhibited a weak dwarf phenotype and kinked gross morphology (**Fig. 4A**). The organs including the inflorescence stems and pedicels were abnormally flexed (**Fig. 4B**). These abnormal phenotypes may be due to the enhanced gravitropic organ bending in the mutant.

Discussion

In the present study, we show that the inflorescence stems of *abcb19-101* displays enhanced gravitropic responses as does the *abcb19-101* hypocotyls.[21] However, this phenotype is inconsistent with the report of impaired gravitropic response of the stem of another *abcb19* allele *mdr1*.[13] This inconsistency may be due to the difference of the ecotypes of the *A. thaliana* plants: the genetic background of *abcb19-101* is Col-0, whereas that of *mdr1* is WS. The discovery of an involvement of ABCB19 in intracellular movements of organelles is unexpected. This suggests that the auxin transporter ABCB19 is associated with the actin-myosin cytoskeleton.

Cytoplasmic streaming is inhibited by treatment with the endogenous auxin indole-3-acetic acid.[22] Other carboxylic acids, including acetic acid, propionic acid, and decanoic acid, have similar effects as indole-3-acetic acid on cytoplasmic streaming[22] and affect the organization of actin cytoskeletons in the treated cells. These events are also supported by a report published ~80 years ago, showing that cytoplasmic streaming was suppressed by treatment with high concentrations of

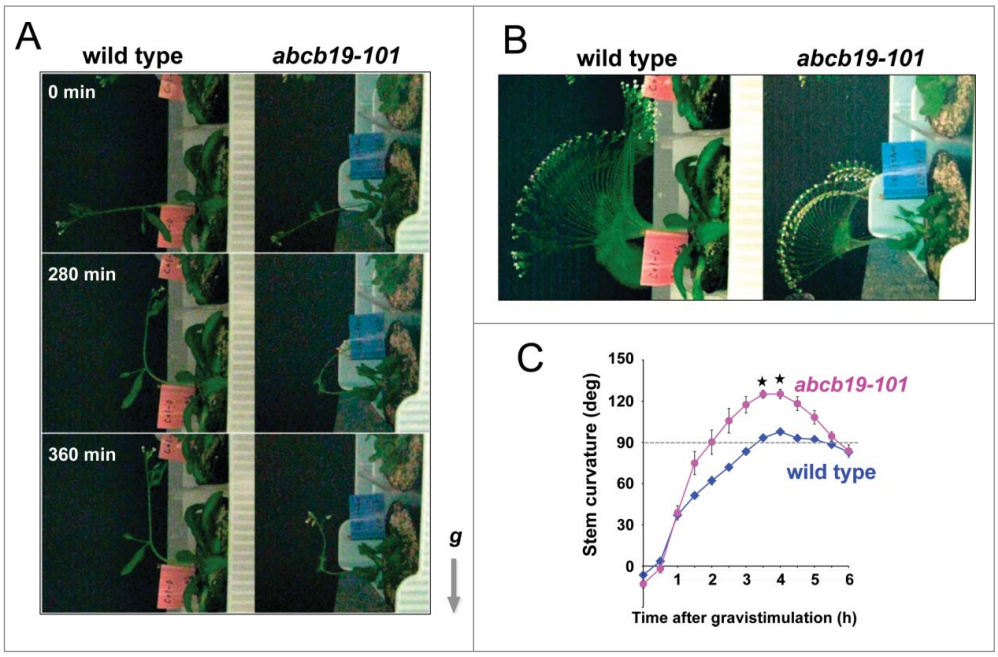

Figure 3. Deficiency of *ABCB19* enhances bending of an inflorescence stem in response to gravity stimuli. (A) Time course of gravitropism of inflorescence stem. **(B)** Maximum intensity projections of time-lapse images during gravitropism of inflorescence stems. Left, wild-type; right, *abcb19-101*. See also Supplementary Movie 2. **(C)** Kinetics of gravitropism of inflorescence stems of wild-type and the *abcb19-101* mutant after placing the plant horizontally. Error bars indicate SE. *, $P < 0.001$ (Student's t test). See also **Table 2**.

Table 2. Gravitropism of the *abcb19-101* inflorescence stem. Absolute values and statistical analysis of the data presented in Fig. 3. *n*, number of data points in the data set; SD, Standard deviation.

	wild type			*abcb19-101*			
	curvature (deg)			curvature (deg)			
Time (min)	average	SD	*n*	average	SD	*n*	*P* value by *t*-test (wild type *versus* *abcb19-101*)
0	−6.5	3.1	15	−12.9	10.5	13	9.76E-01
30	3.6	3.0	15	−1.7	11.2	13	6.49E-01
60	37.2	6.8	15	38.8	18.7	13	1.52E-01
90	51.4	9.2	15	74.9	30.6	13	5.16E-03
120	62.2	11.5	15	90.2	31.8	13	5.03E-03
150	71.9	9.3	15	105.7	32.3	13	4.73E-03
180	83.4	6.4	15	117.4	21.4	13	4.74E-03
210	93.3	4.9	15	125.0	10.4	13	1.21E-04
240	97.8	5.0	15	125.3	12.4	13	5.73E-04
270	93.1	7.3	15	118.2	18.3	13	6.77E-03
300	92.2	6.1	15	108.2	17.6	13	3.65E-02
330	88.4	3.7	15	94.4	11.7	13	3.50E-01
360	83.0	4.1	15	83.8	16.1	13	5.04E-01

auxin.[23] Because *abcb19* causes auxin leakage from the inner tissue cells of hypocotyls to the epidermis[10] and *abcb19* impairs the auxin polar transport in the hypocotyls,[12] *abcb19* may cause a defect in auxin distribution in inflorescence stems. The abnormal auxin distributions may affect the actin cytoskeleton organization, resulting in impaired cytoplasmic streaming. Another possibility is derived from the hypothesis that the actin cytoskeleton regulates polar localization of PIN proteins by potentially participating in the vesicular cycling of the PIN1 protein.[24]

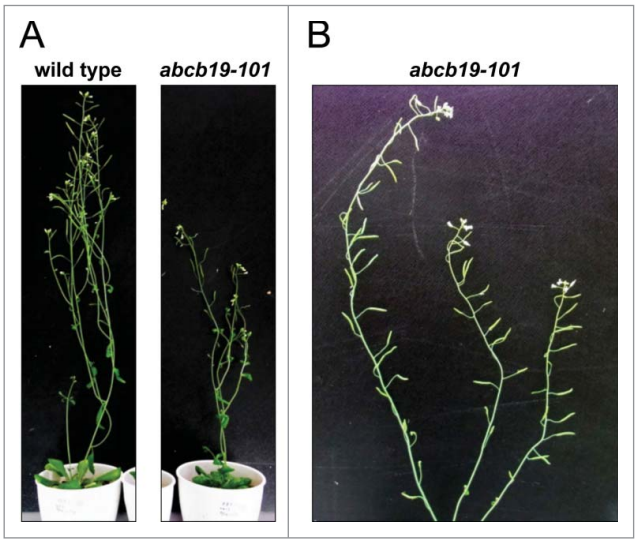

Figure 4. Kinked gross morphology of the *abcb19* mutant. (A) Six-week-old plants of wild-type Col-0 and the *abcb19-101* mutant grown in a growth chamber. **(B)** Inflorescence stems of the *abcb19-101* mutant (9 weeks old).

Materials and Methods

Plant materials and growth conditions

Arabidopsis thaliana L. (Heynh) accession Columbia-0 (CS60000) was used as the wild type. A T-DNA insertion line (*abcb19-101*; SALK_033455)[25] was obtained from the Arabidopsis Biological Resource Center. The T-DNA insertion was confirmed by PCR with primers *abcb19-101*F (CTCAGGCAATTGCTCAAGTTC), *abcb19-101*R (GCAATTGCAATTCTCTGCTTC), and LBa1 (TGGTTCACGTAGTGGGCCATCG). Plants were initially grown on plates of Murashige–Skoog medium containing 1% (w/v) sucrose, 0.5% (w/v) MES-KOH (pH 5.7), and 0.5% (w/v) Gellan gum (Wako), under continuous light at 22°C for 2–3 weeks before being transferred to soil.

Thin sections of the inflorescence stem

Thin sections were prepared as described previously[26] with the upper parts (1–3 cm from the top) of the primary inflorescence stem (14 cm height). The sections were stained with 1% Toluidine Blue[27] and were observed in a bright field with a light microscope (Axioplan 2 Imaging; Zeiss) using objectives (20 × 0.5 NA and 10 × 0.3 NA).

Hand-cut sections of the inflorescence stem

Vertical sections of the inflorescence stem were prepared as described previously.[28] Briefly, double-sided adhesive tape was cut and stuck onto a glass slide. A 4-week-old inflorescence stem segment (less than 1 cm long) was excised from the region 1–2 cm below the apex of the stem and stuck to the tape. The segment was cut longitudinally using a razor blade.

Observation of cytoplasmic streaming in the inflorescence stem

To examine cytoplasmic streaming in the inflorescence stem, the hand-cut vertical sections were observed in a bright field using a light microscopy (LSM 510 META; Zeiss) with a 63 ×

1.2 numerical aperture water-immersion objective. The differential interference contrast images were taken at 1-s intervals for 1 min. For estimation of the velocity, moving plastids in four or five randomly selected sequential frames of 8 (wild-type) and 17 (*abcb19-101*) time-lapse images (143 × 143 μm) were tracked manually using the ImageJ software (http://rsb.info.nih.gov/ij/).

Gravitropism assay of inflorescence stems

Intact plants with 4- to 8-cm primary stems were placed horizontally in non-directional dim light (1 μmol m^{-2} s^{-1}). Photographs were taken automatically every 10 min by a digital camera (Canon). Stem curvature was defined as the angle formed between the growing direction of the apex and the horizontal base line and was measured on the digital images using the ImageJ software.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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