Phosphoinositide 3-Kinases and Their FYVE Domain-containing Effectors as Regulators of Vacuolar/Lysosomal Membrane Trafficking Pathways*

Andrew E. Wurmser, Jonathan D. Gary, and Scott D. Emr‡

From the Division of Cellular and Molecular Medicine and Howard Hughes Medical Institute, University of California at San Diego, School of Medicine, La Jolla, California 92039-0668

Kinases that phosphorylate phosphatidylinositol (PtdIns)³ at specific position(s) of the inositol ring play critical regulatory roles in a diverse array of cellular functions including growth, differentiation, apoptosis, and cytoskeletal rearrangement (1–3). The downstream effects of phosphoinositide (PI) kinases (e.g. p110 PI 3-kinase) are carried out by phosphorylated derivatives of PtdIns, which serve as second messengers that recruit effector proteins to specific subcellular localizations and/or influence their activity (4, 5). In addition to the classical signaling roles of lipid kinases at the plasma membrane, the activity of these kinases is also required for membrane trafficking along the secretory and endocytic pathways (6). Vesicle-mediated delivery within the cell entails: (i) the formation and packaging of cargo into transport intermediates (vesicles), a process requiring the activity of coat proteins; (ii) the docking/fusion of such transport intermediates with the appropriate target organelle, which depends upon SNARE proteins and Rab GTPases; and (iii) the recycling of transport components (e.g. receptors and SNARES) (7–9). The lipid composition of vesicular transport intermediates is also critical, and roles for phosphoinositides, phosphati
dic acid, and lysobisphosphatidic acid have been documented (6, 10, 11). In particular, phosphoinositides, which can be modified at specific sites of the inositol ring, either singly or in combination, represent versatile molecules through which the cell generates distinct second messengers. Here we review recent progress in yeast and mammalian systems, which has converged to clarify the function of 3-phosphoinositides in membrane trafficking.

Requirement for PtdIns-3-P in Vesicular Traffic

The importance of PtdIns-3-P in vesicular transport was first revealed during the study of Golgi to vacuole/lysosome transport in yeast (12). The yeast vacuole, an acidified organelle that contains active hydrolytic enzymes, is the functional analog of the mammalian lysosome. In yeast, dramatic decreases in the cellular levels of PtdIns-3-P (24–26). A human homolog of yeast Vps15p, has been identified as a protein kinase that directly interacts with human Vps34 and stimulates its PtdIns-3-kinase activity (33). In yeast, subcellular frac
tionation data localize Vps15p to a Golgi/endoosome-enriched fraction, suggesting a functional role for PtdIns-3-P in membrane trafficking between the Golgi and endosome (30, 31).

Termination or modification of signals mediated by phosphoinositides have classically been attributed to the action of cytoplasmic phospholipases and phosphatases. For example, in mammalian cells PtdIns-4,5-P₂ is cleaved to distinct second messengers by phospholipase C in response to tyrosine kinase and G-protein-coupled receptor activation (34, 35). PtdIns-4,5-P₂ turnover is also mediated by Type II 5-phosphatases like synaptojanin and OCRL (36, 37). Similarly, cytoplasmic phosphatases may carry out the turnover of 3-phosphoinositides as proteins exhibiting phosphatidylinositol 3,4,5-triphosphate 5-phosphatase (e.g. SHIPs) and PtdIns-3-P 3-phosphatase activity have been identified (38) and purified (39), respectively.

In yeast, dramatic decreases in the cellular levels of PtdIns-3-P occur within minutes after inactivation of Vps34p (32), the result of an arrest in PtdIns-3-P synthesis and the continued function of a PtdIns-3-P turnover pathway. Unlike other phosphoinositides, however, PtdIns-3-P may not be degraded within the cytoplasm of the cell. Interestingly, yeast mutants compromised for vacuolar hydrolase activity exhibit severalfold increases in PtdIns-3-P levels in vivo, indicating that the consumption of PtdIns-3-P requires the activity of lumenal vacuolar hydrolases (40). Cellular levels of PtdIns-4-P and PtdIns-4,5-P₂ are not affected in hydrolase-deficient strains (40). Indeed, the vacuole/lysosome contains candidate lipases and phosphatases (41, 42), which may function in the turn-
over of PtdIns-3-P but not other phosphoinositides.

An intact vacuolar transport pathway is also required to deliver PtdIns-3-P from its site of synthesis at the Golgi/endosome to the vacuole. Impairing endosome-to-vacuole transport through the deletion of VAM3 (vacuolar t-SNARE) (43) or YPT7 (Rab GTPase) (44) causes severalfold increases in PtdIns-3-P, presumably by blocking delivery of vacuole-bound PtdIns-3-P that accumulates in a pre-vacuolar endosomal compartment (40).

Vps15p/Vps34p-mediated synthesis of PtdIns-3-P occurs in the cytoplasmic leaflet of the Golgi/endosome membrane. A mechanism is therefore required to overcome the separation between PtdIns-3-P and the vacuolar hydrolases that are required to degrade it. Morphological studies have shown that mutants which stabilize PtdIns-3-P levels also accumulate luminal vesicles within the endosome or vacuole (40, 45). It is likely that a significant pool of PtdIns-3-P is sorted into these vesicles, which invaginate into the endosome or vacuole. These vesicles, together with PtdIns-3-P, are then degraded by hydrolases in the vacuole lumen (see Fig. 1). Thus, the turnover mechanism for the bulk of PtdIns-3-P is distinct from that of other phosphoinositides. In addition, because PtdIns-3-P is present in endosomal and vacuolar membranes, PtdIns-3-P has the capacity to regulate not only Golgi-to-endosome transport but endosome and possibly vacuole function as well. This is especially likely because the progression of internalized cargo along the endocytic pathway is compromised upon inactivation of Vps34p, consistent with a role for PtdIns-3-P in endosome function (40, 46). Thus, the data predict the existence of Golgi, endosomal, and, possibly, vacuolar effectors functioning downstream of the Vps34 PtdIns-3-Kinase.

**FYVE Domain-containing Proteins as Effectors of Vps34 PtdIns 3-Kinase Signaling**

Several proteins have been postulated to function as downstream effectors of PtdIns-3-kinases (47, 48). One of these, the mammalian EEA1 (early endosome antigen 1) protein, is required for homotypic endosome-endosome fusion in vitro (49). Critical to the involvement of EEA1 in this process is its ability to associate with endosomal membranes (49), an interaction that is inhibited by wortmannin (47). EEA1 thus represents a potential PtdIns-3-P-binding protein.

Recent studies have addressed the capacity of EEA1 to directly bind PtdIns-3-P. In vitro, recombinant EEA1 co-sediments with liposomes containing PtdIns-3-P but not PtdIns phosphorylated at other positions of the inositol ring (50–52). The lipid binding activity is attributable to a cysteine-rich sequence motif encoded by the C terminus of EEA1, referred to as the FYVE (Fab1, YGLO23, Vps27, and EEA1) domain (53, 54). FYVE domains coordinate 2 Zn²⁺ ions via 8 cysteine/histidine residues spaced in a specific manner (CX₄CXₓ₃ₓ₄₋ₓₓₓₓₓₓₓₓₓₓₓₓₓₓCXₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓₓ_xy) (54). This protein motif also contains a basic amino acid patch adjacent to the 3rd cysteine residue (50, 54), which is critical for binding of acidic PtdIns-3-P (50). In addition to binding PtdIns-3-P in vitro, Aequorea victoria green fluorescent protein coupled to the EEA1-FYVE domain localizes to endosomal and vacuolar compartments in yeast (50). This localization is dependent on PtdIns 3-Kinase activity, demonstrating that the FYVE domain is sufficient to mediate membrane association in vivo (50). Conversely, deletion of the FYVE domain of EEA1 (54) or the treatment of cells with wortmannin (47) disrupts the endosomal association of this protein.

As mentioned above, overexpression of Rab5 rescues wortmannin-induced inhibition of homotypic endosome fusion (29), suggesting a possible link between PI 3-kinase signaling and Rab5. Consistent with this, overexpression of Rab5 in its active, GTP-bound state is sufficient to restore endosomal association of EEA1 (49). In fact, in addition to binding PtdIns-3-P, EEA1 acts as an effector of Rab5 as it directly interacts with Rab5-GTP (49). Therefore, by defining EEA1 as a PtdIns-3-P-binding protein in vitro and through in vivo studies in yeast, a molecular mechanism for wortmannin inhibition of endosomal trafficking events in mammalian cells has been resolved.

FYVE domains are not unique to EEA1 but present in the mammalian Hrs and yeast Vac1, Vps27, and Fab1 proteins (53, 54). Like the FYVE domain of EEA1, the FYVE domains of these proteins also bind PtdIns-3-P (50, 51). Thus, FYVE domains function as modular PtdIns-3-P binding motifs. Moreover, these FYVE domain-containing proteins play important roles in membrane trafficking events of the secretory and endocytic pathways. Vac1p, the yeast ortholog of EEA1 (47, 55), functions as a multivalent regulatory protein that interacts with the yeast Rab5 GTPase (Vps21p), the endosomal t-SNARE, Pep12p, and the Sec1p homolog, Vps45p (56–60). PtdIns-3-P, together with the GTP-bound Vps21p, may regulate the ordered series of biochemical interactions between Vac1p and these other proteins, which together are required to ensure the high fidelity of vesicle (CPY-containing) docking/fusion with the vacuole (58). Vps27p, the likely yeast counterpart of mammalian Hrs (61), mediates the maturation of endosomes (e.g., receptor recycling, multivesicular body formation), a process required for endosome fusion with the vacuole (18, 62). An intact FYVE domain is required for Vps27p function (63), underscoring a role for PtdIns-3-P in endosomal maturation. The FYVE domain protein Fab1 regulates a third, distinct membrane trafficking event. Within 10 min after inactivation of Fab1p a 2.5-fold enlargement of the vacuole occurs (64, 65), suggesting a
role for Fab1p in vacuolar membrane efflux/degradation. Point mutations within the FYVE domain of Fab1p also result in enlarged vacuole phenotypes, revealing that this subregion may be essential for Fab1p localization or activity (see below). These results define the Vps34 PtdIns 3-kinase as a regulatory kinase that modulates multiple downstream effectors which act at distinct stages of membrane trafficking to and from the vacuole. Two additional FYVE domain-containing open reading frames are present within the genome and whereas a cellular function has yet to be assigned to these proteins, they also promise to be downstream effectors of Vps34p.

Conversion of PtdIns-3-P, a Signal for Anterograde Traffic to the Vacuole, to PtdIns-3,5-P2, a Signaling Lipid Required for Vacuole Membrane Homeostasis

PtdIns-3,5-P2 is a newly identified phosphoinositide, discovered both in yeast and higher eukaryotic cells (66, 67). This lipid is synthesized directly from a preexisting pool of PtdIns-3-P, indicating the existence of a PtdIns-3-P 5-kinase (66, 67). Thus, yeast and mammalian cells presumably maintain at least two pathways for the turnover of PtdIns-3-P, one that requires the activity of vacuolar/lysosomal hydrolyases and a second mediated by a 5-kinase, which utilizes PtdIns-3-P as a substrate (40, 66, 67).

Sequence comparisons of the catalytic domains of many known phosphoinositide kinases have allowed grouping of these kinases into separate classes, consistent with their substrate specificities (65). This analysis led to the identification of a new subgroup defined by Fab1p (65), a protein essential to the maintenance of normal vacuole morphology (64). The fact that the C-terminal kinase domain of Fab1p diverges from lipid kinases with known activities suggested that Fab1p could have a distinct substrate specificity (65). Cells lacking Fab1p or expressing Fab1p mutants, which contain point mutations within the kinase domain, produce undetectable levels of PtdIns-3,5-P2, without dramatically affecting the levels of other phosphoinositides (65, 68). In addition, purified full-length Fab1p phosphorylates PtdIns-3-P at the 5-position of the inositol ring (68). Fab1p also contains an N-terminal FYVE domain, which functions to bind PtdIns-3-P (see above) (50). Therefore, Fab1p not only functions as a downstream effector of PtdIns-3-P through its FYVE domain (50) but also converts PtdIns-3-P to PtdIns-3,5-P2, a distinct lipid second messenger (65, 67).

What is the function of PtdIns-3,5-P2? Loss of PtdIns-3-P, the immediate product of the Vps34 PtdIns 3-kinase and the Fab1p PtdIns-3-P 5-kinase yields PtdIns-3-P and PtdIns-3,5-P2, respectively. PtdIns-3-P regulates anterograde Golgi-to-vacuole traffic through FYVE domain-containing downstream effectors such as Vac1p and Vps27p. PtdIns-3,5-P2 may be essential to signal the efflux or turnover of vacuolar membranes.

FIG. 2. Biosynthesis and function of 3-phosphoinositides in yeast. The sequential phosphorylation of PtdIns by the Vps34 PtdIns 3-kinase and the Fab1p PtdIns-3-P 5-kinase yields PtdIns-3-P and PtdIns-3,5-P2, respectively. PtdIns-3-P regulates anterograde Golgi-to-vacuole traffic through FYVE domain-containing downstream effectors such as Vac1p and Vps27p. PtdIns-3,5-P2 may be essential to signal the efflux or turnover of vacuolar membranes.

Acknowledgments—We thank Tamara Darsow for the critical reading of the manuscript and Peter Parker and Stephen Dove for sharing unpublished results.

$^{2}$ J. D. Gary and S. D. Emr, unpublished observations.
Minireview: 3-Phosphoinositides in Membrane Traffic

REFERENCES
1. Franke, T. F., Kaplan, D. R., Cantley, L. C., and Toker, A. (1997) Science 275, 665–668
2. Rameh, L. E., Chen, C. S., and Cantley, L. C. (1995) Cell 83, 821–830
3. Stehno, B., Klonowski, K., Stokoe, D., Erdjument-Bromage, H., Painter, G. F., Holmes, A. B., Geifman, P. R., Reese, C. B., McCormick, F., Tempst, P., Coardwell, J., and Hawkins, P. T. (1998) Science 279, 710–714
4. Toker, A., and Cantley, L. C. (1997) Nature 387, 673–676
5. Vanhaesebroeck, B., Leveers, S. J., Panayotou, G., and Waterfield, M. D. (1997) Trends Biochem. Sci. 22, 267–272
6. De Camilli, P., Emr, S. D., McPherson, P. S., and Novick, P. (1996) Science 271, 1533–1539
7. Burd, C. G., Babst, M., and Emr, S. D. (1998) Semin. Cell Dev. Biol. 9, 527–533
8. Novick, P., and Brennewald, P. (1993) Cell 75, 597–601
9. Rothman, J. E. (1998) Nature 372, 55–63
10. Randazzo, P. A., and Kahn, R. A. (1994) J. Cell Biol. 121, 1245–1256
11. Cowles, C. R., Snyder, W. B., Burd, C. G., and Emr, S. D. (1997) EMBO J. 16, 2769–2782
12. Wendland, B., Emr, S. D., and Riezman, H. (1998)Curr. Opin. Cell Biol. 10, 513–522
13. Kinniski, D. J. (1996) J. Biol. Chem. 271, 10807–10810
14. Futter, C. E., Pares, A., Hewlett, L. J., and Hopkins, C. R. (1996) J. Cell Biol. 132, 1011–1023
15. Rothman, J. H., and Stevens, T. H. (1986) Cell 47, 1041–1051
16. Robinson, J. S., Klonowski, K. J., Banta, L. M., and Emr, S. D. (1988) Mol. Cell. Biol. 8, 4936–4948
17. Rothman, J. H., Howald, I., and Stevens, T. H. (1998) EMBO J. 17, 2057–2065
18. Banta, L. M., Klonowski, K. J., Kinniski, D. J., and Emr, S. D. (1998) J. Cell Biol. 107, 1369–1383
19. Herman, P. K., and Emr, S. D. (1998) J. Biol. Chem. 273, 6742–6754
20. Brown, W. J., DeWald, D. B., Emr, S. D., Platner, H., and Balch, W. E. (1995) J. Cell Biol. 129, 781–796
21. Davidson, H. W. (1995) J. Cell Biol. 130, 797–805
22. Shpeter, H., Joly, M., Hartley, J., and Conovera, S. (1996) J. Cell Biol. 132, 1041–1051
23. Valinina, S., Dhand, R., Vanhaesebroeck, B., MacDougall, L. K., Stein, R., Zvelebil, M. J., Domin, J., Panaretou, C., and Waterfield, M. D. (1995) EMBO J. 14, 3389–3398
24. Joly, M., Kazlauskas, A., F., S., and Conovera, S. (1994) Science 263, 684–687
25. Li, G., D'Souza-Schorey, C., Barbieri, M. A., Roberts, R. L., Kippel, A., Williams, L. T., and Stahl, P. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10207–10211
26. Herman, P. K., Stack, J. H., DeModena, J. A., and Emr, S. D. (1991) Cell 64, 2769–2782
27. Stack, J. H., Herman, P. K., Schu, P. V., and Emr, S. D. (1993) EMBO J. 12, 2195–2204
28. Stack, J. H., DeWald, D. B., Takegawa, K., and Emr, S. D. (1995) J. Cell Biol. 129, 321–334
29. Panaretou, C., Domin, J., Cockcroft, S., and Waterfield, M. D. (1997) J. Biol. Chem. 272, 2477–2485
30. Rhee, S. G. (1991) Trends Biochem. Sci. 16, 297–301
31. Sternweiss, P. C., and Smrcka, A. V. (1992) Trends Biochem. Sci. 17, 502–506
32. McPherson, P. S., Garcia, E. P., Slepen, V. I., David, C., Zhang, X., Grabs, D., Sossin, W. S., Bauerfeind, R., Nemoto, Y., and De Camilli, P. (1996) Nature 379, 353–357
33. Attree, O., Olivos, I. M., Okabe, I., Bailey, L. C., Nelson, D. L., Lewis, R. A., McIntyre, R. R., and Nussbaum, R. L. (1992) Nature 359, 239–242
34. Osbisklowski, R., and Parker, P. J. (1997) Trends Biochem. Sci. 22, 427–431
35. Caldwell, K. K., Lips, D. L., Bansal, V. S., and Majerus, P. W. (1991) J. Biol. Chem. 266, 18378–18386
36. Wurmser, A. E., and Emr, S. D. (1998) EMBO J. 17, 4930–4942
37. Randazzo, P. A., and Kolter, T. (1996) Trends Cell Biol. 6, 98–103
38. Kaneko, Y., Toh-e, A., and Oshima, Y. (1982) Mol. Cell. Biol. 2, 127–137
39. Wada, Y., Nakamura, N., Okumura, Y., and Hirata, A. (1997) J. Cell Sci. 110, 1299–1306
40. Weisman, L. S., and Wickner, W. (1997) J. Cell Biol. 142, 651–663
41. Bonangelino, C. J., Catlett, N. L., and Weisman, L. S. (1997) J. Cell Sci. 110, 127–137
42. Odorizzi, G., Babst, M., and Emr, S. D. (1998) Cell 95, 847–858