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The Arabidopsis voltage-dependent anion channel 2 is required for plant growth

Chika Tateda,† Tomonobu Kusano and Yoshihiro Takahashi*

Graduate School of Life Sciences; Tohoku University; Miyagi, Japan

†Current address: The University of Chicago; Center for Integrative Science; Chicago, IL USA

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The voltage-dependent anion channels (VDACs), known as a major group of outer mitochondrial membrane proteins, are present in all eukaryotic species. In mammalian cells, they have been established as a key player in mitochondrial metabolism and apoptosis regulation. By contrast, little is known about the function of plant VDACs. Recently, we performed functional analysis of all *VDAC* gene members in *Arabidopsis thaliana*, and revealed that each AtVDAC member has a specialized function. Especially, in spite of similar subcellular localization and expression profiling of *AtVDAC2* and *AtVDAC4*, both the T-DNA insertion knockout mutants of them, *vdac2–2* and *vdac4–2*, showed severe growth retardation. These results suggest that AtVDAC2 and AtVDAC4 proteins clearly have distinct functions. Here, we introduced the *AtVDAC2* gene into the *vdac2–2* mutant, and demonstrated that the miniature phenotype of *vdac2–2* plant is abolished by *AtVDAC2* expression.

The voltage-dependent anion channels (VDACs), one of the most abundant proteins in outer mitochondrial membrane (OMM), are thought to mediate the transport of metabolites between mitochondria and the cytoplasm.[1] The functional analysis of VDACs has been extensively studied in animals, and now it is recognized that VDAC is involved in many physiological and pathological processes such as male reproduction, the central nervous system, glucose homeostasis, and mitochondrial-mediated apoptosis.[2,3] In contrast, physiological significance of plant VDACs is not well known.[1] Recently, we have performed functional analysis of the plant *VDACs* and revealed that some of *Nicotiana benthamiana VDAC* members and *Arabidopsis thaliana AtVDAC1* are involved in plant innate immunity through regulation of hydrogen peroxide production.[4,5] Moreover, we demonstrated, through characterization of all *Arabidopsis VDAC* members (*AtVDAC1* to *AtVDAC4*), that plant VDACs were also important for vegetative and reproductive growth.[5] According to the phylogenetic analysis, dicotyledonous plant VDACs are clearly classified to two groups: One group is formed by AtVDAC1 and AtVDAC3, the other by AtVDAC2 and AtVDAC4. The former retains the conserved eukaryotic mitochondrial porin signature (MPS) and localizes only in the mitochondrion, while the latter has divergent MPS motif and localizes not only in mitochondria but also in other organelle. In addition, the latter *VDACs*' expression profiles were almost similar.[5] We, therefore, thought that AtVDAC2 and AtVDAC4 have a redundant function *in planta.* However, T-DNA knockout mutant of *AtVDAC2*, *vdac2–2*, showed retarded growth and abnormal pollen development even if *AtVDAC4* gene was highly expressed. Moreover, some of the stress marker genes including *PR* (pathogenesis-related) genes are constitutively expressed in normal growth condition. The *AtVDAC4* knockout plant, *vdac4–2,* showed a similar phenotype with *vdac2–2*.[5] To confirm that the absence of AtVDAC2 is the real causal factor for the severe retarded growth observed in *vdac2–2,* we have generated the complementation plant using a heterozygous T-DNA insertion mutant (*VDAC2/vdac2–2*) because the homozygous knockout mutant (*vdac2–2/vdac2–2*) did not set fertile seed. And yet, we selected the *vdac2–2/vdac2–2* plant expressing HA-tagged *VDAC2* (described as a *35S::VDAC2-HA/vdac2–2*) via self-pollination of the *VDAC2/vdac2–2* heterozygous plant expressing *VDAC2-HA*. As shown in **Figure 1,** T-DNA insertion was confirmed in *vdac2–2* as well as in *35S::VDAC2-HA/vdac2–2* plant (C), and also *AtVDAC2* RNA expression were at null levels (E). In *35S::VDAC2-HA/vdac2–2* plant expressing VDAC2-HA (D), the growth retardation was recovered and the abnormal expression of *AtPR1* gene was totally attenuated (B, E).

Some of the plant *VDACs* could compromise *VDAC1*-disrupted yeast phenotype.[7,8] Overexpression of rice *OsVDAC4* induces apoptosis in the Jurkat T-cell line, which can be blocked by Bcl-2 protein.[9] These findings strongly suggest that VDACs share some common function in eukaryotes. Therefore, we were surprised that AtVDAC2 and AtVDAC4 have distinct function in *A. thaliana*. It is likely that they form a heterocomplex

*Correspondence to: Yoshihiro Takahashi; Email: ytakahashi@ige.tohoku.ac.jp
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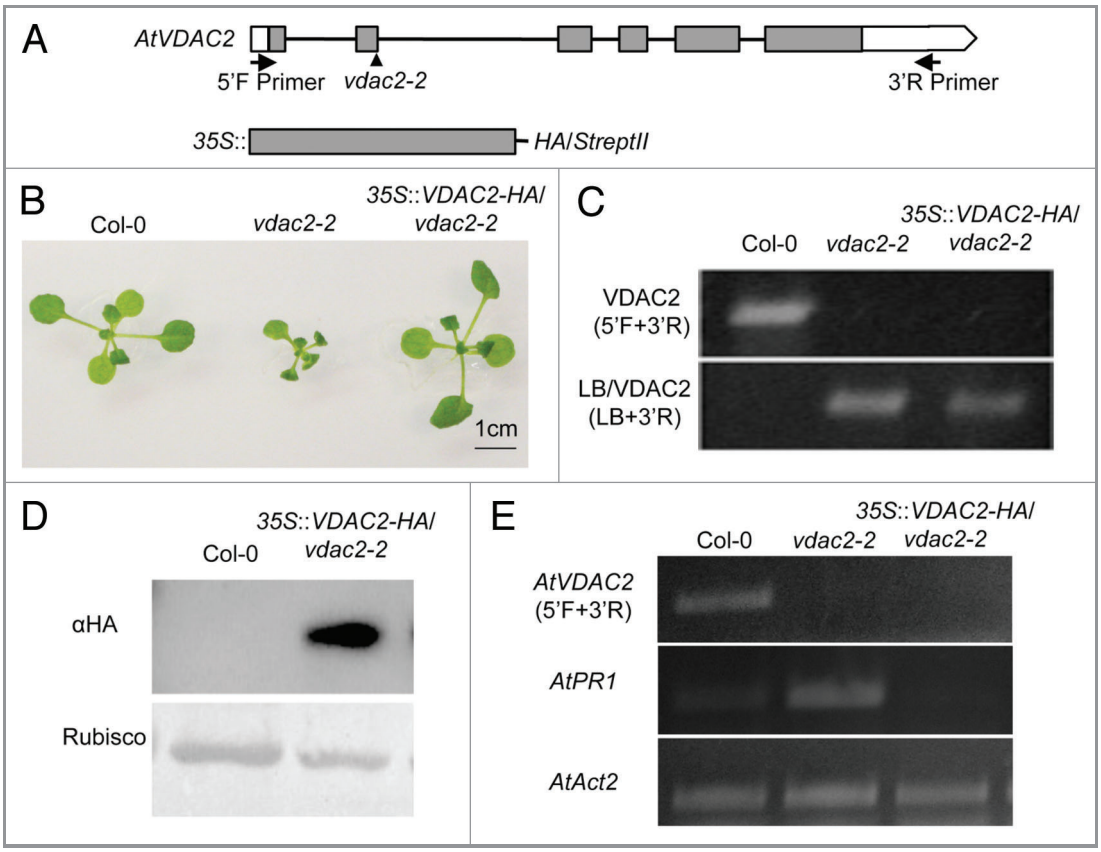

Figure 1. Complementation analysis of *AtVDAC2* knockout plant, *vdac2–2*. (A) Schematic structures of *AtVDAC2* genomic DNA (top) and HA-tag fused *AtVDAC2* construct cloned into pPZP2H3a (+) vector used for making the complementation plant (bottom). Arrows show the positions and orientations of the primers used in PCR (5′F; 5′- GTCGTAATTCTTCTATCTTGATATCTT -3′ and 3′R; 5′- GCGGAACTATTTATTGATTCCA -3′). Arrowhead shows the T-DNA insertion site in *vdac2–2*. Filled boxes, exons; open regions, untranslated regions; black lines, introns. (B) Phenotypes of Col-0, *vdac2–2* and *35S::VDAC2-HA/vdac2–2* plants. All plants were grown at 22°C on 1/2 MS plate for 20 d with 16h light /8h dark condition. Transgenic seeds were selected by 1/2 MS plate with Hygromycin and BASTA, and the other method was followed as described previously.[5] (C) Confirmation of the T-DNA insertion by genomic PCR analysis in Col-0, *vdac2–2* and *35S::VDAC2-HA/vdac2–2* plants. The homozygous line was identified using the left border primer of the T-DNA (LB) and the gene-specific primer (3′R). (D) VDAC2-HA protein detected in *35S::VDAC2-HA/vdac2–2* plants. Total proteins were extracted from 16 d-old plants according to the method by Chinchilla et al.[6] VDAC2-HA protein was detected by anti-HA antibody (Roche, Switzerland). (E) RT-PCR analysis of *AtVDAC2* and *AtPR1* genes in Col-0, *vdac2–2* and *35S::VDAC2-HA/vdac2–2* leaves. *AtAct2* is used as a loading control. The primers information was described previously.[5]

and act as multifunctional channels depend on the site of organelle localization because they show the loose mitochondrial localization. Mammalian VDACs, once thought to be exclusively located in the OMM, are localized to non-mitochondrial cell compartments such as plasma membrane and endoplasmic reticulum.[10,11] In plant VDACs also seem to localize in mitochondria, however, some of the *Lotus japonicus* VDACs are localized in small vesicles at the cell periphery.[7] Maize VDAC identified as a sucrose synthase (SUS)-interacting protein is localized not only in mitochondria but also in nuclei along with SUS.[12] These evidences imply that VDACs have more novel functions. Further studies are needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Kusano T, Tateda C, Berberich T, Takahashi Y. Voltage-dependent anion channels: their roles in plant defense and cell death. Plant Cell Rep 2009; 28: 1301-8; PMID:19585120; http://dx.doi.org/10.1007/s00299-009-0741-z
2. Craigen WJ, Graham BH. Genetic strategies for dissecting mammalian and Drosophila voltage-dependent anion channel functions. J Bioenerg Biomembr 2008; 40:207-12; PMID:18622693; http://dx.doi.org/10.1007/s10863-008-9146-x
3. Shoshan-Barmatz V, De Pinto V, Zweckstetter M, Raviv Z, Keinan N, Arbel N. VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol Aspects Med 2010; 31:227-85; PMID:20346371; http://dx.doi.org/10.1016/j.mam.2010.03.002
4. Tateda C, Yamashita K, Takahashi F, Kusano T, Takahashi Y. Plant voltage-dependent anion channels are involved in host defense against *Pseudomonas cichorii* and in Bax-induced cell death. Plant Cell Rep 2009; 28:41-51; PMID:18953543; http://dx.doi.org/10.1007/s00299-008-0630-x
5. Tateda C, Watanabe K, Kusano T, Takahashi Y. Molecular and genetic characterization of the gene family encoding the voltage-dependent anion channel in *Arabidopsis*. J Exp Bot 2011; 62:4773-85; PMID:21705391; http://dx.doi.org/10.1093/jxb/err113
6. Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JDG, et al. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 2007; 448:497-500; PMID:17625569; http://dx.doi.org/10.1038/nature05999
7. Wandrey M, Trevaskis B, Brewin N, Udvardi MK. Molecular and cell biology of a family of voltage-dependent anion channel porins in *Lotus japonicus*. Plant Physiol 2004; 134:182-93; PMID:14657408; http://dx.doi.org/10.1104/pp.103.031484
8. Elkeles A, Breiman A, Zizi M. Functional differences among wheat voltage-dependent anion channel (VDAC) isoforms expressed in yeast. J Biol Chem 1997; 272:6252-60; PMID:9045642; http://dx.doi.org/10.1074/jbc.272.10.6252
9. Godbole A, Varghese J, Sarin A, Mathew MK. VDAC is a conserved element of death pathways in plant and animal systems. Biochim Biophys Acta 2003; 1642:87-96; PMID:12972297; http://dx.doi.org/10.1016/S0167-4889(03)00102-2
10. Shoshan-Barmatz V, Zalk R, Gincel D, Vardi N. Subcellular localization of VDAC in mitochondria and ER in the cerebellum. Biochim Biophys Acta 2004; 1657:105-14; PMID:15238267; http://dx.doi.org/10.1016/j.bbabio.2004.02.009
11. De Pinto V, Messina A, Lane DJR, Lawen A. Voltage-dependent anion-selective channel (VDAC) in plasma membrane. FEBS Lett 2010; 584:1793-9; PMID:20184885; http://dx.doi.org/10.1016/j.febslet.2010.02.049
12. Subbaiah CC, Huber SC, Sachs MM, Rhoads D. Sucose synthase: expanding protein function. Plant Signal Behav 2007; 2:28-9; PMID:19704804; http://dx.doi.org/10.4161/psb.2.1.3646