Novel type *Arabidopsis thaliana* H^+-PPase is localized to the Golgi apparatus

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Abstract Vacuolar H^+-PPase, a membrane bound proton-translocating pyrophosphatase found in various species including plants, some protozoan and prokaryotes, has been demonstrated to be localized to the vacuolar membrane in plants. Using a GUS reporter system and a green fluorescent protein (GFP) fusion protein, we investigated the tissue distribution and the subcellular localization, respectively, of a novel type H^+-PPase encoded by AVP2/AVPL1 identified in the *Arabidopsis thaliana* genome. We showed that AVP2/AVPL1 is highly expressed at the trichome and the filament of stamen. Furthermore, the fluorescence of GFP-tagged AVP2/AVPL1 showed small dot-like structures that were observed throughout the cytoplasm of various *Arabidopsis* cells under a fluorescent microscope. The distribution of this dot-like fluorescent pattern was apparently affected by a treatment with brefeldin A. Moreover, we demonstrated that most dot-like fluorescent structures colocalized with a Golgi resident protein. These findings suggest that this novel type H^+-PPase resides on the Golgi apparatus rather than the vacuolar membrane.

Key words: AVP2/AVPL1 gene; Golgi apparatus; Green fluorescent protein; Brefeldin A; H^+-PPase; *Arabidopsis thaliana*

1. Introduction

Until recently, vacuolar H^+-PPases, purified and cloned from various plants, and found to have more than 80% amino acid sequence similarity, have been demonstrated to be localized to the tonoplast and to translocate protons into the vacuolar lumen coupled to inorganic pyrophosphate hydrolysis. However, some facultatively aerobic archaeon, photosynthetic bacteria and archaebacteria have also been found to possess H^+-PPase orthologs that have low but significant amino acid sequence similarity with plant V-PPase [1]. Recently, in *Arabidopsis thaliana*, a novel type of H^+-PPase encoded by AVP2/AVPL1, which has only 35% amino acid sequence identity to vacuolar H^+-PPase encoded by AVP1, was identified from the genomic sequence [2,3]. Drozdowicz et al. designated the vacuolar H^+-PPase encoded by AVP1 and the novel H^+-PPase encoded by AVP2/AVPL1 as type-I and type-II H^+-PPases, respectively. The type-II H^+-PPase of *A. thaliana* was demonstrated by the yeast heterologous expression system to have the ability to translocate protons coupled with pyrophosphate hydrolysis in a K^+-independent manner [3]. Since K^+-independent proton-translocating activity has not been observed in the plant vacuole [4,5], it is possible that type-II H^+-PPase is localized to another organelle. In fact, the existence of H^+-PPase in the Golgi apparatus (GA) [6,7], plasma membrane [8,9], mitochondria [10,11] and chloroplast [12,13] has been demonstrated, although the corresponding genes have not been identified.

In this study, we investigated the subcellular localization of the type-II H^+-PPase by using green fluorescent protein (GFP)-tagged type-II H^+-PPase. We showed that the type-II H^+-PPase is targeted mainly to the GA rather than the vacuolar membrane in *Arabidopsis*. This is the first report that demonstrates the localization of the type-II H^+-PPase to the GA.

2. Materials and methods

2.1. Plasmid construction

CaMV35S-sGFP (S65T)-NOS3′/pUC18 vector [14] was kindly provided from Y. Niwa. The vector pMAT137, which is a derivative of pMAT037 [15], was kindly provided from K. Matsuoka. For GUS reporter analysis, the 5′-upstream region of the AVP2/AVPL1 gene was amplified by a PCR with the following primers. AVPLF: 5′-AAGGATCCCCTCTGTTTGTATTGTCCTA-3′ and AVPLR: 5′-AAGGATCCCATCATGTTTTGAGCTGAAT-3′ were used to amplify the 1.4 kb region. AVPLD: 5′-AAGGATCCTTAGTCGATTAGATCTTGAG-3′ and AVPLR were used to amplify the 0.4 kb region. Amplified fragments were subcloned into the *Bam*HI site of binary vector pBI101.1. The construct GFP-AVP2 was generated by a PCR amplification from *A. thaliana* cDNA library using a set of primers, LNBSR: 5′-AATGTACAAGGGAGGTGGAGGTATGATGATGGATGAAGATGTTGAGCA-3′ and LCBSR: 5′-AATGTACATCAGAGGAAAACCGGAGCCA-3′. The amplified DNA fragment was sequenced and was subcloned into the *Bsr*GI site of CaMV35S-sGFP (S65T)-NOS3′/pUC18 vector. The resultant plasmid was designated as GFP-AVP2/pUC18 and was used for transient analysis. The construct AVP2-GFP was generated by a PCR amplification using LNXBA: 5′-AATCTAGAATGATGATGGATGAAGATGT-3′ and LCXBA: 5′-AATCTAGATCAGAGGAAAACCGGAGCCA-3′. The amplified fragment was subcloned into CaMV35S-sGFP (S65T)-NOS3′/pUC18 vector with the *Xba*I site.

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Abbreviations: GFP, green fluorescent protein; MS, Murashige–Skoog

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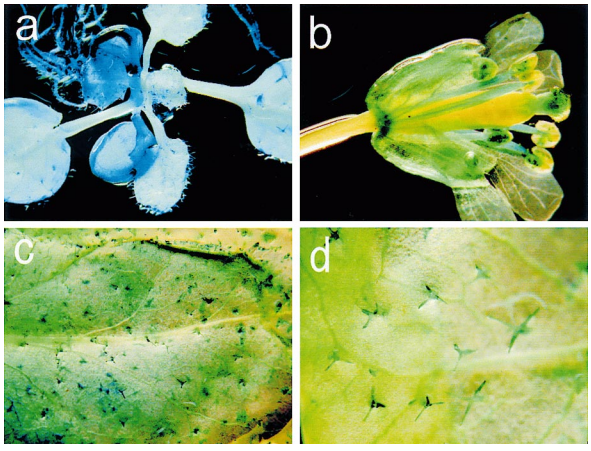

Fig. 1. Histochemical analysis of GUS activity in *Arabidopsis* expressing the β-glucuronidase gene under the control of the 1.4 kb AVP2/AVPL1 promoter. (a) A 10-day-old seedling. Roots and cotyledons were strongly stained. (b) Fully developed flower. Sepals and stamen filaments were strongly stained. (c) Fully developed rosette leaf of 4-week-old plant. (d) High magnification of trichome on a rosette leaf of (c).

The resultant plasmid was designated as AVP2-GFP/pUC18 and used for transient analysis.

For *Agrobacterium*-mediated stable transformation, the *Xba*I–*Eco*RI fragment of GFP-AVP2/pUC18 was subcloned into pMAT137 with the *Xba*I/*Eco*RI sites. The resultant plasmid was used for stable transformation.

2.2. Agrobacterium-mediated stable transformation procedure

All constructs for stable transformation were introduced into *Agrobacterium* C58C1 Rif^r strain [16] by electroporation using a Bio-Rad Gene Pulser II. Fully developed *Arabidopsis* was subjected to *Agrobacterium*-mediated transformation by an in planta vacuum infiltration method [17]. The harvested seeds were screened on Murashige–Skoog (MS) agar plates containing 50 μg/ml kanamycin and 50 μg/ml carbenicillin.

2.3. Histochemical GUS staining

GUS assays were carried out using at least 16 genetically independent transgenic plants of the T1 or T2 generation by the method described by Jefferson et al. [18] with some modification. Briefly, the plant tissues were incubated for 12 h at 37°C in a 100 mM sodium phosphate buffer (pH 7.0) containing 0.1% Triton X-100, 1 mM X-gluc and 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide. The stained tissues were bleached by several replacements with 70% ethanol.

2.4. Culture conditions of Arabidopsis suspension cultured cell

The *Arabidopsis* cell line was kindly provided from Masaaki Umeda. A suspension culture was established from callus tissues obtained from root segments of *A. thaliana* ecotype Col-0 and maintained as described [19].

2.5. Transient expression assays

The plasmid DNA was introduced into *Arabidopsis* suspension cultured cells by the method described by Takeuchi et al. [20]. In the case of leaf mesophyll protoplast, 5 g of fully developed rosette leaves were collected and cut in bulk in distilled water. Cut leaves were incubated in 500 mM mannitol for 1 h. Then, the leaf fragments were transferred to 60 ml of a buffer containing 400 mM mannitol, 1% cellulase, 0.25% macerozyme, 8 mM $CaCl_2$ and 5 mM Mes (pH 5.7), and incubated for 2.5 h. The procedure for transient expression in leaf

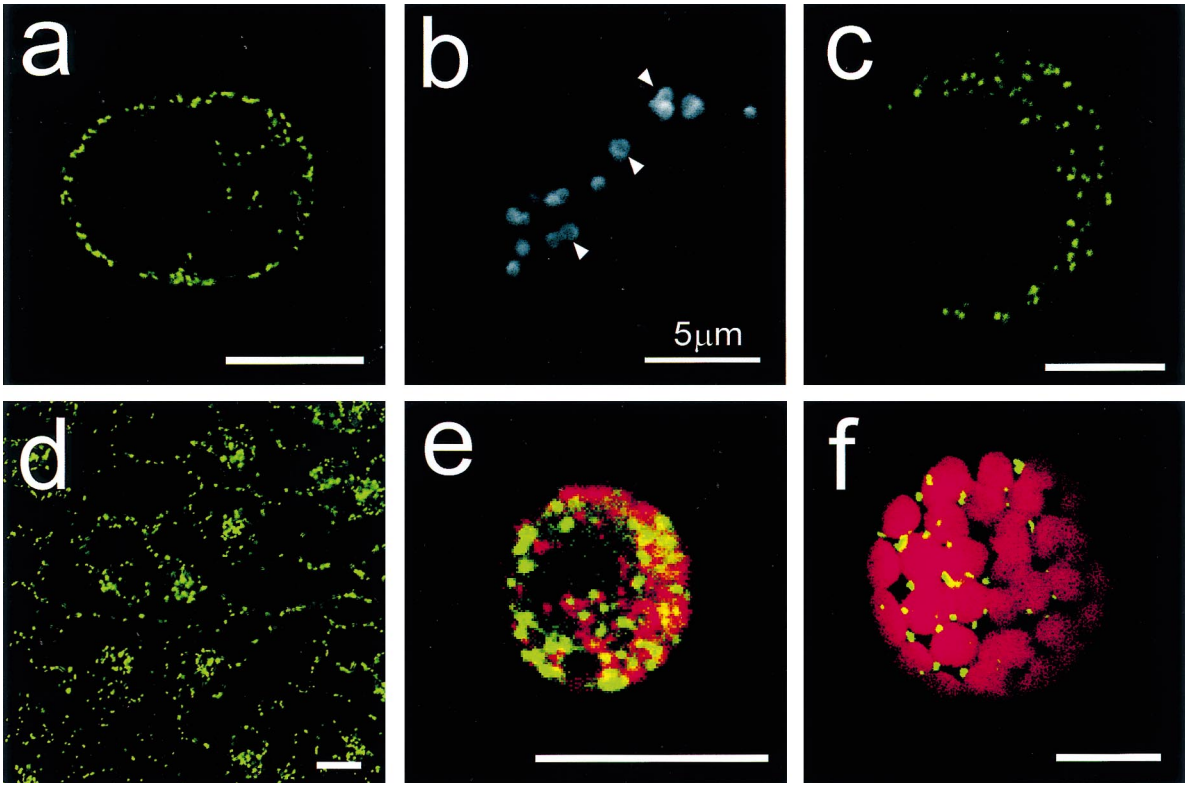

Fig. 2. Distribution pattern of the GFP-tagged type-II H^+-PPase. (a) Dot-like fluorescent structures are observed throughout the cytoplasm in an *Arabidopsis* suspension-cultured cell expressing GFP-AVP2. (b) High magnification of dot-like fluorescent structures. Arrowheads indicate doughnut-shaped or horseshoe-shaped fluorescent structures. (c) The same fluorescent pattern as (a) is observed in cells expressing AVP2-GFP. (d) Dot-like fluorescence of leaf epidermal cells of transgenic plant carrying the construct GFP-AVP2. (e) Dot-like fluorescence of GFP and red fluorescence of Mito-Tracker in an *Arabidopsis* suspension-cultured cell. (f) Dot-like fluorescence of GFP and chlorophyll autofluorescence in leaf protoplast transiently expressing the GFP-AVP2. All bars except (b) indicate 20 μm.

mesophyll cell protoplasts was identical to the method for the suspension culture described above.

2.6. Cell staining reagent

For visualization of mitochondria, GFP-expressing suspension-cultured cells were incubated in MS medium containing 0.4 M mannitol and 50 nM Mito-Tracker Red CM-XRos (Molecular Probes, Inc.) for 15 min. Cells were washed twice with MS medium and observed by confocal laser scanning microscopy (CLSM).

2.7. Brefeldin A (BFA) treatment

BFA was solved in methanol at a concentration of 10 mg/ml as a stock. The suspension-cultured cells expressing the GFP-AVP2 were incubated in MS medium containing 0.4 M mannitol and 0, 1, 10 or 100 μg/ml BFA, respectively. Methanol in the reaction was adjusted to the same concentration (1% v/v) in each experiment. The change of fluorescence was observed by CLSM.

2.8. Immunofluorescent technique

Immunofluorescent staining was performed as described by Paris et al. [21] with some modifications. Protoplasts prepared from suspension-cultured *Arabidopsis* cells or leaf mesophyll tissue were fixed at room temperature for 20 min in a fixation buffer containing 3.7% formaldehyde, 5 mM EGTA, 50 mM potassium phosphate (pH 7.0) and 3% mannitol. Cell adherence to coverslips was improved by pretreatment of coverslips with 1% poly-L-lysine (Sigma). Cell membranes were permeabilized by a 5 min treatment in 0.5% Triton X-100. Prior to incubation with the primary antibodies, non-specific sites were blocked by 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 min. Each antibody was diluted in 0.25% BSA, 0.25% gelatin, 0.05% Nonidet P40, and 0.02% Na azide in PBS and incubated with cells for 1 h at room temperature. Between incubations, the cells were washed for 30 min in buffer alone.

2.9. CLSM

The fluorescent images were observed using a Zeiss Pascal-5 confocal laser microscope. GFP was excited by 488 nm Ar laser. Mito-Tracker Red, chlorophyll autofluorescence and rhodamine were excited by 534 nm green He–Ne laser.

3. Results and discussion

3.1. AVP2/AVPL1 promoter is mainly activated in trichome and filament of stamen

We investigated the tissue distribution of AVP2/AVPL1 expression by using a promoter–GUS reporter system. Approximately 1.4 kb and 0.4 kb 5′-upstream regions of the AVP2/AVPL1 gene were transcriptionally fused to the GUS reporter gene and these reporter constructs were stably introduced into *A. thaliana*. No obvious staining differences were observed between 1.4 kb and 0.4 kb promoter–reporter constructs (data not shown). In young seedlings, cotyledons and roots were more strongly stained than young rosette leaves (Fig. 1a). In rosette leaves, trichomes were extensively stained (Fig. 1a,c,d). Trichome is a huge single cell that is highly vacuolated [22]. In flowers, sepals and stamen filaments were strongly stained, although the anther was hardly stained (Fig. 1b). Stamen filaments develop rapidly with cell enlargement at the final stage of floral development [23]. This staining pattern suggested that the expression of type-II H^+-PPase is up-regulated during cell expansion. The expression pattern of AVP2/AVPL1 was different from that of AVP1 especially in anther. AVP1 is strongly expressed in pollen, but is not expressed in stamen filaments (Mitsuda et al., unpublished data).

3.2. GFP-tagged type-II H^+-PPase produces dot-like fluorescent structures throughout the cytoplasm

To examine the subcellular localization of type-II H^+-PPase, we expressed the GFP-tagged type-II H^+-PPase in *Arabidopsis* suspension-cultured cells and in transgenic plants. GFP was fused to the N-terminus (GFP-AVP2) or C-terminus (AVP2-GFP) of type-II H^+-PPase. In the cells expressing GFP-AVP2, bright clear small dot-like fluorescent structures were observed throughout the cytoplasm under a fluorescent microscope (Fig. 2a). AVP2-GFP also produced the same fluorescent pattern, but with much lower fluorescent intensity than GFP-AVP2 (Fig. 2c). Thereafter, we decided to use GFP-AVP2 for the following experiment. Although we can not rule out the possibility that the type-II H^+-PPase is localized to the dot-like structures due to an influence of GFP fusion, the identical fluorescent pattern of GFP-AVP2 and AVP2-GFP suggests that the localization of both fusion proteins reflects that of the authentic protein. In the transient expression condition, occasionally, GFP fluorescence was observed in the vacuolar lumen (data not shown). This inside-vacuolar staining pattern implies that the vacuole is the final

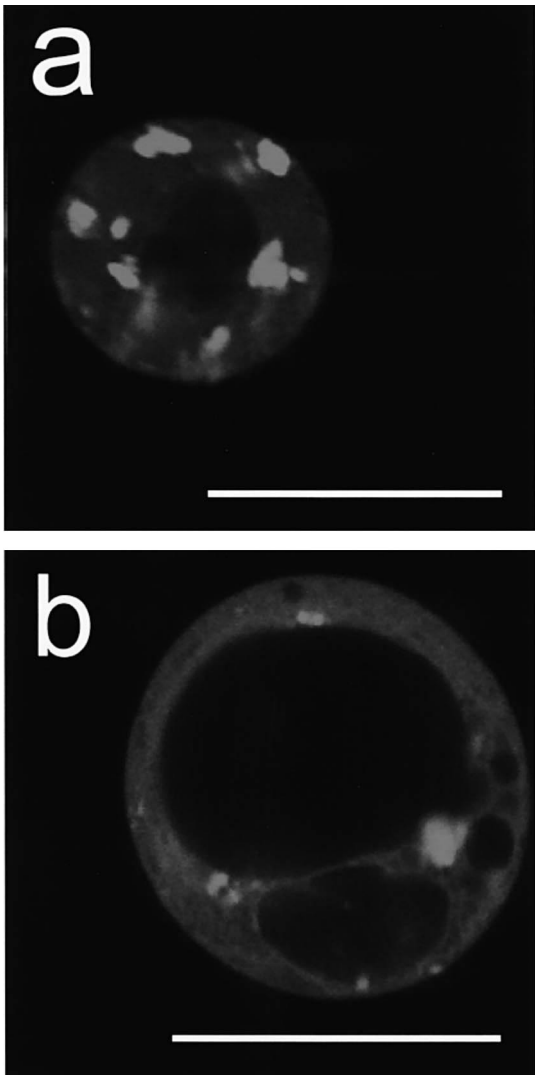

Fig. 3. The effect of BFA treatment on the distribution pattern of GFP-tagged type-II H^+-PPase. The cells expressing the GFP-AVP2 were treated with BFA for 2 h in various concentrations. (a) Most dot-like structures seemed to be fused and form several large aggregated structures. (b) Most dot-like and aggregated structures disappeared and network-like fluorescence could be recognized. Only methanol in the reaction (1% v/v) had no effect on the localization of the protein. All bars indicate 20 μm.

destination of GFP-AVP2 in a transient condition, and we merely observed the transition to the final destination. However, the same fluorescent dot-like structures were observed in the transgenic plant carrying GFP-AVP2 (Fig. 2d), suggesting that this dot-like fluorescent pattern indicates the precise localization of the GFP-AVP2 not dependent on the expression condition.

At a higher magnification, some doughnut-shaped or horseshoe-shaped structures were observed in part of the dot-like fluorescent spots (Fig. 2b). Furthermore, some of these dot-like fluorescent organelles were wiggling or moving along cytoplasmic streaming (data not shown). These features are unique characteristics of the plant GA [24,25]. However, in plant cells, there are some other dot-like organelles such as mitochondria [26–28], chloroplast and peroxisome [29] visualized by GFP fluorescence of the localized proteins. To rule out the possibility that the GFP-tagged type-II H^+-PPase is localized to these organelles, we compared the GFP fluorescence with some fluorescent markers of these organelles.

As shown in Fig. 2e, there is no merged fluorescence of the GFP and Mito-Tracker, which is a fluorescent dye that selectively stains mitochondria. Furthermore, to investigate the chloroplast localization of the protein, we also performed a transient analysis in protoplasts of mesophyll cells isolated from rosette leaves, since suspension-cultured cells have no mature chloroplasts. As in the suspension-cultured cell, dot-like fluorescent patterns were observed throughout the cytoplasm, and never merged with chlorophyll autofluorescence (Fig. 2f).

3.3. *BFA treatment affects the localization of the GFP-tagged type-II H^+-PPase*

We investigated the effect of BFA treatment on the localization of the GFP-tagged type-II H^+-PPase. BFA is a fungal antibiotic that specifically blocks the anterograde protein transport from the endoplasmic reticulum (ER) to the GA and results in redistribution of Golgi resident proteins into the ER [30,31]. In plant cells, this reagent also affects GA structure and the distribution of Golgi resident proteins although some differences from mammalian cells were observed [32–34]. We followed the morphological change of the fluorescent pattern of GFP-tagged type-II H^+-PPase after the addition of BFA. After 2 h incubation at a concentration of 100 μg/ml BFA, the dot-like fluorescent structures disappeared and some aggregated structures (Fig. 3a), and/or network-like structure (Fig. 3b) emerged in most cells. The oval-shaped structure seemed to be the 'BFA compartment' reported previously [32,34]. Although we did not determine whether the network-like structure is ER or not, it is apparent that the distribution of dot-like fluorescent organelle is af-

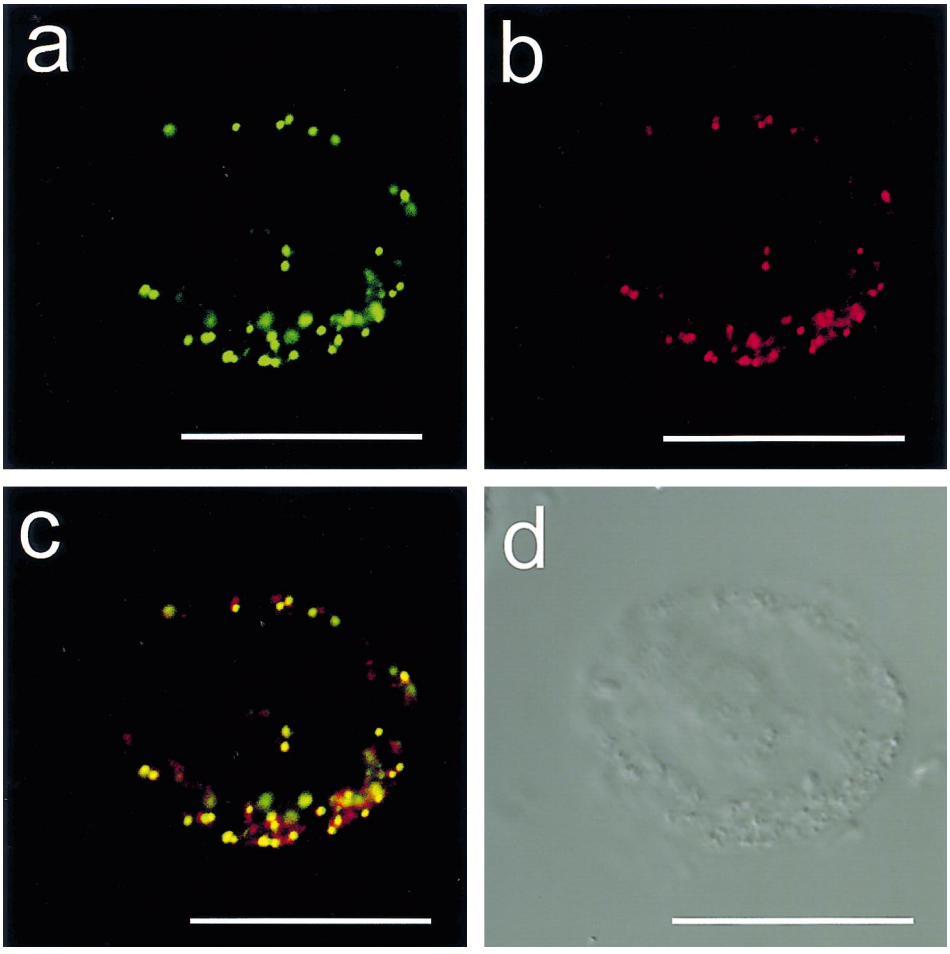

Fig. 4. The GFP-tagged type-II H^+-PPase colocalized with Golgi resident protein, RGP1. (a) Green dots correspond to the GFP signals. (b) Red signals reveal the immunodetected RGP1. (c) Merged image of (a) and (b). Yellow indicates colocalization of the GFP signal and the immunodetected RGP1. (d) Differential interference contrast image of the same cell. All bars indicate 20 μm.

fected by BFA treatment. These data indicate that dot-like fluorescent organelle is apparently BFA sensitive, suggesting that the dot-like fluorescent structure is a GA.

3.4. GFP-tagged type-II H^+-PPase is colocalized with Golgi resident protein

We probed the *Arabidopsis* GA with anti-RGP1 antibodies raised against the *trans*-Golgi localized reversibly glycosylated polypeptide (RGP1) of pea, because pea and *Arabidopsis* have a similar amino acid sequence of RGP1 [35]. In suspension-cultured cells transiently expressing the GFP-AVP2, the most GFP fluorescence was merged with the fluorescence marked by the RGP1 antibody (Fig. 4). This strongly indicates that the dot-like fluorescent organelle is the GA. Therefore, we conclude that type-II H^+-PPase is localized to the GA. Indeed, the existence of H^+-PPase in GA has been demonstrated by measuring the PPi-dependent proton pumping activity [6] and by detecting immunologically [7–9]. V-type H^+-ATPase is actually distributed to the plant GA membrane [7,36–39]. Matsuoka et al. [40] reported that the acidic environment generated by Golgi resident V-ATPase is important for the sorting of soluble vacuolar proteins. It is likely that the type-II H^+-PPase functions as an H^+ pump of GA in combination with GA resident V-ATPase for generating the acidic environment.

The signals responsible for the subcellular localization of both H^+-PPases remain to be identified. The GFP-tagged type-II H^+-PPase may be a useful tool for investigating the Golgi retention mechanism of multi-transmembrane protein in plant cell.

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References

[1] Maeshima, M. (2000) Biochim. Biophys. Acta 1465, 37–51.
[2] Nakanishi, Y. and Maeshima, M. (2000) Plant Physiol. 122, 620.
[3] Drozdowicz, Y.M., Kissinger, J.C. and Rea, P.A. (2000) Plant Physiol. 123, 353–362.
[4] Rea, P.A. and Poole, R.J. (1985) Plant Physiol. 77, 46–52.
[5] Wang, Y., Leigh, R.A., Kaestner, K.H. and Sze, H. (1986) Plant Physiol. 81, 497–502.
[6] Chanson, A., Fichmann, J., Spear, D. and Taiz, L. (1985) Plant Physiol. 79, 159–164.
[7] Oberbeck, K., Drucker, M. and Robinson, D.G. (1994) J. Exp. Bot. 45, 235–244.
[8] Ratajczak, R., Hinz, G. and Robinson, D.G. (1999) Planta 208, 205–211.
[9] Robinson, D.G., Haschke, H.P., Hinz, G., Hoh, B., Maeshima, M. and Marty, F. (1996) Planta 198, 95–103.
[10] Zancani, M., Macri, F., Dal Belin Perufo, A. and Vianello, A. (1995) Eur. J. Biochem. 228, 138–143.
[11] Jiang, S.S., Yang, S.J., Kuo, S.Y. and Pan, R.L. (2000) FEBS Lett. 468, 211–214.
[12] Jiang, S.S., Fan, L.L., Yang, S.J., Kuo, S.Y. and Pan, R.L. (1997) Arch. Biochem. Biophys. 346, 105–112.
[13] Krishnan, V.A. and Gnanam, A. (1988) Arch. Biochem. Biophys. 260, 277–284.
[14] Niwa, Y., Hirano, T., Yoshimoto, K., Shimizu, M. and Kobayashi, H. (1999) Plant J. 18, 455–463.
[15] Matsuoka, K. and Nakamura, K. (1991) Proc. Natl. Acad. Sci. USA 88, 834–838.
[16] Hellens, R., Mullineaux, P. and Klee, H. (2000) Trends Plant Sci. 5, 446–451.
[17] Bechtold, N., Ellis, J. and Pelletier, G. (1993) C.R. Acad. Sci. Paris Life Sci. 316, 1194–1199.
[18] Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987) EMBO J. 6, 3901–3907.
[19] Glab, N., Labidi, B., Qin, L.-X., Trehin, C., Bergounioux, C. and Meijer, L. (1994) FEBS Lett. 353, 207–211.
[20] Takeuchi, M., Ueda, T., Sato, K., Abe, H., Nagata, T. and Nakano, A. (2000) Plant J. 23, 517–525.
[21] Paris, N. and Rogers, J.C. (1996) Plant Physiol. Biochem. 34, 223–227.
[22] Bowman, J.L. (1993) Vegetative development, in: *Arabidopsis*: an Atlas of Morphology and Development (Bowman, J.L., Ed.), pp. 1–82, Springer-Verlag, New York.
[23] Bowman, J.L. (1993) Flowers, in: *Arabidopsis*: an Atlas of Morphology and Development (Bowman, J.L., Ed.), pp. 133–145, Springer-Verlag, New York.
[24] Boevink, P., Oparka, K., Santa cruz, S., Martin, B., Betteridge, A. and Hawes, C. (1998) Plant J. 15, 441–447.
[25] Nebenfuhr, A., Gallagher, L.A., Dunahay, T.G., Frohlick, J.A., Mazurkiewicz, A.M., Meehl, J.B. and Staehelin, L.A. (1999) Plant Physiol. 121, 1127–1141.
[26] Kohler, R.H., Zipfel, W.R., Webb, W.W. and Hanson, M.R. (1997) Plant J. 11, 613–621.
[27] Akashi, K., Grandjean, O. and Small, I. (1998) FEBS Lett. 431, 39–44.
[28] Galvez, S., Roche, O., Bismuth, E., Brown, S., Gadal, P. and Hodges, M. (1998) Proc. Natl. Acad. Sci. USA 95, 7813–7818.
[29] Hayashi, M., Tsugeki, R., Kondo, M., Mori, H. and Nishimura, M. (1996) Plant Mol. Biol. 30, 183–189.
[30] Misumi, Y., Miki, K., Takatsuki, G. and Ikehara, Y. (1986) J. Biol. Chem. 261, 11398–11403.
[31] Lippincott-Schwartz, J., Yuan, L., Bonifacino, J. and Klausner, R. (1989) Cell 56, 801–813.
[32] Saitat-Jeunemaitre, B. and Hawes, C. (1992) J. Cell Sci. 103, 1153–1166.
[33] Saitat-Jeunemaitre, B. and Hawes, C. (1993) Cell Biol. Int. 17, 183–193.
[34] Saitat-Jeunemaitre, B., Boevink, P. and Hawes, C. (1999) Biochimie 81, 597–605.
[35] Dhugga, K.S., Tiwari, S.C. and Ray, P.M. (1997) Proc. Natl. Acad. Sci. USA 94, 7679–7684.
[36] Chanson, A. and Taiz, L. (1985) Plant Physiol. 78, 232–240.
[37] Manadala, S. and Taiz, L. (1985) Plant Phsyiol. 78, 104–109.
[38] Ali, M.S. and Akazawa, T. (1986) Plant Physiol. 81, 222–227.
[39] Herman, E.M., Li, X., Su, R.T., Larsen, P., Hsu, H.-T. and Sze, H. (1994) Plant Physiol. 106, 1313–1324.
[40] Matsuoka, K., Higuchi, T., Maeshima, M. and Nakamura, K. (1997) Plant Cell 9, 533–546.