PLANT SIGNALING & BEHAVIOR
2021, VOL. 16, NO. 5, e1889252 (3 pages)
https://doi.org/10.1080/15592324.2021.1889252

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Check for updates

Evidence for the involvement of *AtPiezo* in mechanical responses

Xianming Fang[a], Yang Zhang[a], Bo Cheng[a], Sheng Luan[b], and Kai He[a]

[a]Ministry of Education Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, Lanzhou, China; [b]Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA

ABSTRACT

The plant-environment interactions are finely tuned by plant endogenous signals and environmental cues. Mechanical forces serve as important exogenous stimuli regulating plant growth and development and shaping plant structures. Studies have shown that mechanosensitive ion channels play essential roles in the responses to mechanical signals in plants. The biological functions of animal Piezos, a group of mechanosensitive ion channels, have been extensively studied and revealed to be required for normal physiological processes. However, little is known about the functions of the homologous genes of animal *Piezo* genes in plants. We have recently pinpointed that *AtPiezo* plays an important role in the root cap in response to mechanical forces in *Arabidopsis thaliana*. Here, we further show that *AtPiezo* responds to mechanical stimuli at the transcriptional level. The results provide additional evidence for the involvement of *Piezo* in mechanical responses in plants.

ARTICLE HISTORY
Received 24 January 2021
Revised 8 February 2021
Accepted 8 February 2021

KEYWORDS
Arabidopsis thaliana; root; mechanosensitive ion channel; AtPiezo; transcription

Under natural conditions, plants encounter continuous challenges from external and internal mechanical forces.[1] The external mechanical forces are effected by wind, rain, touch, neighboring plants, soil contextures and so on.[2,3] The internal mechanical force is mainly exerted by the plant's own weight, cell division, and swelling pressure in the cell.[4] Mechanical force is a ubiquitous factor regulating plant development, which also precisely controls the size and direction of plant growth in order to produce the organs and tissues in a regular and stable manner.[5] How cells sense mechanical force is a long-standing question. In some theories, the deformation of the cell membrane caused by the forces inside and outside the cell is perceived by the mechanosensitive ion channels that are anchored in the membrane.[3,6,7] Upon the activation by mechanical forces, the mechanosensitive ion channels mediate ion flow, thereby affecting the transmission of action potentials, cellular ion balance, and cytosolic Ca^{2+} signals.[6] A number of mechanosensitive ion channels have been identified in plants. MSL8, MSL10, and OSCA1 channels were shown to participate in pollen hydration, root cell swelling, osmosensing and other biological processes.[8-10]

We recently showed the homolog of animal Piezo proteins is also functional in plants. The analyses of *Arabidopsis thaliana AtPiezo* indicated it may play an important role in root cap sensing the mechanical force from the environment. Similarly, a recent preprint report showed *AtPiezo* is required for root cap mechanotransduction in Arabidopsis.[11] Mutation in *AtPiezo* causes impaired rooting ability and disrupted Ca^{2+} response upon mechanical stimuli.[11] In addition, Piezo was revealed by another preprint study to function as a mechanosensitive ion channel in moss.[12]

In our previous study, we found that the roots of Arabidopsis plants appeared helical when the agar concentration of the medium was relatively low when we directly germinated the seeds within the agar medium and cultivated them vertically.[13] Compared to Col-0 wildtype (WT) plants, *atpiezo* mutants showed increased numbers of helical roots and lateral roots. Recent studies have shown that the growth force and external mechanical forces together contribute to the phenotype of helical roots.[14]

In this study, we found that the expression patterns of *AtPiezo* will change upon the stimulation of distinct mechanical stimuli. When the transgenic plants harboring *promoter-AtPiezo::GUS* grew horizontally on the agar medium, the GUS staining was obviously stronger than that in the transgenic plants that vertically grew on the surface of agar medium (Figure 1a). The quantitative analyses on GUS staining also confirmed the up-regulation of *AtPiezo* in the horizontally grown plants (Figure 1b). Moreover, the results of qRT-PCR were in line with the GUS staining (Figure 1c). When the roots of the transgenic grew vertically within the agar medium, GUS staining was significantly stronger in the helical roots than that

CONTACT Sheng Luan sluan@berkeley.edu Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.; Kai He hekai@lzu.edu.cn Ministry of Education Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, Lanzhou 730000, China.

© 2021 Taylor & Francis Group, LLC

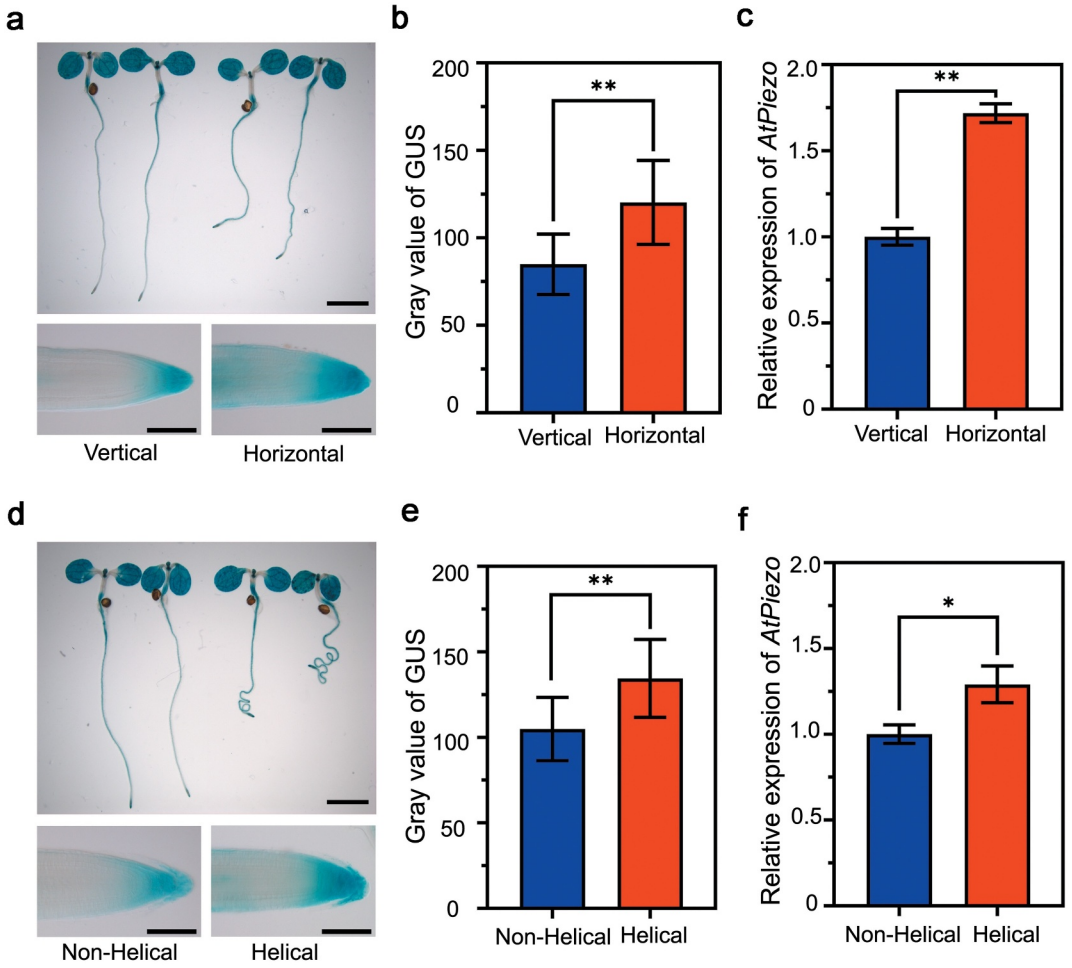

Figure 1. *AtPiezo* responds to mechanical stimulation. (a) Histochemical GUS staining is shown in four-day-old seedling of *pAtPiezo::GUS* lines. The plants that vertically grew on the 1% (w/v) agar medium are shown at left (Vertical). The plants that horizontally grew for 24 h followed by vertical growth for three days on the medium are shown at right (Horizontal). Scale bars = 2 mm. The corresponding root tips are shown in the bottom panels. Scale bars = 0.1 mm. (b) Graph showing the mean GUS intensity of different root tips. Measurements of GUS gray value within a 0.1 mm × 0.1 mm rectangular area in the root tips. Data are presented as mean ± SD (n = 15) and asterisks indicate a significant difference (Unpaired t-test, *P* value < .05). (c) Expression of *AtPiezo* in four-days-old WT roots. Roots were collected under different conditions and *AtPiezo* mRNA levels were measured by qRT-PCR. UBQ10 was used as a normalizer. Data are means ± SD, and asterisks indicate a significant difference (Unpaired t-test, *P* value < .05). (d) Histochemical GUS staining is shown in four-day-old seedlings of *pAtPiezo::GUS* lines. The plants that vertically grew in the 0.8% (w/v) agar medium with non-helical roots are shown at left (Non-Helical). The plants with helical roots are shown at right (Helical). Scale bars = 2 mm. The corresponding root tips are shown in the bottom panels, Scale bars = 0.1 mm. (e) Graph showing the mean GUS intensity of different root tips. Measurements of GUS gray value within a 0.1 mm × 0.1 mm rectangular area in the root tips. Data are presented as mean ± SD (n = 15) and asterisks indicate a significant difference (Unpaired t-test, *P* value < .05). (f) Expression of *AtPiezo* in four-day-old WT roots. Roots were collected under different conditions and *AtPiezo* mRNA levels were measured by qRT-PCR. UBQ10 was used as a normalizer. Data are means ± SD, and asterisks indicate a significant difference (Unpaired t-test, *P* value < .05).

in the non-helical roots (Figure 1d). The higher expression of *AtPiezo* gene in the helical roots growing within the agar medium was verified by the quantitative analyses on GUS staining and qRT-PCR (Figure 1e,f). Taken together, these results indicated that the expression of *AtPiezo* is regulated by mechanical signals.

There are three splice variants of *AtPiezo* gene in Arabidopsis. We cloned the largest splice variant which is a CDS fragment with 7455 bp into a plant expression vector. However, it is still technically difficult to stably transform this plasmid construct into *Agrobacterium* strain at the current point.[13,15] We also cloned *AtPiezo* into pcDNA3.1 vector that was designed for high level stable and transient expression in mammalian hosts, fused with a Venus tag. Of note, when the Hela cells were transfected with *AtPiezo*, the expression of *AtPiezo* caused cell death (Figure 2). By contrast, the expression of Venus alone resulted in no alteration in Hela cells (Figure 2). These results suggested overly or heterologously expressed *AtPiezo* may lead to dysfunctions in mechanical responses which cause the disintegration of the cell ultimately.

In conclusion, our results indicated that *AtPiezo* gene responds to mechanical stimulation at the transcriptional level.

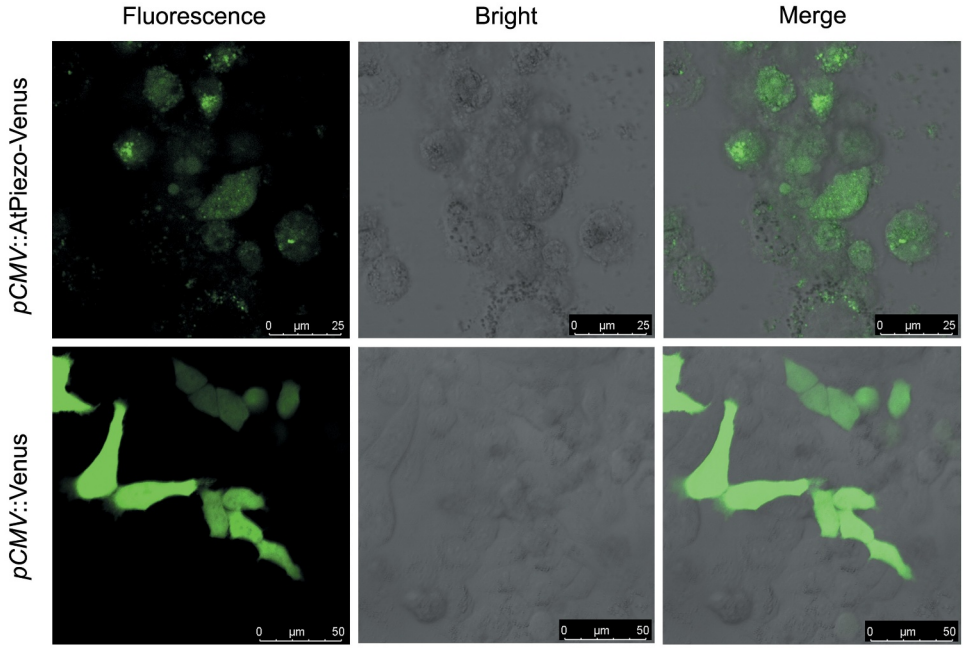

Figure2. Heterologously expressed *AtPiezo* in Hela cells. The *pcDNA3*.1–*AtPiezo* constructs were transfected into Hela cells with Exfect Transfection Reagent. Cells were grown for 24 hours before analysis. All images were obtained using an excitation wavelength of 488 nm and detection wavelength of 505–535 nm by a confocal laser scanning microscope (Leica TCS SP8). All experiments were repeated at least three times and similar results were obtained each time.

Acknowledgments

We thank Yahu Gao, Liang Peng, Liping Guan and Yang Zhao (Core Facility at Life Science Research, Lanzhou University) for technical assistance.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This research was funded by National Natural Science Foundation of China (31870235), the Ministry of Agriculture of the People's Republic of China (2016ZX08009-003-002).

ORCID

Xianming Fang http://orcid.org/0000-0002-0416-7479

References

1. Hamant O, Moulia B. How do plants read their own shapes? New Phytol. 2016;212:333–337. doi:10.1111/nph.14143.
2. Braam J, Davis RW. Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. Cell. 1990;60:357–364. doi:10.1016/0092-8674(90)90587-5.
3. Moulia B, Coutand C, Julien JL. Mechanosensitive control of plant growth: bearing the load, sensing, transducing, and responding. Front Plant Sci. 2015;6:52. doi:10.3389/fpls.2015.00052.
4. Hamant O, Haswell ES. Life behind the wall: sensing mechanical cues in plants. BMC Biol. 2017;15:59. doi:10.1186/s12915-017-0403-5.
5. Hamant O. Widespread mechanosensing controls the structure behind the architecture in plants. Curr Opin Plant Biol. 2013;16:654–660. doi:10.1016/j.pbi.2013.06.006.
6. Hamilton ES, Schlegel AM, Haswell ES. United in diversity: mechanosensitive ion channels in plants. Annu Rev Plant Biol. 2015;66:113–137. doi:10.1146/annurev-arplant-043014-114700.
7. Monshausen GB, Gilroy S. The exploring root–root growth responses to local environmental conditions. Curr Opin Plant Biol. 2009;12:766–772. doi:10.1016/j.pbi.2009.08.002.
8. Yuan F, Yang H, Xue Y, Kong D, Ye R, Li C, Zhang J, Theprungsirikul L, Shrift T, Krichilsky B, et al. OSCA1 mediates osmotic-stress-evoked Ca2+ increases vital for osmosensing in Arabidopsis. Nature. 2014;514:367–371. doi:10.1038/nature13593.
9. Hamilton ES, Jensen GS, Maksaev G, Katims A, Sherp AM, Haswell ES. Mechanosensitive channel MSL8 regulates osmotic forces during pollen hydration and germination. Science. 2015;350:438–441. doi:10.1126/science.aac6014.
10. Basu D, Haswell ES. The mechanosensitive ion channel MSL10 potentiates responses to cell swelling in arabidopsis seedlings. Curr Biol. 2020;30:2716–2728 e2716. doi:10.1016/j.cub.2020.05.015.
11. Patapoutian A, Mousavi SAR, Dubin AE, Zeng W-Z, Coombs AM, Do K, Ghadiri DA, Zhao Y, Ge C. PIEZO ion channel is required for root mechanotransduction in Arabidopsis thaliana. BioRxiv. 2020. doi:10.1101/2020.08.27.270355.
12. Radin I, Richardson RA, Weiner ER, Bascom CS, Bezanilla M, Haswell ES. Regulation of vacuole morphology by PIEZO channels in spreading earth moss. BioRxiv. 2020. doi:10.1101/2020.08.27.269282.
13. Fang X, Liu B, Shao Q, Huang X, Li J, Luan S, He K. AtPiezo plays an important role in root cap mechanotransduction. Int J Mol Sci. 2021;22:467. doi:10.3390/ijms22010467.
14. Yan J, Wang B, Zhou Y, Hao S. Resistance from agar medium impacts the helical growth of Arabidopsis primary roots. J Mech Behav Biomed Mater. 2018;85:43–50. doi:10.1016/j.jmbbm.2018.05.018.
15. Zhang Z, Tong X, Liu SY, Chai LX, Zhu FF, Zhang XP, Zou JZ, Wang XB. Genetic analysis of a Piezo-like protein suppressing systemic movement of plant viruses in Arabidopsis thaliana. Sci Rep. 2019;9:3187. doi:10.1038/s41598-019-39436-3.