Minireview

The phosphatidylinositol 3-phosphate-binding FYVE finger

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Abstract The FYVE zinc finger domain is conserved from yeast (five proteins) to man (27 proteins). It functions in the membrane recruitment of cytosolic proteins by binding to phosphatidylinositol 3-phosphate (PI3P), which is found mainly on endosomes. Here we review recent work that sheds light on the targeting of FYVE finger proteins to PI3P-containing membranes, and how these proteins serve to regulate multiple cellular functions.

Key words: FYVE domain; Phosphatidylinositol 3-kinase; Phosphoinositide; Membrane traffic; Signal transduction

1. Introduction

Phosphoinositides, phosphorylated derivatives of phosphatidylinositol, function as spatial regulators of crucial cellular processes by recruiting cytosolic proteins to specific membrane compartments [1]. Among the most ubiquitous phosphoinositides is phosphatidylinositol 3-phosphate (PI3P), which is generated from phosphatidylinositol by specific isoforms of phosphatidylinositol 3-kinase (PI3K). Even though the levels of PI3P are low (typically 0.2% of phosphatidylinositol in mammalian cells), this phosphoinositide is crucial for endocytic membrane trafficking and other cellular functions. The localisation of PI3P to endosomes and yeast vacuoles [2] suggests that it functions by recruiting effector proteins to these membranes. Two distinct protein domains have been found to bind to PI3P with high affinity and specificity. One is the Phox homology (PX) domain, which is found in proteins that regulate the NADPH oxidase complex, cell survival, and endocytic membrane trafficking [3,4]. The other is the FYVE finger (zinc finger originally observed in **F**ab1p, **Y**OTB, **V**ac1p and **E**EA1) [5], which is the topic of this review. We here discuss the biological role of the FYVE finger in the light of recently accomplished genome sequences, structural determinations and functional data.

2. FYVE finger proteins in the human, nematode and yeast proteomes

Using database searches we find a total of 27 FYVE finger proteins in the human proteome (Fig. 1 and Table 1). This is close to the number reported for the human genome (28) [6], whereas the SMART system [7] reports as many as 48 human FYVE finger proteins. The discrepancy is probably due to a weaker criterion used by SMART, as some of the SMART hits actually represent RING fingers, and SMART also contains some redundant sequences. For *Caenorhabditis elegans*, we find 13 FYVE finger proteins (Table 1), while 15 were reported in the *C. elegans* genome [6]. Again, this is probably due to different criteria for distinguishing between FYVE fingers and FYVE-related fingers. In addition, there appears to be additional proteins in *C. elegans* with distantly related zinc finger domains. In *Saccharomyces cerevisiae*, five FYVE finger proteins can be identified. The number of FYVE finger proteins found in humans, nematodes and yeast thus correlates well with the total estimated numbers of genes in these organisms (42 000, 18 000 and 6000, respectively).

As illustrated by the alignment (Fig. 1), all zinc-coordinating cysteines are absolutely conserved in human FYVE fingers. Apart from the coordinating residues, the R+HHCRxCG motif (in single-letter amino acid code; x means any amino acid) involved in ligand binding (see below) is the most conspicuous feature of the alignment. Additional conserved sequence elements are an N-terminal WxxD motif, and a C-terminal RCV motif, which are both involved in ligand binding (see below). Except for the zinc coordinators, glycine-39 in the alignment is the only residue that is fully conserved in all FYVE fingers. This glycine is positioned at the beginning of β-strand 2, and it presumably plays a critical role in forming the core fold of the domain. It is worth noting that this residue is not conserved in Rabphilin-3A and other FYVE-related zinc fingers [8]. Two of the domains (FENS-1 and MGC20275) have small insertions N-terminal to the first β-strand. Interestingly, both these proteins have a glutamine in position 32 of the alignment, instead of the conserved arginine/lysine. Rabenosyn-5 has a 42-residue insertion after the third β-strand, and its putative yeast homologue, Vac1p, contains a similar insertion [8]. These insertions might be important in substrate recognition and/or in interaction with other proteins. However, so far there are no structural data available to support this.

Based on the occurrence of other functional domains in the

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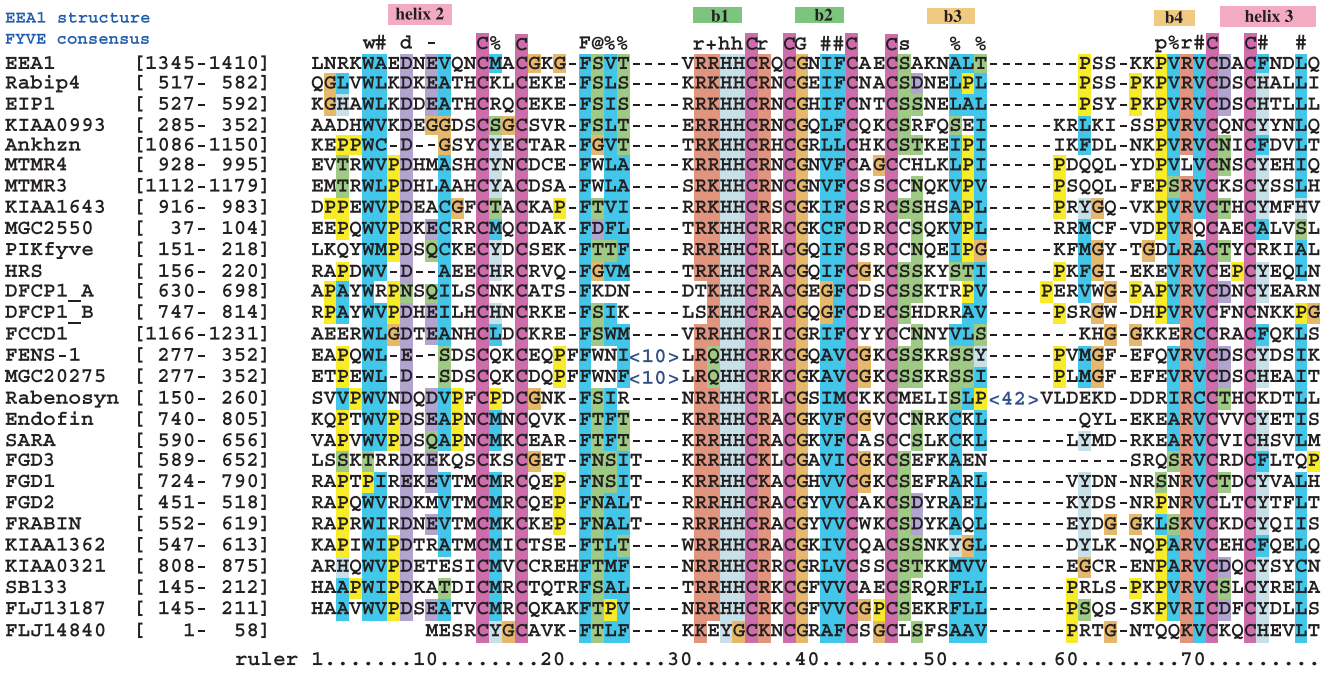

Fig. 1. Colour-coded multiple alignment of the human FYVE fingers (in the absence of a good prediction of the human orthologue, murine FGD2 is shown). Databases were searched using Blastp and Psi-Blastp [50], SMART [7], Pfam [51] and SearchWise [52]. A consensus for the most conserved positions is shown at the top: '#', strongly conserved hydrophobic; '–', strongly conserved acidic; '+', strongly conserved basic; '%', weakly conserved hydrophobic; '@', strongly conserved neutral hydrophilic. β-Strands (b1–4) and α-helices are indicated as defined by the EEA1 crystal structure (PDB entry 1JOC). Amino acid positions in the proteins are indicated in brackets. Three positions where individual proteins have insertions of 10–42 residues are indicated in arrowed brackets. The colour coding is based on a full alignment of all FYVE fingers from man, yeast and *Caenorhabditis elegans*. This full alignment and a table with database accession numbers and additional information is available at http://www.uib.no/aasland/fyve/. The following databases were searched: NCBI's non-redundant protein database (http://www.ncbi.nlm.nih.gov/blast/html/blastcgihelp.html#protein_databases) and the Sptrembl database (http://www.ebi.ac.uk/swissprot/). In some cases the Ensembl database (http://www.ensembl.org) was searched for alternative gene predictions.

FYVE finger proteins, we have divided them into 14 groups (see Table 1 and Fig. 2). For eight of these groups, we find putative homologues in *C. elegans*, while only three yeast proteins can be matched with the human set. This suggests that while the common function of the FYVE finger has been preserved since the origin of unicellular eukaryotes, multicellular organisms have elaborated extensively their use of this functional module. The occurrence of protein domains with known functions related to membrane traffic and PI3P metabolism, and the notable absence of general 'signalling' modules such as SH2, SH3, GTPase and protein kinase domains suggest that FYVE finger proteins typically regulate 'housekeeping' cellular functions rather than agonist-induced processes.

3. Structure of the FYVE finger and its bound ligand

The structural characterisation of FYVE fingers and their interactions with ligands has advanced at a frenetic pace. Initially, the three-dimensional polypeptide fold of a FYVE finger was revealed by the high resolution X-ray diffraction analysis of crystal of the FYVE finger from *S. cerevisiae* Vps27p [9]. The second report of the structure of a FYVE finger arose soon after in the form of the crystal structure of the tandem VHS–FYVE finger pair homodimer from the protein Hrs from *Drosophila melanogaster* [10]. The results of these studies revealed that divergent ~60-residue FYVE finger polypeptide sequences adopt a conserved fold that resembles that of the RING and PHD fingers [11,12]. These are all small domains built around a pair of antiparallel β-strands (Fig. 1, b1 and b2) and two loops on which the four conserved Zn^{2+}-coordinating CxxC motifs are placed. Like the RING finger, the FYVE finger contains a C-terminal α-helix that carries one of the coordinating cysteines. In addition, the FYVE finger contains a second pair of anti-parallel β-strands (Fig. 1, b3 and b4) that protrude from the core structure. Site-directed mutagenesis studies have revealed that the conserved R(R/K)HHCR sequence is a principal site of interaction with PI3P [13,14], and the 3D structure reveals that this segment of the chain contributes to one face of the FYVE finger, along its longest dimension, on the side opposite the C-terminal helix.

The difference between the monomeric nature of the Vps27p structure and the dimeric Hrs FYVE finger structure, combined with crystal contacts with negatively charged groups in lattice-neighbouring Vps27p FYVE finger molecules and buffer-derived citrate anions nestling in surface cavities of the Hrs FYVE homodimer, led the crystallographers to propose conflicting models of how the inositol phosphate headgroup of PI3P would be bound, and additionally how the FYVE finger may rest at the surface of the plasma membrane with semi-conserved hydrophobic side chains seated in the lipid–cytosol interface (reviewed in [15]). Direct measurements of the nature of FYVE finger interactions with lipids were provided by nuclear magnetic resonance (NMR) spectroscopic approaches [16,17]. These reports demonstrated that for the EEA1 FYVE finger expressed on its own, there was stronger binding to micelle-borne PI3P lipids than to soluble PI3P headgroup mimics [16], and provided explicit evidence that

Table 1
Human FYVE finger proteins and their putative nematode and yeast homologues

	Homo sapiens	*Caenorhabditis elegans*	*Saccharomyces cerevisiae*	Structural features	Putative functions
1	EEA1	T10G3.5		N-terminal ZFIRE finger, long coiled-coil regions, Rab5 binding domain(s). Rabenosyn has five NPF motifs. FCCD1 has a similar organisation as EEA1. FCCD1 and Rabip4 contain RUN domains.	EEA1, Rabenosyn-5 and Vac1p are Rab5 effectors in endocytic membrane fusion [22,28,31]. Rabip4 is a Rab4 effector in endocytic recycling [32].
	Rabenosyn-5		Vac1p		
	FCCD1				
	Rabip4	F01F1.4			
	EIP1				
2	Ankhzn	F22G12.4		N-terminal BTB domain (dimerisation?), >15 ankyrin repeats.	Endosome–cytoskeleton interactions [54]?
3	KIAA0993 (partial clone)	T23B5.2, YLN2		WD repeats (implicated in protein/protein interactions). The N-terminus of T23B5.2 has similarity to *beige*/Chediak–Higashi syndrome (lysosomal disorder) protein.	?
4	FENS-1, MGC20275			Seven WD repeats with FYVE finger between repeats 6 and 7.	Endocytic trafficking [44]?
5	MTMR3	YW91		N-terminal region with similarity to myotubularin, including a PI3P phosphatase domain.	MTMR3 is a 3′-phosphatase for PI3P and PI(3,5)P_2. Regulates autophagy [47]?
	MTMR4	F53A2.8			
6	Fgd1	C28C12.10		DH domain, two PH domains flank the FYVE finger (KIAA1362 contains only one PH domain).	GEF for Cdc42, regulation of actin cytoskeleton. Fgd1 is mutated in faciogenital dysplasia [39–42,55].
	Fgd2	C3309.1			
	Fgd3				
	Frabin				
	KIAA1362				
7	SARA	D1022.7		Smad-binding domain, TGFβ receptor-binding domain.	Mediator of TGFβ signalling [36]. $AKAP_{CE}$ recruits PKA to membranes [56].
	Endofin	($AKAP_{CE}$)			
8	PIKfyve	CAA91791.1	Fab1p	DEP domain, chaperonin domain, PI3P 5-kinase domain.	PI3P 5-kinase, formation of multivesicular bodies (MVBs) or membrane recycling from vacuole/lysosome [24,33,57].
9	Hrs	C07G1.5	Vps27p	N-terminal VHS domain, UIM motif(s), coiled-coil region implicated in STAM binding, C-terminal clathrin box motif.	Endosome–lysosome/vacuole trafficking. MVB formation [25,35].
10	DFCP1 (TAFF1)			Double FYVE finger protein. TAFF1 is a shorter splice variant of DFCP1.	Golgi trafficking [44,49]?
11	KIAA0321			Large (1542-residue) protein	?
12	KIAA1643			Large (993-residue) protein	?
13	MGC2550			Small (234-residue) protein	?
14	FLJ14840			N-terminal FYVE finger.	?

The proteins are divided into 14 groups according to their domain organisations.

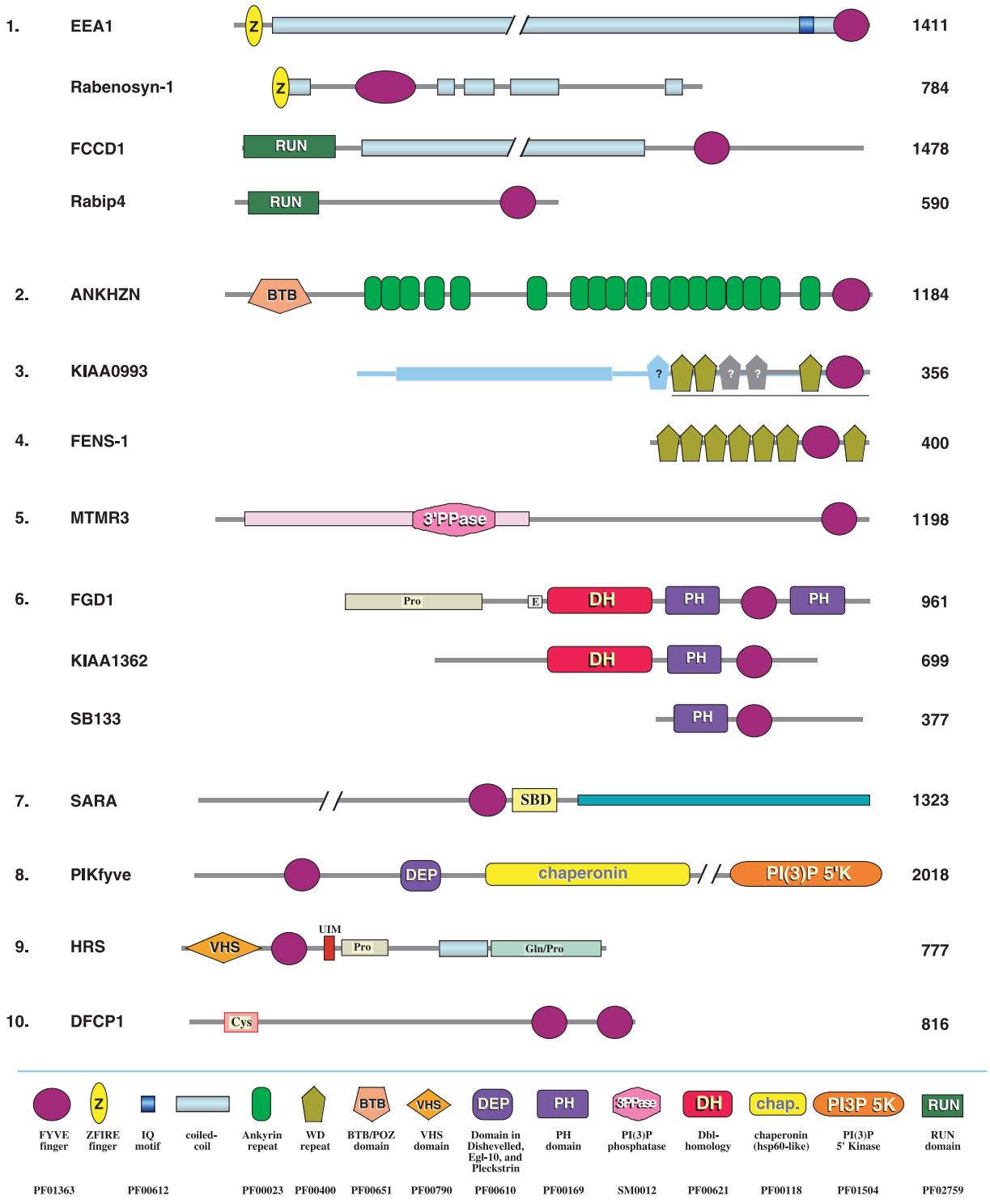

Fig. 2. Cartoon of representative examples of the different classes of human FYVE finger proteins. The Pfam (PF) or SMART (SM) accession numbers for the different domains are given under the figure key. In the case of group 3, the extent of the partial KIAA0993 sequence is underlined, and the domain structure of the larger *C. elegans* homologue T23B5.2 is indicated in blue. 'SBD' is the SMAD-binding domain in SARA and 'UIM' is the ubiquitination motif in Hrs. For further details, see Table 1 and [8].

N-terminal loop residues insert non-specifically under the micelle surface. Subsequent refinement of this picture came in the form of an NMR-derived solution structure of the EEA1 FYVE finger bound to di-C_4-PI3P [17] which provided an (at best) medium resolution picture of the precise mode of interaction of the lipid headgroup phosphates and conserved amino acid side chains. Nevertheless, the NMR work appears to provide corroboration for the basic outline of the interaction model proposed for Vps27p [9].

Greater clarification of the structural biology of FYVE fingers now required a high resolution definition of the ligation coordination of the PI3P headgroup by conserved elements of

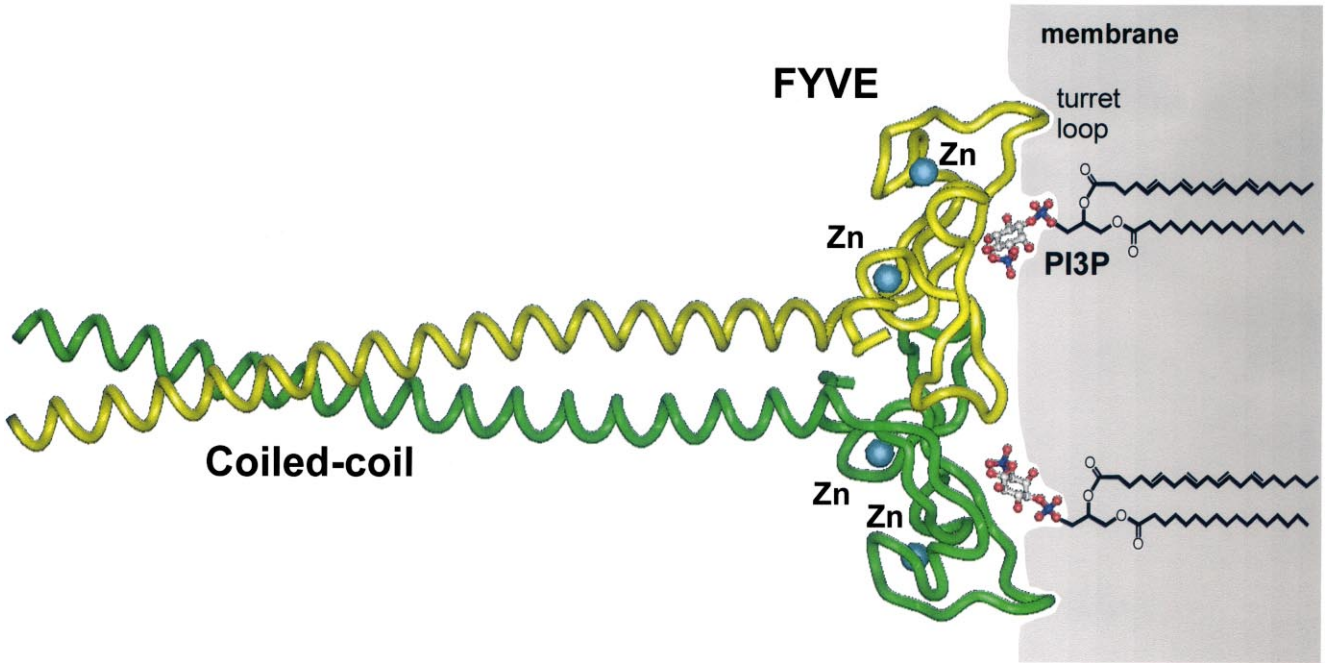

Fig. 3. Crystal structure of the EEA1 FYVE finger and adjacent coiled-coil region. The homodimer nature of this structure contrasts with the NMR observations of the isolated EEA1 FYVE finger. Notably in the new structure, the dimer interface created with the coiled-coil region is contiguous with the contact between the two FYVE fingers. The two PI3P molecules are bound on a common surface directly opposite the coiled-coil stalk and the putative arrangement of the FYVE fingers on the membrane surface is more 'prone' than 'end on' as previously anticipated. The short linking region between the coiled-coil and FYVE finger segments is structurally ordered (forming an α-helix) and residues in this segment assist the stabilisation of the ligand coordination sphere. The interpretation of the NMR data had anticipated FYVE finger dimerisation, but had also suggested ligand-dependent conformational changes of the FYVE finger [16]. Analysis of the EEA1 crystal structure would appear to put strict limits upon any such change, limited perhaps to a few side chain rotamer perturbations [18]. The ligation of the PI3P headgroup involves all three conserved signature motifs (see Fig. 4) [18]. The 3-phosphate is coordinated by basic side chains of the R(R/K)HHCR and RVC motifs (interacting residues underlined) – the latter using a bridging water molecule. The inositol 4-OH and 5-OH/6-OH groups donate H-bonds to the R(R/K)HHCR and WxxD motifs respectively. The 1-phosphate interacts with the first arginine of the R(R/K)HHCR motif. The second basic residue of the R(R/K)HHCR motif makes 'second coordination shell' interactions with other FYVE finger residues that reinforce the direct contacts. Interestingly, side chains involved in direct interaction with the ligand headgroup hydroxyls effectively block the locations that would be occupied by alternative ligands containing phosphates at the 4- or 5-position of the inositol ring, an observation that goes part way to explain the ligand selectivity of the FYVE finger. In all, six residues contact the Ins(1,3)P_2 group, providing a detailed and convincing rationalisation of FYVE finger sequence conservation, ligand specificity, and the results of previously reported mutagenesis studies [13,14]. Alternative phosphoinositide ligands would have to bind in a different orientation and would suffer a significant reduction in binding affinity.

the FYVE finger polypeptide. Satisfaction of this desire has recently been achieved in the form of the 2.2 Å resolution crystal structure of a larger C-terminal segment of EEA1, including the FYVE finger and a substantial portion (40 residues) of the coiled-coil dimerisation domain [18]. In this structure each EEA1 domain of the homodimer is bound by a molecule of inositol 1,3-bisphosphate (Ins(1,3)P_2) – a soluble mimic of PI3P. The resolution of the structure means that this result supersedes all other attempts to model or describe this protein–ligand interaction. It has previously been shown that EEA1 exists as a parallel coiled-coil homodimer in cells [19] and that although essential the FYVE finger is not alone sufficient for localisation to early endosomes [5,20]. Fig. 3 shows a ribbon-style representation of the EEA1 homodimer structure (PDB code 1JOC) and gives an impression of how the quaternary architecture is consistent with the prediction that dimerisation amplifies the weak binding of individual FYVE fingers to the phospholipid ligand, and demonstrates that the structure is ideally configured for location at the flat surface of the membrane, and apparently includes insertion of the so-called 'turret-loop' residues into the membrane surface (see Fig. 3). As shown in Fig. 4, the ligation of the PI3P headgroup involves all the conserved signature motifs of the FYVE domain: R(R/K)HHCR, RVC and WxxD.

4. The functions of FYVE finger proteins

Yeast cells that are defective in the PI3K *VPS34* are unable to produce PI3P. The *vps34* mutation causes one striking cellular defect: membrane trafficking from the Golgi and the plasma membrane via endosomes to the vacuole is impaired [21]. This suggests that, in yeast, the functions of FYVE finger proteins are concerned with endocytic/vacuolar membrane trafficking, and four out of five FYVE finger proteins in *S. cerevisiae* have indeed been proposed to fulfil such a role (the function of the fifth one, YGL023, has still not been investigated). Vac1p regulates the fusion of Golgi- and plasma membrane-derived vesicles with endosomes [22], whereas Fab1p is a PI3P 5-kinase that controls vacuolar membrane homeostasis and the formation of multivesicular bodies (MVBs) [23,24]. The latter function also applies to Vps27p, which belongs to the class E subgroup of vacuolar sorting proteins that are thought to regulate trafficking between endosomes and the vacuole [25]. Vps27p contains two putative

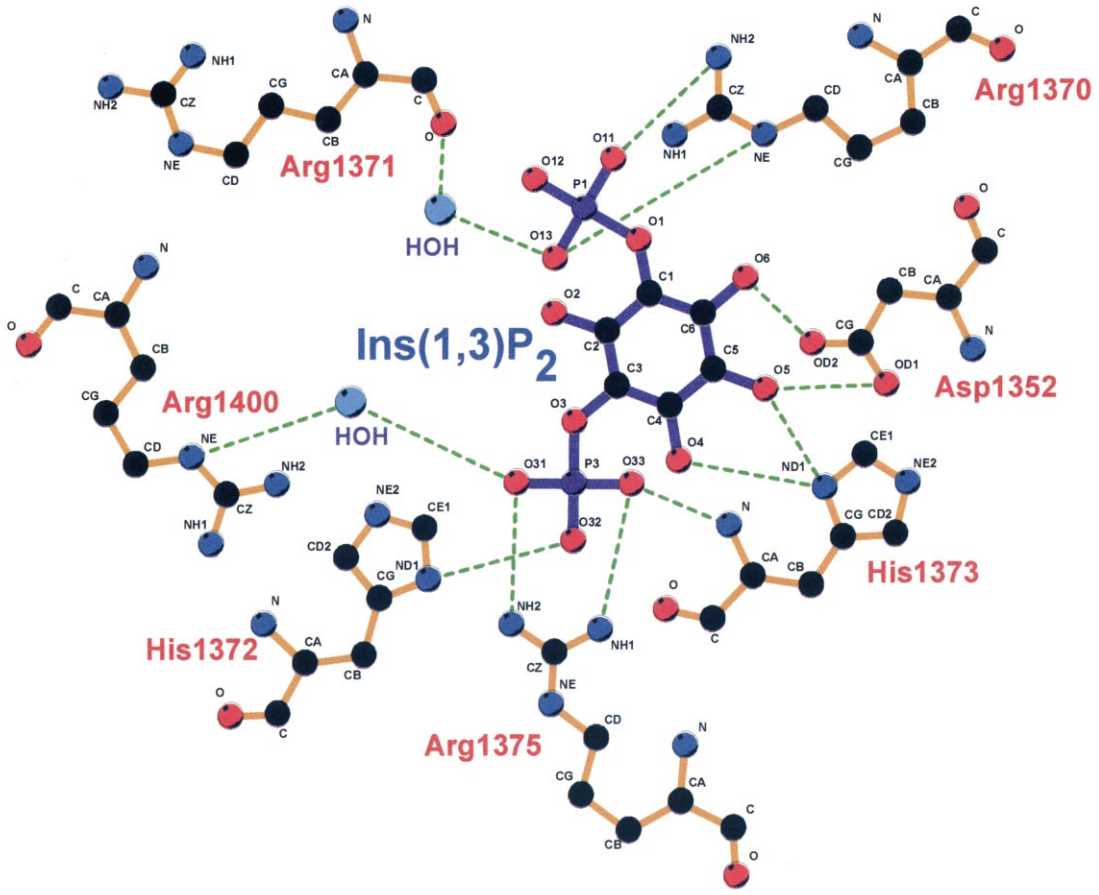

Fig. 4. Schematic representation of the coordination of Ins(1,3)P_2 (a soluble analogue of PI3P) by the conserved sequence motifs of the EEA1 FYVE finger. The picture was composed from the coordinates of PDB entry 1JOC using LIGPLOT [53].

ubiquitin-interacting motifs [26] and could play a role in the sorting of mono-ubiquitinated proteins in endosomes. Vps27p might well function downstream of Pib1p, which is an endosomal ubiquitin ligase that ubiquitinates target proteins [27]. It is interesting to note that yeast FYVE finger proteins regulate distinct trafficking steps such as membrane fusion, receptor sorting and membrane invagination, and that they act consecutively throughout the endocytic pathway. This is consistent with the localisation of PI3P on both endosomes and vacuoles [2].

The role of PI3P and FYVE finger proteins in endocytic membrane trafficking has been conserved from yeast to higher eukaryotes. Rabenosyn-5, the mammalian homologue of Vac1p, and the related protein EEA1 are effectors of the early-endosomal GTPase Rab5, which regulates endocytic membrane fusion [28]. Both Rabenosyn-5, EEA1 and Vac1p contain an N-terminal ZFIRE (zinc finger in Rab effectors) finger, which, in the case of EEA1, has been directly implicated in Rab5 binding. The exact mechanism for how Rabenosyn-5 and EEA1 regulate membrane traffic still remains to be elucidated, but interactions with syntaxin-6 and syntaxin-13, which are thought to mediate endocytic membrane fusion through the engagement in heteromeric complexes with other SNARE proteins, are likely to trigger endocytic membrane tethering and fusion [29–31]. While Rabenosyn-5 and EEA1 are effectors of Rab5, Rabip4 is an effector of another early-endosomal GTPase, Rab4, which regulates endocytic recycling [32]. The interactions of Rabenosyn-5, EEA1 and Rabip4 with different Rab GTPases could contribute to targeting of these proteins to distinct subdomains of early endosomes.

PIKfyve is the mammalian homologue of Fab1p. This protein is localised to early endosomes and MVBs, and overexpression of a mutant with impaired PI3P 5-kinase activity appears to block MVB formation [33]. Presumably, PIKfyve regulates the invagination of endosomal membranes through its PI3P 5-kinase activity, although the mechanism remains obscure. The hepatocyte growth factor-regulated tyrosine kinase substrate Hrs [34] is the mammalian homologue of Vps27p. Like Vps27p it contains a ubiquitin-interacting motif, and the C-terminus of Hrs binds to clathrin [35], suggesting that Hrs could recruit ubiquitinated receptors into clathrin-coated subdomains of early endosomes. This could serve to sort receptors that are destined for late endosomes and lysosomes.

Besides their function in endocytic membrane trafficking, additional roles have evolved for mammalian FYVE finger proteins. The most striking example is SARA (Smad anchor for receptor activation), which is crucial for transforming

growth factor-β (TGFβ) signalling. This protein binds to both the TGFβ receptor and the downstream signalling mediator Smad2, thus facilitating the phosphorylation (and activation) of Smad2 by the activated receptor [36]. Both SARA and the related FYVE finger protein endofin are localised to early endosomes [37], suggesting that TGFβ signalling occurs from these compartments and not from the plasma membrane. It thus appears that the function of the SARA FYVE finger is to localise a signalling complex to endosomes. Besides SARA, there is also evidence that Hrs binds to Smad2 and participates in TGFβ signalling [38], but, since this protein also regulates endocytic membrane trafficking, further work is needed in order to distinguish between its roles in TGFβ receptor signalling and trafficking.

In addition to the established functions of FYVE finger proteins in membrane trafficking and receptor signalling, the Fgd1 family of FYVE finger proteins (see Fig. 2) appear to regulate the actin cytoskeleton. Fgd1, which is associated with the X-linked disease faciogenital dysplasia, is a GDP/GTP exchange factor for the small GTPase Cdc42, which regulates the remodelling of cortical actin [39]. Unlike most other FYVE finger proteins, Fgd1 is not associated with endosomes. Rather, it localises to the plasma membrane, indicating that the interaction with PI3P does not serve as a localisation signal for this protein [39]. It is possible that two pleckstrin homology domains that flank the FYVE finger may target Fgd1 to the plasma membrane by binding to phosphoinositides other than PI3P. The three other members of the Fgd1 family, Fgd2, Fgd3 and Frabin, have been studied in less detail than Fgd1, but preliminary results suggest that they have overlapping functions [40–42]. Besides its atypical localisation, Fgd1 differs from other FYVE finger proteins in that its FYVE finger lacks the conserved tryptophan-5, and it binds to PI3P with low affinity. Interestingly, the Fgd1 FYVE finger also binds to the structurally related phosphoinositide PI5P, whose function is still not known [43]. Therefore, given the lack of endosomal localisation of Fgd1, it is possible that the Fgd1 family of FYVE finger proteins may be regulated by PI5P rather than by PI3P.

5. Conclusion

Recent progress has revealed the full array of FYVE finger proteins in humans and other organisms, and the structural basis for the interaction of FYVE fingers with PI3P has been characterised. The proposed insertion of the hydrophobic 'turret loop' of the FYVE finger into the membrane (Fig. 3) is consistent with the fact that FYVE fingers bind with much higher affinity to membrane-associated PI3P than to its soluble analogues [14,43]. Given that PI3P is mainly found on endosomes, it is not surprising that most FYVE finger proteins are associated with these organelles. However, only a few FYVE fingers (e.g. those of endofin and FENS-1) bind to PI3P with sufficient affinity to function as autonomous membrane targeting domains [37,44]. In other cases (e.g. EEA1 and Hrs), additional domains are required for efficient membrane binding. Coiled-coil dimerisation of EEA1 may increase its avidity for PI3P-containing endosome membranes [45], and an interaction with Rab5 may further stabilise the interaction or target EEA1 to Rab5-containing membrane domains. A few FYVE finger proteins (e.g. MTMR3, Fgd1 and DFCP1/TAFF1) are not found on endosomes [39,46–49]. Presumably, the FYVE fingers of these proteins have too low affinity for PI3P to function as endosome targeting domains, and the functional relevance of their PI3P binding remains elusive.

The functions of many FYVE finger proteins have now been studied, and the majority of these proteins serve as regulators of endocytic membrane trafficking. Through evolution, a few FYVE finger proteins appear to have evolved as regulators of signalling and cytoskeletal functions. Despite recent progress, the exact cellular functions of the FYVE finger proteins remain to be determined, and it will be important to study if their interactions with PI3P play other roles in addition to that of membrane recruitment. With the whole set of FYVE finger proteins in the human proteome available, it should now be possible to determine the roles of these proteins, and of PI3P, in cell biology and human disease.

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