A novel assay reveals preferential binding between Rabs, kinesins, and specific endosomal subpopulations

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Identifying the proteins that regulate vesicle trafficking is a fundamental problem in cell biology. In this paper, we introduce a new assay that involves the expression of an FKBP12-rapamycin-binding domain–tagged candidate vesicle-binding protein, which can be inducibly linked to dynein or kinesin. Vesicles can be labeled by any convenient method. If the candidate protein binds the labeled vesicles, addition of the linker drug results in a predictable, highly distinctive change in vesicle localization. This assay generates robust and easily interpretable results that provide direct experimental evidence of binding between a candidate protein and the vesicle population of interest. We used this approach to compare the binding of Kinesin-3 family members with different endosomal populations. We found that KIF13A and KIF13B bind preferentially to early endosomes and that KIF1A and KIF1Bβ bind preferentially to late endosomes and lysosomes. This assay may have broad utility for identifying the trafficking proteins that bind to different vesicle populations.

Introduction

Organelles in the endomembrane system receive and dispatch vesicles in a highly regulated manner, which is mediated by a complex set of trafficking proteins: motors, SNAREs, tethers, Rabs, coat proteins, cargo adaptors, and others (Vale, 2003; Spang, 2008; Wickner and Schekman, 2008). Identifying the trafficking proteins that associate with different vesicle populations is an essential step in understanding the mechanisms that regulate vesicle trafficking. To accomplish this task, specific vesicle populations can be enriched by subcellular fractionation, immuno-isolation, or fluorescence sorting, and then the proteins present can be identified (Franzusoff et al., 1992; Takamori et al., 2006; Duclos et al., 2011; Zhang et al., 2011; Rhee et al., 2013). Two-color fluorescence microscopy is often used to confirm that a candidate protein binds the relevant vesicle population in vivo, in the appropriate biological context. However, this approach cannot always provide a definitive answer, as visual colocalization is often difficult to evaluate. Alternate approaches such as immuno-EM can be tedious.

Recently, we developed a new strategy to determine which members of a set of candidate kinesins associate with dendritically polarized vesicles in neurons (Jenkins et al., 2012). This “split kinesin” assay involves the expression of separate constructs encoding a kinesin tail fused to the FKBP12-rapamycin–binding (FRB) domain and a kinesin motor domain fused to the FKBP12 domain. These two domains can be inducibly assembled by adding a membrane permeant rapamycin analogue (Belshaw et al., 1996; Kapitein et al., 2010b; Robinson et al., 2010). The kinesin tail contains the cargo-binding domain and can bind vesicles, but it is incapable of influencing their movement because it lacks a motor domain. The kinesin motor domain is constitutively active and translocates into the axon but cannot move vesicles because it lacks a cargo-binding domain. Linking these two components together leads to a dramatic increase in vesicles entering the axon if and only if the expressed kinesin tail binds to the vesicles in question.

This assay offers several advantages. First, the induced change in vesicle trafficking is rapid and unmistakable. Second, the assay allows an unbiased evaluation of interactions between a given vesicle population and all relevant members of a family of trafficking proteins. Finally, the assay works well even if two different kinesins mediate the transport of a given organelle. Despite these advantages, the split-kinesin assay has limitations that restrict its applicability. First, the assay depends on the candidate kinesin's ability to bind to a specific vesicle population and to move vesicles because it lacks a cargo-binding domain. Linking these two components together leads to a dramatic increase in vesicles entering the axon if and only if the expressed kinesin tail binds to the vesicles in question.
unique organization of neurons, which have spatially separate axonal and dendritic domains. Second, the assay requires live imaging to detect the increased vesicle flux that occurs after adding linker drug. This makes these experiments time consuming and technically challenging.

Here, we describe strategies for adapting this assay for use in other cell types, using a readout that does not require live imaging. In most cell types, microtubules originate from a central microtubule-organizing center (MTOC), and their plus ends extend toward the cell periphery. By expressing a candidate protein that can be linked to either a kinesin or dynein, it should be possible to mislocalize vesicles that bind that protein in a predictable manner. To move vesicles toward microtubule plus ends, we used the constitutively active kinesin KIF5C559 (Jenkins et al., 2012), and for transport toward the minus ends, we used the N-terminal fragment of Bicaudal D2 (BicD2), which can link vesicles to dynein (Kapitein et al., 2010a).

Using this approach, we were able to identify the Rabs and kinesins that bind to different endosomal populations, based on drug-induced mislocalization of vesicles when FRB-tagged candidate proteins were linked to active motors. This new assay generates robust and easily interpretable results and, in contrast to conventional, two-color localization experiments, provides direct experimental evidence that a candidate protein binds to a particular vesicle population.

Results and discussion
Linking Rabs to active motors results in mislocalization of endosomes
We set out to develop an assay in which inducible, motor-driven vesicle mislocalization serves as a readout to indicate which of a set of candidate trafficking proteins associates with a given vesicle population. Candidate proteins were tagged with FRB so that they could be linked to a constitutively active motor by rapamycin analogue-induced heterodimerization. We hypothesized that attaching dynein to vesicles would result in their accumulation near the MTOC. Previous work has shown that the localization of mitochondria or peroxisomes can be changed by adding a homotypic tether or by directly cross-linking constitutively active motors to integral membrane proteins present in these organelles (Sengupta et al., 2009; Kapitein et al., 2010a,b; van Spronsen et al., 2013). It is less clear whether linking motors to trafficking proteins that associate only transiently with the cytoplasmic surface of vesicles can produce a distinctive change in vesicle distribution.

As proof of principle, we asked whether linking an FRB-tagged Rab to an unregulated, constitutively active kinesin or dynein produces a distinctive change in the localization of the vesicles that bind that Rab. One potential advantage of directing vesicles toward the minus ends of microtubules is that they are likely to form a single aggregate near the cell center, which should provide a strong signal. We adapted the strategy developed by Kapitein et al., 2010a,b, who investigated dynein-driven peroxisome transport by inducibly linking BicD2, a dynein cargo adaptor, to peroxisomes (Dienstbier and Li, 2009; Kardon and Vale, 2009). We expressed a protein, tandem dimer Tomato (tdTM)–BicD2594–FK506 binding protein (FKBP), consisting of FKBP fused to the dynein-binding fragment of BicD2, which was coexpressed with FRB-Rab5 (Fig. 1 A). In control cells, GFP-Rab5 vesicles were largely distributed in the cell periphery (Fig. 1 B). BicD2 was diffusely distributed throughout the cell; a small amount also appeared to be associated with vesicles (not apparent at the magnification shown in this figure).
In drug-treated cells, the localization of GFP-Rab5 vesicles was profoundly altered. Vesicles were found almost exclusively at the cell center, presumably because they were driven toward the MTOC by dynein. Virtually all of the BicD2 was also concentrated at the cell center, which suggests that molecules of BicD2 that dimerize with FRB-Rab5 remain attached to endosomal vesicles. A comparable experiment was conducted using a kinesin motor domain that was fused to FKBP (Vale et al., 1996; Friedman and Vale, 1999; Jacobson et al., 2006; Cai et al., 2009; Nakata et al., 2011). Endosomes were successfully misdirected to the cell periphery (Fig. S1), but the resulting change was not as obvious as that which resulted from linking vesicles to dynein.

In the experiment just described (and in those that follow), only about half of the transfected cells exhibited the profound changes in vesicle localization illustrated in Figs. 1 and S1. In the other cells, the distribution of endosomes and of BicD2 was no different than in controls. This is not surprising, because the assay depends on the coexpression of multiple proteins at levels appropriate to induce vesicle movement. Because the pattern of vesicle mislocalization induced by association with active motors was never observed in control cells and because this mislocalization requires that the FRB-tagged proteins bind to the labeled vesicles, we interpret positive results as strong evidence of vesicle binding, even if observed in only a fraction of the cells.

Evaluating the selectivity of Rab binding to early and late endosomes

We next asked whether the binding of different FRB-Rabs shows the expected selectivity for different endosome populations. We used two approaches to identify early endosomes: immunostaining to localize EEA1 and uptake of transferrin conjugated to Alexa Fluor 555 (Tf555). LysoTracker red was used to visualize late endosomes and lysosomes.

To determine which of these vesicles bound to Rab7, we expressed BicD2 and FRB-Rab7 (Fig. 2). In control cells, early endosomes were distributed throughout the cell, whereas late endosomes/lysosomes had a more perinuclear labeling pattern (Fig. 2 B). Cells expressing FRB-Rab7 and treated with the linker drug displayed some redistribution of the early endosome markers EEA1 and Tf555. However, most early endosomes...
Identifying the motor proteins associated with different endosomal populations

We next used this assay to investigate the kinesins that are present on different endosomal populations, using multiple approaches to label early and late endosomes (Figs. 3 and S2). We focused on motors of the Kinesin-3 family. Although some of these motors have been previously implicated in endosomal transport (Matsushita et al., 2004; Hoepfner et al., 2005; Blatner et al., 2007; Delevoye et al., 2009; Huckaba et al., 2011; Kanai et al., 2014), there has been no systematic study of whether different Kinesin-3's bind preferentially to different endosomes.

We generated kinesin tails that included the cargo-binding domain but lacked the motor domain and the first coiled coil. These tails were fused to an N-terminal FRB-3myc. Each of these tails was then expressed together with FLAG-BicD2-FKBP to determine whether they bound to different endosomal populations (Fig. 3 A). We used the same three labels of endogenous vesicles as before. In control cells, each of the endosomal markers displayed its characteristic distribution, as described earlier. This distribution was unaffected by expression of any of the kinesin tails (Fig. 3 B).

remained in the cell periphery, indicating that they did not bind FRB-Rab7. The mislocalization of some endosomes labeled with these markers is not surprising, as there is a period during the transition from early to late endosomes when vesicles carry both Rab5 and Rab7 (Rink et al., 2005; Poteryaev et al., 2010). In contrast, adding linker drug caused the accumulation of nearly all LysoTracker red vesicles in cells expressing FRB-Rab7; few if any vesicles could be detected outside of the aggregate. This indicates that FRB-Rab7 binds primarily to late endosomes and lysosomes in this assay, consistent with the known localization of this protein (Bucci et al., 2000). As expected from the results in Figs. 1 and S1, BicD2 also accumulated at the cell center when it was linked to vesicles. Similar experiments using the same vesicle markers and FRB-Rab5 showed preferential binding to early endosomes (Fig. S2). From these experiments, we conclude that this assay correctly reports the selective binding of FRB-Rab7 to late endosomes/lysosomes and FRB-Rab5 to early endosomes. The assay works equally well when endosomes are labeled in living cells or when they are visualized by immunostaining after the experiment has been completed.
In cells expressing KIF1A<sub>tail</sub> or KIF1B<sub>tail</sub> and treated with the linker drug, there was no change in distribution of early endosomes labeled with either Tf555 or EEA1, but there was a profound redistribution of the late endosome marker LysoTracker red. Labeled endosomes formed a compact cluster in the center of the cell; few vesicles could be detected elsewhere. In cells expressing KIF13A<sub>tail</sub> or KIF13B<sub>tail</sub> and treated with linker drug, there was no effect on the distribution of late endosomes/lysosomes, but the addition of linker drug caused EEA1 and Tf555 vesicles to aggregate in the cell center. This shows that different Kinesin-3 family members bind different endosomal populations; KIF1A and KIF1B<sub>β</sub> bind to late endosomes, whereas KIF13A and KIF13B bind early endosomes. These results were confirmed using a series of GFP-tagged proteins to label different endosomal populations (Fig. S3).

**An individual vesicle can bind different kinesins**

The results just described indicate that each endosomal population is capable of binding two different Kinesin-3 family members. This raises the question of whether different kinesins are found on individual endosomes or whether there are subpopulations of early and late endosomes that bind different kinesins. This assay can address this question by using one kinesin tail to label vesicles, then determining whether other FRB-tagged kinesins bind to the same vesicles. As shown in the schematic, vesicles were labeled by expressing GFP-KIF1A<sub>tail</sub>, which was coexpressed with tdtMyc-BicD2<sub>F2</sub>-FKBP and FRB-3myc-KIF1B<sub>α</sub> or FRB-3myc-KIF1B<sub>β</sub>tail. The control cell expressed FRB-3myc-KIF1A<sub>α</sub> but was not exposed to linker drug. The yellow lines outline the cell boundaries. Bar, 25 µm.

**Using the assay to define the vesicle-binding domains of trafficking proteins**

In principle, this assay could also be used to define the vesicle-binding domains of trafficking proteins. To evaluate this possibility, we attempted to define the domain of KIF13B that binds early endosomes (Fig. 5). The KIF13B tail contains a forhead-associated (FHA) domain (residues 423–557) that binds centaurin-α (Tong et al., 2010) and a membrane-associated guanylate kinase (MAGUK) binding stalk (residues 607–831) that interacts with homologues of Drosophila melanogaster discs-large (hDlg; Hanada et al., 2000). Previous studies have implicated the interaction with centaurin-α and the interaction with hDlg as important for the binding of KIF13B to vesicles (Tong et al., 2010; Kanai et al., 2014). We generated an FRB-tagged fragment of the KIF13B tail that contains the FHA domain and the MAGUK binding stalk (KIF13B<sub>442–831</sub>). A second FRB-tagged construct contained the remainder of the tail (KIF13B<sub>832–1,826</sub>). These constructs were each coexpressed with transferrin receptor (TfR)–GFP and FLAG-BicD2<sub>F2</sub>-FKBP (Fig. 5 A). In control cells, TfR–GFP vesicles were distributed throughout the cell (Fig. 5 B). In cells that expressed KIF13B<sub>442–831</sub> and were treated with the linker drug, there was no change in the localization of TfR–GFP vesicles. In cells expressing KIF13B<sub>832–1,826</sub> tail, addition of the linker drug resulted in a pronounced redistribution of TfR–GFP to the cell center. This experiment shows that residues 832–1,826 are sufficient to mediate binding of KIF13B tail to early endosomes and that neither the FHA domain nor the MAGUK binding stalk mediates this interaction.

**Advantages and limitations of this new assay**

The motor-driven vesicle mislocalization assay described here offers a new approach to evaluate protein–vesicle association that has several advantages compared with other methods.
Because binding of a candidate protein causes the vesicles of interest to move to a well-defined end point in the cell, positive interactions result in an unambiguous change in vesicle distribution that is easily interpretable. This makes it possible to evaluate the results by eye, thus allowing rapid screening of multiple combinations of candidate proteins and vesicle populations. Vesicles can be labeled by any convenient method, including the expression of fluorescent proteins, labeling with fluorescent dyes, or immunostaining.

Although the FRB-tagged candidate proteins are overexpressed, we saw little evidence that this led to nonspecific binding. For example, KIF1A and KIF1Bβ never associated with early endosomes and KIF13A and KIF13B never bound late endosomes or lysosomes. Vesicles may change their identity over time, shedding some trafficking proteins and gaining others. Thus, it is possible that an FRB-tagged protein could bind an unlabeled vesicle and induce a change in its localization; if the identity of that vesicle then changed, it could become labeled after it had been misdirected. The mislocalization of some LysoTracker red vesicles that occurred in cells expressing FRB-Rab5 may be an example of this (Fig. S2).

As with any assay, negative results must be interpreted with caution. If the FKBP- and FRB-tagged constructs are not expressed at sufficiently high levels, too few motors will become linked to the vesicles to move them to the intended destination. If a significant fraction of the FKBP-tagged motor becomes linked to FRB-tagged proteins that are not associated with vesicles—either because the expression level of the FRB-tagged protein is too high or because there is a large pool of unbound FRB protein—this could also result in too few vesicle-bound motors to produce effective movements. False negatives could also arise if the FRB-tagged protein cycles off the vesicle membrane quickly or if the FRB epitope becomes inaccessible for linking to FKBP. Despite these potential caveats, the conditions needed to obtain a positive result were not difficult to achieve.

We believe this assay will have broad application for investigating interactions between trafficking proteins and different vesicle populations. Once a set of FRB-tagged constructs has been prepared, they can be screened against a large number of vesicle populations in a remarkably short period of time. This assay may be particularly useful for evaluating the binding of trafficking proteins that are members of large families, such as Rabs, where any given vesicle is likely to bind a limited number of family members. If different family members bind different vesicles in this assay, this establishes that the FRB-tagged proteins are capable of binding and translocating vesicles but do not bind indiscriminately. This assay can also be adapted to explore other aspects of protein–vesicle interactions. By using one GFP-tagged Kinesin-3 to label vesicles, it was possible to establish that a second Kinesin-3 family member also bound to the same vesicle. The assay can also be used to identify the regions of trafficking proteins that mediate vesicle binding.

The role of Kinesin-3 family members in endosomal transport

We show that early and late endosomes bind different members of the Kinesin-3 family with high specificity. KIF13A and KIF13B, two closely related Kinesin-3s, bind early endosomes. KIF1A and KIF1Bβ, two other Kinesin-3s that are also closely related to each other, bind late endosomes and lysosomes. These conclusions are based on consistent results obtained with all

Figure 5. Identifying the domain of KIF13B that binds to endosomes. (A) As shown in the schematic, different regions of KIF13B tail tagged with FRB were coexpressed with tTfM-BicD2ΔFKBP and TIR-GFP. (B) In control cells, TIR-GFP was distributed throughout the cell. After treatment with the linker drug TIR-GFP, vesicles were moved to the cell center in cells expressing FRB-3myc-KIF13B Δ42–1,826 but not FRB-3myc-KIF13B Δ642–831. The yellow lines outline the cell boundaries. Bar, 30 µm.
Table 1. Constructs used in the induced dimerization assay

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Accession numbers were obtained from GenBank.

*aIncludes epitope tag or label (FLAG, myc, GFP, or tdTM), heterodimerization domain (FRB or FKBP), amino acid linker sequence (using single letter code), and trafficking protein (amino acids included).

seven of the markers we used to label these two endosomal populations (Figs. 3 and S3).

Although the work described here is the first systematic investigation of the interaction of Kinesin-3s with different endosomal populations, our findings are consistent with previous studies that implicated one or another Kinesin-3 in different aspects of endosomal trafficking. Kinesin-73, a Drosophila KIF13 homologue, binds Rab5 vesicles in S2 cells (Huckaba et al., 2011), and KIF13B plays a role in tubule formation at sorting endosomes (Delevoye et al., 2009), and KIF13B has been implicated in endocytosis in hepatocytes (Kanai et al., 2014). In contrast, KIF1Bβ binds lysosomes in COS7 cells (Matsushita et al., 2004). KIF16B, a Kinesin-3 we did not examine in this study, is also involved in endocytic trafficking (Hoepfner et al., 2005; Blatner et al., 2007). The new assay we developed allowed a systematic approach to this question, which revealed a pattern of Kinesin-3–endosome binding that appears to be consistent over a range of cell types and species but that was not apparent from earlier studies that examined one kinesin and its interaction with a single population of vesicles.

Conclusions

In the experiments presented here, we introduce a new method to identify protein–vesicle interactions in intact cells. Using an inducible dimerization system to link candidate vesicle-binding proteins to activated motors, the association of a candidate protein with a labeled vesicle is transduced into a predictable, highly distinctive change in vesicle localization. This output provides direct experimental evidence of vesicle binding, in contrast to approaches such as two-color colocalization, which are essentially correlative. Using this approach to investigate trafficking proteins that bind early and late endosomes, we show that the assay has a high specificity, a wide range of utility, and a readout that is easily interpretable.

Materials and methods

Constructs

All constructs were cloned into the pCAG expression vector (Niwa et al., 1991). This expression system consists of a cytomegalovirus–immediate early enhancer combined with a chicken β-actin promoter and has been expressed to steady levels. We generated the following constructs: KIF5C1–1515-tdTM-FKBP (Friedman and Vale, 1999; Jacobson et al., 2006); tdTM-Bicadual2845FKBP; and FLAG-Bicadual2845FKBP (Kapitein et al., 2010a). FRB-tagged Rab proteins were prepared by inserting the FRB-3myc sequence at their N termini. FRB-tagged kinesin tails were engineered by removing the N-terminal motor domain and the first coiled-coiled domain, replacing them with an FRB-3myc domain. Details about linkers, accession numbers, and tags for each of these constructs can be found in Table 1. The following cDNAs were used to label specific vesicle populations: mouse Rab5a (GFP-Rab5; GenBank accession number NM_025887) or Rab7 (GFP-Rab7; GenBank accession number NM_009005) tagged with EGFP at their N termini, human TR tagged with GFP at its C terminus (TR-GFP; Burack et al., 2000; Silverman et al., 2001), human low density lipoprotein receptor tagged by insertion of EGFP downstream of the signal sequence (GFP-LDL receptor; Silverman et al., 2005), and human LAMP1 tagged with a C-terminal GFP (LAMP1-GFP; GenBank accession number J04182).

FRB-tagged constructs included a 3myc epitope tag so that expressing cells could be identified by immunostaining; in preliminary experiments, we established that the great majority of cells that expressed both of the fluorescent constructs also expressed the FRB-tagged protein, based on anti-myc immunostaining.

Cell culture

Rat embryonic fibroblast cells (Heidemann et al., 1999) were grown at 37°C in Dulbecco’s modified Eagle’s medium (Gibco) containing 10% fetal bovine serum, 4.5 g/liter d-glucose, 548 mg/liter glutamine, 110 mg/liter sodium pyruvate, 0.1 g/liter streptomycin, and 100 U/ml penicillin. 1 or 2 d before transfection, cells were trypsinized, and cells were replated on glass coverslips.

Vesicle binding assay

cDNAs encoding each of the components for the vesicle binding assay were expressed by transfection with FuGENE 6 (Promega). Transfected components included the putative vesicle binding protein tagged with FRB, either KIF5C1–1515-tdTM-FKBP, tdTM-Bicadual2845FKBP, and FLAG-Bicadual2845FKBP, and in some cases a GFP-tagged protein that served to label the vesicles of interest. After expression for ~48 h, heterodimerization of the FRB-tagged and FKBP-tagged proteins was induced by treating cells with 100 nM AP21967, a rapamycin analogue (Muthuswamy et al., 1999; Kapitein et al., 2010a). Kinesin tails were designed by removal of the motor and the dimerization domains followed by the addition of an N-terminal FRB-3myc (Table 1). If present, the latter domain could mediate dimerization with endogenous kinesins and hence have a dominant-negative effect (Uchida et al., 2009; Hendricks et al., 2010; Lewis et al., 2011). After 1–3 h with the linker drug, cells were fixed in 4% formaldehyde with 4% sucrose and then mounted in Elvanol (Banker and Goslin, 1998). Control cells were treated identically but not exposed to AP21967. Endogenous lysosomes were labeled by exposing cells for 30 min to 200 nM lysotracker red (DND-99) before fixation (Molecular Probes). Endogenous early endosomes were labeled by exposing cells for 1 h to 25 μg/ml Alexa Fluor 488.
Fluor 55-tagged human transferrin before fixation (Molecular Probes). In some experiments, EEA1-positive endosomes were labeled after fixation by immunostaining with the rabbit monoclonal anti-EEA1 (C45B10, Cell Signaling Technology). Myc-tagged proteins were detected by immunostaining with the mouse monoclonal anti–c-myc 9E10 (M4439, Sigma-Aldrich).

Microscopy
Cells were imaged using an epifluorescence microscope (Axio Observer.Z1; Carl Zeiss) equipped with an LCI Plan Apochromat 40×/1.3 NA or LCI Plan Apochromat 63×/1.4 NA objective. Images of cells were acquired using a camera (AxioCam MRm; Carl Zeiss) and AxioVision software (Carl Zeiss).

Online supplemental material
Fig. S1 shows that linking constitutively active Kinesin-1 to Rab7 endosomes results in their accumulation in the periphery of the cell. Fig. S2 shows that linking FRB-Rab5 to dynein results in the accumulation of early endosomes at the cell center. Fig. S3 shows that different Kinesin-3 family members bind early and late endosomes. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201408056/DC1.

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Figure S1. **Linking Rab7 to a constitutively active kinesin motor domain by inducible dimerization causes late endosomes to accumulate in the cell periphery.** (A) A schematic showing the components that were expressed in this experiment. GFP-Rab7 was used as a vesicle marker and was coexpressed with FRB-3myc-Rab7 and the kinesin motor domain KIF5C559-tdTM-FKBP (Navone et al., 1992; Andrews et al., 1993; Vale et al., 1996; Verhey et al., 1998; Friedman and Vale, 1999; Jacobson et al., 2006; Cai et al., 2009; Wong and Rice, 2010; Nakata et al., 2011). After the addition of the linker drug, the FRB and FKBP domains were fused together, linking the constitutively active motor domain to vesicles that bind FRB-3myc-Rab7. A second diagram shows the predicted redistribution of GFP-Rab7 vesicles to the plus end of microtubules (gray) after the addition of the linker drug. This misdirection can only occur if FRB-3myc-Rab7 and GFP-Rab7 bind to the same vesicles. (B) Representative images showing the distribution of KIF5C559-tdTM-FKBP and GFP-Rab7 in control cells and cells treated with linker drug. In control cells, the kinesin motor domain was mostly diffusely distributed with small accumulations at a few points in the periphery of the cell. GFP-Rab7 vesicles had a mostly perinuclear distribution with no accumulations in the periphery. In the treated cell, GFP-Rab7 vesicles were accumulated in the periphery of the cell, together with the kinesin motor domain. The yellow lines outline the cell boundaries. Bar, 30 µm.
Figure S2. **Rab5 interacts primarily with early endosomal vesicles.** (A) A schematic showing the components of the assay. Endogenous vesicles were labeled by anti-EEA1 staining or by uptake of Tf555 or LysoTracker red. GFP-BicD2<sup>594</sup>-FKBP was expressed together with FRB-3myc-Rab5. (B) Representative cells showing the distribution of vesicles labeled by anti-EEA1 staining, Tf555, or LysoTracker red and of BicD2. In control cells, EEA1- and Tf555-labeled vesicles were found throughout the cell, whereas LysoTracker red labeling was more centrally located; few LysoTracker red vesicles were present in the cell periphery. BicD2 was diffusely distributed throughout the cell. When linker drug was added, EEA1- and Tf555-labeled vesicles accumulated in the center of the cell, forming a single, small spot, whereas the cell periphery was almost completely devoid of early endosomes. LysoTracker red (LysoRed) vesicles were also shifted toward the cell center and distributed in a loose ring with a weakly labeled center; BicD2 occupied the center of this ring. Because early endosomes formed an aggregate of about this size in cells expressing BicD2 and FRB-Rab5, it seemed likely that they occupied the unlabeled zone within the LysoTracker ring. By coexpressing FRB-Rab5 and tdTM-BicD2-FLAG, then labeling vesicles with Tf488, and LysoTracker deep red, we confirmed that early endosomes and BicD2 colocalized in a central spot, which was encircled by vesicles labeled with LysoTracker (not depicted). It could be that overexpressed FRB-Rab5 bound late endosomes. Another possible explanation is that some Rab5-positive early endosomes were misdirected to the cell center, matured, and acquired LysoTracker red labeling and then were displaced centrifugally by later arriving early endosomes. The yellow lines outline the cell boundaries. Bar, 30 µm.
Figure S3. Identifying the kinesins that associate with different populations of endosomes. (A) A schematic showing the components expressed in this assay. Vesicles were labeled with a GFP-tagged marker protein, which was coexpressed with different FRB-tagged kinesin tails and tdTM-BicD2594–FKBP. The addition of linker drug fused the kinesin tail and BicD2, which can bind endogenous dynein. (B) Representative images showing the distribution of vesicles labeled with one of four different marker proteins in cells expressing different FRB-tagged kinesin tails. In control cells, early endosomes (labeled with GFP-Rab5 or TfR-GFP) were distributed throughout cell. Late endosomes (labeled with GFP-Rab7 or Lamp1) were also present throughout the cell, with the majority of vesicles in the center. In cells treated with the linker drug, the early endosome markers Rab5 and TfR were misdirected to the cell center only in cells expressing FRB-3myc-KIF13A\(_\text{tail}\) or FRB-3myc-KIF13B\(_\text{tail}\) tails. The late endosome markers Rab7 and Lamp1 were misdirected only in cells expressing FRB-3myc-KIF1A\(_\text{tail}\) and FRB-3myc-KIF1B\(_\text{tail}\). The control cell illustrated expressed FRB-3myc-KIF1A\(_\text{tail}\) but was not exposed to the linker drug. The yellow lines outline the cell boundaries. Bar, 25 µm.
References


