The dynamic Rab11-FIPs

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Abstract

The Rab11-FIPs (Rab11-family interacting proteins; also known as FIPs) constitute an evolutionarily conserved protein family that act as effector molecules for multiple Rab and Arf (ADP-ribosylation factor) GTPases. They were initially characterized by their ability to bind Rab11 subfamily members via a highly-conserved C-terminal RBD (Rab11-binding domain). Resolution of the crystal structure of Rab11 in complex with FIPs revealed that the RBD mediates homodimerization of the FIP molecules, creating two symmetrical interfaces for Rab11 binding and leading to the formation of a heterotetrameric complex between two FIP and two Rab11 molecules. The FIP proteins are encoded by five genes and alternative splicing has been reported. Based on primary structure, the FIPs were subcategorized into two classes: class I (Rip11, FIP2 and RCP (Rab-coupling protein)) and class II (FIP3 and FIP4). Recent studies have identified the FIPs as key players in the regulation of multiple distinct membrane trafficking events. In this mini-review, we summarize the Rab11-FIP field and discuss, at molecular and cellular levels, the recent findings on FIP function.

The endosomal system

Receptor-mediated endocytosis is the process by which eukaryotic cells internalize many cell surface-localized ligand-bound receptors by the inward budding of vesicles from the plasma membrane. After the internalization of these ligand-bound receptors, vesicles are uncoated, fuse with one another and fuse with pre-existing peripheral structures to form an organelle known as the sorting endosome. In sorting endosomes, a decrease in pH causes many ligands to dissociate from their receptors. From sorting endosomes, most receptors are then ‘sorted’ into membrane domains, which bud-off to form vesicles that are trafficked directly back to the plasma membrane (the ‘fast’ recycling pathway) or are transported to the ERC (endosomal-recycling compartment; sometimes known as the recycling endosome), where they are indirectly returned to the plasma membrane (the ‘slow’ recycling pathway) [1]. In contrast, many ligands, receptor–ligand complexes and the remaining solutes that are destined for degradation are trafficked from sorting endosomes to late endosomes, and ultimately to lysosomes where they are degraded by hydrolytic enzymes [1].

Rab GTPases

Rab GTPases constitute a large family of cellular proteins that regulate all stages of intracellular membrane trafficking in eukaryotic cells [2]. They act as molecular switches by alternating between active (GTP-bound) and inactive (GDP-bound) states. In their active state, Rab proteins are found on the cytosolic face of various intracellular membranous compartments where they regulate specific transport events [2]. When active, Rab proteins interact with downstream ‘effector’ proteins, which then perform diverse cellular functions necessary for the processes of vesicle formation, motility, docking and fusion [2].

Based on distinct subfamily-specific sequence motifs, many Rab proteins have been grouped into subfamilies [3]. Members of the Rab11 subfamily (Rab11a, Rab11b and Rab25) localize to the ERC, and to apical recycling endosomes in polarized cells, and regulate endosomal trafficking through these compartments [4–6]. The currently identified Rab11-effector proteins include the FIPs, Rabphilin-11/Rab11BP [7,8], myosin Vb [9], phosphoinositide 4-kinase β [10] and Sec15 [11].

The FIPs

Approx. 10 years ago, considerable effort was devoted to proteomics approaches to identify the protein machinery downstream of Rab11 GTPases. This led to the identification of an evolutionarily conserved protein family known as the FIPs. There are five members of this protein family [Rip11, FIP2, RCP (Rab-coupling protein), FIP3 and FIP4] [12–15]; however, alternative splicing has also been reported [16–18]. Some members of the FIP family have aliases: RCP is alternatively known as FIP1C; Rip11 as Gaf-1/Gaf1b, pp75 and FIP5; FIP3 as Arfophilin and Eferin; and FIP4 as Arfophilin-2. Although the overall degree of sequence homology between the FIPs is low (14–34% amino acid identity), each of the FIPs has been characterized by the presence of a highly conserved 20 amino acid RBD (Rab11-binding domain), which is located at their C-termini.

Initial in silico analyses of the primary structure of the FIPs revealed the presence of two different types of
putative calcium-binding domains near their N-termini. This formed the basis of classification of the FIPs: class I FIPs (Rip11, FIP2 and RCP) possess phospholipid-binding C2-domains, whereas class II FIPs (FIP3 and FIP4) possess EF-hand domains (Figure 1) [14]. In addition to the RBD and C2/EF-hand domains, each of the FIPs is predicted to have an α-helical coiled-coil structure (Figure 1). Indeed, resolution of the crystal structure of Rab11a in complex with FIP2, and separately in complex with FIP3, revealed that the RBD of the FIPs forms a parallel coiled-coil homodimer, creating two symmetrical interfaces for binding the switch I and switch II regions of two Rab11a-GTP molecules [19–21]. Thus the overall structure is a heterotetrameric complex (Rab11-(FIP)-2-Rab11; Rab11-FIP is Rab11-family interacting protein, also known as FIP), with the FIP forming a central α-helical coiled-coil. Further biophysical studies have confirmed that in addition to the α-helical coiled-coil RBD, there are other regions N-terminal of the RBD that also have α-helical coiled-coil structure that, like the RBD, mediate FIP homodimerization and are necessary to support their cellular functions [22–24].

Functions of FIPs
The existence of multiple Rab11-subfamily effector proteins indicates that this GTPase subfamily is likely to regulate several distinct intracellular membrane trafficking events (Figure 2). In recent years, many of the details of the cellular function of the FIPs have been elucidated. The FIP and Rab11-subfamily-dependent cellular functions that are currently known can be broadly grouped into three categories: recycling of cargo to the cell surface, delivery of membrane to the cleavage furrow/midbody during cell division, and links between Rab11 and molecular motor proteins.

Recycling of cargo to the cell surface
Some of the first indications that the FIPs played a role in endosomal-recycling processes came from studies that monitored the recycling of Tf (transferrin) in BHK (baby-hamster kidney) and HeLa cells [13,25]. These studies revealed that expression of N-terminally truncated class I FIP proteins impeded the recycling of endocytosed Tf to the cell surface. Indeed, it is thought that the N-terminal C2-domains of the class I FIPs target Rab11-FIP complexes, together with their associated cargo, to docking sites on the plasma membrane that are enriched in certain phospholipids [PtdIns(3,4,5)P3 and phosphatidic acid] [26].

More recently, further studies have revealed details of specific cargo trafficked to the cell surface by the individual FIPs. For example, Rip11 is responsible for the translocation of GLUT4 transporter-containing vesicles to the cell surface of adipocytes in response to insulin treatment [27]. Indeed, Rip11 was found to localize to GLUT4-positive intracellular vesicles and perturbation of Rip11 function was found to inhibit insulin-stimulated uptake of 2-deoxyglucose and insertion of GLUT4-containing vesicles into the plasma membrane. This Rip11-dependent trafficking event was also shown to involve the Rab GAP (GTPase-activating protein) AS160, although notably, a direct involvement of Rab11 in the incorporation of GLUT4-containing vesicles into the plasma membrane could not be verified since a Rab11-binding-deficient mutant of Rip11 (Rip11 I630E) could still translocate to the plasma membrane in response to insulin [27].

FIP2 has also been implicated in the recycling of various cargo molecules to the cell surface. Expression of N-terminally truncated FIP2 prevents recycling of the water channel protein AQP2 (aquaporin-2) and the chemokine receptor CXCR2 (CXC chemokine receptor 2) [28,29]. FIP2 has also been implicated in trafficking of the RSV (respiratory syncytial virus) and the GLUT4 and FAT (fatty acid translocase)/CD36 transporters [30,31]. Intriguingly, FIP2 was also recently shown to function in LTP (long-term potentiation) because disruption of FIP2 function blocks calcium-triggered recycling of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors and dendritic spine growth in postsynaptic neurons [32].

Like the other class I FIPs, RCP, which can also bind the Rab4 GTPase [13], has also been implicated in specific plasma membrane recycling events. In macrophages, overexpression of N-terminally truncated RCP blocks recycling from the phagosomal compartment, whereas overexpression of full-length RCP stimulates this recycling event [33]. RCP has also been shown to form a complex with α5β1 integrin and EGFR1 (epidermal growth factor receptor 1) and to drive their recycling to the cell surface [34]. Intriguingly, by this mechanism, RCP regulates α5β1 integrin-dependent cell motility by increasing fibronectin-dependent migration of tumour cells into three-dimensional matrices [34].

Delivery of membrane to the cleavage furrow/midbody during cell division
The completion of cytokinesis in animal cells relies on the co-ordinated delivery of new membrane to the cleavage furrow. In this context, both of the class II FIPs have emerged as key components downstream of Rab11 in the trafficking

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Figure 1 | Schematic representation of the in silico predicted domain architecture and classification of the FIPs
C2, C2-domain; EF, EF-hand domain; PRR, proline-rich region; CC, coiled-coil domain.
of endosomal material during cell division. Both FIP3 and FIP4 localize to the cleavage furrow/midbody during cytokinesis, and perturbation of FIP3 function by siRNA (small interfering RNA)-mediated knockdown or by expression of a Rab11-binding deficient mutant (FIP3 I738E) results in cytokinesis defects [35–37]. Interestingly, both FIP3 and FIP4 bind members of the Arf GTPase family, most notably Arf6 [38,39]. Since Arf6 forms ternary complexes with Rab11 and FIP3 or FIP4, and as Arf6 appears to mediate the recruitment of FIP3 and FIP4 to the cleavage furrow, it is believed that FIP3 and FIP4 couple Rab11 and Arf6 during cell division. In addition, other proteins such as components of the exocyst and centrosomin complexes are also thought to contribute to the recruitment of incoming FIP3- and FIP4-positive endosomal material with the cleavage furrow/midbody during cytokinesis [39,40].

**Figure 2** | Schematic model of the major endosomal trafficking routes controlled by Rab-FIP complexes during interphase

The trafficking routes to/from the ERC for which a direct involvement of a FIP has yet to be demonstrated are indicated with a star symbol. TGN, trans-Golgi network.

**Linking Rab11 to molecular motor proteins**

A further key emerging feature of the functionality of the FIPs is their ability to link Rab11-subfamily GTPases to the molecular motor protein machinery of the cell. FIP2 directly interacts with the actin-based myosin Vb motor protein [41] and this interaction appears to be critical for several aspects of FIP2 function, including regulation of plasma membrane recycling [41], recycling of AQP2 [29], recycling of CXCR2 [28] and trafficking of the AMPA receptor [32]. Rip11 has been shown to directly bind a component of the microtubule-based kinesin II motor complex [42]. This molecular interaction is thought to direct endocytosed receptors through the ERC. FIP3 also binds microtubule-based motor proteins, since it is reported to bind kinesin I [43], and we have recently identified an interaction between FIP3 and cytoplasmic dynein 1 that is necessary for FIP3-dependent pericentrosomal accumulation of the ERC ([24] and C.P. Horgan, S.R. Hanscom and M.W. McCaffrey, unpublished work).

**Regulation of FIP function**

While clarification of the details of regulation of FIP function is in its infancy, some information has emerged. The activities of Rip11 and FIP2 are known to be regulated by phosphorylation [12,44,45], and binding of the C2-domain of Rip11 with phospholipids is magnesium dependent [12]. Finally, RCP has been shown to possess functional PEST (Pro-Glu-Ser-Thr) motifs, which are believed to rapidly target it for calpain-mediated proteolysis after delivery of endosome-derived recycling material to the plasma membrane [46].

**Conclusions**

Over the last decade, significant information regarding the molecular details of FIP function has been forthcoming. Indeed, it is now clear that the FIPs are critical regulators of multiple endosomal-recycling processes (Figure 2). They have also been shown to function in several crucial cellular physiological processes such as cell division, cell migration, responses to insulin, memory processes, immune and inflammatory responses, water reabsorption, fertility, retinal
cell development and various human cancers [16,24,27,28,32–37,47–50]. This repertoire of FIP functions is ever-expanding and as many of these cellular processes are disease-associated, it identifies the FIPs as potential targets for therapeutic intervention in the treatment of human diseases in the future. In the meantime, much work is still required in order to explore comprehensively the molecular details of FIP function. In this regard, we have recently found that some FIP proteins can also serve as effector proteins for another Rab GTPase (E.E. Kelly, C.P. Horgan, C. Adams, T.M. Patzer, J.C. Norman and M.W. McCaffrey, unpublished work). This has led us to speculate that the FIPs may function downstream of a wider spectrum of Rab GTPase proteins, a question that we are currently assessing through a high-throughput screen of Rab11-FIP2 functions in Rab11-interacting proteins. We thank the members of our laboratory for helpful suggestions and a critical reading of this paper. We apologize to colleagues whose primary work has not been cited due to space limitations.

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