Distinct Localization of Two Closely Related Ypt3/Rab11 Proteins on the Trafficking Pathway in Higher Plants*

Takehito Inaba, Yukio Nagano, Takeshi Nagasaki, and Yukiko Sasaki§

From the Laboratory of Plant Molecular Biology, Graduate School of Agricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

Ypt/Rab proteins are Ras-related small GTPases that act on the intracellular membrane through the trafficking pathway, and their function depends on their localization. Approximately 25 genes encoding Ypt3/Rab11-related proteins exist in Arabidopsis, but the reason for the presence of many genes in plants remains unclear. Pea Pra2 and Pra3, members of Ypt3/Rab11, are closely related proteins. Because possible orthologs are conserved among dicots, they can be studied to determine their possible localization. Biochemical analysis revealed that these proteins were localized on distinct membranes in pea. Furthermore, using green fluorescent protein-Pra2 and green fluorescent protein-Pra3 fusion proteins, we demonstrated that these proteins are distinctively localized on the trafficking pathway in tobacco Bright Yellow 2 cells. Pra2 was predominantly localized on Golgi stacks and endosomes, which did not support the localization of Pra on the endoplasmic reticulum (Kang, J. G., Yun, J., Kim, D. H., Chung, K. S., Fujioka, S., Kim, J. L., Dae, H. W., Yoshida, S., Takatsuto, S., Song, P. S., and Park, C. M. (2001) Cell 105, 625–636). In contrast, Pra3 was likely to be localized on the trans-Golgi network and/or the prevacuolar compartment. We concluded that Pra2 and Pra3 proteins are distinctively localized on the trafficking pathway. This finding suggests that functional diversification takes place in the plant Ypt3/Rab11 family.

Ypt/Rab proteins constitute a subfamily of the Ras-related small GTPases and are implicated in the regulatory events of vesicle trafficking (1). Rab regulates vesicle transport from the donor to the acceptor compartment by cooperating with interacting proteins. Different Rabs are localized on distinct vesicles and organelles, and the function of each Rab protein depends on its localization (1). Genome sequences of several model organisms have shown that the Rab family has significantly expanded from yeast to human. The human genome contains 60 Rabs, whereas those of the fruit fly, nematode worm, and yeast have 26, 29, and 11 Rabs, respectively (2). Arabidopsis, a model of higher plants, is one of the organisms whose entire genome has been sequenced (3). Interestingly, despite the small size of its genome, Arabidopsis has more than 50 Ypt/Rab GTPases. Ypt/Rab GTPases are subdivided into subgroups, one of which is Ypt3/Rab11. Yeast and human genomes have two (Ypt31 and Ypt32) and three (Rab11A, Rab11B, and Rab25) genes belonging to Ypt3/Rab11, respectively, whereas Arabidopsis has more than 25 related genes (Fig. 1). Usually, proteins of the Rab subgroup with a similar structure have been believed to have similar functions in yeast and human. However, it has been recently shown that proteins belonging to one subgroup do not always have similar functions (1). The reason that plants have so many Ypt3/Rab11 proteins is still not known. Probably, each Ypt3/Rab11 protein in higher plants has a different localization and function, but evidence is lacking because there have been only a few reports of Ypt3/Rab11 localization in higher plants (4).

Pea Pra2 and Pra3 proteins are members of the Ypt3/Rab11 family (5, 6). Both have some common features as follows. 1) The expression of these genes is negatively regulated by light mediated by photoreceptor phytochromes (7–10). 2) Both proteins are mainly expressed in the etiolated epicotyls (8). 3) Their primary structures are closely related, and the proteins are present on the adjacent branches of the phylogenetic tree (Fig. 1 and Ref. 5). Sequence similarity searches revealed that the possible orthologs of Pra2 and Pra3 proteins were found in the Arabidopsis genome (GenPept accession numbers AAF97325 and BAB11663, respectively) (Fig. 1) and in Lotus japonicus (11) and that Pra2 and Pra3 proteins are probably conserved among dicots. It is interesting to examine whether these similar proteins, Pra2 and Pra3, are localized on distinct subcellular membranes engaging in different functions.

The mechanism of light-regulated expression of the PRA2 gene has been extensively studied (9, 10, 12), but its precise function remains unclear. A recent report (13) postulated that the Pra2 protein is a mediator for the cross-talk between light and brassinosteroids in the etiolation process. In that report, Kang et al. (13) claims that Pra2 is localized on the endoplasmic reticulum (ER) and regulates the activity of the DWF1 protein, which belongs to the P450 family and catalyzes the hydroxylation step of brassinosteroids by direct interaction on the ER. Transgenic analysis led them to conclude that Pra2 and its orthologs are regulators of brassinosteroid biosynthesis. However, Pra2 is a Rab GTPase, and experiments using yeast cells suggest that Pra2 has a function as a Rab GTPase and is likely to be involved in vesicle transport (6). It is important to answer the question of whether the Pra2 protein is localized on the ER.

* This work was supported by Grants-in-aid 12760225 for the Encouragement of Young Scientists and 13017210 for Priority (to Y. N.) from the Japanese Ministry of Education, Culture, Science, Sports, and Technology. This work was also supported by the Japan Society for the Promotion of Science Research for the Future Program Grant JSPS-RFTF 96L006012 (to Y. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipient of Research Fellowships from the Japan Society for the Promotion of Science for Young Scientists.

§ To whom correspondence should be addressed. Tel.: 81-52-789-4165; Fax: 81-52-789-4296; E-mail: sasaki@agr.nagoya-u.ac.jp.

1 The abbreviations used are: ER, endoplasmic reticulum; DTT, dithiothreitol; GFP, green fluorescent protein; RFP, red fluorescent protein; BFA, brefeldin A; TGN, trans-Golgi network; BY, Bright Yellow; SNARE, soluble NSF attachment protein receptors.
and regulates DDWF1 activity without participating in vesicle transport.

In this report, we investigated the subcellular localization of the Pra2 and Pra3 proteins. Using biochemical and cytological methods, we have shown that the Pra2 protein is predominantly localized on Golgi and endosomes, whereas the Pra3 protein is likely to be localized on the trans-Golgi network and/or prevacuolar compartment. Our study yielded no evidence that Pra2 was localized on the ER. Our results indicate that Pra2 and Pra3 were distinctively localized on the trafficking pathway. Furthermore, they suggest that functional diversification takes place in the plant Ypt3/Rab11 family.

**EXPERIMENTAL PROCEDURES**

**Phylogenetic Analysis—Ypt3/Rab11 proteins** were collected using either the TBlastN program against the complete genome sequence of Arabidopsis or the SMART web tool (14). Arabidopsis proteins are distinguished by their GenPept accession numbers. If one protein has more than one accession number, only a representative one is shown. Related proteins from yeast, human, and other plant species are also included. Branch lengths are proportional to sequence divergence and can be measured relative to the bar shown (left bottom). Branch labels record the stability of branches over 1,000 bootstrap replications (indicated by % values). Hs, Homo sapiens; Lj, L. japonicas; Nt, Nicotiana tabacum; Ps, Pisum sativum; Sc, Saccharomyces cerevisiae.

**Sucrose Density Gradient Centrifugation**—The supernatant was overlaid on a cushion buffer containing 50 mM 2mM EDTA, 1 mM DTT, 0.25 M sucrose, 1.5% Polyclar AT, and a proteinase inhibitor mixture (Complete, Roche Molecular Biochemicals). After cutting into pieces in a buffer, the sample was homogenized with a homogenizer (Hitachi). The homogenate was filtered through two layers of Miracloth and then centrifuged at 6,000 × g for 10 min. The supernatant was overlaid on a cushion buffer containing 50 mM glycylglycine-NaOH, pH 7.5, 1 mM EDTA, 1 mM DTT, and 50% (w/v) sucrose. The sample was then centrifuged at 100,000 × g for 2 h and then separated into 0.3-mL fractions. Aliquots (180 µL) of fractions were mixed with a 4 × SDS-PAGE loading buffer (60 µL), boiled for 3 min, and then stored at −20°C. For the enzyme assay, the rest of the samples were stored at −80°C until use.

The experiments described in Fig. 3 were carried out as described above with the exception of some modifications to a buffer. Pea epicytols were homogenated in a buffer containing 50 mM glycylglycine-NaOH, pH 7.5, 1 mM EDTA, 1 mM MgCl₂, 1 mM DTT, 0.25 µM sucrose, 1.5% Polyclar AT, and a protease inhibitor mixture, Complete. The 6,000 × g supernatant was overlaid on a cushion buffer containing 50 mM glycylglycine-NaOH, pH 7.5, 1 mM EDTA, 1 mM MgCl₂, and 50% (w/v) sucrose. After 100,000 × g centrifugation, the interface layer was collected, and the sucrose density was adjusted to 10% (w/w). This microsomal fraction was finally applied to the linear sucrose density gradient (14 ml) of 38–15% (w/w). The gradient was buffered in 50 mM glycylglycine-NaOH, pH 7.5, and 1 mM EDTA containing either 1 mM EDTA (Fig. 3A) or 1 mM MgCl₂ (Fig. 3B). After centrifugation, samples were collected as described above.

**Construction of Plasmids and Transformation**—Green fluorescent protein (GFP) fusion genes were constructed as follows. The desired cDNA was amplified by Ffu DNA polymerase (Stratagene) subcloned into the EcoRI site of a pEZEr-2.1 vector (Invitrogen) and digested with restriction enzymes. Digested fragments were subcloned into the BglII-EcoRI sites of a pMAT-137 vector (a gift from Dr. K. Matsuoka) containing the GFP gene. The fusion gene was driven by the cauliflower mosaic virus 35S promoter-nopaline synthase terminator system. As a result, the desired gene was fused to the C terminus of GFP. The resulting plasmid was transformed into tobacco Bright Yellow 2 (BY-2) cells using Agrobacterium tumefaciens (16).

**Sucrose Density Gradient Centrifugation**—The supernatant was overlaid on a cushion buffer containing 50 mM glycylglycine-NaOH, pH 7.5, 2 mM EDTA, 1 mM DTT, 0.25 M sucrose, 1.5% Polyclar AT, and a protease inhibitor mixture (Complete, Roche Molecular Biochemicals). After cutting into pieces in a buffer, the sample was homogenized with a homogenizer (Hitachi). The homogenate was filtered through two layers of Miracloth and then centrifuged at 6,000 × g for 10 min. The supernatant was overlaid on a cushion buffer containing 50 mM glycylglycine-NaOH, pH 7.5, 1 mM EDTA, 1 mM DTT, and 50% (w/v) sucrose. The sample was then centrifuged at 100,000 × g for 30 min. The interface layer (microsomes) between the upper and lower layers was collected, and the sucrose concentration of the sample was adjusted to 18% (w/w). This microsomal fraction was finally applied to the linear sucrose density gradient (14 ml) of 42–19% (w/w). The gradient was buffered in 50 mM glycylglycine-NaOH, pH 7.5, 1 mM EDTA, and 1 mM DTT. The sample was centrifuged at 100,000 × g for 2 h. After centrifugation, fractions were collected from the bottom to the top of the gradients, and odd-numbered fractions between 13 and 53 were analyzed. ER marker, NADPH-cytochrome c reductase; Golgi marker, inosine diphosphatase.

**Measurement of Enzyme Activities and Immunoblotting**—Enzyme activities, such as inosine diphosphatase and NADPH-cytochrome c
Pra2 and Pra3 Proteins Are Localized on Distinct Subcellular Membranes in Pea—Because the function of Ypt/Rab GTPase depends on its localization, it is necessary to confirm the localization of Pra2 and Pra3 proteins on subcellular membranes. First, we performed biochemical analysis using the growing zone of the etiolated pea epicotyl where both Pra2 and Pra3 were the richest. A crude microsomal fraction was fractionated on a sucrose density gradient buffered in glycylglycine-NaOH (Fig. 2). The distributions of markers in Fig. 2 were similar to those in the previous report (24). Pra2 was strongly observed in fractions 39–45, whereas Pra3 was enriched in fractions 33–39 (Fig. 2), indicating that these proteins are localized on distinct subcellular membranes in pea. Because both the ER and Golgi were distributed over the light density area under this condition, we could not predict the precise localization of Pra2 and Pra3.

It is important to identify the localization of Pra2, because a recent report claims that Pra2 is localized on the ER in onion epidermal cells (13). To demonstrate protein localization on the ER, “Mg2+-shift” experiments are usually performed. The distribution of the ER is disrupted and shifted to a heavier density area in the presence of Mg2+, but not in its absence. We examined the effects of 3 mM MgCl2 on pea epicotyl membranes (25) and found that this method was not suitable for the analysis of pea epicytol membranes, because the distribution of various membranes as well as that of the ER was altered. We employed a method developed by Lord et al. (26) with some modifications. A microsomal fraction homogenized in a buffer containing both 1 mM EDTA and 1 mM MgCl2 was analyzed by sucrose density gradient centrifugation in the presence of either 1 mM EDTA or 1 mM MgCl2. Under the presence of EDTA, four kinds of membranes as well as that of the ER were distributed over various fractions with- out notable disruption of Golgi (Fig. 3A). Western blot analysis of Pra2 and Pra3 showed that their patterns were different from those of the membrane markers (Fig. 3A). In the presence of 1 mM MgCl2, the ER marker distributed over various fractions without remarkable disruption of Golgi (Fig. 3B). Under such a condition, the patterns of Pra2 and Pra3 were similar to those shown in Fig. 3A, suggesting that the membranes on which Pra2 and Pra3 were localized are not the ER, which contradicts the result of the study on onion epidermal cells. However, this information was insufficient to conclude the localization of Pra2 and Pra3.

Pra2 and Pra3 Proteins Are Distinctively Localized on the Trafficking Pathway in Tobacco BY-2 Cells—To further examine the localization of Pra2 and Pra3 proteins, we next tried to conduct cytological analysis using tobacco BY-2 cells. We made stable transformants of tobacco BY-2 cells expressing GFP-Pra2 or GFP-Pra3 proteins and observed the fluorescence of GFP using a confocal laser-scanning microscope. Both GFP-Pra2 and GFP-Pra3 were localized around the perinuclear cytosol with bright-dot staining (Fig. 4, A and B). The dot structure was a representative pattern of Golgi that has been observed in previous reports (27, 28). To confirm whether the dot structure is actually Golgi, we examined the effect of brefeldin A (BFA). BFA treatment (10 μg/ml) caused the redistribu-
tion of GFP-Pra2 to the ER (Fig. 4C, arrowheads). The results indicate that a part of GFP-Pra2 is localized on Golgi sensitive to BFA. BFA treatment could not cause the redistribution of GFP-Pra3 to the ER (Fig. 4D), again confirming that Pra2 and Pra3 are localized on distinct intracellular membranes. Because BFA treatment cannot cause the redistribution of the protein localized in the trans-Golgi network (TGN) to the ER (29), Pra3 might be localized on the TGN.

To confirm the probable localization of Pra3 on the TGN, we introduced a plasmid carrying the AtVTI11-RFP fusion gene into BY-2 cells expressing GFP-Pra3 by particle bombardment. AtVTI11 is believed to be localized on the TGN and the prevacuolar compartment in plant cells (30). In Arabidopsis cells, fluorescent AtVTI11 has been used as a marker of the pathway from the TGN to the prevacuolar compartment (31). Thus, we constructed an AtVTI11-RFP fusion gene and introduced it into tobacco cells expressing GFP-Pra3. As shown in Fig. 5, most of the red signals derived from AtVTI11-RFP overlapped with the green signals of GFP-Pra3 and turned yellow (Fig. 5, merged). The results of our BFA experiments and those shown in this figure suggest that Pra3 is likely to be localized on the TGN and/or the prevacuolar compartment.

Although Pra2 and Pra3 are localized around Golgi, the appearance of an unspecified structure after BFA treatment (Fig. 4C, arrows) suggested that Pra2 was localized on other intracellular membranes. Thus, we also examined the possible localization of Pra2 and Pra3 on the endocytic pathway using FM4–64, which develops a red color. FM4–64 has been used as an endocytic tracer that travels from the plasma membrane to the vacuolar membrane via the endosomes in yeast (18), Arabidopsis (19), and tobacco BY-2 cells (31). After a 60-min chase-period, the red signals derived from the endosomes overlapped with the green signals of GFP-Pra2 and turned yellow (Fig. 6A, arrowheads), indicating that a part of Pra2 is localized on the endosomes as well as on Golgi. After 3 h, FM4–64 arrived at the vacuolar membranes and showed that this drug could be used as a tracer for endocytosis in BY-2 cells (Fig. 6, C and D). In the case of the GFP-Pra3 transformant (Fig. 6B), only a small number of yellow signals was observed, suggesting that Pra3 is not predominantly localized on the endosomes.

To finally confirm the distinct localization between Pra2 and Pra3, we introduced an RFP-Pra3 fusion gene into cells expressing the GFP-Pra2 protein by particle bombardment. As shown in Fig. 7, GFP-Pra2 was apparently distinguishable from RFP-Pra3 again indicating the distinct localization of Pra2 and Pra3 in a cell. Although the artificial localization of fluorescent proteins cannot be completely excluded, reconfirmation of distinct Pra2 and Pra3 localization in BY-2 cells as well as in pea led us to conclude that fluorescent-tagged proteins are likely to be targeted to membranes precisely in BY-2 cells.

Our results from biochemical analysis and those shown in this figure clearly indicate that closely related Pra2 and Pra3 proteins are distinctively localized on the trafficking pathway in both pea and a model system, that of the tobacco BY-2 cell. Pra2 is predominantly localized on Golgi and endosomes, whereas Pra3 is likely to be localized on the TGN and/or the prevacuolar compartment. Our results were not consistent with those of a previous report (13), according to which the Pra2 was localized on the ER.

**DISCUSSION**

In this study, we examined the subcellular localization of two closely related plant Ypt3/Rab11 GTPases, Pra2 and Pra3. A biochemical analysis demonstrated that these proteins are localized on distinct membranes in pea (Fig. 2). Using GFP fusion proteins, Pra2 was shown to be predominantly localized on Golgi and endosomes, whereas Pra3 was likely to be localized on the trans-Golgi network and/or prevacuolar compartment (Figs. 4–6).

Although the Golgi localization of GFP-Pra2 seems to be inconsistent with the results of biochemical analysis, it is well known that the distribution of inosine diphosphatase, a general marker of Golgi, does not fit that of the Golgi protein such as glucuronyltransferase, which is localized on a specific compartment (32). The distinct localization of GFP-Pra2 and RFP-Pra3 (Fig. 7) supports the idea that both proteins are likely to be targeted to membranes precisely in BY-2 cells as observed in pea (Fig. 2), although the possibility of artificial GFP-Pra2 localization on Golgi cannot be completely excluded. We hypothesize that Pra2 is likely to be localized on the specific subcompartment of Golgi and that Pra2 and the Golgi marker were thus observed differently on a sucrose gradient.

This study clearly shows for the first time that functional diversification of Ypt3/Rab11 takes place in plants. In yeast, it has been believed that two isoforms (Ypt31 and Ypt32) have almost identical functions (33, 34). In mammal, Rab11 and Rab25 are expressed in different cells, but their functions were shown to be similar using polarized cells (35). However, our results clearly showed that closely related Pra2 and Pra3 are distinctively localized on the trafficking pathway in the same cell, indicating that they have distinct functions. Such func-
tional diversification of plant Ypt/Rab11 is probably caused by the specific function of the plant Golgi apparatus. In plant cells, matrix polysaccharides such as hemicelluloses are synthesized in Golgi and transported to the cell wall via Golgi-derived vesicles (36); plant cells have to prepare the machinery for delivery of cell wall components and biosynthetic enzymes from Golgi to the plasma membrane. Judging from the fact that Ypt3/Rab11 diversification takes place only in plants, one reason for Ypt3/Rab11 diversification in higher plants might be the necessity of cell wall biogenesis, which is unique to plants. Recent reports also led us to hypothesize other possibilities (37). For example, the inhibition of vesicle trafficking affects the polarized distribution of the efflux carrier of auxin, a plant hormone (37). The implication of vesicle trafficking in such plant hormone transport suggests that Ypt3/Rab11 might be more involved in diverse phenomena in plants than we expected.

Our results also support the proposition of the functional difference of the Ypt3/Rab11 family among different organisms. Ypt3/32 in yeast seems to be localized on the intra-Golgi (33), whereas Rab11 in human was localized on the endosomes (38, 39), providing grounds for proposing a functional difference between Ypt3 and Rab11 (1). However, this is still controversial because no t-SNAREs, a partner of Ypt/Rab, were localized on intra-Golgi stacks in yeast (40). Our finding of Pra2 localization on both endosomes and Golgi suggests that Pra2 protein has a distinct function from both yeast Ypt3 and human Rab11. The result that Pra2 could not complement either temperature- or cold-sensitive ypt3f1 mutants (6) is consistent with this idea. In addition to Ypt3/Rab11, unique diversification within the Rab5 family also takes place. Arabidopsis has Ara6 GTPase, which resembles Rab5 best but has a unique structure (19). Ueda et al. (19) demonstrated that Ara6 and Ara7, a putative authentic ortholog of human Rab5, are differently regulated by the distribution, expression, and localization levels (19). Their findings and our results encourage us to investigate the functional difference of Ypt/Rab GTPase between plants and other organisms.

A recent report (13) showed that Pra2 is localized on the ER and regulates an essential step of brassinosteroid biosynthesis by interacting with DDWF1, which belongs to the P450 family and catalyzes the hydroxylation step of brassinosteroid biosynthesis. Although our finding did not support the existence of Pra2 on the ER (Fig. 3), there is a possibility that Pra2 is a multifunctional protein. A minor fraction of Pra2 might be localized on the ER or be recruited to the ER by additional factors where Pra2 can interact with DDWF1. However, phylogenetic analysis of DDWF1 relatives showed that a gene orthologous to pea DDWF1 is not present in the current data base of the Arabidopsis genome (data not shown). In addition, possible DDWF1 orthologs in tobacco were induced by the inoculation of a phytopathogen (41) or the treatment of a fungal elicitor (42), suggesting that these genes might be involved in the resistance to phytopathogens. The report claimed that suppression of NtRab11D, a proposed ortholog of Pra2 in tobacco, is responsible for reduced brassinosteroid biosynthesis (3). However, our phylogenetic analysis showed that NtRab11D is a Pra3 ortholog (Fig. 1). In view of these facts, further studies would be required to demonstrate that Pra2 is a multifunctional protein that regulates brassinosteroid biosynthesis by interacting with DDWF1.

In summary, we demonstrated that closely related Pra2 and Pra3 proteins are distinctively localized on the trafficking pathway. Our results suggest that functional diversification takes place in the plant Ypt3/Rab11 family.

Acknowledgments—We thank Drs. Ken Matsuoaka and Takashi Ueda for advice throughout our work. We also thank Drs. K. Nakamura and K. M. Mao for transformation of BY-2 cell. M. Maeshima for vacuolar-type ATPase antibody, T. Sugiyama for plasma membrane-type ATPase antibody, H. Mori for membrane fractionation, and Y. Kitaura for confocal microscopy. We would also like to acknowledge Dr. N. Matsuda for critical reading of our manuscript and Dr. M. Takeuchi for helpful advice. Dr. J. Chappell is acknowledged for allowing us to read his manuscript prior to publication.

REFERENCES
1. Chavrier, P., and Goud, B. (1999) Curr. Opin. Cell Biol. 11, 466–475
2. Bock, J. B., Matern, H. T., Peden, A. A., and Scheller, R. H. (2001) Nature 409, 839–841
3. The Arabidopsis Genome Initiative (2000) Nature 408, 798–815
4. Ueda, T., Anai, T., Takayama, H., Hirata, A., and Uchimiya, H. (1996) Mol. Gen. Genet. 250, 533–539
5. Naganuma, N., Mura, T., Matsuno, R., and Sasaki, Y. (1993) Plant Cell Physiol. 34, 447–455
6. Matsuda, N., Ueda, T., Sasaki, Y., and Nakano, A. (2000) Cell Struct. Funct. 25, 11–20
7. Yoshida, K., Naganuma, N., Mura, T., and Sasaki, Y. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6636–6640
8. Naganuma, Y., Okada, Y., Narita, H., Asaka, Y., and Sasaki, Y. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6314–6318
9. Inaba, T., Naganuma, Y., Sakakibara, T., and Sasaki, Y. (1999) Plant Physiol. (Bethesda) 120, 491–499
10. Inaba, T., Naganuma, Y., Reid, J. B., and Sasaki, Y. (2000) J. Biol. Chem. 275, 19723–19727
11. Berg, S., Brandstrup, B., Jensen T. J., and Poulsen, C. (1997) Plant J. 11, 237–240
12. Naganuma, N., Inaba, T., Furuhashi, H., and Sasaki, Y. (2001) J. Biol. Chem. 276, 22238–22243
13. Kang, J. G., Yun, J., Kim, D. H., Chung, K. S., Fujioke, S., Kim, J. I., Dae, H. W., Yoshida, S., Takatsuto, S., Song, P. S., and Park, C. M. (2001) Cell 105, 625–636
14. Schultz, J., Copley, R. R., Doerks, T., Ponting, C. P., and Bork, P. (2000) Nucleic Acids Res. 28, 231–234
15. Thompson, J. H., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) Nucleic Acids Res. 25, 4876–4882
16. Matsuoka, K., and Nakamura, K. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 834–838
17. Kitaura, Y., Matsumoto, S., Satoh, H., Hitomi, K., and Maki, M. (2001) J. Biol. Chem. 276, 14053–14058
18. Yahara, N., Ueda, T., Sato, K., and Nakano, A. (2001) Mol. Biol. Cell 12, 221–238
19. Ueda, T., Yamaguchi, M., Uchimiya, H., and Nakano, A. (2001) EMBO J. 20, 4730–4741
20. Matsuoka, K., Watanabe, N., and Nakamura, K. (1995) Plant J. 8, 877–889
21. Fujita, M., and Asahi, T. (1985) Plant Cell Physiol. 26, 389–395
22. Matsuura-Endo, C., Maeshima, M., and Yoshida, S. (1992) Plant Physiol. (Bethesda) 100, 718–722
Distinct Localization of Ypt3/Rab11 in Higher Plants

23. Nagao, T., Sasakawa, H., and Sugiyama, T. (1987) Plant Cell Physiol. 28, 1181–1186
24. Ali, M. S., Nishimura, M., Mitsui, T., Akazawa, T., and Kojima, K. (1985) Plant Cell Physiol. 26, 1119–1133
25. Ahmed, S. U., Bar-Peled, M., and Raikhel, N. V. (1997) Plant Physiol. (Bethesda) 114, 325–336
26. Lord, J. M., Kagawa, T., Moore, S. T., and Beevers, H. (1973) J. Cell Biol. 57, 659–667
27. Nebenführ, A., Gallagher, L. A., Dunahay, T. G., Frohlick, J. A., Mazurkiewicz, A. M., Meek, J. B., and Staehelin, L. A. (1999) Plant Physiol. (Bethesda) 121, 1127–1141
28. Takeuchi, M., Ueda, T., Sato, K., Abe, H., Nagata, T., and Nakano, A. (2000) Plant J. 23, 517–525
29. Chege, N. W., and Pfeffer, S. R. (1990) J. Cell Biol. 111, 893–899
30. Zheng, H., von Mollard, G. F., Kovaleva, V., Stevens, V., and Raikhel, N. V. (1999) Mol. Biol. Cell 10, 2251–2264
31. Kim, D. H., Eu, Y. J., Yoo, C. M., Kim, Y. W., Pih, K. T., Jin, J. B., Kim, S. J., Stenmark, H., and Hwang, I. (2001) Plant Cell 13, 287–301
32. Hobbs, M. C., Delarge, M. H., Baydoun, E. A., and Brett, C. T. (1991) Biochem. J. 277, 653–658
33. Benil, M., Döring, F., Robinson, D. G., Yang, X., and Gallwitz, D. (1996) EMBO J. 15, 6460–6475
34. Jedd, G., Mulholland, J., and Segev, N. (1997) J. Cell Biol. 137, 563–580
35. Wang, X., Kumar, R., Navarre, J., Casanova, J. E., and Goldenring, J. R. (2000) J. Biol. Chem. 275, 29138–29146
36. Taiz, L., and Zeiger, E. (1998) Plant Physiology, 2nd Ed, p. 419, Sinauer Associates, Inc., Sunderland, MA
37. Geldner, N., Friml, J., Stierhof, Y. D., Jurgens, G., and Palme, K. (2001) Nature 413, 425–428
38. Sünnichen, B., De Renzi, S., Nielsen, E., Rietdorf, J., and Zerial, M. (2000) J. Cell Biol. 149, 901–913
39. Wilcke, M., Johannes, L., Galli, T., Mayau, V., Goud, B., and Salamero, J. (2000) J. Cell Biol. 151, 1207–1220
40. Pelham, H. R. (1998) Trends Cell Biol. 8, 45–49
41. Czernic, P., Huang, H. C., and Marco, Y. (1996) Plant Mol. Biol. 31, 255–265
42. Ralston, L., Kwon, S. T., Schoenbeck, M., Ralston, J., Schenk, D. J., Coates, R., and Chappell, J. (2001) Arch. Biochem. Biophys. 393, 222–235
Distinct Localization of Two Closely Related Ypt3/Rab11 Proteins on the Trafficking Pathway in Higher Plants
Takehito Inaba, Yukio Nagano, Takeshi Nagasaki and Yukiko Sasaki

J. Biol. Chem. 2002, 277:9183-9188.
doi: 10.1074/jbc.M111491200 originally published online December 26, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M111491200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 21 of which can be accessed free at http://www.jbc.org/content/277/11/9183.full.html#ref-list-1