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Polarization-Dependent Selective Transport to the Apical Membrane by KIF5B in MDCK Cells

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SUMMARY

Microtubule-based vesicular transport is well documented in epithelial cells, but the specific motors involved and their regulation during polarization are largely unknown. We demonstrate that KIF5B mediates post-Golgi transport of an apical protein in epithelial cells, but only after polarity has developed. Time-lapse imaging of EB1-GFP in polarized MDCK cells showed microtubule plus ends growing toward the apical membrane, implying that plus end-directed N-kinesins might be used to transport apical proteins. Indeed, time-lapse microscopy revealed that expression of a KIF5B dominant negative or microinjection of function-blocking KIF5 antibodies inhibited selectively post-Golgi transport of the apical marker, p75-GFP, after polarization of MDCK cells. Expression of other KIF5B dominant negatives did not alter p75-GFP trafficking. Immunoprecipitation experiments demonstrated an interaction between KIF5B and p75-GFP in polarized, but not in subconfluent, MDCK cells. Our results demonstrate that apical protein transport depends on selective microtubule motors and that epithelial cells switch kinesins for post-Golgi transport during acquisition of polarity.

INTRODUCTION

The generation and maintenance of epithelial asymmetry is critical to the function of numerous tissues and organs. A key event in epithelial polarization is establishment of polarized transport routes to apical and basolateral regions of the plasma membrane (Rodriguez-Boulan et al., 2005). Apical and basolateral membrane proteins are synthesized in the endoplasmic reticulum, transferred to the Golgi, and segregated into different post-Golgi transport intermediates at the trans-Golgi network for export to the cell surface. Additional sorting can occur in post-Golgi endosomes, tubular transport intermediates, and at the plasma membrane (Ellis et al., 2006). Basolateral sorting signals include tyrosine, dileucine, and monoleucine motifs (Bonifacino and Traub, 2003) that mediate interactions with vesicle adaptor proteins AP1B, AP4, and other clathrin-associated adaptors (Heilker et al., 1999; Kirchhausen et al., 1997; Rodriguez-Boulan et al., 2005). Apical sorting is mediated by N- and O-linked glycans, glycosphatidylinositol anchors, and short cytoplasmic motifs (Potter et al., 2006; Schuck and Simons, 2004). Production, transport, and delivery of apical transport intermediates appear to depend on microtubule (MT) motors such as dynein and kinesin (Rodriguez-Boulan et al., 2005). Selective use of kinesins in transport of axonal and dendritic membrane proteins has been well documented in neuronal cells (Hirokawa and Takemura, 2005; Wozniak et al., 2004), but little is known about the kinesins mediating transport of apical and basolateral proteins in epithelial cells. Early reports described a key role of microtubules in apical transport of membrane proteins in MDCK and Caco-2 cells (Eilers et al., 1989; Rindler et al., 1987) and suggested that dynein and kinesin participate in apical delivery in perforated MDCK cells (Lafont et al., 1994). In MDCK and Caco-2 cells, MTs are reorganized from centrosomally nucleated, radial arrays into longitudinal bundles (minus ends oriented apically) and arrays of mixed polarity underlying the apical pole and overlying the basal membrane (Bacallao et al., 1989; Gilbert et al., 1991) in a process regulated by Par1 (Cohen et al., 2004). MT reorganization is accompanied by relocation of the Golgi to apical regions of the cytoplasm (Bacallao et al., 1989). Because many MT minus ends are oriented toward the apical membrane, it is thought that minus end-directed MT motors, such as dynein and C-kinesins (MT minus end-directed motors), are involved in delivering proteins to this surface. Indeed, inhibition of dynein (Lafont et al., 1994; Tai et al., 1999) or the C-kinesin KIFC3 (Noda et al., 2001) results in decreased delivery of apical markers influenza hemagglutinin, rhodopsin, and annexin XIIIb. Interestingly, inhibition of the N-kinesin (MT plus end directed), KIF5, also inhibits delivery of hemagglutinin to the apical membrane (Lafont et al., 1994), suggesting that plus end-directed kinesins can function in apical transport. In addition, kinesin II family members KIF3A/B and KIF17 can deliver proteins to the apical primary cilium in polarized epithelial cells (Fan et al., 2004; Jenkins et al.,...
These observations are not easily reconciled with our current knowledge of MT orientation in polarized epithelial cells.

By using time-lapse fluorescence microscopy to follow post-Golgi trafficking of an apical membrane protein fused to GFP, p75-GFP, we found that rates of emptying from the Golgi increased 1.4-fold after polarization. In addition, the velocities at which post-Golgi structures containing p75-GFP moved were 2.5-fold faster, on average, than in subconfluent cells. Together, these results indicated a change in molecular motor “signature” in transport of vesicles containing p75-GFP after polarization. To test this directly, we expressed KIF-specific dominant-negative constructs in MDCK cells and analyzed whether post-Golgi transport of p75-GFP was dependent on the same or different kinesin family members before and after polarization. Our studies demonstrate a polarization-dependent switch in the kinesin used for post-Golgi transport of p75-GFP and show that KIF5B is involved in biosynthetic trafficking of p75-GFP in polarized cells. Time-lapse imaging of cells expressing GFP-tagged EB1, a MT plus end-binding protein, revealed a population of MTs with plus ends oriented apically that could be used in apical membrane trafficking by the plus end-directed motor, KIF5B. We also show that p75-GFP and KIF5B interact in polarized MDCK cells expressing p75-GFP, but not in subconfluent cells. Inhibition of KIF5B had no significant effect on trafficking of several other apical membrane proteins, suggesting that apical transport is mediated by more than one kinesin family member in polarized epithelial cells.

RESULTS

Post-Golgi Transport of an Apical Membrane Protein Is Faster after Polarization of MDCK Cells

The neurotrophin receptor p75 is recognized widely for its activity in brain but is also expressed by nonneuronal cells and has been implicated in differentiation of a variety of epithelial tissues (Huber and Chao, 1995; Passino et al., 2007; Sariola et al., 1991). In polarized epithelial cells, p75 localizes to the apical membrane via a mechanism involving O-glycan signals and galectin-3 (Yeaman et al., 1997; Delacour et al., 2006), but molecular motors involved in its apical translocation have not yet been identified.

We expressed GFP-tagged (or YFP) p75 neurotrophin receptor (p75-GFP) in MDCK cells by intranuclear microinjection of cDNA encoding p75-GFP. p75-GFP could be detected in the endoplasmic reticulum and the Golgi, but not yet at the plasma membrane, by 50 min after injection (Kreitzer et al., 2003). At this time, newly expressed p75-GFP was chased into the Golgi by a 20°C temperature block (Matlin and Simons, 1983), and its plasma membrane transport was followed by time-lapse fluorescence microscopy after shifting to a temperature permissive for transport out of the Golgi (33°C). Imaging of post-Golgi transport was performed at 33°C because of technical considerations (Supplemental Experimental Procedures, see the Supplemental Data available with this article online) and had no significant effect on exit of cargo from the Golgi or rates of transport from the Golgi to the plasma membrane (Kreitzer et al., 2000). Similarly, incubation cells at 20°C and 33°C had no detectable effect on MT or Golgi organization (Figure S8).

In polarized cells, p75-GFP exited the Golgi with a half time of ~85 min (±5% SEM, n = 26) and was redistributed completely to the apical membrane by ~180 min after release of the Golgi block (Figure 1). The rate of p75-GFP Golgi-to-plasma membrane transport was 1.4x faster than what we observed in subconfluent MDCK cells (11/2 = 120 min ± 4.5% SEM, n = 26, Figure 1; Kreitzer et al., 2000). A transient increase in Golgi-associated p75-GFP after release of the 20°C block was often observed and likely reflects influx of residual ER-associated p75 into the Golgi. An increase in Golgi-associated p75-GFP could also be detected in a few individual nonpolarized cells but was not observed in averaged data.

Figure 1. Rates of p75-GFP Emptying from the Golgi in Subconfluent and Polarized MDCK Cells

Subconfluent and polarized cells expressing p75-GFP were imaged by time-lapse microscopy for 3–4 hr after release of the Golgi block. For polarized cells, 3D image stacks were acquired at each time point. Golgi exit rates were calculated by measuring the ratio of Golgi total p75-GFP fluorescence (Experimental Procedures). Data represent pooled results from three recordings for each data set shown. Error bars represent standard error of the mean (SEM).

Velocity of Post-Golgi Carriers Containing p75-GFP Is Faster in Polarized Cells

We measured the velocities of post-Golgi carriers containing p75-GFP in subconfluent and polarized cells. Post-Golgi carriers were imaged in the first 45 min after release of the Golgi block by spinning disk confocal microscopy for 2–5 min at 1 s intervals. In both subconfluent and polarized MDCK cells, we observed numerous pleiomorphic carriers containing p75-GFP undergoing saltatory movements along curvilinear paths in the cytoplasm, indicative of MT-based movement (Figures 2A–2C; Movies S1 and S2). In nonpolarized cells, post-Golgi carriers containing p75-GFP moved with an average velocity of 0.56 μm/s (SD 0.23 μm/s, n = 30 carriers taken from 4 cells) and
a maximum velocity of 0.96 mm/s. By contrast, in polarized cells, p75-GFP carriers moved with an average velocity of 1.4 mm/s (SD = 0.46 mm/s, n = 30 carriers from 4 cells) and a maximum velocity of 2.56 mm/s. Figure 2D shows a histogram displaying the velocity distribution of carriers containing p75-GFP in subconfluent and polarized MDCK cells. Together, the increased rate of Golgi exit, delivery to the plasma membrane, and velocity of post-Golgi carriers containing p75-GFP after polarization suggest a change in machinery involved in Golgi-to-plasma membrane transport. Alterations in the rates of Golgi emptying and the velocity of vesicles containing p75-GFP suggest two possible mechanisms by which trafficking of this cargo could change during polarization: (1) different motor proteins are used to transport these vesicles before and after polarization or (2) the activity of the motor responsible for p75 transport is altered during polarization.

Expression Profiling of Kinesin Family Members in Epithelial Cells
In subconfluent MDCK cells, post-Golgi trafficking of p75-GFP involves kinesin-mediated transport (Kreitzer et al., 2000). In polarized MDCK cells, p75 is targeted to the
apical membrane by a MT-dependent mechanism (Kreitzer et al., 2003) also presumed to be kinesin driven. However, the specific kinesin family member(s) involved in post-Golgi transport of p75 in either subconfluent or polarized cells have not yet been identified. To facilitate identification of KIFs involved in post-Golgi transport of p75, we first determined the complement of KIFs expressed in different epithelial cells. We prepared mRNA from 10 human epithelial cell lines and primary cultures and performed reverse transcription and PCR amplification with KIF-specific primers. Surprisingly, of the 41 KIFs in the human genome, ~70%–80% are expressed at the mRNA level in all epithelial cell lines and primary cultures tested (Figure 3A; Table S1). Protein expression was confirmed by western blotting and immunofluorescence staining for KIFs to which antibodies were available (Figure S1). These data demonstrate that epithelial cells express numerous kinesin family members that could be used to transport different cargoes from the Golgi to the cell surface.

**Microtubule Plus Ends Are Oriented toward the Apical Membrane in MDCK Cells**

Current models of MT organization in polarized epithelial cells posit that MT minus ends are oriented toward the apical membrane (see Introduction). However, this view is based entirely on static images of MTs in fixed cell preparations. To determine whether plus end-directed kinesins might participate in apical transport, we first examined whether dynamic MTs with plus ends oriented apically might exist in polarized MDCK cells. To visualize MT growing ends directly, we microinjected cDNA encoding the MT plus end-binding protein, EB1, tagged with GFP, into fully polarized MDCK cells and performed time-lapse fluorescence imaging. Because overexpression of EB1 can alter MT dynamics (Bu and Su, 2001; Ligon et al., 2003), we
analyzed only cells expressing EB1-GFP at low levels (in which EB1-GFP tracked MT tips but did not label the length of MTs). Images acquired in the top 1–3 μm of polarized cells revealed that a significant number of MTs labeled with EB1-GFP are oriented with their plus ends toward the apical membrane (Figures 4A and 4B; Movies S3–S5). Similarly, endogenous EB1 decorated MT plus ends that were oriented apically and often protruded above tight junctions in polarized cells (Figure 4C).

**KIF5B Transports p75-YFP to the Apical Membrane in Polarized, but Not Subconfluent, MDCK Cells**

Of the KIFs expressed in epithelial cells, four stood out as candidate transporters of vesicular cargoes to the apical membrane domain based on previous studies in MDCK cells (see Introduction): the kinesin 1 family member, KIF5B; the kinesin 2 family members, KIF3A/B and KIF17; and the kinesin 14 family member, KIFC3. We prepared expression constructs containing either the cargo-binding tail domain alone or the cargo-binding tail and all or a portion of the coiled-coil domain of these KIFs for use as selective inhibitors of KIF-cargo interaction in cells (Figure 3B). Previous studies have used motorless KIFs (deleted for the MT-binding domain) or KIF tail domains (MT-binding domain and portions of the coiled-coil domains deleted) as dominant-negative inhibitors of KIF-dependent, vesicle transport (Bi et al., 1997; Le Bot et al., 1998; Noda et al., 2001; Setou et al., 2002; Skoufias et al., 1994).
We coexpressed p75-YFP and constructs encoding CFP (or RFP)-tagged KIF tail domains in polarized and subconfluent MDCK cells and performed time-lapse imaging of p75-YFP after release of the Golgi block. In control polarized cells, the majority of p75-YFP exited the Golgi and was delivered to the apical membrane by 3 hr after release of the Golgi block. In polarized cells expressing RFP-KIF5B-T, however, a significant pool of p75-YFP remained in the Golgi during this time (Figure 5A). Analysis of p75-YFP exit from the Golgi revealed a 45% decrease in the rate of Golgi emptying in the presence of KIF5B-Tail as compared with control (no KIF-Tail, see Figure 1) or KIFC3-Tail-expressing (Figure 5B) cells. p75-YFP exiting the Golgi in the presence of KIF5B-Tail was delivered in a nonpolarized manner to both apical and basolateral membranes (Figure 5A; Movie S6). By contrast, in subconfluent cells, expression of RFP-KIF5B-T had no effect on Golgi exit and plasma membrane delivery of p75-YFP (Figure 5C). Expression of RFP-KIF5-C-Tail in nonpolarized cells did induce perinuclear clustering of mitochondria (Figure 5A), confirming that overexpressed KIF5B-Tail is functionally active in nonpolarized cells. These data demonstrate that different kinesin family members are used in post-Golgi trafficking of a single cargo before and after epithelial polarization.

As a second test of the role of KIF5B in p75 post-Golgi trafficking, we microinjected KIF5-specific, function-blocking antibodies H1 or SUK4 (Ingold et al., 1988; Lippincott-Schwartz et al., 1995) into MDCK cells, and we monitored redistribution of p75-GFP in living (by time-lapse imaging) or fixed cells. As described previously (Kreitzer et al., 2000), injection of KIF5-specific IgG (SUK4 or H1) had no effect on transport of p75-GFP in subconfluent MDCK cells (not shown). By contrast, in polarized cells, injected anti-KIF5 IgG, like overexpressed KIF5B-Tail, also inhibited emptying of p75-GFP from the Golgi (Figure 5A). However, antibody injection more consistently resulted in accumulation of p75-GFP vesicles in the cytoplasm rather than Golgi retention 3 hr after release of the Golgi block (Figure S2). The somewhat different effects of anti-KIF5 IgG as compared with the KIF5B tail construct on p75-GFP trafficking likely reflect a difference in the inhibitory mechanism of these two perturbants. Overexpressed KIF5-T likely competes with endogenous KIF5 for interaction sites at Golgi membrane domains containing “budding” p75 vesicles; injected antibodies (which target the KIF5 head) inhibit the activity of KIF5 but do not likely inhibit binding to nascent p75-containing vesicles. Thus, vesicles may bud from the Golgi but are not transported appropriately through the cytoplasm. This is consistent with our observations of p75-GFP trafficking in nonpolarized cells: injection of a more broadly reactive kinesin antibody, HD (Rodionov et al., 1991; Wright et al., 1993), did not block completely budding of p75-GFP vesicles from the Golgi but did inhibit their movement away from the Golgi toward the cell periphery (Kreitzer et al., 2000).

KIF5B Interacts with p75-GFP in Polarized Cells but Not in Nonpolarized Cells

We next tested whether KIF5B interacts with p75-GFP in MDCK cells by using an MDCK cell line that stably expresses p75-GFP (Kreitzer et al., 2000). To enrich for post-Golgi carriers containing p75-GFP, cells were incubated at 20°C for 2 hr and then shifted to 37°C for 30 min so that post-Golgi transport intermediates would be released into the cytoplasm. We prepared lysates from both subconfluent and polarized cells for immunoprecipitation with GFP antibodies. Immune complexes were isolated and probed by western blot for endogenous KIF5. Consistent with functional studies described above, KIF5 coimmunoprecipitated with p75-GFP in lysates prepared from polarized cells but not in lysates prepared from subconfluent cells (Figure 5D). Similarly, we found that endogenous KIF5 colocalized with post-Golgi carriers containing p75-GFP in polarized cells but not in subconfluent cells (Figure S3). Because KIF5 protein level did not change during polarization (see total lysates in Figure 5D), our data suggest that the interaction between p75-GFP and KIF5 is regulated and occurs only after cells have polarized.

Transport of p75-YFP Is Mediated by KIF5B Selectively in Polarized MDCK Cells

Among other KIFs that could participate in biosynthetic trafficking of p75-GFP in polarized MDCK cells, we were particularly interested in the minus end-directed kinesin, KIFC3, because it was described to inhibit apical delivery of annexin XIIIb and influenza hemaglutinin in MDCK cells (Noda et al., 2001). We injected cDNAs encoding p75-YFP and a dominant-negative, HA-tagged KIFC3-T into polarized MDCK cells and analyzed Golgi exit and targeted delivery of p75-YFP to the cell surface as described above. In cells expressing HA-KIFC3-T, we observed no change in emptying of p75-YFP from the Golgi or in targeted delivery to the apical membrane (Figure 5A). Similarly, coexpression of p75-GFP with RFP-tagged, dominant-negative tail constructs encoding plus end-directed KIFs, RFP-KIF3A-T, and RFP-KIF17-T (either motorless or Tail-only) had no effect on post-Golgi transport and apical delivery of p75-GFP in polarized cells (Figure S4). Thus, of the dominant-negative KIF tails tested (representing examples of three different kinesin subfamilies), only KIF5-T inhibited post-Golgi transport and apical targeting of p75-GFP. Although overexpressing KIF5B-T is sufficient to inhibit p75 post-Golgi transport, we cannot rule out the possibility that additional, untested KIFs may also be involved in this process.

KIF5B Is Not a General Motor for Apical Transport in Polarized MDCK Cells

Next, we investigated whether KIF5B was used for trafficking of other apical markers including prominin-YFP (Corbell et al., 1999), YFP-GPI (Keller et al., 2001), and the ABC transporter BSEP-YFP (Wakabayashi et al., 2004). Polarized MDCK cells were injected with cDNAs encoding prominin-YFP, YFP-GPI, or BSEP-YFP in the
Figure 5. Polarization-Dependent, Selective Transport of p75-YFP by KIF5B
(A) Time-lapse images of polarized MDCK cells expressing p75-YFP, p75-YFP, and CFP-KIF5B-T or p75-YFP and HA-KIFC3-T. In all conditions, p75-YFP is found in the Golgi after the 20°C block (5 min 33°C). 4 hr later, (245 min 33°C), p75-YFP was delivered to the apical membrane in control and HA-KIFC3-expressing cells. By contrast, ~50% of p75-YFP remains in the Golgi in cells expressing CFP-KIF5B-T. Images show maximum XY projections of Z series. Lower panels show orthogonal views of injected cells at the end of each recording. Insets show corresponding CFP-KIF5B-T fluorescence and HA-KIFC3-T immunostained after the time lapse. Far right panels show p75-GFP in cells coinjected with H1 IgG at the start (5 min 33°C) and end (245 min 33°C) of a time lapse.

(B) Polarized cells expressing p75-GFP and KIF5B-T or KIFC3-T were imaged by time-lapse microscopy for 3.5 hr after release of the Golgi block. p75-GFP exit from the Golgi was analyzed as described in Figure 1. KIFC3-T had no effect on emptying of p75-GFP from the Golgi. Error bars: SEM for KIF5B-T-, SD for KIFC3-T-expressing cells.

(C) Post-Golgi transport of p75-YFP in subconfluent MDCK cells in the absence or presence of CFP-KIF5B-T. Expression of CFP-KIF5B-T does not inhibit transport of p75-YFP to the plasma membrane in subconfluent cells as compared with polarized cells.

(D) p75-GFP associates with KIF5 in polarized but not subconfluent MDCK cells. KIF5 was present in approximately equal amounts in lysates used for IP (total lysate). Tubulin was blotted as a loading control.
absence or presence of CFP-KIF5B-T. One hour after injection, cells were incubated at 20°C to accumulate newly synthesized material in the Golgi, and exit from the Golgi was imaged by time-lapse fluorescence microscopy as described above. For each additional apical membrane protein tested, Golgi emptying and apical membrane targeting was unaffected by overexpressed CFP-KIF5B-T (Figure 6). We also found that expression of CFP-KIF5B-T in polarized MDCK cells had no effect on post-Golgi trafficking of several YFP-tagged basolateral membrane proteins including E-cadherin (Figure S5), low-density lipoprotein receptor, and neural cell adhesion marker (not shown). Taken together, these data demonstrate that KIF5B participates selectively in transport of p75 to the apical surface.

DISCUSSION

The results presented here provide the first direct evidence of a polarization-dependent switch in kinesin-mediated selective transport of apical cargo from the Golgi to the plasma membrane of epithelial cells. Several clues to the presence of a “kinesin switch” were evident from our initial observations of Golgi-to-plasma membrane trafficking of the apical cargo, p75, in subconfluent and polarized MDCK cells. First, exit of p75-GFP from the Golgi is 1.4× faster in polarized cells as compared with subconfluent cells. Second, the velocity of individual post-Golgi carriers containing p75-GFP is 2.5× faster in polarized cells. The average rate of p75-GFP vesicle movement that we observed in polarized cells was quite high (1.4 μm/s) but well within the range of vesicle velocities observed previously (Brady et al., 1982; Hirschberg et al., 1998; Vale et al., 1985).

When considered alone, the more rapid rate of Golgi emptying after polarization could be attributed to either use of a faster motor or to an increase in the rate of vesicle budding from the Golgi. However, when considered with data demonstrating a change in velocity of post-Golgi vesicles, a more likely scenario involves a switch in the motor used to transport p75-GFP after polarization. This switch could be mediated through polarization-dependent regulation of factors that control, either directly or indirectly, KIF-cargo binding. This idea is supported by data showing that interaction of kinesin with vesicular cargo can be regulated by phosphorylation (Donelan et al., 2002; Morfini et al., 2002). To date, it is not known whether phosphorylation induces phosphorylation of specific KIF-vesicle interactions; however, studies in PC12 cells did demonstrate a correlation between NGF-stimulated...
mechanism to regulate kinesin-vesicle interactions (Lee and Hollenbeck, 1993). Alternatively, the same motor may be used for transport of these post-Golgi vesicles before and after polarization, but either motor activity (MT binding and processivity) or the number of motors per vesicle could change after polarization to promote vesicle movement. In support of this, biophysical studies showed that increased kinesin density attenuates the inhibitory effects of viscous drag (similar to that encountered cytoplasmically) on MT gliding in vitro (Hunt et al., 1994).

To distinguish between these possibilities, it was essential to identify the kinesin responsible for transporting p75 in epithelial cells. To this end, we first screened for human KIFs expressed in epithelial cells. More than 40 kinesin family members are present in both human and mouse genomes, but only a subset of these are likely to be expressed in any given tissue type. By using primer-specific PCR, we found that ~70%–80% of kinesin family members are expressed in a variety of human epithelial cells. Of these, ~10%–20% had nonoverlapping expression in the different epithelial cells analyzed. These tissue-specific kinesins might perform functions specific to individual epithelia, including the transport of a tissue-specific complement of membrane proteins.

Expression of such a large number of kinesins raises the possibility that numerous KIFs could participate in post-Golgi transport of different cargoes in epithelia. This idea is supported by the well-documented, large number of KIFs expressed in neurons and their selectivity for distinct cargoes (see Introduction). An alternative, but not mutually exclusive, idea is that expression of a large number of KIFs in any one cell provides functional redundancy and that some or all "cargoes" can be transported by more than one KIF. Indeed, mitochondria positioning has been shown to be mediated by both KIF5B and KIF1B in neurons (Nangaku et al., 1994; Tanaka et al., 1998). Similarly, KIF5 and KIFC3 have both been shown to participate in apical trafficking of influenza-HA in polarized MDCK cells (Lafont et al., 1994; Noda et al., 2001). Whether KIFs that interact with the same cargo perform distinct or overlapping roles in the mechanics of cargo transport and organelle positioning remains unknown at this time.

To identify the kinesin family member involved in biosynthetic trafficking of p75, we coexpressed p75-YFP with CFP-tagged, KIF-specific dominant-negative inhibitory tail domains and evaluated effects of these KIF tails on post-Golgi transport and apical targeting of p75-YFP. We tested four KIFs identified from previous work as candidate apical transporters: KIF5 (Lafont et al., 1994), KIF3 (Fan et al., 2004), KIF17 (Jenkins et al., 2006), and KIFC3 (Noda et al., 2001). These KIFs were all expressed in epithelial cells at both mRNA and protein levels. Of these, only KIF5B tail inhibited post-Golgi transport of p75-YFP in polarized cells. In support of the kinesin switch hypothesis, expression of dominant-negative KIF5B-tail constructs or injection of KIF5-specific, function-blocking antibodies inhibited post-Golgi transport of p75-GFP in polarized cells but had no significant effect on p75-GFP trafficking in subconfluent cells. Furthermore, KIF5B coimmunoprecipitated with p75-GFP only in polarized cells. These results are consistent with our previous work showing that microinjection of KIF5-specific, function-blocking antibody, SUK4, had no effect on post-Golgi transport of p75-GFP in subconfluent cells (Kreitzer et al., 2000). Because MTs are dramatically reorganized during polarization (Bacallao et al., 1989; Gilbert et al., 1991), a switch in kinesin's cargo-selection machinery or motor activity may occur to facilitate establishment of new trafficking routes to the plasma membrane. Studies are underway to determine whether phosphorylation of KIF5 changes during polarization and whether this affects binding of KIF5 to vesicles containing p75-GFP.

Because KIF5 is a plus-end-directed kinesin, we revisited the issue of MT organization in polarized epithelia by 3-dimensional time-lapse microscopy. In cells expressing EB1-GFP, we observed a population of dynamic MTs growing from the centrosome toward the apical membrane. 3-dimensional reconstructions revealed a MT array similar to that described in primary rat hepatocytes (Novikoff et al., 1989) in which many MTs emanate from the centrosome and extend apically and apico-laterally. From our recordings, we could not determine whether these MTs remain nucleated or are ejected from the centrosome (Keating et al., 1997) or the extent of the MT array with apically oriented plus ends. Importantly, however, we clearly demonstrate that there are MTs capable of supporting plus-end-directed transport to the apical membrane. Whether cargoes carried by KIF5 utilize dynamic (EB1-labeled) or stabilized (primarily not labeled by EB1) MTs for apical transport is unknown. There is evidence that some KIFs interact preferentially with post-translationally modified, stabilized MTs (Ikegami et al., 2007; Liao and Gundersen, 1998; Reed et al., 2006), suggesting that targeted transport by kinesins could be influenced by the type of MT tracks present in cells.

One important, unsolved question is how kinesins recognize selectively the vesicular cargoes they transport. One possibility is that KIFs "select" their cargoes by interacting directly with distinct sorting signals carried by the cargo itself. Alternatively, cargo selection could be mediated via differential interaction of KIFs with adapter proteins that recognize the vesicles containing proteins with distinct sorting signals. Although a direct interaction between the dynemin light chain, Tctex-1, and the COOH-terminal tail of rhodopsin (vesicle-associated) has been shown (Tai et al., 1999), to our knowledge there are no definitive examples demonstrating direct interactions between a KIF and a vesicle-bound transmembrane protein. There are, however, numerous reports of KIF interactions with vesicle-bound cargo that are mediated through different vesicle adaptors. For example, KIF13A mediates vesicular transport of mannose-6-phosphate receptors in MDCK cells through an interaction with the vesicle adaptor AP1 (Nakagawa et al., 2000), whereas...
KIF17, via the PDZ scaffolding protein LIN-10, mediates transport of NMDA receptor subunits to dendrites (Setou et al., 2000). The diversity of sorting motifs suggests that numerous factors, motot recognition being only one, can contribute to protein sorting and membrane targeting. Identifying the KIFs used in transport of multiple cargoes to the same cellular destination may ultimately help solve this problem.

Along these lines, we tested whether expression of dominant-negative KIF5B-Tail altered trafficking of other apical membrane protein markers in polarized MDCK cells. The reporters we used represent well-characterized, distinct classes of apical membrane proteins that utilize either direct (nontranscytotic) or indirect (transcytotic) routes from the Golgi complex to the apical surface of MDCK cells. These include: (1) p75-neurotrophin receptor, a nonlipid raft-associated protein that utilizes O-glycans as apical targeting signals and requires galactin-3 for apical sorting (Delacour et al., 2006; Yeaman et al., 1997), (2) bile salt export pump (BSEP), an N-glycosylated apical endosome (Wakabayashi et al., 2004), (3) lectin-3 for apical sorting (Delacour et al., 2006; Yeaman et al., 2000) was provided by S. Tsukita (Kyoto University), and HA-tagged, mouse KIFC3 Tail (aa 1–515) was provided by A. Musch (Well-Cornell), a construct identical to this inhibited apical delivery of hemaglutinin and annexin XIb in MDCK cells (Noda et al., 2001). KIFSB, KIF3A, and KIF17 Tails were amplified by PCR of hemaglutinin and annexin XIIIb in MDCK cells (Noda et al., 2001). cDNAs were subjected to SDS-PAGE and immunoblotting with mouse anti-GFP serum or control IgG for 12 hr at 4°C. Protein A-Sepharose 3 ml/C0/C15 for YFP-GPI, 50 μg/ml/C0/C15 for p75-GFP, 50 μg/ml/C0/C15 for YFP-GPI, 50 μg/ml/C0/C15 for prominin-YFP, and 5–10 μg/ml/C0/C15 for KIF-Tails. After injection, cells were maintained at 37°C for 60 min to allow for expression of cDNAs. Monoclonal anti-KIF5 IgGs, H1 (provided by P. Leopold, Weill-Cornell Medical College), and SUK4 or mouse IgG (all at 9 mg/ml/C0/C15) were co-injected with p75-p75-GFP cDNA.

**Expression Constructs**

p75-GFP was described previously (Kreitzer et al., 2000). Prominin-YFP was provided by K. Zhang (University of Utah), YFP-GPI was provided by K. Simons (EMBL, Heidelberg, Germany), BSEP-YFP was provided by Y. Wakabayashi (NICHID, NIH), EB1-GFP (Mimori-Kiyosue et al., 2000) was provided by S. Tsukita (Kyoto University), and HA-tagged, mouse KIFC3 Tail (aa 1–515) was provided by A. Musch (Well-Cornell). A construct identical to this inhibited apical delivery of hemaglutinin and annexin XIib in MDCK cells (Noda et al., 2001). KIF5B, KIF3A, and KIF17 Tails were amplified by PCR from human A549 cells and cloned into mammalian Gateway (Invitrogen). cDNA concentrations were: 5 μg/ml/C0/C0 for p75-GFP, 50 μg/ml/C0/C0 for YFP-GPI, 50 μg/ml/C0/C0 for prominin-YFP, and 5–10 μg/ml/C0/C0 for KIF-Tails. After injection, cells were maintained at 37°C for 60 min to allow for expression of cDNAs. Monoclonal anti-KIF5 IgGs, H1 (provided by P. Leopold, Weill-Cornell Medical College), and SUK4 or mouse IgG (all at 9 mg/ml/C0/C15) were co-injected with p75-p75-GFP cDNA.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Microinjection**

Madine Darby canine kidney (MDCK) cells were cultured as described previously (Kreitzer et al., 2003). Cells were seeded on sterilized cover-slips at ~13,000 cells/cm² or ~130,000 cells/cm² and grown for 36 hr or 4–5 days for analysis of subconfluent cells or polarized cells, respectively. Cells were pressure microinjected intranuclearly with cDNAs in HKCI (10 mM HEPES, 140 mM KCl) [pH 7.4] via a Narishige microinjection micro manipulator (Narishige, Greenwale, NY). cDNA concentrations were: 5 μg/ml/C0/C0 for p75-GFP, 50 μg/ml/C0/C0 for YFP-GPI, 50 μg/ml/C0/C0 for prominin-YFP, and 5–10 μg/ml/C0/C0 for KIF-Tails. After injection, cells were maintained at 37°C for 60 min to allow for expression of cDNAs. Monoclonal anti-KIF5 IgGs, H1 (provided by P. Leopold, Well-Cornell Medical College), and SUK4 or mouse IgG (all at 9 mg/ml/C0/C15) were co-injected with p75-p75-GFP cDNA.

**RT-PCR**

We designed two sets of primers specific for each human kinesin family member (available upon request). Specificity was determined by BLAST analysis against the human genome. We prepared total RNA (with Trizol*, Invitrogen) or oligo-dT purified polyA mRNA from human epithelial cell lines and primary cultures including Caco2 (ATCC® HTB-38, T-84 (ATCC® CCL-248), A549 (ATCC® CCL-185), NHBE (Cambrex # CC-2540), 184B5 (ATCC®CRCL-8799), MCF-7 (ATCC® HTB-22), A498 (NCI-60 collection, provided by P. Giannakou, Well-Cornell), NHEK-A, NHEK-B, and A431 (ATCC® CRL-1555, provided by K. Green, Northwestern University), and MSKleuk1 (provided by A. Dannenberg and K. Subbaramaiah, Well-Cornell). cDNA was prepared by reverse transcription with Superscript II and oligo-dT (Invitrogen) and KIFs were amplified by PCR. GAPDH or actin primers were included as controls.

**Immunoprecipitation**

p75-GFP was immunoprecipitated from subconfluent or polarized MDCK cells stably expressing p75-GFP (Kreitzer et al., 2000) with rabbit anti-GFP (Novus Biologicals, Littleton, CO). Cells were lysed in HEPES 50 mM, 150 mM NaCl, 1.5 mM MgCl2, 0.1 mM CaCl2, 10% glycerol, 1% Triton X-100 (pH 7.5), 30 min after release of a 2 hr Golgi block at 20°C. 0.5 mg total protein was incubated with 1 μl of rabbit anti-GFP serum or control IgG for 12 hr at 4°C. Protein A-Sepharose 4B (Amersham, Uppalsa, Sweden, 100 μl of a 10% slurry) was added, and lysates were incubated 1 hr at 4°C and washed 3x in lysis buffer. Immunocomplexes were eluted by boiling in SDS sample buffer and subjected to SDS-PAGE and immunoblotting with mouse anti-GFP (1:1000, Roche, Indianapolis, IN) or mouse anti-KIF5 (H1 and H2, 1:500).

**Time-Lapse Imaging and Analysis**

After microinjection, newly synthesized protein was accumulated in the TGN at 20°C for 2.5 hr in bicarbonate-free DMEM with 5% FBS, 20 mM HEPES, and 100 μM cycloheximide. Cells were transferred to Recording Medium (Hanks Balanced Salt Solution with 20 mM HEPES, 1% FBS, 4.5 g/l glucose, essential and nonessential
Developmental Cell
A Kinesin Switch for Apical Transport in Epithelia

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