PH-domain-dependent selective transport of p75 by kinesin-3 family motors in non-polarized MDCK cells

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Summary

A key process during epithelial polarization involves establishment of polarized transport routes from the Golgi to distinct apical and basolateral membrane domains. To do this, the machinery involved in selective trafficking must be regulated during differentiation. Our previous studies showed that KIF5B selectively transports vesicles containing p75-neurotrophin receptors to the apical membrane of polarized, but not non-polarized MDCK cells. To identify the kinesin(s) responsible for p75 trafficking in non-polarized MDCK cells we expressed KIF-specific dominant-negative constructs and assayed for changes in post-Golgi transport of p75 by time-lapse fluorescence microscopy. Overexpression of the tail domains of kinesin-3 family members that contain a C-terminal pleckstrin homology (PH) domain, KIF1A or KIF1Bβ, attenuated the rate of p75 exit from the Golgi in non-polarized MDCK cells but not in polarized cells. Analysis of p75 post-Golgi transport in cells expressing KIF1A or KIF1Bβ with their PH domains deleted revealed that vesicle transport by these motors depends on the PH domains. Furthermore, purified KIF1A and KIF1Bβ tails interact with p75 vesicles and these interactions require the PH domain. Knockdown of canine KIF1A also inhibited exit of p75 from the Golgi, and this was rescued by expression of human KIF1A. Together these data demonstrate that post-Golgi transport of p75 in non-polarized epithelial cells is mediated by kinesin-3 family motors in a PH-domain-dependent process.

Key words: Epithelial polarization, Kinesin, Membrane trafficking

Introduction

The establishment and maintenance of epithelial polarity is critical to the function of numerous tissues and organs (Bryant and Mostov, 2008). During epithelial differentiation, dramatic reorganization of microtubule (MT) networks promotes the establishment of polarized transport routes to apical and basolateral regions of the plasma membrane (Musch, 2004). Membrane proteins are synthesized in the endoplasmic reticulum, transferred to the Golgi and segregated into different transport intermediates at the trans-Golgi network for export (Rodriguez-Boulan et al., 2005). Additional sorting can occur in post-Golgi endosomes, tubular transport intermediates and at the plasma membrane (Ellis et al., 2006). Time-lapse microscopy of fluorescent membrane proteins in fibroblasts and in non-polarized and polarized epithelial cells have shown that vesicular transport intermediates move through the cytoplasm along MTs (Hirschberg et al., 1998; Kreitzer et al., 2000; Kreitzer et al., 2003; Schmoranzer et al., 2003; Toomre et al., 1999). Biochemical studies also showed that in polarized epithelial cells, MT disruption results selectively in mis-targeting of apical membrane proteins (Breitfeld et al., 1990; Eilers et al., 1989; Gilbert et al., 1991; Kreitzer et al., 2003; Rindler et al., 1987). This selective effect on targeting of apical proteins appears to involve mislocalization of the apical t-SNARE, syntaxin-3, in response to MT depolymerization (Kreitzer et al., 2003). Despite this, specific molecular effectors of microtubule-based, targeted membrane transport in epithelia are largely unknown. However, production, transport and apical delivery of post-Golgi vesicles appear to depend on MT motors (Musch, 2004; Rodriguez-Boulan et al., 2005) and a role for both MT plus-end- and minus-end-directed kinesins as transporters to the apical membrane has been demonstrated (Fan et al., 2004; Jaulin et al., 2007; Jenkins et al., 2006; Noda et al., 2001).

Over forty kinesin family members have been identified in humans, and ~80% of these are expressed in epithelial cells of diverse origin (Jaulin et al., 2007). Kinesin family (KIF) proteins share significant sequence homology in their microtubule and ATP-binding domains and general similarity in overall architecture. KIF tail domains are highly divergent, enabling specific kinesins to interact with and transport distinct cargo in cells (Hirokawa and Noda, 2008). Although selective use of KIFs in targeted transport of axonal and dendritic membrane proteins has been well documented (Hirokawa and Noda, 2008; Wozniak et al., 2004), significantly less is known about KIFs used to transport apical and basolateral proteins in epithelial cells. Moreover, very little is known about whether kinesin utilization is regulated to promote membrane polarity during epithelial differentiation. However, the large complement of kinesin family motors and their selective interactions with cargoes and cargo adaptors suggests that kinesins can orchestrate domain-selective targeting of vesicles during polarization.

We demonstrated recently that KIF5B, a member of the kinesin-1 family, selectively transports p75 neurotrophin receptor to the apical membrane in polarized but not in non-polarized MDCK cells (Jaulin et al., 2007). The identity of the kinesin(s) involved in post-Golgi trafficking of p75 before polarization is not yet known. To this end, we tested the effects of overexpressed, kinesin tail domains on post-Golgi trafficking of p75 in non-polarized MDCK cells. Using this dominant-negative approach, in combination with siRNA-mediated knockdown, we identified kinesin-3 family members KIF1A and KIF1Bβ as selective transporters of p75 in non-polarized, but not polarized, MDCK cells. In addition, purified

KIF1A and KIF1Bβ tails interact with vesicles containing p75 in vitro and these interactions depend on the lipid-binding PH domains in KIF1A and KIF1Bβ tails. Thus kinesin-3 family members interact, through their PH domains, with vesicles containing p75, to mediate Golgi to plasma membrane transport of this protein in non-polarized MDCK cells whereas kinesin-1 mediates post-Golgi trafficking of p75 after polarization. This switch in kinesin utilization by a single cargo suggests that kinesin-cargo interactions are regulated during epithelial differentiation and may contribute to when and how plasma membrane domains with distinctive protein and lipid compositions are formed during polarization.

Results

Post-Golgi transport of p75 to the plasma membrane is mediated by KIF1A in non-polarized MDCK cells

We showed previously that post-Golgi transport of p75 is mediated by different kinesins before and after MDCK cell polarization, with KIF5B acting as the motor for p75 transport in polarized cells (Jaulin et al., 2007). In non-polarized cells, post-Golgi transport of p75 was inhibited by microinjection of a broadly reactive, function-blocking kinesin antibody, HD (Rodionov et al., 1991; Rodionov et al., 1993) but the specific kinesin(s) that mediates this transport has not yet been identified (Kreitzer et al., 2000). To address this issue, we expressed KIF-specific inhibitory constructs encoding the cargo-binding tail domains of different kinesins (Fig. 1) and assessed whether they affected both p75-GFP exit from the Golgi and delivery to the plasma membrane in non-polarized MDCK cells.

We assayed exit of p75 from the Golgi by time-lapse fluorescence microscopy followed by quantitative analysis of Golgi-associated p75-GFP over time. Non-polarized (sub-confluent) MDCK cells were microinjected with cDNAs encoding p75-GFP and sialyltransferase-mRFP (ST-mRFP) as a marker of the Golgi. One hour after injection, newly synthesized p75-GFP could be detected in the ER and Golgi. At this time, cells were incubated at 20°C for 2.5 hours to accumulate p75-GFP in the Golgi [Fig. 2A, 0 minutes 37°C (Matlin and Simons, 1983)]. We monitored exit of p75-GFP from the Golgi at 37°C in cells expressing p75-GFP alone and cells coexpressing myc-tagged KIF tails by time-lapse microscopy. In control cells, p75-GFP exited the Golgi with a half time ($t_{½}$) of ~120 minutes. Four hours after release of the Golgi block, most p75-GFP had emptied from the Golgi (Fig. 2A,B). By contrast, p75 exit from the Golgi ($t_{½}$) was attenuated 44% in cells coexpressing dominant-negative KIF1A-Tail (KIF1A-T; Fig. 2A,B). KIF1A was identified originally as an anterograde, axonal transporter of synaptic vesicle precursors (Okada et al., 1995b), however, it is also expressed in epithelial cells (Figs 2E, 4B, 5). We detected no significant change in exit of p75 from the Golgi in cells expressing KIF3A-T (Fig. 2A,B), KIF5B-T [not shown (see Jaulin et al., 2007)] or a motorless version of KIF1C (KIF1C-ML; supplementary material Fig. S5). KIF3A-T, KIF5B-T and KIF1C-ML are known to act as dominant-negative inhibitors of KIF3A, KIF5B and KIF1C, respectively, in cells (Dorner et al., 1998; Jaulin et al., 2007; Nishimura et al., 2004).

To test whether expression of KIF1A-T also inhibited delivery of p75-GFP to the plasma membrane, we measured the ratio of surface-associated p75-GFP to total p75-GFP in fixed, un-permeabilized cells 4 hours after release of the Golgi block. Surface-associated p75-GFP was detected by immunofluorescence staining with an antibody raised against the p75 extracellular domain as described previously (Kreitzer et al., 2003) and in the Materials and Methods. Similar to its effect on exit of p75 from the Golgi, expression of KIF1A-T inhibited the appearance of p75-GFP at the cell surface by 45% as compared with controls (see Fig. 6C). As expected, neither KIF3A-T nor KIF5B-T affected delivery of p75-GFP to the plasma membrane in this assay.

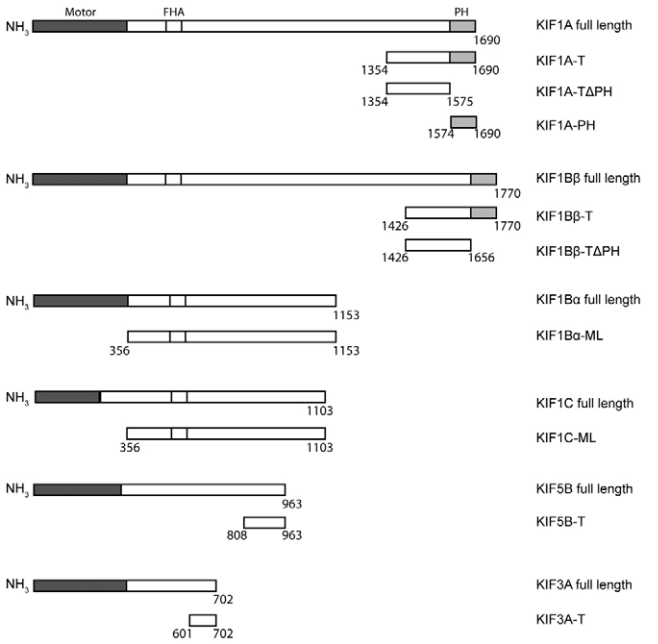

Fig. 1. Kinesin expression constructs. Schematic representation of full-length KIF1A, KIF1Bβ, KIF1Bα, KIF1C, KIF5B, KIF3A and KIF-specific tail constructs used as dominant-negative inhibitors.

Interestingly, KIF1A-T expression did not alter Golgi exit of p75 in polarized MDCK cells (Fig. 2C,D). This is consistent with our previous study demonstrating that different kinesins are used for post-Golgi trafficking of p75-GFP before and after epithelial polarization (Jaulin et al., 2007). Quantitative analysis of KIF1A protein levels in non-polarized and polarized MDCK cells revealed a 32±8.7% (±s.d., in three experiments) increase in KIF1A expression after polarization. Thus, altered KIF1A protein levels during polarization cannot account for the observed loss of KIF1A utilization in transport of p75 vesicles after polarization. This implicates an as yet unknown mechanism by which functional association of kinesin(s) with p75-containing vesicles is regulated during polarization.

KIF1A is not a general motor for transport of apical proteins in non-polarized MDCK cells

To test whether KIF1A-mediated post-Golgi transport is cargo-selective, we measured the effect of KIF1A-T expression on post-Golgi transport of two additional apical markers, GFP-GPI (Keller et al., 2001) and the ABC transporter BSEP-YFP (Wakabayashi et al., 2004). We expressed myc-KIF1A-T with GFP-GPI or BSEP-YFP in non-polarized MDCK cells and monitored exit of these proteins from the Golgi by time-lapse microscopy as described above. Expression of KIF1A-T had no significant effect on post-Golgi trafficking of either protein (Fig. 3) demonstrating that KIF1A is a selective vesicle transporter.

The selective inhibition of p75 trafficking by overexpressed KIF1A-T suggests that p75 is segregated from other apical markers upon exit from the Golgi. To determine if this is indeed the case,

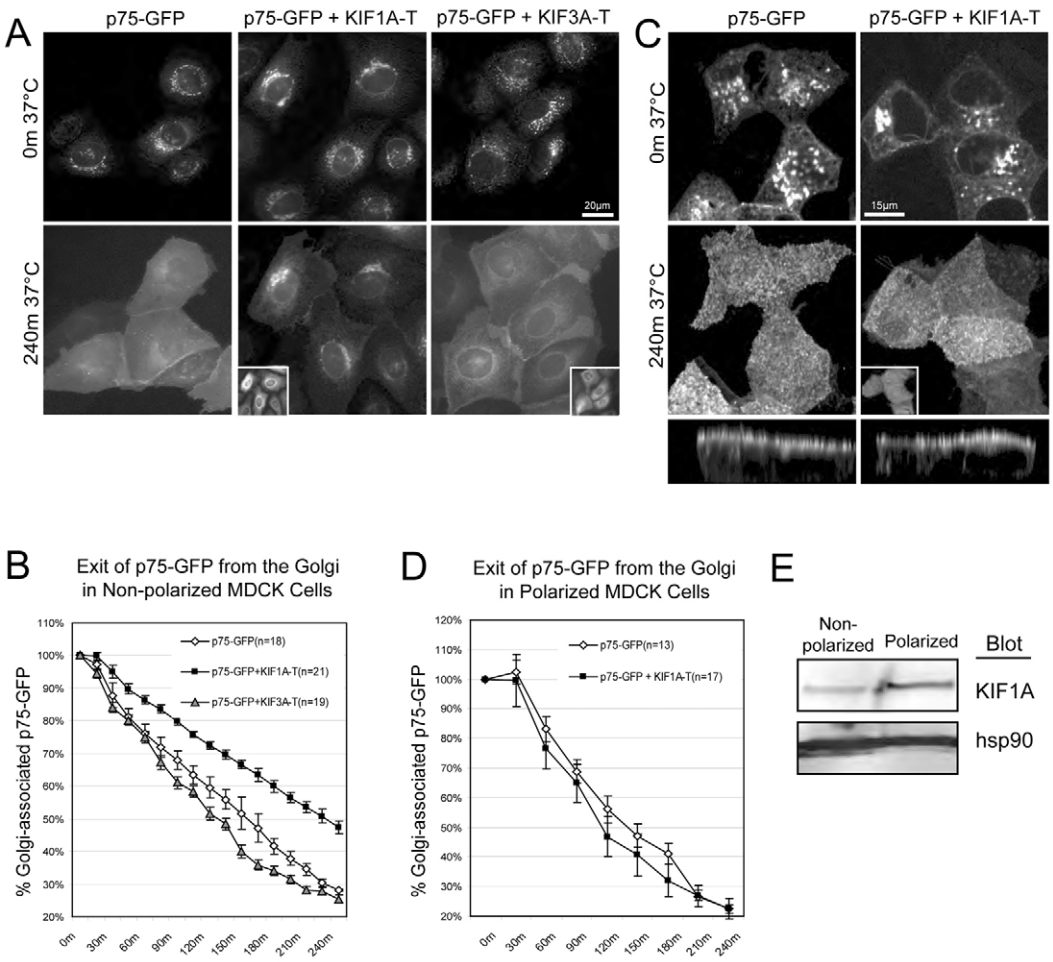

Fig. 2. KIF1A mediates post-Golgi transport of p75-GFP in non-polarized, but not in polarized, MDCK cells. (**A**) First and last frames from time-lapse recordings of non-polarized MDCK cells expressing p75-GFP, p75-GFP and myc-KIF1A-T or p75-GFP and myc-KIF3A-T. Upper panels show p75-GFP in the Golgi immediately after release of the 20°C temperature block (0 minutes 37°C). Lower panels show p75-GFP localization 240 minutes later. Insets: corresponding anti-myc immunostaining in fixed cells after the recording. (**B**) Quantification of Golgi-associated p75-GFP in each frame of time-lapse recordings for cells expressing p75-GFP alone or coexpressing myc-KIF1A-T or myc-KIF3A-T. Data were pooled from three independent experiments for each condition. (**C**) First and last frames from time-lapse recordings of polarized MDCK cells expressing p75-GFP or p75-GFP and myc-KIF1A-T. Upper and middle panels show p75-GFP in the Golgi immediately after release of the 20°C block (0 minutes 37°C) and at the apical membrane 240 minutes later. Images show maximum XY projections of Z series. Inset: myc-KIF1A-T immunostaining in cells fixed after the time-lapse recordings. Lower panels: orthogonal views of cells at the end of each recording. (**D**) Quantification of Golgi-associated p75-GFP in each Z-series of time-lapse recordings of polarized MDCK cells. Data were pooled from two independent experiments for each condition. (**E**) Western blot of endogenous KIF1A and HSP90 as a control in lysates prepared from non-polarized and polarized MDCK cells. Error bars indicate ±s.e.m.

we performed high resolution time-lapse imaging of cells coexpressing p75-CFP and one of three additional apical markers, GPI-YFP (Fig. 3C), BSEP-YFP or prominin-YFP (not shown). Proteins were expressed by microinjection as described above and images were acquired 5-30 minutes after release of the Golgi block. In all cases, p75-CFP segregated from other markers into distinct post-Golgi transport intermediates (Fig. 3C, insets a′,b′).

KIF1A knockdown inhibits post-Golgi transport of p75 in non-polarized cells

We amplified full-length KIF1A by PCR from MDCK cells. Canine KIF1A cDNA encoded a protein of 1690 amino acids, with a predicted molecular mass of 191 kDa, in accord with the observed size of KIF1A on western blots of MDCK lysates. Sequence alignment showed that canine KIF1A shares 97% identity with human KIF1A and contains the conserved kinesin motor domain [amino acids (a.a.) 3-362], a forkhead-associated domain (FHA; a.a. 515-572), and a carboxyl-terminal pleckstrin homology (PH) domain (a.a. 1576-1675; supplementary material Fig. S1). We used this sequence information to design specific siRNAs that target canine, but not human, KIF1A. We transfected MDCK cells with five siRNAs, two of which reduced KIF1A protein levels significantly [as determined by immunoblot (Fig. 4B) and immunofluorescence (supplementary material Fig. S2)] as compared with controls. Transfection of siRNAs targeting KIF1A did not affect protein levels of the closely related motor, KIF1Bβ (Fig. 4B). In addition, we observed no apparent changes in Golgi organization, localization of resident Golgi markers (supplementary material Fig. S3), cell morphology or proliferation in KIF1A-depleted cultures.

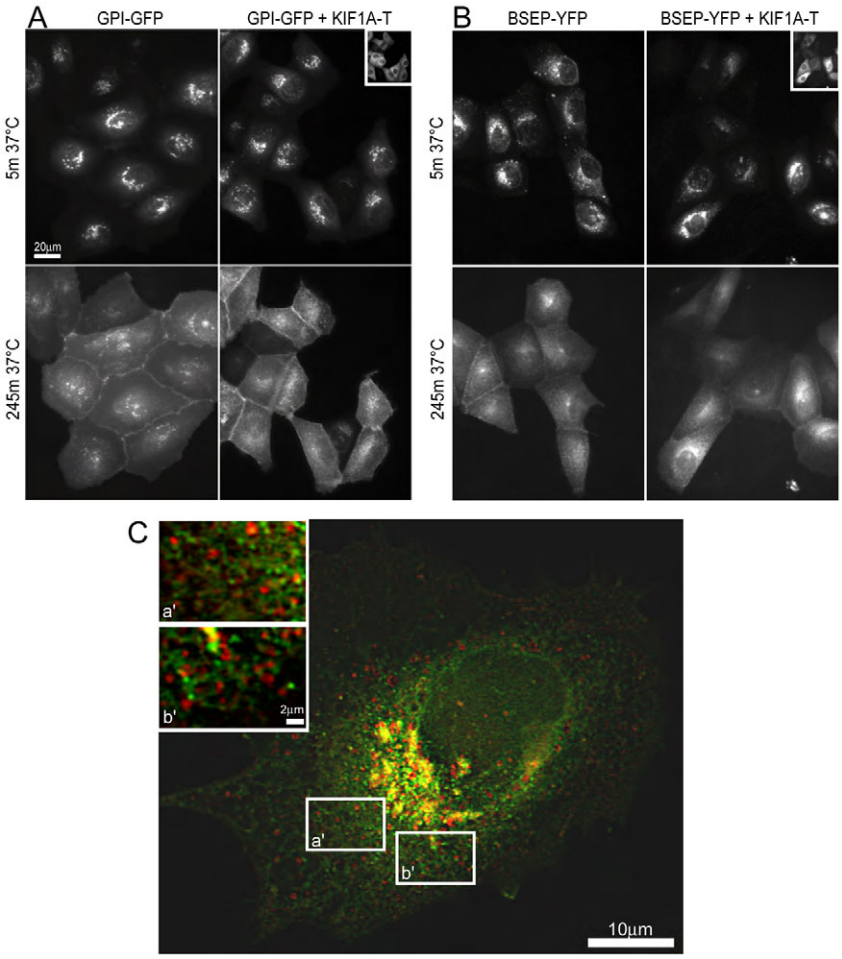

Fig. 3. KIF1A is not a general motor for apical cargo transport to the plasma membrane in non-polarized MDCK cells. First and last frames from time-lapse recordings of non-polarized MDCK cells expressing (**A**) GPI-GFP or GPI-GFP and myc-KIF1A-T, or (**B**) BSEP-YFP or BSEP-YFP and CFP-KIF1A-T. Upper panels show GPI-GFP or BSEP-YFP in the Golgi after the 20°C block (5 minutes 37°C). Lower panels show the localizations of the apical markers 4 hours later (245 minutes 37°C). Corresponding CFP-KIF1A-T fluorescence or myc-KIF1A-T immunostaining is shown in the insets. (**C**) p75-YFP (green) and GPI-CFP (red) coexpressed in non-polarized MDCK cells. Image shown was taken from a time-lapse series acquired 16 minutes after release of the 20°C Golgi block. Boxed regions a′ and b′ are shown at high magnification in the insets and illustrate sorting of p75 and GPI into distinct vesicle populations.

As an independent test of KIF1A function in p75 trafficking, we examined post-Golgi transport of p75-GFP in KIF1A siRNA-transfected cells by time-lapse imaging. Depletion of KIF1A, like overexpressed KIF1A-T, inhibited export of p75-GFP from the Golgi, after release of the 20°C block, by 43% (Fig. 4A,C). This was not due to off-target effects of the KIF1A siRNA as inhibition of p75 trafficking was efficiently rescued by expression of human full-length KIF1A (hKIF1A; Fig. 4A,C). Consistent with selective inhibition of p75 trafficking by overexpressed myc-KIF1A-T, KIF1A knockdown had no detectable effect on exit of either GPI-GFP (supplementary material Fig. S4) or BSEP-GFP (not shown) from the Golgi.

We also tested whether depletion of KIF1A inhibited exit of p75 from the Golgi in polarized MDCK cells. Again, consistent with results shown in Fig. 2, we observed no inhibition of p75 exit from the Golgi in polarized cells expressing KIF1A siRNA despite continued depletion of KIF1A protein (not shown). Polarization appeared to be slower in KIF1A-depleted cells, however, we observed a similar delay in cells transfected with control siRNA as well.

KIF1A colocalizes with vesicles containing p75 in non-polarized MDCK cells

To investigate the mechanism by which KIF1A mediates post-Golgi transport of p75 transport, we next examined the localization of endogenous KIF1A in cells expressing p75-GFP. Non-polarized MDCK cells were injected with cDNA encoding p75-GFP, fixed 15 minutes after release of the Golgi block and immunostained for KIF1A. At this time, the majority of p75-GFP localizes to the Golgi but p75-GFP post-Golgi vesicles are also observed. Numerous KIF1A puncta colocalized with vesicles containing p75-GFP (Fig. 5). By contrast, no colocalization between p75-GFP and endogenous KIF1A was observed in polarized MDCK cells (Fig. 5). This suggests that attenuation of p75-GFP post-Golgi trafficking by KIF1A-T expression is due to inhibition of a functional association of endogenous KIF1A with p75-GFP vesicles.

KIF1A and KIF1Bβ act cooperatively in post-Golgi transport of p75 in non-polarized MDCK cells

KIF1A is a member of the kinesin-3 family, which also includes KIF1Bα and KIF1Bβ (encoded by alternatively spliced forms of the same gene), KIF1C, KIF13A, KIF13B, KIF14, KIF16B and KIF28 (Miki et al., 2005). Among these, KIF1A and KIF1Bβ are most similar in that they contain lipid-binding PH domains at their C-terminus; KIF16B contains a lipid-binding PX domain and was shown to be involved in trafficking of endocytic vesicles (Hoepfner et al., 2005). To test whether KIF1Bβ also participates in post-Golgi transport of p75, we coexpressed p75-GFP and the C-terminal tail domain of KIF1Bβ (KIF1Bβ-T), and recorded p75-GFP post-Golgi transport after release of the Golgi block. Expression of KIF1Bβ-T inhibited p75 exit from the Golgi ($t_{½}$) by 44% (Fig. 6A,B), similar to what was observed in cells expressing

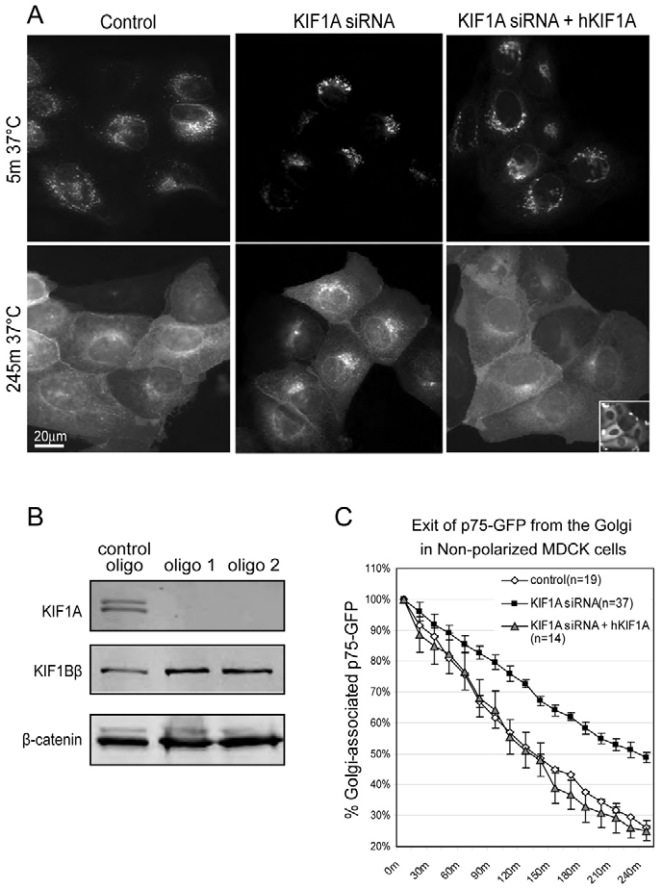

Fig. 4. Knockdown of KIF1A inhibits post-Golgi transport of p75 in non-polarized MDCK cells. (**A**) First and last frames from time-lapse recordings of p75-GFP in non-polarized MDCK cells treated with control siRNA, KIF1A siRNA (oligo 1) or KIF1A siRNA rescued by microinjecting cDNA encoding human myc-KIF1A (hKIF1A). Inset shows corresponding human myc-KIF1A immunostained after the time-lapse recordings. (**B**) Immunoblot showing KIF1A, KIF1Bβ and β-catenin in non-polarized MDCK cells transfected with control siRNA or two different KIF1A siRNAs. (**C**) Quantification of Golgi-associated p75-GFP in each frame of time-lapse recordings of non-polarized MDCK cells treated with control siRNA, KIF1A siRNA, or KIF1A siRNA coexpressing myc-hKIF1A. Data represent pooled results from three recordings for each data set shown. Error bars indicate ±s.e.m.

KIF1A-T. In cells transfected with KIF1A siRNA, expression of myc-KIF1Bβ-T resulted in further inhibition of p75 export from the Golgi (56%; Fig. 6A,B), suggesting that KIF1A and KIF1Bβ act synergistically in p75 trafficking. High resolution imaging of cells coexpressing p75-GFP with both myc-KIF1A-T and RFP-KIF1Bβ-T also revealed an ~3.5-fold decrease in the number of motile p75 vesicles (classified as vesicles moving in at least 10 sequential frames of the time-lapse sequence) as compared with controls. In addition, the mean velocity of vesicles that we did observe in cells coexpressing myc-KIF1A-T and RFP-KIF1Bβ-T was 0.21±0.12 μm/second (n=27), 2.4-fold slower than in controls [0.51± 0.19 μm/second (n=21)] (see also Jaulin et al., 2007). These data suggest that budding of p75 from the Golgi is inhibited when both motors are perturbed and vesicles that do bud are probably transported by a different kinesin.

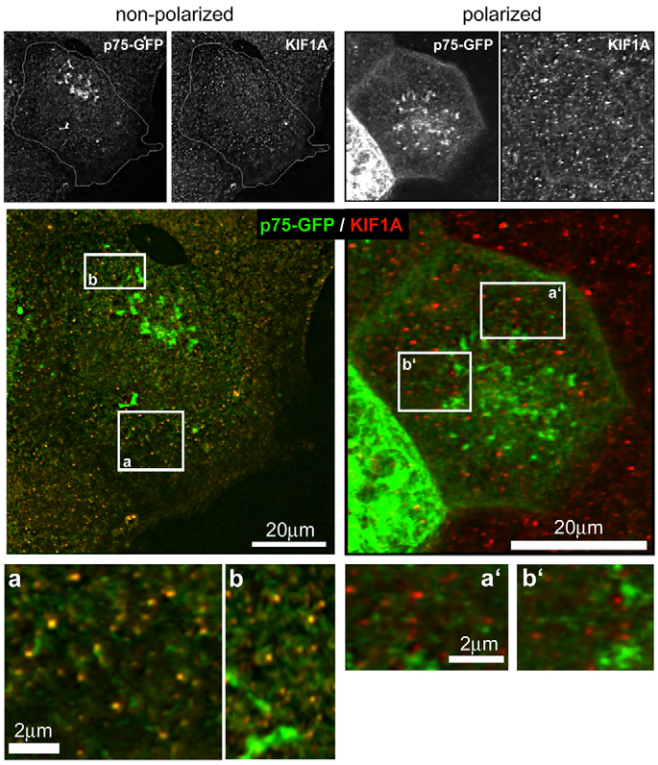

Fig. 5. KIF1A colocalizes with vesicles containing p75-GFP in non-polarized cells but not in polarized cells. Localization of microinjected p75-GFP and endogenous KIF1A in non-polarized or polarized MDCK cells. Cells were fixed 15 minutes after release of the Golgi block and immunostained for KIF1A. Color overlay images show enlargements of grayscale images. Boxed regions are further enlarged in bottom panels. Enlargement of boxed region b was rotated 90°.

KIF1A and KIF1Bβ associate with p75 vesicles prepared from non-polarized MDCK cells

The PH domain of UNC-104, the C. *elegans* homolog of KIF1A and KIF1Bβ, was shown *in vitro* to bind preferentially to synthetic vesicles enriched in phosphatidylinositol (4,5)-bisphosphate and to be essential for axonal transport of synaptic vesicles (Klopfenstein et al., 2002; Klopfenstein and Vale, 2004). This raised the question of whether the PH domains of KIF1A and KIF1Bβ are required to mediate interactions between the motor and p75 vesicles. To test this, we generated GST-tagged KIF1A and KIF1Bβ tail domains (KIF1A-T, KIF1Bβ-T), and KIF1A and KIF1Bβ tails deleted of their PH domains (KIF1A-TΔPH, KIF1Bβ-TΔPH) and determined whether they interact with vesicles containing p75 isolated from non-polarized MDCK cells. To enrich for Golgi-derived vesicles, non-polarized MDCK cells stably expressing p75-GFP were incubated at 20°C for 3 hours to accumulate newly synthesized p75-GFP in the Golgi. Cells were then transferred to 37°C for 30 minutes. At this time, we homogenized the cells and used sucrose gradient density centrifugation to isolate vesicles containing p75-GFP. Previously, we showed that numerous post-Golgi vesicles have been produced by this time and can be observed moving through the cytoplasm toward the membrane (Jaulin et al., 2007; Kreitzer et al., 2003) and negative-stain electron microscopy verified the presence of vesicular profiles in these fractions (data not shown). Fractions containing post-Golgi vesicles were incubated

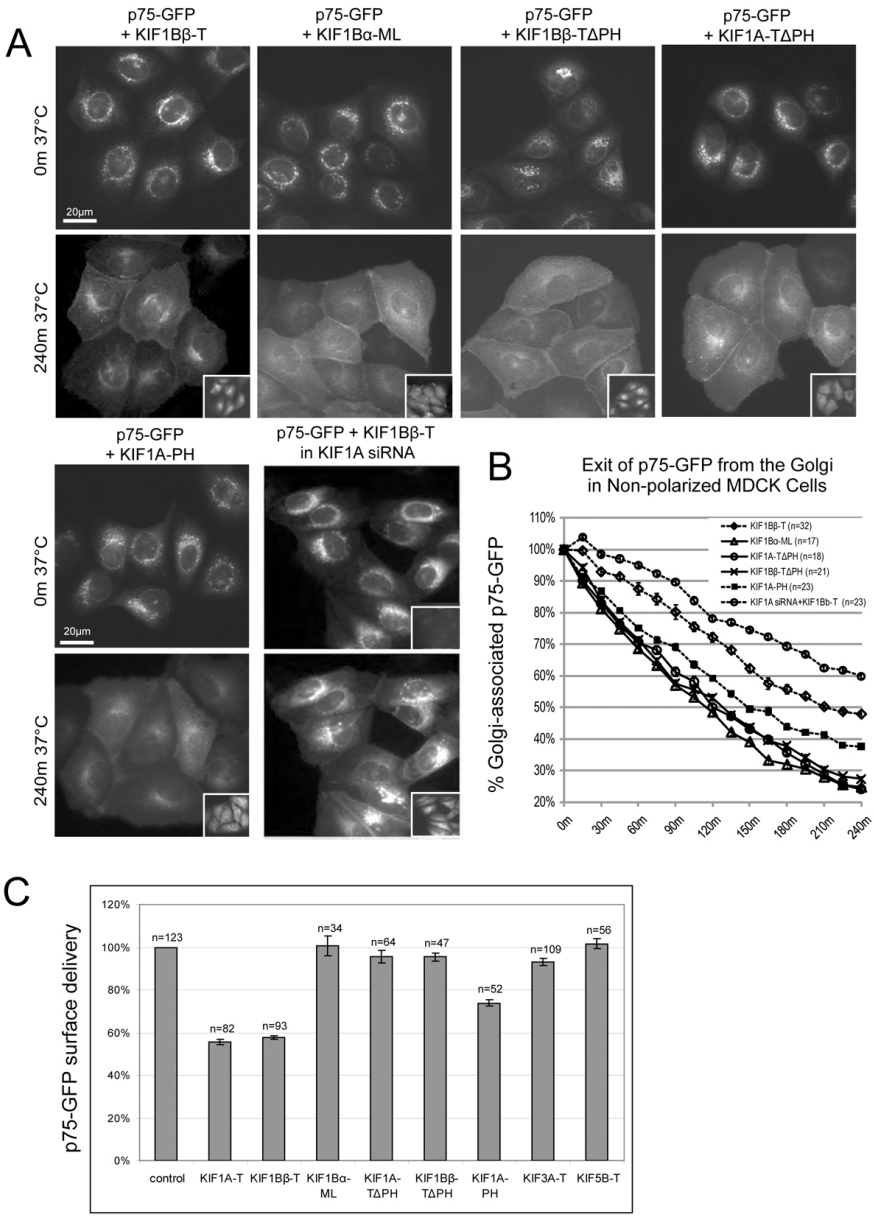

Fig. 6. PH-domain-dependent post-Golgi transport of p75-GFP by KIF1A and KIF1Bβ in non-polarized MDCK cells. (**A**) First (0 minutes 37°C) and last (240 minutes 37°C) frames from time-lapse recordings of non-polarized MDCK cells expressing p75-GFP with myc-KIF1Bβ-T, myc-KIF1Bα-ML, myc-KIF1A-TΔPH, myc-KIF1Bβ-TΔPH, myc-KIF1A-PH or myc-KIF1Bβ-T in KIF1A-depleted cells. Insets in the 240-minute panels show immunostaining of myc-KIF-Tails after the time-lapse recordings. Inset in 0-minute panel of KIF1A siRNA-treated cells shows anti-KIF1A immunostaining and loss of KIF1A signal in siRNA-treated cells. (**B**) Quantification of Golgi-associated p75-GFP in each frame of time-lapse recordings of non-polarized MDCK cells coexpressing the indicated KIF-tail constructs. Data represent mean values from indicated number of cells taken from two or three recordings for each data set shown. Error bars indicate s.e.m. (**C**) Surface-associated p75-GFP in non-polarized MDCK cells expressing p75-GFP alone (control) or p75-GFP with myc-KIF1A-T, KIF1Bβ-T, myc-KIF1Bα-ML, myc-KIF1A-TΔPH, myc-KIF1Bβ-TΔPH or myc-KIF1A-PH, KIF3A-T, KIF5B-T. Cells were fixed 4 hours after release of the Golgi block. Surface-associated p75 was detected with antibodies against the p75 ectodomain in unpermeabilized cells. Surface (immunolabeled):total (GFP) p75 fluorescence intensity was calculated. Values obtained in controls were normalized to 100%. Data represent mean values from the indicated number of cells taken from two independent experiments. Error bars indicate ±s.d.

with GST-tagged KIF1A-T, KIF1Bβ-T, KIF1A-TΔPH, KIF1Bβ-TΔPH and KIF5B-T (Fig. 1) immobilized on glutathione beads. After extensive washes to remove unbound vesicles, vesicle-kinesin tail complexes were separated by SDS-PAGE and immunoblotted for p75-GFP. In these experiments KIF1A-T and KIF1Bβ-T, but not KIF1A-TΔPH, KIF1Bβ-TΔPH or KIF5B-T, pulled down vesicles containing p75-GFP (Fig. 7). These data demonstrate directly the PH-domain-dependent association of KIF1A and KIF1Bβ with p75-containing vesicles.

PH domains of KIF1A and KIF1Bβ are necessary for p75 transport in non-polarized MDCK cells

We next tested whether the PH domains of KIF1A and KIF1Bβ are required for p75 transport in non-polarized MDCK cells. We analyzed post-Golgi trafficking of p75-GFP in cells expressing myc-KIF1A-TΔPH or myc-KIF1Bβ-TΔPH as described above and found no significant change in exit of p75 from the Golgi under these conditions (Fig. 6A,B). Similarly, overexpression of motorless KIF1Bα (KIF1Bα-ML; Fig. 6A,B) or KIF1C (KIF1C-ML; supplementary material Fig. S5), which lack a PH domain, had no effect on p75 trafficking.

Consistent with Golgi exit analysis, expression of KIF1Bβ-T resulted in 42% reduction in the p75-GFP delivery to the cell surface as compared with controls. By comparison, expression of KIF1A-TΔPH or KIF1Bβ-TΔPH did not significantly reduce the amount of p75-GFP at the cell surface 4 hours after release of the Golgi block (Fig. 6C). These results reveal an essential role of the PH domain in transport of p75 by KIF1A and KIF1Bβ in non-polarized MDCK cells. We also tested if expression of the KIF1A PH domain alone (KIF1A-PH) affected p75 export from the Golgi and delivery to the plasma membrane. In these experiments, exit of p75 from the Golgi was inhibited 18% (Fig. 6A,B) and surface-associated p75-GFP was reduced 26% 4 hours after release of the Golgi block (Fig. 6C). The inhibitory effect of overexpressed

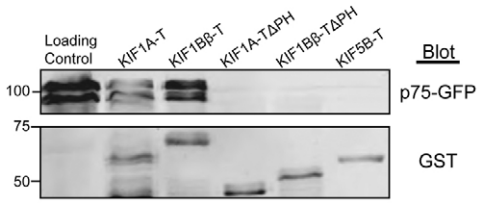

Fig. 7. PH-domain-dependent interaction of p75 vesicles with KIF1A and KIF1Bβ tails. Immunoblot showing p75-GFP and GST-tagged KIF1A-T, KIF1Bβ-T, KIF1A-TΔPH, KIF1Bβ-TΔPH and KIF5BT tails in GST pull-downs. Purified post-Golgi vesicles containing p75-GFP (1 ml) were incubated with 1 μg of the indicated GST-KIF-tails immobilized on glutathione beads. 100 μl of purified vesicles was used as loading control. p75-GFP vesicles are pulled down only by KIF1A-T and KIF1Bβ-T containing a PH domain. The p75 doublet on these blots is probably due to degradation during the vesicle purification procedure and is not generally observed when cell lysates are prepared for standard immunoblot analysis.

KIF1A-PH domain on p75 post-Golgi trafficking was less robust than the effects of KIF1A-T or KIF1Bβ-T, suggesting that additional regions of the kinesin tails are important for vesicle binding. These results are consistent with biochemical analyses described above and demonstrate that KIF1A and KIF1Bβ mediate p75 transport in a PH-domain-dependent manner.

Discussion

Transport of newly synthesized membrane and secreted proteins to the plasma membrane is mediated by the interaction of kinesin family motors with vesicular cargo emerging from the Golgi. As cells develop polarity, these interactions must be regulated to ensure that proteins are delivered to the correct membrane domain. Despite a vast body of work documenting selective transport of different cargo by specific kinesins (Hirokawa and Takemura, 2005; Wozniak et al., 2004), it is not known whether motor-cargo selection is regulated during cell polarization. To address this question, we screened for kinesins involved in trafficking of an apical marker, p75 neurotrophin receptor, before and after epithelial polarization. We took advantage of the diversity in cargo-binding kinesin tail domains and their activity as dominant-negative inhibitors (Bi et al., 1997; Jaulin et al., 2007; Le Bot et al., 1998; Noda et al., 2001; Setou et al., 2002; Skoufias et al., 1994). Here, we provide direct evidence that kinesin-3 family motors, KIF1A and KIF1Bβ, mediate post-Golgi trafficking of p75, but only in non-polarized epithelial cells. In a previous study, we showed that post-Golgi trafficking of p75 in polarized cells is mediated instead by KIF5B (Jaulin et al., 2007). Together, these data demonstrate that kinesin utilization in post-Golgi vesicular transport is indeed regulated during differentiation.

KIF1A and KIF1Bβ were identified originally as kinesins that transport synaptic vesicle precursors in neurons (Okada et al., 1995b; Zhao et al., 2001). Both *Kif1A* and *Kif1B* knockout mice die shortly after birth and the number of synaptic vesicle precursors was reduced significantly in the nervous systems of these animals (Yonekawa et al., 1998; Zhao et al., 2001). Similarly, UNC-104, a homolog of KIF1A in *C. elegans*, is also required for the transport of synaptic vesicle precursors (Hall and Hedgecock, 1991). Although KIF1A and KIF1B have not yet been reported to mediate vesicular trafficking in non-neuronal cells, they are expressed in epithelial cells and thus could be used to transport cargoes from the Golgi to the cell surface (Fig. 4B) (Jaulin et al., 2007).

We used time-lapse fluorescence microscopy to follow trafficking of newly synthesized p75-GFP from the Golgi to the plasma membrane in non-polarized MDCK cells. In cells expressing the tail domains of KIF1A or KIF1Bβ, both Golgi exit and surface delivery of p75 was inhibited. No other KIF-specific tails induced measurable inhibition of p75 trafficking in these assays. Furthermore, post-Golgi trafficking of two additional apical markers, GPI-GFP and BSEP-GFP, was not altered by expression of the KIF1A tail or by siRNA-mediated depletion of KIF1A. Importantly, we also showed that p75-containing post-Golgi vesicles purified from non-polarized MDCK cells bind selectively to purified KIF1A and KIF1Bβ tail domains in in vitro pull-down assays. Together, these data show that kinesin-cargo interactions are selective in epithelial cells, even before polarization.

Both KIF1A and KIF1Bβ contain a conserved C-terminal pleckstrin homology (PH) domain, and studies on the *C. elegans* homolog, UNC-104, demonstrated that these PH domains interact preferentially with vesicles enriched in phosphatidylinositol 4,5-bisphosphate (Klopfenstein et al., 2002) and that the PH domain was necessary for axonal transport of synaptic vesicles (Klopfenstein and Vale, 2004). Consistent with this, we found that overexpression of constructs encoding the tail domains of KIF1Bα, a KIF1B splice variant lacking a PH domain, or KIF1A and KIF1Bβ constructs deleted of their PH domains, does not affect p75-GFP trafficking in non-polarized MDCK cells. This indicates that the PH domain of KIF1A and KIF1Bβ is necessary for post-Golgi transport of vesicles containing p75. Moreover, purified KIF1A and KIF1Bβ tails interact with p75-contaning vesicles and these interactions require the PH domain. Interestingly, expression of the PH domain alone only partially inhibited p75 transport from the Golgi, suggesting that additional regions in the kinesin tails may be required to mediate efficient vesicle interaction and cargo transport. This is consistent with reports showing that the PH domain of UNC-104 is not sufficient to bind and transport vesicles in vivo (Klopfenstein and Vale, 2004).

These results, together with our previous work (Jaulin et al., 2007), show that a single vesicular cargo is transported by different kinesin family motors before and after polarization. To our knowledge, this is the first experimental evidence demonstrating differentiation-dependent regulation of kinesin-cargo interactions and kinesin-mediated post-Golgi transport. Although the mechanism underlying this switch in kinesin utilization remains to be determined, it probably arises in response to the dramatic reorganization, stabilization and post-translational modification of MT arrays accompanying epithelial polarization (Bacallao et al., 1989; Gilbert et al., 1991). These structural changes might demand a switch in kinesins' cargo-selection machinery or motor activity to accommodate polarized trafficking routes to the plasma membrane established during differentiation.

One possible mechanism through which kinesin utilization could be regulated during polarization involves kinesin post-translational modification. In support of this, studies in PC12 neurons showed that kinesin phosphorylation was induced by NGF treatment and that this correlated with neurite outgrowth (Lee and Hollenbeck, 1995). Several additional reports (although not studied in the context of polarization) document that phosphorylation of kinesin heavy and light chains affects motor binding to vesicular cargo as well as motor interactions with MTs in neurons (Donelan et al., 2002; Guillaud et al., 2008; Morfini et al., 2002; Okada et al., 1995a; Sato-Yoshitake et al., 1992; Stagi et al., 2006). No similar information is available from

studies in epithelia, in part because so few kinesin-cargo pairs have been identified in these cells. With the knowledge that KIF1A, KIF1Bβ and KIF5B mediate p75 transport before and after epithelial polarization, respectively, we are now in a position to address these important questions in the context of morphological and functional differentiation.

Another mechanism by which kinesin-cargo interactions may be regulated involves selective use of the many kinesin and vesicle adaptor proteins in cells (Caviston and Holzbaur, 2006). Although numerous studies show that adaptor proteins contribute to selective kinesin-vesicle transport, there are no data regarding whether either adaptor expression or use affects motor-cargo interactions during differentiation. We have not yet determined precisely how p75 vesicles interact with KIF1A/1Bβ or KIF5B. However, overexpression of the cytoplasmic domain of p75 did not affect post-Golgi trafficking of p75-GFP (our unpublished results), suggesting the interaction of KIF1A/1Bβ or KIF5B with these vesicles is mediated indirectly through an adaptor or scaffold of some kind. We are pursuing studies aimed at identifying possible adaptors that mediate kinesin-p75 vesicle interactions in non-polarized and polarized epithelial cells.

Regulation of kinesin-cargo interactions by Rab GTPases offers a third attractive mechanism to control selective transport by motor proteins during polarization. This large family of GTPases are well known for their function as regulators of motor-vesicle interaction and vesicle motility on microtubules (Caviston and Holzbaur, 2006). Several Rab proteins, including Rab3 (Saxena et al., 2005; van IJzendoorn et al., 2002), Rab11 (Castle et al., 2002; Li et al., 2007; Pelissier et al., 2003; Wakabayashi et al., 2004) and Rab27A (Saxena et al., 2005) have been implicated in regulating delivery of apical cargoes to the plasma membrane. In addition, Rab6 was shown to 'mark' exocytic vesicles for transport by kinesin-1 (KIF5B) to the cell periphery (Grigoriev et al., 2007) and Rab3 regulates the binding of KIF1A and KIF1Bβ to pre-synaptic vesicles (Niwa et al., 2008). However, it is not known whether Rab expression and Rab-regulated kinesin-vesicle interactions are affected by differentiative cues. This is an obvious topic of interest and we are currently investigating whether several Rab candidates regulate interaction of p75 vesicles with either KIF1A/1Bβ or KIF5B in non-polarized and polarized epithelial cells.

A final interesting finding presented here is the apparent cooperation of KIF1A and KIF1Bβ in post-Golgi transport of p75 in epithelial cells. This is reminiscent of earlier reports showing that knockout of *Kif1A* or *Kif1B* genes in mice produced similar phenotypes; lethality due to defective transport of synaptic vesicle precursors (Yonekawa et al., 1998; Zhao et al., 2001). Both KIF1A and KIF1Bβ interact with the Rab3 GEF (known as DENN/MADD), which promotes transport of Rab3 in its GTP-bound, and presumably vesicle-bound, state (Niwa et al., 2008). It is also interesting that kinesin-3 and kinesin-1 family motors mediate transport of some common cargoes, including mitochondria (Nangaku et al., 1994; Rodionov et al., 1993; Tanaka et al., 1998) and axonal vesicles (Elluru et al., 1995; Okada et al., 1995b; Sato-Yoshitake et al., 1992; Zhao et al., 2001). Most noteworthy, KIF5 (kinesin-1) protein levels in brain are increased in KIF1A knockout mice (Yonekawa et al., 1998). And, in *C. elegans*, expression of a mutant KIF5-cargo adaptor, UNC-116 (JIP3), can suppress the effects of KIF1A knockout on synaptic vesicle transport (Byrd et al., 2001). Together, these data suggest that KIF1A/Bβ and KIF5B have overlapping, and perhaps compensatory transporter functions in cells. How interactions of organelles with either KIF1A/1Bβ or KIF5 are regulated physiologically is still unclear. However, our data showing that transport of p75 is mediated selectively by KIF1A and KIF1Bβ before polarization and by KIF5B after polarization suggest that differentiative cues trigger a switch in kinesin utilization by specific vesicular cargoes. This probably occurs to facilitate establishment of polarized trafficking routes to the plasma membrane as MT arrays are remodeled during epithelial differentiation. It may also be crucial to generation and maintenance of epithelial membrane polarity and, thus, the specific functions of epithelial organs.

Materials and Methods

Cell culture and microinjection

Madine Darby canine kidney (MDCK) cells were cultured in DMEM (4.5g/l glucose) supplemented with 10% FBS and 20 mM HEPES. Cells were seeded on sterilized coverslips at 13,000 cells/cm^2 or 130,000 cells/cm^2 and grown for 36 hours or 4–5 days for analysis of non-polarized cells or polarized cells, respectively. Cells were pressure-microinjected intranuclearly with cDNAs in HKCl (10 mM HEPES, 140 mM KCl, pH 7.4) using a Narishige micromanipulator (Narishige, Greenvale, NY). The concentrations of cDNAs in the needle were: 5 μg/ml p75-GFP, 50 μg/ml GFP-GPI, 50 μg/ml YFP-BSEP, 10 μg/ml ST-RFP and 5-10 μg/ml KIF-Tails. After injection, cells were maintained at 37°C for 60 minutes to allow for expression of cDNAs. For KIF1A rescue in cells transfected with KIF1A siRNA, cDNAs encoding p75-GFP and full-length, human myc-KIF1A were co-injected.

Sequencing of canine KIF1A

Primers specific for dog KIF1A were designed based on conserved regions of mammalian KIF1A genes (human, mouse, cow). Total RNA was prepared from MDCK cells using Trizol (Invitrogen, Carlsbad, CA). cDNA was prepared by reverse transcription with Superscript III and oligo(dT) (Invitrogen) and dog KIF1A was amplified in overlapping fragments by PCR, followed by sequencing. The primer for 5′ UTR is: 5′-CAGTGACGCCGGAGAGGTGTT-3′. The 3′ reverse primer is: 5′-TTCAGACCCGCATCTGGGCAGA-3′.

Expression constructs and siRNAs

p75-GFP was described previously (Kreitzer et al., 2000). ST-mRFP was provided by E. Rodriguez-Boulan (New York, NY). GFP-GPI was provided by K. Simons (EMBL, Heidelberg, Germany). BSEP-YFP was provided by Y. Wakabayashi (NICHD, NIH), KIF5B-Tail (a.a. 808-963) and KIF3A-Tail (a.a. 601-702) constructs were described previously (Jaulin et al., 2007). KIF1A-T, KIF1Bα-T, KIF1Bβ-T, KIF1Bα-ML, KIF1C-ML, KIF1A-TΔPH, KIF1BβT-ΔPH and KIF1A-PH constructs were amplified by PCR from human A549 cells and cloned into mammalian Gateway™ expression vectors (Invitrogen, Carlsbad, CA) as described by the manufacturer. KIF1A-T (a.a. 1354-1690) and KIF1A-TΔPH (a.a. 1354-1575) forward primer: 5′-GTCCGGGGCGAGGAGAACC-3′. KIF1A-T and KIF1A-PH (a.a. 1574-1690) reverse primer: 5′-TTCAGACCCGCATCTGGGCAGA-3′. KIF1A-TΔPH reverse primer: 5′-CTACGGGCTGACTCGGATCTCC-3′. KIF1A-PH forward primer: 5′-TGATCGTTTCCAAGAAGGGGTACC-3′. KIF1Bβ-T (a.a. 1426-2770) and KIF1 BβT-ΔPH (a.a. 1426-1656) forward primer: 5′-TGCGGGGAGAAGAGA-ACTTAGCAG-3′. KIF1Bβ-T reverse primer: 5′-TTAGTATTTCGACTGGCT-CGGGCAT-3′. KIF1BβT-ΔPH reverse primer: 5′-CACTGAGCTTGGTCTAATTT-CTTCAA-3′. KIF1Bα-ML (a.a. 356-1153) forward primer: 5′-TCGACCCCAA-TGCCAAACTGGTTCG-3′ and reverse primer: 5′-CCACCATGATTTCAAG-TTTGTCCAGTAA-3′. KIF1C-ML (a.a. 356-1103) forward primer: 5′-GACCCTAATGCCCGGCTGATTAGA-3′ and reverse primer: 5′-AGCAG-CGTCTCGGGCGTCTTCC-3′. Full-length KIF1A DNA was purchased from Open Biosystem (Huntsville, AL) and subcloned into pRK5-Myc vector using *Bam*HI and *Xba*I sites.

siRNA oligos targeting canine, but not human, KIF1A were designed with siDesign tools (Dharmacon) and purchased from Shanghai GenePharma (Shanghai, China). KIF1A siRNAs include: (1) rUGA AGG AGA CGG AGA AGA UdTdT (sense) and rAUC UUC UCC GUC UCC UUC AdTdT (antisense), (2) rGCG UGG AGC UCA AGA AGA AdTdT (sense) and rUUC UUC UUG AGC UCC ACG CdTdT (antisense). Control siRNA was supplied by Shanghai GenePharma: rUUC UUC GAA CGU GUC ACG UdTdT (sense) and rACG UGA CAC GUU CGG AGA AdTdT (antisense). Cells were trypsinized and electroporated with 2.5 μg siRNA in 100 μl Nucleofector Solution V using the Amaxa Nucleofector System (Cologne, Germany). Cells were plated and used 2 days after transfection.

Imaging and analysis

For KIF1A immunostaining, cells were fixed in 2% PFA for 1 minute and permeabilized in –20°C methanol before staining with goat anti-KIF1A (1:100; Santa Cruz Biotechnology). For post-acquisition immunostaining of myc-KIF-Tails

and Golgi, cells were fixed in –20°C methanol and stained with mouse anti-myc IgG (Cell Signaling Technology). Fluorescently conjugated secondary antibodies were from Jackson Immunoresearch. Human anti-Golgi and TGN (G3 and G4, provided by A. Gonzalez, Pontifica Universidad Catolica de Chile, Santiago, Chile) were used at 1:400 dilution.

Time-lapse imaging and analysis of protein exit from the Golgi was conducted as described previously (Kreitzer et al., 2000). Briefly, after release of the Golgi block, cells were transferred to recording medium (Hanks balanced salt solution with 1% FBS, 10 mM HEPES, pH 7.0, and 100 μg/ml cycloheximide) and placed in a temperature-controlled recording chamber on a Nikon TE-2000U inverted microscope (Nikon, Inc., Greenvale, NY). Images of p75-GFP and ST-mRFP were acquired at 15-minute intervals for 4 hours with a ×20 water immersion objective and an ORCAIIER CCD camera (Hamamatsu Photonics, Hamamatsu, Japan). All devices were controlled by MetaMorph™ (Molecular Devices, Inc., Downingtown, PA). Golgi exit rates were determined by measuring the integrated fluorescence intensity of Golgi-associated p75-GFP at each time point. Golgi regions were identified by ST-mRFP fluorescence. For analysis of p75-GFP trafficking in polarized cells, image stacks (1 μm Z-sections through the entire cell depth) were acquired at each time point using a Yokagawa spinning disk confocal scan-head (Yokagawa, Perkin Elmer, LAS). Golgi-associated fluorescence from each image plane was measured and summed. For high resolution imaging to monitor vesicle budding from the Golgi and to measure vesicle velocities, images were acquired with a 60× water immersion objective at 1.5-second intervals for 2 minutes. Mean velocities were measured by calculating displacement over time for vesicles that moved continuously in at least 10 sequential frames of the time-lapse sequence. Post-acquisition analysis and processing was performed with MetaMorph™.

Surface-associated p75-GFP was determined in cells fixed at 0 minutes and 4 hours after release of the Golgi block. Cells were fixed in 2% PFA at room temperature for 2 minutes and were not permeabilized. p75-GFP at the cell surface was immunolabeled with a monoclonal antibody that recognizes an extracellular epitope of p75, as described previously (Kreitzer et al., 2003). Cy5-labeled anti-mouse antibodies were used to visualize surface-associated p75-GFP. Images of p75-GFP-injected cells were acquired using identical settings and exposure times for all samples in a single experiment. The integrated fluorescence intensities in GFP and anti-p75 images were measured and the ratio of anti-p75:p75-GFP intensities were calculated for each group of injected cells. Values were normalized to control cells expressing p75-GFP alone, which was set to 100% surface delivery of p75 at 4 hours after release of the Golgi block.

Bacterial protein expression and purification

Recombinant GST-KIF tails were produced in BL21 *E. coli*. One liter cultures were grown to A_{600}=1.0 and then induced by addition of 0.3 mM isopropyl 1-thio-D-galactopyranoside (IPTG) for 12 hours at 25°C. Cells were flash frozen, thawed and lysed by sonication in PBS (with 5 mM $MgCl_2$ and 1 mM EGTA 0.2 mg/ml lysozyme). Lysates were cleared by centrifugation at 12,000 ***g*** in a Beckman rotor JA-20. Supernatants were incubated with glutathione-Sepharose (Amersham, Piscataway, NJ) for 1 hour at 4°C. After extensive washing, immobilized GST-KIF tails were resuspended in PBS with 20% glycerol and 2 mM dithiothreitol (DTT) and either used immediately or stored at –80°C.

Vesicle purification, GST pull down and western blotting

Purification of p75-GFP vesicles was conducted essentially as described previously (Cramm-Behrens et al., 2008). Briefly, MDCK cells stably expressing p75-GFP were incubated at 20°C for 3 hours and then for 30 minutes at 37°C to enrich for Golgi-derived vesicles. Cells were scraped into 1 ml lysis buffer (35 mM Pipes, 5 mM $MgCl_2$, 1 mM EGTA, 0.5 mM EDTA, 4 mM DTT, 250 mM sucrose) and homogenized by 20 passes through a 26-gauge needle. After centrifugation at 2200 ***g*** for 5 minutes at 4°C, the supernatants were loaded on top of a sucrose step gradient (0.8-2.0 M: 2 ml 0.8 M, 3.5 ml 1.16 M, 4 ml 1.3 M, 1 ml 2 M). After ultracentrifugation at 47,000 ***g*** for 2.5 hours at 4°C, 1 ml fractions were collected from the bottom of each gradient. Vesicle fractions 8, 9 and 10 contained p75-GFP and vesicle profiles were observed by negative-stain electron microscopy. Vesicles were incubated with 1 μg purified GST-KIF tails on glutathione-Sepharose beads for 1 hour at 4°C. The beads were washed four times with 1 ml of ice-cold wash buffer (35 mM Pipes, 5 mM $MgCl_2$, 1 mM EGTA, 0.5 mM EDTA, 4 mM DTT), separated by SDS-PAGE and analyzed by western blotting. Mouse anti-KIF1A (BD Transduction Laboratories, San Jose, CA) was used at 1:100. Rabbit anti-KIF1B (Bethyl Laboratories, Montgomery, TX) was used at 1:100. Rabbit anti-GST (Cell Signaling Technology, Danvers, MA) was used at1:100. Rabbit anti-HSP90 (Stressgen, Ann Arbor, MI) was used at 1:1000. Rabbit anti-GFP antibody antibody (Novus Biologicals, Littleton, CO) was used at 1:100. Rabbit anti-β-catenin antibody (Sigma, St Louis, MO) was used at 1:200.

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Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/123/10/1732/DC1

References

Bacallao, R., Antony, C., Dotti, C., Karsenti, E., Stelzer, E. H. and Simons, K. (1989). The subcellular organization of Madin-Darby canine kidney cells during the formation of a polarized epithelium. *J. Cell Biol.* **109**, 2817-2832.

Bi, G. Q., Morris, R. L., Liao, G., Alderton, J. M., Scholey, J. M. and Steinhardt, R. A. (1997). Kinesin- and myosin-driven steps of vesicle recruitment for Ca^{2+}-regulated exocytosis. *J. Cell Biol.* **138**, 999-1008.

Breitfeld, P. P., McKinnon, W. C. and Mostov, K. E. (1990). Effect of nocodazole on vesicular traffic to the apical and basolateral surfaces of polarized MDCK cells. *J. Cell Biol.* **111**, 2365-2373.

Bryant, D. M. and Mostov, K. E. (2008). From cells to organs: building polarized tissue. *Nat. Rev. Mol. Cell Biol.* **9**, 887-901.

Byrd, D. T., Kawasaki, M., Walcoff, M., Hisamoto, N., Matsumoto, K. and Jin, Y. (2001). UNC-16, a JNK-signaling scaffold protein, regulates vesicle transport in C. elegans. *Neuron* **32**, 787-800.

Castle, A. M., Huang, A. Y. and Castle, J. D. (2002). The minor regulated pathway, a rapid component of salivary secretion, may provide docking/fusion sites for granule exocytosis at the apical surface of acinar cells. *J. Cell Sci.* **115**, 2963-2973.

Caviston, J. P. and Holzbaur, E. L. (2006). Microtubule motors at the intersection of trafficking and transport. *Trends Cell. Biol.* **16**, 530-537.

Cramm-Behrens, C. I., Dienst, M. and Jacob, R. (2008). Apical cargo traverses endosomal compartments on the passage to the cell surface. *Traffic* **9**, 2206-2220.

Donelan, M. J., Morfini, G., Julyan, R., Sommers, S., Hays, L., Kajio, H., Briaud, I., Easom, R. A., Molkentin, J. D., Brady, S. T. et al. (2002). Ca^{2+}-dependent dephosphorylation of kinesin heavy chain on beta-granules in pancreatic beta-cells. Implications for regulated beta-granule transport and insulin exocytosis. *J. Biol. Chem.* **277**, 24232-24242.

Dorner, C., Ciossek, T., Muller, S., Moller, P. H., Ullrich, A. and Lammers, R. (1998). Characterization of KIF1C, a new kinesin-like protein involved in vesicle transport from the Golgi apparatus to the endoplasmic reticulum. *J. Biol. Chem.* **273**, 20267-20275.

Eilers, U., Klumperman, J. and Hauri, H. P. (1989). Nocodazole, a microtubule-active drug, interferes with apical protein delivery in cultured intestinal epithelial cells (Caco-2). *J. Cell Biol.* **108**, 13-22.

Ellis, M. A., Potter, B. A., Cresawn, K. O. and Weisz, O. A. (2006). Polarized biosynthetic traffic in renal epithelial cells: sorting, sorting, everywhere. *Am. J. Physiol. Renal. Physiol.* **291**, F707-F713.

Elluru, R. G., Bloom, G. S. and Brady, S. T. (1995). Fast axonal transport of kinesin in the rat visual system: functionality of kinesin heavy chain isoforms. *Mol. Biol. Cell* **6**, 21-40.

Fan, S., Hurd, T. W., Liu, C. J., Straight, S. W., Weimbs, T., Hurd, E. A., Domino, S. E. and Margolis, B. (2004). Polarity proteins control ciliogenesis via kinesin motor interactions. *Curr. Biol.* **14**, 1451-1461.

Gilbert, T., Le Bivic, A., Quaroni, A. and Rodriguez-Boulan, E. (1991). Microtubular organization and its involvement in the biogenetic pathways of plasma membrane proteins in Caco-2 intestinal epithelial cells. *J. Cell Biol.* **113**, 275-288.

Grigoriev, I., Splinter, D., Keijzer, N., Wulf, P. S., Demmers, J., Ohtsuka, T., Modesti, M., Maly, I. V., Grosveld, F., Hoogenraad, C. C. et al. (2007). Rab6 regulates transport and targeting of exocytotic carriers. *Dev. Cell* **13**, 305-314.

Guillaud, L., Wong, R. and Hirokawa, N. (2008). Disruption of KIF17-Mint1 interaction by CaMKII-dependent phosphorylation: a molecular model of kinesin-cargo release. *Nat. Cell. Biol.* **10**, 19-29.

Hall, D. H. and Hedgecock, E. M. (1991). Kinesin-related gene unc-104 is required for axonal transport of synaptic vesicles in C. elegans. *Cell* **65**, 837-847.

Hirokawa, N. and Takemura, R. (2005). Molecular motors and mechanisms of directional transport in neurons. *Nat. Rev. Neurosci.* **6**, 201-214.

Hirokawa, N. and Noda, Y. (2008). Intracellular transport and kinesin superfamily proteins, KIFs: structure, function and dynamics. *Physiol. Rev.* **88**, 1089-1118.

Hirschberg, K., Miller, C. M., Ellenberg, J., Presley, J. F., Siggia, E. D., Phair, R. D. and Lippincott-Schwartz, J. (1998). Kinetic analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells. *J. Cell Biol.* **143**, 1485-1503.

Hoepfner, S., Severin, F., Cabezas, A., Habermann, B., Runge, A., Gillooly, D., Stenmark, H. and Zerial, M. (2005). Modulation of receptor recycling and degradation by the endosomal kinesin KIF16B. *Cell* **121**, 437-450.

Jaulin, F., Xue, X., Rodriguez-Boulan, E. and Kreitzer, G. (2007). Polarization-dependent selective transport to the apical membrane by KIF5B in MDCK cells. *Dev. Cell* **13**, 511-522.

Jenkins, P. M., Hurd, T. W., Zhang, L., McEwen, D. P., Brown, R. L., Margolis, B., Verhey, K. J. and Martens, J. R. (2006). Ciliary targeting of olfactory CNG channels requires the CNGB1b subunit and the kinesin-2 motor protein, KIF17. *Curr. Biol.* **16**, 1211-1216.

Keller, P., Toomre, D., Diaz, E., White, J. and Simons, K. (2001). Multicolour imaging of post-Golgi sorting and trafficking in live cells. *Nat. Cell Biol.* **3**, 140-149.

Klopfenstein, D. R. and Vale, R. D. (2004). The lipid binding pleckstrin homology domain in UNC-104 kinesin is necessary for synaptic vesicle transport in Caenorhabditis elegans. *Mol. Biol. Cell* **15**, 3729-3739.

Klopfenstein, D. R., Tomishige, M., Stuurman, N. and Vale, R. D. (2002). Role of phosphatidylinositol(4,5)bisphosphate organization in membrane transport by the Unc104 kinesin motor. *Cell* **109**, 347-358.

Kreitzer, G., Marmorstein, A., Okamoto, P., Vallee, R. and Rodriguez-Boulan, E. (2000). Kinesin and dynamin are required for post-Golgi transport of a plasma-membrane protein. *Nat. Cell Biol.* **2**, 125-127.

Kreitzer, G., Schmoranzer, J., Low, S. H., Li, X., Gan, Y., Weimbs, T., Simon, S. M. and Rodriguez-Boulan, E. (2003). Three-dimensional analysis of post-Golgi carrier exocytosis in epithelial cells. *Nat. Cell Biol.* **5**, 126-136.

Le Bot, N., Antony, C., White, J., Karsenti, E. and Vernos, I. (1998). Role of xklp3, a subunit of the Xenopus kinesin II heterotrimeric complex, in membrane transport between the endoplasmic reticulum and the Golgi apparatus. *J. Cell Biol.* **143**, 1559-1573.

Lee, K. D. and Hollenbeck, P. J. (1995). Phosphorylation of kinesin in vivo correlates with organelle association and neurite outgrowth. *J. Biol. Chem.* **270**, 5600-5605.

Li, B. X., Satoh, A. K. and Ready, D. F. (2007). Myosin V, Rab11, and dRip11 direct apical secretion and cellular morphogenesis in developing Drosophila photoreceptors. *J. Cell Biol.* **177**, 659-669.

Matlin, K. S. and Simons, K. (1983). Reduced temperature prevents transfer of a membrane glycoprotein to the cell surface but does not prevent terminal glycosylation. *Cell* **34**, 233-243.

Miki, H., Okada, Y. and Hirokawa, N. (2005). Analysis of the kinesin superfamily: insights into structure and function. *Trends Cell. Biol.* **15**, 467-476.

Morfini, G., Szebenyi, G., Elluru, R., Ratner, N. and Brady, S. T. (2002). Glycogen synthase kinase 3 phosphorylates kinesin light chains and negatively regulates kinesin-based motility. *EMBO J.* **21**, 281-293.

Musch, A. (2004). Microtubule organization and function in epithelial cells. *Traffic* **5**, 1-9.

Nangaku, M., Sato-Yoshitake, R., Okada, Y., Noda, Y., Takemura, R., Yamazaki, H. and Hirokawa, N. (1994). KIF1B, a novel microtubule plus end-directed monomeric motor protein for transport of mitochondria. *Cell* **79**, 1209-1220.

Nishimura, T., Kato, K., Yamaguchi, T., Fukata, Y., Ohno, S. and Kaibuchi, K. (2004). Role of the PAR-3-KIF3 complex in the establishment of neuronal polarity. *Nat. Cell Biol.* **6**, 328-334.

Niwa, S., Tanaka, Y. and Hirokawa, N. (2008). KIF1Bbeta- and KIF1A-mediated axonal transport of presynaptic regulator Rab3 occurs in a GTP-dependent manner through DENN/MADD. *Nat. Cell Biol.* **10**, 1269-1279.

Noda, Y., Okada, Y., Saito, N., Setou, M., Xu, Y., Zhang, Z. and Hirokawa, N. (2001). KIFC3, a microtubule minus end-directed motor for the apical transport of annexin XIIIb-associated Triton-insoluble membranes. *J. Cell Biol.* **155**, 77-88.

Okada, Y., Sato-Yoshitake, R. and Hirokawa, N. (1995a). The activation of protein kinase A pathway selectively inhibits anterograde axonal transport of vesicles but not mitochondria transport or retrograde transport in vivo. *J. Neurosci.* **15**, 3053-3064.

Okada, Y., Yamazaki, H., Sekine-Aizawa, Y. and Hirokawa, N. (1995b). The neuron-specific kinesin superfamily protein KIF1A is a unique monomeric motor for anterograde axonal transport of synaptic vesicle precursors. *Cell* **81**, 769-780.

Pelissier, A., Chauvin, J. P. and Lecuit, T. (2003). Trafficking through Rab11 endosomes is required for cellularization during Drosophila embryogenesis. *Curr. Biol.* **13**, 1848-1857.

Rindler, M. J., Ivanov, I. E. and Sabatini, D. D. (1987). Microtubule-acting drugs lead to the nonpolarized delivery of the influenza hemagglutinin to the cell surface of polarized Madin-Darby canine kidney cells. *J. Cell Biol.* **104**, 231-241.

Rodionov, V. I., Gyoeva, F. K. and Gelfand, V. I. (1991). Kinesin is responsible for centrifugal movement of pigment granules in melanophores. *Proc. Natl. Acad. Sci. USA* **88**, 4956-4960.

Rodionov, V. I., Gyoeva, F. K., Tanaka, E., Bershadsky, A. D., Vasiliev, J. M. and Gelfand, V. I. (1993). Microtubule-dependent control of cell shape and pseudopodial activity is inhibited by the antibody to kinesin motor domain. *J. Cell Biol.* **123**, 1811-1820.

Rodriguez-Boulan, E., Kreitzer, G. and Musch, A. (2005). Organization of vesicular trafficking in epithelia. *Nat. Rev. Mol. Cell Biol.* **6**, 233-247.

Sato-Yoshitake, R., Yorifuji, H., Inagaki, M. and Hirokawa, N. (1992). The phosphorylation of kinesin regulates its binding to synaptic vesicles. *J. Biol. Chem.* **267**, 23930-23936.

Saxena, S., Singh, M., Engisch, K., Fukuda, M. and Kaur, S. (2005). Rab proteins regulate epithelial sodium channel activity in colonic epithelial HT-29 cells. *Biochem. Biophys. Res. Commun.* **337**, 1219-1223.

Schmoranzer, J., Kreitzer, G. and Simon, S. M. (2003). Migrating fibroblasts perform polarized, microtubule-dependent exocytosis towards the leading edge. *J. Cell Sci.* **116**, 4513-4519.

Setou, M., Seog, D. H., Tanaka, Y., Kanai, Y., Takei, Y., Kawagishi, M. and Hirokawa, N. (2002). Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature* **417**, 83-87.

Skoufias, D. A., Cole, D. G., Wedaman, K. P. and Scholey, J. M. (1994). The carboxyl-terminal domain of kinesin heavy chain is important for membrane binding. *J. Biol. Chem.* **269**, 1477-1485.

Stagi, M., Gorlovoy, P., Larionov, S., Takahashi, K. and Neumann, H. (2006). Unloading kinesin transported cargoes from the tubulin track via the inflammatory c-Jun N-terminal kinase pathway. *FASEB J.* **20**, 2573-2575.

Tanaka, Y., Kanai, Y., Okada, Y., Nonaka, S., Takeda, S., Harada, A. and Hirokawa, N. (1998). Targeted disruption of mouse conventional kinesin heavy chain, kif5B, results in abnormal perinuclear clustering of mitochondria. *Cell* **93**, 1147-1158.

Toomre, D., Keller, P., White, J., Olivo, J. C. and Simons, K. (1999). Dual-color visualization of trans-Golgi network to plasma membrane traffic along microtubules in living cells. *J. Cell Sci.* **112**, 21-33.

van IJzendoorn, S. C., Tuvim, M. J., Weimbs, T., Dickey, B. F. and Mostov, K. E. (2002). Direct interaction between Rab3b and the polymeric immunoglobulin receptor controls ligand-stimulated transcytosis in epithelial cells. *Dev. Cell* **2**, 219-228.

Wakabayashi, Y., Lippincott-Schwartz, J. and Arias, I. M. (2004). Intracellular trafficking of bile salt export pump (ABCB11) in polarized hepatic cells: constitutive cycling between the canalicular membrane and rab11-positive endosomes. *Mol. Biol. Cell* **15**, 3485-3496.

Wozniak, M. J., Milner, R. and Allan, V. (2004). N-terminal kinesins: many and various. *Traffic* **5**, 400-410.

Yonekawa, Y., Harada, A., Okada, Y., Funakoshi, T., Kanai, Y., Takei, Y., Terada, S., Noda, T. and Hirokawa, N. (1998). Defect in synaptic vesicle precursor transport and neuronal cell death in KIF1A motor protein-deficient mice. *J. Cell Biol.* **141**, 431-441.

Zhao, C., Takita, J., Tanaka, Y., Setou, M., Nakagawa, T., Takeda, S., Yang, H. W., Terada, S., Nakata, T., Takei, Y. et al. (2001). Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bbeta. *Cell* **105**, 587-597.