Kinesins comprise a large family of microtubule-based motor proteins, of which individual members mediate specific types of motile processes. Using the ezrin domain of the protein-tyrosine phosphatase PTPD1 as a bait in a yeast two-hybrid screen, we identified a new kinesin-like protein, KIF1C. KIF1C represents a member of the Unc104 subfamily of kinesin-like proteins that are involved in the transport of mitochondria or synaptic vesicles in axons. Like its homologues, the 1103-amino acid protein KIF1C consists of an amino-terminal motor domain followed by a U104 domain and probably binds to target membranes through carboxyl-terminal sequences. Interestingly, KIF1C was tyrosine-phosphorylated after peroxovanadate stimulation when overexpressed in 293 or NIH3T3 fibroblasts or in native C3C12 cells. Using immunofluorescence, we found that KIF1C is localized primarily at the Golgi apparatus. In brefeldin A-treated cells, the Golgi membranes and KIF1C redistributed to the endoplasmic reticulum (ER). This brefeldin A-induced flow of Golgi membranes into the ER was inhibited in cells transiently overexpressing catalytically inactive KIF1C. In conclusion, our data suggest an involvement of tyrosine phosphorylation in the regulation of the Golgi to ER membrane flow and describe a new kinesin-like motor protein responsible for this transport.

Proteins destined for secretion, plasma membrane, or intracellular organelles are synthesized in the endoplasmic reticulum (ER) and transported along the secretory pathway through the Golgi complex by coated vesicular carriers. Adjacent to the stacked cisternae of the Golgi complex, regions of the ER membrane bud off to form vesicles. These vesicles then seem to fuse with a tubular network at the cis face of the Golgi apparatus. Vesicles are formed from the ER by the action of the coat protein II complex, and they incorporate vesicle targeting molecules (known as v-SNAREs). These proteins interact with corresponding t-SNAREs, which are present on the target membranes of the cis-Golgi network. Proteins not only leave the ER near Golgi membranes but emerge at sites that are distributed apparently randomly over the cell. These sites are marked by little groups of vesicles and tubules (vesicular-tubular clusters). The vesicular-tubular clusters, containing the secreted proteins, travel along microtubules (MTs) to the Golgi apparatus, where they fuse with the cis-Golgi network. This flow of membranes into the Golgi complex needs to be counterbalanced by a reverse (Golgi to ER) membrane transport pathway that involves coat protein I-coated vesicles traveling along MTs (for reviews, see Refs. 1 and 2).

Intracellular transport along microtubules is controlled by two microtubule-based mechaenochemical motor protein superfamilies: the dyneins and the kinesin-like proteins (KLPs) or kinesin family proteins (KIFs). Nucleation of microtubule growth by the microtubule-organizing center, which in many cell types is located close to the nucleus, results in a radially polarized array of MT with their fast growing (plus) ends at the periphery of the cell and their slow growing (minus) ends at the cell center. Dyneins and some members of the kinesin superfamily drive movement toward MT minus ends, whereas conventional kinesin and most kinesin-related proteins are involved in the movement of membrane-bound organelles and vesicles toward the plus ends of microtubules. In addition to their function in vesicle transport, motor proteins of both superfamilies are also involved in mitosis and meiosis. Recently, dynein was identified by Presley et al. (3) as the motor protein mediating the vesicle transport from the ER to the Golgi complex.

Kinesins share a conserved motor domain of ~340 amino acids, and similarities have been used to construct molecular phylogenies of the KLPs with so far eight subfamilies and numerous outgroups being described (4, 5). Outside of the motor domain, the amino acid sequences of the KLPs differ from each other, reflecting their different functions. Like kinesin, most KLPs have a central alpha-helical region. This region is judged to mediate the homodimerization of kinesin heavy chains (KHCs). The tail of kinesin is associated with two kinesin light chains and thought to bind the cargo and/or accessory proteins. An integral membrane protein, kinectin, has been identified to act as the link between kinesin and its cargo (6). Very few associating proteins have been identified for KLPs. Candidates include KAP3 for KIF3A/3B regulating membrane binding of the KIF3 complex (7) and the serine/threonine phos-
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Experimental Procedures

Yeast Two-hybrid Screen—The ezrin-like domain of PTPD1 (Ref. 12; amino acids 1–345) was cloned into the LexA fusion protein vector pBTHST110 (Clontech) by S. Hollenberg and J. Cooper and transfected into Saccharomyces cerevisiae strain L40 (MATa trp1 leu2 his3 lys2::lexA HIS3 URA3::lexA lacZ; Ref. 13), generating the L40 lexA-D1ezrin strain. A skeletal muscle cDNA library fused to the GAL4 activation domain in the pGAD10 vector (CLONTECH) was transfected into the L40 lexA-D1ezrin strain. The anti-phosphotyrosine 4G10 antibody 4G10 was obtained from Upstate Biotechnology, Inc., and the phosducin antibody 5a, kindly provided by J. Cooper, was lyed in 1 ml of lysis buffer/10-cm plate (1% Triton X-100, 50 mM HEPES, pH 7.5, 10 mM glycerol, 150 mM NaCl, 1.5 mM MgCl2, 1 mM EGTA, 10 mM sodium pyrophosphosphate, 100 mM NaF, 1 mM sodium orthovanadate, 10 μM protinin, 1 mM phenylmethylsulfonyl fluoride), and the lysates were precleared by centrifugation at 13,000 rpm for 15 min at 4 °C. The lysates were adjusted for equal protein concentration, and the appropriate antibody (2 μl of serum or 2 μg of antibody) and protein A-Sepharose (Amersham Pharmacia Biotech) were added and incubated for at least 3 h at 4 °C. The immunoprecipitates were washed extensively with HNTG buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 100% glycerol, 10 mM NaF, 1 mM sodium orthovanadate), separated on an 8% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane, and incubated with the respective antibody. Bound antigens were visualized using the ECL system (Amersham Pharmacia Biotech) in conjunction with horseradish peroxidase-conjugated goat-anti-mouse or goat-anti-rabbit antibodies (BioRad, Sigma). Before reprobing, blots were incubated for 30 min in 62.5 mM Tris-HCl pH 6.8, 2% SDS, and 0.1% β-mercaptoethanol at 50 °C.

Immunofluorescence—For immunofluorescence studies, NIH3T3 cells grown on uncoated glass coverslips were fixed for 20 min in cold methanol at −20 °C, washed with phosphate-buffered saline, and incubated for 15 min with 0.1% NaN3 and 0.1 μM glycine in phosphate-buffered saline to block autofluorescence. Nonspecific antibody binding was blocked for 45 min with PBG (phosphate-buffered saline with 0.045% fish gelatin) containing 5% normal goat serum and 1% bovine serum albumin. Incubation with the primary antibody was done for 1.5 h at 37 °C after dilution in PBG containing 5% normal goat serum; the affinity-purified KIF1C antibody was used at a concentration of 20 ng/μl unless otherwise stated, and the 58K antibody (Sigma) was diluted as recommended by the manufacturer. After five washes with PBG, primary antibody binding was detected with isotype-specific secondary antibody conjugated with either DTAf or Cy3 (Dianova). The coverslips were mounted in PermaFlour (ImmuneTech). Triton extraction for immunofluorescence under MT-stabilizing conditions was performed prior to fixation for 5 min on ice in a buffer containing 100 mM Pipes, pH 7.0, 5 mM MgSO4, 10 mM EGTA, 2 mM dithiothreitol, 10% glycerol, 0.75% Triton X-100, 10 μM taxol (Sigma), 10 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride. Lysate under MT-destabilizing conditions was performed in the lysis buffer described above lacking EGTA and with the addition of 80 μM Colcemid (21).

RESULTS

Cloning of KIF1C—PTP1D1 belongs, together with PTPPEZ (22), PTPH1 (23), PTPM2G (24), and PTPNAS (25), to the group of cytosolic protein-tyrosine phosphatases containing an ezrin-like domain. This domain is homologous to the amino-terminal portion of the cytoskeleton-associated proteins of the band 4.1 superfamily, and in the case of the ERM proteins (ezrin, radixin, and moesin) mediates the association with the membrane-spanning protein CD44 (26), thereby linking the cytoskeleton with the plasma membrane. By contrast, the functional relevance of this ezrin-like domain in PTPs has not yet been described. To identify proteins binding to the ezrin-like domain of PTP1D1, we employed the yeast two-hybrid system (for a review, see Ref. 27). A human skeletal muscle cDNA library was screened using the ezrin-like domain of PTP1D1 (amino acids 1–345) fused to the lexA DNA binding domain as the bait. Out of 105 clones screened, nine clones were found to interact with the ezrin-like domain of PTP1D1 in yeast. Sequence analysis suggested that the clones encoded cDNAs derived from the same gene. None of the clones isolated contained a full-length open reading frame, but all of...
them encoded an overlapping sequence of 96 amino acids (amino acids 714–809), apparently sufficient for binding PTPD1. The missing 5'-end of the cDNA was isolated from a human hippocampus library by conventional screening using the K1 cDNA obtained in the two-hybrid screen (base pairs 1196–4082) as a probe. The sequence showed greatest homology to the kinesin-like motor protein KIF1B (28), and in accordance with the nomenclature introduced by Nangaku et al. (28) and Okada et al. (29), this protein was named KIF1C.

The complete cDNA of KIF1C has a size of 4.1 kilobase pairs and contains a single open reading frame coding for a 1103-amino acid protein with a calculated molecular weight of 122,000 (Fig. 1). KIF1C belongs to the family of kinesin-like proteins as evidenced by the presence of the kinesin signature (amino acids 242–253) in the amino-terminal motor domain. In addition, the protein contains a P-loop sequence (amino acids 97–104) characteristic for ATP- or GTP-binding proteins (30) and the consensus sequence of the nucleotide binding motif for kinesins (31, 32). Like all MT-dependent motor proteins, KIF1C is capable of binding microtubules in vitro and in intact cells (data not shown). Close to the amino-terminal motor domain, KIF1C contains a U104 domain, which is also found in the other kinesins belonging to the Unc104 subfamily and in the AF-6 and Cno gene products (33). The function of the U104 domain has not yet been defined.

Sequence analysis places KIF1C in the Unc104 subfamily of the kinesin-like proteins. This subfamily consists of the murine proteins KIF1A (29) and KIF1B (28), the human protein ATSV (34), the C. elegans protein Unc104 (35, 36) and the Drosophila protein KLP38B (8). More recently, in a polymerase chain reaction screen, a partial amino acid sequence of the motor domain of murine KIF1C was identified and showed 98% homology to human KIF1C (37). In Fig. 2, a sequence alignment of the three known mammalian members of this subfamily is depicted. KIF1C shows a high degree of homology to KIF1A and KIF1B in the motor and U104 domain but significantly lower homology to these proteins in the central and carboxyl-terminal regions. While most kinesin-like proteins form homodimers by folding an α-helical coiled-coil in the stalk domain (4), the proteins of the Unc104 subfamily are judged to exist predominantly as globular monomers due to the low probability of forming coiled-coils (28, 29). Using the algorithm of Lupas (38), we found that KIF1C also has only a low probability to form coiled-coil structures (data not shown). This implies that KIF1C, similar to KIF1A and KIF1B, may act as a monomeric motor.

Expression of KIF1C—To study the size of the transcript and the tissue distribution of KIF1C, Northern blot analysis using the cDNA fragment K9 (base pairs 2252–3126) as a probe was
performed. A KIF1C transcript of approximately 4.2 kilobase was detected in all tissues examined but with especially high expression in heart and skeletal muscle (Fig. 3A).

For Western blot analysis, a rabbit polyclonal antiserum against KIF1C was raised against a glutathione S-transferase-KIF1C fusion protein containing the intervening region between the motor domain and the PTPD1 binding domain of KIF1C. When screening various cell lines for KIF1C protein expression, we detected KIF1C as a protein of approximately 135 kDa (Fig. 3B). The discrepancy between the calculated molecular weight and the one observed in SDS-PAGE could be caused by posttranslational modifications of KIF1C. Interestingly, the human KIF1C has a slightly higher mobility than the rodent protein. This may be due to differences in the number of amino acids or, alternatively, to posttranslational modifications. The size of the protein in human cell lines corresponds to the size of the protein expressed after transfection of a KIF1C expression construct into 293 cells, indicating that we indeed cloned the complete sequence of KIF1C.

Binding of KIF1C to PTPD1—As described above, KIF1C was isolated as a cDNA encoding a protein capable of binding to the ezrin-like domain of PTPD1. Nine cDNAs were isolated, which all differ in length from each other and thus are independent and not derived from clonal amplification of the same original cDNA. They all contain a region in the carboxyl-terminal half of KIF1C, encoding the amino acids 714–809. This demonstrates that these 96 residues are sufficient to bind PTPD1 (Fig. 4A). While the two-hybrid system has now been successfully used many times to demonstrate protein-protein binding, it is also known to be capable of producing false positives. To confirm the interaction of PTPD1 and KIF1C in a different system, we examined the association under the condition of overexpression within cells. Therefore, we expressed KIF1C alone or together with PTPD1 transiently in 293 cells. After immunoprecipitations using the PTPD1 antiserum, the precipitated proteins were separated by SDS-PAGE, trans-
KIF1C protein in human, mouse, and rat cell lines. 30 μg of protein of PC12, C2C12, NIH3T3, RD, and 293 cells were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted using the KIF1C antiserum. KIF1C immunoprecipitated from overexpressing 293 cells was used as a control. Size markers are indicated in kDa, and KIF1C is marked by an arrow.

ferred to nitrocellulose, and blotted with the affinity-purified KIF1C antibody. As shown in Fig. 4B, KIF1C coprecipitated with PTPD1. The specificity of the KIF1C interaction was investigated in a similar coexpression experiment with a related tyrosine phosphatase, PTPH1, also containing an amino-terminal ezrin-like domain. However, no coprecipitation of KIF1C with PTPH1 could be observed, although KIF1C was expressed (Fig. 4B). Furthermore, we tested the ezrin-like domains of PTPBAS, PTPMEG, and PTPD2 and did not find an association with KIF1C (data not shown). The binding of KIF1C is therefore specific for PTPD1, and future work is necessary to show the physiological relevance of this interaction.

Tyrosine Phosphorylation of KIF1C—Since KIF1C was identified by its association with a PTP, we investigated whether KIF1C itself can be tyrosine-phosphorylated. We therefore expressed KIF1C in 293 cells and stimulated the cells with POV. In a parallel experiment, we coexpressed KIF1C with the cytosolic tyrosine kinase c-Src. As shown in Fig. 5A, KIF1C was tyrosine-phosphorylated upon treatment with POV and also upon coexpression with c-Src. To prove equal amounts of KIF1C expression in the experiment, we reprobed the filter with KIF1C antibody. The lower band probably represents an unphosphorylated degradation product.

To confirm these data in a cell line stably overexpressing KIF1C, a NIH3T3-derived cell line was generated by infection with a retrovirus encoding KIF1C. The 3T3/X/KIF1C cells were stimulated with POV before lysis, and KIF1C was immunoprecipitated. As observed in 293 cells, KIF1C was again tyrosine-phosphorylated (Fig. 5B). The phosphorylated proteins detected in addition to KIF1C could represent associated proteins.

We next investigated the tyrosine phosphorylation state of endogenous KIF1C in undifferentiated mouse C2C12 cells. KIF1C was immunoprecipitated from cleared Triton X-100 lysates and analyzed by Western blot. As shown in Fig. 5C, KIF1C is tyrosine-phosphorylated after POV stimulation and to a low extent also in unstimulated cells, as observed in longer exposures of the same blot. These data indicate that the motor protein KIF1C is tyrosine-phosphorylated in intact cells, which can be enhanced by treatment of the cells with the PTP inhibitor POV. As a tyrosine kinase potentially capable of phosphorylating KIF1C, c-Src was identified.

Localization of KIF1C in NIH3T3 Cells—To elucidate the
functions of KIF1C, we performed immunofluorescence experiments utilizing the affinity-purified antibody against endogenous KIF1C. Studies in NIH3T3 fibroblasts revealed that KIF1C is localized mainly on a tubular structure close to the nucleus, which is reminiscent of the stacks of the Golgi apparatus (Fig. 6A). In a control experiment, the Golgi apparatus was detected using a monoclonal antibody against the protein 58K (Fig. 6C) which is described to be located specifically at the cis/medial site of the Golgi apparatus by binding simultaneously to microtubules and the Golgi membranes (39, 40). To prove that KIF1C indeed colocalizes with the Golgi, we performed double staining of KIF1C and 58K. Conventional microscopy (Fig. 6, D and E) as well as laser confocal microscopy (data not shown) clearly demonstrated the colocalization of KIF1C with the Golgi stacks. Treatment of cells with taxol and the microtubule-disrupting agent colcemid leads to a fragmentation of the Golgi membrane system into numerous vesicle-like structures of varying size that are distributed throughout the cytoplasm (41, 42). This characteristic scattered pattern was also found for KIF1C after incubation of the cells for 4 h either with taxol or with colcemid (data not shown).

We postulated KIF1C to be a microtubule-based motor protein. Thus, KIF1C should associate with the microtubular cytoskeleton and should not be extractable from the cell while the microtubules are intact. Therefore, we lysed the cells under MT-stabilizing conditions to extract cytosolic proteins prior to fixation and then stained the cytoskeleton and the associating proteins with the KIF1C antiserum. As depicted in Fig. 6B, a signal specific for KIF1C can be observed at a structure resembling the Golgi. When cells were lysed in the presence of calcium ions under MT-destabilizing conditions, however, all KIF1C protein was extracted (data not shown). These data show that KIF1C localized at the Golgi apparatus is associated with the microtubular cytoskeleton.

We also wanted to investigate the behavior of KIF1C under the influence of BFA, which has been shown to induce a rapid redistribution of the Golgi membranes into the ER (43, 44). Removal of the drug results in the sorting of Golgi components out of the ER and reformation of the Golgi. To investigate whether KIF1C remains associated with the membranes of the Golgi and cycles with these membranes to the ER, we examined its distribution in cells treated with BFA. A typical time course of the effect of BFA on KIF1C is depicted in Fig. 6, F and G. After 2 min of incubation of the cells with the drug, Golgi membranes and KIF1C redistributed into the ER via tubular processes that extended toward the cell periphery. After a 20-min treatment with BFA, KIF1C showed the punctuate pattern characteristic of ER staining. This occurred in a similar way as described for the Golgi marker protein 58K (45). Removal of the drug after 30 min resulted in the reassembly of the Golgi apparatus and the relocalization of KIF1C to the Golgi within 2 h (Fig. 6H).

Inhibition of the Flow of Golgi Membranes into the ER by Transient Overexpression of Catalytically Inactive KIF1C—

Golgi to ER transport is MT-dependent and occurs toward the plus ends of microtubules (40). Since all proteins of the Unc104 subfamily are described to be MT plus end-directed motors (28, 29), we wondered whether KIF1C functions as a vesicle-transporting motor protein in the membrane flow from the Golgi to the ER. A lysine to alanine mutation in the P-loop has been described for other ATP-binding proteins to abolish ATP binding (30, 46). KIF1C-K103A carries such a mutation and is catalytically inactive, while its other properties should not be affected. Consequently, overexpressed KIF1C-K103A should compete with and replace endogenous

**Fig. 6. Intracellular localization of KIF1C.** The localization of KIF1C was determined by immunofluorescence analysis (A–H) in NIH3T3 cells. The cells were fixed with methanol and stained either with the KIF1C antibody (A, B, D, F, G, and H) or with the 58K antibody (C and E) followed by DTAF goat anti-rabbit or Cy3 goat anti-mouse antibody, respectively. Cells in B were extracted with Triton X-100 under MT-stabilizing conditions prior to fixation. For BFA treatment, cells were incubated with 5 μg/ml BFA for 2 min (F), 20 min (G), or 30 min followed by a recovery period of 2 h in BFA-free medium (H).
KIF1C on Golgi membranes. We overexpressed KIF1C-K103A transiently in NIH3T3 cells and analyzed these cells by immunofluorescence. A double staining of the cells using the 58K antibody and the affinity-purified KIF1C antibody was performed. The concentration of the KIF1C antiserum was chosen to detect KIF1C only in overexpressing cells. As shown in Fig. 7A, overexpressed KIF1C-K103A primarily localized to the Golgi compartment but was also found diffusely distributed in the cytoplasm due to the high expression level. The morphology of the Golgi apparatus as evidenced by the localization of the marker protein 58K was not significantly altered when compared with untransfected cells. Like KIF1C-K103A, ΔNT-KIF1C blocked the BFA-induced flow of Golgi membranes to the ER (data not shown).

Interestingly, overexpressed wild-type KIF1C did not localize to the Golgi apparatus but was found distributed over the cell and accumulated in cell periphery (Fig. 7E). The Golgi structure in the transfected cells was not altered compared with untransfected cells (Fig. 7F). Upon treatment with BFA, the discrete Golgi structure disappeared and formed the diffusive ER-like pattern in transfected as well as in untransfected cells (Fig. 7G, H). These data clearly demonstrate that cells overexpressing a transport inactive form of KIF1C are resistant to BFA-induced morphological changes.

**FIG. 7. Inhibition of the BFA induced retrograde flow of Golgi membranes to the ER by transient expression of catalytically inactive KIF1C.** NIH3T3 cells transiently overexpressing KIF1C-KA (A–D) or KIF1C (E–H) were double stained with an antiserum against the Golgi marker protein 58K (right panels) and the KIF1C antiserum (5 ng/µl; left panels). The cells were either left untreated (A, B, E, and F) or treated for 15 min with BFA before staining (C, D, G, and H). Note the disappearance of the Golgi complex as a compact structure in untransfected and KIF1C-expressing cells, while the morphology of the Golgi in KIF1C-KA-transfected cells remained intact after BFA treatment.

KIF1C: A New Motor Protein Involved in Golgi to ER Transport

In the present study, we used the ezrin-like domain of PTPD1 in a functional screen to detect possible targets of the phosphatase, and we identified KIF1C, a protein belonging to the family of kinesin-like proteins. The association observed in the yeast two-hybrid screen was verified in vitro and in 293 cells transiently overexpressing both proteins. Although the evaluation of the physiological relevance of this association is still in progress, this association of a kinesin-like motor protein with a PTP is an important finding and may lead to new insights into the regulation of intracellular membrane flow by tyrosine phosphorylation.

A number of kinesin-like proteins have recently been identified by a variety of methods, including a systematic polymerase chain reaction screen of a murine brain cDNA library (47). In this screen the mammalian members of the Unc104 subfamily, namely KIF1A (29) and KIF1B (28), were cloned and further characterized. Sequence analysis of KIF1C revealed a high similarity with KIF1B in the motor domain but weaker homology in the central or tail domains. We therefore consider KIF1C to be a new member of the Unc104 subfamily with similar properties as described for KIF1B, which is a plus end-directed microtubule-dependent motor protein. Although we have not yet demonstrated the motor activity of KIF1C itself, there are several hints suggesting such an activity; next to the high homology between KIF1C and KIF1B in the motor domain and the presence of the conserved kinesin nucleotide binding motifs (32), immunoprecipitations of β-tubulin from lysates of 293 cells resulted in a coprecipitation of KIF1C. In addition, KIF1C associated with purified MT (data not shown). After lysis of NIH3T3 cells using MT-stabilizing conditions prior to fixation, we could still detect KIF1C in immunofluorescence experiments, whereas it was depleted after lysis of the cells under MT-stabilizing conditions, a behavior that has also been observed for the motor protein dynein (21). Finally, transient overexpression of wild-type KIF1C in NIH3T3 fibroblasts resulted in an accumulation of KIF1C in the cell periphery. This is similar to experiments using neurons, where accumulation of motor proteins on the proximal or distal site of ligated axons indicates plus or minus end-directed movement (48). It supports the idea that KIF1C indeed is a plus end-directed motor protein that moves under the conditions of overexpression along MTs to the cell periphery. In vitro data suggested that the variable tail domain of KHC is responsible for vesicle cargo binding (49). Since the amino-terminally truncated ΔNT-KIF1C protein, lacking the motor and the U104 domain, is still capable of binding Golgi membranes, our data indicate that for
KIF1C is ubiquitously expressed in motor protein that belongs to the Unc104 subfamily of KLPs. In the cell it mainly localizes to the Golgi apparatus and probably is involved in the membrane trafficking from the Golgi to the ER. Future work is necessary to determine the role of the observed tyrosine phosphorylation and the physiological relevance of the association of KIF1C with the PTPD1.

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**Supplementary Material**

KIF1C, an essential motor protein involved in Golgi to ER transport. *J. Cell Biol.* 119, 1121–1131

**Figures**

1. A mechanism of vesicle transport from the Golgi to the ER
2. In conclusion, KIF1C is a ubiquitously expressed motor protein involved in Golgi to ER transport.
3. The mechanism of vesicle transport from the Golgi to the ER has been a subject of controversial experiments. Based on microinjection of anti-KHC antibody into fibroblasts, it was suggested that this transport is mediated by kinesin (50). However, by using antisense oligonucleotide techniques, Feiguin et al. demonstrated that kinesin is responsible for maintaining the Golgi structure in astrocytes but not for the BFA-induced Golgi to ER transport. By expression of a dominant negative KHC mutant in fibroblasts, Nakata and Hirokawa demonstrated that KHC is involved in the anterograde transport of lysosomes but not in the retrograde Golgi to ER transport. The different techniques that have been used could account for these in part contradictory results. The microinjected antibody against KHC may cross-react with other KLPs due to the high homology between the kinesin superfamily members. It is therefore possible that other proteins of the kinesin superfamily are involved in the Golgi to ER pathway that are not affected by the antisense approach and the dominant negative mutant but are blocked by the microinjected antibody (53). We used a similar technical approach as Nakata and Hirokawa by transiently expressing a catalytically inactive form of the protein of interest. Consequently, our data support their result that KHC is not involved in the Golgi to ER membrane flow and suggest a newly identified motor protein, KIF1C, for this process.

The predicted cycling of KIF1C between Golgi and ER must be tightly regulated to prevent an imbalance between the two compartments. One possible regulatory element could be the observed tyrosine phosphorylation of KIF1C. Based on in vitro experiments, a basal tyrosine phosphorylation of KHC has already been suggested by Matthies et al. (11), but the physiological role of this phosphorylation remained unclear. By contrast, an extensive in vivo phosphorylation on serine has been observed for kinesin and kinesin-like proteins as well as for dynactin by several groups (9, 54–59). Serine phosphorylation of kinesin by protein kinase A and protein kinase C had no effect on the velocity of microtubule translocation of kinesin in vitro (54) but remarkably reduced the affinity of kinesin to vesicles (55). We found that a part of the cellular pool of KIF1C was tyrosine-phosphorylated even in serum-starved cells. This tyrosine phosphorylation was enhanced by treatment of the cells with the PTP inhibitor PUV. Since tyrosine phosphorylation and dephosphorylation are regulative elements in many cellular processes (60), we speculate that the observed tyrosine phosphorylation of KIF1C could be an important regulator of either its motor activity or its association with distinct vesicles and thereby its localization to different compartments within the cell.

In conclusion, KIF1C is a ubiquitously expressed motor protein that belongs to the Unc104 subfamily of KLPs. In the cell it mainly localizes to the Golgi apparatus and probably is involved in the membrane trafficking from the Golgi to the ER. Future work is necessary to determine the role of the observed tyrosine phosphorylation and the physiological relevance of the association of KIF1C with the PTPD1.
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