Complementation Analysis in PtdInsP Kinase-deficient
Yeast Mutants Demonstrates That Schizosaccharomyces pombe and Murine Fab1p Homologues Are Phosphatidylinositol 3-Phosphate 5-Kinases*

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Phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P2) is widespread in eukaryotic cells. In Saccharomyces cerevisiae, PtdIns(3,5)P2 synthesis is catalyzed by the PtdIns3P 5-kinase Fab1p, and loss of this activity results in vacuolar morphological defects, indicating that PtdIns(3,5)P2 is essential for vacuole homeostasis. We have therefore suggested that all Fab1p homologues may be PtdIns3P 5-kinases involved in membrane trafficking. It is unclear which phosphatidylinositol phosphate kinases (PIPkins) are responsible for PtdIns(3,5)P2 synthesis in higher eukaryotes. To clarify how PtdIns(3,5)P2 is synthesized in mammalian and other cells, we determined whether yeast and mammalian Fab1p homologues or mammalian Type I PIPkins (PtdIns4P 5-kinases) make PtdIns(3,5)P2 in vivo. The recently cloned murine (p235) and Schizosaccharomyces pombe FAB1 homologues both restored basal PtdIns(3,5)P2 synthesis in Δfab1b cells and made PtdIns(3,5)P2 in vitro. Only p235 corrected the growth and vacuolar defects of fab1 S. cerevisiae. A mammalian Type I PIPkin supported no PtdIns(3,5)P2 synthesis. Thus, FAB1 and its homologues constitute a distinct class of Type III PIPkins dedicated to PtdIns(3,5)P2 synthesis. The differential abilities of p235 and of SpFab1p to complement the phenotypic defects of Δfab1 cells suggests that interaction(s) with other protein factors may be important for spatial and/or temporal regulation of PtdIns(3,5)P2 synthesis. These results also suggest that p235 may regulate a step in membrane trafficking in mammalian cells that is analogous to its function in yeast.

In eukaryotes, phosphoinositides play important roles in several cell functions. In particular, they have been implicated in the membrane trafficking events by which membranes and proteins are sorted into vesicles and targeted to various cell compartments (1). Golgi-to-vacuole trafficking of membranes and proteins in Saccharomyces cerevisiae has provided a very informative system for the study of phosphoinositide-dependent membrane trafficking, because it is not essential for growth and so lends itself to mutational analysis. The phosphatidylinositol 3-phosphate (PtdIns3P)† that is made by the phosphatidylinositol kinase Vps34p is essential for the targeting of proteins from the Golgi to the yeast prevacuolar compartment and also for the prevacuolar compartment-to-vacuole trafficking step (2).

This functional sequence was recently extended by the demonstration that the yeast FAB1 gene, the protein product of which is necessary for membrane efflux from the vacuole, encodes a PtdIns3P 5-kinase (3, 4). Phenotypes caused by mutations in FAB1 (temperature-sensitive growth and massive enlargement of the yeast vacuole, which also fails to acidify) had been identified earlier, but it had been predicted that the Fab1p protein encoded by FAB1 (which we shall term ScFab1p) would be a PtdIns4P 5-kinase (5). Because PtdIns(3,5)P2 has been found in all eukaryote cells so far examined (6, 7), we predicted that ScFab1p would be the first member of a novel family of PtdInsP kinases (PIPkins) that would be dedicated to PtdIns(3,5)P2 synthesis; the PtdIns3P 5-kinases of other organisms would be their Fab1p homologues (3). We suggested that an appropriate generic name for this group of enzymes would be “Type III PIPkins,” to distinguish them from Type I PIPkins (which are PtdIns4P 5-kinases) and Type II PIPkins (which are PtdIns5P 4-kinases) (for a recent review, see Ref. 8).

Shisheva et al. (9) have recently suggested that p235, a murine protein that displays extensive homology to ScFab1p, has PtdIns 5-kinase activity. Moreover, Tolias et al. (10) observed that a recombinant Type I PIPkin can make

† The abbreviations used are: PtdIns3P, phosphatidylinositol 3-phosphate; PtdIns(3,5)P2, phosphatidylinositol (3,5)-bisphosphate; PtdIns3P 5-kinase, phosphatidylinositol 3-phosphate 5-kinase; PtdIns(4,5)P2, phosphatidylinositol 4,5-bisphosphate; PIPkins, phosphatidylinositol phosphate kinases; GroPDIns, glycerophosphoinositol; GroInsP3P, glycerophosphoinositol 3-phosphate; GroIns4P, glycerophosphoinositol 4-phosphate; GroIns(3,5)P2, glycerophosphoinositol (3,5)-bisphosphate; HPLC, high pressure liquid chromatography; SC, synthetic complete; ORF, open reading frame.

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‡‡ The first two authors contributed equally to this work.
PtdIns(3,5)P₂ from PtdIns3P in lipid kinase assays and suggested that enzymes of this type may make PtdIns(3,5)P₂ in vivo in mammalian cells.

Taken alone, however, such enzyme assays in vitro do not unambiguously define the biological substrate specificities of inositol lipid kinases. In order to determine what lipids the Fab1p-like kinases make in vivo, we therefore determined in what ways the expression of a mammalian Type Iβ PIPkin, the murine Fab1p-like protein p235, or the Schizosaccharomyces pombe Fab1p (SpFab1p) would change the endogenously synthesized phosphoinositide complements of yeasts carrying mutants of MSS4 (which encodes PtdIns4P 5-kinase) and of yeasts in which the FAB1 gene was knocked out. The conditional-lethal msst-1 mutants that we used have a reduced PtdIns(4,5)P₂ content (our data); such mutants show defects in their actin cytoskeleton (11, 12), and ∆fab1 cells make no PtdIns(3,5)P₂ (3, 4). The utility of this type of approach has already been demonstrated by the finding that expression of a Type I PIPkin, but not of a Type II PIPkin, corrects the temperature-sensitive growth defect of msst-1 mutant yeast (12).

**EXPERIMENTAL PROCEDURES**

[32P]Gro₃₇.5 at 2 °C. [32P]Gro₃ was prepared by dephosphorylating [32P]Gro₃₇.5 and of glycerophosphoinositol (Gro₃₇.5) at 2 °C. Glycerophosphoinositol (Gro₃₇.5,6) was prepared by dephosphorylating [14C]inositol-labeled yeast were stored in buffered solutions (10–15 mM HEPES KOH, pH 7.5) at ~80 °C. [32P]Gro₃₇.5P₅ was prepared by dephosphorylating [32P]Gro₃₇.5P₅ using washed erythrocyte ghosts as described previously (21).

Expression of Epoxy-tailed p235 and SpFab1p—Plasmids pRM2 and pRM23 were introduced into strain fab1Δ-1 using standard techniques and transformants were selected on SC medium lacking uracil (SC-Ura). Liquid cultures were grown overnight in SC-Ura plus 2% (w/v) raffinose, supplemented with all other amino acids. 200 ml of SC-Ura containing 2% (w/v) raffinose was then inoculated to 4 × 10³ cells/ml and grown for 12–16 h. Cells were diluted to 4 × 10⁶ cells/ml in SC-Ura 2% (w/v) galactose, grown for a further 5 h, harvested, and lysed using a GlaCol Bio-Nebuliser. GST-fusion proteins were affinity-purified on glutathione-Sepharose as before (3), except that lysate buffers contained 5% (v/v) glycerol.

**RESULTS**

Functional Complementation of ScFab1 Inactivation—We tested whether expression of the presumptive murine Fab1p-like protein p235 of Shisheva et al. (9); termed PIKfyve in the Mouse Genome Database (MGI 1335106) (49)), is the only mammalian Fab1p homologue so far reported, or of SpFab1p (the Fab1p homologue of S. pombe) would correct some or all of the defects caused by deletion or mutation of ScFab1 in S. cerevisiae. In pFABCEN cells, which express a single copy of the ScFab1 gene behind its own promoter,
PtdIns(3,5)P$_2$ synthesis is restored (Fig. 1, right), the vacuoles revert to a multilobed morphology similar to that of wild-type cells (Fig. 2 a). Expression of p235 rescued several of the phenotypic defects of fab1 mutants. p235-expressing fab1-1 cells grew at the restrictive temperature (Fig. 2 b). Moreover, p235 restored the ability of fab1 mutants, which make no PtdIns(3,5)P$_2$ (3), to make a basal concentration of PtdIns(3,5)P$_2$ similar to that present in wild-type cells (Fig. 1, right; Fig. 2 c). However, hyperosmotic stress (0.9 M NaCl for 1–10 min) provoked no increase in PtdIns(3,5)P$_2$ synthesis in S. cerevisiae and S. pombe (6) and also in fab1 cells that overexpress exogenous ScFab1p (3). The negative result obtained with p235 is consistent with the fact that none of the mammalian cell lines we have tested have shown enhanced PtdIns(3,5)P$_2$ synthesis when hyperosmotically stressed (6).  

Under some experimental conditions in vitro, p235 synthesizes a phosphoinositide that appears to be PtdIns$_5$P$_2$ (9, 49). However, we never detected any PtdIns$_5$P$_2$ in yeast lipids that were analyzed by an HPLC method that resolves deacylated PtdIns$_3$P$_2$, PtdIns$_4$P$_2$ and PtdIns$_5$P$_2$, even in the p235-expressing cells (Fig. 2 c, left panel). Moreover in vitro, recombinant p235 phosphorylated PtdIns$_3$P$_2$ much more readily than PtdIns or PtdIns$_4$P$_2$ under the experimental conditions under which we previously defined the PtdIns$_3$P$_5$-kinase activity of ScFab1p (Fig. 3 a). When PtdIns$_5$P$_2$ spots from such PtdIns$_3$P$_5$-kinase assays were deacylated, the resulting GroPIns$_5$P$_2$ co-chromatographed with GroPIns(3,5)P$_2$, confirming that PtdIns(3,5)P$_2$ was synthesized (Fig. 3 c). p235 preferentially synthesized PtdIns(3,5)P$_2$, even when the only PtdIns$_3$P$_2$ present was a contaminant present in commercially available PtdIns and  

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PtdIns4P preparations. We conclude that p235 serves solely as a PtdIns3P 5-kinase in vivo and that its main activity in kinase assays in vitro is the 5-phosphorylation of PtdIns3P.

Expression of SpFab1p also restored the ability of θfab1 cells to make a basal concentration of PtdIns(3,5)P2 similar to that present in wild-type cells (~5% of the concentration of PtdIns(4,5)P2) and about half of that present in pFABCEN cells (Fig. 1, right; Fig. 4a, left panel). SpFab1p-expressing cells showed a modest increase in PtdIns(3,5)P2 synthesis in response to hyperosmotic stress (Fig. 4a, right panel). There was an approximately 2-fold elevation in PtdIns(3,5)P2 concentration, as compared with a 14-fold increase in cells expressing ScFAB1 from a single-copy plasmid (Fig. 1, right). This behavior is similar to that of strains carrying the fab1-2 temperature-sensitive allele of ScFAB1, which at restrictive temperatures show only a 2-fold stimulation in PtdIns(3,5)P2 in response to hyperosmotic shock (3). In contrast to the results with p235, expression of SpFab1p neither complemented the temperature-sensitive growth phenotype of fab1-1 cells (data not shown) nor corrected the vacuolar morphology of fab1-1::LEU2 cells (Fig. 4b). However, when recombinant SpFab1p was assayed for lipid kinase activity in vitro, it was an active PtdInsP kinase that showed a marked preference for PtdIns3P as a substrate (Fig. 3c). Deacetylation of the product and co-chromatography with aGroPIns(3,5)P2 standard confirmed that the PtdInsP2 synthesized was PtdIns(3,5)P2 (Fig. 3b).

**PIPkin-Ib Restores PtdIns(4,5)P2 Synthesis in mss4 Mutants**—We examined the effect of expressing a Type I PIPkin and the Fab1p proteins in strain YOC808, which is wild-type for the FAB1 gene but harbors the temperature-sensitive MSS4 allele mss4-1. YOC808 cells fail to grow at 37 °C, at least partly because of defects in their actin cytoskeleton, and this fault is overcome by overexpression of a mammalian Type I PIPkin (12). To confirm that the mss4-1 lesion limits PtdIns(4,5)P2 synthesis, the PtdIns(4,5)P2 content of YOC808 cells was analyzed at 23 °C and after 2 h at the restrictive temperature (38 °C). Even at the lower temperature, the mss4-1 cells contained about one-quarter of the [3H]PtdIns(4,5)P2 of wild-type cells (Fig. 5a, *versus* b; Fig. 6, left). When the temperature was raised, the wild-type cells increased their PtdIns(4,5)P2 complement but PtdIns(4,5)P2 declined further in the mss4-1 cells (Fig. 6, left). Expression of Type Ib PIPkin in these cells restored their PtdIns(4,5)P2 content to a level somewhat higher than that of wild-type cells, confirming that the Type Ib PIPkin is an active PtdIns4P 5-kinase in yeast (Fig. 5c). Expression of a mammalian Type II PIPkin, described as a PtdIns5P 4-kinase (25), had no effect on the PtdIns(4,5)P2 complement (not shown). We have never detected PtdIns5P in yeast (see above), so this was to be expected. Overexpression of ScFab1p, SpFab1p or p235 also had no major effect on the PtdIns(4,5)P2 content of mss4-1 cells (Fig. 5, d–f, respectively; Fig. 6, left), indicating that these enzymes make no contribution to PtdIns(4,5)P2 synthesis.

**Type I PIPkins Do Not Make PtdIns(3,5)P2 in Vivo and Do Not Phenotypically Rescue fab1 Cells**—The fab1-1::LEU2 strain, which contains an inactivated FAB1 allele, has a wild-type MSS4 gene and hence a normal PtdIns(4,5)P2 complement. However, it makes no PtdIns(3,5)P2 either under basal conditions or when osmotically stressed (Fig. 1, right; Fig. 7a, left panel). These cells have enlarged vacuoles that occupy much of the cell and fail to correctly acidify (3–5). When expressing a mammalian Type Ib PIPkin, they still made no PtdIns(3,5)P2 (even when hyperosmotically stressed; Fig. 1 and Fig. 7a, right panel), and their vacuolar phenotype remained abnormal (Fig. 7b). Moreover, the Type I PIPkin did not correct the temperature-sensitive growth phenotype of fab1-1 cells (data not shown).

**ScFab1p and Its Homologues Are Very Similar Both in Overall Organization and in Amino Acid Sequence, Thus Defining a Family of Closely Related Proteins**—Having suggested that ScFab1p and its orthologues in other organisms will make up a “Type III” family of PIPkins that specifically 5-phosphorylate PtdIns3P (3), we compared the molecular organizations and amino acid sequences of the available full-length ScFab1p-like proteins. The sequences of five close relatives of ScFab1p have been reported so far: from S. pombe (SpFab1p; the two overlapping clones AL023534 and AL021838), Caenorhabditis elegans (CeFab1p; AL023817), Drosophila melanogaster (DmFab1p; AL035311), mouse (p235; AF102777), and Arabidopsis thaliana (AtFab1p; AL035525).

Fig. 8a compares the overall domain arrangements of these proteins, and Fig. 8b and c, shows detailed alignments of their FYVE and kinase domains, respectively. All of the sequences include a C-terminal PtdInsP kinase domain, an N-terminal FYVE zinc finger domain, and a central domain with similarities to a conserved sequence motif present in Cct1p and its homologues (26): sequence conservation is most striking in the FYVE and kinase domains. FYVE domains, at least some of which specifically bind the ScFab1p substrate PtdIns3P (even when hyperosmotically stressed; Fig. 1 and Fig. 7a, right panel), and their cyclic phenotype remained abnormal (Fig. 7b). Moreover, the Type I PIPkin did not correct the temperature-sensitive growth phenotype of fab1-1 cells (data not shown).

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Asp-2196, and Asp-2216. In protein kinase A, the Lys residue equivalent to Lys-2059 of ScFab1p interacts with the α-phosphate group of ATP (37) and is essential for kinase activity (32). Substitution of Lys-2059 with Met abolishes ScFab1p PtdIns3P5-kinase activity (data not shown).

Although ScFab1p was initially designated as a likely Type I PIPkin (38), the kinase domains of all of the Type III PIPkins lack an "insert" region that is present in the Type I and Type II PIPkins (38, 39). At least in the recombinant Type II PIPkin, this insert is structurally disordered (35). A second region of divergent sequence in the kinase domains of the PIPkins is again disordered in the Type II PIPkin (35). By analogy with protein kinases (32, 40, 41), this may be a substrate-binding activation loop that is involved in defining the substrate specificity of the Type II PIPkin (35). The relevant sequence in the Type III PIPkins (Thr-2200 to Gly-2238 in ScFab1p) is indicated by the plus signs in Fig. 8c. Alongside that region of the sequence comparison of the different Type III PIPkins, we also show the relevant parts of a mammalian Type I PIPkin, of Mss4p (functionally equivalent to a Type I PIPkin.

### Table I

Plasmids and S. cerevisiae strains used in this study (for further details, see under "Experimental Procedures")

| Plasmid       | Parent plasmid or genotype | Markers | Ref. |
|---------------|----------------------------|---------|------|
| pYO2144       | TRP1, pGAP; 2 μm origin    |         | 12   |
| pRS416        | URA3; ARS CEN              |         | 14   |
| pEG-KT        | URA3; pGAL1-CYC1; 2 μm origin |       | 15   |
| pTT102U       | URA3; pADH1; 2 μm origin   |         | 16   |
| pEMY105       | ScFAB1 gene; 2 μm origin   |         | 5    |
| pYO2145       | Murine PIPkin-1β gene; 2 μm origin | | 12   |
| pYO2146       | Human PIPkin-IIa gene; 2 μm origin |       | 12   |
| pFABCEN       | ScFAB1 gene; ARS CEN       |         |      |
| pRS416        | ScFAB1 ORF; 2 μm origin    |         |      |
| pRM19         | ScFAB1 ORF; 2 μm origin    |         |      |
| pRM22         | ScFAB1 ORF; 2 μm origin    |         |      |
| pRM23         | ScFAB1 ORF; 2 μm origin    |         |      |
| pRM24         | p235s ORF; 2 μm origin     |         |      |
| Strain        |                           |         |      |
| YPH499        | MAT a ura3 lys2 ade2 trp1 his3 leu2 |         | 14   |
| YOC808        | MATa ade2 leu2 lys2 trp1 ura3 mss4; HIS3 ade3; mss4-1::LEU2 | | 12   |
| 616-3-2       | MATa fab1-1 ade3 trp1 can1 cyh1 gal1 |         | 5    |
| 663D-1-3      | MATa fab1-Δ1 his7 leu2 ura3 gal1 |         | 5    |
| 6742-1-3      | MATa fab1-LEU2 ade2 his3 leu2 trp1 ura3 suc2 | | 17   |
| FYS33         | MATa his3 ura3 leu2 lys2 trp1 GAL2 |         | 5    |
| SEY6120       | MATa leu2 ura3 his3 trp1 lys2 suc2 GAL |       | 18   |
| SDY500        | ade3 leu2 ura3 his3 trp1 fab1-1 |         |      |

FIG. 4. SpFab1p catalyzes PtdIns(3,5)P2 synthesis in vivo but does not suppress all Δfab1 defects. a, GroPInsP2 levels from shocked (right) and nonshocked (left) fab1::LEU2 cells expressing plasmid pRM19. Cells were labeled at 24 °C in inositol-free SC-Trp medium containing 10 μCi/ml [3H]inositol for five cell divisions. They were challenged with medium (basal) or 0.9 M NaCl for 10 min before being killed. Deacylated lipid samples were spiked with [14C]GroPIns(3,5)P2 and [14C]GroPIns(4,5)P2 standards (open circles) and were separated using gradient 2 (24). b, SpFAB1 did not suppress the mutant vacuolar phenotype of fab1 cells. Fluorescence images (rhodamine channel) are shown of FM4–64-stained fab1::LEU2 strains transformed with pYO2144 (fab1::LEU2) or pRM19 (fab1::LEU2 + SpFab1p).
in S. cerevisiae), and of a mammalian Type II PIPkin. The sequences of this “activation loop” are very different in each PIPkin family but are well conserved throughout any one family. For the Type III PIPkins, the consensus sequence is T(F/Y)T(W/L)DKKLE(S/T/M)WVK XX G(I/L)(V/L)G: this motif has a well maintained pattern of hydrophobicity and of charge (including three conserved basic residues) in all Type III PIPkins. This motif may be involved in defining PtdIns3P as the substrate for 5-phosphorylation.

DISCUSSION

Recent studies suggest that mammalian Type I PIPkins can act as PtdIns3P 5-kinases in vitro (10, 25, 42). On the basis of these experiments, Tolias et al. (10) have suggested that the Type I PIPkins are responsible for the synthesis of PtdIns(3,5)P2 that has been observed to occur in mammalian cells (6, 7). We directly addressed this substrate specificity issue by expressing the murine Type Ib PIPkin in D fab1 MSS4 and FAB1 mss4-1 yeast. Our data show that the murine Type Ib PIPkin is expressed in yeast in a functional state because this enzyme restores wild-type levels of PtdIns(4,5)P2 to FAB1 mss4-1 yeast as well as complementing the mss4-1 phenotype (12). In contrast, the murine Type Ib PIPkin is unable to synthesize any PtdIns(3,5)P2 when expressed in D fab1 MSS4 yeast and failed to complement the D fab1 phenotype. This suggests that the ability of Type I PIPkins to synthesize PtdIns(3,5)P2 in vitro is not a biologically relevant activity; although there appears to be a modest increase in

**FIG. 5.** ScFab1p, SpFab1p, and p235 do not enhance PtdIns(4,5)P2 levels in mss4-1 cells. mss4-1 strains expressing the mammalian Type Ib PIPkin, ScFab1p, SpFab1p, and p235 were grown at 23 °C in selective inositol-free SC medium containing 10 μCi/ml [3H]inositol. Cultures were grown to a cell density of 1 × 106 cells/ml and then split into four samples. Two samples were grown at 23 °C for a further 2 h, and the other two were grown at the restrictive temperature for 2 h, after which their lipids were extracted and analyzed. HPLC chromatograms are shown of the GroPInsP₈ at 23 °C of wild-type (YPH499) (a), mss4-1 (YOC808) (b), mss4-1 expressing Type Ib PIPkin (c), mss4-1 expressing ScFab1p (d), mss4-1 expressing SpFab1p (e), and mss4-1 expressing p235 (f).

**FIG. 6.** PtdIns(3,5)P2 and PtdIns(4,5)P2 in mss4-1 temperature-sensitive cells. The strain used was YOC808 carrying the mss4-1 temperature-sensitive allele and, as indicated, transformed with 2-μm plasmids containing the Type Ib PIPkin (pYO2145), ScFAB1, SpFAB1, or p235 ORFs. S. cerevisiae YPH499 was the parental strain of YOC808. Cells were incubated at the restrictive (38 °C) or nonrestrictive (23 °C) temperature for 2 h before lipid extraction. Values, calculated as in Fig. 1, are representative of multiple independent experiments performed in duplicate (mean ± S.E.; n = 2).
PtdIns(3,5)P₂ levels in FAB1 mss4-1 cells expressing the murine Type I PIPkin relative to FAB1 mss4-1 cells containing an empty vector, this ability to increase the steady-state levels of PtdIns(3,5)P₂ requires that the cells also express a wild-type FAB1 gene, i.e. no increase occurs in fab1 MSS4 cells. The increase in the steady-state levels of PtdIns(3,5)P₂ in the FAB1 mss4-1 cells is therefore unlikely to result from direct synthesis of PtdIns(3,5)P₂ by the murine Type I PIPkin.

Because the Type I PIPkins do not synthesize PtdIns(3,5)P₂ in vivo, the identity of the mammalian PtdIns3P 5-kinase appeared unresolved. The similarity of a cDNA encoding p235, a PIPkin homologue, to the yeast FAB1 gene suggested to us that the protein product of this gene might fulfil this function. Other studies have shown that this enzyme can act as a PtdIns...
zymes restored basal PtdIns(3,5) synthesis to levels similar to the pEGKT promoter of pYO2144 (Table I) and to the high copy plasmids pYO2144 (data not shown). This suggests that the p235-catalyzed activity reflects the conditions of subcellular localization (probably in the Golgi/lysosome/endosome continuum) in most or all eukaryotes, including mammals. This would be consonant with the fact that synthesis of PtdIns3P, the substrate of the Type III PIPkins, by Vps34p and other Type III phosphoinositide 3-kinases is essential for protein trafficking to vacuolar/lysosomal compartments both in yeast (43–45) and in mammalian cells (46–48).

The absence of a hyperosmotic stress-induced increase in PtdIns(3,5)P2 in p235-expressing cells suggests that this enzyme is not involved in the stress response in mammalian cells and also demonstrates that basal PtdIns(3,5)P2 synthesis is sufficient to rescue all the known phenotypic defects of fab1-1 yeast. In contrast, SpFab1p partly restored the hyperosmotic activation of PtdIns(3,5)P2 synthesis, but it neither complemented the temperature-sensitive growth of fab1-1 cells nor restored the morphology of Δ fab1 cells, suggesting that an additional regulatory interaction (separate from its PtdIns3P 5-kinase activity and involved in the response of the cell to stress) is conserved between S. cerevisiae and S. pombe; the different regulatory properties seen with SpFab1p and p235 might merely have reflected a difference in their expression levels or rates of degradation. However, although expression of FAB1 from a single-copy plasmid was sufficient to complement the phenotypic defects of Δ fab1 and fab1-1 cells (see Fig. 2, a and b), expression of the SpFab1p ORF from constitutively active promoters of the high copy plasmids pYO2144 (Table I) and pVT102U (data not shown) and from the galactose regulated promoter of pEGKT in 2% (w/v) galactose (data not shown) failed to rescue these defects. Thus, because both enzymes restored basal PtdIns(3,5)P2 synthesis to fab1 cells and complemented at least one of the other functions of ScFab1p, this trivial explanation seems much less likely than a real difference in the functional properties of the two enzymes.

In support of our experimental data, the results of the sequence comparison establish that the six known proteins of the Fab1p family that includes the experimentally untested proteins of Arabidopsis, Caenorhabditis, and Drosophila are very closely related, consistent with the idea that they fulfill the same function in each of their host organisms. This conclusion, combined with the earlier demonstrations of the specific PtdIns3P 5-kinase activity of ScFab1p (3), makes it clear that ScFab1p and its homologues do indeed make up a new family of Type III PIPkins that synthesize PtdIns(3,5)P2; we suggest that this could be abbreviated to PIPkin-III, with an appropriate prefix to indicate species (e.g. ScPIPkin-III for Fab1p).

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