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Research Article

Rab11-FIP1A regulates early trafficking into the recycling endosomes

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ABSTRACT

The Rab11 family of small GTPases, along with the Rab11-family interacting proteins (Rab11-FIPs), are critical regulators of intracellular vesicle trafficking and recycling. We have identified a point mutation of Threonine-197 site to an Alanine in Rab11-FIP1A, which causes a dramatic dominant negative phenotype when expressed in HeLa cells. The normally perinuclear distribution of GFP-Rab11-FIP1A was condensed into a membranous cisternum with almost no GFP-Rab11-FIP1A(T197A) remaining outside of this central locus. Also, this condensed GFP-FIP1A(T197A) altered the distribution of proteins in the Rab11a recycling pathway including endogenous Rab11a, Rab11-FIP1C, and transferrin receptor (CD71). Furthermore, this condensed GFP-FIP1A(T197A)-containing structure exhibited little movement in live HeLa cells. Expression of GFP-FIP1A(T197A) caused a strong blockade of transferrin recycling. Treatment of cells expressing GFP-FIP1A(T197A) with nocodazole did not disperse the Rab11a-containing recycling system. We also found that Rab5 and EEA1 were accumulated in membranes by GFP-Rab11-FIP1A but Rab4 was unaffected, suggesting that a direct pathway may exist from early endosomes into the Rab11a-containing recycling system. Our study of a potent inhibitory trafficking mutation in Rab11-FIP1A shows that Rab11-FIP1A associates with and regulates trafficking at an early step in the process of membrane recycling.

1. Introduction

Rab small GTPases are essential proteins in intracellular vesicle trafficking. The Rab protein family contains more than 40 mammalian Rabs, and each exists in both a GDP bound state and a GTP bound state [1]. In the GTP bound state, a Rab is considered active and is capable of binding effector proteins to do downstream work. When a Rab is bound to GDP, it is inactive and thus unavailable for protein transport. It is thought that Rab proteins not only define sub-populations of endosomal membranes, but also participate in trafficking through these compartments [2]. Thus, Rab4 and Rab5 are important in early endosome transport, Rab7 is involved in late endosome transport, and Rab11a participates in recycling endosome transport. In some contexts, Rab proteins may overlap at key transition points in trafficking, as for Rab4 and Rab5 at the transition between early and sorting endosomes or for Rab4 and Rab11a between sorting and recycling endosomes [3].

The Rab11 family of Rab proteins are essential regulators of endosomal trafficking and specifically, trafficking through recycling endosomes [4,5]. This family consists of Rab11a, Rab11b, and Rab25 [6]. Previous work identified a family of effectors, which are capable of binding the Rab11 family and acting as effectors in endosomal trafficking. This family of effectors, designated as the Rab11 Family Interacting Proteins (Rab11-FIPs) includes Rab11-FIP1 with multiple splice isoforms, Rab11-FIP2, Rab11-FIP3, Rab11-FIP4, and Rab11-FIP5 [7,8]. All members of the Rab11-FIPs bind the Rab11 family members through a conserved carboxyl-terminal amphipathic alpha-helical domain [7,9]. Beyond that, there is great variety in the Rab11-FIP protein structure. Rab11-FIP1C, Rab11-FIP1B, Rab11-FIP2, and Rab11-FIP5 contain amino-terminal C2 domains [10]. Rab11-FIP3 and Rab11-FIP4 contain ERM (ezrin-radixin-moesin) domains and 2 EF-hand motifs for interaction with Arf5 or Arf6 [11]. Rab11-FIP2 interacts with both Rab11 family members and MYO5A and MYO5B [12], which are also Rab11 interacting proteins [13,14]. While there is variety in protein structure, the Rab11-FIP proteins are generally flexible. This has been shown through crystal structure of the carboxyl terminus of Rab11-FIP2 [15] and is readily seen by the lack of domain structure in the Rab11-FIP1 isoforms Rab11-FIP1A and Rab11-FIP1B.

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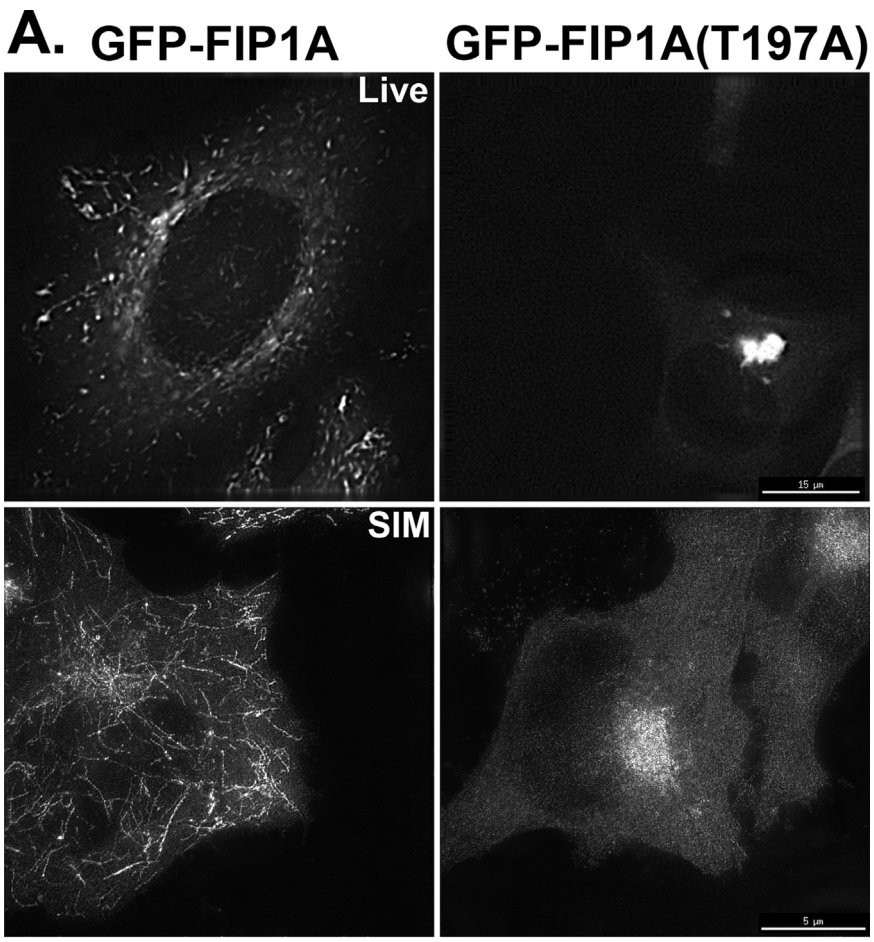

B.

	Vesicles	1-2 large foci	2+ large foci
FIP1A-WT	94%	0	6%
FIP1A(TA)	2%	80%	18%

Fig. 1. *GFP-Rab11-FIP1A(T197A) localizes to a collapsed perinuclear membrane cisternum.* (A) HeLa cells were transfected with either GFP-Rab11-FIP1A or GFP-Rab11-FIP1A (T197A) and imaged live for deconvolution. Separately, cells were transfected, fixed, and imaged by Structured Illumination Microscopy (SIM). Wild-type GFP-Rab11-FIP1A shows vesicles concentrated in the perinuclear region as well as extended tubules throughout the cell, typical of vesicular proteins involved in endosomal trafficking. Mutant GFP-Rab11-FIP1A(T197A), however, was redistributed almost entirely to the perinuclear area and shows no tubule localization. See Supplementary Videos 1 and 2. (B) The distribution of GFP-Rab11-FIP1A and GFP-Rab11-FIP1A(T197A) was evaluated in 50 cells and the distribution of transfected protein was scored as located in diffuse vesicles, in one to two condensed puncta, or in multiple large puncta.

Recent investigations have focused on the characterization of an increasingly complex network of recycling compartments that manifest a dynamic structure ranging from discrete vesicles to tubule vesicular elements and polymorphic tubules. Rab11-FIP proteins define discrete subdomains within endosomal recycling pathways [16]. Mutations in the actin motor MYO5B or motorless tail constructs of MYO5B inhibit trafficking through the recycling system [17,18]. We and others have also reported that specific mutations in Rab11-FIPs can also potently inhibit trafficking of cargoes through the recycling system [10,19,20]. Two mutations in Rab11-FIP2 are known that cause a disruption of endosomal trafficking. One mutation truncates the amino-terminal 128 amino acids including the C2 domain, Rab11-FIP2(129-512). This truncation mutation blocks trafficking through the plasma membrane recycling system and accumulates Rab11a in a collapsed membrane cisternum [7,10,20]. The second mutation, Rab11-FIP2 (SARG), causes a similar blockade in trafficking at a somewhat later stage in recycling by changing two amino acids (Serine 227 to Alanine and Arginine 413 to Glycine) [20]. Constructs of Rab11-FIP1C and Rab11-FIP5 lacking amino-terminal C2-domains also demonstrate significant inhibition of recycling endosome trafficking and accumulation of Rab11a [8,21,22].

While the Rab11-FIP1 gene encodes multiple splice isoforms [23], these protein products have very distinct localizations and likely distinct functions [16,24]. Rab11-FIP1A, Rab11-FIP1B, and Rab11-FIP1C reside on different membranes within the endosomal system indicating that they may interact with Rab11a at different points in recycling. Rab11-FIP1C can also interact with Rab14 at a site overlapping with the Rab11a binding site [25,26] and Rab11-FIP1C has been implicated in HIV trafficking in combination with Rab14 [27].

Our previous studies demonstrated that transferrin enters a Rab11-FIP1A-containing compartment early in the process of recycling [16]. While we have observed Rab11-FIP1A at different points in the cell, its function within the hierarchy of membrane recycling remains unclear. Although Rab11-FIP1A lacks an amino terminal C2-domain, our recent studies have shown that the protein associates with membranes enriched in phosphatidylserine [24]. The current work seeks to elucidate a specific role for Rab11-FIP1A in recycling through identification of potential

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